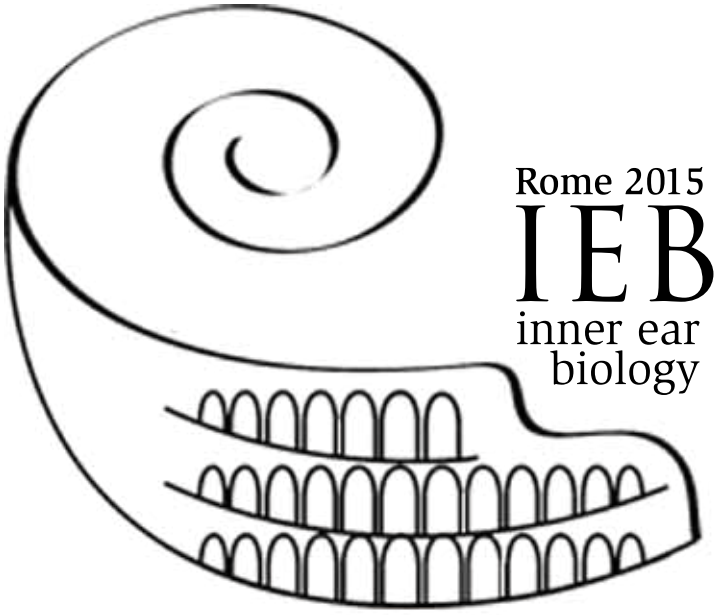

Gemelli
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Università Cattolica del Sacro Cuore

AudioLab
UCSC - Rome

Symposium & Workshop
52nd Inner Ear Biology
12-15 September 2015 - Rome, Italy

Chairs: Gaetano Paludetti and Diana Troiani

Program and Abstract book

Contents

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Dear Friends and Collegues,

It is with great pleasure that we warmly welcome all Participants at the 52nd Inner Ear Biology Workshop and Symposium.

First of all, we are grateful for the great number of contributions sent by Delegates from all around the world, about 80 oral presentations and 150 posters.

We are sure that together we can reach the goal of promoting the dialogue between basic research and clinical practice. As is customary, in conjunction with the Workshop there will be an opening Symposium day focused on the perspectives of inner ear research "From Lab to Clinic".

In addition to the outstanding Target Lectures, this year we have introduced a short oral presentation also for posters, in order to improve interactive discussion between authors and audience. We hope you will approve this innovation.

We believe that you will also enjoy our beautiful city. Rome will welcome you with its magnificent history, its churches and basilicas, its museums and piazzas. At the same time, "la città eterna" is honored to host such outstanding researchers. We have planned an attractive social program for all delegates and accompanying persons.

We would like to express our thanks to the pharmaceutical and manufacturing industries for their generous support, to the Organizing Secretariat "Meet and Work" for its active organizational contribution, to colleagues and friends for their untiring help, support and advice in planning and arranging this meeting.

We hope that you will enjoy the Congress and that your interaction with your colleagues from many different countries will stimulate a creative exchange of ideas and will be personally rewarding.

Yours sincerely,

Gaetano Paludetti and Diana Troiani

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Catholic University
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Conflicts of Interest declared:

*[1] Research support, stock shareholder and consultation fees - Inception 3, Inc.
*[2] Research resources in kind - Cochlear
*[3] Research support - L-3 Technologies; consultation fees - Otonomy Inc. and L-3 Technologies; stock shareholder – Otonomy Inc.
*[4] Preclinical research grant - MedEl
*[5] Research support – Cochlear Europe Ltd
*[6] Grants and research support – Amplifon, Audionova, Audibel, Audiologika, Central Acoustic 2000; consultation fees - COTER srl

SYMPOSIUM

"From lab to clinic: The opportunities and challenges for hearing rehabilitation and inner ear therapies"

Saturday 12 September 2015

Auditorium University Campus

08.30	Registration
09.00	Welcome addresses

SESSION I

Chairman: Marco de Vincentiis
Moderators: Alessandro Martini, Maurizio Barbara

09.15	S1	NEUROTRANSMISSION AND NEUROPROTECTION IN THE COCHLEA Jean-Luc Puel (Montpellier, France)
09.40	S2	OXIDATIVE STRESS AS A TARGET OF ACQUIRED SENSORINEURAL HEARING LOSS Allen Ryan (San Diego, USA)
10.05	S3	INFLAMMATORY AND IMMUNE RESPONSES IN THE COCHLEA: FROM THE PERSPECTIVE OF BASIC RESEARCH TOWARD CLINICS Masato Fujioka (Tokyo, Japan)
10.30	S4	CONNECTING INNER EAR CONNEXINS STRUCTURE TO FUNCTION AND DYSFUNCTION Fabio Mammano (Rome, Italy)
10.55 - 11.15		Coffee break

SESSION II

Chairman: Marco de Vincentiis
Moderators: Paolo Gasparini, Diego Zanetti

11.15	S5	LINKING GENES UNDERLYING DEAFNESS TO CLINICAL PERSPECTIVES Christine Petit (Paris, France)
11.40	S6	A PERSPECTIVE ON PROSPECTS AND CHALLENGES FOR HAIR CELL REGENERATION Andrew Forge (London, UK)
12.05	S7	ADDRESSING CHALLENGES FOR THE CLINICAL APPLICATION OF HUMAN STEM CELLS Marcelo Rivolta (Sheffield, UK)
12.30	S8	SENSORIMOTOR PROCESSING OF SPEECH Luciano Fadiga (Ferrara, Italy)
13.00 - 14.00		Lunch

SESSION III

Chairman: Agostino Serra
Moderators: Fabrizio Ottaviani, Stefano Berrettini

14.00	S9	BRAIN AND NEUROIMAGING Renzo Manara (Salerno, Italy)
14.25	S10	EFFECT OF CROSS-MODAL PLASTICITY ON AUDITORY FUNCTION IN CONGENITAL DEAFNESS Andrej Kral (Hannover, Germany)
14.50	S11	MOLECULAR UNDERPINNINGS OF TINNITUS/HYPERACUSIS Marlies Knipper (Tübingen, Germany)
15.15	S12	PRESBYCUSIS IN EXPERIMENTAL ANIMALS AND IN MEN Josef Syka (Prague, Czech Republic)
15.40 - 16.00		Coffee break

SESSION IV

Chairman: Agostino Serra
Moderators: Roberto Filipo, Domenico Cuda

16.00	S13	NEW INSIGHT ON HEARING AIDS AND AGING Mark Laureyns (Antwerp, Belgium)
16.25	S14	PRESENT STATUS AND FUTURE DEVELOPMENTS OF ACOUSTIC IMPLANTS (MIDDLE EAR IMPLANTS) Thomas Lenarz (Hannover, Germany)
16.50	S15	A THREE DIMENSIONAL PERCEPT IN THE ABSENCE OF FUSION: WHAT COCHLEAR IMPLANTS IN CHILDREN HAVE TAUGHT US ABOUT THE DEVELOPING AUDITORY SYSTEM Blake Papsin (Toronto, Canada)
17.15	S16	OPTOGENETIC STIMULATION OF THE AUDITORY NERVE FOR RESEARCH AND FUTURE COCHLEAR IMPLANTS Tobias Moser (Göttingen, Germany)
17.40	S17	NEW INSIGHT ON COCHLEAR IMPLANTS Naida CI: the combination of Advanced Bionics and Phonak technology Patrick Boyle - *Advanced Bionics* Hearing Implant Technology Carl Van Himbeeck - *Cochlear* Cochlear implants in single-sided deafness: What can we learn? Reinhold Schatzer - *Med-El*
18.30 - 20.00		Welcome Reception

52nd INNER EAR BIOLOGY WORKSHOP

Sunday 13 September 2015

Catholic University
Giovanni XXIII Building, 1st floor
Lazzati Room

SESSION I

Chairman: Ettore Cassandro

DEVELOPMENTAL BIOLOGY AND REGENERATION Moderators: Isabel Varela-Nieto, Elisabetta Genovese		
08.00	TL1	**TARGET LECTURE** IDENTIFICATION OF PROGENITOR CELLS IN THE COCHLEA: LGR5 POSITIVE SUPPORTING CELLS Albert S.B. Edge Fuxin Shi, Lingxiang Hu (Boston, USA)
08.25	O1	THE EXPRESSION OF NETRIN-1 RECEPTORS IN SENSORY EPITHELIUM AND SPIRAL GANGLION CELLS OF THE NEONATAL COCHLEA Norio Yamamoto, Kohei Yamahara, Takayuki Nakagawa, Juichi Ito (Kyoto, Japan)
08.38	O2	THE ROLE OF SOX2 IN INNER EAR DEVELOPMENT Martina Dvorakova[1], Romana Bohuslavova[1], Bernd Fritzsch[2], Josef Syka[1], Tatyana Chumak[1], Gabriela Pavlinkova[1] ([1]Prague, Czech Republic; [2]Iowa City, USA)
08.51	O3	ROLES OF HGF/MET SIGNALING IN THE MOUSE COCHLEA Toru Miwa[1,2], Takahiro Ohyama[2], Shumei Shibata[3], Ryosei Minoda[1], Eiji Yumoto[1] ([1]Kumamoto, Japan; [2]Los Angeles, USA; [3]Fukuoka, Japan)
09.04	O4	ATOH1 INDUCED AUDITORY SENSORY EPITHELIUM REGENERATION *IN VITRO* Ning Cong, Juanmei Yang, Dongdong Ren, Zhao Han, Yibo Huang, Wenwei Luo, Fanglu Chi (Shanghai, China)
09.17	O5	WNT-RESPONSIVE CELLS MITOTICALLY REGENERATE HAIR CELLS IN THE NEONATAL MOUSE UTRICLE Alan G. Cheng[1], Tian Wang[1], Renjie Chai[1,2], Grace Kim[1], Nicole Pham[1] ([1]Stanford, USA; [2]Nanjing, China)
09.30	O6	REGIONAL DIFFERENCES OF MOUSE UTRICLE HAIR CELLS PROLIFERATION AND DIFFERENTIATION AND ESTABLISHMENT OF THE PLANAR CELL POLARITY Xiao-Yu Yang, Xiang Liu, Rui Ma, Juan-Mei Yang, Fang-Lu Chi, Dong-dong Ren (Shanghai, China)
09.42	O7	A HIERARCHY OF SIGNALING MECHANISMS INITIATES AND COORDINATES INNER EAR HAIR CELL PLANAR POLARITY Michael R. Deans (Salt Lake City, USA)
09.50	O8	DO YOU HEAR WHAT I HEAR? REJUVENATING THE SENSORY EPITHELIUM OF THE INNER EAR Ksenia Gnedeva, A. James Hudspeth (New York, USA)

POSTER SESSIONS

Lazzati Room

10.10 - 11.00	POSTER SESSION I (details on page 19)
	DEVELOPMENTAL BIOLOGY AND REGENERATION **Moderators:** Marta Roccio, Giovanni Almadori

Bausola Room

10.10 - 11.00	POSTER SESSION II (details on page 20)
	AGING AND VESTIBULAR DISORDES **Moderators:** Raekil Park, Pasqualina M. Picciotti

Open Coffee

Lazzati Room

SESSION II

Chairman: Desiderio Passali

MOLECULAR BIOLOGY AND CELL PHYSIOLOGY (I) **Moderators:** Anthony Gummer, Renata Sisto		
11.00	TL2	**TARGET LECTURE** THE IDENTIFICATION AND LOCALIZATION OF COMPONENTS OF THE MECHANOTRANSDUCTION COMPLEX IN HAIR CELLS Bechara Kachar (Bethesda, USA)
11.25	O9	ON THE CHLORIDE DEPENDENCE OF PRESTIN CHARGE MOVEMENT Joseph Santos-Sacchi, Lei Song (New Haven, USA)
11.38	O10	ADAPTATION INDEPENDENT MODULATION OF AUDITORY HAIR CELL MECHANOTRANSDUCTION CHANNEL OPEN PROBABILITY IMPLICATES A ROLE FOR THE LIPID BILAYER Anthony Peng, Anthony Ricci (Stanford, USA)
11.51	O11	CYCLIC GMP-GATED CNGA3 AND CNGB1 IN HAIR CELLS Marian J Drescher, Dakshnamurthy Selvakumar, Dennis G Drescher (Detroit, USA)
12.04	O12	EPITHELIAL GAP JUNCTION NETWORK IN THE HUMAN COCHLEA – AN ULTRASTRUCTURAL AND CONFOCAL LASER IMMUNOHISTOCHEMISTRY STUDY Wei Liu[1], Rudolf Glueckert[2], Göran Laurell[1], Annelies Schrott-Fischer[2], Helge Rask-Andersen[1] ([1]Uppsala, Sweden; [2]Innsbruck, Austria)
12.17	O13	ANALITYCAL APPROACHES TO THE DIAGNOSIS AND TREATMENT OF AGE–RELATED HEARING LOSS: REDOX STATUS AND PROTEOMICS A. Serra, V. Calabrese, L. Maiolino, S. Cocuzza, P. Di Mauro (Catania, Italy)
12.30	O14	THE REDOX PROTEIN P66SHC MEDIATES COCHLEAR ENDOTHELIAL DYSFUNCTION AND EARLY NOISE-INDUCED HEARING LOSS IN MICE G. Pani, S.L.M. Eramo, F. Paciello, R. Rolesi, A.R. Fetoni, G. Paludetti, D. Troiani (Rome, Italy)
12.43	O15	MOLECULAR BASIS OF HEREDITARY DEAFNESS: A THEORETICAL APPROACH Francesco Zonta[1], Damiano Buratto[2], Guang Yang[1], Fabio Mammano[2,3] ([1]Shanghai, China; [2]Padova, Italy; [3]Rome, Italy)

13.00-13.15		Group photo
13.15-14.00		Lunch time

Lazzati Room

SESSION III

Chairman: Carlo Antonio Leone

GENETICS OF HEARING **Moderators:** Ignacio del Castillo, Rosamaria Santarelli		
14.00	TL3	**TARGET LECTURE** GENETICS OF HEARING LOSS: PAST, PRESENT AND FUTURE Paolo Gasparini (Trieste, Italy)
14.25	O16	GENETIC POLYMORPHISMS IN INNER EAR DISORDERS: BACKGROUND AND PRELIMINARY RESULTS Maurizio Simmaco, Luigi Volpini, Isotta Musy, Davide Leonardo, Simonetta Monini, Maurizio Barbara (Rome, Italy)
14.38	O17	THE P.S178L HOT-SPOT MUTATION OF *TBC1D24* LEAD TO DOMINANT, PROGRESSIVE AND NON-SYNDROMIC HEARING IMPAIRMENT THROUGH A GAIN-OF-FUNCTION MECHANISM Xiuhong Pang, Luping Zhang, Lingxiang Hu, Hao Wu and Tao Yang (Shanghai, China)
14.51	O18	INCOMPLETE COMPENSATION OF ANGULIN1/LSR TO RESCUE DFNB42, CAUSED BY MUTATION IN ANGULIN2/ILDR1 Shin-ichiro Kitajiri, Tomohito Higashi, Tatsuya Katsuno, Mikio Furuse (Kyoto, Japan)
15.04	O19	NOVEL MUTATIONS IN DFNB59, THE GENE ENCODING PEJVAKIN, IN SUBJECTS WITH AUTOSOMAL RECESSIVE NON-SYNDROMIC HEARING IMPAIRMENT AND AUDITORY NEUROPATHY SPECTRUM DISORDER María Domínguez[1], Montserrat Rodríguez-Ballesteros[1], Marta Gandía[1], Elena Gómez-Rosas[1], Manuela Villamar[1], Elona Cama[2,3], Pietro Scimemi[2,3], Nanna Dahl Rendtorff[4], Miguel Ángel Moreno-Pelayo[1], Lisbeth Tranebjaerg[4], Carmen Medá[5], Rosamaria Santarelli[2,3], Ignacio del Castillo[1] ([1]Madrid, Spain; [2]Padova, Italy; [3]Treviso, Italy; [4]Copenhagen, Denmark; [5]Palma de Mallorca, Spain)
15.17	O20	OPA1-RELATED AUDITORY NEUROPATHY: SITE OF LESION AND OUTCOME OF COCHLEAR IMPLANTATION Rosamaria Santarelli[1,2], Chiara La Morgia[3], Pietro Scimemi[1,2], Elona Cama[1,2], Leonardo Caporali[3], Maria Lucia Valentino[3], Rocco Liguori[3], Valerio Carelli[3] ([1]Padova, Italy; [2]Treviso, Italy; [3]Bologna, Italy)
15.30	O21	COCHLEAR GAP JUNCTION PLAQUE, STABILIZED MACROMOLECULAR COMPLEX COMPOSED OF SPECIFIC CONNEXINS Kazusaku Kamiya[1], Ichiro Fukunaga[1], Kaori Hatakeyama[1], Toru Aoki[1], Ayumi Fujimoto[1], Atena Nishikawa[1], Takashi Anzai[1], Osamu Minowa[2], Katsuhisa Ikeda[1] ([1]Tokyo, Japan; [2]Tsukuba, Japan)
15.43	O22	NEXT GENERATION SEQUENCING FOR THE STUDY OF HEREDITARY HEARING LOSS: PSIP1/LEDGF AS A NEW GENE CAUSING SENSORINEURAL PROGRESSIVE HEARING LOSS AND VARIABLE EYE PHENOTYPES Giorgia Girotto[1], Deborah I. Scheffer[2], Anna Morgan[1], Diego Vozzi[1], Elisa Rubinato[1], Mariateresa Di Stazio[1], Enrico Muzzi[1], Stefano Pensiero[1], Anne B. Giersch[2], David P. Corey[2], Paolo Gasparini[1] ([1]Trieste, Italy; [2]Boston, USA)
15.56	O23	THE EXPRESS LANE FROM LAB TO CLINIC: HIGH-THROUGHPUT SEQUENCING IN HEARING IMPAIRED PATIENTS DISCLOSES INFORMATIVE MUTATIONS AT LIGHTNING SPEED Barbara Vona[1], Michaela A. H. Hofrichter[1], Cordula Neuner[1], Jörg Schröder[1], Oliver Bartsch[2], Ulrich Zechner[2], Wafaa Shehata-Dieler[1], Indrajit Nanda[1], Thomas Haaf[1] ([1]Würzburg, Germany; [2]Mainz, Germany)

POSTER SESSIONS

Lazzati Room

16.10 - 17.00	POSTER SESSION III (details on page 21)
	MOLECULAR BIOLOGY **Moderators:** Dennis Drescher, Guido Conti

Bausola Room

16.10 - 17.00	POSTER SESSION IV (details on page 22)
	COCHLEAR IMPLANTS **Moderators:** Helge Rask Andersen, Gaetano Paludetti

Open Coffee

Lazzati Room

SESSION IV

Chairman: Franco Trabalzini

OTOTOXICITY AND NOISE INDUCED HEARING LOSS **Moderators:** Jochen Schacht, Diana Troiani		
17.00	TL4	**TARGET LECTURE** CIRCADIAN RHYTHM AND NOISE-INDUCED HEARING LOSS Barbara Canlon Vasiliki Basinou, Jung-sub Park, Chrsitopher Cederroth (Stockholm, Sweden)
17.25	O24	AMINOGLYCOSIDE ANTIBIOTICS ALTER MITOCHONDRIAL DYNAMICS Gao Wei, Ann Kendall, Jochen Schacht (Ann Arbor, USA)
17.38	O25	ROLE OF STAT1 IN CISPLATIN AND GENTAMICIN INDUCED AUDITORY HAIR CELL LOSS Daniel Bodmer, Soledad Levano (Basel, Switzerland)
17.51	O26	THE EFFECTS OF CISPLATIN AND AMINOGLYCOSIDES ON INNER EAR MITOCHONDRIA Agnieszka J. Szczepek, Kaitlyn Badley, Birgit Mazurek (Berlin, Germany)
18.04	O27	METFORMIN PROTECTS AGAINST CISPLATIN INDUCED OTOTOXICITY BY REDUCING ROS PRODUCTION AND MODULATING INTRACELLULAR CALCIUM Jiwon Chang, Su Kyoung Park, Hyung-Jong Kim, Sehee Lee, Hak Hyun Jung, June Choi, Gi Jung Im (Seoul, South Korea)
18.17	O28	SENSORINEURAL HEARING LOSS AFTER PNEUMOCOCCAL MENINGITIS IN INFANTS: AN ANIMAL MODEL Michael Perny[1], Denis Grandgirard[1], Marta Roccio[1], Fabian Liechti[1], Stephen L Leib[1,2]*, Pascal Senn[1,3]* (*Co-Lead of the project) ([1]Bern,Switzerland; [2]Spiez, Switzerlan; [3]Genève, Switzerland)
18.30	O29	ANTI-EXCITOXICITY AGENTS REDUCE NOISE-INDUCED LOSS OF HAIR CELL-AUDITORY NERVE SYNAPSES AND TINNITUS Richard Altschuler[1], David Dolan[1], Karin Halsey[1], Ariane Kanicki[1], Diane Prieskorn[1], Cathy Martin[1]; Sue DeRemer[1], Susan Shore[1], and Josef Miller[1,2] ([1]Ann Arbor, USA; [2]Stockholm, Sweden)
18.43	O30	THE ROLE OF JNK ISOFORMS IN NOISE-INDUCED HEARING LOSS Allen F Ryan, Kwang Pak, Joseph Hardeman (La Jolla, USA)
18.56	O31	SEPTIN4 KNOCKOUT MICE SHOW SUSCEPTIBILITY TO INTENSE SOUND EXPOSURE Takushi Miyoshi[1], Atsuhiro Yoshida[1,2], Yosuke Tona[1], Takayuki Nakagawa[1], Juichi Ito[1,3], Norio Yamamoto[1] ([1]Kyoto, Japan; [2]Kurashiki, Japan; [3]Moriyama, Japan)

19.10		End of daily sessions

Monday 14 September 2015

Lazzati Room

SESSION V

Chairman: Antonio Pirodda

OTOPROTECTION AND DRUG DELIVERY SYSTEMS **Moderators:** Annaliese Schrott-Fischer, Gaetano Paludetti		
08.00	TL5	**TARGET LECTURE** LOCAL DELIVERY OF DEXAMETHASONE INTO THE SCALA TYMPANI PROTECTS AGAINST ELECTRODE INSERTION TRAUMA (EIT)-INDUCED HEARING LOSS, DAMAGE TO AUDITORY NEUROSENSORY CELLS AND INCREASES IN IMPEDANCE Thomas R. Van De Water Esperanza Bas, Jorge Bohorquez, Christine Dinh, Stefania Goncalves, Adrien Eshraghi, Roland Hessler (Miami, USA)
08.25	O32	A HIGH-THROUGHPUT SCREEN IDENTIFIES SMALL MOLECULES THAT PROTECT AGAINST CISPLATIN- AND NOISE-INDUCED HEARING LOSS Tal Teitz, Jie Fang, Asli Goktug, Justine Bonga, Shiyong Diao, Yinmei Zhou, Cheng Cheng, Taosheng Cheng, Jian Zuo (Memphis, USA)
08.38	O33	DELIVERY OF LOW LEVEL LASER TO THE HUMAN COCHLEA: A CADAVERIC STUDY Irumee Pai[1], Dan Jiang[1], Patrick Boyle[2], Alec Fitzgerald-O'Connor[1] ([1]London, UK; [2]Cambridge, UK)
08.51	O34	GENE THERAPY AND DRUG DELIVERY TO IMPROVE AUDITORY NERVE SURVIVAL IN THE DEAFENED COCHLEA Andrew Wise (Melbourne, Australia)
09.04	O35	HEARING LOSS AND NATURAL ANTIOXIDANTS: A NOVEL PARADIGM FOR NEUROPROTECTION Cesare Mancuso, Anna Rita Fetoni, Fabiola Paciello, Sara Eramo, Rolando Rolesi, Gaetano Paludetti, Diana Troiani (Rome, Italy)
09.17	O36	COENZYME Q10 AND VITAMINE TREATMENT PREVENT CISPLATIN OTOTOXICITY IN RAT Laura Astolfi[1], Edi Simoni[1], Filippo Valente[1], Alice Cani[1], Vincenza Cascella[1], Pietro Giordano[1], Stavros Hatzopoulos[2], Alessandro Martini[1] ([1]Padova, Italy; [2]Ferrara, Italy)
09.30	O37	OTOPROTECTIVE EFFECT OF A DEXAMETHASONE-LIPOSOME GEL INJECTED IN THE MIDDLE EAR AFTER COCHLEAR IMPLANTATION IN AN ANIMAL MODEL Elisabeth Mamelle, Naila El Kechai, Amelie Bochot, Florence Agnely, Olivier Sterkers, Evelyne Ferrary, Yann Nguyen (Paris, France)
09.42	O38	SPIRAL GANGLION CELL DEGENERATION FOLLOWING HAIR CELL LOSS: SIMULTANEOUS RATHER THAN RETROGRADE Dyan Ramekers, Huib Versnel, Emma M. Smeets, Steven Kroon, Sjaak FL Klis, Wilko Grolman (Utrecht, the Netherlands)
09.50	O39	SUDDEN DEAFNESS: IS IT AN OTOLOGIC EMERGENCY? A PREDICTIVE MODEL IN PATIENTS TREATED WITH INTRATYMPANIC STEROIDS G. Attanasio[1], E. Di Porto[2], F.Y. Russo1, E. Covelli[1], R. Filipo[1] ([1]Rome, Italy; [2]Naples, Italy)

POSTER SESSIONS

Lazzati Room

10.05 - 11.00	POSTER SESSION V (details on page 23)
	NOISE INDUCED HEARING LOSS, OTOTOXICITY AND IMMUNOSYSTEM **Moderators:** Huawei Li, Bruno Sergi

Bausola Room

10.05 - 11.00	POSTER SESSION VI (details on page 24)
	GENETICS OF DEAFNESS **Moderators:** Byung Yoon Choi, Eva Orzan

Open Coffee

Lazzati Room

SESSION VI

Chairman: Antonio Della Volpe

REGENERATION, STEM CELLS AND GENE THERAPY **Moderators:** Hubert Loewenheim, Nicola Quaranta		
11.00	TL6	**TARGET LECTURE** STRATEGIES FOR INDUCTION OF REGENERATION IN MAMMALIAN INNER EAR Takayuki Nakagawa Norio Yamamoto (Kyoto, Japan)
11.25	O40	STROMAL STEM CELLS AS A TOOL IN COCHLEAR REGENERATION: DIFFERENT ADULT SOURCES AND POSSIBLE CROSSTALK WITH THE NEUROGENIC NICHE W. Lattanzi, A.R. Fetoni, R. Rolesi, M. Barba, F. Paciello, L. Di Pietro, S.L.M. Eramo, V. Quercia, V. Pino, F. Michetti, D. Troiani, G. Paludetti (Rome, Italy)
11.51	O41	ADIPOSE-DERIVED STEM CELLS IMPROVE SURVIVAL OF AUDITORY NEURONS IN AN ANIMAL MODEL OF SENSORY HEARING LOSS Andreas Radeloff, Philipp Schendzielorz, Kristen Rak, Johannes Voelker, Katrin Froelich, Rudolf Hagen (Würzburg, Germany)
12.04	O42	MOUSE DERIVED CELL TRANSPLANTATION INTO THE MOUSE OTOCYST *IN VIVO* Hiroki Takeda, Ryosei Minoda, Toru Miwa, Takao Yamada, Momoko Ise, Eiji Yumoto (Kumamoto, Japan)
12.17	O43	EFFICIENT DELIVERY OF CRISPR/CAS9 PROTEINS INTO MAMMALIAN INNER EAR *IN VIVO* AND NEURON STEM CELLS IN VITRO Yilai Shu[1,2], John A. Zuris[3], David B. Thompson[3], Yong Tao[1], Wenyan Li[1,2], Zhengmin Wang[2], Huawei Li[2], David R. Liu[3], Zheng-Yi Chen[1] ([1]Boston, USA; [2]Shanghai, China; [3]Cambridge, USA)
12.30	O44	RNA SEQ ANALYSIS OF REGENERATED HAIR CELLS IN THE POSTNATAL MOUSE COCHLEA Tetsuji Yamashita[1], Ken Sugino[2], David Finkelstein[1], Fei Zheng[1], Bradley Walters[1], Jian Zuo[1] ([1]Memphis, USA; [2]Ashburn, USA)
12.43	O45	CELL CYCLE REACTIVATION OF LGR5+ COCHLEAR PROGENITOR CELLS IN NEONATAL FUCCI MICE BY A GSK3 SMALL MOLECULE INHIBITOR Marta Roccio[1], Stefan Hahnewald[1], Michael Perny[1], Pascal Senn[1,2] ([1]Bern, Switzerland; [2]Geneva, Switzerland)
12.56	046	MURINE “MINI EARS” FOR HIGH-CONTENT SCREENING IN HEARING RESEARCH Mirko Jaumann[1], Aurélie Dos-Santos[1], Marcus Müller[2], Hubert Löwenheim[2] ([1]Tübingen, Germany; [2]Oldenburg, Germany)

13.10-13.20		Spoeldling Award Cerimony
13.20-14.15		Lunch time

Lazzati Room

SESSION VII

Chairman: Giampiero Ricci

IMMUNOMEDIATED DISEASES AND COCHLEAR IMPLANTS **Moderators:** Thomas Lenarz, Sandro Burdo		
14.15	O47	KNOCKOUT OF THE AUTOIMMUNE HEARING LOSS TARGET CTL2/SLC44A2 RESULTS IN SPIRAL GANGLION CELL LOSS, HAIR CELL LOSS AND PROGRESSIVE HEARING LOSS Pavan Kommareddi[1], Thankam Nair[1], Bala Naveen Kakaraparthi[1], Maria M. Galano[1], Danielle Miller[1], Irina Laczkovich[1], Trey Thomas[1], Lillian Lu[1], Kelli Rule[1], Lisa Kabara[1], Ariane Kanicki[1], Elizabeth D. Hughes[1], Julie M. Jones[1], Mark Hoenerhoff[1], Susan G. Fisher[2], Richard A. Altschuler[1], David Dolan[1], David C. Kohrman[1], Thomas L. Saunders[1], Thomas E. Carey[1] ([1]Ann Arbor, USA; [2]Philadelphia, USA)
14.28	O48	UNILATERAL SNHL AS POSSIBLE IMMUNE-BASED INNER EAR DISEASE (AIED) Francesca Atturo, Roberta Colangeli, Simonetta Monini, Maurizio Barbara (Rome, Italy)
14.41	O49	THE IMMUNE RESPONSE OF THE COCHLEA TO AN IMPLANTED ELECTRODE IN THE CONTEXT OF AN OTOTOXIC INSULT Leila Abbas[1], Daniela I. Cacciabue-Rivolta[1], Daniel Smyth[2], Marcelo N. Rivolta[1] ([1]Sheffield, UK; [2]Mechelen, Belgium)
14.54	O50	RESPONSE PROFILES OF MURINE SPIRAL GANGLION NEURONS ON MULTI-ELECTRODE ARRAYS Stefan Hahnewald[1], Anne Tscherter[1], Emanuele Marconi[1], Jürg Streit[1], Hans Rudolf Widmer[1], Carolyn Garnham[2], Heval Benav[2], Marcus Mueller[3], Hubert Löwenheim[3], Marta Roccio[1], Pascal Senn[1,4] ([1]Bern, Switzerland; [2]Innsbruck, Austria; [3]Tübingen, Germany; [4]Geneva, Switzerland)
15.07	O51	THE DEVELOPMENT OF TEAMWORKING IN THE PATIENT WITH COCHLEAR IMPLANT TO IMPROVE SAFETY AND FINAL OUTCOME Daniele Frezza[1], Martina Antniutti[1], Franco Trabalzini[2] ([1]Treviso, Italy; [2]Siena, Italy)
15.20	O52	OUTCOME OF COCHLEAR IMPLANTATION IN CHILDREN CONSIDERING MOLECULAR GENETIC ETIOLOGY Joo Hyun Park[1], Ah Reum Kim[2], Ja- Won Koo[2], Sun O Chang[3], Byung Yoon Choi[2] ([1]Goyang, South Korea; [2]Seongnam, South Korea; [3]Seoul, South Korea)
15.33	O53	COATED COCHLEAR IMPLANT ELECTRODES TO SUPPRESS INTRACOCHLEAR INFLAMMATORY RESPONSE AND FIBROSIS Susanne Braun[2], Johannes Fischer[1], Katharina Niedermeier[1],Jochen Steinhoff[1], Sören Schilp[2], Thomas Stark[1] ([1]Munich, Germany; [2]Innsbruck, Austria and Starnberg, Germany)
15.47	O54	NANOMATERIALS-BASED STRATEGIES FOR PIEZOELECTRIC COCHLEAR IMPLANTS Serena Danti, Luca Bruschini, Gianni Ciofani, Massimiliano Labardi, Carlos Mota, Luisa Trombi, Claudio Ricci, Delfo D'Alessandro, Giuseppe C. Gallone, Stefano Berrettini (Pisa, Italy)

POSTER SESSIONS

Lazzati Room

16.00 - 16.45	POSTER SESSION VII (details on page 25)
	STEM CELLS AND GENE THERAPY **Moderators:** Marcelo Rivolta, Anna Rita Fetoni

Bausola Room

16.00 - 16.45	POSTER SESSION VIII (details on page 26)
	CELL PHYSIOLOGY **Moderators:** Jonathan Gale, Giorgia Girotto

Tuesday 15 September 2015

Lazzati Room

SESSION VIII

Chairman: Claudio Grassi

PHYSIOPATHOLOGY OF AUDITORY PATHWAYS AND TINNITUS **Moderators:** Wei Sun, Jacopo Galli		
08.00	TL8	**TARGET LECTURE** COCHLEAR AND CENTRAL MECHANISMS OF TINNITUS AND THERAPEUTIC APPROACHES Arnaud Norena (Marseille, France)
08.25	O55	THE DIFFERENTIAL EAR EFFECTS OF LONG-TERM UNILATERAL DEAFNESS ON SPEECH PERCEPTION: PSYCHOACOUSTIC COMPARISONS Jae Young Byun, Min Young Kwak, Dong Hyun Kim, Yong-Hwi An, Hyun Joon Shim (Seoul, South Korea)
08.38	O56	CONTRALATERAL STIMULATION EFFECT ON SHORT AND LONG LATENCY DISTORTION PRODUCT OTOACOUSTIC EMISSIONS IN PATIENTS AFFECTED BY PARKINSON DISEASE Renata Sisto, Arturo Moleti, Valerio Pisani, Stefano Di Girolamo, Teresa Botti, Filippo Sanjust, Luigi Cerini (Rome, Italy)
08.51	O57	SENSITIVITY OF ELECTROCOCHLEOGRAPHY VS. OTOACOUSTIC EMISSIONS TO DISRUPTED COCHLEAR HOMEOSTASIS IN MENIÈRE PATIENTS Fabrice Giraudet, Thierry Mom, Paul Avan (Clermont-Ferrand, France)
09.04	O58	DOES BLOCKING MICROGLIAL ACTIVATION PREVENT TINNITUS ONSET? Alessandro Venturino, Paola Perin, Vittorio Bertone, Gloria Colombo, Adriano Oda, Gabriele Sanchini, Vincenzo Vitale, Alessia Capetta, Roberto Pizzala (Pavia, Italy)
09.17	O59	LOUDNESS AFFECTED BY HIGH DOSES OF SALICYLATE AND NOISE EXPOSURE – A BEHAVIORAL MODEL FOR HYPERACUSIS AND TINNITUS Wei Sun, Chao Zhang, Anaam Alkharabsheh (Buffalo, USA)
09.30	O60	TREATMENT OF ACUTE SALICYLATE-INDUCED TINNITUS WITH TNF-α BLOCKER IN AN ANIMAL MODEL David Chang-Wei Huang[1] and Tien-Chen Liu[2] ([1]Taichung, Taiwan; [2]Taipei, Taiwan)
09.43	O61	PHENOTYPIC CHARACTERISTICS OF THE T961G MITOCHONDRIAL MUTATION Filippo Mazzei, Rosaria Turchetta, Maria Patrizia Orlando, Giancarlo Altissimi, Giancarlo Cianfrone (Rome, Italy)
09.50	O62	DISTINCT ROLES OF EPS8 IN THE FUNCTIONAL MATURATION OF COCHLEAR AND VESTIBULAR HAIR CELLS Sergio Masetto[1], Walter Marcotti[2] ([1]Pavia, Italy; [2]Sheffield, UK)

Bausola Room

SESSION IX

Chairman: Elio Marciano

AGING AND VESTIBULAR DISORDERS **Moderators:** Lukas Rüttiger, Vito E. Pettorossi		
08.00	TL9	**TARGET LECTURE** PATHOPHYSIOLOGY, GENETICS AND SOCIAL IMPACT OF PRESBYCUSIS Helena Caria (Lisbon, Portugal)
08.25	O63	AGE MATTERS: CENTRAL COMPENSATION OF COCHLEAR FUNCTION LOSS Lukas Rüttiger, Dorit Möhrle, Ksenia Varakina, Dan Bing, Marlies Knipper (Tübingen, Germany)

08.38	O64	AGE-RELATED DIFFERENCES IN HEARING FUNCTION AND COCHLEAR MORPHOLOGY BETWEEN MALE AND FEMALE FISCHER 344 RATS Jiří Popelář[1], Francesca Chiumenti[2], Zuzana Balogová[1], Tetyana Chumak[1], Josef Syka[1] ([1]Prague, Czech Republic; [2]Padova, Italy)
08.51	O65	STRESS GRANULES AND MECHANISMS OF RNA TRIAGE DURING COCHLEAR STRESS Ana Cláudia Gonçalves, Naila Haq, Emily Towers, Lisa Nolan, Sally Dawson, Jonathan Gale (London, UK)
09.04	O66	SELECTIVE SEROTONIN REUPTAKE INHIBITOR (SSRI) AND VESTIBULAR FUNCTION Kensuke Kiyomizu[1,2], Hironori Fujii[3], Hiroaki Shimogori[3)] Keiji Matsuda[2], Koji Torihara[2], Kensei Yoshida[1], Tetsuya Tono[2] ([1]Nobeoka, Japan; [2]Miyazaki, Japan; [3]Ube, Japan)
09.17	O67	CHRONIC CEREBERO-SPINAL VENOUS INSUFFICIENCY IN MENIERE' DISEASE: DIAGNOSIS AND ENDOVASCULAR TREATMENT G. Attanasio[1], A. Bruno[2], L. Califano[2], F. Ciciariello[1], M. Viccaro, E. Masci[1], L. Cagnoni[1], D. Mastrangelo[2], F. Salafia[2], V. Giugliano[2], P.P. Cavazzuti[3], B. Bernardo[2], R. Filipo[1] ([1]Rome, Italy; [2]Benevento, Italy; [3]Bologna, Italy)
09.30	O68	CELLULAR STRESS RESPONSE AND VITAGENE'S ROLE IN VESTIBULAR DISEASE A. Serra, V. Calabrese, L. Maiolino, S. Cocuzza, P. Di Mauro (Catania, Italy)
09.43	O69	LONG-TERM EFFECTS IN THE VESTIBULAR SYSTEM ARE INFLUENCED BY SEX CIRCULATING HORMONES Cristina V. Dieni, Roberto Panichi, Mariangela Scarduzio, Silvarosa Grassi, Vito E. Pettorossi (Perugia, Italy)
09.56	O70	POTENTIAL BIOMARKERS IN MÉNIÈRE DISEASE: A PROTEOMICS-DRIVEN APPROACH Ettore Cassandro[1], Antonio Concolino[2], Giuliano Sequino [1], Alfonso Scarpa[1], Giuseppe Chiarella[2] ([1]Salerno, Italy; [2]Catanzaro, Italy)

Open Coffee

POSTER SESSIONS

Bausola Room

10.10 - 10.55	POSTER SESSION IX (details on page 27)
	PHYSIOPATHOLOGY OF AUDITORY PATHWAYS AND TINNITUS **Moderators:** Regis Nouvian, Gabriella Cadoni

Bausola Room

10.55 - 11.40	POSTER SESSION X (details on page 28)
	OTOPROTECTION AND DRUG DELIVERY SYSTEMS **Moderators:** Jean Luc Puel, Roberto Albera

Lazzati Room

SESSION X

Chairman: Giancarlo Cianfrone

MOLECULAR BIOLOGY AND CELL PHYSIOLOGY (II) **Moderators:** Jonathan Ashmore, Joseph Santos Sacchi		
10.05	O71	APOPTOTIC REGULATORS IN THE AUDITORY SYSTEM: KEY FACTORS IN HEARING LOSS Rachel A Burt (Parkville, Australia)

10.18	O72	SOUND RESPONSE MEDIATED BY THE TRP CHANNELS NOMPC, NANCHUNG, AND INACTIVE IN CHORDOTONAL ORGANS OF DROSOPHILA LARVAE Wei Zhang[1], Zhiqiang Yan[1,2], Lily Yeh Jan[1], Yuh Nung Jan[1] ([1]San Francisco, USA; [2]Shanghai, China)
10.31	O73	ACID-SENSING ION CHANNELS IN THE COCHLEAR AFFERENT NEURONS Enrique Soto[1], Rosario Vega[1], Antonia González-Garrido[1], Francisco Mercado[2], Ivan López[3] ([1]Puebla, Mexico; [2]Mexico DF, Mexico; [3]Los Angeles, USA)
10.44	O74	DISRUPTION OF ADAPTOR PROTEIN 2μ (AP-2μ) IN COCHLEAR HAIR CELLS IMPAIRS SYNAPTIC VESICLE REPLENISHMENT AND HEARING SangYong Jung[1#], Tanja Maritzen[2#], Carolin Wichmann[1#], Zhizi Jing[1], Andreas Neef[1], Natalia H Revelo[1], Hanan Al-Moyed[1], Sandra Meese[1], Sonja M Wojcik[1], Iliana Panou[1], Haydar Bulut[2], Peter Schu[1], Ralf Ficner[1], Ellen Reisinger[1], Silvio O Rizzoli[1], Jakob Neef[1], Nicola Strenzke[1], Volker Haucke[2], Tobias Moser[1] ([1]Göttingen, Germany; [2]Berlin, Germany)
10.57	O75	MANIPULATIONS OF PHOSPHATIDYLINOSITOL 4,5-BISPHOSPHATE METABOLISM EFFECTS MECHANOELECTRICAL TRANSDUCTION CURRENTS IN MAMMALIAN INNER HAIR CELLS Thomas Effertz, Anthony Ricci (Stanford, USA)
11.10	O76	MOLECULAR CONSTITUENTS OF THE MECHANOTRANSDUCITON MACHINERY OF COCHLEAR HAIR CELLS Ulrich Mueller, Bo Zhao, Wei Xiong, Nicolas Grillet (La Jolla, USA)
11.23	O77	A MULTIPHOTON MICROSCOPY INVESTIGATION ON THE ROLE OF NAD(P)H AND PLASMA MEMBRANE FLUIDITY IN NOISE-INDUCED OXIDATION OF OUTER HAIR CELLS G. Maulucci, D. Troiani, S.L.M. Eramo, F. Paciello, M.V. Podda, G. Paludetti, M. Papi, A. Maiorana, V. Palmieri, M. De Spirito, A.R. Fetoni (Rome, Italy)
11.37	O78	FURTHER CHARACTERIZATION OF THE STRIATED ORGANELLE AND APICAL MITOCHONDRIA IN RAT INNER EAR HAIR CELLS Anna Lysakowski, Robstein L Chidavaenzi (Chicago, USA)
11.50	O79	CLOSING THE LOOP ON THE OLIVOCOCHLEAR REFLEX: OUTER HAIR CELLS AND TYPE II SPIRAL GANGLION AFFERENTS DRIVE CONTRALATERAL SUPPRESSION Gary D. Housley[1], Kristina E. Froud[1], Ann Chi Yan Wong[1], Jennie M.E. Cederholm[1], Matthias Klugmann[1], Shaun L. Sandow[2], Jean-Pierre Julien[3], Allen F. Ryan[4] ([1]Sydney, Australia; [2]Maroochydore, Australia; [3] Québec City, Canada; [4] La Jolla, USA)

12.10-13.10		IEB Business Meeting and Closing

52nd INNER EAR BIOLOGY WORKSHOP

Sunday 13 September 2015

Catholic University
Giovanni XXIII Building, 1st floor

POSTER SESSIONS

Lazzati Room

10.10 - 11.00	**POSTER SESSION I**
DEVELOPMENTAL BIOLOGY AND REGENERATION **Moderators:** Marta Roccio, Giovanni Almadori	
P1	BMI1 REGULATES THE PROLIFERATION OF COCHLEAR SUPPORTING CELLS VIA THE CANONICAL WNT SIGNALING PATHWAY Renjie Chai[1,2], Xiaoling Lu[3], Shan Sun[3], Wenyan Li[3], Yan Chen[3], Lei Wang[3], Huawei Li[3] ([1]Nanjing, China; [2]Nantong, China; [3]Shanghai, China)
P2	DYNAMIC EXPRESSION OF LGR6 IN THE DEVELOPING AND MATURE MOUSE COCHLEA Yanping Zhang[1,2, 4], Yan Chen[1,2], Wenli Ni[3], Luo Guo[1,2], Xiaoling Lu[3], Liman Liu[2], Wen Li[1,2], Shan Sun[1,2], Lei Wang[4], Huawei Li[2,3,5] (Shanghai, China)
P3	BMI1-MEDIATED REGULATION OF THE SPHERE-FORMING CAPACITY OF THE MURINE ORGAN OF CORTI Mohamed Bassiouni[1], Aurélie Dos Santos[1], Marcus Müller[2], Hubert Löwenheim[2] ([1]Tübingen, Germany; [2]Oldenburg, Germany)
P4	ELECTROPORATION-MEDIATED TRANSUTERINE GENE TRANSFER INTO MOUSE OTOCYSTS (EUGO) UTILIZING NEPA21 ELECTOROPORATOR Ryosei Minoda, Hiroki Takeda, Toru Miwa and Eiji Yumoto (Kumamoto City, Japan)
P5	THE NOGO RECEPTOR 1 IS A PROMISING TARGET IN THE INNER EAR Laura Holtmann[1], Katharina Schulz[2], Stephan Lang[1], Stefan Hansen[1] ([1]Essen, Germany; [2]Düsseldorf, Germany)
P6	NEUREGULIN-1 SUPPORTS SPIRAL GANGLION OUTGROWTH AND SCHWANN CELL PROLIFERATION IN THE INNER EAR Stefan Hansen[1], Diana Lang[2], Laura Holtmann[1], Stephan Lang[1] ([1]Essen, Germany; [2]Düsseldorf, Germany)
P7	MINIATURE PIGS: A LARGE ANIMAL MODEL OF COCHLEAR IMPLANTATION Weiwei Guo, Tao Cong, Shi-Ming Yang (Beijing, China)
P8	NOTCH SIGNALING ACTS AS A NEGATIVE REGULATOR FOR THE PROLIFERATION OF PROGENITORS IN MAMMALIAN COCHLEAE Wenyan Li, Hui Jiang, Wenli Ni, Jingfang Wu (Shanghai, China)
P9	THE ROLE OF PARTICULATE GUANYLYL CYCLASE B (GC-B) FOR AUDITORY FUNCTION IN MICE Steffen Wolter[1], Dorit Möhrle[1], Dennis Zelle[1], Marlies Knipper[1], Robert Feil[1], Hannes Schmidt[2], Lukas Rüttiger[1] ([1]Tübingen, Germany; [2]Berlin, Germany)
P10	THE PROMOTER AND MULTIPLE ENHANCERS OF THE *POU4F3* GENE REGULATE GENE EXPRESSION IN HAIR CELLS Masatsugu Masuda[1,3], Yan Li[1], Kwang Pak[1], Eduardo Chavez[1], Lina Mullen[1] and Allen F Ryan[1,2] ([1]La Jolla, USA; [2]Tokyo, Japan)
P11	EXTRACELLULAR NUCLEOTIDE SIGNALING DURING THE EMBRYONIC DEVELOPMENT OF THE CHICKEN INNER EAR: A MOLECULAR AND ELECTROPHYSIOLOGICAL STUDY Fabián Galindo[1], Eduardo Monjaraz[1], Jorge Cebada[2], Amira Flores[1] (Puebla, México)
P12	ROLE OF P63 IN INNER EAR DEVELOPMENT: MORPHOLOGIC ABNORMALITIES AND CLINICAL FINDINGS Ernesto Bruno, Alessandro Terrinoni, Andrea Viziano, Alessandro Micarelli, Sara De Fazio, Simone Mauramati, Marco Alessandrini, Fabrizio Ottaviani (Rome, Italy)
P13	DIFFERENTIAL GENE EXPRESSION IN THE HUMAN COCHLEA AND VESTIBULAR SYSTEM DURING THE FIRST TRIMESTER OF PREGNANCY Suvarna Dash-Wagh, Sara Hägg, Beata Kostyszyn, Elisabet Åkesson, Mats Ulfendahl (Stockholm, Sweden)

Bausola Room

10.10 - 11.00	**POSTER SESSION II**
AGING AND VESTIBULAR DISORDES **Moderators:** Raekil Park, Pasqualina M. Picciotti	
P14	EPIGENETICS AND AGING Ken-ichi Watanabe, Kimihiro Ohkubo (Tokyo, Japan)
P15	AGE-RELATED DIFFERENCE IN THE EFFECTS OF PARVALBUMIN DEFICIENCY ON ACOUSTIC STARTLE RESPONSE AND PREPULSE INHIBITION IN MICE Jana Burianová[1], Beat Schwaller[2], Josef Syka[1] ([1]Prague, Czech Republic;[2]Fribourg, Switzerland)
P16	AUDITORY FUNCTION, HOMEOSTASIS AND AGEING IN *DROSOPHILA MELANOGASTER* Camille Tardieu, Liza Malong, Nicholas Boyd-Gibbins, Ryan Kavlie, Jonathan Gale, Joerg Albert (London, UK)
P17	PRESBYCUSIS AS BIOLOGICAL MARKER OF AGING PROCESS: THE ROLE OF NAT2 AND GRM7 GENES J. Chora[1], T.D. Matos[1], G. Fialho[1], H. Caria[1,2] ([1] Lisbon, Portugal; [2] Setúbal, Portugal)
P18	UNILATERAL INTRATYMPANIC ADMINISTRATION OF GENTAMICIN CREATES OPTIMAL VESTIBULAR DISORDER MODEL IN GUINEA PIGS Makoto Chiba, Tsukasa Ito, Chikako Shinkawa, Seiji Kakehata (Yamagata-shi, Japan)
P19	EFFECT OF INTRA-TYMPANIC ISOSORBIDE ON ENDOLYMPHATIC HYDROPS IN NEW ANIMAL MODEL Minbum Kim (Seo-Gu Incheon, South Korea)
P20	VESTIBULAR COMPENSATION AFTER COCHLEAR IMPLANTATION: AN ANIMAL RESEARCH Yong-Ho Park (Daejeon, South Korea)
P21	QUANTIFICATION OF THE ENDOLYMPHATIC HYDROPS IN MÉNIÈRE'S DISEASE USING CONTRAST ENHANCED SMALL ANIMAL MRI M. Müller[1], J.G. Mannheim[2], A.M. Schmid[2], H. Kumagami[3], S. Vollmer[4], U. Gottwald[4], B.J. Pichler[2], H. Löwenheim[2] ([1]Oldenburg, Germany; [2]Tübingen, Germany; [3] Nagasaki, Japan; [4]Berlin, Germany)
P22	INFLUENCE OF EXTERO- AND PROPRIOCEPTIVE AFFERENTS OF THE PLANTAR SURFACE IN DETERMINING SUBJECTIVE VISUAL VERTICAL IN PATIENTS WITH UNILATERAL VESTIBULAR DYSFUNCTION Mario Faralli, Vito Enrico Pettorossi, Giampietro Ricci (Perugia, Italy)
P23	AFTER EFFECTS OF GALVANIC STIMULATION ON SELF MOTION PERCEPTION DEPEND ON THE INTENSITY AND INTERVAL OF STIMULATION PATTERNS Aldo Ferraresi, Roberto Panichi, Chiara Occhigrossi, Silvarosa Grassi, Vito Enrico Pettorossi (Perugia, Italy)
P24	SELF-MOTION PERCEPTION IN VESTIBULAR COMPENSATION Roberto Panichi[1], Mario Faralli[1], Chiara Occhigrossi[1], Aldo Ferraresi[1], Adolfo M Bronstein[2], Vito Enrico Pettorossi[1] ([1]Perugia, Italia; [2]London, UK)
P25	PSYCHIATRIC COMORBIDITY IN PATIENTS WITH DIZZINESS AND THE THERAPY OF PSYCHOTROPIC DRUGS Kensuke Kiyomizu[1,2], Keiji Matsuda[2], Koji Torihara[2], Yasushi Ishida[2], Kensei Yoshida[1], Tetsuya Tono[2] ([1]Nobeoka, Japan; [2]Miyazaki, Japan)
P26	CERVICAL NEURO-MUSCULAR SYNDROME PRESENTS AS VERTIGO. CASE REPORT Aikaterini Drylli, Georgios Markogiannakis, Georgios Psilovasilopoulos, Ioannis Mylonakis, Christos Kelesis, Vasileios Varsos (Athens, Greece)
P27	CLINICAL CHARACTERISTICS OF ACUTE VESTIBULAR NEURITIS ACCORDING TO THE INVOLVEMENT SITE Ryung Chae (Seoul, South Korea)
P28	EPIDEMIOLOGY OF VERTIGO ON HOSPITAL EMERGENCY Ana Rita Lameiras, Luís Reis, Ricardo Santos, Pedro Escada (Lisbon, Portugal)
P29	OCULAR VESTIBULAR EVOKED MYOGENIC POTENTIAL TESTING FOR THE PROGNOSIS OF BELL'S PALSY Hyun Joon Shim, Eun Woong Ryu, Moon Suh Park, Seung Geun Yeo, Jae Yong Byun (Seoul, South Korea)
P30	EFFECT OF MICROPRESSURE TREATMENT ON ENDOLYMPHATIC HYDROPS M. Barbara, E. Covelli, L. Volpini, S. Monini (Rome, Italy)

Lazzati Room

16.10 - 17.00	**POSTER SESSION III**
MOLECULAR BIOLOGY **Moderators:** Dennis Drescher, Guido Conti	
P31	FURTHER CHARACTERIZATION OF THE RECENTLY DESCRIBED *SLC26A4* C.918+2T>C MUTATION AND REPORTING OF A NOVEL VARIANT OF UNKNOWN CLINICAL SIGNIFICANCE A.C. Gonçalves[1], R. Santos[2], A. O'Neill[2], J. O'Neill [2], P. Escada[2], F. Fialho[1], H. Caria[1,2] ([1]Lisbon, Portugal; [2]Setúbal, Portugal)
P32	DIFFERENTIAL ROLE OF NO-SENSITIVE GYANYLYL CYCLASE ISOFORMS NO-GC1 AND NO-GC2 IN AUDITORY FUNCTION IN ADULT MICE Dorit Möhrle[1], Nicole Eichert[1], Steffen Wolter[1], Evanthia Mergia[2], Doris Koesling[2], Andreas Friebe[3], Marlies Knipper[1], Lukas Rüttiger[1] ([1]Tübingen, Germany; [2]Bochum, Germany; [3]Würzburg, Germany)
P33	REPRODUCTION OF VARIOUS TYPES OF COCHLEAR GAP JUNCTION PLAQUES IN HUMAN CELL LINE Kaori Hatakeyama, Katsuhisa Ikeda, Kazusaku Kamiya (Tokyo, Japan)
P34	CAVEOLINS ACCUMULATES AT THE ORGAN OF CORTI IN GJB2 ASSOCIATED DEAFNESS Takashi Anzai, Kazusaku Kamiya, Katsuhisa Ikeda (Tokyo, Japan)
P35	SAMPLING OF HUMAN PERILYMPH AND PROTEOME ANALYSIS OF PERILYMPH BY MASS SPECTROMETRY Heike Schmitt, Andreas Pich, Giorgio Lilli, Günter Reuter, Thomas Lenarz (Hanover, Germany)
P36	A TRANSGENIC MOUSE MODEL (BLEV) FOR VISUALIZATION OF THE DIFFERENTIAL USAGE OF BDNF EXON IV AND VI Dario Campanelli[1], Wibke Singer[1], Eleonora Passeri[1], Hyun-Soon Geisler[1], Da Guo[1], Florian Mayer[1], Jing Hu[1], Verena Bautze[2], Jörg Strotmann[2], Ulrike Zimmermann[1], Lukas Rüttiger[1], Rama Panford-Walsh[1], Marlies Knipper[1] ([1]Tübingen, Germany; [2]Stuttgart, Germany)
P37	ANALYSES OF THE DIFFERENTIAL USAGE OF BDNF EXON IV AND VI UNDER DIFFERENT SOUND EXPOSURE PARADIGMS USING A TRANSGENIC BDNF MOUSE LINE (BLEV) Giulia Asola, Wibke Singer, Dario Campanelli, Hyun-Soon Geisler, Ulrike Zimmermann, Lukas Rüttiger, Rama Panford-Walsh, Marlies Knipper (Tübingen, Germany)
P38	DELETION OF EHD4 DISTURBS HEARING FUNCTION Yan Zhu[1], Jens Schaller[2], Markus Plomann[2], Marlies Knipper[1], Lukas Rüttiger[1] ([1]Tübingen, Germany; [2]Cologne, Germany)
P39	PEX5 DEFICIENCY RESULTS IN HEARING LOSS Jae-Young Lim[1], Min-Soo Kim[1], Joon No Lee[1], Se-Jin Kim[1], Seong-Kyu Choe[1], Channy Park[2], Raekil Park[1*] ([1]Iksan, Jeonbuk, South Korea; [2]Los Angeles, USA)
P40	HOTSPOT-MUTATION ANALYSIS OF THE EGFR, KRAS, BRAF PATHWAY IN SPORADIC VESTIBULAR SCHWANNOMAS Igor Stenin[1], Stefan Hansen[2], Karl Schäfer[2], Jörg Schipper[1] ([1]Duesseldorf, Germany; [2]Essen, Germany)
P41	EXPRESSION PATTERN OF GRHL2, AN AGE-RELATED HEARING IMPAIRMENT GENE, IN COMMON MARMOSET (CALLITHRIX JACCHUS) INNER EAR Makoto Hosoya[1], Masato Fujioka[1], Takashi Inoue[2], Hideyuki Okano[1], Kaoru Ogawa[1] ([1]Tokyo, Japan; [2]Kanagawa, Japan)
P42	ENDOPLASMIC RETICULUM STRESS MAY PARTICIPATE IN THE PATHOGENESIS OF AGE-RELATED HEARING LOSS AND CISPLATIN-INDUCED OTOTOXICITY Wenwen Wang, Yu Sun, Weijia Kong (Wuhan, China)
P43	EXPRESSION PATTERN OF WOLFLAMIN, THE WOLFRAM SYNDROME 1 GENE (WFS1) PRODUCT, IN COMMON MARMOSET (*CALLITHRIX JACCHUS*) INNER EAR Noriomi Suzuki[1], Masato Fujioka[1], Makoto Hosoya[1], Naoki Oishi[1], Takashi Inoue[2], Hideyuki Okano[1], Kaoru Ogawa[1] ([1]Tokyo, Japan; [2]Kanagawa, Japan)

Bausola Room

16.10 - 17.00	**POSTER SESSION IV**
COCHLEAR IMPLANTS **Moderators:** Helge Rask Andersen, Gaetano Paludetti	
P44	SURGICAL OUTCOMES OF COCHLEAR IMPLANT IN PATIENTS WITH BONY COCHLEAR NERVE CANAL ATRESIA AND STENOSIS Yun Suk An[1], Tae Su Kim[2], Kwang-Sun Lee[3] ([1]Seongnam, South Korea; [2]Chuncheon, South Korea; [3] Ulsan, South Korea)
P45	THE DETERMINATION OF MOST COMFORTABLE LEVELS IN PATIENTS WITH A COCHLEAR IMPLANT WITH A HEARING LOSS AFTER MENINGITIS Y.O. Radionova, N.N. Petrova, D.S. Klyachko (Saint-Petersburg, Russia)
P46	INTRAINDIVIDUAL COMPARISON OF PSYCHOPHYSICAL PARAMETERS BETWEEN PERIMODIOLAR AND LATERAL-TYPE ELECTRODE ARRAYS IN PATIENTS WITH BILATERAL COCHLEAR IMPLANTS Junhui Jeong[1], Ji Hye Heo[1], Young Joon Seo[2], In Seok Moon[1], and Jae Young Choi[1] ([1]Seoul, South Korea; [2]Wonju, South Korea)
P47	COCHLEAR IMPLANTS IN RECIPIENTS WITH SINGLE SIDED DEAFNESS: DIRECTIONAL HEARING, SPEECH INTELLIGIBILITY, LOUDNESS BALANCE AND PITCH MATCHING Mark Praetorius, Maria Rösli-Khabas, Sebastian Hoth (Heidelberg, Germany)
P48	OUTCOMES OF COCHLEAR IMPLANTATIONS IN PATIENTS WITH NARROW BONY COCHLEAR NERVE CANAL Juyong Chung[1], Jeong Hun Jang[2], Jae-Jin Song[3], So Young Kim[3], Jun Ho Lee[3], Seung Ha Oh[3], Sun O Chang[4] ([1]Iksan, South Korea; [2]Taegu, South Korea; [3]Seoul, South Korea; [4]Jongno-Gu, Seoul, South Korea)
P49	COCHLEAR IMPLANT DEVICE EXTRUSION SUCCESSFULLY MANAGED WITH ADVANCED LOCAL FLAP Min Kim, Woo Jin Kim, Jong Won Lee, Shi Nae Park, Sang Won Yeo (Seoul, South Korea)
P50	ELECTRICALLY EVOKED COMPOUND ACTION POTANTIAL RECORDING AS A PREDICTIVE FACTOR FOR SPEECH PERCEPTION IN COCHLEAR IMPLANT PATIENTS: A SYSTEMATIC REVIEW Ruben van Eijl, Patrick Buitenhuis, Inge Stegeman, Sjaak Klis, Wilko Grolman (Utrecht, The Netherlands)
P51	VALUE OF COMPUTATIONAL IMAGINE ANALYSIS IN DIAGNOSIS OF COCHLEAR OSSIFICATION Xinbo Xu, Hanbing Zhang, Xiaojie Ma, Xiao Han (Jinan, China)
P52	INNER EAR STRUCTURES DAMAGE DURING COCHLEAR IMPLANTATION. ANALYSIS OF INSERTION FORCES AND CONE BEAM CT IN TEMPORAL BONE SPECIMENS Daniele De Seta[1-2], Guillaume Kazmitcheff[1], Renato Torres[1], Evelyne Ferrary[1], Olivier Sterkers[1], Yann Nguyen[1] ([1]Paris, France; [2]Rome, Italy)
P53	TRANSDUCTION OF A COMPLEX SIGNAL THROUGH THE NORMAL COCHLEA AND THROUGH THE COCHLEAR IMPLANT Mona Ibrahim Morad, Mohamed Eid Ibrahim, Mohamed Aziz Mohamed Talaat, Mirhan Khamis Eldeeb, Hicham G. Elmongui (Alexandria, Egypt)
P54	COCHLEAR OPTOGENETICS AND µLED IMPLANT APPLICATION Christian Wrobel[1], Marcus Jeschke[1], Daniel Keppeler[1], Victor H Hernandez[1,2], Anna Gehrt[1], Gerhard Hoch[1], Christian Goßler[3], Ulrich T Schwarz[3], Patrick Ruther[3], Michael Schwaerzle[3], Abhishek Raj[3], Roland Hessler[4], Tim Salditt[1], Nicola Strenzke[1], Sebastian Kügler[1], Tobias Moser[1] ([1]Goettingen, Germany; [2]Guanajuato, Mexico; [3]Freiburg, Germany; [4]Innsbruck, Austria and Starnberg, Germany)
P55	PREOPERATIVE INTRATYMPANIC GLUCOCORTICOID HYDROGELS: EFFECTS IN A COCHLEAR IMPLANT MODEL Clemens Honeder, Chengjing Zhu, Julia Clara Gausterer,Hanna Schöpper, Elisabeth Engleder, Lukas Landegger, Manuel Walter, Franz Gabor, Christoph Arnoldner (Vienna, Austria)
P56	GUIDED AUDITORY NEURON GROWTH ON TOPOGRAPHICALLY MODIFIED NANOCRYSTALLINE DIAMOND Yixiao Cai, Fredrik Edin, Wei Liu, Helge Rask-Andersen, Mikael Karlsson and Hao Li (Uppsala, Sweden)
P57	TRANSLATIONAL TOOLS FOR ANIMAL STUDIES IN COCHLEAR IMPLANT RESEARCH Jonathon Kirk[1], Daniel Smyth[2], Kristien Verhoeven[2], Claudiu Treaba[1] ([1]Centennial, USA; [2]Mechelen, Belgium)

P58	EFFECTS OF LOCALLY APPLIED GERANYLGERANYLACETONE (GGA) ON ELECTRODE INSERTION TRAUMA IN COCHLEA IMPLANTED GUINEA PIGS Jochen Tillein[1,2], Felix Schmidt[1], Claudia Settevendemie[1], Susanne Braun[2], Sebastian Strieth[1], Timo Stöver[1] ([1]Frankfurt, Germany; [2]Starnberg, Germany)
P59	PMDS BIOCOMPATIBILITY IN PC12 NEURONAL CELL LINE Edi Simoni, Filippo Valente, Alice Cani, Laura Astolfi, Alessandro Martini (Padua, Italy)
P60	THE CONTROLLED-RELEASE EFFECT OF HYALURONIC ACID ON DRUG DELIVERY TO THE COCHLEAR SPIRAL GANGLION Yozo Inagaki, Sho Kanzaki, Masato Fujioka, Naoki Oisi, Kaoru Ogawa (Tokyo, Japan)

Monday 14 September 2015

Lazzati Room

10.05 - 11.00	**POSTER SESSION V**
NOISE INDUCED HEARING LOSS, OTOTOXICITY AND IMMUNOSYSTEM **Moderators:** Huawei Li, Bruno Sergi	
P61	NRF2 ATTENUATES NOISE-INDUCED HEARING LOSS BY PREVENTING OXIDATIVE DAMAGE OF COCHLEA Yohei Honkura, Shohei Murakami, Tetsuaki Kawase, Yukio Katori, Hozumi Motohashi (Sendai, Japan)
P62	NOISE-INDUCED COCHLEAR F-ACTIN DEPOLYMERIZATION IS MEDIATED VIA ROCK2/P-ERM SIGNALING Yu Han, Xian-Ren Wang, Jun Chen, Su-Hua Sha (Charleston, USA)
P63	COCHLEAR RESPONSE TO ACUTE AND CHRONIC NOISE EXPOSURE IN ADENOSINE RECEPTOR-DEFICIENT MICE Srdjan M Vlajkovic[1], Song Y Paek[1], Michelle Quinn[1], Detlev Boison[2], Gary D. Housley[3], Peter R. Thorne[1] ([1]Auckland, New Zealand; [2]Portland, USA; [3]Sydney, Australia)
P64	REDUCED CONNEXIN26 IN MATURE COCHLEA INCREASES SUSCEPTIBILITY WITH ACOUSTIC TRAUMA Sen Chen, Xingxing Zhou, Yu Sun, Weijia Kong (Wuhan, China)
P65	NUTRITIONAL THERAPY AND OCCUPATIONAL HEARING LOSS Natalya Petrova, Viia Panshina (St.Petersburg, Russia)
P66	OTOTOXICITY OF STREPTOCOCCUS SPECIES: AN *IN VITRO* MODEL Magdalena Solyga[1,4], Michael Perny[1], Marta Roccio[1], Denis Grandgirard[1], Stephen L. Leib[1,2], Pascal Senn[1,3] ([1]Bern, Switzerland; [2]Spiez, Switzerland; [3]Genève, France; [4]Cranfield, UK)
P67	TRANSTYMPANIC MODEL OF CISPLATIN-INDUCED OTOTOXICITY: COMPARISON OF THE COCHLEA AND THE VESTIBULE Angela Callejo[1,2], Amandine Durochat[3], Christian Chabbert[3,4], Ivan Domenech Juan[2,5], Jordi Llorens[1,5], Sophie Gaboyard-Niay[3] ([1]Barcelona, Spain; [2]Barcelona,Spain; [3]Montpellier, France; [4]Marseille, France; [5]Llobregat, Spain)
P68	REDOX IMBALANCE IN STYRENE OTOTOXICITY AND ACOUSTIC TRAUMA: IN VIVO PROTECTIVE EFFECT OF QTER R. Rolesi, F. Paciello, S.L.M. Eramo, A.R. Fetoni, G. Paludetti, D. Troiani (Rome, Italy)
P69	HYDROGEN PEROXIDE IN INNER EAR CELLS AS AN OXIDATIVE STRESS MODEL Alice Cani, Edi Simoni, Filippo Valente, Laura Astolfi, Alessandro Martini (Padua, Italy)
P70	WNT ACTIVATION PROTECTS AGAINST NEOMYCIN-INDUCED HAIR CELL DAMAGE IN THE MOUSE COCHLEA Renjie Chai[1,2], Liman Liu[3], Yan Chen[3], Yanping Zhang[3], Yingzi He[3], Wenli Ni[3], Dan You[3], Shan Sun[3], Makoto M. Taketo[4], Lei Wang[3], Huawei Li[3] ([1]Nanjing, China; [2]Nantong, China; [3]Shanghai, China; [4]Kyoto, China)

P71	CONNEXIN 43 ACTS AS A PRO-APOPTOTIC MODULATOR IN CISPLATIN-INDUCED AUDITORY CELL DEATH Yeon Ju Kim, Young Sun Kim, Jong Joo Lee, Oak-Sung Choo, Yun-Hoon Choung (Suwon, South Korea)
P72	MATRIX METALLOPROTEINASE INHIBITOR ATTENUATES COCHLEAR LATERAL WALL DAMAGE INDUCED BY INTRATYMPANIC INSTILLATION OF ENDOTOXIN Chul Ho Jang, Young Ho Choi, Yong Beom Cho (Gwangju, South Korea)
P73	FETAL THYMUS GRAFT ENABLES RECOVERY FROM AGE-RELATED HEARING LOSS AND EXPANSION OF CD4-POSITIVE T CELLS EXPRESSING IL-1 RECEPTOR TYPE 2 AND REGULATORY T CELLS Hiroshi Iwai, Muneo Inaba (Osaka, Japan)
P74	IFN/IFNAR1 SIGNALING IS CRUCIAL IN THE CONTROL OF VIRAL INFECTION IN THE COCHLEAR SENSORY EPITHELIUM Yushi Hayashi[1,2], Koji Onomoto[3,4], Mitsutoshi Yoneyama[3,4], Takashi Fujita[4], Nobuyuki Tanaka[2] ([1]Tokyo, Japan; [2]Kawasaki, Japan; [3]Chiba, Japan; [4]Kyoto, Japan)
P75	IMMUNE-RESPONSE OF GUINEA PIG COCHLEAE AFTER GRAFTING HUMAN CELLS AND ITS PREVENTION BY BONE MARROW DERIVED STROMAL CELLS Masaaki Ishikawa, Hiroe Ohnishi, Desislava Skerleva, Tatsunori Sakamoto, Norio Yamamoto, Juichi Ito, Takayuki Nakagawa (Kyoto, Japan)

Bausola Room

10.05 - 11.00	**POSTER SESSION VI**
GENETICS OF DEAFNESS **Moderators:** Byung Yoon Choi, Eva Orzan	
P76	IN VITRO EVALUATION OF PATHOGENIC POTENTIAL OF THE NOVEL *TMPRSS3* MUTATIONS AND GENOTYPE-PHENOTYPE CORRELATION Ah Reum Kim[1,4], So Ra Im[2], Sang Min Park[2], Woo Jin Park[2], Joo Hyun Park[3], Byung Yoon Choi[4] ([1]Seoul, South Korea; [2]Buk-gu, Gwangju, South Korea; [3]Siksa-dong, Ilsandong-gu, Goyang-si, Gyeonggi-do, South Korea; [4]Seongnam, South Korea)
P77	GENE EXPRESSION STUDY OF THE VESTIBULAR SYSTEM OF THE *IGF1* DEFICIENT MOUSE Lourdes Rodríguez-de la Rosa[1], Silvia Murillo-Cuesta[1], Julio Contreras[1], Joaquín Dopazo[1,2], Isabel Varela-Nieto[1], Marta Milo[3] ([1]Madrid, Spain; [2]Valencia, Spain; [3]Sheffield, UK)
P78	IDENTIFICATION OF A NOVEL RECESSIVE MUTATION OF MYO7A IN PROFOUND HEARING LOSS BY TARGETED EXOME SEQUENCING Jihye Rhee[1], Ah Reum Kim[2], Byung Yoon Choi[1,2] ([1]Seoul, South Korea; [2]Kyunggi, South Korea)
P79	ROLE OF *SYNJ2* IN HIGH FREQUENCY PROGRESSIVE HEARING LOSS Elisa Martelletti[1], Annalisa Buniello[1,2], Johanna C. Pass[1,2], Neil J. Ingham[1,2], Jacqueline K. White[2], Karen P. Steel[1,2] ([1]London, UK; [2]Cambridge, UK)
P80	POLYMORPHISMS IN GENES INVOLVED IN OXIDATIVE STRESS IN PATIENTS WITH MÉNIÈRE'S DISEASE Masaaki Teranishi[1], Yasue Uchida[1], Naoki Nishio[1], Ken Kato[1], Hironao Otake[1], Tadao Yoshida[1], Michihiko Sone[1], Saiko Sugiura[2], Fujiko Ando[2], Hiroshi Shimokata[2],Tsutomu Nakashima[2] ([1]Nagoya, Japan; [2]Aichi, Japan)
P81	A RETROSPECTIVE STUDY ON GJB2 ALLELES IN PATIENTS WITH NONSYNDROMIC SENSORINEURAL HEARING IMPAIRMENT: GENOTYPE/AUDITORY PHENOTYPE CORRELATION AND DESCRIPTION OF RARE VARIANTS Ilaria Stanghellini, Silvia Palma, Cristina Falcinelli, Maria Consolatrice Guarnaccia, Elisabetta Genovese, Antonio Percesepe (Modena, Italy)
P82	SYNDROMIC SENSORINEURAL HEARING LOSS AND MITOCHONDRIAL DNA MUTATIONS IN MELAS AND CPEO PATIENTS Gabriella Cadoni, Serenella Servidei, Monica Giuliani, Guido Primiano, Luca Liberati, Pasqualina Picciotti, Guido Conti (Rome, Italy)

P83	A HAYSTACK FULL OF NEEDLES: THE COMPLICATED INTERPRETATION OF RARE GENETIC VARIANTS IN A GERMAN FAMILY WITH AUTOSOMAL DOMINANT NON-SYNDROMIC HEARING LOSS Michaela A. H. Hofrichter, Barbara Vona, Erdmute Kunstmann, Indrajit Nanda, Thomas Haaf (Würzburg, Germany)
P84	CHARACTERISTCS OF INNER EAR HEARING LOSS DEPENDING ON DIFFERENT MUTATIONS OF GJB2 GENE Judith Arnolds[1], Sven Saleik[1], Patrick Munder[1], Bernd Wollnik[2], Dagmar Wieczorek[1] Alma Kuechler[1] Diana Arweiler-Harbeck[1] ([1]Essen, Germany; [2]Cologne, Germany)
P85	DIAGNOSTIC YIELD OF A TARGETED GENE SEQUENCING APPROACH WITHIN A REGIONAL UNIVERSAL NEWBORN HEARING SCREENING AND CHILDHOOD SURVEILLANCE PROGRAM: A 2 YEAR EXPERIENCE IN FRIULI VENEZIA GIULIA, ITALY Eva Orzan[1], Giorgia Girotto[2], Marco Gregori[1], Raffaella Marchi[1], Caterina Marchese[1], Lorenzo Monasta[3], Anna Morgan[2], Martina La Bianca[4], Diego Vozzi[4], Paolo Gasparini[2,4] (Trieste, Italy)
P86	A NEW TARGETED RE-SEQUENCING PANEL FOR UNVEILING THE GENETIC CAUSES OF AGE RELATED HEARING LOSS (ARHL) Anna Morgan[1], Diego Vozzi[2], Dragana Vuckovic[1], Martina La Bianca[1], Angela D'Eustacchio[2], Maria Pina Concas[2], Mario Pirastu[2], Paolo Gasparini[1], Giorgia Girotto[1] ([1]Trieste, Italy; [2]Sassari, Italy)
P87	DFNB1 LOCUS ANALYSIS IN SÃO TOMÉ AND PRÍNCIPE POPULATION Cristina Caroça[1,2], Tiago Morim de Matos[1,2], Graça Fialho[1], João Paço[1], Helena Caria[1,2] ([1]Lisbon, Portugal; [2]Setúbal, Portugal)
P88	PRESBYCUSIS AND MTDNA J. Chora[1], M. Flook[1], T.D. Matos[1], G Fialho[1], H. Caria[1,2] ([1]Lisbon, Portugal; [2]Setúbal, Portugal)
P89	CLASSIFICATION, PATHOPHYSIOLOGY AND AUDIOLOGICAL PATTERNS OF AGE-RELATED HEARING LOSS R. Santos[1], A.R. Lameiras[1], A. O'Neill[1], P. Escada[1], J. O'Neill[1], G. Fialho[1], H. Caria[1,2] ([1]Lisbon, Portugal; [2]Setúbal, Portugal)
P90	AGE-RELATED HEARING LOSS: IS THIS A PREVENTABLE CONDITION? M. Aparício[1], T.M. Matos[1], P. Arguello[2], M. Antunes[1], A. O'Neill[1], P. Escada[1], J. O'Neill[1], F. Fialho[1], H. Caria[1,2] ([1]Lisbon, Portugal; [2]Setúbal, Portugal)

Lazzati Room

16.00 - 16.45	**POSTER SESSION VII**
STEM CELLS AND GENE THERAPY **Moderators:** Marcelo Rivolta, Anna Rita Fetoni	
P91	HYALURONIC ACID PRETREATMENT FOR SENDAI VIRUS-MEDIATED COCHLEAR GENE TRANSFER Takaomi Kurioka[1], Kunio Mizutari[1], Katsuki Niwa[1], Makoto Inoue[2], Yasuji Ueda[2], Akihiro Shiotani[1] ([1]Saitama, Japan; [2]Ibaraki, Japan)
P92	A SIMPLE STEPWISE METHOD FOR HAIR CELL INDUCTION FROM HUMAN INDUCED PLURIPOTENT STEM CELLS Hiroe Ohnishi, Desislava Skerleva, Shin-ichiro Kitajiri, Tatsunori Sakamoto, Norio Yamamoto, Juichi Ito, Takayuki Nakagawa (Kyoto, Japan)
P93	EVALUATION OF THE EFFECT OF HUMAN ADIPOSE-DERIVED STEM CELLS (ASCS) ON SPIRAL GANGLION NEURONS TO IMPROVE COCHLEAR IMPLANTATION Philipp Schendzielorz, Yingjun Zhi, Anna Lundershausen, Kristen Rak, Johannes Völker, Agmal Scherzad, Katrin Frölich, Rudolf Hagen and Andreas Radeloff (Würzburg, Germany)
P94	COMPARISON OF THE DIFFERENTIATION OF NEURAL STEM CELLS AND PRIMARY NEURONS FROM THE RAT COCHLEAR NUCLEUS BY SPONTANEOUS CALCIUM ACTIVITY C. Voelker, K. Rak, J. Voelker, P. Schendzielorz, R. Hagen, A. Radeloff (Wuerzburg, Germany)
P95	HUMAN DERMAL FIBROBLASTS DEMONSTRATE POSITIVE IMMUNOSTAINING FOR NEURON- AND GLIA- SPECIFIC PROTEINS Cynthia.J. Janmaat, Karien E de Rooij, Simon C. de Groot, Heko Locher, John C.M.J. de Groot, Johan H.M. Frijns, Margriet A.Huisman (Leiden, the Netherlands)

P96	HYPERBARIC OXYGEN TREATMENT OF BONE MARROW DERIVED HUMAN MESENCHYMAL STEM CELLS Jennifer Schulze, Gerrit Paasche, Hans Lamm, Thomas Lenarz, Athanasia Warnecke (Hanover, Germany)
P97	DIFFERENTIATION OF MOUSE IPS CELL INTO CX26-POSITIVE CELL AND FORMATION OF INTER CELLULAR CX26-GAP JUNCTION PLAQUE Ichiro Fukunaga[1], Kaori Hatakeyama[1],Toru Aoki[1], Atena Nishikawa[1], Ayumi Fujimoto[1], Osamu Minowa[2], Katsuhisa Ikeda[1], Kazusaku Kamiya[1] ([1]Tokyo, Japan; [2]Tsukuba, Japan)
P98	CHEMICAL INDUCTION OF SENSORY CELL DIFFERENTIATION IN AN OTIC STEM CELL-BASED *IN VITRO* ASSAY Aurélie Dos Santos[1], Mirko Jaumann[1], Andrea Müller[1], Fiona Speichinger[1], Marcus Müller[2], Hubert Löwenheim[2] ([1]Tübingen, Germany; [2]Oldenburg, Germany)
P99	INDUCTION OF SENSORY NEURONS BY SMALL MOLECULES Rouknuddin Ali, Evelina Blomberg, Anna Falk, Lars Ährlund-Richter, Mats Ulfendahl (Stockholm, Sweden)
P100	PURIFICATION AND NEUROGLIAL DIFFERENTIATION OF MULTIPOTENT HAIR-FOLLICLE-BULGE-DERIVED STEM CELLS Timo Schomann, Fleur ten Tije, Shirley Man, Carlijn N.E. Peerboom, Johan H.M. Frijns, Margriet A. Huisman (Leiden, the Netherlands)
P101	INTENSIVE SUPPORTING CELL PROLIFERATION AND MITOTIC HAIR CELL GENERATION BY GENETIC REPROGRAMMING IN NEONATAL MOUSE COCHLEA Wenli Ni, Chen Lin, Luo Guoa, Wenyan Li, Huawei Li (Shanghai, China)
P102	ISOLATION AND CHARACTERIZATION OF NEURAL STEM CELLS FROM THE INFERIOR COLLICULUS Johannes Voelker, Christine Voelker, Kristen Rak, Philipp Schendzielorz, Rudolf Hagen, Andreas Radeloff (Wuerzburg, Germany)
P103	SCREENING FOR SURFACE MARKERS THAT WOULD ALLOW FOR THE PROSPECTIVE IDENTIFICATION AND PURIFICATION OF HESC-DERIVED OTIC PROGENITORS Sarah L. Boddy, Darrell M. Barrott, Paul J. Gokhale, Marcelo N. Rivolta (Sheffield, UK)

Bausola Room

16.00 - 16.45	**POSTER SESSION VIII**
CELL PHYSIOLOGY **Moderators:** Jonathan Gale, Giorgia Girotto	
P104	THE HAIR CELL SYNAPTIC COMPLEX AND NEUROJUNCTIONAL CONNECTIVITY Dennis G. Drescher, Neeliyath A. Ramakrishnan, Marian J. Drescher (Detroit, USA)
P105	VLGR1-MEDIATED SIGNALING PATHWAY IS IMPORTANT FOR HEARING Haibo Du, Qiaoxia Hu, Yanfei Wang, Jinpeng Sun, Zhigang Xu (Shandong, China)
P106	HYDROGEN SULFIDE INDUCES STORE-OPERATED CA2+ ENTRY THROUGH ACTIVATION OF TRPV4 IN OUTER HAIR CELLS Narinobu Harada[1], Yukari Ito[1], Ippei Takashima[2], Akio Ojida[2], Fumiko Sekiguchi[3], Atsufumi Kawabata[3] ([1]Osaka, Japan; [2]Fukuoka, Japan; [3]Osaka, Japan)
P107	THE BIOPHYSICAL PROPERTIES OF $I_{K,L}$ IN MAMMALIAN VESTIBULAR TYPE I HAIR CELLS AND HOW THEY ARE AFFECTED BY THE NERVE CALYX Elisa Tavazzani, Paolo Spaiardi, Marco Manca, Jacopo Magistretti, Giancarlo Russo, Ivo Prigioni, Sergio Masetto (Pavia, Italy)
P108	SODIUM-ACTIVATED POTASSIUM CURRENT IS ACTIVATED BY THE ACID SENSING IONIC CHANNELS MEDIATED Na^+ INFLUX IN AFFERENT VESTIBULAR NEURONS Blanca Cervantes[1], Rosario Vega[2], Enrique Soto[2] ([1]Madrid, Spain; [2]Puebla, México)
P109	STAGGERING ALONG THE COCHLEA: DOES THE SHAPE OF AN INNER HAIR CELL MATTER? Anwen Bullen, Antonio M Garcia de Diego, Andrew Forge, Jonathan Ashmore (London, UK)

P110	ACTIN FILAMENT NETWORK REGULATES EXOCYTOSIS AT THE HAIR CELL RIBBON SYNAPSE Marie Guillet, Gaston Sendin, Jérôme Bourien, Jean-Luc Puel and Régis Nouvian (Montpellier, France)
P111	HISTAMINE TYPE 4 RECEPTOR ANTAGONIST JNJ7777120 INHIBITS SODIUM CURRENT OF VESTIBULAR AFFERENT NEURONS OF THE RAT Rosario Vega, Enrique Soto, Emilio Salceda, Eduardo Salinas, Emmanuel Seseña (Puebla, México)
P112	DETECTION OF "HIDDEN" AUDITORY NERVE FIBER LOSS Charlène Batrel, Antoine Huet, Gilles Desmadryl, Jean-Luc Puel, Jérôme Bourien (Montpellier, France)
P113	INTRACELLULAR VESICLE TRAFFIC IN THE OUTER HAIR CELL OF THE GUINEA-PIG COCHLEA Anthony W. Gummer, Susanne Badum, Csaba Harasztosi (Tübingen, Germany)
P114	*EPS8* REGULATES K+ CHANNELS EXPRESSION IN MOUSE COCHLEAR BUT NOT VESTIBULAR HAIR CELLS Paolo Spaiardi, Elisa Tavazzani, Valeria Zampini, Marco Manca, Jacopo Magistretti, Giancarlo Russo, Sergio Masetto, Ivo Prigioni (Pavia, Italy)
P115	THE MYELIN PROTEIN ZERO-DEFICIENT MOUSE AS A MODEL FOR AUDITORY NEUROPATHY K. Rak, J. Völker, S. Schendzielorz, J. Groh, R. Martini, R. Hagen, A. Radeloff (Wuerzburg, Germany)
P116	MICROTUBULE MESHWORK REMODELING IN THE AUDITORY NEUROPATHY AUNA1 Cément Surel[1], Marie Guillet[1], Marc Lenoir[1], Jérôme Bourien[1], Benjamin Delprat[1], Marci Lesperance[2], Jean-Luc Puel[1], Régis Nouvian[1] ([1]Montpellier France; [2]Ann Arbor, USA)
P117	NEURONAL ENCODING OF SOUND IN NOISE Antoine Huet, Gilles Desmadryl, Jean-Luc Puel, Jérôme Bourien (Montpellier, France)
P118	VASCULAR DEGENERATION IN THE COCHLEA DURING MCMV INFECTION IN A MOUSE MODEL Mattia Carraro[1], Ali Almishaal[2], Elaine Hillas[2], Matthew Firpo[2], Albert Park[2], Robert Harrison[1] ([1]Toronto, Canada; [2]Salt Lake City, USA)

Tuesday 15 September 2015

Bausola Room

10.10 - 10.55	**POSTER SESSION IX**
PHYSIOPATHOLOGY OF AUDITORY PATHWAYS AND TINNITUS **Moderators:** Regis Nouvian, Gabriella Cadoni	
P119	POSSIBLE ROLE OF HERPESVIRIDAE FAMILY VIRUSES IN THE PATHOGENESIS AND EVOLUTION OF SENSORINEURAL HEARING LOSS Walter Di Nardo, Roberta Anzivino, Eugenio De Corso, Paola Cattani, Rosaria Santangelo, Gaetano Paludetti (Rome, Italy)
P120	TRANSIENT INDUCTION OF DEAFNESS BY OPTOGENESIS TARGETING THE ENDOCOCHLEAR POTENTIAL IN THE INNER EAR Mitsuo P Sato[1,2], Taiga Higuchi[1], Fumiaki Nin[1], Takamasa Yoshida[1,3], Karin Hori[1], Satoru Uetsuka[1,2], Samuel Choi[1], Shuichi Sakamoto[1], Hirohide Takebayashi[1], Katsumi Doi[2], Kenji Tanaka[4], Hiroshi Hibino[1] ([1]Niigata, Japan; [2]Osaka, Japan; [3]Kyushu, Japan; [4]Tokyo, Japan)
P121	AUDITORY CORTEX PLASTICITY IN A RAT MODEL OF NOISE-INDUCED HEARING LOSS: EFFECT OF ANODAL TRANSCRANIAL DIRECT CURRENT STIMULATION F. Paciello, F. Zanin, R. Rolesi, A.R. Fetoni, G. Paludetti, C. Grassi, D. Troiani, M.V. Podda (Rome, Italy)
P122	COCHLEAR MICROPHONIC: A GREAT COMEBACK AS NON INVASIVE TOOL FOR INTRACRANIAL PRESSURE MONITORING Blandine Lourenço, Fabrice Giraudet, François Longeras, Xavier Durando, Jean Gabrillargues, Laurent Sakka, Paul Avan (Clermont-Ferrand, France)

P123	CORRELATION BETWEEN MORPHOLOGY OF COCHLEAR EFFERENTS AND MEDIAL OLIVOCOCHLEAR REFLEX ACTIVITY IN MICE Shi Nae Park, So Young Park, Sang A Bak, Jung Mee Park, Woo Jin Kim, Sang Won Yeo (Seoul, South Korea)
P124	ACOUSTIC TRAUMA AT THE ONSET OF HEARING AFFECTS AUDITORY TEMPORAL RESOLUTION IN ADULT RAT Natalia Rybalko, Daniel Šuta, Jiří Popelář, Josef Syka (Prague, Czech Republic)
P125	ACTIVIN SIGNALING DISRUPTION IN THE COCHLEA DOES NOT INFLUENCE HEARING IN ADULT MICE Lukas Horvath[1], Daniel Bodmer[2], [2]Vesna Radojevic, [1]Arianne Monge Naldi ([1]Zurich, Switzerland; [2]Basel, Switzerland)
P126	**A** NEW PATHOLOGICAL COCHLEAR PROFILE Marion Souchal, Fabrice Giraudet, Paul Avan (Clermont-Ferrand, France)
P127	APPLYING A VERBOTONAL METHOD TO REHABILITATION OF THE HEARING IMPAIRED CHILDREN Ivanka Jurjević- Grkinić, Boška Munivrana, Vesna Mijić (Zagreb, Croatia)
P128	HYPERACUSIS AND TINNITUS DEVELOPMENT OVER AGE AND THE INFLUENCE OF NOISE EXPOSURE IN THE RAT BEHAVIORAL MODEL Sathish Kumar Singaravelu[1], Dorit Möhrle[1], Kun Ni[2], Dan Bing[3], Ksenia Varakina[1], Mirjam Rieger[1], Marlies Knipper[1], Lukas Rüttiger[1] ([1]Tübingen, Germany; [2]Shanghai, China; [3]Wuhan, China)
P129	I KNOW WHAT YOU HEAR: OBJECTIFICATION AND DIFFERENTIAL DIAGNOSIS OF VASCULAR PULSATILE TINNITUS BY TRANSCANAL SOUND RECORDING AND SPECTRO-TEMPORAL ANALYSIS Jae-Jin Song[1,] Gwang Seok An[2], Inyong Choi[3], Dirk De Ridder[4, 5], So Young Kim[1], Hyun Seok Choi[1], Joo Hyun Park[6], Byung Yoon Choi[1], Ja-Won Koo[1], Kyogu Lee[2] ([1]Seongnam, South Korea; [2]Seoul, South Korea; [3]Boston, USA; [4]Dunedin, New Zealand; [5]Antwerp, Belgium; [6]Goyang, South Korea)
P130	IS TINNITUS RETRAINING THERAPY USING VIDEO EDUCATION ON SMARTPAD AND SOUND SELF-THERAPY ALSO EFFECTIVE AS CONVENTIONAL ONE? Yoonjoong Kim, Moo Kyun Park, Jun Ho Lee, Seung Ha Oh, Myung-Whan Suh, Young Ho Kim (Seoul, South Korea)
P131	RELATIONSHIP BETWEEN TINNITUS AND SUICIDAL BEHAVIOR IN KOREAN MEN AND WOMEN: A CROSS-SECTIONAL STUDY Jae-Hyun Seo (Bucheon-si, South Korea)
P132	CLINICAL CHARACTERISTICS AND THERAPEUTIC EFFICACY OF PALATAL MYOCLONIC TINNITUS Woo Jin Kim, Jung Min Kim, So Young Park, Sang-Won Yeo, Shi Nae Park (Seoul, South Korea)

Bausola Room

10.55 - 11.40	**POSTER SESSION X**
OTOPROTECTION AND DRUG DELIVERY SYSTEMS **Moderators:** Jean Luc Puel, Roberto Albera	
P133	PROTECTION OF INNER HAIR CELLS FROM LETHAL INJURY: PREVENTING PROGRESSION TO PROFOUND HEARING LOSS Fitim Fetahu, Andrew Forge, Ruth Taylor (London, UK)
P134	MOLECULAR TARGETS FOR ANTICANCER REDOX CHEMOTHERAPY AND SIDE EFFECTS: THE ROLE OF CURCUMIN ON PSTAT3 AND NRF2 SIGNALLING A.R. Fetoni, F. Paciello, D. Mezzogori, S.L.M. Eramo, R. Rolesi, G. Paludetti, D. Troiani (Rome, Italy)
P135	ACTIONS OF DENDROGENIN B IN THE MOUSE COCHLEA Silvia Murillo-Cuesta[1], Néstor Vallecillo[1], Julio Contreras[1],Adelaida Celaya[1], RafaelCediel[1], Brigitte Malgrange[2], Michaël R. Paillasse[3], Isabel Varela-Nieto[1] ([1]Madrid, Spain; [2]Liège, Belgium; [3]Toulouse, France)
P136	NEUROPROTECTIVE AND NEUROREGENERATIVE POTENTIAL OF DENDROGENIN B IN *IN VITRO* MODELS OF HEARING LOSS Elodie Bacquié[1], Nicolas Caron[1], Debadutta Deb[2], Néstor Vallecillo[3], Bénédicte Franco[2], Philippe Lefebvre[2], Philippe de Medina[1], Brigitte Malgrange[2], Isabel Varela-Nieto[3], Michaël R. Paillasse[1] ([1]Toulouse, France, [2]Liège, Belgium; [3]Madrid, Spain)

P137	REDUCTION OF NOISE-INDUCED COCHLEAR SENSITIVITY LOSS BY INHIBITION OF MITOCHONDRIAL FISSION Alfred Nuttall, Sarah Foster, Beth Kempton, Teresa Wilson (Portland, USA)
P138	EFFICACY OF DIFFERENT ROUTES OF ADMINISTRATION FOR OTOPROTECTIVE AGENTS IN NOISE-INDUCED HEARING LOSS: SYSTEMIC VERSUS TRANS-TYMPANIC MODALITY S.L.M. Eramo, R. Rolesi, A. Di Pino, F. Paciello, G. Paludetti, A.R. Fetoni (Rome, Italy)
P139	ROSMARINIC ACID UP-REGULATES ENDOGENOUS ANTIOXIDANT DEFENCES THROUGH THE ACTIVATION OF NRF2/HO-1 PATHWAY AND PROTECTS AGAINST NOISE-INDUCED HEARING LOSS F. Paciello, A.R. Fetoni, R. Rolesi, S.L.M. Eramo, C. Mancuso, D. Troiani, G. Paludetti (Rome, Italy)
P140	GLYCEROL MONOOLEATE NANOPARTICLES *IN VITRO* BIOCOMPATIBILITY FOR INNER EAR DRUG DELIVERY FilippoValente[1], Edi Simoni[1], Helena Bysell[2], Alice Cani[1], Andrea Fornara[2], Laura Astolfi[1], Alessandro Martini[1] ([1]Padua, Italy; [2]Stockholm, Sweden)
P141	EFFECT OF LOCALLY APPLIED DEXAMETHASONE ON SPIRAL GANGLION NEURON SURVIVAL AND SIZE IN VIVO Verena Scheper[1], Mareike Hütten[1], Maciek Wilk[1], Claude Jolly[2], Jürgen Groll[3], Thomas Lenarz[1], Roland Hessler[2] ([1]Hannover, Germany; [2]Innsbruck, Austria; [3]Würzburg, Germany)
P142	DEVELOPMENT OF A NEUROTROPHIC IMPLANT FOR SEVERE HEARING LOSS Heike Janssen[1], Wiebke Konerding[1], Pavel Mistrik[2], Anandhan Dhanasingh[2], Jana Schwieger[1], Jens Tornøe[3], Lars U. Wahlberg[3], Thomas Lenarz[1], Andrej Kral[1], Verena Scheper[1] ([1]Hanover, Germany; [2]Innsbruck, Austria; [3]Ballerup, Denmark)
P143	DEVELOPMENT OF A RESEARCH SYSTEM FOR DRUG DELIVERY TO THE INNER EAR Daniel Smyth[1], Alec Salt[2], Jonathon Kirk[3], Jared Hartsock[2] ([1]Mechelen, Belgium; [2]St. Louis, USA; [3]Denver, USA)

Saturday 12 September 2015

Symposium "From lab to clinic: The opportunities and challenges for hearing rehabilitation and inner ear therapies"

S1
NEUROTRANSMISSION AND NEUROPROTECTION IN THE COCHLEA

Jean-Luc Puel
Institute for Neurosciences of Montpellier Inserm U1051 and University of Montpellier, France

Human hearing covers a large range of sound pressure levels (i.e., 0-120 dB sound pressure level; SPL). Sound pressure level is encoded in the firing rate of the auditory nerve fibers (ANFs): low sound-pressure levels activate the high-spontaneous rate (SR) fibers (SR > 18 spikes/s), and increasing levels gradually recruit medium- (0.5 < SR < 18 spikes/s) and then low-SR (SR < 0.5 spike/s) fibers. Intense sound stimulation results in various structural changes leading to functional auditory impairment. Early studies have attempted to correlate the functional impairment with stereocilias of the hair cells. More recently, Liberman's group reported direct evidence showing that massive inner hair cell (IHC) ribbon synapse loss can be a primary result of sound exposure, even when there is no loss of sensory hair cells (Kujawa and Liberman, 2009; Lin et al., 2011).

One feature of the cochlea's acute response to acoustic overstimulation is a massive swelling below the IHCs, most probably resulting from excess of glutamate release from the inner hair cells. In cochlea'guinea pigs, swelling of auditory nerve terminals can be prevent by intracochlear perfusion of glutamate antagonists (kynurenate, quinoxaliones, GYKI) during the noise exposure, and can be mimicked by perfusion of glutamate agonist (i.e., kainate and AMPA; Puel, 1995). In contrast to noise exposure, intracochlear of AMPA disrupted all the IHC-auditory nerve synapses along the tonotopic axis. More intriguing, SGN regenerated their terminals and formed new functional synapses within 5 days after AMPA perfusion. This synaptic repair was also confirmed by the full recovery of CAP thresholds and amplitudes. The discrepancies between noise-induced loss of afferent fibers and synaptic repair after AMPA perfusion can be reconciled if we consider that noise exposure induce additional injury of the presynaptic element. Finally, our presentation will discuss adapted pharmacological strategies to protect and/or promote the repair of ribbons synapses in excitotoxicity-related hearing loss.

S2
OXIDATIVE STRESS AS A TARGET OF ACQUIRED SENSORINEURAL HEARING LOSS

Allen F. Ryan[1], Volker Noack[1,2], Kwang Pak[1], Rahul Jalota[1], Yun-Hoon Choung[1,3]
Departments of Otolaryngology, [1]UCSD and San Diego VA Medical Center, La Jolla, CA USA, [2]Ruhr University, Bochum, Germany and [3]Ajou University, Seoul, South Korea

Extensive data link the formation of reactive oxygen species (ROS) to cochlear damage and hearing loss. In particular, many animal studies have demonstrated a protective effect of antioxidants against hair cell (HC) damage and hearing loss due to noise or ototoxic drugs. These data strongly suggest that there is potential for anti-oxidants to prevent HC damage. While only a limited number of controlled clinical trials have been conducted, some results suggest effectiveness of anti-oxidant therapy. We performed *in vitro* studies to investigate the time course of ROS production in HCs, the interaction of ROS formation with other intracellular processes that lead to HC death, and the ability of different redox compounds to influence sensory cell survival.

We assessed the production of ROS in living HCs during treatment with low-dose gentamicin, levels comparable to that seen in the *in vivo* cochlea. We found that ROS production peaks 24 hours prior to noticeable HC damage, providing a window of opportunity for intervention with anti-oxidant therapy.

We also tested, individually and in combination, anti-oxidant treatment, inhibition of Ras-cdc42-JNK signaling and inhibition of apoptosis, to determine whether synergistic protection against gentamicin toxicity might be achieved. We found that targeting additional aspects of HC damage or death signaling in combination with an anti-oxidant can be more effective than targeting any one process alone.

Another issue of importance in the use of anti-oxidants as HC protectants is the wide variety available for potential use. To address this, we used micro-explants of organ of Corti from mice expressing GFP in all HCs as a screening assay. We screened an 84-compound commercial library of anti-oxidants and pro-oxidants, to identify those with the potential to alter the process of gentamicin-induced HC death. We found that only a limited number of these factors were able to influence HC damage. This suggests that some anti-oxidant therapies may be more effective than others in protecting cochlear HCs.

Supported by grants from the US Veterans Administration and the NIH/NIDCD.

S3
INFLAMMATORY AND IMMUNE RESPONSES IN THE COCHLEA: FROM THE PERSPECTIVE OF BASIC RESEARCH TOWARD CLINICS

Masato Fujioka
Department of Otolaryngology-HNS, Keio University, School of Medicine, Tokyo, Japan

The inner ear was previously assumed to be an "immune-privileged" organ due to the existence of its tight junction-based blood-labyrinth barrier. However, studies performed during the past decade revealed that the mesenchymal region of the cochlea, including its lateral wall, is a common site of inflammation. In the presenting talk, I will first give a brief review of literatures describing immune/inflammatory cells in the cochlear mesenchymal region; then I will introduce our previous works describing the changes

of those cells in respond to the damages. Finally, I will show our new data indicating how the immunologic tolerance is essential in the inner ear of mice and the intolerance to the hair cells leads to bilateral, gradually developed hearing impairment. We anticipate that the findings coming up from these basic research would shed light on the significance of the immune-mediated symptoms in the inner ear and the findings may lead to novel therapeutics against sensorineural hearing impairment in the clinic.

S4
CONNECTING INNER EAR CONNEXINS STRUCTURE TO FUNCTION AND DYSFUNCTION
Fabio Mammano
Institute of Cell Biology and Neurobiology - National Research Council, Rome, Italy

Numerous studies have revealed that mutations in connexin genes lead to severe and debilitating diseases. In many cases, single point mutations lead to dramatic effects on connexin trafficking, assembly and channel function. The only structure resolved at near–atomic resolution (3.5 Å) is that of a (homomeric homotypic) gap junction channel formed by human Cx26 protomers. However, the mechanisms involved in the etiology of diseases linked to Cx26 (hearing impairment with or without dermatological manifestations) remain elusive. In this talk I will summarize the results of critical experiments performed on HeLa transfectants, cochlear organotypic cultures and live mice using a variety of biophysical methods. I will also discuss data interpretation in the light of multi–scale simulations *in silico* ranging from the atomic detail of connexin structure to mathematical modeling of cell networks and associated signaling mechanisms. Further details will be provided in a talk by Francesco Zonta (Session II, Sunday Sept. 13th, 12:43).
Supported by Fondazione Telethon, Grant GGP13114.

S5
LINKING GENES UNDERLYING DEAFNESS TO CLINICAL PERSPECTIVES
Christine Petit
Professor at College de France (Chair of Genetics and Cellular Physiology), Professor at Institut Pasteur, Paris, France

Severe to profound deafness affects one newborn child out of 700. It affects one additional child out of 1000 before adulthood, and 2.3% of the general population between 60 and 70. Moreover, 40% of the people past the age of 65 suffer from hearing loss impeding their conversational exchanges. More than 87 genes causative for monogenic forms of human non-syndromic (isolated) deafness, and 150 genes responsible for syndromic forms have now been identified. This genetic approach, coupled with interdisciplinary analyses of genetically engineered mouse mutants, has shed light on the underlying pathogenic mechanisms of most of these deafness forms, allowing to classify them into a small number of well-characterized pathogenic categories. On the basis of this knowledge, sources of bias in the audiometric evaluations have been revealed. Also the potential benefit, inefficiency or likely deleterious effects of the auditory prostheses (hearing aids and/or cochlear implants) in each of these deafness forms can now be anticipated. In parallel, taking advantage of these mouse models, the restoration of hearing by using different strategies can be tested rigorously and is providing promising results. These different points will be illustrated and discussed.

S6
A PERSPECTIVE ON PROSPECTS AND CHALLENGES FOR HAIR CELL REGENERATION
Andrew Forge, Ruth Taylor
UCL Ear Institute, London, UK

It has been shown there is a limited capacity to regenerate hair cells in the vestibular system of some mammals. The regenerated hair cells appear to arise by direct non-mitotic conversion of supporting cells, and *in vivo* they become innervated. There is evidence from studies of mouse vestibular organs that it may be possible to induce supporting cell conversion either through transfection with the *Atoh-1* gene, or through pharmacological manipulation of the Notch-Delta signalling pathway. Recent observations of immature hair bundles in utricular maculae from elderly human patients suggest that a limited capacity to regenerate hair cells may exist throughout life in the vestibular system of humans. In human vestibular tissues maintained in explant cultures, following exposure to aminoglycoside to ablate hair cells, application of procedures to induce supporting cell conversion results in the appearance of many cells that express hair cell markers. While electrophysiological characterisation is yet to be performed, results indicate that regenerating hair cells in human vestibular organs may be a feasible prospect as a therapy for some vestibular disorders. In the organ of Corti, however, while there are reports that a limited degree of supporting cell to hair cell conversion might be inducible in the immediate aftermath of an event that causes hair cell loss, there may be certain challenges to developing a regenerative therapy for hearing impairment. Supporting cells in the organ of Corti acquire particular specialisations during late stages of cochlear maturation, but following hair cell loss these are retained and there is no de-differentiation to a more immature status that might be susceptible to manipulation of pathways that are normally active during development. Subsequently, over time, the organ of Corti can become replaced by a non-specialised squamous-like epithelium, during the formation of which those supporting cells that normally surround the hair cells, and which might be thought to be the precursors of new hair cells, may be lost. This tissue re-organisation has other implications for the application of regenerative therapies. It may occur in patches scattered along the organ of Corti, such that different cellular environments may exist along an individual cochlea, and the rate and extent of its development may be affected by genetic background so there are likely to be differences between individual patients with hearing loss. It is also apparent from experimental studies of animal cochleae and of the temporal bones from hearing impaired people that in many conditions where there is hair cell loss other tissues and structures, for example the tectorial membrane or stria vascularis which are important for auditory function, are also adversely affected.Under such conditions regenerating hair cells may not be an effective means to restore hearing.

S7
ADDRESSING CHALLENGES FOR THE CLINICAL APPLICATION OF HUMAN STEM CELLS
Marcelo N. Rivolta
Centre for Stem Cell Biology, Department of Biomedical Sciences, University of Sheffield, UK

The development of a clinical application for stem cells would require overcoming a series of challenges such as a safe and efficient

manufacture of the appropriate cell type under GMP conditions. Protocols have been developed to induce differentiation of human embryonic stem cells (hESCs) along otic lineages and these have been shown in animal models to be capable of rescuing auditory function. However, the generation of otic progenitors *in vitro* is still inefficient, producing relative low yields in a heterogeneous population. We are currently developing new, more efficient methods for the manufacture of otic cells. To achieve this objective, we created a reporter line by using two enhancers that drive SOX2 expression to the nasal and otic placodes (NOP-1 and NOP-2). Using this reporter, we have modified our previous monolayer method based on FGF signalling by incorporating differential manipulation of Wnt signalling. This new process improves the yield of otic progenitors from ~20% to ~55%, as measured by the co-expression of eGFP and several otic markers. We are also using this platform to identify relevant surface antigens that could be used for their prospective purification. Recently, we have directed our attention to the status of the original, undifferentiated hESC population that is fed into the manufacturing process. A variable degree of heterogeneity has been described within the pluripotent hESC compartment. Cell fate decisions in stem cell populations can be substantially affected by these variations, generating lineage bias and becoming an important source of variability. We have therefore explored if fluctuations within the pluripotent state affect the differentiation outcome. More specifically, if these fluctuations generate subsets of cells that are primed to favourably differentiate into otic lineages. To address these questions we sorted hES cells for stage specific embryonic antigen 3 (SSEA3) and subjected the positive and negative fractions to otic differentiation. SSEA3 is a sensitive marker of the more pristine, pluripotent state. Our data showed that the SSEA3+ and SSEA3- populations had a differential response to the same differentiation cues, with the SSEA3+ fraction being more efficient at producing otic phenotypes.

Supported by the Medical Research Council and the EUFP7 Consortium OTOSTEM.

S8

SENSORIMOTOR PROCESSING OF SPEECH

Luciano Fadiga

University of Ferrara and IIT@UNIFE Center for Translational Neurophysiology of Speech and Communication, Ferrara, Italy

Despite the resonance evoked by the famous 'motor theory of speech perception' conceived by Alvin Liberman almost fifty years ago, a strong debate still survives on the possibility that speech understanding does not rely on sensory processing alone. In my presentation I will provide evidence that Liberman was substantially right and that a motor framework for speech understanding does exist. The sensorimotor association at the basis of speech processing is just an extension of a more general "mirror" mechanism, as proven by recent TMS data, patients studies, and computational models, all converging in the same direction.

S9

BRAIN AND NEUROIMAGING

Renzo Manara

University of Salerno, Department of Medicine and Surgery, Salerno, Italy

Advances in magnetic resonance imaging (MRI) have enabled the non-invasive detailed anatomical and functional investigation of the human auditory cortex. The developmental process implies a morphological and functional maturation encompassing gyrification, myelination and neural connectivity changes that can be charted by conventional MRI from the intrauterine life to adulthood. Functional MRI provides interesting insights about the tonotopic organization of the human acoustic cortex and the role of the distinct acoustic areas in sound information processing. According to these findings, the auditory cortex presents a hierarchical organization with a core of primary auditory areas that receive ascending projections from the auditory portion of the thalamus, and is surrounded by non-primary belt and parabelt regions. Recent post-processing analyses (voxel based analysis, cortical thickness analysis, tract based spatial statistics, seed based analysis, tractography etc.) provide further information about the anatomical and functional connectivity and about the (ultra)structural organization that might be impaired in several clinical conditions.

S10

EFFECT OF CROSS-MODAL PLASTICITY ON AUDITORY FUNCTION IN CONGENITAL DEAFNESS

Andrej Kral

Institute of AudioNeuroTechnology & Dept. of Experimental Otology, ENT Clinics, Medical University Hannover, Germany

Deafness interferes with the maturation of the auditory cortex (Kral et al., 2005, Cereb Cortex). Early chronic cochlear implant (CI) stimulation can induce cortical maturation both in humans and in congenitally deaf cats (CDCs) (Kral, 2013, Neuroscience). One possible measure of auditory plasticity is the reorganization of aural preference following monaural CIs (Kral et al., 2013, Brain), showing a sensitive period of ~3 months in cats. A substantial reduction of binaural information following developmental unilateral hearing was found in cortical neurons (Kral et al., 2015, Audiol Neurootol). Consequently, auditory maturation requires experience. Additional to reduced synaptic plasticity, loss of acuity in feature representation, deficits in integrative function of the cortical column and deficits in corticocortical, particularly top-down, interactions, close the sensitive periods (Kral 2013, Neuroscience). Cross-modal plasticity recruits auditory resources for non-auditory tasks (Lomber et al., 2010, Nat Neurosci). Despite of cross-modal reorganization in some auditory areas the extend of the underlying reorganization of corticortical connections (Barone et al., 2013, PLoS One) indicates that this only moderately limits auditory processing capacity. Electrophysiological recordings in the dorsal auditory cortex of CDCs demonstrate that the cross-modally reorganized secondary auditory areas maintain a predominance of dormant auditory inputs additional to moderate cross-modal reorganization.

Supported by Deutsche Forschungsgemeinschaft (Cluster of Excellence Hearing4all)

S11

MOLECULAR UNDERPINNINGS OF TINNITUS/ HYPERACUSIS

Marlies Knipper, Dan Bing, Dario Campanelli, Lewis Sze Chim Lee, Dorit Möhrle, Kun Ni, Yan Zhu, Wibke Singer, Lukas Rüttiger

Department of Otolaryngology, Head and Neck Surgery, Hearing Research Centre Tübingen, Molecular Physiology of Hearing, University of Tübingen, Germany

Hearing disorders (Age-and Noise dependent hearing loss, Tinnitus

or Hyperacusis) are expected to increase over the next decades due to demographic changes and altered leisure behavior. We here summarize the current knowledge of different cochlear damage profiles in the context of different brain responses and possible resulting auditory disorders. On the basis of this current knowledge new possible research directions and requests for future medical and industrial research profiles are suggested.
Supported by the Marie Curie Research Training Network CavNET MRTN-CT-2006-035367, the Deutsche Forschungsgemeinschaft DFG-Kni-316-4-1 and Hahn Stiftung (Index AG).

S12
PRESBYCUSIS IN EXPERIMENTAL ANIMALS AND IN MEN
Josef Syka
Institute of Experimental Medicine, Academy of Sciences, Department of Auditory Neuroscience, Prague, Czech Republic

With the prolongation of human life, the number of people suffering from age-related hearing loss is continuously increasing, yet there is no efficient method of treatment with the exception of hearing aids or cochlear implants. The aim of this presentation is to characterize the changes both in the peripheral and central parts of the auditory system that accompany presbycusis, with the ultimate goals of understanding the mechanisms underlying this process and developing methods of better diagnosis and treatment. The age-related apoptotic decrease in the number of outer and inner hair cells in the inner ear is well known, in addition to this recent data also demonstrates a significant age-related decrease in the number of ribbon synapses.. Changes in the central auditory system associated with aging have so far been described to a lesser extent. Our data shows that aging in the central auditory system of rats is accompanied with a non-significant decrease in the number of neurons, however, a significant loss appears among special groups of neurons, like SMI-32 – immuno-reactive neurons. The results of animal experiments show that one of the significant age-related changes is the deterioration of inhibitory transmission in the central auditory pathway. Age-related changes in the auditory system are also present on the level of calcium-binding proteins. Aging is accompanied by a worsening of temporal resolution as demonstrated by increased gap detection thresholds in human subjects and experimental animals.

S13
NEW INSIGHT ON HEARING AIDS AND AGING
Mark Laureyns
Amplifon CRS International, Antwerp, Belgium

The ageing population presents specific needs for hearing care. Recently the relation between hearing loss and cognitive decline has been confirmed in multiple studies. Taking cognition into account for hearing care (Cognitive Hearing Science) proves to be very interesting domain. We will present an update on the relation hearing loss and cognition and how the evaluation of cognitive aspects leads to innovative new concepts of hearing care.
When using a new adaptive version of the reading span test – intended to evaluate working memory capacity - and comparing this with the self-perceived ability to stay concentrated for a long time during a conversation, this provides valuable information on how to select and set the features of hearing aids and can call for intensive counselling the ageing patient.

S14
PRESENT STATUS AND FUTURE DEVELOPMENTS OF ACOUSTIC IMPLANTS (MIDDLE EAR IMPLANTS)
T. Lenarz, H. Maier
Department of Otorhinolaryngology, Medical University of Hannover (MHH), Germany

Acoustic implants are used to serve patients with either conductive or mixed hearing loss. They produce vibratory energy to stimulate the inner ear and couple either to the bone (bone-anchored hearing aids), to the ossicular chain (middle ear implants) or directly into the perilymph (DACS = direct acoustic cochlear stimulation).
The different classes of devices serve patients with different degrees of conductive and mixed hearing loss. BAHA is indicated for patients with mainly pure conductive loss, middle ear implants for patients with moderate–to-severe mixed hearing loss and DACS devices for patients with profound-to-severe mixed hearing loss.
The overlap with cochlear implants is given for patients with residual hearing loss up to 80 dB HL across all frequencies. Therefore also patients with pure sensorineural hearing loss can benefit from acoustic implants.
Current developments are ongoing in the area of coupling modes to the ossicular chain and the inner ear, as well as the development of intraoperative biological measures to test the efficiency of the various stimulation modalities.
Future developments will go towards integrated electro-mechanical devices, providing intracochlear stimulation with a combination of electrical and vibratory stimulation by integrated mechanical transducers into the cochlear implant electrode. This will provide a wide range of stimulation modes and flexibility, to enable the adjustment of the signal processing to the progression of the hearing loss. Even multichannel mechanical stimulators can be realized using optoacoustic effects for locally restricted direct mechanical stimulation of hair cells.

S15
A THREE DIMENSIONAL PERCEPT IN THE ABSENCE OF FUSION: WHAT COCHLEAR IMPLANTS IN CHILDREN HAVE TAUGHT US ABOUT THE DEVELOPING AUDITORY SYSTEM
Blake Papsin
Department of Otolaryngology-HNS, The Hospital for Sick Children, Toronto, Ontario, Canada

This presentation will explore what we have learned about the central processing of poor fidelity auditory stimuli such as those provided by current cochlear implants in children with severe to profound sensorineural hearing loss. Our group has been fascinated by the implanted human's ability to use incomplete primary source data to reassemble the auditory environment reasonably correctly. Even more fascinating is the finding that bilateral auditory inputs in these children fuse incompletely if at all yet they are able to lateralize sounds correctly. This capacity is undoubtedly related to the importance of correct sensory reassembly for survival. The study of children with cochlear implants has allowed us a wonderful opportunity to study the developmental processes which underlie this ability.
The contributions of the auditory brainstem and central auditory centres in addition to the other non-auditory cortical processors will be discussed and a model of sensory reassembly presented.

S16
OPTOGENETIC STIMULATION OF THE AUDITORY NERVE FOR RESEARCH AND FUTURE COCHLEAR IMPLANTS
Tobias Moser (for the Göttingen Cochlear Optogenetics Program)
Institute for Auditory Neuroscience, University of Göttingen Medical Center, Göttingen, Germany; Auditory Neuroscience Group, German Primate Center, Göttingen, Germany; Bernstein Focus for Neurotechnology, University of Göttingen, Göttingen, Germany; Auditory Neuroscience Group, Max-Planck-Institutes for Experimental Medicine, Göttingen, Germany

When hearing fails, speech comprehension can be restored by auditory prostheses. However, sound coding with current prostheses, based on electrical stimulation of auditory neurons, has limited frequency resolution due to broad current spread. Optical stimulation can be spatially confined and may therefore improve frequency and intensity resolution. We have established optogenetic stimulation of the auditory pathway in rodents using virus-mediated expression of channelrhodopsins to render spiral ganglion neurons light-sensitive. Optogenetic stimulation of spiral ganglion neurons activated the auditory pathway, as demonstrated by recordings of single neuron and neuronal population responses at various stages of the auditory system. We approximated the spatial spread of cochlear excitation by recording local field potentials in the inferior colliculus in response to suprathreshold optical and electrical stimuli, which suggested a better frequency resolution for optogenetic than for electrical stimulation. Moreover, we found activation of neurons in primary auditory cortex and were able to restore auditory activity in deaf mice. In a collaborative effort we develop and characterize flexible µLED-based multichannel intracochlear stimulators. My presentation will review recent progress in optogenetic stimulation of the auditory system and its potential for future application in research and hearing restoration.

S17
NEW INSIGHT ON COCHLEAR IMPLANTS
Patrick Boyle - *Advanced Bionics*; Carl Van Himbeeck - *Cochlear;* Reinhold Schatzer - *Med-El*

S17A
NAIDA CI: THE COMBINATION OF ADVANCED BIONICS AND PHONAK TECHNOLOGY
Patrick Boyle
Advanced Bionics GmbH

Communication in background noise is still a major problem for CI recipients. Hence, major research directions include the new beamforming systems and improved combinations of acoustic and electrical hearing. These are available in the new Naida CI sound processors.
The AB/Phonak voice stream technology enables the wireless bi-directional exchange of audio signals between bilateral sound processors. This makes possible a truly binaural beamformer (StereoZoom), combining two ipsilateral and two contralateral microphones into a full 4-microphone beamforming array. Such systems provide a substantially sharper front focus than today's 2-microphone beamformers. Improving ease of use for the CI recipient, through automatic activation of beamforming in speech-in-noise situations, is another important field of innovation.
Another effective option to improve hearing performance for CI recipients with low-frequency residual hearing is complementary acoustic amplification, either with EAS processors, combining electric and acoustic stimulation in one ear, or by bimodal fitting, where the contralateral ear is fitted with a hearing aid. For those without residual hearing, the unique Phantom current steering technology can improve low-frequency perception by shifting the electrical excitation in the apical direction; beyond the end of the electrode array.
Together the techniques outlined above will, improve performance in some of the most difficult listening situations. They will add naturalness and sound quality, while making it easier for the CI recipient to use these various algorithms.

S17B
HEARING IMPLANT TECHNOLOGY
Carl Van Himbeeck
Cochlear Technology Centre Europe

The presentation will give a brief overview of the latest hearing implant technology developments at Cochlear spanning a broad range of implantable hearing aid technologies using different stimulation modes. The presentation will cover recent developments on wireless technologies, bimodal stimulation, hearing preservation, electrode development and an overview of Cochlear's choice of implantable hearing solutions.

S17C
COCHLEAR IMPLANTS IN SINGLE-SIDED DEAFNESS: WHAT CAN WE LEARN?
R. Schatzer[1], K. Vermeire[2], S. Zirn[3], P. Van de Heyning[4], C. Zierhofer[5]
[1]MED-EL, Innsbruck, Austria; [2]Long Island Medical Center, New Hyde Park, NY, USA; [3]Department of Oto-Rhino-Laryngology, University of Freiburg Medical Center, Freiburg, Germany; [4]Department of Otorhinolaryngology, University Hospital Antwerp, Antwerp, Belgium; [5]Institute of Mechatronics, University of Innsbruck, Innsbruck, Austria

Single-sided deafness (SSD) has become the most recent indication for treatment with a cochlear implant (CI), receiving regulatory approval in several countries including the EU. CI recipients with normal hearing on the contralateral ear can uniquely compare stimuli across electrical and acoustic stimulus domains. The resulting findings may not only reveal factors that facilitate the perceptual integration of the CI in SSD users, but also inform the design of cochlear implants in general.
Studies comparing acoustic and electric pitch percepts in experienced SSD MED-EL CI recipients revealed that electrode place pitch matches in experienced CI users do not deviate consistently from Greenwood's map for acoustic hearing and correspond to model predictions; with long electrode arrays, place pitch does not shift significantly over time, minimizing the need for perceptual adaptation; low pitch percepts are elicited only by applying a correspondingly low pulse rate on apical electrodes; and that the slopes of rate-pitch functions match those of normal acoustic frequency-pitch functions only on electrodes in the apical turn.
These results support the approach of representing low frequency information via explicit rate code on apical electrodes in MED-EL fine-structure sound coding strategies. The relatively small

perceptual adaptation required in implant recipients with long electrode arrays has been shown to facilitate a faster implant learning curve and higher asymptotic levels of speech recognition in MED-EL patients.

Another study measured wave V latencies in the auditory brainstem of SSD implant users for both acoustic (ABR) and electrical (EABR) stimuli. EABR latencies combined with the channel-specific CI processor group delays, representing the total wave V latency from the acoustic signal through the CI to the brainstem, were comparable to normal acoustic latencies.

Similar wave V latencies for acoustic and electrical stimulation modalities may improve binaural hearing and support better ITD sensitivity and spatial release from masking in SSD CI users.

Mimicking normal frequency-place maps, temporal coding, and frequency-specific latencies in a CI system may not only facilitate a perceptual integration of the CI in SSD implant recipients, but also support faster learning curves in traditional uni- and bilateral implant recipients.

Sunday 13 September 2015

52nd Inner Ear Biology Workshop
Oral Communications

SESSION I
DEVELOPMENTAL BIOLOGY AND REGENERATION

TL1

IDENTIFICATION OF PROGENITOR CELLS IN THE COCHLEA: LGR5-POSITIVE SUPPORTING CELLS

Fuxin Shi, Lingxiang Hu, Albert S.B. Edge
[1]Harvard Medical School, Boston, MA, USA; [2]Massachusetts Eye and Ear Infirmary, Boston, MA, USA

Wnt signaling is required for the differentiation of hair cells during embryogenesis. Wnt stimulates otic progenitors to express transcription factor, Atoh1, which is required for hair cell development. Lgr5, a downstream target of the Wnt pathway and a protein that marks intestinal epithelial stem cells, is expressed in Lgr5-positive cells that gave rise to hair cells based on lineage tracing. Lgr5 continued to be expressed in the postnatal cochlea in a specific subset of supporting cells. In vitro analysis showed that Lgr5-positive cells had distinct phenotypes from the other (Sox2-positive) supporting cells and differentiated to hair cells at a higher rate, consistent with these cells playing a role as hair cell progenitors. The in vitro studies also showed that hair cells did not differentiate from Lgr5-negative cells. Hair cell replacement seen following ototoxic damage in neonatal ears was due to supporting cell transdifferentiation to hair cells, directly, or after cell division in a spontaneous response to damage, without pharmacological intervention. The response to damage was accompanied by Wnt release and was blocked by inhibition of Wnt signaling. Both cell division and hair cell differentiation were increased by treatment with an inhibitor of gamma-secretase. Based on lineage tracing, upregulation of Wnt signaling in the newborn inner ear, even in the absence of damage, specifically targeted the Lgr5-expressing cells, leading to proliferation, and the cells transdifferentiated to hair cells after increasing expression of Atoh1, which was downstream of Wnt. These data suggest that manipulation of signaling pathways increases regeneration of hair cells and that Lgr5-positive cells act as hair cell progenitors in the cochlea.

O1

THE EXPRESSION OF NETRIN-1 RECEPTORS IN SENSORY EPITHELIUM AND SPIRAL GANGLION CELLS OF THE NEONATAL COCHLEA

Norio Yamamoto, Kohei Yamahara, Takayuki Nakagawa, Juichi Ito
Graduate School of Medicine, Kyoto University,/ Department of Otolaryngology, Head and Neck Surgery, Kyoto, Japan

Background. We have demonstrated that insulin-like growth factor 1 (IGF1) protects cochlear hair cells of neonatal mice against aminoglycoside (Hayashi et al. Molecular and Cellular Neuroscience 2013). We have identified two genes whose expressions were increased by IGF1 treatment, using microarray and quantitative RT-PCR (qRT-PCR) (Hayashi et al. Neuroscience Letters 2014). One of such gene was netrin-1 (*Ntn1*). NTN1 is known to mediate an anti-apoptotic survival effect other than their conventional roles, axon guidance and cell migration through 6 canonical receptors, UNC5A, UNC5B, UNC5C, UNC5D, DCC, and NEOGENIN. Recent studies have indicated a crucial role for NTN1 receptors, especially UNC5B and DCC, in mediating survival effects of NTN1. We have demonstrated that administration of NTN1 resulted in the maintenance of both inner hair cells and outer hair cells numbers after aminoglycoside treatment as observed in IGF1. These results indicated that NTN1 is the probable effector of IGF1 signal and that NTN1 can be the novel and potent treatment of sensorineural hearing loss. As a step to identify the effector cells of NTN1, we tried to identify the physiological expression of NTN1 receptors in the cochlea using *in situ* hybridization for NTN1 receptors in the cochlea.
Methods. *In situ* hybridization was performed for each NTN1 receptor on 12 μm sections from mouse cochleae at P2. A partial length mouse cDNA sequence of each receptor was used to generate digoxigenin-labeled sense and antisense RNA probes. Corresponding sense probes were carried out in this experiment as controls and yielded little hybridization signal.
Results. Among 6 canonical receptors, Only *Unc5b* is expressed in the sensory epithelium including hair cells as previously reported (Matilainen et al, Int. J. Dev. Biol 2007). However, all canonical NTN1 receptors except *Unc5a* are expressed in spiral ganglion cells.
Conclusions. We found that NTN1 receptors are highly expressed in sensory epithelium and spiral ganglion cells of the cochlea in the physiological condition.
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O2

THE ROLE OF SOX2 IN INNER EAR DEVELOPMENT

Martina Dvorakova[1], Romana Bohuslavova[1], Bernd Fritzsch[2], Josef Syka[3], Tatyana Chumak[3], Gabriela Pavlinkova[1]
[1]Department of Molecular Pathogenetics, Institute of Biotechnology, Academy of Sciences, Prague, Czech Republic; [2]Department of Biology, University of Iowa, Iowa City, Iowa, USA; [3]Department of Auditory Neuroscience, Institute of Experimental Medicine, Academy of Sciences, Prague, Czech Republic

Introduction. Neurons and sensory cells of the inner ear derive from a common embryonic epithelial neurosensory domain of the otocyst. Transcription factors Islet1 and Sox2 are overlappingly expressed in these common neurosensory precursors. Sox2 is necessary for the specification of neurosensory cells through the

direct activation of differentiating transcription factors such as Neurog1 and Atoh1. Later in development, Sox2 activates negative regulators of these factors and therefore becomes downregulated. Islet1 is likewise expressed in the early otocyst, predominantly in the region of future cochleovestibular ganglion. Our aim is to determine function and possible interactions between Sox2 and Islet1 during inner ear neurosensory development.

Methods. We used a mouse model with the conditional deletion of *Sox2* in Islet1 expressing cells. *Sox2* gene was flanked by two loxP sites generating a conditional null allele in the cells expressing *Cre* recombinase under the *Islet1* promoter. Embryonic cells were visualized by immunolabelling of specific markers: Sox2 for supporting sensory cells, Myosin7A for hair cells and Tubulin for neuronal fibers. Additionally, the expression of *Islet1* and *Pax2* genes was analyzed by immunohistochemistry. The inner ear morphology was viewed by three-dimensional reconstruction from optical sections using confocal microscopy.

Results. The conditional *Sox2* deletion was postnatally lethal and the mutants die shortly after birth. The development of the inner ear was severely affected by *Sox2* deletion with apparent morphological defects of the vestibular system and undetectable sensory cells either in the cochlea or in the vestibular system. The innervation of the inner ear was impaired in the mutants compared to control littermates and no spiral ganglion neurons innervating the mutant cochlea were detectable. In addition to the neurosensory defects, mutant mice showed the motor neuron defects as early as embryonic day 10.5.

Conclusion. We show that Sox2 expression overlap with Islet1 in cells which are necessary for later formation and differentiation of sensory cells and neurons in the inner ear. The neuronal projections are impaired and the sensory cells do not differentiate in the inner ear of $Sox2^{-/-}$ mutants. Thus, Sox2 and Islet1 are essential for neurosensory development of the inner ear.

O3
ROLES OF HGF/MET SIGNALING IN THE MOUSE COCHLEA

Toru Miwa[1,2], Takahiro Ohyama[2], Shumei Shibata[3], Ryosei Minoda[1], Eiji Yumoto[1]

[1]Department of Otolaryngology-Head and Neck Surgery, Kumamoto University, Kumamoto, Japan; [2]Department of Otolaryngology, Keck School of medicine, University of Southern California, Los Angeles, CA, USA; [3]Department of Otolaryngology-Head and Neck Surgery, Kyushu University, Fukuoka, Japan

Objective. Mutations of Hepatocyte growth factor (HGF) gene cause the nonsyndromic hearing loss in human, and conditional knockout mouse of HGF dysregulation shows hearing disorders (Schultz et al., 2009). The cMet receptor tyrosine kinase (MET) is the only known cell-surface receptor for the HGF-mediated signaling pathway. Although the HGF/MET signaling pathway has been studied in diverse organs and cell types, roles of HGF/MET on inner ear development remain to be elucidated.

We investigated the roles of HGF/MET signaling in the developing inner ear utilizing inner ear-specific HGF and MET conditional knockout (cKO) mice by the Pax2cre transgenic mice (HGFfx/fx;Pax2cre, METfx/fx;Pax2cre) and neural crest cell-specific MET cKO mice by the Wnt1cre transgenic mice (METfx/fx;Wnt1cre).

Methods. The expression patterns of HGF and MET in the developing mouse inner ear were characterized by using in situ hybridization and immunohistochemistry. To determine the auditory function, Auditory Brain Responses (ABRs) were measured, and the features of morphology were examined by HE staining and immunohistochemistry.

Results. HGF signal was detected in stria vascularis (SV), and cMet signal was detected in lateral side of SV and neural crest cells (NCCs). HGFfx/fx; Pax2cre, METfx/fx; Pax2cre and METfx/fx; Wnt1cre mice had severe hearing loss and each SV was thinner than those of control mice. And the SV of each cKO mice lacked intermediate cells.

Conclusions. We investigated the roles of HGF/MET signaling in the developing inner ear utilizing inner ear-specific cKO mice and neural crest cell-specific cKO mice. HGF/MET signaling contributed to develop the intermediate layer of SV and was crucial for hearing.

O4
ATOH1 INDUCED AUDITORY SENSORY EPITHELIUM REGENERATION *IN VITRO*

Ning Cong[1], Juanmei Yang[2], Dongdong Ren[2], Zhao Han[2], Yibo Huang[2], Wenwei Luo[2], Fanglu Chi[2, 3, 4]

[1]Research Center, Eye Ear Nose & Throat Hospital, Fudan University,Shanghai 200031, China; [2]Department of Otology and Skull Base Surgery, Eye Ear Nose & Throat Hospital, Fudan University, Shanghai 200031, China; [3]Shanghai Auditory Medical Center, Shanghai, China; [4]Key laboratory of Hearing Science, Ministry of Health, Shanghai, China

Objective. To investigate whether new hair cells can integrate into organ of Corti (OC), and if so, what is the possible mechanism of the integration.

Methods. The basilar membrane of P2 (Postnatal 2 days) rat was explanted and cultured in three sections as basal, middle and apex in vitro. Atoh1 gene was introduced into the cultured basilar membrane by adenovirus with co-expression of EGFP (Ad-atoh1-EGFP), and adenovirus encoding only EGFP (Ad-EGFP) was used as control. New hair cells were identified by Myosin7a expression together with EGFP. Scanning electron microscopy(SEM) was used to observe the morphological change. Section-co-culture was used to determine the difference appearance from the basal to the apex during new hair cell production and the possible cue of the new hair cell integration into OC. The movement of new hair cells was recording by live cell scan array for 24h.

Results. 1) New hair cells were identified in non-sensory epithelial regions on both side of OC, involving the greater epithelial region (GER) and the lateral epithelial region (LER). 2) Stereocilum like protuberances were found in staircase pattern on top of ectopic hair cells, accompanied with lateral links among them. 3) The fates of atoh1 overexpressed cells were not only hair cell-like cells, but also supporting cell-like cells, indicated by GFAP expression. 4) New hair cells were recorded integrating into OC. Furthermore, the integration was faster in the basal section than in the apex. 5) None integration was found in simply LER without OC, although sporadic hair cells were still recorded after atoh1 overexpression. 6) None tendentious movement was found in non-bordering co-culture with LER and OC.

Conclusion. 1) GER and LER are both potential sources for new hair cells in the inner ear. 2) Atoh1 overexpression cells may trans-differentiated into both new hair cells and new supporting

cells. 3) The integration of new hair cell into OC acted in a basal-apex gradient such as convergent extension movement during development. 4) The integration of new hair cell into OC relied on the existence of cell connection. The cue of the integration may diffuse through the cell junction.

O5

WNT-RESPONSIVE CELLS MITOTICALLY REGENERATE HAIR CELLS IN THE NEONATAL MOUSE UTRICLE

Alan G. Cheng[1], Tian Wang[1], Renjie Chai[1,2], Grace Kim[1], Nicole Pham[1]

[1]Department of Otolaryngology-Head and Neck Surgery, Stanford University School of Medicine, Stanford, California 94305, USA; [2]Key Laboratory for Developmental Genes and Human Disease, Ministry of Education, Institute of Life Sciences, Southeast University, Nanjing 210096, China

Non-mammalian species naturally regenerate hair cells via two main mechanisms: mitotic regeneration and direct transdifferentiation. In the mature mammalian cochlea, no regeneration has been reported after damage, while a vestigial level of non-mitotic hair cell regeneration occurs in the mature mammalian utricle. Using the transgenic mouse strain Pou4f3-DTR, where the human diphtheria toxin receptor (DTR) is expressed in hair cells, we selectively ablated hair cells in neonatal mouse utricle. After administration of diphtheria toxin (DT) on postnatal day 1 (P1), progressive hair cell loss occurs before a partial but significant recovery in hair cell density is observed. In comparison to the P7 organs, hair cell densities in the striolar and extrastriolar regions of the P30 utricle are significantly higher. When the thymidine analog EdU is applied between P4-6, robust labelling is noted in the striolar region of DT-damaged utricle whereas the undamaged organ is mitotically quiescent. Using in situ hybridization and a reporter mouse strain, we detected an upregulation of the Wnt target gene Lgr5 in the striolar region of the DT-damaged utricle. Lgr5 is undetectable in the undamaged, postnatal utricle or in the extrastriolar region of the DT-damaged utricle. We conducted fate-mapping experiments using the Cre-loxp approach using Lgr5-CreERT2; Rosa26-tdTomato and Lgr5-CreERT2; Rosa26-tdTomato; Pou4f3-DTR mice. Without DT-mediated damage, tamoxifen administration only induced rare tdTomato-labeling. After DT-induced hair cell damage, tamoxifen led to tdTomato-labeling of Lgr5-positive striolar supporting cells. At P7, majority of tdTomato-labeled cells resided in the supporting cell layer and did not express the hair cell marker MyosinVIIa. When mitotically labelling was conducted concurrently with lineage tracing, almost all proliferative cells resided in the striolar support cell layer at P7 also. At P30 when the hair cell number has recovered, lineage-traced, tdTomato-positive cells expressed MyosinVIIa and resided in the hair cell layer. EdU-labeled divided cells also expressed MyosinVIIa, suggesting Lgr5-expressing cells mitotically regenerated hair cells. Regenerated hair cells exhibit Shank1- and Ctbp2-positive synapses but disarrayed stereocilia. Lastly, stabilizing beta-catenin to enhance Wnt signalling resulted in increased number of mitotically regenerated hair cells. Together, these data characterizes Lgr5-positive cells as Wnt-responsive hair cell progenitors in the neonatal mouse utricle.

O6

REGIONAL DIFFERENCES OF MOUSE UTRICLE HAIR CELLS PROLIFERATION AND DIFFERENTIATION AND ESTABLISHMENT OF THE PLANAR CELL POLARITY

Xiao-Yu Yang [1,2,3],Xiang Liu[1,2,3],Rui Ma[1,2,3],Juan-Mei Yang[1,2,3], Fang-Lu Chi[1,2,3], Dong-dong Ren[1,2,3]

[1]Department of Otology and Skull Base Surgery, Eye & Ent Hospital of Fudan University,Shanghai 200031,China; [2]Shanghai Auditory Medical Center, Shanghai, China; [3]Key laboratory of Hearing Science, Ministry of Health,Shanghai, China

Objective. Cochlear sensory epithelial cells stop proliferation from apical turn and exit the cell cycle firstly, while the earliest differentiated area of cochlear hair cell is the junctional zone of middle turn and basal turn. Up to now, there are few researches have been done about the characteristics of how the vestibular hair cells proliferate and differentiate. Planar cell polarity is a special kind of cell arrangement regularity. Vestibular organ has special inner cell polarity, while the hair cells arrangement is closely correlated with the function of vestibular. Therefore, the aims of this study are: 1) To discover the proliferation and differentiation of vestibular hair cells in different regions, 2) To discover how the planar cell polarity is established in utricle.

Method. Mice's utricle is used as the model. 1) Edu is injected into pregnant C57BL/6 mice from E11.5 to E16.5,embryos were harvested at E18.5. For Math1-GFP mice,we havest utricle from E11.5 to E18.5. Also, Edu is injected into pregnant Math1-GFP mice on day E12.5, E13.5 and E14.5, then embyos are harvested on that day. Using confocal scanning, Edu/Myosin7a double positive cell is counted.We use Edu to label cell proliferation, P27 to label cell existing cell cycle, Math1-GFP to label cell differentiation and anti-oncomodulin to label the striola region. 2) we use scanning electron microscopy(SEM) to observe the directions of planar cell polarity of hair cells in different early embryonic days.3)we use Pard6B, γ-tubulin and Prickle to indicate hair cell polarity.

Results. 1) The amount of Edu/Myosin7a double positive cells peaks on E11.5 in medial extrastiola(MES) and striola zone of utricle. In lateral extrastriola(LES), the amount peaks on E13.5. The differentiation of hair cells that marked with Math1-GFP earliest starts from E11.5 in striola, while the differentiation starting time in MES is earlier than LES. 2) On E12.5, P27 and Math1 positive cells mainly occur in striola. On E13.5, P27 and Math1 positive cells occur in striola and MES. Edu positive cells decrease first in striola and then in MES. 3) The planar cell polarity of hair cells is established before stereocilia bundles formation in the striola zone on E13.5. The line of polarity reversal(LPR) exists starts to be established with the PCP proteins expression on E14.5.

Conclusion. 1) The feature of utricle exiting cell cycle and starting to differentiate: First, precursors in striola exit cell cycle and start to differentiate and then in MES region, at last in LES region. 2) The rule of establishment of utricle planar cell polarity: subcellular planar polarity of hair cell is established first in striola and MES region on E12.5 cellular polarity and tissure polarity are established on E14.5 with PCP proteins expression. The PCP of urticle formation is in accordance with the regional diffrences of urticle hair cells proliferation and differentiation.

O7
A HIERARCHY OF SIGNALING MECHANISMS INITIATES AND COORDINATES INNER EAR HAIR CELL PLANAR POLARITY
Michael R. Deans
University of Utah School of Medicine, Division of Otolaryngology, Dept. of Neurobiology and Anatomy, Salt Lake City, UT United States

The mechanosensory hair cells of the inner ear have emerged as one of the primary models for studying the development of planar polarity in vertebrates. Planar polarity is the polarized organization of cells or cellular structures in the plane of an epithelium. For hair cells, planar polarity is manifest in the polarized organization of the stereociliary bundle, the structure that enables hair cells to detect sound and motion. I will discuss the significance of planar polarity on vestibular and auditory function and the molecular mechanisms associated with development of planar polarity at three distinct anatomical levels. The seminar will be based upon a series of projects that we have conducted using knockout and transgenic mouse models in addition to transcriptional profiling of vestibular hair cells with opposite bundle polarities.

O8
DO YOU HEAR WHAT I HEAR? REJUVENATING THE SENSORY EPITHELIUM OF THE INNER EAR
Ksenia Gnedeva, A. James Hudspeth
The Rockefeller University, New York, NY, USA

At birth the human organ of Corti contains approximately 15,500 hair cells. No additional cells develop throughout life, and once lost, these cochlear sensory receptors cannot be restored naturally. In contrast, nearly half of the hair cells in the vestibular sensory epithelium of the murine utricle are generated during the first week after birth. As in the auditory organ of the human inner ear, however, supporting cells in the murine utricle rapidly lose the capacity to multiply and generate new hair cells.
We used the murine utricle as a model system to study the genes involved in maintenance of hair cell production early in life and during the age-dependent decline in regenerative capacity. Using RNA-sequencing, we identified 104 transcription factors that show a significant change in the level of expression with age. Two *SoxC* genes, *Sox4* and *Sox11*, are strongly downregulated in the utricle following the epoch of hair cell production. *Sox4* and *Sox11* are known to play a major role in neurogenesis; in the developing visual system, for example, they orchestrate the production of retinal ganglion cells.
We demonstrated that *SoxC* genes are expressed in actively dividing supporting cells on the growing periphery of the organ and in newly differentiated hair cells. The expression of both genes disappears in the mature sensory epithelium. We also found that conditional inner-ear knockout of only a single allele of each of the three *SoxC* genes in mice results in notable vestibular ataxia and partial deafness. The loss of both copies of the *Sox4* and *Sox11* genes in the developing inner ear causes severely stunted vestibular and cochlear sensory organs with no hair cells. Finally, using adenoviruses as a delivery vectors, we showed that *Sox4* and *Sox11* genes can reactivate supporting cell proliferation and hair cell production when overexpressed *in vitro* in mature, normally quiescent utricles. Taken together these findings suggest that *SoxC* genes play an important role in inner-ear development and are essential for hair cell formation and supporting cell proliferation.

SESSION II
MOLECULAR BIOLOGY AND CELL PHYSIOLOGY (I)

TL2
THE IDENTIFICATION AND LOCALIZATION OF COMPONENTS OF THE MECHANOTRANSDUCTION COMPLEX IN HAIR CELLS
Bechara Kachar
National Institute on Deafness and other Communicative Disorders, National Institutes of Health, Bethesda MD, USA

Stereocilia convert mechanical stimuli into electrical signals through mechanoelectrical transduction (MET) by molecular complexes built around tip-link filaments made of CHD23 and PCDH15 that connect adjacent stereocilia in the direction of optimal mechanosensitivity. The upper tip-link density (UTLD) is presumed to contain a cluster of motor proteins that maintain a resting tension on the tip link. Previous studies showed that MYO7A, SANS, and Harmonin-b form the core components of the UTLD molecular complex (Grati and Kachar, 2011). In this complex, MYO7A is likely the motor element that pulls on CDH23 to exert tension on the tip-link. The MET channel is located at or near the lower tip link insertion site (Beurg et al., 2009). Transmembrane channel-like 1 and 2 (TMC1 and TMC2) are essential for MET (Kawashima et al., 2011) and are hypothesized to be components of the MET complex, but evidence for their predicted spatiotemporal localization in stereocilia is lacking. We now show that TMC1 and TMC2 localize along the length of immature stereocilia. However, as hair cells develop, the two proteins localize predominantly to the tips of the second row and shorter stereocilia at the presumed site of the MET channel complex. Both TMCs are absent from the tips of the tallest stereocilia, where MET activity is not detectable. These data are consistent with TMC1 and TMC2 being components of the stereocilia MET channel complex. In this presentation we will highlight these findings and discuss interactions of these proteins with other MET proteins and ideas on the molecular-scale architecture of the entire MET complex and how it is integrated with the stereocilia membrane and actin core.

O9
ON THE CHLORIDE DEPENDENCE OF PRESTIN CHARGE MOVEMENT
Joseph Santos-Sacchi, Lei Song
Yale University School of Medicine, Surgery (Otolaryngology), Neurobiology and Cellular and Molecular Physiology, New Haven, CT USA

The OHC drives cochlear amplification through a voltage-dependent process whereby the motor protein prestin changes conformation resulting in electromotility. The voltage-sensor of prestin can be characterized by measuring nonlinear capacitance (NLC), a consequence of voltage-sensor charge movement within the OHC lateral plasma membrane. Chloride has been observed to play a critical role in prestin's activity, influencing both the operating voltage range and estimated charge movement (Qmax, the total sensor charge moved) as measured by Boltzmann fits to NLC. Typically, NLC is measured by admittance analysis at frequencies near 1kHz. By measuring NLC at various frequencies, we find that

NLC frequency dependence is chloride dependent. Derived Qmax markedly rolls off at frequencies above our lowest admittance interrogation frequency of 195 Hz. In order to interrogate lower frequencies, we equivalently integrated voltage step-induced currents. We find that Qmax asymptotes as we increase integration times, indicating that Qmax has been markedly underestimated by high frequency admittance techniques. By fitting our data to the *meno presto* model (Santos-Sacchi and Song, Biol Chem. 289:10823-30, 2014) we arrive at the conclusion that chloride level does not influence Qmax, but works by changing the kinetics of prestin's molecular transitions. These observations run counter to current thought, and provide clues about prestin's molecular workings.

Supported by NIH NIDCD R01 DC008130.

O10

ADAPTATION INDEPENDENT MODULATION OF AUDITORY HAIR CELL MECHANOTRANSDUCTION CHANNEL OPEN PROBABILITY IMPLICATES A ROLE FOR THE LIPID BILAYER

Anthony Peng[1], Anthony Ricci[1,2]

[1]Department of Otolaryngology, Head &Neck surgery, School of Medicine, Stanford University, Stanford, CA, USA; [2]Department of Molecular and Cellular Physiology, School of Medicine, Stanford University, Stanford, CA, USA

The auditory system is able to detect movement down to atomic dimensions. This sensitivity is partly attributed to mechanisms associated with gating of hair cell mechanotransduction (MET) channels. MET channels, located at the tops of stereocilia, are poised to detect tension induced by hair bundle deflection. Hair bundle deflection generates a force by pulling on tip-link proteins connecting adjacent stereocilia. How the force is translated to the channel remains unknown. The resting open probability (P_{open}) of MET channels determines the linearity and maximal sensitivity to mechanical stimulation. Classically, P_{open} is regulated by a calcium and voltage sensitive adaptation mechanism where lowering extracellular calcium or depolarization increases P_{open}. Recent data demonstrated that fast adaptation is independent of both calcium and voltage, thus requiring an alternative explanation for P_{open} changes caused by calcium and voltage (Peng et al., 2013). Here we characterize an adaptation independent mechanism whereby the resting P_{open} is modulated by divalent ions interacting with the local lipid environment. Sensitivity to specific divalent ions suggests a non EF-hand or calmodulin based binding site. Voltage and divalent ions modulate P_{open} by a common mechanism lying within the membrane's electric field, ruling out a tip-link role. GsMTx4, a lipid mediated modifier of cationic stretch-activated channels, eliminated the voltage and divalent sensitivity, with minimal effects on adaptation confirming independent mechanisms. These data identify an adaptation independent lipid based modulation of the MET channel's open probability. It demonstrates that the voltage and calcium effects work through a common mechanism and sugges this mechanism is at least modulated by the lipid environment. We postulate this additional mechanism is critical for allowing an extension of the dynamic range of the system while maintaining adaptation kinetics at their maximal rates.

O11

CYCLIC GMP-GATED CNGA3 AND CNGB1 IN HAIR CELLS

Marian J. Drescher[1], Dakshnamurthy Selvakumar[1], Dennis G. Drescher[1,2]

[1]Laboratory of Bio-otology, Department of Otolaryngology, Wayne State University School of Medicine, Detroit, MI 48201, USA; [2]Department of Biochemistry and Molecular Biology

Objective. Sensory transduction in photoreceptors, primary olfactory neurons and chemosensory receptor cells is mediated by combinations of sensory-specific CNG alpha and beta subunits. cGMP-gated CNGA3 has previously been identified in stereocilia of inner ear hair cells and found to directly interact with proteins required for mechanotransduction: tip link protein cadherin 23 +68 or alternatively, myosin VIIa (Selvakumar et al., J. Biol. Chem. 288: 7215-7229, 2013). The present investigation was undertaken to identify the CNG beta subunit(s) in hair cells that would combine with CNGA3, determine CNG subunit protein-protein interactions (PPI), and ascertain the underlying cGMP pathway.

Methods. Primers targeting CNG beta subunit(s) crossing introns were applied in RT-PCR to cDNA of purified saccular hair cells and rat organ of Corti. Direct PPI were determined with pull-down assays and kinetic constants obtained with surface plasmon resonance (SPR). CNG protein complexes were elucidated with co-immunoprecipitation. Scanning electron microscopy (SEM) and calcium imaging were carried out.

Results. In heterologous systems, heteromeric channels formed from beta and alpha CNG subunits exhibit remarkably different physiology compared to alpha subunit homomeric channels. The identity of beta subunit(s) that combine with CNGA3 to form a hair cell CNG channel has been hitherto unknown. We now report that primers designed to target CNG beta subunits in RT-PCR applied to saccular and cochlear hair cell models, in fact, only elicited amplification of a rod photorephotoreceptor-type CNGB1 cDNA and not olfactory CNGB1 (differing only in the amino terminus), or cone photoreceptor-like CNGB3. A cGMP-pathway has been elucidated in saccular hair cells required for cGMP-gating of the CNGA3 + CNGB1 channel comprising a membrane GC with identity to both retinal GC-E and GC-F, Ca^{2+}-regulated activating proteins GCAP1 and GCAP2, transducins 1 and 2, PDE6C, and opsin.

Conclusions. The combination of CNGA3 with CNGB1 found in inner ear hair cells is considerably more subject to the inhibitory action of L-*cis*-diltiazem (a feature of MET) compared to the CNGA3 monomeric channel (Zhong et al., PNAS 100: 5509–5513, 2003). Furthermore, this combination of CNG subunits elicits increased channel cAMP-sensitivity and would be subserved by the elucidated CNG subunit PPIs and hair-cell cGMP pathway.

O12

EPITHELIAL GAP JUNCTION NETWORK IN THE HUMAN COCHLEA – AN ULTRASTRUCTURAL AND CONFOCAL LASER IMMUNOHISTOCHEMISTRY STUDY

Wei Liu[1], Rudolf Glueckert[2], Göran Laurell[1], Annelies Schrott-Fischer[2], Helge Rask-Andersen[1]

[1]Department of Surgical Sciences, Head and Neck Surgery, Section of Otolaryngology and Departments of Otolaryngology, Uppsala University Hospital, Uppsala, Sweden; [2]Department of Otolaryngology, Medical University of Innsbruck, Austria

The cochlea contains two principally different types of gap

junction (GJ) networks. One *epithelial network* is located between epithelial cells in the organ of Corti while two *connective tissue networks* are situated in the lateral wall and spiral limbus. We analyzed GJs and connexin-26/30 (Cx26, Cx30) expression in the human GJ epithelial network including the spiral prominence (SP) using transmission electron microscopy (TEM) and confocal laser immunohistochemistry. GJs were residential between all types of neighbouring epithelial cells, except between sensory and supporting cells, above the whole length of the basilar membrane, including the inner and outer sulcus epithelial cells, as well as spiral prominence epithelial cells that sit above the lateral anchoring part of the basilar membrane. Few GJs were found in the inter-dental cells. Epithelial GJs were solitary expressed; either as Cx30 or Cx26 where the former dominated. Outer pillars were coupled through Cx30 expressing GJs. There were no expressions in hair cells or nerve elements. Basal plasma membrane, facing the basilar membrane, showed focal electron-densities and horizontal GJ Cx30 plaques that were believed to represent hemi-channels. The epithelial GJ networks congregated at the SP with root cells projecting into the sub-epithelial space. The findings may suggest that K^+-recycling from the basal poles of
the hair cells may involve both cellular and extra-cellular routes in man.

O13

ANALITYCAL APPROACHES TO THE DIAGNOSIS AND TREATMENT OF AGE-RELATED HEARING LOSS: REDOX STATUS AND PROTEOMICS

A. Serra[1], V. Calabrese[2], L. Maiolino[1], S. Cocuzza[1], P. Di Mauro[1]
[1]ENT Department, University of Catania, Italy; [2]Department of Chemistry, Biochemistry and Molecular Biology, University of Catania , Italy

Objective. Aging is an inevitable passage of living organisms. In humans, aging is characterized by the appearance of gray hair, a decline in vision and hearing, wrinkles in the skin, and a decline in the physical strength of muscles and bones. While the progression of aging can easily be observed in individuals over years, it appears to be one of the most complex biological events. The turning point in aging research was the remarkable discovery that life span could be genetically controlled by mutating specifi c genes in the nematode *Caenorhabditis elegans.* Since then, a plethora of research activities has been carried out to shed more light on the mechanisms that underlie the process of aging.
Methods. In the present study, we exploit recent advances in the redox biology of aging hearing loss and discuss the potential of proteomics approaches as innovative tools for monitoring at the proteome level the extent of protein oxidative.
Results. The hormetic dose – response challenges long-standing beliefs about the nature of the dose – response in a low-dose zone, having the potential to signifi cantly aff ect the design of preclinical studies and clinical trials as well as strategies for optimal patient dosing in the treatment of numerous diseases age-related, as presbycusis.
Conclusion. Our study using the recent developments in proteomics technologies will help to dissect and fully understand the underlying pathways involved in the different aspects of aging-related hearing loss. Nonetheless, with the continued advances in proteomics, the study of the proteome during aging is entering a brand new phase of discovery.

O14

THE REDOX PROTEIN P66SHC MEDIATES COCHLEAR ENDOTHELIAL DYSFUNCTION AND EARLY NOISE-INDUCED HEARING LOSS IN MICE

G. Pani[1], S.L.M. Eramo[2], F. Paciello[3], R. Rolesi[3], A.R. Fetoni[3], G. Paludetti[3], D. Troiani[2]
[1]Institute of General Pathology, [2]Institute of Human Physiology, [3]Department of Head and Neck Surgery, Università Cattolica, Medical School, Rome, Italy

p66Shc, a member of the ShcA protein family, is essential for cellular response to oxidative stress, and elicits the formation of mitochondrial Reactive Oxygen Species (ROS), which promote endothelial damage, compromise vasomotor function and participate in proinflammatory cascades. Accordingly, mice lacking the p66 isoform display increased resistance to oxidative tissue damage and to cardiovascular disorders. We investigated the potential role of p66shc in noise-induced hearing loss (NIHL) and age-related hearing loss (ARHL), diseases tightly linked to oxidative stress. p66shc expression and serine phosphorylation were induced following noise exposure in the rat cochlea, together with markers of oxidative stress, inflammation and ischemia. Importantly, p66Shc knock-out (p66 KO) 126 SvEv adult mice were less vulnerable to acoustic trauma with respect to wild type controls, as indicated by preserved auditory function and by remarkably lower levels of oxidative stress and of VEGF in the highly vascularised cochlear lateral region. Of note, decline of auditory function observed in 12 month old WT controls was markedly attenuated in p66KO mice consistent with delayed inner ear senescence. Collectively, we have identified a pivotal role for p66Shc and ROS-induced vascular dysfunction in a common pathogenic cascade shared by noise-induced and age-related hearing loss.

O15

MOLECULAR BASIS OF HEREDITARY DEAFNESS: A THEORETICAL APPROACH

Francesco Zonta[1], Damiano Buratto[2], Guang Yang[1], Fabio Mammano[2,3,4]
[1]Shanghai Institute for Advanced Immunochemical Studies (SIAIS), ShanghaiTech University, Shanghai, China; [2]Department of Physics and Astronomy, G. Galilei, Padova University, Padova, Italy; [3]Venetian Institute of Molecular Medicine, Padova, Italy; [4]CNR Institute of Cell Biology and Neurobiology, Rome, Italy

Objective. Gap junction channels, formed by connexin proteins, are the main direct way of communication between adjacent cells. They can also function as membrane channels when undocked; in this case they are referred to as hemichannels. In the inner ear they promote ATP release sustaining long range intracellular calcium signal propagation and allow diffusion of calcium mobilizing second messengers across coupled cells. Mutations of GJB2 gene, encoding connexin 26 protein are the cause of nearly half of Caucasian and Japanese cases of hereditary deafness.
Methods. By use of molecular dynamics simulations we built a theoretical model to understand the physiology and pathology of gap junction channels at the molecular level. In particular, we analyzed the effects of two different point mutations of connexin 26 protein, which are linked to hereditary deafness. The first is located in the first transmembrane helix of Cx26 (Cx26M34T), the second in one of the extracellular loops (Cx26C169Y).
Results. Experimental results indicate that channels formed by

Cx26M34T retain only 11% of the wild type conductance. Our simulations show that the quaternary structure of the Cx26M34T channel is altered at the level of the pore funnel. As a consequence, the free energy barrier encountered by permeating ions in channels formed by Cx26M34T is significantly higher than in the wild type and thus the unitary conductance is reduced. The absence of Cys169, instead, disrupts a disulfide bridge, which is fundamental in maintaining the structure of the extra cellular region. As a consequence, the mutant protein shows an alteration in the spatial positioning of two important residues (Asn176 and Thr177), which have been identified as crucial in the intermolecular interactions that keep the structure of the gap junction channel. This theoretical prediction has been confirmed by immunofluorencence microscopy experiments, showing that CxC169Y proteins do not form gap junction channels in HeLa cells, even though they are correctly targeted to the plasma membrane.
Conclusions. By using numerical simulations, we are able to provide an interpretative model, at the molecular level, of the pathological behavior of gap junction channels associated with hereditary deafness.

SESSION III
GENETICS OF HEARING

TL3
GENETICS OF HEARING LOSS: PAST, PRESENT AND FUTURE

P. Gasparini[1,2,3]
[1]Dept. of Medical Sciences, University of Trieste, Trieste, Italy, [2]Institute of Maternal and Child Health-IRCCS "Burlo Garofolo", Trieste, Italy, [3]Division of Experimental Geenetics, Sidra, Doha, Qatar

Hereditary hearing loss (HHL) is a common disorder that affects from 1 to 3 in 1000 newborns. Despite the high level of genetic heteorgeneity, several cases are due to mutations in *GJB2* gene, even though its prevalence could vary between ethnical groups. According to the population studied, more than two thirds of patients cannot benefit from a genetically proven diagnosis because this gene accounts for only 10-50% of HL. Actually, all other forms of HHL belong to very rare subtypes with a subtype-specific prevalence below 1:1,000. Their number definitely exceeds 150, leading to an unsolvable genetic diagnostic dilemma at the moment. Routine genetic tests are available for only few hearing loss genes, although more than 65 monogenic genes have been so far described (Hereditary Hearing Loss Homepage, *http://hereditaryhearingloss.org*).
Despite the very high prevalence of Age Related Hearing Loss (ARHL), a significant heritability and the huge impact on the quality of life and high societal costs, genetic analyses of ARHL are highly underrepresented among genetic studies of multifactorial disorders. In part, this is due to an assumption that there are few genetic factors with a major contribution, but also ARHL studies are underfunded when compared to other, often less common, complex diseases affecting the ageing population. Furthermore, as a heterogeneous disease it is a difficult problem. The state-of-the art on the genetic bases of both HHL and ARHL will be presented and discussed with a perspective view on future possible diagnostic and therapeutical interventions.

O16
GENETIC POLYMORPHISMS IN INNER EAR DISORDERS: BACKGROUND AND PRELIMINARY RESULTS

Maurizio Simmaco, Luigi Volpini, Isotta Musy, Davide Leonardo, Simonetta Monini, Maurizio Barbara
Sapienza University, Sant'Andrea Hospital, NESMOS Department, Rome Italy

Menière's disease (MD) and sudden sensorineural hearing loss (SSNHL) still present many shadows in term of pathogenesis and therapeutical protocols. For both diseases the etiology is thought to be multifactorial, with association between environmental factors and genetic predisposition. In the present study 30 subjects have been enrolled: 10 Menière patients with definite disease according to the AAO-HNS criteria; 10 patients with SSNHL and 10 subjects as control. In this report, several genetic polymorphisms have been investigated in the literature as possible candidates forp redisposing a subject to the development of these inner ear disorders. From the analysis of recent literature data, some specific susceptibility genes for MD and SSNH have been taken into account. Eighteen SNPs have been selected. Genomic DNA was extracted from peripheral blood leukocytes by Helix Extraction System (Diatech) and X-Tractor Gene (Corbett Robotics). Genotyping assays for HCFC1(rs2266886), KCNE1 112G/A (rs1805127), IL1 -889C/T (rs1800587), MTHFR 677C/T (rs1801133), PRKCH 1425G/A (rs2230500), MTHFR 1298A/C (rs1801131), GPIa 807C/T (rs1126643), MTR 2756G/A (rs1805087), KCNE3 198T/C (rs2270676), fatt V 1691G/A (rs6025), eNOS G894T (rs1799983), IL4R 576Q/R (rs180275), CFH 402Y/H (rs1061170), IL-6 C-572G (rs1800796), ADD1 G460W (rs4961), eNOS-T786C (rs2070744), MMP-1-1607G/2G (rs1799750) and AQP3 -C105G (rs591810) were developed, using a mass spectrometer based technology (Sequenom MassArray Analyzer).
The results showed an association between polymorphisms of genes HCFC1 and AQP3 with both inner ear diseases, while eNOS T786C in recessive homozygous and ADD1 in heterozygous were only associated to SSNHL. These preliminary results would indicate that some of the examined polymorphism are more prevalent in the diseased population than in the normal one. Further studies are needed in order to better elucidate the genetic basis of both diseases and eventually support the development of personalized therapeutic protocols.

O17
THE P.S178L HOT-SPOT MUTATION OF *TBC1D24* LEAD TO DOMINANT, PROGRESSIVE AND NON-SYNDROMIC HEARING IMPAIRMENT THROUGH A GAIN-OF-FUNCTION MECHANISM

Xiuhong Pang, Luping Zhang, Lingxiang Hu, Hao Wu and Tao Yang
Xinhua Hospital, Shanghai Jiaotong University School of Medicine, Shanghai, China

Mutations in *TBC1D24* have been linked to a variety of epileptic syndromes and recently to syndromic deafness DOORS syndrome and non-syndromic deafness DFNB86. All *TBC1D24* mutations reported previously were inherited in the recessive mode and resulted in congenital, severe-to-profound deafness. By linkage analysis of a dominant Chinese Han family segregated with late-onset, progressive and non-syndromic hearing impairment, we

identified a 2.07 Mb candidate region on chromosome 16p13.3 that contains *TBC1D24*. Whole-exome sequencing identified a heterozygous p.Ser178Leu variant of *TBC1D24* as the only candidate mutation segregating with the hearing impairment within the family. In perinatal and adult mouse cochlea, expression of *Tbc1d24* was detected in the spiral ganglion neurons, implying of an important function of *TBC1D24* in this region. Interestingly, the p.Ser178Leu mutation of *TBC1D24* has been subsequently reported as the cause of a Caucasian family with similar dominant, non-syndromic hearing impairment, suggesting that it is probably a hot-spot mutation with a rather consistent phenotype. Since all truncating mutations of *TBC1D24* were phenotypically normal in heterozygosis, we speculated that the dominantly inherited p.Ser178Leu mutation most likely cause the non-syndromic hearing impairment through a gain-of-function mechanism. This hypothesis were supported by our *in vitro* study in which exogenous expression of wild type and the p.S178L mutant TBC1D24 both provoked the extension and the branching of the mouse cortical neurons, while the latter produced a much stronger effect.

O18

INCOMPLETE COMPENSATION OF ANGULIN1/LSR TO RESCUE DFNB42, CAUSED BY MUTATION IN ANGULIN2/ILDR1

Shin-ichiro Kitajiri, Tomohito Higashi[1], Tatsuya Katsuno, Mikio Furuse[1,2,3]

[1]Kobe University Graduate School of Medicine, Department of Physiology and Cell Biology, Kobe, Japan; [2]National Institute for Physiological Sciences, Division of Cerebral Structure, Okazaki, Japan; [3]National Institute for Physiological Sciences, Department of Physiological Sciences, Okazaki, Japan

Tricellular tight junctions (tTJs) are specialized structures of tight junctions to obliterate the narrow extracellular space at tricellular contacts, where the corners of three epithelial cells meet. Tricellulin is the first molecule identified localize at tTJs, and responsible to barrier function at tTJ. The mutations in tricellulin cause human deafness DFNB49, and knockin mice showed profound deaf. In addition to tricellulin, LSR, ILDR1 and ILDR2 are localized at tTJ, and they recruit tricellulin into tTJ. These three molecules are named angulin family (angulin1/LSR, angulin2/ILDR1, angulin3/ILDR2). Among them, angulin2/ILDR1 cause human deafness DFNB42. To reveal the pathophysiology of DFNB42 deafness, we analyzed the mice with targeted disruption of Ildr1, which encodes angulin2/ILDR1. The Ildr1$^{-/-}$ mice showed profound deaf with progressive degeneration of cochlea hair cells. In the absence of angulin2/ILDR1, angulin1/LSR upregulated at organ of Corti, and tricellulin was recruited into tTJ. This is the first example of compensation among angulin family. But, organ of Corti with compensational recruited tricellulin by angulin1/LSR can not be functional and degenerates, indicates that angulin2/ILDR1 have unique function.

O19

NOVEL MUTATIONS IN *DFNB59*, THE GENE ENCODING PEJVAKIN, IN SUBJECTS WITH AUTOSOMAL RECESSIVE NON-SYNDROMIC HEARING IMPAIRMENT AND AUDITORY NEUROPATHY SPECTRUM DISORDER

María Domínguez[1,2], Montserrat Rodríguez-Ballesteros[1,2], Marta Gandía[1,2], Elena Gómez-Rosas[1,2], Manuela Villamar[1,2], Elona Cama[3,4], Pietro Scimemi[3,4], Nanna Dahl Rendtorff[5], Miguel Ángel Moreno-Pelayo[1,2], Lisbeth Tranebjaerg[5,6,7], Carmen Medá[8], Rosamaria Santarelli[3,4], Ignacio del Castillo[1,2]

[1]Servicio de Genética, Hospital Universitario Ramón y Cajal, IRYCIS, Madrid, Spain; [2]Centro de Investigación Biomédica en Red de Enfermedades Raras (CIBERER), Madrid, Spain; [3]Department of Neurosciences, University of Padova, Italy; [4]Audiology and Phoniatrics Service, Treviso Regional Hospital, Treviso, Italy; [5]Clinical Genetics Clinic, The Kennedy Center, University Hospital, Copenhagen, Denmark; [6]Department of Cellular and Molecular Medicine, University of Copenhagen, Copenhagen, Denmark; [7]Department of Otorhinolaryngology, Head & Neck Surgery and Audiology, Bispebjerg Hospital, Rigshospitalet, Copenhagen, Denmark; [8]Unidad de Prevención de Enfermedades del Oído, Conselleria de Salut, Illes Balears, Palma de Mallorca, Spain

Autosomal recessive non-syndromic hearing impairment (AR-NSHI) is genetically heterogeneous, with over 60 genes currently known to be involved. Up to 11 different mutations in the *DFNB59* gene, encoding pejvakin, have been reported in 14 families with AR-NSHI from diverse geographic origins. In one Iranian family the hearing impairment showed features of auditory neuropathy (AN) (abnormal or absent auditory brainstem responses but normal otoacoustic emissions). This clinical feature was in accordance with the expression pattern of the *DFNB59* gene (organ of Corti, spiral ganglion, and the first three relays of the afferent auditory pathway) and with the phenotype observed in a knock-in mouse model. However, AN was not observed in any of the other reported cases with two mutant *DFNB59* alleles.

We have screened a cohort of 140 Spanish familial cases of AR-NSHI, and a cohort of 84 subjects (40 from Spain, 23 from Italy, 21 from Denmark) with isolated AN in whom mutations in the *OTOF* (otoferlin) gene had been excluded. In familial cases, all siblings and their parents were genotyped for microsatellite markers D2S148, D2S2173, D2S324, and D2S2310, closely linked to *DFNB59*. We sequenced all exons and intron/exon boundaries of one affected subject from all families showing compatibility with linkage to these markers, and those of all the cases with AN. We found four novel causative mutations in two cases: one in the AR-NSHI cohort (0.7%) and one in the cohort with isolated AN (1.2%). Affected subjects from the Spanish family S269 had a severe NSHI, and were compound heterozygous for mutations c.671T>G (p.Leu224Arg) and c.880delC (p.His294Ilefs*43). Italian subject E1471 had profound NSHI and the clinical profile of AN. He was compound heterozygous for mutations c.880C>G (p.His294Asp) and c.950delT (p.Phe317Serfs*20). All these mutations segregate with the disorder in the families, and they were not found in either 50 ethnically matched controls with normal hearing or in genomic databases. Our results confirm that mutations in *DFNB59* cause AN, but indicate that they account for a very small proportion of cases. Likewise, mutations in *DFNB59* are a rare cause of AR-NSHI in Spain, as also concluded from screenings of other populations.

O20

OPA1-RELATED AUDITORY NEUROPATHY: SITE OF LESION AND OUTCOME OF COCHLEAR IMPLANTATION

Rosamaria Santarelli[1,2], Chiara La Morgia[3,4], Pietro Scimemi[1,2], Elona Cama[1,2], Leonardo Caporali[3,4], Maria Lucia Valentino[3,4], Rocco Liguori[3,4], Valerio Carelli[3,4]

[1]Department of Neurosciences, University of Padova, Italy; [2]Audiology and Phoniatrics Service, Treviso Regional Hospital, Treviso, Italy; [3]Department of Biomedical and Neuromotor Sciences (DIBINEM), University of Bologna, Italy; [4]IRCCS Institute of Neurological Sciences of Bologna, Bellaria Hospital, Bologna, Italy

Dominant optic atrophy (DOA) is associated with pathogenic mutations in the nuclear gene encoding for the *OPA1* protein in about 60-70% of cases. Besides optic atrophy, patients carrying *OPA1* mutations present with hearing loss, ataxia, sensorimotor neuropathy, external ophthalmoplegia and mitochondrial myopathy. In this study we characterized the hearing dysfunction in *OPA1*-linked disorders and provided effective rehabilitative options to improve speech perception.

We studied two groups of *OPA1* subjects, one comprising 11 patients (13-79 years) carrying *OPA1* mutations inducing haploinsufficiency (*OPA1*-H), the other, 10 subjects (5-58 years) harboring *OPA1* missense mutations (*OPA1*-M). Both groups underwent pure tone and speech perception evaluation, and otoacoustic emissions (OAEs) and auditory brainstem response (ABR) recording. Cochlear potentials were recorded through transtympanic electrocochleography from the *OPA1*-M group and were compared to recordings obtained from 20 normally-hearing controls and from 19 subjects with cochlear hearing loss. Eight *OPA1*-M patients underwent cochlear implantation. Speech perception measures and electrically-evoked auditory nerve and brainstem responses were obtained after one year of cochlear implant use.

Nine out of 11 patients included in the *OPA1*-H group had normal hearing function. In contrast, all but one *OPA1*-M subject displayed impaired speech perception, abnormal ABRs and presence of OAEs consistent with auditory neuropathy. In electrocochleography recordings, cochlear microphonic had enhanced amplitudes while summating potential showed normal latency and peak amplitude consistent with preservation of both outer and inner hair cell activities. After cancelling the cochlear microphonic, the synchronized neural response seen in both normally-hearing controls and hearing-impaired subjects was replaced by a prolonged, low-amplitude negative potential that decreased in both amplitude and duration during rapid stimulation consistent with neural generation. The use of cochlear implant improved speech perception in all patients. Brainstem potentials were recorded in response to electrical stimulation in five subjects out of six, whereas no compound action potential was evoked from the auditory nerve through the cochlear implant.

These findings indicate that underlying the hearing impairment in patients carrying *OPA1* missense mutations is a disordered synchrony in auditory nerve fiber activity resulting from neural degeneration affecting the terminal dendrites. Cochlear implantation improves speech perception and synchronous activation of auditory pathways by by-passing the site of lesion.

O21

COCHLEAR GAP JUNCTION PLAQUE, STABILIZED MACROMOLECULAR COMPLEX COMPOSED OF SPECIFIC CONNEXINS

Kazusaku Kamiya[1], Ichiro Fukunaga[1], Kaori Hatakeyama[1], Toru Aoki[1], Ayumi Fujimoto[1], Atena Nishikawa[1], Takashi Anzai[1], Osamu Minowa[2], Katsuhisa Ikeda[1]

[1]Department of Otorhinolaryngology, Juntendo University School of Medicine, Tokyo, Japan; [2]BioResource Center, RIKEN, Tsukuba, Japan

Objectives. Hereditary deafness affects about 1 in 2000 children and GJB2 gene mutation is most frequent cause for this disease. GJB2 encodes connexin26 (Cx26), a component in cochlear gap junction. To elucidate the molecular pathway for the gap junction dysfunction, biochemical analysis of gap junction plaques (GJPs) with multiple Cx26 mutant mouse models are needed.

Methods. We analyzed macromolecular change of gap junction plaques with two different types of Cx26 mutation as major classification of clinical case, one is a model of dominant negative type, Cx26R75W+ and the other is conditional gene deficient mouse, Cx26f/fP0Cre as a model for insufficiency of gap junction protein.

Results. Gap junction composed mainly of Cx26 and Cx30 in wild type mice formed large planar GJPs. In contrast, Cx26R75W+ and Cx26f/fP0Cre showed drastically disrupted GJPs. It is demonstrated that Cx26-dependent gap junction plaque (GJP) disruption occurs as the earliest change during embryonic development, resulting in a drastic reduction in the protein levels, and is associated with excessive endocytosis with increased expression of Caveolins. GJP disruption with the functional defects was also clearly reproduced with human Cx26 and Cx30 cDNAs in vitro (Kamiya, J Clin Invest, 2014,;124(4):1598–1607). The GJP disruption accompanied with Cx protein reduction were also observed in Brn4 deficient mice, a model of DFN3 non-syndromic deafness.

Conclusions. In the present study, we demonstrated a new molecular pathology in most common hereditary deafness with different types of Connexin26 mutations and also in other type of hereditary deafness DFN3 model.

O22

NEXT GENERATION SEQUENCING FOR THE STUDY OF HEREDITARY HEARING LOSS: PSIP1/LEDGF AS A NEW GENE CAUSING SENSORINEURAL PROGRESSIVE HEARING LOSS AND VARIABLE EYE PHENOTYPES

Giorgia Girotto[1], Deborah I. Scheffer[2], Anna Morgan[1], Diego Vozzi[3], Elisa Rubinato[1], Mariateresa Di Stazio[1], Enrico Muzzi[3], Stefano Pensiero[3], Anne B. Giersch[2], David P. Corey[2], Paolo Gasparini[1,3]

[1]Dep. Medical Sciences, University of Trieste, Trieste, Italy, [2]Dep. Neurobiology and Howard Hughes Medical Institute, HMS, Boston, US, [3]Inst. Maternal and Child Health-IRCCS "Burlo Garofolo", Trieste, Italy

Hereditary Hearing Loss (HHL) is an extremely genetically heterogeneous disorder that prompted us to develop a powerful diagnostic algorithm characterized by screening of 113 HHL genes by targeted re-sequencing (TS) followed, in negative cases, by whole exome sequencing (WES) to detect causative mutation in new genes.

TS of 113 different HHL genes was based on Ion Torrent PGM™

(LifeTechnologies) (4.356 amplicons ensuring approximately 92,6 % coverage of 744,38Kb of target region) while WES protocol was performed with Ion Proton™(LifeTechnologies) (293.903 amplicons ensuring approx. >99% coverage of 57.742.646 of target region).
This strategy allowed us to identify several new mutations/genes in 20 families from Italy and Qatar.
In particular, we characterized 20 families out of 33 (60%) with mutations in 15 known and new HHL genes (*BDP1-Girotto et al. 2014, TBLY1* pers.comm.). The WES strategy also lead to the identification of *PSIP1/LEDGF* as a novel gene causing sensorineural progressive HHL restricted to the medium-high frequencies and a variable eye phenotype (i.e. uveitis, optic neuropathy) in an Italian pedigree. Further clinical examinations of the affected members showed normal auditory brainstem responses amplitude indicating that the auditory nerve is not involved, while vestibular evoked potentials indicated some differences in terms of visual acuity and optic nerve functionality.
A frameshift deletion leading to a premature stop codon (c.1554_1555del, p.E518Dfs*2, p.T519X) and truncation of the last 12 amino acids segregated with the disease. Our additional studies using different methodological approaches (i.e. cDNA analysis, RNA Seq, immunolabeling, etc.) demonstrate that: 1) this deletion does not lead to mRNA degradation 2) Psip1 is expressed in the nuclei of all hair cells and supporting cells of both mouse cochlea and vestibular system.
These findings strongly suggest an important role for *PSIP1* in HHL as well as in eye defect, and it also highlights the importance of next generation sequencing technologies to better understand the genetic basis of HHL.

O23
THE EXPRESS LANE FROM LAB TO CLINIC: HIGH-THROUGHPUT SEQUENCING IN HEARING IMPAIRED PATIENTS DISCLOSES INFORMATIVE MUTATIONS AT LIGHTNING SPEED

Barbara Vona[1], Michaela A. H. Hofrichter[1], Cordula Neuner[1], Jörg Schröder[1], Oliver Bartsch[2], Ulrich Zechner[2], Wafaa Shehata-Dieler[3], Indrajit Nanda[1], Thomas Haaf[1]
[1]Institute of Human Genetics, University of Würzburg, Germany; [2]Institute of Human Genetics, University Medical Centre, Johannes Gutenberg University of Mainz, Germany; [3]Comprehensive Hearing Centre, Department of Otorhinolaryngology, Plastic, Aesthetic and Reconstructive Surgery, University Hospital, Würzburg, Germany

The post-genomic era has delivered an unprecedented advancement of knowledge about the types of genes involved in non-syndromic hearing loss (NSHL), as well as their mutational spectrum. This breakthrough has been driven by high-throughput sequencing technologies ranging from small targeted gene panels to clinical and whole exomes that provide a cost-effective start toward personalized medicine and therapies for NSHL patients. We report our experiences using a combination of panel types that has allowed for the successful identification of likely causative pathogenic mutation(s) in 45% of investigated cases.
We describe several examples demonstrating the promise and powers of high-throughput sequencing in NSHL patients. Firstly, analysis of parent-child clinical exome trios optimized variant filtering and quickly disclosed a novel, likely pathogenic *de novo* mutation in the gene *CEACAM16* (OMIM *614591) responsible for autosomal dominant NSHL (DFNA4B). Such investigations for *de novo* mutations are important, as the *de novo* mutation rate for NSHL has yet to be determined. Recently, the gene *MYO1A* (OMIM: *601478) was disqualified as an autosomal dominant NSHL gene (DFNA48) due to discordant co-segregation in three families. High-throughput sequencing of validated hearing loss genes allows for rigorous testing for potential co-segregation incompatibilities. We present additional families supporting disqualification of this gene. Alternatively, high-throughput testing of known hearing loss genes allowed for the correct assignment of *PDZD7* (OMIM: *612971) as an autosomal recessive NSHL gene when biallelic mutations are present. This gene has been largely overlooked for NSHL and has been primarily implicated as an Usher syndrome associated gene.
We highlight one of several limitations that high-throughput sequencing technologies have yet to overcome. The gene *STRC* (OMIM *606440) causes autosomal recessive NSHL (DFNB16) and accounts for approximately 6% of all NSHL. Testing of *STRC* via high-throughput sequencing approaches is complicated by a tandem duplication of chromosome 15q15.3 that contains functional *STRC* and its non-processed pseudogene counterpart sharing 98.9% genomic sequence identity. We introduce our testing strategy to overcome this limitation for understanding *STRC* mutational fallout.
Although high-throughput sequencing remains in its infancy, it has already permitted remarkable navigation through genetically heterogeneous NSHL and holds great promise for future translational medicine.

SESSION IV
OTOTOXICITY AND NOISE INDUCED HEARING LOSS

TL4
CIRCADIAN RHYTHM AND NOISE-INDUCED HEARING LOSS

Vasiliki Basinou, Jung-sub Park, Chrsitopher Cederroth, Barbara Canlon
Department of Physiology and Pharmacology, Karolinska Institute, Stockholm, Sweden

Remarkably little is known of how acoustic trauma is affecting the cochlea across the circadian cycle. We have recently shown that noise-induced damage to the cochlea is more severe when the exposure occurs at night compared to the day. Moreover, we identified a new network of proteins, the circadian network known to regulate cellular function in a 24-hour period, potentially involved in the regulation of circadian sensitivity to noise trauma. These novel findings in mice add another level of complexity on how the auditory system reacts to noise insults. This presentation will discuss the mechanisms underlying these day and night differences in sensitivity to noise trauma and establish a solid ground on which to explain how circadian rhythms can modify hearing sensitivity to noise trauma. Specifically, recovery from day noise trauma coincided with a greater induction of brain-derived neurotrophic factor (BDNF) mRNA transcripts in the cochlea compared to night trauma. *In vivo* administration of the selective TrkB receptor agonist 7,8-dihydroxyflavone (DHF) in the night,

but not in the day, lead to a complete auditory recovery after noise and maintenance of inner hair cell synaptic integrity. Our findings demonstrate that noise trauma during the night contributes to more severe consequences compared to noise exposure given during the day and that this daily variance in noise sensitivity is adjusted by a self-sustained circadian cochlear clock gating the protective functions of TrkB on synaptic integrity. These findings highlight the coupling of circadian rhythmicity and TrkB receptor for the successful prevention and treatment of noise-induced hearing loss.

O24
AMINOGLYCOSIDE ANTIBIOTICS ALTER MITOCHONDRIAL DYNAMICS

Gao Wei, Ann Kendall, Jochen Schacht
Kresge Hearing Research Institute, Department of Otolaryngology, University of Michigan, Ann Arbor, MI, USA

Objective. An involvement of mitochondria in cochlear pathologies is well established (Böttger and Schacht, Hear. Res. 303:12-19, 2013). There is also suggestive evidence that the formation of reactive oxygen species (ROS), which is a decisive factor in hair cell damage, originates in mitochondria. In the case of aminoglycoside ototoxicity, direct effects of these drugs on the mitochondrial redox balance have been reported (Dehne et al., 2002; Jensen-Smith et al., 2012; Majumder et al., 2012). Our recent studies (Matt et al., Proc. Natl. Acad. Sci. US 109:10984-10989, 2012 & Shulman et al., J. Biol. Chem. 289:2318-2330, 2014) more specifically point to the mitochondrial ribosome as a target of aminoglycoside actions resulting in mitochondrial dysfunction. An important mechanism to maintain mitochondrial functionality under stress is the process of mitochondrial dynamics (fission and fusion), the equilibrium between the generation of new organelles and the removal or repair of damaged ones. Disruption of these processes may cause developmental deficiencies or neurodegenerative disorders, and mediate the neurotoxic or ototoxic actions of environmental stresses.
Methods. Here we report on the role of mitochondrial dynamics in aminoglycoside ototoxicity. The basic model is a 72-h culture of explants of the early postnatal murine organ of Corti in which micromolar concentrations of gentamicin cause a dose-dependent loss of outer hair cells in a base-to-apex gradient. We evaluate ROS generation, mitochondrial fusion and fission and hair cell death.
Results. Our salient findings are:
1. mitochondrial dynamics is disrupted by aminoglycoside antibiotics;
2. disturbance of mitochondrial dynamics precedes hair cell death;
3. ROS formation is upstream of disturbances of mitochondrial dynamics;
4. mitochondria-targeting scavengers prevent cell death;
5. restoration of mitochondrial dynamics protects against gentamicin-induced hair cell death.

Conclusion. These results give new insights into the complexity of aminoglycoside actions and routes to cell death. They may also open new avenues to the design of protective strategies.
Supported by research grant R01 DC003685 and core grant P30 DC005188 from the National Institutes on Deafness and Other Communication Disorders, National Institutes of Health.

O25
ROLE OF STAT1 IN CISPLATIN AND GENTAMICIN INDUCED AUDITORY HAIR CELL LOSS

Daniel Bodmer, Soledad Levano
University Hospital Basel, Switzerland

Objective. Hair cell damage is a side effect of cisplatin and aminoglycoside use. The inhibition or attenuation of this process is a target of many investigations. There is growing evidence that STAT1 deficiency decreases cisplatin-mediated ototoxicity; however, the role of STAT function and the molecules that act in gentamicin-mediated toxicity have not been fully elucidated.
Methods and Results. We used mice lacking STAT1 to investigate the effect of STAT1 ablation in cultured organs treated with cisplatin and gentamicin. Here we show that ablation of STAT1 decreased cisplatin toxicity and attenuated gentamicin-mediated hair cell damage. While cisplatin increased serine phosphorylation of STAT1 in WT mice and diminished STAT3 expression in WT and $STAT1^{-/-}$ mice, gentamicin increased tyrosine phosphorylation of STAT3 in $STAT1^{-/-}$ mice. The early inflammatory response was manifested in upregulation of TNF-α and IL-6 in cisplatin-treated explants, and the increase of TNF-α in gentamicin-treated explants. Expression of the anti-inflammatory cytokine IL-10 was altered in cisplatin-treated explants, upregulated in WT explants, and downregulated in $STAT1^{-/-}$ explants. Cisplatin triggered the activation of c-Jun. Activation of Akt was observed in gentamicin-treated explants from $STAT1^{-/-}$ mice. Increased levels of the autophagy proteins Beclin-1 and LC3-II were observed in $STAT1^{-/-}$ explants.
Conclusions. These data suggest STAT1 is a central apoptotic actor in mediated ototoxicity. Gentamicin and cisplatin activate different downstream factors in their toxicity pathway. Although cisplatin and gentamicin triggered inflammation and activated apoptotic factors, the absence of STAT1 allowed the cells to overcome the fate of toxicity.

O26
THE EFFECTS OF CISPLATIN AND AMINOGLYCOSIDES ON INNER EAR MITOCHONDRIA

Agnieszka J. Szczepek[1], Kaitlyn Badley[1], Birgit Mazurek[2]
[1]ORL Department, Charite University Hospital, Berlin, Germany; [2]Tinnitus Center, Charite University Hospital, Berlin, Germany

Ototoxic medications are known to negatively affect mitochondria in the hair cells and to induce oxidative stress and consecutive cell loss. We were interested in early changes that may lead to mitochondrial dysfunctions. To address this question, we compared the effects, which cisplatin and genatmicin have on genes encoding components of mitochondrial structures and genes encoding components of mitochondrial energy metabolism. Cochlear tissues explants containing organ of Corti, spiral limbus and spiral ganglion neurons were exposed to to 30uM cisplatin or 0.2mM gentamicin for a period of 4 hours (n=10 for gentamicin, n=10 for cisplatin and n=15 for controls). After that time, tissues were lysed and RNA was purified. Commercial RT² Profiler PCR Array for Rat Mitochondria and Rat Mitochondrial Energy Metabolism (both from Qiagen, Hilden, Germany) were used to compare the changes in gene expression between exposed explants and unexposed controls and between the two types of ototoxic exposure. Only two-fold changes were considered significant. The RT² Profiler PCR Array for Rat Mitochondria showed the Grpel1 gene as being over-expressed in the cisplatin group vs. control group. For Rat Mitochondrial Energy

Metabolism PCR Array, the Atp6v0d2 gene was over-expressed and Atp6v1g3 gene was under-expressed for cisplatin group vs. control group. Gentamicin group compared to control group had two under-expressed Rat Mitochondrial Energy Metabolism genes, Atp5g3 and Ndufb6. Taken together, our results demonstrate early changes occurring in cochlear membranous tissues after exposure to gentamicin or to cisplatin in the gene expression pattern affecting the mitochondrial structure and metabolism. In addition, we demonstrate that each ototoxic substance induces individual changes. The results including further time points as well as protein expression studies will be presented.

O27
METFORMIN PROTECTS AGAINST CISPLATIN INDUCED OTOTOXICITY BY REDUCING ROS PRODUCTION AND MODULATING INTRACELLULAR CALCIUM

Jiwon Chang[1], Su Kyoung Park[1], Hyung-Jong Kim[1], Sehee Lee[2], Hak Hyun Jung[2], June Choi[2], Gi Jung Im[2]
[1]Department of Otolaryngology-Head and Neck Surgery, Kangnam Sacred Heart Hospital, Hallym University College of Medicine, Seoul, South Korea; [2]Department of Otolaryngology-Head and Neck Surgery, Korea University College of Medicine, Seoul, South Korea

Metformin, an antidiabetic drug with a potent anticancer property, is known to prevent oxidative stress-induced cell death in several cell types through a mechanism dependent on the mitochondria. In the present study, we investigated the influence of metformin on cisplatin ototoxicity in auditory cell line.
Cell viability was determined by MTT, and oxidative stress and apoptosis were assessed by flow cytometry analysis, Hoeschst 33258 staining, ROS measurement, and western blotting. Intracelluar calcium concentration changes were detected with calcium imaging. Pretreatment with 100uM of metformin prior to application of 20 uM of cisplatin significantly decreased the late apoptosis in HEI-OC1 cells and also, significantly attenuated the cisplatin-induced increase in reactive oxygen species (ROS). Metformin inhibited the activation of caspase-3 and the expression of poly-ADP-ribose polymerase. Also, pretreatment with metformin prevented the elevation of intracellular calcium concentration induced by cisplatin. When we analyzed the activation of caspase 8, 9, 3 in time and dose dependent manner, caspase 3 and 9 were most activated in 48 hour. JNK and ERK were significantly phosphorylated in 15-30 minutes and in 48 hours.
We propose that metformin has protective effects against cisplatin induced ototoxicity by inhibiting the increase of intracellular calcium and preventing apoptosis and ROS production.

O28
SENSORINEURAL HEARING LOSS AFTER PNEUMOCOCCAL MENINGITIS IN INFANTS: AN ANIMAL MODEL

Michael Perny[1,2,3,4], Denis Grandgirard[1,3], Marta Roccio[1,4], Fabian Liechti[2,3], Stephen L. Leib[1,3,5]*, Pascal Senn[1,4,6]* (*Co-Lead of the project)
[1]Laboratory for Regenerative Neuroscience, DCR, University of Bern, Switzerland; [2]Graduate School for Cellular and Biomedical Sciences, University of Bern, Switzerland; [3]Neuroinfection Laboratory, Inst. for Infectious Diseases, University of Bern, Switzerland; [4]University Department of Otorhinolaryngology, Head and Neck Surgery, Inselspital, Bern, Switzerland; [5]Biology Division, Spiez Laboratory, Swiss Federal Office for Civil Protection, Spiez, Switzerland; [6]Dept. of Otorhinolaryngology, Head & Neck Surgery, Hôpitaux Universitaires de Genève, Switzerland

Background. Bacterial meningitis (BM) is the most common cause of acquired profound bilateral sensorineural hearing loss (SNHL) in childhood. Bilateral SNHL occurs in up to 45% of BM patients with *Streptococcus pneumoniae* infection. The aim of the project is to characterize in detail the pathogenesis of the inner ear damage in an infant rat model of pneumococcal meningitis, and to investigate the relationship between the infection, inflammation and the resulting hearing loss. This model has been shown relevant in the development of therapeutic and regenerative approaches for postmeningitic sequelae including hearing loss.
Methods. 11 day old male Wistar rats were inoculated intracisternally with increasing doses of live *Streptococcus pneumonia* serotype 3. Antibiotic therapy was initiated 18 hours postinfection with ceftriaxone (Rocephine® 100 mg/kg, s.c. bid) and continued for 3 days. Samples of cerebrospinal fluid (CSF) were collected by puncture of the cisterna magna to determine the bacterial titers and measure inflammatory cytokines (Luminex®). Three weeks after the infection, hearing sensitivity was determined with the auditory brainstem response (click-ABR). Cell loss of hair cells and auditory neurons and afferent ribbon synapse density were quantified histopathologically at different locations in the cochlea.
Results. Infected animals showed significantly higher levels of the pro-inflammatory cytokines IL-1β, IL-6, TNF-α, IFN-γ and the anti-inflammatory cytokine IL-10 after 18 hours, depending on the inoculum concentration. Increasing inoculum concentrations significantly elevated the hearing thresholds in a dose-dependent manner and the reduced neuronal density showed a base-to-apex gradient. Furthermore, we found a base-to-apex gradient of predominantly outer hair cell loss upon infection. Ribbon synapse quantification is currently under examination.
Conclusions. The present data demonstrates that the infant rat model of pneumococcal meningitis allows for controlling the extent of disease-associated hearing loss. The initial inoculum concentration can be used as a key regulator to manipulate the hearing threshold, the spiral ganglion neuronal density and hair cell loss. Thus, this well controlled disease model will allow us to manipulate the degree of SNHL and to develop specific regenerative approaches for different disease severities.

O29

ANTI-EXCITOXICITY AGENTS REDUCE NOISE-INDUCED LOSS OF HAIR CELL-AUDITORY NERVE SYNAPSES AND TINNITUS

Richard Altschuler[1], David Dolan[1], Karin Halsey[1], Ariane Kanicki[1], Diane Prieskorn[1], Cathy Martin[1]; Sue DeRemer[1], Susan Shore[1], Josef Miller[1,2,3]

[1]Kresge Hearing Research Institute, University of Michigan, Ann Arbor,MI, USA; [2]Karolinska Institutet, Stockholm, Sweden

Vulnerability of the synapse of the auditory nerve on the inner hair cell after noise exposure and excitotoxicity is well documented. The high incidence of tinnitus following noise exposure has fueled speculation that the loss of these synapses may be a key peripheral factor underlying the initiation of tinnitus.

Objective. In this study we tested the hypothesis that loss of inner hair cell-auditory nerve synapses would lead to tinnitus and that anti-excitotoxic agents that prevent this loss would reduce noise-induced tinnitus.

Methods. Inner hair cell synaptic ribbons (CTBP2 density), ABR (4, 8 & 20kHz) input-output functions, and tinnitus (reduced gap detection without loss of pre-pulse Inhibition) were assessed in normal Wistar rats and groups of rats following noise exposure (117 dB Octave Band Noise centered at 4 kHz for 3 hours), including those: untreated, treated with a combination of agents that reduce excitoxicity and Ca^{++} uptake in the auditory nerve (Piribedil & Memantine), and treated with Piribedl and Memantine, plus the antioxidant formulation vitamins A, C & E plus the competitive Ca^{++} blocker, magnesium (ACEMg).

Results. Following noise exposure CTBP2 density was significantly reduced and tinnitus increased in untreated animals compared to unexposed rats. The reduction in CTBP2 density was reversed by Piribedal and Mementine but still below that in unexposed animals. In animals treated with Peribidal, Mementine and ACEMg, CTBP2 density was equivalent to unexposed subjects and tinnitus was reduced compared to untreated exposed animals.

Conclusions. These findings are consistent with the hypothesis that noise-induced loss of the auditory nerve—hair cell synapse play a significant part in initiation of tinnitus and that anti-excitotoxic agents may be effective in mitigating this factor.

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[3]This author may benefit from commercial development of ACEMg for prevention of noise-induced hearing loss and is under a University of Michigan COI management plan.

O30

THE ROLE OF JNK ISOFORMS IN NOISE-INDUCED HEARING LOSS

Allen F. Ryan, Kwang Pak, Joseph Hardeman

University of California, San Diego, Surgery La Jolla, CA, USA and Veterans Administration Medical Center, San Diego, CA USA

Substantial evidence links activation of cJun terminal kinase (JNK) to hair cell (HC) damage, including that induced by noise. This includes JNK activation during HC damage and reduced HC loss after application of JNK inhibitors. However, three isoforms of JNK are each encoded by a separate gene. These can be expressed in different cell types and have different or even opposing roles in cellular damage. We assessed the role of each JNK isoform in noise-induced hearing loss.

Mice deficient in the *jnk1, jnk2* or *jnk3* gene were exposed to a two-octave band of noise (4-16 kHz) at 105 dB SPL, for 30 minutes. Controls were wild-type (WT) C57BL/6, the background strain of the knockout (KO) mice. ABR thresholds and amplitude growth functions were obtained at 8, 16 and 32 kHz immediately before and after noise exposure, and at 1 and 2 weeks. HC counts were obtained from the mid-basal turn after final ABRs.

Immediately after noise exposure, both JNK1 and JNK2 KO mice exhibited significantly reduced threshold shift when compared to WT mice at 8, 16 and 32 kHz. JNK1 KO thresholds were reduced to a greater extent than observed for JNK2 KOs at all frequencies, although this difference was not statistically significant. When permanent threshold shift was assessed at 2 weeks, JNK2 KOs showed significantly less hearing loss than WTs at all frequencies tested, while JNK1 KOs showed reduced thresholds at 32 kHz. Immediate and permanent threshold shifts in JNK3 KO mice were similar to those in WTs. HC loss at 4 weeks was significantly less than in WTs for both JNK1 and JNK2 KO mice, but not for mice deficient in JNK3. Preliminary data suggest that asymptotic ABR amplitudes were higher post-noise in JNK3 KOs than in WTs, JNK1 or JNK2 deficient mice.

The results suggest that while both JNK1 and JNK2 are involved in immediate threshold shifts, JNK2 may play a greater role in permanent hearing loss. JNK3 is not involved in hearing loss, but this neural isoform may contribute to the loss of afferent synapses on inner HCs.

Supported by NIH/NIDCD and VA research grants.

O31

SEPTIN4 KNOCKOUT MICE SHOW SUSCEPTIBILITY TO INTENSE SOUND EXPOSURE

Takushi Miyoshi[1], Atsuhiro Yoshida[1, 2], Yosuke Tona[1], Takayuki Nakagawa[1], Juichi Ito[1, 3], Norio Yamamoto[1]

[1]Department of Otolaryngology, Head and Neck Surgery, Graduate School of Medicine, Kyoto University, Kyoto, Japan; [2]Department of Otolaryngology / Head and Neck Surgery, Kurashiki Central Hospital, Kurashiki, Japan; [3]Shiga Medical Center Research Institute, Moriyama, Japan

Background. Septin is a highly-conserved guanosine-5'-triphosphate (GTP)-binding protein family originally reported in cell-division mutant of yeast. Thirteen subtypes of Septin are found in mammals, and form cytoskeleton-like hetero-oligomers. Septins interact with other cytoskeletons, and play important roles in the following processes, other than cell division: cell rigidity, membrane compartmentalization and protein-reaction scaffold. Yoshida et al. examined the Septin4, 5, and 7 in the cochlea and found that they were mainly localized in the supporting cells (pillar and Deiter's cells) of the organ of Corti (OC). However, Septin4 and 5 knockout mice showed no audiological phenotypes under natural conditions, and Septin7 knockout mice were embryonic lethal. As Septins contribute to cell rigidity, we hypothesized that Septin knockout may alter the mechanical property of the OC, and attempted to evaluate this with intense sound exposure.

Methods. Septin4$^{-/-}$ or wild-type mice on CBA/J-background were anesthetized and exposed to 8-kHz octave band noise at 120 dB for 1 h. Threshold shifts at 10, 20, and 40 kHz were evaluated immediately after exposure and at days 1, 3, 7, and 14. Threshold shifts were compared between Septin4$^{-/-}$ (14 ears, 7 mice) and the wild-types (20 ears, 10 mice). Immunohistological analysis was

conducted on the OCs dissected after exposure, and the synaptic ribbons and hair cells were counted using anti-CtBP2 and anti-Myo7a antibodies.
Results. The average threshold shifts at day 14 were 40.0 dB (10 kHz), 39.6 dB (20 kHz), and 41.8 dB (40 kHz) for Septin4$^{-/-}$, and 17.3 dB (10 kHz), 17.0 dB (20 kHz), and 19.8 dB (40 kHz) for wild-types. Two-way repeated-factorial analysis of variance (ANOVA) revealed p values <0.01 for all frequencies. Synaptic ribbons evaluated by anti-CtBP2 antibody were slightly decreased at the basal turns of OCs in Septin4$^{-/-}$. Myo7a-positive cells did not show significant decrease in Septin4$^{-/-}$ or in wild-types.
Conclusion. Septin4$^{-/-}$ mice showed susceptibility to intense sound. Considering that Septins contribute cell rigidity and were expressed in pillar and Deiter's cells, knockout of Septin4 may alter the rigidity of the OC, and induce excessive vibrations.

SESSION V
OTOPROTECTION AND DRUG DELIVERY SYSTEMS

TL5
LOCAL DELIVERY OF DEXAMETHASONE INTO THE SCALA TYMPANI PROTECTS AGAINST ELECTRODE INSERTION TRAUMA (EIT)-INDUCED HEARING LOSS, DAMAGE TO AUDITORY NEUROSENSORY CELLS AND INCREASES IN IMPEDANCE

Esperanza Bas[1], Jorge Bohorquez[1,2], Christine Dinh[1], Stefania Goncalves[1], Adrien Eshraghi[1], Roland Hessler[3], Thomas R. Van De Water[1]
[1]Cochlear Implant Research Program, University of Miami Ear Institute, USA; [2]Department of Biomedical Engineering, University of Miami, USA; [3]MED-EL, Innsbruck, Austria

The insertion of a cochlear implant electrode array can initiate an inflammatory cascade that if not controlled will result in increases in hearing thresholds, loss of auditory hair cells via programmed cell death, damage to neural elements and initiation of fibrosis within the scala tympani. Perfusion of a dexamethasone/artificial perilymph solution into the scala tympani immediately following electrode insertion and continuing for a period of 1 week provided protection against both EIT-induced hearing and auditory hair cell loss for up to 1 month post-EIT. Studies in a guinea pig model of EIT-induced hearing loss have recently shown that incorporation of dexamethasone into the silicone of the cochlear implant electrode arrays is effective in protecting against permanent increases in hearing thresholds, loss of auditory hair cells, damage to neural elements and prevents fibrosis within the scala tympani over a post-EIT period of 3 months. When dexamethasone is mixed into medical grade silicone it is released in a bioactive form and has been shown to be effective in preventing fibrosis around implanted cardiac leads. *In vitro* studies have shown that dexamethasone can be added to a polymer base and released into the media where it effectively protects organ of Corti explants against the ototoxic effects of an inflammatory cytokine (i.e. TNF-alpha). This otoprotective effect is mediated by up- and down- regulation of anti- and pro- cell death members of the *Bcl-2* family. Recent results from an EIT model using cochlear explants has demonstrated activation of wound healing that involved an inflammatory response with increases in inflammatory cytokines, inducible enzymes, cell adhesion molecules and chemokines. These EIT-explants next entered into a proliferative-fibrosis phase with increases in fibrosis associated growth factors, e.g. CTGF and TGF-B1. Treatment of these EIT-cochlear explants with dexamethasone prevented trauma-induced increases of both inflammatory and fibrosis associated factors. These *in vitro* results explain the mechanisms behind our in *vivo* observations in our guinea pig model of EIT-induced hearing and hair cell losses. Taken together these *in vitro* and *in vivo* results provide strong support for future clinical trials that test drug-eluting cochlear implant electrode arrays in patients where preservation of hearing and prevention of scala tympani fibrosis are important factors.

O32
A HIGH-THROUGHPUT SCREEN IDENTIFIES SMALL MOLECULES THAT PROTECT AGAINST CISPLATIN- AND NOISE-INDUCED HEARING LOSS

Tal Teitz[1], Jie Fang[1], Asli Goktug[2], Justine Bonga[1], Shiyong Diao[1], Yinmei Zhou[3], Cheng Cheng[3], Taosheng Cheng[2], Jian Zuo[1]
Dept. of Developmental Neurobiology[1], Chemical Biology and Therapeutics[2], and Biostatistics[3], St. Jude Children's Research Hospital, Memphis, TN, USA

Objective. There are no FDA-approved otoprotective drugs. We aim to use an unbiased, cell-based high-throughput screen to develop small molecules that protect against cisplatin- and noise-induced hearing loss.
Methods. We used the HEI-OC1 cell line derived from neonatal mouse cochleae where cisplatin-induced cell death could be blocked by effective test compounds. Cell death was quantified by Caspase-3/7 Glo and cell viability was monitored by Cell Titer Glo. A library of 4,375 unique biologically-active compounds (including 844 FDA-approved drugs) was screened using robots. The top 157 compounds were validated by dose responses. The best 13 compounds were tested in neonatal (P3) mouse cochlear explant cultures. In each adult FVB mouse, one ear was treated, via local delivery (transtympanic injection), with 5 µl of a compound (250 µM) and the other ear with DMSO (0.5%) alone in a double-blind, random manner. Cisplatin was injected intraperitoneally at 30 mg/kg 2 hrs post-compound treatment, and hair cell counts were analyzed one day post-cisplatin treatment. The compound was locally delivered immediately after 100 dB SPL, 8-16 kHz octave-band noise exposure for 2 hrs. ABR thresholds were measured 7 and 14 days post-noise exposure.
Results. We identified >10 compounds with protection against cisplatin-induced cell death in HEI-OC1 cells (0.1-7.6 µM IC_{50} [half maximal inhibitory concentration]) and cochlear explants (0.15-30 µM IC_{50}). The top compound (SJZuo-4) displays 150 nM IC_{50} and >30 µM LD_{50} [median lethal dose] in cochlear explants. SJZuo-4 protects against cisplatin-induced outer hair cells loss in vivo 24-hrs post-cisplatin treatment. At 7 and 14 days post-noise treatment, SJZuo-4 treated ears exhibited ~12 dB significantly better hearing than control ears at 8 and 16 kHz.
Conclusions. We have identified >10 small molecule compounds that protect against cisplatin-induced cell loss in HEI-OC1 cells and cochlear explants. Our top compound (SJZuo-4) protects against cisplatin-induced outer hair cell loss and noise induced hearing loss in vivo in adult mice. These compounds appear to target a diverse

range of previously under-appreciated biological pathways in otoprotection and provide starting points for further development of in vivo chemical probes for otoprotection in the clinic.

O33
DELIVERY OF LOW LEVEL LASER TO THE HUMAN COCHLEA: A CADAVERIC STUDY

Irumee Pai[1], Dan Jiang[1], Patrick Boyle[2], Alec Fitzgerald-O'Connor[1]
[1]Hearing Implant Centre, St. Thomas' Hospital, London, United Kingdom; [2]Global Research, Advanced Bionics, Cambrdige, United Kingdom

Objective. To evaluate the feasibility of low level laser therapy for otoprotection by measuring the amount of light energy delivered to the cochlea via a permeatal route in cadaveric temporal bones.
Methods. Near-infrared 808nm diode laser was applied permeatally to four ears in two fresh-frozen cadaveric heads. The light energy reaching the cochlea was measured with a photodiode detector placed in the most lateral portion of the internal auditory canal and expressed as a percentage of the laser output. The angulation of the laser probe that allowed maximal transmission of light energy was noted in each case.
Results. The maximum amount of light energy reaching the cochlea was remarkably consistent, and was between 83% and 87% in the four ears studied. It was noted that transmission was highly dependent on precise angular positioning of the laser probe. The optimal angulation was 15 degrees above horizontal in the axial plane and 15 degrees posterior-to-anterior in the sagittal plane in three cases. The figures were 15 degrees and zero degree respectively in the other case.
Conclusion. Low level laser can be delivered to the human cochlea efficiently and non-invasively. Our result suggests that further investigation into its potential therapeutic value in otoprotection is warranted.

O34
GENE THERAPY AND DRUG DELIVERY TO IMPROVE AUDITORY NERVE SURVIVAL IN THE DEAFENED COCHLEA

Andrew Wise
Bionics Institute, Melbourne, Victoria, Australia

Auditory neurons, the target cells of the cochlear implant, undergo progressive degeneration following deafness, ultimately leading to cell death. The delivery of drugs to the cochlea, such as neurotrophins, has been shown to protect auditory neurons and promote regrowth of their peripheral fibres. However, an effective strategy to safely deliver therapeutics is yet to be established thus limiting the clinical potential of this approach.
This talk will describe experiments that have examined the use of two different clinically applicable approaches (gene therapy and nanotechnology) that aim to deliver drugs to the cochlea for long-term auditory neuron protection and hair cell regeneration. In these experiments we have used adult guinea pigs deafened by aminoglycoside drugs. Animals received treatment with gene therapy with an inoculation of an adenoviral vector containing genes for neurotrophins or an inoculation with a control vector. In another set of experiments, animals were implanted with nanoengineered particles. One ear received particles loaded with neurotrophins and the other ear received control particles. Both approaches resulted in significant auditory nerve survival.
The preservation and regrowth of auditory neurons may lead to improvements in clinical outcomes for cochlear implant recipients. In addition to neural protection and regrowth, the safe and effective delivery of therapeutic drugs to the inner ear may also enable the preservation or restoration of residual sensory function that is known to deteriorate following cochlear implantation. The use of gene therapy may enable to regeneration of lost sensory hair cells. The application of drugs to protect and maybe even restore both neural and sensory elements is likely to be a key factor in improved clinical outcomes for cochlear implant recipients in the future.
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O35
HEARING LOSS AND NATURAL ANTIOXIDANTS: A NOVEL PARADIGM FOR NEUROPROTECTION

Cesare Mancuso[1], Anna Rita Fetoni[2], Fabiola Paciello[2], Sara Eramo[3], Rolando Rolesi[2], Gaetano Paludetti[2], Diana Troiani[3]
[1]Institute of Pharmacology, [2]Department of Head and Neck Surgery and [3]Institute of Human Physiology, Università Casttolica, Medical School, Rome, Italy

Hearing loss recognizes several causes ranging from direct and prolonged acoustic trauma to administration of drugs such as streptomycin and cisplatin. The mechanisms through which these agents induce hearing loss are well understood and the role played by excessive production of free radicals, namely reactive oxygen species, and the consequent oxidative stress are no longer matter of debate. Through the adaptive stress response, cells, in particular neural cells, try to counteract free radical-induced damage and increase lifespan. The heme oxygenase-1/biliverdin reductase system (HO-1/BVR) is a main player in the cell stress response because it catabolyzes heme, toxic if produced in excess or under condition of redox imbalance, and generates bilirubin, a strong free radical scavenger. Several approaches have been proposed to activate the HO-1/BVR system and prevent/contrast neuronal damage, one of the more reliable consists in the supplementation with natural antioxidants such as ferulic acid (FA), curcumin (CUR), Coenzyme Q (CoQ), rosmarinic acid (RA) etc. Our studies demonstrated that the sub-chronic systemic administration of both FA and CUR and CoQ and RA improved auditory function in rodents exposed to noise or cisplatin with a composite mechanism of action which include an efficient free radical scavenging activity and reduced oxidative stress, particularly evident at earlier time points. Furthermore, a sustained induction of HO-1, through the nuclear translocation of the transcription factor Nrf2, is an adjunctive mechanism of neuroprotection which becomes significant at later time points. In conclusion, systemic administration of (poly)phenols and other natural antioxidants is a novel strategy to improve auditory function in subjects exposed to acoustic trauma or ototoxic drugs.
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O36

COENZYME Q10 AND VITAMINE TREATMENT PREVENT CISPLATIN OTOTOXICITY IN RAT

Laura Astolfi[1], Edi Simoni[1], Filippo Valente[1], Alice Cani[1], Vincenza Cascella[1], Pietro Giordano[1], Stavros Hatzopoulos[2], Alessandro Martini[3]

[1]Bioacoustics Research Laboratory, Department of Neurosciences, University of Padua, Italy; [2]ENT & Audiology Department, University Hospital of Ferrara, Italy; [3]ENT Surgery - Department of Neurosciences, University of Padua, Italy

Studies in the literature have demonstrated that cisplatin (CDDP) causes a high level of oxidative stress, resulting in an increase of free radicals production, which damage the inner ear and causing hearing loss. In particular this drug induces considerable damage to the organ of Corti, with involvement of the outer hair cells, more intense in the basal cochlear turn, reflecting hearing loss at high frequencies. Data from animal studies show that antioxidants can compensate against ototoxicity CDDP-induced. Acuval400® is a dietary supplement composed of various antioxidant agents and minerals. The present study had two aims the first was evaluate the ototoxic effect of two different way of CDDP administration, the second was evaluate the protective effect of an added dosage of Coenzyme Q10 terclatrate (Q-TER) to an Acuval 400® solution, in response to CDDP-induced ototoxicity in Sprague-Dawley rat model.

Thirty Sprague Dawley rats were divided into six groups: 1) not treated animals, 2) animals treated with Acuval 400® and Q-TER, 3-4) CDDP treated animals only with a bolus (14 mg/kg) and also with Acuval 400® and Q-TER, 5-6) CDDP treated animals with three consecutive doses (4,6 mg/kg) and also with Acuval 400® and Q-TER.

The Acuval 400® and Q-TER solution was administered orally 5 times: 24 and 2 hrs prior to CDDP, and then daily for three days. The auditory function was assessed by measuring Auditory Brainstem responses, from 4 to 32 kHz, before CDDP administration and 4 days after treatment.

Animals receiving the Acuval 400® and Q-TER treatment did not present any side effects; animals treated with the CDDP bolus showed the same hearing threshold alterations of the animals treated with three CDDP dosages. The Acuval 400® and Q-TER treatment was able to prevent CDDP ototoxicity only in animals treated with three consecutive doses of CDDP.

The data of this study suggest that the administration of CDDP in one bolus instead of three consecutive doses cause the same hearing loss, and the Acuval 400® and Q-TER solution, administered orally, protects from ototoxicity CDDP-induced when applied in several lower doses according to human chemotherapy protocols.

O37

OTOPROTECTIVE EFFECT OF A DEXAMETHASONE-LIPOSOME GEL INJECTED IN THE MIDDLE EAR AFTER COCHLEAR IMPLANTATION IN AN ANIMAL MODEL

Elisabeth Mamelle[1,2,3], Naila El Kechai[4], Amelie Bochot[4], Florence Agnely[4], Olivier Sterkers[1,2,3], Evelyne Ferrary[1,2,3], Yann Nguyen[1,2,3]

[1]AP-HP, Pitié-Salpêtrière Hospital, Otolaryngology Department, Unit of Otology, Auditory Implants and Skull Base Surgery, Paris, France; [2] Sorbonne University, UPMC Univ. Paris 06, Paris, France; [3] Inserm, Paris 6 UMPC Univ, UMR S-1159, "Minimally Invasive Robot-based Hearing Rehabilitation", Paris France; [4] Université Paris Sud, Faculté de Pharmacie, Institut Galien Paris Sud, CNRS UMR 8612, Châtenay-Malabry, France

Introduction. During cochlear implantation, the electrode insertion, performed with no visual feedback beyond the entry into the cochlea, is supposed to generate cochlear cell damages that could lead to lower speech performance. However, few local therapies for the inner ear are available to limit intracochlear damages. In this study, our goal was to evaluate, in guinea pigs, the impact on postoperative hearing of a local application of a hyaluronic acid gel formulation bearing liposomes encapsulating dexamethasone phosphate.

Material and Methods. First we assessed the dexamethasone (15 mg/ml)- liposomal (80 mM), hyalorunic acid gel (2.28%) biodistribution in the cochlea after its injection in the bulla (120 µl) (15 guinea pigs). Perilymph fluids were collected through a hand-drilled cochleostomy in the basal turn of the cochlea. A micropapillary pipette sampled 2 µL of perilymph. The concentration of both dexametahsone sodium phosphate and its active form dexamethasone were measured in perilymph samples using high-pressure liquid chromatography combined with mass spectrometry detection at Day-2, Day-7 and Day-15 (n=6; n=4; n= 15 respectively). Secondly a manual technique was performed for cochlear implantation of 12 guinea pigs, with a 5 mm length array of 0.4 mm diameter. At the end of the procedure, hyaluronic acid gel containing drug free liposomes or loaded with dexamethasone was injected. ABR thresholds were recorded at Day-0, Day-2 and Day-7 after the cochlear implantation.

Results. Dexamethasone was detected in perilymph fluid until Day-15 in its active form when the gel was applied in bulla for a passive diffusion through the round window. After cochlear implantation, ABR thresholds showed a hearing recovery at Day-7 after the initial loss observed at Day-2 when dexamethasone was administrated compared to the drug free liposome gel ($p<0.05$).

Conclusion. This study showed the benefit of a dexamethasone loaded hyaluronic acid gel containing liposomes, to prevent the postoperative ABR threshold shift. Furthermore, we demonstrated the interest of this original local therapy to allow prolonged concentration in the perilymph fluid through the round window.

O38

SPIRAL GANGLION CELL DEGENERATION FOLLOWING HAIR CELL LOSS:SIMULTANEOUS RATHER THAN RETROGRADE

Dyan Ramekers[1,2], Huib Versnel[1,2], Emma M. Smeets[1], Steven Kroon[1], Sjaak F.L. Klis[1,2], Wilko Grolman[1,2]

[1]University Medical Center Utrecht, Department of Otorhinolaryngology and Head & Neck Surgery, Utrecht, the Netherlands;[2]Brain Center Rudolf Magnus, Utrecht, the Netherlands

Severe damage to the organ of Corti leads to degeneration of the cochlear spiral ganglion cells (SGCs) which form the auditory nerve. This degeneration starts at the level of synaptic connection of the peripheral processes of SGCs with the cochlear hair cells. It is thought that from this point SGC degeneration progresses in a retrograde fashion; that peripheral processes degenerate first, followed by the SGCs and their axons with a delay of several weeks to many months. Since evidence for this course of events in the literature is not unambiguous, we here aimed to provide a comprehensive account of the course of SGC degeneration in the guinea pig cochlea after ototoxic treatment. Histological analysis of six healthy and twenty-three deafened cochleas (using kanamycin and furosemide) showed that the degeneration of SGCs and their

peripheral and central processes was simultaneous rather than sequential. The course of the degeneration process may vary among species, and may depend on the cause of deafness, but the present findings at least indicate that gradual retrograde degeneration is not an elemental process following damage to the organ of Corti. In additional experiments, we found that protection against degeneration with brain-derived neurotrophic factor (BDNF) was slightly less effective for the peripheral process than for the SGC soma. Strategies to prevent SGC degeneration after hair cell loss should consider the differential effects on the various neural elements.

O39

SUDDEN DEAFNESS: IS IT AN OTOLOGIC EMERGENCY? A PREDICTIVE MODEL IN PATIENTS TREATED WITH INTRATYMPANIC STEROIDS

G. Attanasio[1], E. Di Porto[2], F.Y. Russo [3], E. Covelli [4], R. Filipo[5]
[1]Head and Neck Department, Policlinic Umberto I of Rome, Italy; [2]Department of Economics and Statistics, Federico II University of Neaples, Italy; [3]Department of Sensory Organs, Sapienza University of Rome, Italy; [4]ENT Unit, NESMOS Department, Medicine and Psychology and Medicine, Sapienza University of Rome, Italy; [5]Sapienza University of Rome, Italy

Idiopathic sudden sensorineural hearing loss (ISSNHL), although not a frequent disease, is the subject of great interest for the presence of a high percentage of recovery. The boundary between spontaneous recovery and efficacy of early medical therapy is still controversial. Systemic steroid therapy remains that of election, but the last decade has increasingly used the intra-tympanic route of administration ensuring a local organ-specific treatment, a high dosage of the drug and an absence of systemic side effects. The present retrospective study has been conducted to evaluate the impact of two variables, DELAY (defined as the time interval between the onset of ISSNHL and the beginning of therapy) and DELAY SQUARE on SUCCESS rate, according to the Furuhashi criteria, for the different audiometric curve shapes with which the ISSNHL can occur.
381 patients with unilateral ISSNHL underwent first-line treatment with intratympanic infiltration of prednisolone, once a day, for three consecutive days. The results showed a non-linear configuration for the variable DELAY. As supposed, DELAY was negatively correlated with the variable SUCCESS, moreover DELAY SQUARE showed a positive correlation almost significant in every sample. These two evidences suggested that the probability of therapeutic success declines as time passes in absence of the treatment, but during the first days following the onset of the SSNHL the probability of success decreases much faster. Following our predictive model derived by the Probit regressions and considering an adult subject of about 50 years old, one day of delay led to 2.8% of decreased in the probability of success. This probability declined to 4.4% for flat audiometric curves and to 5.6% for up-sloping curves. In profound hearing loss the chance of success was lower than the other curves also with few days of delay, and the correlation DELAY/SUCCESS, while remaining inversely proportional, reduced to 1.3% for each day of delay. The results suggested that in patients with ISSNHL an early treatment is highly recommended and that in most cases the ISSNHL should be considered an otologic emergency.

SESSION VI
REGENERATION, STEM CELL AND GENE THERAPY

TL6

STRATEGIES FOR INDUCTION OF REGENERATION IN MAMMALIAN INNER EARS

Norio Yamamoto, Takayuki Nakagawa
Kyoto University, Department of Otolaryngology, Head and Neck Surgery, Kyoto, Japan

Sensorineural hearing loss (SNHL) is a common disability in the world; however, at present, options for the treatment of SNHL are very limited. Previous studies including human temporal bone analyses have revealed that the degeneration of the cochlea is a common mechanism of SNHL. A major problem for the development of novel treatments for SNHL has been the limited regeneration capacity in mammalian cochlear cells. However, recent progress in basic studies has led to several effective strategies for the induction of regeneration in the mammalian cochlea, including promotion of self-repair, trans-differentiation, de-differentiation, cell transplantation and technological regeneration. In this targeted lecture, several possible strategies for regenerative medicine for SNHL will be introduced in accordance with the stage of cochlear degeneration.

O40

STROMAL STEM CELLS AS A TOOL IN COCHLEAR REGENERATION: DIFFERENT ADULT SOURCES AND POSSIBLE CROSSTALK WITH THE NEUROGENIC NICHE

W. Lattanzi[1,2], A.R. Fetoni[3], R. Rolesi[3], M. Barba[1], F. Paciello[3], L. Di Pietro[1], S.L.M. Eramo[13], V. Quercia[1], V. Pino[1], F. Michetti[1,2], D. Troiani[4], G. Paludetti[3]
[1]Institute of Anatomy and Cell Biology, Università Cattolica del Sacro Cuore, Rome, Italy; [2]Latium Musculoskeletal Tissue Bank, Rome, Italy; [3]Institute of Otolaryngology, Head and Neck Surgery, "A. Gemelli" University Hospital, Università Cattolica del Sacro Cuore, Rome, Italy; [4]Institute of Physiology - Università Cattolica del Sacro Cuore, Rome, Italy

Although a therapeutic use of somatic stem cells is already feasible in several anatomical sites, the nervous tissue, including sensory epithelia, is generally refractory to regeneration/repair, due to an unfriendly environment. Converging evidence is suggesting the feasibility of cochlear regenerative strategies based on somatic stem cells isolated from the stroma of adult organs (namely, mesenchymal or stromal stem cells). These cells, similar to those originally found in the bone marrow stroma, are capable of extensive *in vitro* multilineage commitment, and exert a number of trophic features, even though their effective *in vivo* plasticity is far from being demonstrated.
We have recently demonstrated that adipose-derived stem cells, implanted in the cochlea upon noise-induced cochlea, are able to migrate at the site of tissue damage and express trophic factors. Others reported a putative active role of neural-inuced stromal cells in inducing neuroepithelial cell regeneration.
The aim of the present study is to provide a comprehensive description of the possible similarities and crosstalk between stromal stem cells and neural stem cells niches, in order to provide

some clues towards the definition of a stronger rationale for successful cell-based cochlear regeneration.
Extended comparison of cellular immunophenotype, gene expression profile and differentiation potential, along with documented effects of their *in vivo* regenerative potential is provided, focusing on migration paths, lineage commitment, environmental signals, cell-matrix crossotalk, and underlying molecular networks.
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O41
ADIPOSE-DERIVED STEM CELLS IMPROVE SURVIVAL OF AUDITORY NEURONS IN AN ANIMAL MODEL OF SENSORY HEARING LOSS
Andreas Radeloff, Philipp Schendzielorz, Kristen Rak, Johannes Voelker, Katrin Froelich, Rudolf Hagen
Comprehensive Hearing Center, Department of Oto-Rhino-Laryngology, Plastic, Aesthetic and Reconstructive Head and Neck Surgery, University of Wuerzburg, Germany

Cochlear implantation (CIs) is very successful in restoring speech perception capabilities of deaf patients in quiet. However, there are still limitations in speech understanding in noise and perception of music in the vast majority of CI patients. There is evidence that a limited number of effective spectral channels originating from a poor electrode-neuron interface is one important reason for this problem. The application of neurotrophic factors via pump systems or genetically engineered cells has been suggested to overcome the issue, but both are not suitable for the use in humans.
We recently showed that adipose-derived stem cells (ASCs), multipotent stem cells that can be harvested from adult adipose tissue, produce relevant amounts of neurotrophins and, thus, could serve as neurotrophin source. In coculture, moreover, ASCs enhanced the survival and neuritogenesis of auditory neurons *in vitro*.
For the recent study, an autologous transplantation of ASCs was performed in the guinea pig model. For this, ASCs were isolated individually from the neck adipose tissue of each animal, and both ears were deafened by a local application of gentamicin. After one week, deafness was confirmed by auditory brain-stem responses, and 5 x 10^5 ASCs were applied to the basal turn of the right cochlea whereas the left side served as a control. Two, 4 and 8 weeks after transplantation the animals were euthanized and the cochleae were histologically evaluated. There was a markedly improved survival of auditory neurons in the treated ears with a number of survived neurons that was more than twice as high compared to the control ears in the basal turn of the cochlea after 2 weeks. The effect slightly decreased over time, but remained statistically significant in the basal part of the cochlea, where the transplanted cells were situated. At all time points, there was a gradient evident with a stronger effect in the basal and a weaker effect in the apical parts of the cochlea. Similarly, the density of peripheral dendrites increased in the transplanted ears.
These results support the assumption that ASCs could play a role for CI in the future.

O42
MOUSE DERIVED CELL TRANSPLANTATION INTO THE MOUSE OTOCYST *IN VIVO*
Hiroki Takeda, Ryosei Minoda, Toru Miwa, Takao Yamada, Momoko Ise, Eiji Yumoto
Department of Otolaryngology-Head and Neck Surgery, Faculty of Life Sciences, Kumamoto University, Kumamoto City, Japan

Introduction. Mouse otocyst is an attractive experimental target for investigating treatment modalities about inner ear diseases and/or studying inner ear development. Herein, we have demonstrated in vivo cell transplantation into mouse otocyst with two types of cells; mouse-derived pluripotent stem (m-iPS) cells and EGFP expression otocystic cells which is obtained from EGFP expression transgenic mice (C57BL/6-Tg; CAG-EGFP: hereinafter referred to as EGFP mice).
Methods. We used wild type timed pregnant CD-1 mice at E11.5 for recipient. The timed pregnant mice underwent the injection of m-iPS cells (RIKEN; iPS-MEF-Ng-20D-17) which has EGFP expression with nanog promoter and of EGFP-otocystic cells which were obtained from EGFP mice at E11.5. Regarding EGFP-otocystic cells, the otocysts were extracted from EGFP mice, subsequently the otocysts were dissociated into single cells. After injection of m-iPS and EGFP-otocystic cells, thet1t11hirokihihit treated embryos were evaluated at E13.5, E15.5 and E18.5.
Results. The injected m-iPS cells were detectetable in a space of treated inner ear at E13.5 and E15.5, but not detected at E18.5. Those cells attached to lining cells of the developing inner ear, but not migrate into the inner ear epithelium. Whereas, the injected EGFP-otocystic cells were detectable in a space of treated inner ear at E13.5, and the cells were found in the lining epithelium of the inner ear at E15.5.
Conclusion. The m-iPS cells did not invade into normal developing inner ear epithelium, meanwhile, EGFP-otocystic cells which were obtained from E11.5 mice migrated into the treated inner ear epithelium. These findings suggest that precise time matching of the stage of recipient mice and the stage of donor cells is required to achieve successful cell transplantation in the otocyst. Furthermore, we preliminary injected m-iPS cells into Cx30-/- mice at E11.5, the injected m-iPS cells aggregated and destroyed the structure of inner ear, suggesting tumorigenesis.

O43
EFFICIENT DELIVERY OF CRISPR/CAS9 PROTEINS INTO MAMMALIAN INNER EAR IN VIVO AND NEURON STEM CELLS IN VITRO
Yilai Shu[1,2,3,4], John A Zuris[5,6], David B Thompson[5,6], Yong Tao[1,2], Wenyan Li[1,2,3,4], Zhengmin Wang[3,4], Huawei Li[3,4], David R Liu[5,6], Zheng-Yi Chen[1,2]
[1]Department of Otology and Laryngology, Harvard Medical School, Boston, Ma, USA; [2]Eaton-Peabody Laboratory, Massachusetts Eye and Ear Infirmary, Boston, Ma, USA; [3]Department of Otology and Skull Base Surgery, Eye and Ear, Nose, Throat Hospital, Shanghai Medical College, Fudan University, Shanghai, China; [4]Key Laboratory of Health Ministry for Hearing Medicine, Shanghai, China; [5]Department of Chemistry & Chemical Biology, Harvard University, Cambridge, Massachusetts, USA; [6]Howard Hughes Medical Institute, Harvard University, Cambridge, Massachusetts, USA

Introduction. One of the major hurdles to study mammalian

inner ear is the lack of efficient delivery of genes and proteins into the inner ear. We studied the use of the application of liposomal reagents, developed for DNA and RNA transfection, complexed with supercharged GFP proteins to deliver Cre to mouse inner ear *in vivo*. We further studied the strategy for genome-editing proteins complexed with RNA for delivery in the both neonatal and adult inner ear *in vivo* and neuron stem cells *in vitro.*

Methods. Supercharged GFP proteins carrying Cre recombinase, (-30)GFP-Cre, were injected into P1 Rosa-tdTomato$^{f/f}$ cochlea, with tdT^{+} cells identified by immunolabeling. Gene-editing enzyme Cas9 and guideRNA complexed by liposomal reagents, were injected to neonatal and adult Atoh1-GFP mouse cochlea *in vivo* and neuron stem cells *in vitro*. Gene editing was assessed by disappearance of signal in the target cells and by new generation sequencing (NGS) to identify indels in the target genes. We also tested a procedure which culture whole adult mammalian cochlea.

Results. Microinjection of (-30)GFP-Cre and Lipofectamine 2000 into Rosa-tdTomato$^{f/f}$ cochlea resulted in tdTomato signal in 90% of hair cells near the injection site, indicating highly efficient functional Cre protein uptake by hair cells. No tdT^{+} was seen in control cochlea. After delivering of Cas9 and gRNA (against GFP) complexed by Lipo2000 into neonatal and adult Atoh1-GFP mouse cochlea, 20% of hair cells showed disappearance of GFP signal and NGS showed indels in the GFP gene, a demonstration of CRISPR-mediated gene editing in hair cells. All hair cells survived with interact stereocilia and hearing was preserved perfectly. And disappearance of GFP of neuron stem cells reached 35%. The adult cochlear can be cultured and preserved extremely well after 18 days.

Conclusion. Supercharged proteins and Liposomal formulation can be used to efficiently deliver genome-editing proteins into neonatal and adult mouse hair cells *in vivo,* resulting in specific gene editing. This technology should enable the editing of gene mutations to correct genetic deafness, and to study inner ear protein functions for both neonatal and adult mammalian inner ear. Combining with our adult culture system it is possible to model human deafness phenotype in vitro as well. The delivery system can be developed to carry unlimited combinations of proteins in biophysiology study and be used as protein-based therapy in regeneration in mammalian inner ear. It also can be used to regenerate or correct mutation for hair cells and neuron cells through stem cells.

O44
RNA SEQ ANALYSIS OF REGENERATED HAIR CELLS IN THE POSTNATAL MOUSE COCHLEA

Tetsuji Yamashita[1], Ken Sugino[2], David Finkelstein[3], Fei Zheng[1], Bradley Walters[1], Jian Zuo[1]

[1] Dept. of Developmental Neurobiology, [3] Dept. of Computational Biology, St. Jude Children's Research Hospital, Memphis, TN, USA; [2] Janelia Farm Research Campus, HHMI, Ashburn, VA, USA

Objective. Recent studies demonstrated that mammalian cochleae exhibit limited spontaneous or forced hair cell (HC) regeneration at neonatal ages in vivo. However, newly regenerated HCs are immature. The aim of this study is to identify genes and pathways that are important for HC regeneration and maturation in the postnatal mammalian cochlea.

Methods. We used the mouse models where induced ectopic expression of Atoh1 in neonatal cochlear supporting cells (SCs) led to new, immature HC formation at adult ages. From cochleae of mice aged between postnatal day 21 (P21) and P76, we manually isolated ~20-130 fluorescently labelled cells for each of the following cell types: 1) new regenerated immature HCs; 2) endogenous mature SCs; 3) endogenous mature outer HCs (OHCs). We then performed RNA Seq on amplified samples, and comparative transcriptome profile analysis.

Results. Independent replicates of each cell source were nearly identical; new HCs expressed a number of known HC and SC markers that have been confirmed by immunostaining previously; new HCs resembled SCs more than OHCs, supporting our previous characterization by immunostaining and electrophysiology.

We found that 109 genes encoding transcription factors (TFs) that were differentially expressed in these new HCs vs. in endogenous mature SCs and OHCs. Interestingly, most of the top 30 TFs are not identified by gene expression profiling in chicken, zebrafish, or mammalian utricle HC regeneration. Gene set enrichment analysis also identified the Notch pathway as activated in new HCs compared to that in SCs, and the TGFβ and MAPK pathways as activated in new HCs compared to those in OHCs. All three pathways have been implicated in the normal development of the vertebrate inner ear, and in HC regeneration in chickens and zebrafish lateral lines.

Conclusions. We have identified ~30 candidate TFs and 3 signaling pathways that likely play key roles in HC regeneration and maturation in postnatal mouse cochleae. We are currently validating these RNA Seq results by independent analysis using Fluidigm single cell qRT-PCR, in situ hybridization and immunostaining, and further testing the roles of these ~30 genes and 3 pathways in HC regeneration and maturation ex vivo and in vivo.

O45
CELL CYCLE REACTIVATION OF LGR5+ COCHLEAR PROGENITOR CELLS IN NEONATAL FUCCI MICE BY A GSK3 SMALL MOLECULE INHIBITOR

Marta Roccio[1], Stefan Hahnewald[1], Michael Perny[1,3], Pascal Senn[1,2]

[1]Laboratory of Inner Ear Research, Department of Clinical Research, University of Bern and University Department of Otorhinolaryngology, Head & Neck Surgery, Inselspital, Bern, Switzerland; [2]Department of Otorhinolaryngology, Head & Neck Surgery University Hospital Geneva (HUG), Switzerland;[3]Laboratory of Neuroinfectiology, Institute of Infectious Diseases (IFIK), University of Bern, Switzerland

Due to the lack of regenerative capacity of the mammalian auditory epithelium, sensory hair cell loss results in permanent hearing deficit. Despite this, a population of tissue resident stem/progenitor cells has been recently described. Identification of methods to trigger their activity could lead to exploitation of their potential therapeutically.

Here we validate the use of transgenic mice reporting cell cycle progression (FUCCI), and stemness (Lgr5-GFP), as a valuable tool to identify regulators of cell cycle re-entry of supporting cells within the auditory epithelium. The FUCCI system allows for monitoring of cell cycle progression in a dynamic fashion, relying on the mutual expression of two fluorescently tagged probes: Geminin-AG, labeling S/G2/M by green fluorescence and Cdt1-KO2, labeling G0/G1 cells with red fluorescence.

Hair cell progenitors, characterized by the expression of Sox2 and Lgr5, have been previously demonstrated by genetic means to be Wnt responsive. In this study, a small molecule compound

(CHIR99021) was used to inhibit GSK3 activity and thereby activate canonical Wnt signaling. This led to a significant increase in the fraction of proliferating sphere-forming cells, labeled by the FUCCI markers and in the percentage of Lgr5-GFP+ cells, as well as a selective increase in the fraction of S-G2-M cells in the Lgr5+ population. Using whole mount cultures of the Organ of Corti from FUCCI animals we detected a significant increment in the fraction of proliferating Sox2+supporting cells and appearance of novel MyoVIIa+/Edu+ hair cells.
In conclusion, the combination of stem cell and cell cycle reporters utilized, provides a robust mean to identify novel regulators of auditory organ regeneration and to clarify the contribution of stem cell activity.

O46
MURINE "MINI EARS" FOR HIGH-CONTENT SCREENING IN HEARING RESEARCH

Mirko Jaumann[1], Aurélie Dos-Santos[1], Marcus Müller[2], Hubert Löwenheim[2]
[1] University of Tübingen Medical Center, Department of Otorhinolaryngology - Head & Neck Surgery, Hearing Research Center, Tübingen, Germany; [2] University of Oldenburg, Department of Otorhinolaryngology, Head & Neck Surgery, European Medical School, Oldenburg, Germany

Mammalian *in vitro* models to study ototoxicity, otoprotection or otoregeneration have utilized inner ear cell lines, tissue culture and to a certain extent whole organ culture. Tissue cultures have a limited application as a validation tool for the increasing number of potential ototoxic and otoprotective agents that arise from the increasing number of screening efforts using inner ear derived cell lines or the zebrafish lateral line. Since cell lines and non-mammalian models for ototoxicity may behave distinct from primary cells in response to drug treatment, we developed an *in vitro* standardized assay for ototoxic drug screening derived from murine organ of Corti progenitor cells, representing "Mini Ears".
We isolated cells from the postnatal day 0 organ of Corti of NMRI mice that are known to give rise to stem cell derived otospheres (Oshima et al., 2007, J Assoc Res Otolaryngol). From these spheres, differentiated epithelial islands - "Mini Ears" - populated with hair- and supporting cell-like cells can be obtained. Primary cells were cultured in a proliferative environment for 5 days in vitro (DIV). The cells were then plated on 96-well plates for differentiation for 14 DIV. "Mini ears" were fixed, immunohistochemically stained, and analyzed using an ImageXpress Micro XLS High-Content Screening microscope (Molecular Devices).
Varying culture conditions and testing markers for hair and supporting cell–like cells, we found reproducible conditions for the generation of "Mini-Ears". A fully automated data acquisition algorithm that identifies, images, and analyses "Mini Ears" with no user intervention in up to 5 different channels and z-stacks, now provides a screening platform to conduct "Mini Ear" based high-content screening for ototoxicity, otoprotection, and otoregeneration.

SESSION VII
IMMUNOMEDIATED DISEASES AND COCHLEAR IMPLANTS

O47
KNOCKOUT OF THE AUTOIMMUNE HEARING LOSS TARGET CTL2/SLC44A2 RESULTS IN SPIRAL GANGLION CELL LOSS, HAIR CELL LOSS AND PROGRESSIVE HEARING LOSS

Pavan Kommareddi[1]*, Thankam Nair[1]*, Bala Naveen Kakaraparthi[1], Maria M. Galano[1], Danielle Miller[1], Irina Laczkovich[1], Trey Thomas[1], Lillian Lu[1], Kelli Rule[2], Lisa Kabara[1], Ariane Kanicki[1], Elizabeth D. Hughes[3], Julie M. Jones[5], Mark Hoenerhoff[2], Susan G. Fisher[6], Richard A. Altschuler[1], David Dolan[1], David C. Kohrman[1,5], Thomas L. Saunders[3,4], Thomas E. Carey[1]
*[1]Kresge Hearing Research Institute, Department of Otolaryngology, Head and Neck Surgery, University of Michigan, Ann Arbor, MI, USA; [2]Unit for Laboratory Animal Medicine, University of Michigan, Ann Arbor MI, USA; [3]Department of Internal Medicine, Division of Molecular Medicine and Genetics, University of Michigan, Ann Arbor, MI, USA; [4]Biomedical Research Core Facilities, Transgenic Animal Model Core, University of Michigan, Ann Arbor, MI, USA; [5]Department of Human Genetics, University of Michigan, Ann Arbor, MI, USA; [6]Department of Clinical Sciences and Temple Clinical Research Institute, Temple University School of Medicine, Philadelphia, PA; *Contributed equally to the work.*

SLC44A2 (solute carrier 44a2), also known as CTL2 (choline transporter-like protein 2), is expressed in many supporting cell types in the cochlea and is implicated in autoimmune hearing loss and hair cell survival. To determine the importance of SLC44A2/CTL2 in auditory function, we created mice with deletion of Slc44a2. In mice with the mixed C57BL/6-129 background, homozygous deletion of *Slc44a2* exons 3-10 (*Slc44a2*$^{\Delta/\Delta}$) resulted in high frequency hearing loss and hair cell death. To reduce effects associated with age-related hearing loss (ARHL) in these strains, mice carrying the *Slc44a2*$^{\Delta}$ allele were backcrossed to the ARHL-resistant FVB/NJ strain, and evaluated after backcross seven (N7) (99% FVB). *Slc44a2*$^{\Delta/\Delta}$ mice produced abnormally spliced *Slc44a2* transcripts that contain a frameshift and premature stop codons. Neither full-length SLC44A2 nor a putative truncated protein could be detected in *Slc44a2*$^{\Delta/\Delta}$ mice, suggesting a likely null allele. Auditory brainstem responses (ABR) of mice carrying the *Slc44a2*$^{\Delta}$ allele on an FVB/NJ genetic background were tested longitudinally between the ages of 2 to 10 months. By 6 months of age, *Slc44a2*$^{\Delta/\Delta}$ mice exhibited hearing loss at 32 kHz, but at 12 and 24 kHz had sound thresholds similar to wild-type *Slc44a2*$^{+/+}$ and heterozygous *+/Slc44a2*$^{\Delta}$ mice. After 6 months of age, *Slc44a2*$^{\Delta/\Delta}$ mutants exhibited progressive hearing loss at all frequencies and *+/Slc44a2*$^{\Delta}$ mice exhibited moderate threshold elevations at high frequency. Histologic evaluation of *Slc44a2*$^{\Delta/\Delta}$ mice revealed extensive hair cell and spiral ganglion cell loss, especially in the basal turn of the cochlea. We conclude that *Slc44a2* function is required for long-term hair cell survival and maintenance of hearing.

O48

UNILATERAL SNHL AS POSSIBLE IMMUNE-BASED INNER EAR DISEASE (AIED)

Francesca Atturo, Roberta Colangeli, Simonetta Monini, Maurizio Barbara

Sant'Andrea Hospital, NESMOS Department, Sapienza University, Rome Italy

Introduction. Autoimmune inner ear disease (AIED) is recognized as a syndrome of bilateral asymmetric sensorineural hearing loss (SNHL) and/or dizziness, with rapid progression over days to months, probably due to specific or aspecific antibodies or immune cells which are attacking the inner ear. The most rational strategy for a correct diagnosis of AIED should consider, in addition to the typology of SNHL, the response to steroid treatment, as well as the results of specific and aspecific immunological laboratory tests. In the present study, the serological evidence of positivity for aspecific immunological tests not only in bilateral AIED but also in sudden and progressive unilateral SNHL.

Materials and Methods. Patients suffering from unilateral or bilateral SNHL, sudden or progressive, symmetric or asymmetric were included, while patients with Menière's disease, retrocochlear pathologies or aged >65 years were excluded. All the patients underwent a battery of blood exams to evaluate the immunological response: ANA, ENA screening, anti-thyroperoxidase (anti-TPO), anti-thyroglobulin and antibody against smooth muscle (ASMA). The patients were clinically divided into two groups: Group A (39 patients) affected by bilateral SNHL and Group B (33 patients) with unilateral SNHL. A quantitative scale has been assigned to ANA, ENA and ASMA assay. A statistical two pair sample test (t-test) has been applied for the evaluation of eventual statistical differences.

Results. In both groups a significant positivity of aspecific immunological test has been assessed, i.e. 66.7 %. In both A and B groups, the same percentage of positivity was found. ASMA and ANA were the most frequent positive antibodies in both groups, without statistical differences between the individual titration levels.

Discussion. Our results have shown that the progressive forms, uni- or bilateral, possess the greater positivity to aspecific immunological tests. A high positivity of ANA and ASMA was evidenced in uni- and bilateral SNHL, with similar titration levels. The authors suggest to include also the unilateral SNHL in the clinical suspicion of AIED, especially when progressive in tipology, and to always assess these cases with ANA and ASMA titration assay.

O49

THE IMMUNE RESPONSE OF THE COCHLEA TO AN IMPLANTED ELECTRODE IN THE CONTEXT OF AN OTOTOXIC INSULT

Leila Abbas[1], Daniela I. Cacciabue-Rivolta[1], Daniel Smyth[2], Marcelo N. Rivolta[1]

[1]Centre for Stem Cell Biology, Department of Biomedical Science, Unversity of Sheffield, Western Bank, Sheffield, UK; [2]Cochlear Technology Centre, Gebouw I, 2800 Mechelen, Belgium

In cochlear implantation, the response of the host tissue to the prosthetic device can be a critical factor in determining the outcome of the procedure. In cases where fibrosis and osteoneogenesis is rife, the functioning of the implant may be severely impaired and can result in rejection and surgical resection of the device.

We are using a gerbil model for cochlear implantation in which animals are deafened beforehand in a 'one-shot' approach, consisting of a single dose each of kanamycin and furosemide. This gives a comprehensive abrogation of both inner and outer hair cells from base to apex of the cochlea and consequently results in a substantial and irreversible raising of ABR thresholds. A week after this treatment, a fully implantable electrical stimulator is surgically implanted into the deafened animal and its behavioural and neurophysiological responses are measured.

However, it has been noted by previous workers that an ototoxic lesion may also lead to an immune response in the cochlea, activating innate defensive pathways in order to clear away the damage to the organ of Corti. The logical sequela to this is to ask whether we are implanting animals that are already 'primed' to defend against a foreign body – will this lead to an enhancement of any fibrotic reaction directed towards the implant? And which cells are mediating any inflammatory response, can we dissect out an acute versus a chronic response if either occurs? Our initial data would suggest that there is scant immune response 7d after the ototoxic treatment. We also do not see great swathes of fibrosis in the cochleae of either naïve or lesioned animals in the months after stimulator implantation.

Our goal is to combine the use of a cochlear prosthetic in conjunction with an otic neural progenitor graft to restore the function of the auditory nerve in a 'double ablated' animal, which has had an ototoxic lesion and an ouabain treatment in order to obliterate both sensorineural elements of the hearing pathway. These animals may well require immunosuppression with cyclosporine to prevent rejection of the transplanted human cells, so it will also be of great interest to examine the effects of this immunomodulation on any fibrotic response directed towards the implant.

O50

RESPONSE PROFILES OF MURINE SPIRAL GANGLION NEURONS ON MULTI-ELECTRODE ARRAYS

Stefan Hahnewald[1,7], Anne Tscherter[2], Emanuele Marconi[1], Jürg Streit[2], Hans Rudolf Widmer[3,7], Carolyn Garnham[4], Heval Benav[4], Marcus Mueller[5], Hubert Löwenheim[5,8], Marta Roccio[1,7*], Pascal Senn[1,6,7*]

[1]Inner Ear Research Laboratory, University Departments of Clinical Research and Otorhinolaryngology, Head & Neck Surgery, Inselspital, University of Bern, Switzerland; [2]Department of Physiology, University of Bern, Switzerland;[3]Laboratory for Neural Repair, University Department of Neurosurgery, Inselspital, Bern, Switzerland; [4]MEDEL, Innsbruck, Austria; [5]Hörforschungszentrum, University Department of Otorhinolaryngology, Tübingen, Germany; [6]Department of Clinical Neurosciences, Service of Otorhinolaryngology, Head & Neck Surgery, University Hospital of Geneva (HUG), Switzerland; [7]Regenerative Neuroscience Cluster, Department of Clinical Research, University of Bern, Switzerland

Objective. Cochlear implants (CIs) have become the gold standard treatment for deafness. These neuroprosthetic devices feature a linear electrode array, surgically inserted into the cochlea, and function by directly stimulating the auditory neurons located within the spiral ganglion, bypassing lost or not-functioning hair cells. The final goal of the study is to characterize response profiles of Spiral Ganglion Neurons (SGNs) growing in intimate contact with an electrode array, in view of designing novel CI devices and stimulation protocols featuring a gapless interface with auditory neurons.

Approach. We have characterized SGNs responses to extracellular stimulation using multi-electrode arrays (MEAs). This setup allows in our view to optimize *in vitro* many of the limiting interface aspects between CI and SGNs.
Main results. Early postnatal mouse SGN explants were analyzed after 6 to 18 days in culture. Different stimulation protocols were compared with the aim to lower the stimulation threshold and the energy needed to elicit a response. In the best case, a four-fold reduction of the energy was obtained by lengthening the biphasic stimulus from 40 μs to 160 μs. Similarly, quasi monophasic pulses were more effective than biphasic and the insertion of an interphase gap moderately improved efficiency. Finally, the stimulation with an external electrode mounted on a micromanipulator showed that the energy needed to elicit a response could be reduced by a factor of five with decreasing its distance from 40μm to 0μm from the auditory neurons.
Significance. This study is the first to analyze SGNs on MEAs. Our findings may help to improve CI stimulation and to reduce energy consumption, a significant factor in the development of fully implantable devices.

O51
THE DEVELOPMENT OF TEAMWORKING IN THE PATIENT WITH COCHLEAR IMPLANT TO IMPROVE SAFETY AND FINAL OUTCOME

Daniele Frezza[1], Martina Antoniutti[2], Franco Trabalzini[3]
[1]Ear Surgery Department, Ca' Foncello Hospital, Treviso, Italy; [2]Educational and Training Department, Ca' Foncello Hospital, Treviso, Italy; [3]Otology and Skull base Surgery Department – Santa Maria alle Scotte Hospital - Siena University, Italy

Objective. Creating a Team of Health Care Professionals that works with patients with cochlear implant is today necessary. It is important to follow patients from selection to surgery treatment until rehabilitation, granting psychological support to them and their families. By using Non Technical Skills it is possible to assure appropriate and secure threatment and also a therapeutic alliance with the patient and his family.
Methods. Deafness is a deep and serious disability, which today affects adults and children and reppresents more and more often an indication of the cochlear implant.
Organizational standards and adeguate Patient Safety process are necessary to reduce high economic costs of procedure and also require a new vision of integration between Health Care Team and the development of an alliance between patient and his family.
The Authors are creating an experimental model of Team development dedicated to the Cochlear Implant by verifying its applicability and the its benefits in Patient Safety, Appropriateness, Organizational Model and Alliance with the patient and his family.
Results. This work has permitted the creation of 3 types of teams with different aims:
Team of Health Care Professionals to prevent adverse events;
Team of Health Care Professionals and Patient to create therapeutic alliance;
Team of Health Care Professionals, Patient and his Family to improve patient quality life.
Conclusions. As provided for by the Guidelines of the Ministry of Health the partecipation of patient in the Health Care Process becomes indispensable for effective therapy.
Adverse events both immediate or remote, related to a process involving many Heath Care Professionals could be reduced and prevented through the development of a Team that constantly use Non Technical Skills.
Introducing this innovation in to the Team that involves the patient and his family, in order to improve the final outcome, it is possible to enhance the therapeutic alliance which is essential in a patient who will be folllowed during all his entire life.

O52
OUTCOME OF COCHLEAR IMPLANTATION IN CHILDREN CONSIDERING MOLECULAR GENETIC ETIOLOGY

Joo Hyun Park[1], Ah Reum Kim[2], Ja- Won Koo[2], Sun O Chang[3], Byung Yoon Choi[2]
[1]Department of Otorhinolaryngology, Head and Neck Surgery, Dongguk University Ilsan Hospital, Goyang, Korea; [2]Department of Otorhinolaryngology, Head and Neck Surgery, Seoul National University Bundang Hospital, Seongnam, Korea; [3]Department of Otorhinolaryngology-Head and Neck Surgery, Kangbuk Samsung hospital, Seoul, Korea

We have evaluated the etiologic spectrum of Korean cochlear implantees(Ci) by incorporating the targeted resequencing method of 204 candidate deafness genes(TRS-204) and a phenotype-driven candidate gene approach previously. About 55% of Cis are proven to have causative variants and we reported that substantial portion of unsolved cases are predicted to have non-genetic or complex etiology rather than a Mendelian genetic disorder. We aimed to compare outcomes of CI depending on whether genetic diagnosis was performed. Ninety-three Cis consented to molecular genetic testing(MGT) and underwent at least one MGT. Patients with a characteristic phenotypic marker were subject to Sanger sequencing to detect variants in corresponding candidate genes and patients without markers were tested on *GJB2*. Next, TRS-204 was applied in *GJB2*-negative cases without any phenotypic marker to detect single nucleotide variants and copy number variations. Fifty-three were revealed to carry pathogenic variants of deafness gene and 42 patients remained genetically undiagnosed. Among them, Cis who had a follow-up duration of more than 12 months and results of speech evaluation were included. We compared audiologic performance of pre-, postCI 3, 6 12 and 18 months after CI of undiagnosed group (n=34) with that of the control group who had a definite autosomal recessive(AR) genotype (n=38). Diagnosed group included 11 patients with *SLC26A4* mutation that were known to be expect excellent result from CI. They had higher CAP score than undiagnosed group and diagnosed group with other variants at postCI 3, 6, 12 and 18 month as well as baseline. Undiagnosed group was also subdivided according to the presence of inner ear anomaly(IEA). Undiagnosed group with IEAs showed significantly lower CAP score than the others at postCI 6, 12 and 18 month. PostCI CAP scores in diagnosed group except *SLC26A4* mutation and undiagnosed group without IEA were not different. Audiologic outcome of Cis who had AR genotype confirmed by MGT was not greatly different from that of genetically undiagnosed Ci with normal inner ear who are expected to have non-genetic or complex etiology rather than a Mendelian genetic disorder. These results will be helpful for counseling patients with severe to profound SNHL.

O53

COATED COCHLEAR IMPLANT ELECTRODES TO SUPPRESS INTRACOCHLEAR INFLAMMATORY RESPONSE AND FIBROSIS

Susanne Braun[2], Johannes Fischer[1], Katharina Niedermeier[1], Jochen Steinhoff[1], Sören Schilp[2], Thomas Stark[1]
[1]Technical University Munich, Klinikum Rechts der Isar, ENT Department, Munich, Germany; [2]MED-EL GmbH Innsbruck, Austria and Starnberg, Germany

Objectives. Cochlear implant (CI) research continuously strives for improving electrodes and inventing new electrode types to enable the best possible performance of CI recipients. One goal is to minimize intra-cochlear chronic inflammation and fibrosis due to foreign body reaction after implantation that leads to an increase in electrode impedance and a decrease of the dynamic fitting range of loudness levels. Additionally, chronic inflammation in the middle to long term may cause a destruction of sensorineural structures thus influencing the performance of implantees with standard indications as well as electric-acoustic stimulation (EAS) candidates due to loss of residual hearing.
While pharmacological intervention with glucocorticoids is a promising approach to dampen postoperative inflammatory reactions, prevent fibrosis, and preserve residual hearing a non-pharmacological approach was chosen, namely a passivating coating for the electrode array thereby making it less detectable by the immune system.
Methods. 20 normal hearing guinea pigs (Dunkin Hartley) were chronically implanted with custom-made electrodes (MED-EL) either coated with a highly biocompatible polymer (n=12 COATED) or common silicone arrays (n=12 NON-COATED). Impedance and ABR-measurements were performed after 1, 2, 5, 9, 12, and 16 weeks post-implantation. This was followed by histological examination of the cochleae. Evaluation included measurement of fibrosis in the cochlea, various inflammatory markers and immunohistological analysis of degeneration of spiral ganglion neurons (GAP43).
Results. Although there was no significant difference between the two groups regarding hearing thresholds animals with coated implants showed smaller shifts of postoperative hearing thresholds at higher frequencies (4 – 32 kHz). Impedance measurements revealed no statistically significant difference between the two groups. No correlation was observed between postoperative hearing loss and impedance increase. Histological analysis revealed significantly reduced chronic lymphoplasmatic inflammation ($p = 0.012$) in the basal turn of cochleae implanted with coated implants. No significant difference was observed concerning fibrosis and new bone formation. Immunohistological GAP43 staining demonstrated a significantly lower amount of degenerated spiral ganglion neurons in the basal turn of the cochleae implanted with coated electrodes ($p = 0.003$).
Conclusion. The highly biocompatible coating seems to be a promising improvement for future electrodes possibly in combination with anti-inflammatory drugs like dexamethasone.

O54

NANOMATERIALS-BASED STRATEGIES FOR PIEZOELECTRIC COCHLEAR IMPLANTS

Serena Danti[1], Luca Bruschini[1], Gianni Ciofani[2], Massimiliano Labardi[3], Carlos Mota[1], Luisa Trombi[1], Claudio Ricci[1], Delfo D'Alessandro[1], Giuseppe C. Gallone[4], Stefano Berrettini[1]
[1]OtoLab, Azienda Ospedaliero-Universitaria Pisana (AOUP), Pisa, Italy; [2]Istituto Italiano di Tecnologia (IIT), Pontedera (PI), Italy; [3]Istituto per i Processi Chimico-Fisici, CNR, Pisa, Italy; [4]Dept. of Civil and Industrial Engineering, University of Pisa, Italy

As of today, cochlear implants (CIs) represent the only option for deaf people affected by profound or severe sensorineural hearing loss (SNHL). Although conventional CIs can bring patients back to an acceptable hearing, on the other hand they suffer from some important disadvantages impacting the quality of life and hearing after implantation and for such reasons a new class of CIs is desirable. The physical properties of piezoelectric materials, which polarize in response to mechanic deformation, entitle them to replace the function of hair cells. The development of a working piezoelectric CI requires that the electric charge generated by the piezo-materials must be self-sufficient to stimulate the cochlear neurons. First studies using a basic piezoelectric polymer (polyvinylidene fluoride, PVDF) have shown the proof-of-concept, but the device sensitivity was still insufficient.
The aim of this study is to fabricate novel piezoelectric substrates based on piezo-polymers (PVDF and PVDF-TrFE) doped with ceramic nanoparticles (barium titanate nanospheres, carbon nanotubes) to obtain composite materials with enhanced performance. Several micro-fabrication strategies were investigated to produce thin piezoelectric polymer/ceramic composite scaffolds, in the form of electrospun nanofibers and micro/nano films to be placed on the basilar membrane, including spin coating, hot-press, and co-axial electrospinning. Presence of the piezoelectric crystallographic phases of the composite materials were assessed via X-ray diffraction. The piezoelectric constants were investigated with a bench set-up in which the material deformation is measured under an applied current. Finally, the substrates were cultured *in vitro* with neuronal-like cell lines and mesenchymal stem cells (MSCs) to assess their potential ability to interact with inner ear resident cells.
The fabrication parameters were tuned to optimize the piezoelectric response of the materials. Increasing barium titanate nanoparticle concentration up to 20% (w/w) in PVDF nanofiber ribbons enhanced inverse (deformation) but not direct (polarization) piezoelectric effect. Interaction with neuronal-like cells was not affected by barium titanate concentration. Differently, the presence of carbon nanotubes in PVDF-TrFE films increased performance and MSC adhesion with respect to plain PVDF-TrFE films.
Improvements in piezoelectric CIs could substantially impact the quality of life of people affected by profound SNHL and reduce the healthcare costs.

SESSION VIII
PHYSIOPATHOLOGY OF AUDITORY PATHWAYS AND TINNITUS

TL8
COCHLEAR AND CENTRAL MECHANISMS OF TINNITUS AND THERAPEUTIC APPROACHES

Arnaud Norena
Laboratoire de Neurosciences Intégratives et Adaptatives, CNRS et Aix-Marseille Université, Marseille, France

Tinnitus is supposed to result from an aberrant pattern of neural activity generated in the absence of sound. Cochlear tinnitus refers to tinnitus-related activity generated (and recordable) at the cochlear level. An increase of the endocochlear potential, for example, can result in a cochlear tinnitus (i.e., an increase of the cochlear spontaneous firing rate). Central tinnitus, on the other hand, requires the active participation of central mechanisms. This may be the case when the cochlear spontaneous firing rate is reduced, due to cochlear damages for instance. Actually, recent models of tinnitus suggest that the tinnitus-related central mechanisms are triggered by the reduction of sensory inputs produced by cochlear damages. This family of models suggests that tinnitus is a by-product of an adaptive mechanism adapting central sensitivity to the distribution of sensory inputs. According on whether tinnitus-related activity is cochlear or central different therapeutic approaches should be proposed. Cochlear activity should be reduced in cochlear tinnitus while central sensitivity should be decreased in central tinnitus.

O55
THE DIFFERENTIAL EAR EFFECTS OF LONG-TERM UNILATERAL DEAFNESS ON SPEECH PERCEPTION: PSYCHOACOUSTIC COMPARISONS

Jae Young Byun[1], Min Young Kwak[2], Dong Hyun Kim[2], Yong-Hwi An[2], Hyun Joon Shim[2]
[1]Kyung Hee University, College of Medicine, Kyung Hee University Hospital at Gangdong, Department of Otolaryngology, Head and Neck Surgery, Seoul, South Korea; [2]Eulji University School of Medicine, Eulji Medical Center, Department of Otorhinolaryngology, Head and Neck Surgery, Seoul, South Korea

Aim. Though a few electrophysiological studies reported that the compensatory activation after long-term unilateral deafness would be induced in right rather than left auditory cortex, the differential ear effects on psychoacoustic performances remain still unclear in human. The present study aimed to evaluate whether left-sided and right-sided deafness had differential effects on auditory spectral resolution, temporal resolution, and speech perception ability.
Subjects and Methods. Subjects included 16 unilateral deaf subjects (UDSs) with unilaterally normal-hearing threshold (<20 dB HL) who became opposite-side deaf more than 10 years (Nine with left-sided unilateral deafness and 7 with right-sided unilateral deafness). Sixteen normal-hearing subjects (NHS) matched in age and sex were enrolled as a control group. Three different psychoacoustic measurements were performed monaurally: 1) spectral-ripple discrimination (SRD), 2) temporal modulation detection (TMD), and 3) speech recognition threshold (SRT) in noise.
Results. The right ears of left UDSs were not different form right ears of NHSs in SRD thresholds, TMD thresholds or SRTs, whereas left ears of right UDSs showed poorer performances than left ears of NHSs in SRD and SRT in noise ($p = 0.019$ and $p = 0.044$). The Kruskal-Wallis test showed a group effect on the SRD ($p = 0.025$), and the post-hoc test revealed a significant poorer performance in the left ear of right UDSs compared to right ear of left UDSs and left ears of NHSs ($p = 0.016$ and $p = 0.032$). When we compared between both ears of NHSs, there were no significant differences in the SRD thresholds, TMD thresholds, or SRTs in noise ($P > 0.05$).
Conclusions. We found that different functional change after unilateral hearing deprivation was induced depending on the site. And right UDS would have more handicaps in spectral resolution and possibly in speech perception than left UDS.

O56
CONTRALATERAL STIMULATION EFFECT ON SHORT AND LONG LATENCY DISTORTION PRODUCT OTOACOUSTIC EMISSIONS IN PATIENTS AFFECTED BY PARKINSON DISEASE

Renata Sisto [1], Arturo Moleti [2], Valerio Pisani[2,3], Stefano Di Girolamo[2], Teresa Botti[1], Filippo Sanjust[1], Luigi Cerini[1]
[1] INAIL Research, Monteporzio Catone, Rome, Italy; [2] University of Rome "Tor Vergata", Rome, Italy; [3] I.R.C.C.S. "Santa Lucia" Foundation, Rome, Italy

Objective. Objective of this study is the investigation of cochlear functionality, and in particular of the functionality of the medial olivocochlear efferent system (MOC), in patients at their first diagnosis of Parkinson disease and after a pharmacological treatment with levodopa at therapeutic dosage. A speculative parallelism has been developed between the present human study and animal studies in which knockout and wildtype mice data are compared. In Maison et al. (2012), knockout mice with targeted deletion of different dopamine receptors have been employed. Specific knockout mice may be considered an experimental model of patients at their first diagnosis, whilst wildtype mice may be assimilated to controls or to patients after pharmacological treatment.
Methods. Patients at their first diagnosis of Parkinson disease (PD) and age-sex matched control healthy subjects (CTRL), were enrolled in this study. Parkinson patients have been retested after a treatment with levodopa that is transformed in dopamine at the level of the central nervous system. DPOAEs have been recorded alternatively with and without a contralateral suppression stimulus of 80 dB (CAS). High-resolution DPOAE spectra have been measured using an advanced chirp technique. A suitable time-frequency wavelet analysis has been applied to separate long- and short-latency DPOAE components.
Results. Statistically significant differences were found between PD and CTRL groups suggesting a degradation of cochlear functionality in patients affected by Parkinson disease. An enhancement of the DPOAE short latency component has been found as consequence of levodopa treatment particularly in individual subjects. As regards the CAS effect, statistically significant differences have been found between PD and CTRL groups showing interesting similarity between Parkinson and aging effects.
Conclusions. Otoacoustic Emissions can be an important objective test of peripheral auditory pathway, able of revealing hidden pathogenetic mechanisms at the basis of neurological degenerative disorders.

O57

SENSITIVITY OF ELECTROCOCHLEOGRAPHY VS. OTOACOUSTIC EMISSIONS TO DISRUPTED COCHLEAR HOMEOSTASIS IN MENIÈRE PATIENTS

Fabrice Giraudet, Thierry Mom, Paul Avan
University of Auvergne, School of Medicine, Laboratory of Neurosensory Biophysics, INSERM UMR 1107, Clermont-Ferrand, France

Introduction. The responses of cochlear hair cells to sound stimuli are highly sensitive to the resting environment and operating point of their stereocilia bundles, adjusted for optimal operation. Cochlear hydrops, a hallmark of Menière's disease (MD), likely disrupts stereocilia operation, with fluctuating hearing sensitivity as an expected result. For several decades, its objective diagnosis has rested upon electrocochleography. The increased size of the summating potential SP relative to the compound action potential of the cochlear nerve AP has been thought to reflect an exaggerated depolarization of hair cells in relation to a deformed, inflated scala media. Recently, otoacoustic emission (OAE) changes with body tilt have shown an abnormal behavior in the presence of MD symptoms (Avan et al, Hear Res. 2011, 277, 88-95). Here, the outcomes of SP and OAE tests performed concomitantly were compared in typical MD patients.

Results. Considering that the OAE phase shift with body tilt (upright/supine) does not exceed 40 degrees in a normal population, the OAE test showed a sensitivity of 75% and a specificity of 91% similar to that of the previous cohort of patients. The concomitantly measured SP/AP ratio had a sensitivity of 60% and a specificity of 94%, as usually reported in the literature. Short-term instability of the SP/AP ratio was conspicuous, on a time scale of a few min. Objective diagnosis based upon the combination of OAEs and SP/AP reached an improved sensitivity, 94%.

Conclusion. Discrepancies between the outcomes of the SP/AP and otoacoustic tests, observed in both models, suggest that these tests do not react to the same aspects of disrupted cochlear homeostasis; notably, otoacoustic emissions seem much more immediately sensitive to any acute change in cochlear fluid hydrostatic pressure. The use of the two tests might thus enrich the study of the cochlear environment in patients with hydropic ears, with the long-term goal of better tracking the correlates of the symptoms, and ultimately, the efficiency of a therapy.

O58

DOES BLOCKING MICROGLIAL ACTIVATION PREVENT TINNITUS ONSET?

Alessandro Venturino[1], Paola Perin[1], Vittorio Bertone[3], Gloria Colombo[1], Adriano Oda[1], Gabriele Sanchini[1], Vincenzo Vitale[1], Alessia Capetta[1], Roberto Pizzala[2]
[1]University of Pavia, Dept. of Brain and Behavioral Sciences, Pavia, Italy; [2]University of Pavia, Dept. of Molecular Medicine, Pavia, Italy; [3]University of Pavia, Dept. of Biology and Biotechnology, Pavia, Italy

Tinnitus is a phantom auditory perception that can affect quality of life similarly to chronic pain. Although several risk factors (Kim et al. 2015) and brain areas involved (Chen et al. 2015; Lowe and Walton 2015) have been identified, tinnitus etiogenesis has not been clarified. Similarly, in animal models, it is still unclear whether tinnitus induced with different experimental protocols is due to similar mechanisms, also considering that the reliability of tinnitus induction protocols is not optimal, and a variable fraction of treated animals do not develop the symptom (Koehler and Shore 2013).

Changes in neural plasticity at several stages of the auditory system have been observed in correlation with tinnitus (Henry et al. 2014). Since neural plasticity is affected by both neuronal activity and microenvironmental conditions, we focused on glial responses in tinnitus, given that glial activation is a well-known nervous tissue response to insult and strongly impacts on the microenvironment. In particular, we studied tinnitus-associated microglia changes in DCN, a structure which is known to be necessary for tinnitus onset (Brozoski and Bauer 2005) and in higher auditory stations.

Tinnitus was induced (in 9/11 treated rats) with unilateral cochlear destruction, noise trauma (7/8) or salicylate (4/6), and microglia was observed with Iba-1 immunofluorescence in rat brain slices by confocal microscopy. Although all treatments were able to induce tinnitus (tested as in Turner 2006), noise trauma and salicyilate treatment increased microglial density without inducing activation, whereas cochlear destruction increased both microglia density and activation. After noise trauma, microglia became less uniformly distributed, showing a cluster in the DCN region corresponding to noise trauma frequencies.

Microglia activation after cochlear destruction was necessary for tinnitus onset: animals treated with minocycline starting 2 hours after surgery did not develop microgliosis nor behavioural signs of tinnitus. On the other hand, tinnitus induced with salicylate or noise trauma was observed regardless of minocycline treatment. Our results suggest that DCN microglia activation is sufficient, although not necessary, for the onset of tinnitus.

O59

LOUDNESS AFFECTED BY HIGH DOSES OF SALICYLATE AND NOISE EXPOSURE – A BEHAVIORAL MODEL FOR HYPERACUSIS AND TINNITUS

Wei Sun, Chao Zhang, Anaam Alkharabsheh
Center for Hearing & Deafness, State University of New York at Buffalo, Buffalo, NY, USA

Tinnitus and hyperacusis are common disorders in otology clinic. The causes of tinnitus and hyperacusis are still not clear. High doses of salicylate are known to cause sensorial hearing loss and tinnitus in human. Noise induced hearing loss is also reported related with tinnitus and hyperacusis. Therefore. tinnitus and hyperacusis have been induced by high doses of salicylate or noise exposure in animal models to study the sources of these diseases. In this study, we established an operant conditioning based behavioral task in rats and measured their loudness perception changes before and after high doses of salicylate injection (250 mg/kg, i.p.) or noise exposure. We found that high doses of salicylate induced a significant increase to loudness response in rats, suggesting a hyperacusis behavior. A rapid increase of loudness response was also detected in some rats, suggesting loudness recruitment. The reaction time of the rats was also measured during the loudness tests before and after salicylate exposure or noise exposure The reaction time level functions are highly correlated to the loudness response functions. Our studies confirmed that increased sound sensitivity, which is commonly seen in patients with tinnitus and hyperacusis, can be induced by high doses of salicylate and noise exposure. As salicylate can enhance the evoked potentials recorded from the central auditory system (CAS), our study suggests the increased sound sensitivity is related to plasticity change in the CAS. The loudness change induced by

salicylate and noise exposure may be related with hypersensitivity in the CAS.

O60
TREATMENT OF ACUTE SALICYLATE-INDUCED TINNITUS WITH TNF-α BLOCKER IN AN ANIMAL MODEL

David Chang-Wei Huang[1], Tien-Chen Liu[2]
[1]Department of Otolaryngology, Kuang-Tien General Hospital, Taichung, Taiwan; [2]Department of Otolaryngology, National Taiwan University Hospital, Taipei, Taiwan

Objectives. Salicylate-induced tinnitus was known to augment TNF-α gene expression in the cochlea of mice. We assumed this pro-inflammatory cytokine may directly induce tinnitus or via modulating the NMDA receptor. Therefore, we tried to observe treatment outcome of TNF-α blocker and its interaction with NMDA receptor.
Method. Thirty 3-month-old male C57BL/6 mice were randomly divided into TNF-α blocker treatment group (n=15) and saline-treated control group (n=15). ABR were performed before and after study to record hearing threshold. 5-days Active avoidance task training was performed first and the tinnitus score was determined. Tinnitus baseline test was measured 1-hour before intraperitoneal injection of 300mg/kg sodium salicylate. Two treatment strategies were as followed: 30mg/kg TNF-α blocker (Enbrel) or saline administration 1-hour after salicylate injection. Then, tinnitus score test was repeatedly performed immediately and for consecutive 2 days after treatment. Eventually, mice were euthanized to determine the geneexpression level of TNF-αR1/2 and NR2B in cochlea.
Results. Enbrel may significant decrease salicylate-induced ABR hearing threshold shift. The tinnitus score (saline/Enbrel) were significant decreased on day2 and day3. The relative quantification gene expression for TNF-αR1/2 to β-actin was significantly decreased in treatment group. Moreover, TNF-αR2 had dominant gene expression to TNF-αR1 in control group. One the other hand, NR2B and DREAM protein gene expression were also significantly decreased in treatment group.
Conclusions. TNF-α blocker revealed reduction of salicylate-induced acute tinnitus in animal model. TNF-αR2 played more dominant role than TNF-αR1. Finaly,the NMDA receptor is also modulated by TNF-α blocker.

O61
PHENOTYPIC CHARACTERISTICS OF THE T961G MITOCHONDRIAL MUTATION

Filippo Mazzei, Rosaria Turchetta, Maria Patrizia Orlando, Giancarlo Altissimi, Giancarlo Cianfrone
Department of Sensory Organs, University of Rome "La Sapienza", Rome, Italy

Introduction. Audiological findings in genetic Hearing Loss (HL), and in particular in those of mitochondrial origin, are extremely varied. In non-syndromic HL the mtDNA gene 12S rRNA gene is often involved. Such mutations can be sporadic or show incomplete penetrance, since not all individuals carrying the mutations have HL.
Object. This presentation describes the different phenotypic features of mithochondrial mutations in HL, in particular those of a family with the T961G mutation.
Methods. All members of a family with T961G 12S rRNA mutation underwent audiological and radiological investigations, including Pure Tone Audiometry, Otoacoustic Emissions, Auditory Evoked Potentials, and CT scan of the petrous bone.
Results. Six members of the family had the T961G mitochondrial mutation; four of them had different degrees of HL, CT alterations were visible in three (mainly Enlarged vestibular aqueduct and dysplastic chocleas), while the remaining two had normal hearing and CT scans.
Conclusions. T961G 12S rRNA mutation can show different phenotypic features: according to our findings HL is highly probable, and the anatomical inner ear malformations might represent a challenge in treatment of these patients.

O62
DISTINCT ROLES OF *EPS8* IN THE FUNCTIONAL MATURATION OF COCHLEAR AND VESTIBULAR HAIR CELLS

Sergio Masetto[1], Walter Marcotti[2]
[1]Department of Brain and Behavioral Sciences, University of Pavia, Italy; [2]Department of Biomedical Science, University of Sheffield, UK

Several genetic mutations have been discovered in recent years, which produce hearing impairment with or without vestibular dysfunction. In mice *Esp8*, a gene involved in actin remodeling, has been implicated in the normal stereocilia growth in both the cochlear and vestibular hair cells. However, its suppression causes deafness but no obvious vestibular deficits. Eps8 is also required for the functional maturation of the basolateral membrane ion channels in the cochlear inner hair cells (IHCs). Since it is currently unknown if *Esp8* deletion also affects ion channel expression in vestibular hair cells, we have investigated the biophysical properties of vestibular Type I and Type II hair cells from the semicircular canals of wild type (WT) and *Eps8*-knockout (KO) mice. We found that vestibular hair cells of *Eps8*-KO mice acquire the normal pattern of voltage-dependent ion channels, including the low-voltage activated K^+ current ($I_{K,L}$), which is characteristic of Type I cells. Consistent with this finding, the voltage response to injected sinusoidal currents mimicking the transducer current was normal in *Eps8*-KO vestibular hair cells, but significantly altered in *Eps8*-KO IHCs, which would affect their ability to tune to the physiological stimulus. We conclude that Eps8 is indirectly involved in regulating the expression of basolateral membrane ion channels in IHCs, but not in vestibular cells. This might explain why *Eps8* deletion results in profound deafness but no obvious vestibular problems.

SESSION IX
AGING AND VESTIBULAR DISORDERS

TL9
PATHOPHYSIOLOGY, GENETICS AND SOCIAL IMPACT OF PRESBYCUSIS

Helena Caria[1,2]
[1]BioISI, Biosystems & Integrative Sciences Institute, Faculty of Science of the University of Lisbon, Portugal; [2] ESS/IPS, School of Health, Polytechnic Institute of Setúbal, Portugal

Presbycusis or age-related hearing loss (ARHL) is a multifaceted

common phenomenon, with a broad spectrum of causes, environmental and/or genetic, having cognitive and psychosocial consequences. ARHL is the most common sensory impairment in the elderly, affecting approximately 60% of all individuals over 65 years in the world. Physical health plays an important role in the link between frailty and overall health (e.g. health status, familial and clinical history, sensory function), but due to the physiology complexity of ARHL, its mechanisms are not fully understood as well as the relation between ARHL and health status.

The existence of susceptibility genetic factors predisposing for presbycusis implies that ARHL is not an inevitable condition, but a complex disease with possible treatment and prevention what is particularly relevant to the field of active aging.

From the established association between pathophysiological mechanisms and alterations of cochlear morphology underlying ARHL and a specific pattern of audiometric evaluation with clinical relevance, we discuss physiological, genetics and clinical aspects, taking into consideration also the results obtained with Portuguese population with ARHL.

Thus, we expect to contribute to the promotion of active ageing and open the possibility of more efficient preventive or therapeutic approaches.

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O63
AGE MATTERS: CENTRAL COMPENSATION OF COCHLEAR FUNCTION LOSS

Lukas Rüttiger, Dorit Möhrle, Ksenia Varakina, Dan Bing, Marlies Knipper

University of Tübingen, Department of Otolaryngology, Tübingen, Germany

Objective. During aging and as a consequence of even moderate auditory overexposure inner ear physiology and good hearing sensitivity is weakened. On the functional side recent studies showed a close relation between aging, loss of inner hair cell synaptic contacts, and ABR wave amplitudes in the mouse and in the rat. Recovery from TTS after mild noise exposure with "full recovery" of normal audiometric threshold sensitivity is nevertheless accompanied by a loss of cochlear function at supra-threshold sensation levels and a loss of IHC synaptic contacts in an age related manner. Here, it was our purpose to find out how the peripheral (cochlear) loss of function is reflected in central auditory processing.

Methods. Different age groups of rats were exposed to temporally desensitizing sound, leading exclusively to a mild temporary threshold shift. Hearing thresholds recovered completely within few days and remained stable over a long life span. However, as already described by others, supra-threshold ABR wave-I responses (reflecting auditory nerve responses) were reduced weeks following the sound exposure. However, it is still unclear if and how the reduced information output from the cochlea can be centrally compensated to keep up the central auditory processing (as reflected in ABR wave IV).

Results and Conclusions. Peripheral and central auditory responses were analyzed and compared to hair cell function (otoacoustic emissions) and quantification of CtBP2 positive staining at the IHC synapse (giving an estimate for the number of afferent contacts). The data will be discussed considering the IHC ribbon loss observed over age, the change of ABR wave amplitudes and latencies, the capacity to centrally compensate for the peripheral loss after noise induced damage of the ear during aging and the risk to generate tinnitus and hyperacusis during age.

Supported by grants from the Deutsche Forschungsgemeinschaft (FOR 2060 project FE 438/5-1) and Action on Hearing Loss (RNID G54-Ru).

O64
AGE-RELATED DIFFERENCES IN HEARING FUNCTION AND COCHLEAR MORPHOLOGY BETWEEN MALE AND FEMALE FISCHER 344 RATS

Jiří Popelář[1], Francesca Chiumenti[2], Zuzana Balogová[3], Tetyana Chumak[1], Josef Syka[1]

[1]Institute of Experimental Medicine, Academy of Sciences of the Czech Republic, Prague, Czech Republic; [2]Faculty of Medicine, University of Padova, Italy; [3]Department of Otorhinolaryngology and Head and Neck Surgery, 1st Faculty of Medicine, Charles University in Prague, University Hospital Motol, Prague, Czech Republic

Objective. Structural and functional changes in the auditory system during aging were compared in male and female rats of the Fischer344 strain (F344).

Methods. Hearing function in males and females of different ages (1-30 months) was assessed by recording auditory brainstem responses (ABRs) and distortion products otoacoustic emissions (DPOAEs). Neural adaptation was tested by recording ABRs elicited by a series of four clicks with inter-click intervals of varying lengths. Temporal discrimination ability was tested by recording ABR to gaps of different durations embedded in noise. Cochlear morphology was evaluated using the cochlear surface preparation.

Results. Similar hearing thresholds were found in young (1-month-old) males and females. In 8-30-month-old males the ABR thresholds were higher than those measured in age-matched females. The amplitudes of the individual waves of the click-evoked ABRs were smaller in aged males than in aged females.

In spite of smaller ABR amplitudes in old males, the interclick interval, in which the ABR amplitude in response to the later clicks was equal to the ABR amplitude to the first click, was the same in both aged males and females (12 ms). The gap duration, after which the ABR amplitude in response to the second noise stimulus (after the gap) reached the ABR amplitude in response to the beginning of the noise, was similar in both males and females.

Amplitudes of DPOAEs in males decreased with age faster than in females; in males older than 20 months the DPOAEs were practically absent. Hair cell loss in old F344 rats corresponded with hearing loss. No difference in the number of inner hair cell (IHC) ribbon synapses was found between males and females. However, the size of the IHC ribbon synapses was significantly smaller in males than in females. There were no differences in the cochlear efferent innervation between old males and females.

Conclusions. The present results demonstrate more pronounced age-related changes in the cochlear morphology, hearing thresholds and ABR amplitudes in F344 males compared with females. However, the neural adaptation and temporal resolution abilities on the brainstem level were comparable in both males and females.

Supported by grants P304/12/G069 and P304/12/1342.

O65
STRESS GRANULES AND MECHANISMS OF RNA TRIAGE DURING COCHLEAR STRESS

Ana Cláudia Gonçalves, Naila Haq, Emily Towers, Lisa Nolan, Sally Dawson*, Jonathan Gale* *[*equal contribution]*
UCL Ear Institute, London, UK

Stress granules (SGs) are cytoplasmic aggregates of proteins and mRNA formed as a consequence of cellular stress. RNAs sequestered by SGs are effectively silenced, allowing the translation of essential proteins to continue. Dysregulation of SG-formation has recently been linked to age-related disease, in support of the hypothesis that SGs play a critical role during in survival during cellular stress.

An RNA-immunoprecipitation (RIP-seq) study in our laboratory identified RNAs bound to two different SG-RNA-binding proteins, TIA-1 and Caprin-1, during either heat-shock (HS) or arsenite stress (AS) in the inner ear-derived UB/OC-2 cell line. Here, we used an immuno-RNA-FISH protocol to determine the cellular localization of these RNAs during stress. A fluorescent polyA$^+$ mRNA probe indicated co-localization of RNA with both TIA-1 and Caprin-1 in the SGs formed after HS and arsenite stress. The RIP-seq indicated enriched binding of several RNAs to SG during both HS and AS, including PTGES3, a glucocorticoid and steroid receptor chaperone. Consistent with the RIP-seq, our immuno-RNA-FISH demonstrated cellular re-distribution of this mRNA to SGs during stress. mRNAs encoding Hsp70 are thought to be selectively excluded from SGs and our immuno-RNA-FISH data are consistent with that observation under both HS and AS. We also assessed Hsp70-mRNA and Hsp70 protein levels using qPCR and immunolabelling respectively. One hour of HS increased Hsp70 gene expression significantly with a maximum level reached between 1-2h after the end of the stress. Hsp70 protein expression corresponded to these data with ~1 hour delay. Other RIP-seq candidates are currently under investigation.

The SG-aging profile in the cochlea is being investigated by: (i) characterising changes in the expression of known SG-proteins (e.g. TIA-1, Caprin-1); (ii) localising individual mRNAs to TIA-1 labelled-SGs using immuno-RNA-FISH. We observe clear changes in SG formation in both the organ of Corti and the spiral ganglion neurons. We are using the same approaches to determine the effect of aminoglycoside-treatment on SG formation *in vivo*.

By combining our RIP-seq data with qPCR and immuno-RNA-FISH we have demonstrated that we can detect RNAs sequestered by SGs and determine those RNAs that are preferentially translated during stress.

O66
SELECTIVE SEROTONIN REUPTAKE INHIBITOR (SSRI) AND VESTIBULAR FUNCTION

Kensuke Kiyomizu[1,2], Hironori Fujii[3], Hiroaki Shimogori[3], Keiji Matsuda[2], Koji Torihara[2], Kensei Yoshida[1], Tetsuya Tono[2]
[1]Yoshida Hospital, Department of Psychiatry, Nobeoka, Japan; [2]Miyazaki Medical College, University of Miyazaki, Department of Otorhinolaryngology, Miyazaki, Japan; [3]University of Yamaguchi, Department of Otorhinolaryngology, Ube, Japan

Objective. It is well known that SSRI is effective for patients with dizziness and depression. However, there are only few papers about vestibular function for those patients. We therefore performed vestibular ocular reflex tests in rotation (VOR tests) and utilized Dizziness Handicap Inventory (DHI) questionnaire before and after SSRI therapy.

Methods. Out of 97 depressive patients in 820 patients with dizziness in our psychiatric hospital, the subjects were 9 patients (3 men, 6 women) (mean age, 57.7 years) experiencing dizziness and depression with the following conditions; 1) regular treatment including anti-vertigenous drugs, physical therapy, minor tranquilizer or sleep medications were not effective, 2) non-acute phase, 3) non-prescription of antidepressants, 4) signature of consent form approved by the ethics committee in University of Yamaguchi. VOR tests were performed using video-oculograph (Nystamo 21 type 2, IRN-2, Morita Manufacturing corporation, Tokyo). Eye movements were monitored using an infrared eye camera installed in the goggles. Head movements were transduced to d.c. signals (range 0–5 V) by a small angular velocity sensor (Gyrostar, Murata Corporation, Japan). The chair was not automatic but it was manually rotated at a frequency of 0.3–0.6 Hz and the angular velocity ranged from -130 to 130 deg/s like a pendulum. Eye and head movements were recorded for more than 40 s and the recordings of more than 30 s were used for subsequent analysis.

Results. After about 4 weeks of using additional SSRI treatment, DHI scores were improved significantly (57.4→31.8). In VOR tests, DP% scores (vestibular function) were improved significantly (26.6→9.46).

Conclusions. In basic research, Dr. Shimogori in our group examined how SSRI affects the vestibular system using guinea pig model. In this clinical research, we believe that SSRI could possibly affect the vestibular system directly by the activation of cyclic AMP-responsive element blinding protein-brain-derived neurotrophic factor (CREB-BDNF) system.

O67
CHRONIC CEREBERO-SPINAL VENOUS INSUFFICIENCY IN MENIERE' DISEASE: DIAGNOSIS AND ENDOVASCULAR TREATMENT

G. Attanasio[1], A. Bruno[2], L. Califano[3], F. Ciciariello[4], M. Viccaro, E. Masci[7], L. Cagnoni[7], D. Mastrangelo[2], F. Salafia[3], V. Giugliano[5], P.P. Cavazzuti [6], B. Bernardo[2], R. Filipo[7]
[1]Head and Neck Department, Policlinic Umberto I of Rome, Italy; [2]Vascular Surgery Department CLINICA GEPOS Telese Terme, Benevento, Italy; [3]SSD Audiology and Phoniatrics, "G. Rummo" Hospital, Benevento, Italy; [4]Department of Cardiovascular, Respiratory, Geriatric and Morphologic Sciences, Sapienza University of Rome, Italy; [5]Radiology Department Gepos Clinic Telese Terme, Benevento, Italy; [6]Department of Otolaryngology-Head and Neck Surgery, Maggiore Hospital, Bologna, Italy; [7]Department of Sensory Organs, Sapienza University of Rome, Italy

Results of a previous study (Filipo et al, 2013) demonstrated the presence of chronic cerebrospinal venous insufficiency (CCSVI) and vascular abnormalities in patients with Meniere's disease using Echo-Color Doppler examination of the cerebrospinal venous flow. The aim of the study was to evaluate by the means of Doppler ultrasound, MRI and phlebography the relationship between Meniere's Disease and CCSVI and to test whether Percutaneous Transluminal Angioplasty (PTA) is an effective procedure in improving symptoms. 172 patients diagnosed with definite Meniere's Disease (AAO 1995) who had gained no benefit by

medical therapy, underwent echo-enhanced color Doppler (ECD) sonography using protocol to check for CCSVI. One-hundred healthy subjects matched for age and gender acted as controls. An ultrasound diagnosis of CCSVI was made in 150 patients (87.2%). In the healthy population, was found in only 10% of cases. In 60 patients venography confirmed the CCSVI diagnosis. These patients were treated by angioplasty of the Internal Jugular Vein, then re-tested respect the baseline scales of Meniere's diseases. PTA proved to be effective in 80% of patients, with significant improvement of audiological and vestibular function at 24 month follow-up.

O68

CELLULAR STRESS RESPONSE AND VITAGENE'S ROLE IN VESTIBULAR DISEASE

A. Serra[1], V. Calabrese2, L. Maiolino[1], S. Cocuzza[1], P. Di Mauro[1]
[1]ENT Department, University of Catania, Italy; [2]Department of Chemistry, Biochemistry and Molecular Biology, University of Catania, Italy

Vestibular diseases are often characterized by hearing loss and recurrent episodic vertigo. Increasing evidence suggests that oxidative stress is involved in the development of endolymphatic hydrops and that cellular damage and apoptotic cell death might contribute to the sensorineural hearing loss found in later stages of vetibular disease. While excess reactive oxygen species (ROS) are toxic, regulated ROS, however, play an important role in cellular signaling. The ability of a cell to counteract stressful conditions, known as cellular stress response, requires the activation of pro-survival pathways and the production of molecules with anti-oxidant, antiapoptotic or pro-apoptotic activities. Among the cellular pathways conferring protection against oxidative stress, a key role is played by vitagenes, which include heat shock proteins (Hsps) as well as the thioredoxin/thioredoxin reductase system.
In this study we tested the hypothesis that in MD patients measurable increases in markers of cellular stress response and oxidative stress in peripheral blood are present. This study also explores the hypothesis that changes in the redox status of glutathione, the major endogenous antioxidant, associated with abnormal expression and activity of carbonic anhydrase can contribute to increase oxidative stress and to disruption of systemic redox homeostasis which can be associated to possible alterations on vulnerable neurons such as spiral ganglion neurons and consequent cellular degeneration.
We therefore evaluated systemic oxidative stress and cellular stress response in patients suffering from vestibular diseases and hearing loss in age-matched healthy subjects. Systemic oxidative stress was estimated by measuring protein oxidation, such as protein carbonyls (PC) and 4-hydroxynonenal (HNE) in lymphocytes of patients with respect to control group.
In conclusion, patients affected by various forms of vestibular diseases are under condition of systemic oxidative stress and the induction of vitagenes Hsp70 is a maintained response in counteracting the intracellular pro-oxidant status generated by decreased content of GSH as well as expression f Trx. The search for novel and more potent inducers of vitagenes will facilitate the development of pharmacological strategies to increase the intrinsic capacity of vulnerable ganglion cells to maximize antidegenerative mechanisms,such as stress response and thus cytoprotection.

O69

LONG-TERM EFFECTS IN THE VESTIBULAR SYSTEM ARE INFLUENCED BY SEX CIRCULATING HORMONES

Cristina V. Dieni, Roberto Panichi, Mariangela Scarduzio, Silvarosa Grassi, Vito E. Pettorossi
University of Perugia, Department of Experimental Medicine, Sector of Physiology and Biochemistry, Perugia, Italy

We investigated the possible influence of sex and estrous cycle on the synaptic responses and plasticity of neurons in the medial vestibular nucleus (MVN). We evaluated the field potential evoked in the MVN by vestibular afferent stimulation in brain stem slices of male and female rats during proestrus (PE) and diestrus (DE). In PE females the field potential had a lower threshold and higher amplitude than in DE females and in males and the stimulus-response curve was shifted to the left. In addition the exogenous administration of 17β-estradiol (E_2) in DE females and males, enhanced the field potential amplitude to values close to those of PE females. This suggests an influence of the circulating E_2 in central vestibular responses. Moreover, the plasticity of the vestibular neurons was remarkably influenced. The local synthesised sex neurosteroid E_2 is normally involved in the induction of LTP and the neurosteroids testosterone (T) or α-dihydrotestosterone (DHT) in the LTD. We found that the probability of induction of LTP and LTD varied depending on the circulating E_2 level. Moreover, the administration of T, that is precursor of both E_2 (estrogenic pathway) and DHT (androgenic pathway) induced mostly LTD in PE females and no effect in DE females, while it only provoked LTP in males. This suggests that the high level of circulating E_2 may interfere with the local conversion of T, by inhibiting the neural estrogenic pathway and facilitating the androgenic one. We concluded that the interaction of sex circulating and neural synthesised steroids may explain the different sex expression of clinical symptoms observed in human vestibular pathology.

O70

POTENTIAL BIOMARKERS IN MÉNIÈRE DISEASE: A PROTEOMICS-DRIVEN APPROACH

Ettore Cassandro[1], Antonio Concolino[2], Sequino Giuliano[1], Scarpa Alfonso[1], Giuseppe Chiarella[3]
[1]Department of Medicine and Surgery, University of Salerno, Italy; [2]Department of Experimental and Clinical Medicine, Laboratory of proteomics and mass spectrometry, "Magna Graecia" University, Catanzaro, Italy; [3]Department of Experimental and Clinical Medicine, Audiology and Phoniatrics Unit, "Magna Graecia" University, Catanzaro, Italy

The pathophysiology of Ménière Disease (MD) is largely unknown, consequences are on inaccuracy in terms of epidemiology, diagnosis and treatment. We have used a proteomics-driven approach to identify potential biomarkers of MD. To this end, we selected 20 MD patients, from whose whole blood was obtained plasma, and compared to plasma from healthy donors. Two-dimensional gel electrophoresis, followed by in-gel tryptic digestion of the selected spots and LC-MS/MS analysis, allowed us to identify a set of proteins whose expression appears to be differentially modulated in patients vs. controls. In particular: complement factor H (CHF) and B, fibrinogen alpha and gamma chains, beta-actin and pigment epithelium derived factor are over expressed; on the other hand, the levels of beta-2 glycoprotein-1 (beta-2GP1), vitamin D binding protein (VDBP) and apolipoprotein-1 are significantly decreased

in the plasma of MD-affected individuals. Among the identified proteins, there are few involved in ear-related disorders. CFH, and its related proteins, are strongly activated in otitis media with effusion, underscoring the role played by the alternative complement pathway in the development of inflammation in this particular disease. The presence of beta-2GP1 antibodies has been recently detected in patients with idiopathic sudden sensorineural hearing loss. VDBP is primarily involved in actin scavenging system, thus protecting cells from the toxic effect of intravascular actin polymerization. Beta actin is a ubiquitous protein involved in the formation of filaments that are a major component of the cytoskeleton. This protein has been identified as a potential candidate autoantigen in autoimmune inner ear disease. Moreover, beta actin mutations altering depolymerization dynamics are associated with autosomal dominant deafness, and dystonia and with non-syndromic hearing loss. Even though preliminary and not necessarily linked directly to the molecular pathogenesis of the disease, our original findings suggest that a molecular signature, represented by the plasma protein profile previously described, might represent a potentially powerful, innovative and not invasive tool for early diagnosis and clinical management of MD patients. Moreover we think that our findings uncover a potentially starring role for some proteins in the development and fate of MD, whose pathogenesis still remains unclear.

SESSION X
MOLECULAR BIOLOGY AND CELL PHYSIOLOGY (II)

O71
APOPTOTIC REGULATORS IN THE AUDITORY SYSTEM: KEY FACTORS IN HEARING LOSS

Rachel A. Burt
Murdoch Childrens Research Institute, Parkville, Australia

Background. Acquired hearing loss (including age-related, noise-induced and drug-induced loss) is a significant public health issue. The molecular mechanisms resulting in this condition are poorly understood. However, it is clear that programmed death (apoptosis) of cells within the cochlea is often involved. Through better understanding of the regulation of this process we hope to identify therapeutic targets for prevention and treatment of acquired hearing loss.
Objectives. To understand the molecular regulation of cell death in the ear so as to identify drug targets for prevention and treatment of hearing loss.
Methods. A range of functional genomics approaches are being applied to elucidate the regulation of auditory apoptosis. Primarily, a panel of engineered mouse strains harbouring mutations in apoptotic regulators is being assessed for hearing loss, to better understand cell death in the auditory system.
Results. Mutations at particular points of the intrinsic pathway of apoptosis have a profound effect on the auditory system. Deficiencies of certain apoptotic regulators can disrupt development of the auditory system or result in hearing loss in the adult.
Conclusion. Tightly regulated apoptosis is required for both development and maintenance of hearing. Targeting of apoptotic regulators will likely prove useful in prevention of cell death and resultant hearing loss in the ear. We are actively pursuing this as a strategy for development of novel therapies for acquired hearing impairment.

O72
SOUND RESPONSE MEDIATED BY THE TRP CHANNELS NOMPC, NANCHUNG, AND INACTIVE IN CHORDOTONAL ORGANS OF DROSOPHILA LARVAE

Wei Zhang[1*], Zhiqiang Yan[1,2*], Lily Yeh Jan[1], and Yuh Nung Jan[1]
*[*authors contributed equally to this work]*
[1]Howard Hughes Medical Institute and Departments of Physiology, Biochemistry, and Biophysics, University of California, San Francisco, CA, USA; [2]School of Life Sciences, Fudan University, Shanghai, China

Mechanical stimuli, including tactile and sound signals, convey a variety of information important for animals to navigate the environment and avoid predators. Recent studies have revealed that Drosophila larvae can sense harsh or gentle touch with dendritic arborization (da) neurons in the body wall and can detect vibration with chordotonal organs (Cho). Whether they can also detect and respond to vibration or sound from their predators remains an open question. Here we report that larvae respond to sound of wasps and yellow jackets, as well as to pure tones of frequencies that are represented in such natural sounds, with startle and burrowing behaviors. The larval response to sound vibration requires Cho neurons and, to a lesser extent, class IV da neurons. Our calcium imaging and electrophysiological experiments reveal that Cho neurons, but not class IV da neurons, are excited by natural sounds or pure tones, with tuning curves and intensity dependence appropriate for the behavioral responses. Furthermore, our study implicates the transient receptor potential (TRP) channels NOMPC, NANCHUNG, and INACTIVE, but not the dmPIEZO channel, in the mechanotransduction and/or signal amplification for the detection of sound by the larval Cho neurons. These findings indicate that larval Cho, like their counterparts in the adult fly, use some of the same mechanotransduction channels to detect sound waves and mediate the sensation akin to hearing in Drosophila larvae, allowing them to respond to the appearance of predators or other environmental cues at a distance with behaviors crucial for survival.

O73
ACID-SENSING ION CHANNELS IN THE COCHLEAR AFFERENT NEURONS

Enrique Soto[1], Rosario Vega[1], Antonia González-Garrido[1], Francisco Mercado[2], Ivan López[3]
[1]Instituto de Fisiología, Benemérita Universidad Autónoma de Puebla, México; [2]Dirección de Investigaciones en Neurociencias, Instituto Nacional de Psiquiatría Ramón de la Fuente Muñiz, México D.F, México; [3]Department of Head and Neck Surgery, David Geffen School of Medicine at UCLA, CA, USA

Acid-sensing ion channels (ASICs) are activated by an increase in the extracellular proton concentration. There are four genes (ASIC1-4) that encode six subunits, and they are involved in diverse neuronal functions, such as mechanosensation, learning and memory, nociception, and modulation of retinal function. In this study, we characterize the ASIC currents in primary cultured

spiral ganglion neurons (SGNs) of the rodent cochlea bnu using whole cell voltage clamp. The ASIC currents were carried by Na^+, exhibit fast activation and desensitization, display a pH50 of 6.2 and were blocked by amiloride. The ASIC currents were further characterized using several pharmacological tools. Gadolinium and acetylsalicylic acid reduced these currents, and FMRFamide, zinc (at high concentrations) and N,N,N',N'–tetrakis-(2-piridilmetil)-etilendiamina (TPEN) increased them, indicating that functional ASICs are composed of the subunits ASIC1, ASIC2 and ASIC3. The aminoglycosides neomycin and streptomycin reduced the desensitization rate of the ASIC current in SGNs, indicating that ASICs may contribute to the ototoxic action of aminoglycosides. RT-PCR of the spiral ganglion revealed significant expression of all ASIC subunits. The expression of the subunits ASIC1a, ASIC2a, ASIC2b and ASIC3 in SGNs were determined by immunohistochemistry. In functional current clamp sturecords only a few SGNs exhibited action potential firing in response to an acidic stimulus, protons in the extracellular solution modulated SGN activity during electrical sinusoidal stimulation. Our results show that extracelular changes in the pH (proton concentration) modulate the excitability of SGNs via ASICs.

O74
DISRUPTION OF ADAPTOR PROTEIN 2μ (AP-2μ) IN COCHLEAR HAIR CELLS IMPAIRS SYNAPTIC VESICLE REPLENISHMENT AND HEARING

SangYong Jung[1,2*], Tanja Maritzen[3*], Carolin Wichmann[2,5*], Zhizi Jing[2,6], Andreas Neef[1,7], Natalia H. Revelo[2,8], Hanan Al-Moyed[2,5], Sandra Meese[2,9], Sonja M. Wojcik[10], Iliana Panou[1,2], Haydar Bulut[3], Peter Schu[11], Ralf Ficner[2,8], Ellen Reisinger[2,12], Silvio O. Rizzoli[2,7,13], Jakob Neef[1,2], Nicola Strenzke[2,6], Volker Haucke[3,4], Tobias Moser[1,2,13] *[*authors contributed equally to this work]*
[1]Institute for Auditory Neuroscience and InnerEarLab, Department of Otolaryngology, University Medical Center Göttingen, Germany; [2]Collaborative Research Center 889, University of Göttingen, Germany; [3]Leibniz Institut für Molekulare Pharmakologie (FMP), Berlin, Germany; [4]NeuroCure Cluster of Excellence & Collaborative Research Center 958, Freie Universität Berlin, Germany; [5]Molecular Architecture of Synapses Group, InnerEarLab, Department of Otolaryngology, University Medical Center Göttingen, Germany; [6]Auditory Systems Physiology Group, InnerEarLab, Department of Otolaryngology, University Medical Center Göttingen, Germany; [7]Bernstein Group Biophysics of Neural Computation, Max Planck Institute for Dynamics and Self-Organization, 37077 Göttingen, Germany,[8]Department of Neuro- and Sensory Physiology, University Medical Center Göttingen, Germany; [9]Department of Molecular Structural Biology, Institute for Microbiology and Genetics, University of Göttingen, Germany; [10]Department of Molecular Neurobiology, Max Planck Institute for Experimental Medicine, Göttingen, Germany; [11]Department of Cellular Biochemistry, University Medical Center Göttingen, Germany,[12]Molecular Biology of Cochlear Neurotransmission Group, InnerEarLab, Department of Otolaryngology, University Medical Center Göttingen, Germany; [13]Center for Nanoscale Microscopy and Molecular Physiology of the Brain, University of Göttingen, Germany

Active zones (AZs) of inner hair cells (IHCs) indefatigably release hundreds of vesicles per second, requiring each release site to replenish vesicles at tens per second. Here, we report that the endocytic adaptor protein 2μ (AP-2μ) is required for release site replenishment and hearing. We show that hair-cell-specific disruption of AP-2μ slows IHC exocytosis immediately after fusion of the readily releasable pool of vesicles, despite normal abundance of membrane-proximal vesicles and intact endocytic membrane retrieval. Sound-driven postsynaptic spiking was reduced in a use-dependent manner and the altered interspike interval statistics suggested a slowed replenishment of release sites. Sustained strong stimulation led to accumulation of endosome-like vacuoles, fewer clathrin-coated endocytic intermediates and vesicle-depletion of the membrane-distal synaptic ribbon in AP-2μ-deficient IHCs, indicating a further role of AP-2μ in clathrin-dependent vesicle reformation on a timescale of many seconds. Finally, we characterized the interaction of AP-2 with its cargo otoferlin. We propose that binding of AP-2 to otoferlin facilitates replenishment of release sites via speeding clearance of exocytosed material, in addition to a role of AP-2 in endocytic recycling.

O75
MANIPULATIONS OF PHOSPHATIDYLINOSITOL 4,5-BISPHOSPHATE METABOLISM EFFECTS MECHANOELECTRICAL TRANSDUCTION CURRENTS IN MAMMALIAN INNER HAIR CELLS

Thomas Effertz[1], Anthony Ricci[1,2]
[1]Department of Otolaryngology, Head &Neck surgery, School of Medicine, Stanford University, Stanford, CA, USA; [2]Department of Molecular and Cellular Physiology, School of Medicine, Stanford University, Stanford, CA, USA

Mechanoelectrical-transduction (MET) underlies our ability to perceive sound. MET-currents are directly transduced by mechanically gated channels residing at the tips of sensory hair cell stereocilia, which comprise the sensory hair bundle. Previous data showed that bullfrog hair cells require phosphatidylinositol 4,5-bisphosphate (PIP_2) for adaptation, where Hirono et al. 2004 suggested PIP_2 interacted with mysosin motors to regulate force at the channel. We investigated the role of PIP_2 in mammalian (ma) MET using rat inner hair cells (IHC). As previously described (Hirono et al. 2004), we blocked phosphatidylinositol 4-kinase (PI4K) with phenylarsine oxide (PAO), which reduces PIP_2 levels. We used piezo driven stiff probes and a custom build fluid-jet system to stimulate IHCs (P7-P9). PAO treated IHCs showed a reduction of maMET-current amplitude, a loss of adaptation, an increase in baseline current, and a broadening of the current-displacement plots. The loss of adaptation was apparent in the time domain (current reduction over time during constant stimulation) and with paired-pulse stimulation (comparing activation curves acquired at resting position and after pre-displacement). Fluid-jet stimulation combined with high speed, confocal Ca^{2+} imaging found no loss of functional stereocilia but an increase in baseline Ca^{2+} levels as indicated by the decrease in Ca^{2+} signal with depolarization. Often Ca^{2+} signals would increase with hair bundle deflection but remain high for longer periods of time following stimulation. All effects were reduced or eliminated by including an excess of PIP_2 in the patch electrode. Changes in lipid geometry from a cylindrical PIP_2 to an inverse-conical DAG can gate TRP-channels (Hardie and Franze 2012). Other MET-channels (like TREK-1 or TRAAK) are either modulated by PIP_2 or require PIP_2 for their function (Hansen 2015). Our data show that PIP_2 is essential for maMET-channel

adaptation, where other effects on mechanosensitivity may simply result from this loss. PIP_2 may interact directly with the maMET-channel or with other transmembrane interacting proteins to: alter mechanical force relay, cause lipid geometry changes that alter energy required for channel conformational changes or downstream effects of PIP_2 second messaging cascade.
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O76
MOLECULAR CONSTITUENTS OF THE MECHANOTRANSDUCITON MACHINERY OF COCHLEAR HAIR CELLS
Ulrich Mueller, Bo Zhao, Wei Xiong, Nicolas Grillet
The Scripps Research Institute, La Jolla, CA, USA

Hair cells are the mechanosensory cells of the inner ear. Mechanotransduction channels in hair cells are gated by tip links. The molecular composition of the mechanotranduction machinery of cochlear hair cells is only partially understood. Here we present data demonstrating that several components of the mechanotransduction machinery of hair cells are encoded by genes that are linked to inherited forms of deafness in humans. Using genetically modified mice, we provide insights into the mechanisms by which mutations in genes for several of these components such as LHFPL5, TMIE, and TMC1/2 cause hearing loss. We also provide evidence that alternative splicing leads to functional diversification in components of the transduction machinery and that this complexity is critical for regulating channel properties in different hair cells along the cochlea's tonotopic axis. Our findings define basic features of the mechanotransduction machinery of cochlear hair cells and highlight that a subset of genetic forms of hearing loss can be classified as "mechanotransduction diseases".

O77
A MULTIPHOTON MICROSCOPY INVESTIGATION ON THE ROLE OF NAD(P)H AND PLASMA MEMBRANE FLUIDITY IN NOISE-INDUCED OXIDATION OF OUTER HAIR CELLS
G. Maulucci[1], D. Troiani [2], S.L.M. Eramo[2], F. Paciello[3], M.V. Podda[2], G. Paludetti[3], M. Papi[1], A. Maiorana[1], V. Palmieri[1], M. De Spirito[1], A.R. Fetoni[3]
[1]Institute of Physics, [2]Institute of Human Physiology, [3]Department of Head and Neck Surgery, Università Cattolica, Medical School, Rome, Italy

The common basis for OHC dysfunction and loss by acoustic over-stimulation is represented by reactive oxygen species (ROS) overload that may affect the membrane structural organization through lipid peroxidation. We investigated in OHC different functional zones the mechanisms linking metabolic functional state (NAD(P)H intracellular distribution) to the generation of lipid peroxides and to the physical state of membranes by two photon fluorescence microscopy. In OHCs of control animals, a more oxidized NAD(P)H redox state is associated to a less fluid plasma membrane structure. Acoustic trauma induces a topologically differentiated NAD(P)H oxidation in OHC rows, which is damped between 1 and 6 h. Peroxidation occurs after ~4 h from noise insult, while ROS are produced in thefirst 0.2 h and damage cells for a period of time after noise exposure has ended (~7.5 h) when a decrease of fluidity of OHC plasma membrane occurs. OHCs belonging to inner rows, characterized by a lower metabolic activity with respect to other rows, show less severe metabolic impairment.Our data indicate that plasma membrane fluidity is related to NAD(P)H redox state and lipid peroxidation which may represent key targets for therapeutic rescuing plan from noise insults.
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O78
FURTHER CHARACTERIZATION OF THE STRIATED ORGANELLE AND APICAL MITOCHONDRIA IN RAT INNER EAR HAIR CELLS
Anna Lysakowski[1], Robstein L. Chidavaenzi[2]
Department of Anatomy and Cell Biology, University of Illinois at Chicago, IL, USA

Objectives. We have been characterizing the striated organelle, a cytoskeletal specialization found in the apical region of both types of vestibular and in cochlear inner hair cells [1]. This organelle may interact with stereociliar rootlets and nearby mitochondria and thus affect mechanotransduction.
Methods. We use confocal, EM immunogold and EM tomography methods, coupled with Western blots, co-immunoprecipitation and mass spectrometry.
Results. We have previously described the localization of α2- and β2-spectrin in the striated organelle (SO). Recently, we have found another protein, nebulin, closely associated with the SO. Nebulin is the largest known actin-bundling protein (600-900kDa). Each molecule has the capacity to bind 200 actin monomers. Extensively studied in vertebrate skeletal muscle thin filaments, nebulin has been investigated for its role in determining the length of actin filaments and in regulating actin-myosin interactions in a calcium-calmodulin dependent manner. Hence an integral role in various cytoskeletal assemblages is implied. On that basis, we sought to determine its presence and distribution in the mechanically sensitive inner ear auditory and vestibular hair cells. Nebulin is co-extensive with, but extends beyond, the two spectrin isoforms in both the cuticular plate and SO. We have also been examining the relationship of subcuticular mitochondria to the striated organelle. In central vestibular type I hair cells, these mitochondria are especially large. The crista junctions in some appear to be polarized toward the SO and stereociliar rootlets. We are intrigued by this relationship and are attempting to further characterize the mitochondria in both vestibular and cochlear hair cells. In conclusion, the striated organelle and its associated elements continue to be intriguing structures.

O79

CLOSING THE LOOP ON THE OLIVOCOCHLEAR REFLEX: OUTER HAIR CELLS AND TYPE II SPIRAL GANGLION AFFERENTS DRIVE CONTRALATERAL SUPPRESSION

Gary D. Housley[1], Kristina E. Froud[1], Ann Chi Yan Wong[1], Jennie M.E. Cederholm[1], Matthias Klugmann[1], Shaun L. Sandow[2], Jean-Pierre Julien[3], Allen F. Ryan[4]

[1]UNSW Australia, Translational Neuroscience Facility & Dept. Physiology, Sydney, Australia; [2]University of the Sunshine Coast, Inflammation and Healing Cluster, Faculty of Science, Health, Education and Engineering, Maroochydore, Australia; [3]Laval University, Institut Universitaire en santé mentale de Québec, Department of Psychiatry and Neuroscience, Quebec,Canada; [4]University of California San Diego, and Veterans Administration Medical Center, Departments of Surgery & Neurosciences, La Jolla, CA, USA

Objective. When sound intensity increases, the medial olivocochlear (MOC) reflex turns down the 'cochlear amplifier' to dynamically balance the input of each ear. Here we sought to resolve the outstanding question of the sensory driver for this reflex.

Methods. In the mouse cochlea, the type II spiral ganglion neurons (SGNs) that innervate the outer hair cells (OHCs) exclusively express the type III intermediate filament peripherin (Prph). The cochlear innervation of Prph knockout (KO) mice and wildtype (WT) mice was compared using neurofilament 200 immunofluorescence and serial blockface tomography scanning electron microscopy (Zeiss – Gatan 3View). Auditory brainstem responses (ABR) and distortion product otoacoustic emissions (DPOAE) were used to evaluate hearing function.

Results. The outer spiral bundles were absent in PrphKO mice and no type II SGN afferent synapses were idenfied (0 / 54 PrphKO OHC recontructions, n = 3), whereas virtually all WT OHC reconstructions had afferent boutons (49 / 56 WT OHCs, n = 4). The distribution of MOC efferent boutons on PrphKO OHCs were comparable to WT controls (132 / 54 PrphKO OHCs, n = 3; 132 / 56 WT OHCs, n = 4). While baseline hearing of the PrphKO mice was indistinguishible from the WT controls (ABR and cubic (2f1-f2) DPOAE input-output functions), remarkably, the PrphKO mice lacked contralateral suppression. Brief presentation (up to 1 minute) of 82 – 95 dB SPL band-pass noise presented to one ear failed to elicit a transient suppression of the quadratic (f2-f1) DPOAE in the opposite ear (22 / 22 PrphKO mice, across 3 studies). WT mice exhibited consistent contralateral suppression (23 / 24 WT mice, across 3 studies).

Conclusions. Type II SGN fibres are required for contralateral suppression, indicating that the OHC – type II SGN sensory pathway is a distinct auditory transmission channel. This establishes the PrphKO mouse model as a new tool for evaluation of MOC reflex-mediated unmasking of hearing in noise, sound localization, noise- and age-related hearing loss.

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52nd Inner Ear Biology Workshop

Poster presentations

POSTER SESSION I
DEVELOPMENTAL BIOLOGY AND REGENERATION

P1

BMI1 REGULATES THE PROLIFERATION OF COCHLEAR SUPPORTING CELLS VIA THE CANONICAL WNT SIGNALING PATHWAY

Renjie Chai[1,2], Xiaoling Lu[3,4], Shan Sun[3,4], Wenyan Li[3,4], Yan Chen[3,4], Lei Wang[5], Huawei Li[3,4,5]
[1]Key Laboratory for Developmental Genes and Human Disease, Ministry of Education, Institute of Life Sciences, Southeast University, Nanjing, China; [2]Co-innovation Center of Neuroregeneration, Nantong University, Nantong, China; [3]Department of Otorhinolaryngology, Hearing Research Institute, Affiliated Eye and ENT Hospital of Fudan University, Shanghai, China; [4]Central laboratory, Affiliated Eye and ENT Hospital of Fudan University, Shanghai, China; [5]Institutes of Biomedical Sciences, Fudan University, Shanghai, China

Cochlear supporting cells (SCs), which include the cochlear progenitor cells, have been shown to be a promising resource for hair cell (HC) regeneration, but the mechanisms underlying the initiation and regulation of postnatal cochlear SC proliferation is not yet fully understood. Bmi1 is a member of the Polycomb protein family and has been reported to regulate the proliferation of stem cells and progenitor cells in multiple organs. In this study, we investigated the role of Bmi1 in regulating SC proliferation. We first showed that knockout of Bmi1 significantlyinhibited the proliferation of SCs andLgr5-positive progenitor cellsafter neomycin injury*in vitro* suggesting that Bmi1 is required for the initiation of SC proliferation. We also showed that Bmi1 deficiency significantly reduced the sphere-forming ability of the organ of Corti andLgr5-positive progenitor cells.Next, we found that Lgr5 expression in$Bmi1^{-/-}$ mice was significantly down-regulated compared to wild-typecontrols.Thisdemonstrated that Bmi1 knockout significantly inhibited the Wntsignaling pathway and indicatedthat Wnt signaling might beinvolved in the inhibition of SC proliferationin $Bmi1^{-/-}$ mice.Furthermore, the exogenousWnt agonist Biorescued the down-regulation of SC proliferationin $Bmi1^{-/-}$mice suggesting thatBmi1 knockout inhibits the proliferation of SCs via down-regulation of the canonical Wnt signaling pathway.Our findings demonstrate that Bmi1 playsan important role in regulating the proliferation of cochlear SCsand Lgr5-positive progenitor cells via the Wnt signaling pathway, and this suggests that Bmi1 might be a new therapeutic target for HC regeneration.

P2

DYNAMIC EXPRESSION OF LGR6 IN THE DEVELOPING AND MATURE MOUSE COCHLEA

Yanping Zhang[1,2,4], Yan Chen[1,2], Wenli Ni[3], Luo Guo[1,2], Xiaoling Lu[3], Liman Liu[2], Wen Li[1,2], Shan Sun[1,2], Lei Wang[4], Huawei Li[2,3,5]
[1]Research Center, Affiliated Eye and ENT Hospital of Fudan University, Shanghai, China; [2]Key Laboratory of Hearing Medicine, Ministry of Health, Affiliated Eye and ENT Hospital of Fudan University, Shanghai, China; [3]Department of Otorhinolaryngology, Affiliated Eye and ENT Hospital of Fudan University, Shanghai, China; [4]Institutes of Sciences, Fudan University, Shanghai, China; [5]State Key Laboratory of Medical Neurobiology, Fudan University, Shanghai, China

The Wnt/β-catenin signaling pathway plays important roles in mammalian inner ear development. Lgr5, one of the downstream target genes of the Wnt/β-catenin signaling pathway, has been reported to be a marker for inner ear hair cell progenitors. Lgr6 shares approximately 50% sequence homology with Lgr5 and has been identified as a stem cell marker in several organs. However, the detailed expression profiles of Lgr6 have not yet been investigated in the mouse inner ear. Here, we first used Lgr6-EGFP-Ires-CreERT2 mice to examine the spatiotemporal expression of Lgr6 protein in the cochlear duct during embryonic and postnatal development. Lgr6-EGFP was first observed in one row of prosensory cells in the middle and basal turn at embryonic day 15.5 (E15.5). From E18.5 to postnatal day 3 (P3), the expression of Lgr6-EGFP was restricted to the inner pillar cells (IPCs). From P7 to P15, the Lgr6-EGFP expression level gradually decreased in the IPCs and gradually increased in the inner border cells (IBCs). At P20, Lgr6-EGFP was only expressed in the IBCs, and by P30 Lgr6-EGFP expression had completely disappeared. Next, we demonstrated that Wnt/β-catenin signaling is required to maintain the Lgr6-EGFP expression in vitro. Finally, we demonstrated that the Lgr6-EGFP-positive cells isolated by flow cytometry could differentiate into myosin 7a-positive hair cells after 10 days in-culture, and this suggests that the Lgr6-positive cells might serve as the hair cell progenitor cells in the cochlea.

P3

BMI1-MEDIATED REGULATION OF THE SPHERE-FORMING CAPACITY OF THE MURINE ORGAN OF CORTI

Mohamed Bassiouni[1], Aurélie Dos Santos[1], Marcus Müller[2], Hubert Löwenheim[2]
[1]Tübingen Hearing Research Centre, Department of Otolaryngology, Head and Neck Surgery, Eberhard Karls University Tübingen, Germany; [2]Department of Otolaryngology Head and Neck Surgery, Carl von Ossietzky University Oldenburg, Germany

The oncogene Bmi1 is essential for the normal self-renewal

potential of adult stem cells in the central and peripheral nervous system (reviewed in Park et al., J Clin Invest. 2004). This also includes sensory organs as the retina (Chatoo et al., Stem Cells. 2010). Expression of Bmi1 was furthermore observed in otic stem cell-derived spheres, obtained from the postnatal organ of Corti (Waldhaus et al., PlosOne. 2012). This implies that Bmi1 plays a role in regulating otic stem cell self-renewal.
Using immunohistochemical methods, we investigated Bmi1 expression in the mouse cochlea at three developmental time points; embryonic day (E) 13.5, postnatal day (p)0, and p28. In addition, a Bmi1-GFP reporter mouse line was used, where exon 2 of Bmi1 is replaced by GFP, resulting in a null allele (Hosen et al., Stem Cells. 2010). Bmi1 expression was detected in the developing embryonic and postnatal cochlear duct epithelium and spiral ganglion cells. Furthermore, Bmi1 expression persisted in the functionally-mature cochlea at postnatal day (p) 28, but was down-regulated in the stria vascularis. The neonatal Bmi1-null cochlea lacked Bmi1 expression, but showed normal development of the organ of Corti.
To determine whether Bmi1 plays a role in the capacity of the mouse organ of Corti to form otic spheres, we isolated cells from the organ of Corti of neonatal mice at p0. Using the otosphere assay, we examined wildtype, heterozygous, and homozygous Bmi1-null mice. Bmi1-deficient organ of Corti specimens gave rise to significantly less spheres compared to their wildtype littermates.
These findings indicate that Bmi1 is involved in the regulation of the sphere-forming capacity of the auditory sensory epithelium, but is not essential for the development of the organ of Corti. The exact mechanism, through which Bmi1 exerts this function, still needs to be determined.

P4

ELECTROPORATION-MEDIATED TRANSUTERINE GENE TRANSFER INTO MOUSE OTOCYSTS (EUGO) UTILIZING NEPA21 ELECTOROPORATOR

Ryosei Minoda, Hiroki Takeda, Toru Miwa, Eiji Yumoto
Department of Otolaryngology-Head and Neck Surgery, Kumamoto University, Graduate School of Medicine, Kumamoto City, Japan

Mouse otocysts are an attractive experimental target for developing treatment modalities for congenital inner ear diseases and for studying inner ear development. There is a paucity of reports on successfully induced gene transfer into the otocysts in vivo. We have previously reported that electroporation-mediated gene transfers of the Cx30 gene into the otocysts of Cx30 knock-out mice successfully prevented putative profound hearing loss (Miwa, Molecular therapy, 2013). We previously utilized a CUY21 EDIT electoroporator (Nepa Gene), which generates a single type of pulses. More recently, it has been reported that the NEPA21 electoroporator (Nepa Gene) induces less collaborate damage to tissue and more efficient expression levels in tissue. NEPA21 pulses consist of two types of pules: poring pulses; and, transfer pulses. We can also adjust those attenuation rates. We herein report on survival rates and expression rates after Gene transfer via the NEPA21 on various conditions.
Materials and Methods. Gene transfer was achieved via Electroporation-Mediated Transuterine Gene Transfer into Otocysts (EUGO) as follows. At 11.5 days post-coitum, the uteruses of pregnant mice were exposed by a low-midline laparotomy, placed on a transparent surgical stage and illuminated from below with a fiber-optic beam to identify the location of the otocysts. EGFP plasmid vectors were microinjected into one side of the otocysts. Utilizing the NEPA21, the plasmid filled otocysts were then electroporated under various stimulation conditions. At E13.5, EGFP expression levels in the inner ears were assessed.
Results. Overall survival rate of embyos was 58.62%. Overall incidence of EGFP expression in treated otocysts was 20%. The expression rates of the treated otocysts, otocysts which underwent 25V of poring pulses showed highest expression rate: 50%.
Conclusions. Changes of poring pulses and transfer pulses had effects on survival rates of treated embyos and expression rates in the otocysts. The NEPA21 gives us a chance to change pulse conditions almost infinitely. Determination of optimal conditions of these parameters is necessary for achieving higher survival rates of the embryos and higher expression rates in the otocysts; our target is to elucidate optimal settings.

P5

THE NOGO RECEPTOR 1 IS A PROMISING TARGET IN THE INNER EAR

Laura Holtmann[1], Katharina Schulz[2], Stephan Lang[1], Stefan Hansen[1]
[1]Department for Otorhinolaryngology, University Hospital Essen, Germany; [2]Department for Otorhinolaryngology, University Hospital Düsseldorf, Germany

Objective. During the last decade Nogo-proteins have become of extensive interest in the field of neuronal regeneration. The best studied member is Nogo-A and, like the other isoforms Nogo-B and -C, originate from the reticulon 4 gene. All three consists of a 66-amino acid segment called Nogo-66, which is the interaction partner for the Nogo receptor 1 (NgR1). NgR1 is a receptor complex which is additionally formed by the transmembrane proteins p75NTR and LINGO1. Beneath Nogo-A the NgR1 is activated by the myelin components myelin-associated protein (MAG), which is also present in the inner ear, and oligodendrocyte myelin glycoprotein (OMGP) within the central nervous system. The activation of NgR1 induces a growth cone collapse and a growth arrest in outgrowing neurites by destabilization of the actin cytoskeleton. The blockage of the NgR1 or disruption of the Nogo-NgR1-interaction increases neurite outgrowth, and enhances regeneration and plastic rearrangements of nerve fiber connections after CNS injuries.
Methods. Here, we demonstrate the expression of NgR1 during hearing development in cryosections of the mouse cochlea. Additionally, we cultured spiral ganglion neurons of newborn mice and analyzed the growth behavior of spiral
ganglion neurites with activation or blockage of NgR1.
Results. NgR1 expression was present during hearing development mainly in the spiral ganglion soma and less intense in the central and peripheral projections. However, restricted to the first postnatal week, there was a strong expression of NgR1 in the inner pillar cells. In the spiral ganglion cell culture NgR1-blocking with Nogo-66(1-40) resulted in an extended neurite outgrowth while receptor activation through MAG caused significantly reduced neurite length.
Conclusion. Further knowledge of Nogo function in the inner ear could reveal a promising tool for the regeneration, maintenance, and guided resprouting of spiral ganglion neurites.

P6

NEUREGULIN-1 SUPPORTS SPIRAL GANGLION OUTGROWTH AND SCHWANN CELL PROLIFERATION IN THE INNER EAR

Stefan Hansen[1], Diana Lang[2], Laura Holtmann[1], Stephan Lang[1]
[1]Department for Otorhinolaryngology, University Hospital Essen, Germany; [2]Department for Otorhnolarygology, University Hospital Düsseldorf, Germany

Objective. Schwann cells enwrap axons of peripheral nerves and play a critical role in the axonal regeneration process by providing trophic support. After nerve injury they can dedifferentiate and promote neurite outgrowth by releasing various factors, like adhesion molecules, integrins and neurotrophic factors. It has been demonstrated that neuregulin-1 (NRG1) is an essential axo-glial signal protein required for peripheral nerve development and nerve repair. NRG1 signaling is critical in Schwann cell precursor generation. NRG1 is a trophic factor that contains an epi dermal growth factor (EGF)-like domain that signals by stimulating ErbB receptor tyrosine kinases. In the inner ear NRG1 is expressed by spiral ganglion neurons, whereas ErbB2 and ErbB3 are expressed by supporting cells of the organ of Corti. Furthermore, it has been shown that neuregulin/ErbB signaling is essential in long-term functional regulation of the auditory nerve, maybe through reciprocal neuron-glial interactions (Watanabe et al., Hansen et al., Stankovic et al.).
Methods. Beneath immunohistochemical expression analysis of NRG1 during hearing development in the mouse we studied the effect of NRG1 administration on neurite outgrowth and Schwann cell development in vitro as well as blocking of ErB-receptor activation in a spiral ganglion/Schwann cell co-culture.
Results. The immunohistochemical analysis showed a change in the expression of NRG1 in the modiolus during the development of hearing. In the cell culture the addition of NRG1 caused a concentration-dependent increase in axonal outgrowth and proliferation of immature Schwann cells, while the panErbB receptor blocker (Cl-1033) induced a neurite growth inhibition.
Conclusion. NRG1 seems to be an important factor in the development of the auditory nerves by Schwann cell interaction and could play a critical role in future regenerative therapeutic strategies in the inner ear.

P7

MINIATURE PIGS: A LARGE ANIMAL MODEL OF COCHLEAR IMPLANTATION

Weiwei Guo, Tao Cong, Shi-Ming Yang
Department of Otolaryngology, Head & Neck Surgery, Institute of Otolaryngology of PLA, Chinese PLA General Hospital, Beijing, China

Objective. To investigate the suitability of the miniature pig as an animal model of cochlear implantation (CI).
Methods. Micro-CT scanning and three-dimensional reconstructions of the inner ear were completed in two animals. Photographs of the procedures and measurements of the inner ear were made. The CI procedure was simulated in 10 animals. Electrically evoked auditory brain stem responses (EABRs) and radiographic images were evaluated during or
after the CI procedure.
Results. Morphological examination and measurements of the inner ears of the miniature pigs were completed by micro-CT scanning. The EABRs were evoked during the CI procedure, and a radiographic image after the CI procedure revealed that the electrode was located in the scala tympani of the first and second turns.
Conclusion. Compared with traditional animal models, greater similarities of the inner ear between miniature pigs and humans make this animal a potentially useful model for applications in CI.

P8

NOTCH SIGNALING ACTS AS A NEGATIVE REGULATOR FOR THE PROLIFERATION OF PROGENITORS IN MAMMALIAN COCHLEAE

Wenyan Li, Hui Jiang, Wenli Ni, Jingfang Wu
Fudan University, Affiliated Eye and Ear, Nose, Throat Hospital, Department of Otorhinolaryngology, Shanghai, China

Inner ear sensory epithelium consists of a mosaic of hair cells and supporting cells, generated from the same progenitors in the prosensory domain during development. It has been reported that the formation of the mosaic HC and SC pattern is mediated by lateral inhibition through the Notch signaling pathway. In current study, we show that the inhibition of Notch at E10.5 can prolong the proliferation process of the progenitor cells and lead to a widened sensory epithelium and numerous hair cells, furthermore, the inhibition of Notch at E14.5 can initiated the proliferation of supporting cells and mitotic generation of hair cells. We conclude that Notch signaling acts as a negative regulator for the proliferation of progenitors in mammalian cochleae and plays an important role on maintaining the homeostasis of cochlear sensory epithelium on cell number and structures, which may provide a new route for the hair cell regeneration process in mammalian cochleae through manipulating Notch signaling.

P9

THE ROLE OF PARTICULATE GUANYLYL CYCLASE B (GC-B) FOR AUDITORY FUNCTION IN MICE

Steffen Wolter[1], Dorit Möhrle[1], Dennis Zelle[1], Marlies Knipper[1], Robert Feil[2], Hannes Schmidt[3], Lukas Rüttiger[1]
[1]University of Tübingen, Department of Otolaryngology, Tübingen, Germany; [2]University of Tübingen, Interfaculty Institute of Biochemistry, Tübingen, Germany; [3]Max Delbrück Center for Molecular Medicine, Developmental Neurobiology, Berlin, Germany

cGMP signaling triggered by the binding of C-type natriuretic peptide (CNP) to its receptor guanylyl cyclase B (GC-B; NPR2; NPRB) has been linked by genetic evidence to a remarkable variety of physiological functions like skeletal bone growth, female fertility, cardiac growth, fat metabolism and gastrointestinal function. For the nervous system it has been recently demonstrated that the CNP/GC-B/cGMP/cGMP-dependent protein kinase I (cGKI) signaling pathway is essential for sensory axon branching at the dorsal root entry zone of the spinal cord and at the rhombomeres of the hindbrain during embryonic development. Also axons from spiral ganglion neurons bifurcate at the border of the embryonic hindbrain and consequently extend daughter branches that finally innervate the anteroventral, posteroventral, and dorsal cochlear nuclei (aVCN, pVCN, DCN). The absence of GC-B impairs axonal bifurcation and results in a blurred tonotopic organization of central auditory circuits in mice.

Hearing measurements were conducted on six week old GC-B knock-out mice and their wild-type littermates, using evoked auditory brainstem responses (ABR) and distortion product otoacoustic emission (DPOAE). Data were analyzed with respect to ABR/DPOAE thresholds, ABR wave amplitudes/latencies and DPOAE amplitudes/growth functions. Histological correlates of the auditory phenotype were verified by applying immunohistochemistry on fixed sections of cochlea and brain tissue.

Here, we describe that the lack of GC-B in addition leads to a subtle peripheral phenotype which is manifested in hearing threshold loss and altered wave amplitudes and latencies of evoked ABR. Our preliminary results indicate that this deficit is related to a combined cochlear and central phenotype.

The functional shortfalls on hearing and central plasticity are still unknown and will be further investigated with respect to local cGMP-cascade activation. We expect implications in sound localization, a progressive aging phenotype and an increased risk for tinnitus.

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P10

THE PROMOTER AND MULTIPLE ENHANCERS OF THE *POU4F3* GENE REGULATE GENE EXPRESSION IN HAIR CELLS

Masatsugu Masuda[1,3], Yan Li[1], Kwang Pak[1], Eduardo Chavez[1], Lina Mullen[1], Allen F Ryan[1,2]

Departments of [1]Surgery, Otolaryngology and [2]Neurosciences, VA Medical Center and UCSD School of Medicine, La Jolla, CA, USA; [3]Department of Otolaryngology, Kyorin University, Tokyo, Japan

Noncoding regulatory DNA of genes control their expression, including the promoter immediately 5' to the transcription start site, and enhancers/repressors that can be 5' or 3' to the coding region or within introns. Few enhancers that target gene expression to hair cells (HCs) have been identified. We evaluated the control of *pou4f3* gene expression in HCs, since it is expressed only in HCs within the inner ear, and continues to be expressed throughout life. Cross-species bioinformatic analysis was conducted 20kb 5' to 20 kb 3' to coding sequences, to identify potential regulatory regions. An 8.5 kb genomic DNA fragment 5' to the start codon of the *pou4f3* gene, linked to eGFP as a reporter in transgenic mice, drove expression in all embryonic and neonatal HCs, and in adult vestibular and inner HCs, but not in adult outer HCs. To define the DNA elements involved, various portions of the 8.5 kb were deleted from the 5' end to create 0.4 kb, 0.8 kb, 1.6 kb, 2.5 kb, 6.4 kb and 7.2 kb transgenics, and eGFP expression patterns were assessed. The results indicate that the region from 6.4 kb to 7.2 kb contains regulatory sequence sufficient to produce expression in vestibular HCs and neonatal basal outer HCs, although none of the sequence was conserved across mammalian species. The region from 7.2 kb to 8.5 kb contains regulatory sequence that directs expression to inner and apical outer HCs. This region also includes ~285 bp that is highly conserved across mammals and contains paired Atoh1 binding sites. Deletion of the region from 0.4 to 5.5 kb 5' to the *pou4f3* ATG did not affect HC expression. To assess the role the region from 1 to 0.4 kb, it was replaced with the minimal promoter of the *Ela1* gene, which does not contribute to organ specificity. HC expression was maintained, but at a drastically reduced level, indicating no role in HC targeting. A region of highly conserved sequence, 5' to the 8.5 kb, contained POU4F3 and GFI1 binding sites. This region could possibly be involved in maintaining POU4F3 expression in adult outer HCs.

P11

EXTRACELLULAR NUCLEOTIDE SIGNALING DURING THE EMBRYONIC DEVELOPMENT OF THE CHICKEN INNER EAR: A MOLECULAR AND ELECTROPHYSIOLOGICAL STUDY

Fabián Galindo[1], Eduardo Monjaraz[1], Jorge Cebada[2], Amira Flores[1]

[1]Physiology Institute, Benemérita Universidad Autónoma de Puebla; [2]Medicine Faculty, Benemérita Universidad Autónoma de Puebla

Extracellular adenosine triphosphate (ATP) released from cellular sources plays an important role in a variety of physiologic process in the inner ear. ATP can activate P2X and P2Y receptors to influence cell functions; however, there are few reports about the role of ATP during development.

Objective. The aim of this work was to study the modulation of spontaneous vestibular and auditory afferent activity by ATP through its receptors during development.

Methods. To achieve this study we used an *in vitro* preparation of the chicken inner ear of 15, 18 and 21 embryonic days. This work was performed using multiunit and single-unit extracellular recordings from the posterior branch of the vestibular nerve and the auditory nerve.

Results. 2-MeSATP, a P2Y agonist (100nM to 1mM) increased significantly the vestibular afferent activity but this effect was abolished when RA2 (100μM), a P2Y antagonist was present. αβ-meATP, a P2X agonist (1μM to 1mM), increased notably the discharge frequency of the vestibular afferents mainly in E15 and E18 and this effect diminished by PPADS (30 and 300 μM), a P2X antagonist. When Mg^{2+} was present in the solution (2mM), the effect of P2X agonist also decreased. Vestibular evoked activity by mechanical stimuli did not changed by the presence of the P2Y or P2X agonist or antagonist. The auditory nerve activity showed significant increases by the microinjection of ATP (1μM to 1mM), 2-MeSATP and UTP (10μM to 1mM) and perfusion of RA2 (100μM) decreased the spontaneous and agonist evoked electrical activity but not the mechanical-evoked activity. Blocking the glutamate ionotropic receptors with a combination of MK-801, 7-Clk and CNQX (10 μM), abolished the purinergic agonist effect. RT-PCR revealed the presence of $P2X_3$, $P2Y_1$, $P2Y_2$ and $P2Y_6$ mRNA receptors in the vestibular system with more important presence during early stage (E15); however, in the auditory system we found only the $P2Y_1$, $P2Y_2$ and $P2Y_6$ mRNA.

Conclusions. Our results suggest that ATP may be participating as a neuromodulator of the vestibular and auditory afferent transmission from embryonic stage and its effect probably is mediated by glutamate release.

P12
ROLE OF P63 IN INNER EAR DEVELOPMENT: MORPHOLOGIC ABNORMALITIES AND CLINICAL FINDINGS

Ernesto Bruno[1], Alessandro Terrinoni[2], Andrea Viziano[1], Alessandro Micarelli[1], Sara De Fazio[1], Simone Mauramati[1], Marco Alessandrini[1], Fabrizio Ottaviani[1]
[1]Department of Clinical Sciences and Translational Medicine, University of Rome "Tor Vergata", Rome, Italy; [2]Biochemistry Laboratory Istituto Dermopatico Dell'Immacolata, c/o Department of Experimental Medicine and Surgery, University of Rome "Tor Vergata", Rome, Italy

Ectodermal dysplasia (ED) syndromes are a cluster of inherited human diseases characterized by developmental abnormalities of ectodermally derived structures. A large subset of autosomal dominant ED syndromes are caused by heterozygous mutations in the Tumor Protein p63 (TRP63) gene. p63 is a protein belonging to the p53 family of transcription factors, playing a fundamental role in organogenesis.
Conductive – as well as sensorineural – hearing loss is present in part of ED patients. Thus, we have investigated the role of p63 in the development of the inner ear. Our studies, using gene expression analysis and morphological examination of gene-targeted mice, demonstrated that the isoform TAp63 is essential for normal development of the cochlea by means of activation of the Notch signaling pathway.
The morphologic abnormalities in p63(-/-) mice embryos were investigated with immunofluorescence staining. Furthermore, due to the embryological and cytoarchitectural similarities between the Organ of Corti and the vestibular receptors, we searched for similar alterations in the vestibular maculae of the same murine models. Fluorescent antibodies against MyosinVIIa (MyoVIIa), a contractile hair cell protein, and Connexin 26 (Cx26), expressed in the supporting cells, were used to evaluate the distribution of these elements in the inner ear. Both proteins showed an abnormal distribution: Cx26 loses its normal basal layer specificity, while MyoVIIa staining shows supernumerary hair cells detached from the underlying supporting layer. This phenotype demonstrated to be consistent with previous studies, resembling the one observed in the vestibule and cochlea of Hes5(-/-) mice.
We provided further clinical insight by a thorough vestibular examination in a patient affected by an ED syndrome. The subject, a 18-year old male, suffered from mild-to-moderate mixed hearing loss with previous ventilation tube positioning and no personal history of vertigo or dizziness. Bedside examination showed normal otoscopy, absence of lateropulsion and no spontaneous or positional nystagmus. Caloric testing showed mild bilateral hyporeflectivity, while the video-head impulse test (vHIT) showed a bilateral reduction in vestibulo-ocular reflex gain. These findings could suggest a sort of subclinical impairment of the vestibular organs in subjects suffering from ED syndromes, underlying morphological abnormalities possibly counterparting those found in murine models.

P13
DIFFERENTIAL GENE EXPRESSION IN THE HUMAN COCHLEA AND VESTIBULAR SYSTEM DURING THE FIRST TRIMESTER OF PREGNANCY

Suvarna Dash-Wagh[1], Sara Hägg[2], Beata Kostyszyn[1], Elisabet Åkesson3, Mats Ulfendahl[1]
[1]Department of Neuroscience, Karolinska Institutet, Stockholm, Sweden; [2]Department of Medical Epidemiology and Biostatistics, Karolinska Institutet, Stockholm, Sweden; [3]Department of Neurobiology, Care Sciences and Society, Karolinska Institutet, Stockholm, Sweden

The morphogenesis of the hearing and balancing organs in the inner ear is a set of complex yet coordinated processes regulated by the spatiotemporal expression of several genes. Deciphering the gene profile is important for understanding the development and functions of these organs. Embryological and genetic studies in animals have served as a valuable tool to understand their development. However, little is known about the genes regulating differentiation of the human inner ear during the first trimester of pregnancy. Comparative and functional genomics using microarrays provide insights into the developing inner ear. We compared the differentially expressed genes in the cochlea, vestibular organ and spiral ganglion in the human inner ear during gestation weeks 5.5-11. A total of 4,498 probe sets were significantly altered, and more than half these (2,852) were unique to the inner ear. The spiral ganglion was the tissue type with greatest deviation compared to the cochlea and vestibule, and showed differential expression on 2,357 probe sets including 1,403 unique genes. For further characterization, functional annotation of the separate gene lists were performed using DAVID software. The most enriched annotations for spiral ganglion seemed to involve synapse and neuronal differentiation. While for the cochlea, deafness, tight junction and neuron differentiation, and for vestibule ear development and cell junction categories were the most enriched annotations. The study provides insights into the genes specifying the development of inner ear in human, contributing to our understanding of mechanisms of deafness and to the development of new therapeutic approaches for hearing disabilities.

POSTER SESSION II
AGING AND VESTIBULAR DISORDES

P14
EPIGENETICS AND AGING

Ken-ichi Watanabe, Kimihiro Ohkubo
Dept. of Otolaryngology, Nippon Medical School, Tokyo, Japan

Introduction. Epigenetic is to be involved in genetic and differentiation, such as, cancer, aging, metabolic disease, abnormal differentiation. Distinctive mechanism is the methylation of modifications and DNA of the histone protein. We examined methylation of DNA using aged mice in this study.
Material and Methods. We were using the female mice (C57/BL6). All procedures were performed under anesthesia by xylazinhydrochloride administration 5% ketaminhydrochloride and 2%. This experiment was conducted with the permission of the facility animal experimentation ethics committee.

Animals were divided into the young group (8 weeks of age) and the age groups (132 weeks). After deep anesthesia the animals were rapidly excised on both sides of the inner ear bone capsule and cooled in dry ice.
The inner ear tissues were homogenized, DNA extraction kit (Norgen Genomic DNA Isolation Kit, # 24700) was used to extract DNA. We performed bisulfite process (Epigenetek Inc.) in some specimens. These products inducible nitric oxide synthase (iNOS, Quiagen, Inc.) as primers, it was measured by qPCR (ΔΔ CT method).
Result. It were no significant differences in qPCR at a young age and age group in qPCR.
Consideration. From previous reports epigenetics has been known to cause modification of the methylation and histone proteins of the DNA in various diseases related to aging. Histone protein modifications primarily due to acetylation and methylation. In this study, it has been speculated that some oxidative stresses including iNOS, however, it was not clear. In the future, we are using a next-generation sequencer, and is expected to further advance the research.

P15
AGE-RELATED DIFFERENCE IN THE EFFECTS OF PARVALBUMIN DEFICIENCY ON ACOUSTIC STARTLE RESPONSE AND PREPULSE INHIBITION IN MICE
Jana Burianová[1], Beat Schwaller[2], Josef Syka[1]
[1]Department of Auditory Neuroscience, Institute of Experimental Medicine of the Czech Academy of Sciences, Prague, Czech Republic; [2]Anatomy Unit, Department of Medicine, University of Fribourg, Switzerland

Objective. In comparison with wild type mice, parvalbumin-deficient (PV-/-) mice exhibit differences in locomotor behavior (Farré-Castany, 2007) as well as in the strength of the acoustic startle response (ASR) and prepulse inhibition (PPI) (Popelář et al. 2013). However, whether these differences change over time remains unknown.
Methods. In the present study the strength of the ASR and PPI was examined in 1, 5, and 16-month-old parvalbumin-deficient mice and compared with age-matched controls, C57/BL/6J. The ASR in response to a broad-band noise impulse or a tone pip (4-16 kHz) and the PPI of ASR elicited by a broad-band noise impulse or 8 kHz tones over a range of 20-80 dB SPL were recorded. Hearing function in the mice was evaluated by recording the auditory brainstem responses (ABRs).
Results. The hearing thresholds in both strains were similar: the difference in thresholds did not exceed 10 dB. The ASR and PPI of the 1-month-old mice were comparable in both strains. However, the parvalbumin deficiency was manifested in the middle aged mice by a less efficient PPI when 8 kHz tones were used. In old mice, this pattern was found to be reversed. The PV (-/-) mice exhibited more efficient PPI (particularly when using a broad-band noise impulse) and more pronounced ASR in 120 dB pips.
Conclusions. These results demonstrate the partially beneficial effect of parvalbumin deficiency on the ASR and PPI in old mice. However, the underlying mechanisms are largely unknown and deserve to be explored in more detail.
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P16
AUDITORY FUNCTION, HOMEOSTASIS AND AGEING IN *DROSOPHILA MELANOGASTER*
Camille Tardieu, Liza Malong, Nicholas Boyd-Gibbins, Ryan *Kavlie, Jonathan Gale, Joerg Albert UCL Ear Institute, London, UK*

Homeostasis maintains the normal function of sensory organs across the life span. Here we use the fruit fly *Drosophila* to study the homeostasis of hearing with a specific emphasis on the core machinery responsible for converting sound into neuronal excitation - the auditory transducer complex.
Firstly, our Laser-Doppler vibrometric analyses of auditory mechanics and extracellular nerve recordings indicate that there are both biomechanical and electrophysiological changes in the fly ear throughout ageing. These analyses also reveal a phase of "auditory maturation" that precedes the age-related decline. Using *de novo* synthesis of NompC (TRPN1) - a core component of the fly's auditory transducer apparatus - in a *nompC* null mutant background, we find functional recovery of auditory transduction. These results suggest that core components of the fly's auditory transducer machinery are under dynamic or homeostatic regulation. To study the possible proteostasis of NompC directly, Fluorescence Recovery After Photobleaching (FRAP) experiments were performed in live animals. FRAP analysis demonstrated a virtually complete turnover of NompC molecules within 24 hours, indicating the translocation of new channels to the transducer sites in the distal cilium.
Findings suggest a new line of investigation into the mechanisms of hearing loss, which explores the possibility that a breakdown of transducer proteostasis is responsible for the observed age-related functional decline. In order to provide a more complete understanding of how ageing alters protein expression in the auditory organ, we use high-throughput RNA-sequencing combined with complementary quantitative real time PCR (qPCR) and bioinformatic analyses. We not only show an age-related decrease in the expression of transducer channel candidates (NompC, Inactive and Nanchung) and their transporter dTulp, but also increased expression consistent with the phase of maturation in young flies.
Our study intends to offer a deeper comprehension of how the homeostatic machinery of the auditory system copes with endogenous, e.g. age-related, or also exogenous, e.g. noise-induced perturbations. We aim to help create new tools, and recovery strategies that can protect the auditory system from damage.

P17
PRESBYCUSIS AS BIOLOGICAL MARKER OF AGING PROCESS: THE ROLE OF NAT2 AND GRM7 GENES
J. Chora[1], T.D. Matos[1], G. Fialho[1], H. Caria[1,2]
[1]BioISI, Biosystems & Integrative Sciences Institute Faculty of Science of the University of Lisbon, Portugal; [2]ESS/IPS, School of Health, Polytechnic Institute of Setúbal, Portugal

Presbycusis or age-related hearing loss (ARHL), the most common sensory impairment in the elderly, affects approximately 60% of all individuals over 65 years in the world. A strong association between ARHL, depression and cognitive functional decline is described and after a certain age a shift in the hearing threshold is defined internationally for men and women. Physical health plays an important role in the link between frailty and overall health (e.g. health status, familial and clinical history, sensory function).

For example, blood pressure can be a marker for hypertension, in which oxidative stress play a key pathological role. This and other physiological parameters can be considered to be biological age markers for chronological age identifying specific biological mechanisms. We have analyzed the NAT2 and GRM7 genes in the elderly individuals of the Portuguese population (n≅400), since both genes code for proteins that play an important role into the oxidative metabolism, and have been previously associated with ARHL. Our results, comprehending audiological evaluation, other epidemiological data and genetic analysis concerning the prevalence of variants in NAT2 and GRM7 genes, will be presented and discussed also considering socio-demographic parameters of the elderly individuals of the sample. We will present the statistical analysis concerning the existence of natural patterns of hearing decrease considering both the shape and the magnitude of the audiological curve. In conclusion, we will debate biological and clinical aspects, taking into consideration both the genetic results from the Portuguese population with ARHL and the social dimensions of ageing. Thus, this study expects to contribute to the promotion of active ageing and, in consequence, to the elimination of some ageism present in societies. It also shows that presbycusis can be used as biomarker through the study of this genes.

P18

UNILATERAL INTRATYMPANIC ADMINISTRATION OF GENTAMICIN CREATES OPTIMAL VESTIBULAR DISORDER MODEL IN GUINEA PIGS

Makoto Chiba, Tsukasa Ito, Chikako Shinkawa, Seiji Kakehata
Yamagata University, Faculty of Medicine, Department of Otolaryngology, Head and Neck Surgery, Yamagata-shi, Japan

Objectives. Gentamicin is the default standard for creating vestibular disorder models. However, whether gentamicin is best administered systemically or locally has yet to be rigorously studied from the standpoints of optimal administration method, optimal dosage and mortality rates. We thus compared the efficacy of the systemic intraperitoneal administration method versus the local intratympanic administration method in terms of effect on vestibular damage and mortality rate in guinea pigs. Vestibular damage was measured by the caloric test and damage to the hair cells.
Methods. Guinea pigs were divided into a single intraperitoneal administration group and 4 intratympanic administration subgroups for a total of 5 groups. The intraperitoneal group (Group 1) received daily intraperitoneal injections of gentamicin (40 mg/ml) for a period of 10 consecutive days, at a daily dose of 125 mg/kg body weight. The intratympanic administration group was further divided into 4 subgroups with Group 2A treated with gentamicin-soaked gelatin sponges (40/100/200/300 mg/ml) placed in the tympanic cavity of both ears; Group 2B treated with an intratympanic gentamicin (40 mg/ml) injection given to each ear separately under 2 hours of anesthesia; Group 2C treated with an intratympanic gentamicin (40 mg/ml) injection given to each ear separately under 6 hours of anesthesia; and Group 2D treated with an intratympanic gentamicin (40 mg/ml) injection on the left ear only under 2 hours of anesthesia. Each animal underwent caloric test 2 weeks later and was then sacrificed for morphological study.
Results. An examination of the vestibular damage revealed moderate to high levels of damage in Groups 1, 2B, 2C and 2D, however the mortality rate was also high in Groups 1, 2B and 2C. In contrast, both vestibular damage and mortality rate were low in Group 2A. Groups 2B and 2D exhibited moderate hair cell loss while Group 2C exhibited extremely high hair cell loss.
Conclusion. These results suggests that the best vestibular disorder model which balances vestibular damage and mortality rate is achieved by intratympanic administration of gentamicin unilaterally as demonstrated by Group 2D.

P19

EFFECT OF INTRA-TYMPANIC ISOSORBIDE ON ENDOLYMPHATIC HYDROPS IN NEW ANIMAL MODEL

Minbum Kim
Catholic Kwandong University, International St. Mary's Hospital, Department of Otorhinolaryngology-Head & Neck Surgery, Seo-Gu Incheon, Korea

Objectives. The aim was to investigate efficacy and safety of intra-tympanic injection of isosorbide (IT-ISB) in new animal model.
Methods. Forty male guinea pigs were used. Isosorbidewas administered via intratympanic injection. Intracochlear concentration was analyzed by HPLC-RI. In normal animals, change of ABR threshold and middle ear mucosa was observed after IT- ISB for evaluation of toxicity. 'Chronic hydrops model' was made 12 weeks after endolymphatic sac ablation. 'Acute attack model' was induced by desmopressin injection (SQ,100ug/Kg) 4 weeks after sac ablation. Histologic section was made to confirm improvement of endolymphatic hydrops after IT-ISB. Vestibular function was evaluated with animal rotator before and after IT-ISB in both models.
Results. (1) Isosorbide can rapidly pass through the round window membrane (RWM) after round window perfusion (RWP). Over a 6-hour, RWP for 30 mins can deliver higher concentrations of isosorbide into perilymph than those achieved with PO. (2) Similar intracochlear concentration was measured between 50% and 100% IT-ISB, however, significantly lower concentration was observed after 25% IT-ISB ($p = 0.004, 0.005$). (3) In terms of toxicity, neither ABR and middle ear mucosa change was observed 1week after 25, 50% IT-ISB. However, swollen middle ear mucosa and otorrhea was observed after 100% IT-ISB (2/5). (4) In chronic hydrops model, hydrops was reduced histologically after IT-ISB. However, no significant change of vestibular function was induced. In acute attack model, not only histologic reduction of hydrops but also recovery of symmetric vestibular response was observed after IT-ISB. **Conclusion.** Isosorbide can rapidly pass through the round window membrane after IT. IT-50% ISB was safe and effective. The improvement of hydrops and the recovery of vestibular function were observed in new acute hydrops model after IT-ISB. IT-ISB could be a treatment option for acute attack of endolymphatic hydrops.

P20

VESTIBULAR COMPENSATION AFTER COCHLEAR IMPLANTATION: AN ANIMAL RESEARCH

Yong-Ho Park
Chungnam National University, Otolaryngology, Daejeon, Korea

Dizziness or vertigo could be occurred commonly after cochlear implantation surgery especially in early stage. Although the surgical approaches for the cochlea might disturb the cochlear homeostatsis but it could affect vestibular organs also. In this study, we aimed

to investigate the effect of cochleostomy on vestibular organ through the time sequence after cochleostomy using the functional and morphological studies in animal model. As a result, functional losses were observed in early stage and those findings were sustained until twenty days after cochleostomy. Histopathological findings were showed the mild peripheral vestibular organ damages. Increased c-fos immunoreactivities of vestibular nucleus were observed in early stage after cochleostomy and those immunoreactivities were decreased with time course. Cochleostomy would be a risk factor for peripheral vestibular organ damage and it could bring functional impairment of peripheral vestibular organs. Functional compensation would be happen through the increased vestibular nucleus activities.

P21

QUANTIFICATION OF THE ENDOLYMPHATIC HYDROPS IN MÉNIÈRE'S DISEASE USING CONTRAST ENHANCED SMALL ANIMAL MRI

M. Müller[1], J.G. Mannheim[2], A.M. Schmid[2], H. Kumagami[3], S. Vollmer[4], U. Gottwald[4], B.J. Pichler[2], H. Löwenheim[2]
[1]Department of Otolaryngology, Head and Neck Surgery, Carl von Ossietzky University Oldenburg, Germany; [2] Werner Siemens Imaging Center, Department of Preclinical Imaging and Radiopharmacy, Eberhard Karls University Tübingen, Germany; [3]Department of Otolaryngology-Head and Neck Surgery, Nagasaki University Graduate School of Biomedical Sciences, Nagasaki, Japan; [4]Bayer Pharma AG, Global Drug Discovery, Berlin, Germany

Morbus Ménière is characterized by hearing loss, tinnitus and vertigo. It is assumed that the expansion of volume in the endolymphatic system can cause these symptoms. Hydrops usually affects the ductus cochlearis and the sacculus in the labyrinthine membrane. Recent advances in MR imaging have allowed the volumetric quantification of the endolymphatic hydrops and hence allow *in vivo* diagnosis. Eight guinea pigs underwent a unilateral surgical obliteration of the ductus endolymphaticus. To quantify volume change of the fluid spaces in the inner ear, guinea pigs were *i.v.* injected with a gadolinium based contrast agent. MR images were acquired at a voxel size of 0.102 x 0.102 x 0.3 mm^3. MR imaging was performed one week before, 1, 3, 5 and 7 weeks after obliteration. After the last MRI scan, the inner ear function of the animals was examined using electrocochleography, and inner ears were fixed. 1 week after obliteration volume of scala media had increased in 5 of 9 animals at the expense of the volume of the scala vestibuli (max. 3.1 to 1.5 mm^3). Three weeks after obliteration 2 out of those 5 animals showed still an increase of the volume of the scala. For one animal that didn't show a volume change in week 1, an increase of volume was detected in week 3 (decrease of scala vestibuli 2.7 to 1.1 mm^3). For the remaining 4 animals, that showed a volume increase in week 1, the volume decreased back to baseline levels. Five and 7 weeks after obliteration 2 animals had an enlargement of the scala media. Volume of the scala tympani was stable over time. The electrophysiological examination did not show a significant difference between the control and the obliterated ear. Histological staining showed a good correlation with the MRI results. To our knowledge this is the first *in vivo* study showing the onset of the endolymphatic hydrops one week after surgical obliteration. However, further studies are required to optimize the surgery to obtain a more stable hydrops. Nevertheless, this animal model showed a high potential of being a suitable tool to study potential therapies.

P22

INFLUENCE OF EXTERO- AND PROPRIOCEPTIVE AFFERENTS OF THE PLANTAR SURFACE IN DETERMINING SUBJECTIVE VISUAL VERTICAL IN PATIENTS WITH UNILATERAL VESTIBULAR DYSFUNCTION

Mario Faralli[1], Vito Enrico Pettorossi[2], Giampietro Ricci[1]
[1]Perugia Hospital, Perugia, Italy; [2] University of Perugia, Dept of Experimental Medicine, Sector Physiology and Biochemistry, Perugia, Italy

Subjective visual vertical (SVV) refers to individual ability to indicate what is a perfectly vertical line in specific experimental conditions. Although the otolith organs play a key role in the perception of verticality, the contribution of other systems (visual, proprioceptive) cannot be overlook ed. Aim of this study was to test the hypothesis that extero- proprioceptive signals, particularly from the plantar surface of the foot, can influence the evolution of SVV following unilateral acute vestibular dysfunction (AVD). In the event of unilateral AVD, the subject tilts the upper end of a bar towards the affected ear, shifting by several degrees with respect to the gravitational axis. SVV was studied in 40 consecutive patients with unilateral AVD. It was first measured 1-2 days after onset of symptoms. Baseline and provocative tests were performed: In the baseline tests patient stands in direct contact with the floor, while in provocative test patient stands on a soft support between the feet and the floor. Based on a comparison between baseline and provocative test, the patients were divided into three groups: group A: patients showing larger deviation of SVV in provocative test ($p<0.05$); group B: patients showing a larger deviation in the baseline test ($p < 0.05$); group C: patients showing no significant changes ($p>0.05$). The tests were repeated at 30, 90 and 180 days. At the end, a persistent alteration in SVV perception was noted in 87% of the patients of group B, 31% of the patients of group C but none in the patients from group A, all of whom were able to correct the perception error since the second examination (90 days). The study demonstrates that normalisation of SVV in subjects with unilateral AVD seems to be influenced by the possibility of exploiting extra-vestibular sensory information, particularly extero- and proprioceptive information from the plantar surface.

P23

AFTER EFFECTS OF GALVANIC STIMULATION ON SELF MOTION PERCEPTION DEPEND ON THE INTENSITY AND INTERVAL OF STIMULATION PATTERNS

Aldo Ferraresi, Roberto Panichi, Chiara Occhigrossi, Silvarosa Grassi, Vito Enrico Pettorossi
University of Perugia, Dept of Experimental Medicine, Sector Physiology and Biochemistry, Perugia, Italy

It has been recently shown that motion perception is influenced by previous movement experience, for enhancing the dynamic sensitivity of the vestibular system. As a result of adaptive mechanism, perception of fast rotation persistently increases and that of slow rotation decreases when fast and slow whole body rotation are delivered in sequence (asymmetric rotation) (Pettorossi et, al J Physiol, 2012). Moreover, repetitive slow rotations cancel these after-effects.

The mechanisms underneath these behavioural long term changes may reside in synaptic plasticity of the vestibular nuclei neurons.

In fact, we showed in vitro experiments that high frequency stimulation (HFS) induces LTP and low frequency stimulation (LFS) cancels already settled LTP (Grassi et al, J Neurosci. 1996). Moreover, HFS with short train intervals (1 sec) induced LTP while HFS with longer train intervals (>6 sec) elicited LTD (Scarduzio et al Neurosci. 2012).
To know whether the plasticity observed in vitro experiments can be responsible of the behavioural findings, we simulate these experiments by using galvanic stimulation in vivo. In 10 subjects we examined the motion perception during 4 cycles of whole body asymmetric rotations in the dark after conditioning monopolar galvanic stimulation with different intensity (0.5 or 1 mA) and different interval between two consecutive stimuli (1 sec or 7 sec).
We found that high intensity (1 mA) and short interval (1 sec) galvanic stimulation induced long lasting enhancement of motion perception (53±15%) during rotation toward the cathode and reduction (20±11%) in the opposite direction. Conversely, low intensity (0.5 mA) or longer stimulus intervals (7 sec) had the reverse effect or cancelled the previous conditioned responses. This demonstrates that the vestibular responsiveness showed different after-effects depending on the intensity or frequency of the conditioning stimulation patterns. The characteristics of the facilitator and inhibitor galvanic patterns are similar to those used in vitro for inducing LTP and LTD, respectively. This findings suggest that the behavioural adaptive mechanism occurring during sequences of vestibular stimulation reflects the plastic events taking place within the vestibular circuitry at level of the vestibular nuclei.

P24
SELF-MOTION PERCEPTION IN VESTIBULAR COMPENSATION

Roberto Panichi[1], Mario Faralli[2], Chiara Occhigrossi[1], Aldo Ferraresi[1], Adolfo M Bronstein[3], Vito Enrico Pettorossi[1]
[1] University of Perugia, Dept of Experimental Medicine, Sector Physiology and Biochemistry, Perugia, Italy; [2]Perugia Hospital, Perugia, Italy; [3] University of London, Imperial College Sci Technol & Med, Academy Dept Neurootology, London, England

The self-motion perception was examined in thirty patients at 1 week, 6 and 12 months after an acute episode of vestibular neuritis that induced an unilateral vestibular deficit. The motion perception was evaluated by considering the reproduction in space of remembered visual target during two series of 4 cycles of opposite directed whole body asymmetric rotations in the dark (back and forth sinusoidal composed by fast half cycle followed by slow half cycle). Compared with normal subjects, all patients showed an altered motion perception. The error in space reproduction normally induced by asymmetric rotation, was increased at the end of first series of stimulus with half cycle toward the healthy side and decreased at the end of second series with fast half cycle toward the damaged side. This directional preponderance was remarkable at 1 week after the acute episode and gradually decreased in the following tests, but it was still abnormal 12 months later. The comparison of the motion perception with the responses to caloric test, head shaking test and the subjective visual vertical evidenced a dissociate time course of compensation in ~ 50% of patients with a slower recover of motion perception. Moreover, DHI was also administered at 12 months for evaluating the Quality of Life (QoL) affected by dizziness. The DHI score was still high at 12 months showing a moderate/severe handicap and correlated with the directional preponderance of the self-motion perception. Conversely, correlation was not observed for the other test values. We concluded first, that the compensation of motion perception is a more demanding and variable process than that of the other vestibular symptoms, suggesting a different mechanism taking place in the central nervous system. Second, the subjective perception of quality of life powerfully depends on the level of the recovery of self-motion perception rather than on the balance of ocular reflex vestibular responses and subjective vertical perception. The persistent self-motion perception imbalance may explain the high score of the DHI in vestibular neuritis patients and testing self-motion perception may be helpful to better understand the reason of the dizziness persistence and confirm the DHI results.

P25
PSYCHIATRIC COMORBIDITY IN PATIENTS WITH DIZZINESS AND THE THERAPY OF PSYCHOTROPIC DRUGS

Kensuke Kiyomizu[1,2], Keiji Matsuda[2], Koji Torihara[2], Yasushi Ishida[3], Kensei Yoshida[1], Tetsuya Tono[2]
[1]Yoshida Hospital, Department of Psychiatry, Nobeoka, Japan; [2]Miyazaki Medical College, University of Miyazaki, Department of Otorhinolaryngology, Miyazaki, Japan; [3]Miyazaki Medical College, University of Miyazaki, Department of Psychiatry, Miyazaki, Japan

Objective. We reported neuro-otological findings in psychiatric patients with nystagmus (Eur Arch Otorhinolaryngol. 268(12):1713-9 2011). In this study, we investigated 3 types of *psychogenic dizziness (PsD)* (narrow type, wide type and *psychiatric (Psy) comorbidity*) and the therapy of psychotropic drugs.
Methods. The 847 patients (272 men, 575 women, age range, 7-95; mean age ± SD 60.3 ± 18.0 years) with dizziness were classified as otolaryngologic (Otola) disorders (D): dizziness of unknown cause (DUC) in 354 (41.8%), otogenic vertigo (OV) in 169 (20.0%), Meniere's disease (MD) in 119 (14.0%), chronic cerebral insufficiency in 111 (13.1%),
BPPV in 54 (6.4%) and other types of diseases in 40 (4.7%).
Results. PsD narrow type was revealed in 150 (17.7%). Psy comorbidity was revealed in 554 (65.4%). Of 554 patients, various types of Psy D were found, such as anxiety or panic D (F41) in 310 (56.0%), mood D (F3) in 101 (18.4%), adjustment D or post-traumatic stress D (F43) in 27 (4.9%), dissociative D (F44) in 7 (1.3%), other neurotic D (F48) in 18 (3.2%), organic mental D (F0) in 47 (8.5%) and schizophrenia (F2) in 26 (4.7%). These patients were not only treated by otolaryngologists, but also received Psy therapy, and 432 (78%) of these patients were prescribed psychotropic drugs. Minor tranquilizer was prescribed in 300 (69.4%), sleep medication in 142 (32.9%), antidepressant in 102 (23.6%). major tranquilizer in 75 (17.4%), anti- epileptic drugs in 27 (6.3%), lithium carbonate in 6 (1.4%), anti-
Parkinson's disease drugs in 18 (4.2%), other drugs in 32 (7.4%).
Conclusions. We believe that collaboration between psychiatrists and otolaryngologists in the hospital and/or doctors in local area can improve the mental condition and the quality of life of patients who are suffering from dizziness with psychiatric comorbidity.

P26

CERVICAL NEURO-MUSCULAR SYNDROME PRESENTS AS VERTIGO. CASE REPORT

Aikaterini Drylli, Georgios Markogiannakis, Georgios Psilovasilopoulos, Ioannis Mylonakis, Christos Kelesis, Vasileios Varsos

Neurosurgery Clinic, General Hospital Athens. Hellinic Red Cross''Korgialeneio-Benakeio", Athens, Greece

Introduction. Vertigo involves a perceived movement either of one's own body, such as swaying or rotation, or of the environment, or both. Alongside headache, instability and vertigo are among the more common symptoms with which patients present to physicians in general. Their lifetime prevalence is approximately 20% to 30%.
Case Report. We report the case of a 73-year-old man, without particular past medical history, presenting for a period of 1,5 years with symptoms of vertigo, instability and cervical headache, which temporary improved after receiving medication and bed rest. The symptomatology was further complicated with the appearance of numbness and right upper extremity weakness for the last six months. On admission to the Neurosurgery Clinic, the neurological examination indicated a mild impairment of muscle strength with accompanying numbness of the right upper limb, without deficit of deep sensation or recto-bladder disorders. The MRI of cervical spine demonstrated a posterior right prolapse of intervertebral disk A5-A6 and A6-A7 and cervical spinal stenosis in the corresponding levels. The patient underwent an anterior cervical diskectomy at accessed levels A5-A6 and A6-A7, with synchronous placement of fitting cages and spondylodesis with plate / screws of vertebral bodies A5, A6 and A7. The patient was discharged from the clinic with significant improvement of his symptoms.
Conclusion. Vertigo and instability can be caused by disturbances of factors associated with the balance system, either of sensory, visual, vestibular, neurologic, or muscular origin. Function of all these components deteriorates with ageing. The diagnosis of Cervical Neuro-Muscular Syndrome is based on focused interview of the patient's history and an intensive clinical examination. Imaging of the neck can be of particular significance in certain cases.

P27

CLINICAL CHARACTERISTICS OF ACUTE VESTIBULAR NEURITIS ACCORDING TO THE INVOLVEMENT SITE

Ryung Chae

Department of Otolaryngology-Head and Neck Surgery, Kangbuk Samsung Hospital, Sungkyunkwan University School of Medicine, Seoul, South Korea

Objectives. Vestibular neuritis(VN) mostly involves the superior portion of the vestibular nerve. Inferior vestibular neuritis(IVN) is a relatively minor subtype. This study aimed to describe the clinical features of VN involving the inferior vestibular labyrinth and its afferents only.
Methods. We retrospectively reviewed the clinical characteristics of patients with IVN and compared them with those of superior VN(SVN) and total(superior and inferior) VN(TVN), which were also diagnosed on the basis of symptoms and the clinical vestibular response measurements. Caloric response, cVEMP, vHIT responses were measured in 133 patients with VN. The patients were classified into three group: (1)TVN group, who showed asymmetric responses in both caloric and cVEMP responses;(2)SVN group, who showed asymmetric caloric responses but symmetrical cVEMP response;(3) IVN group, who showed asymmetric cVEMP responses but symmetrical caloric responses. Furthermore, we studied correlation between cVEMP, vHIT and Caloric responses.
Results. Of the 133 patients with VN, 16(12.03%) were classified as having IVN. IVN patients with down beating nystagmus were 3(18.75%). The time to remission of nystagmus with IVN(10.26±7.94days) was significantly shorter than with TVN and SVN(21.0±33.3days,21.5±26.8days).Durationofhospitalizationand follow up period of patients with IVN(3.6±1.6days, 31.3±40.6days) were shorter than those of SVN(4.6±1.5days,63.7±83.6days) and TVN(4.6±1.6days,59.0±79.9days). CP value of patients with IVN(10.4±5.6) was lower than those of patients with TVN and SVN(76.5±28.5,81.7±25.5). The amplitudes of spontaneous nystagmus and HSN of patients with IVN(5.1±3.8,8.1±5.3)were smaller than those of patients with TVN(8.3±5.2,11.3±6.1)and SVN(10.1±6.5,13.2±6.8). Furthermore, the rates of horizontal and anterior semicircular canals deficit in canal paresis group were 81.9%, 76.1%, and 58.6% of the cases with canal paresis showed catch up saccade. 25.7% of the patients with cVEMP asymmetry showed posterior semicircular canal deficit.
Conclusion. Inferior vestibular neuritis(IVN) is minor subtype of VN. Clinical charateristics are different from those of SVN and TVN. We found that the utricular and saccular nerves recover first, and ampullary nerves recover subsequently. We confirmed cVEMP and vHIT testing are helpful to diagnose the site of VN a degree.

P28

EPIDEMIOLOGY OF VERTIGO ON HOSPITAL EMERGENCY

Ana Rita Lameiras[1], Luís Reis[1,2], Ricardo Santos[1], Pedro Escada[1,2,3]

[1]Centro Hospitalar de Lisboa Ocidental EPE / Hospital Egas Moniz, ENT Service, Lisbon, Portugal; [2]CEFITEC, Faculty of Sciences and Technology, New University of Lisbon, Portugal; [3]FCM/UNL, Department of Anatomy, Faculty of Medical Sciences, New University of Lisbon, Portugal

Introduction. Vertigo is one of the most frequent motives in seeking specialized medical care in an emergency. The aim of this study is to evaluate the epidemiological characteristics associated with vertigo in a sample of patients who used the health care Emergency Department of Otorhinolaryngology of the Egas Moniz Hospital during a period of four years and, in particular, analyse the possible seasonality of vertigo.
Material and Methods. The project was conceived as a descriptive epidemiological study of population type, retrospective, during the period from 2010 to 2013. A total population of 40173 patients attended during this period the Emergency Department of Otolaryngology. The primary objective is to realize the relevance of the balance disorders in the urgency of this specialty. The parameters studied included age, gender, annual number of cases (total and percentage), date of crisis of vertigo, seasonal distribution by seasons and annual proportion of hospitalized cases.
Results. A total of 4347 patients (10.8 percent) sought medical attention due to dizziness/balance disorders over the four years of the study. There has been an annual increase in the number of cases between 7.6% and 17%. Women were more often affected (68,3%) and crises occurred more often in individuals between 60 and 79 years of age (40%). The cases were distributed heterogeneous

between the seasons, having more episodes of dizziness in summer and autumn and with an increasing trend between 2010 and 2013. The number of hospitalizations has increased annually over this period.
Discussion. The epidemiology of vertigo and vestibular disorders is still a specific field to study, because it may be useful for clinical decision-making and health care planning.
Conclusion. The study revealed that cases of vertigo in urgency rise annually and are more frequent in women, in the elderly population and in Summer and Autumn.

P29
OCULAR VESTIBULAR EVOKED MYOGENIC POTENTIAL TESTING FOR THE PROGNOSIS OF BELL'S PALSY
Hyun Joon Shim[1], Eun Woong Ryu[2], Moon Suh Park[2], Seung Geun Yeo[2], Jae Yong Byun[2]
[1]Eulji University School of Medicine, Eulji Medical Center, Department of Otorhinolaryngology-Head and Neck Surgery, Seoul, South Korea; [2]Kyung Hee University, College of Medicine, Kyung Hee University Hospital at Gang dong, Department of Otolaryngology, Head and Neck Surgery, Seoul, South Korea

Background and Objectives. Several studies have reported about asymptomatic involvement of vestibular nerve in cases of Bell's palsy. The basic hypothesis for these consequence is the close proximity of the vestibular and facial nerves in the internal auditory canal (IAC). The aim of this study was to investigate the correlation between the prognosis and ocular vestibular evoked myogenic potential (oVEMP) in Bell's palsy.
Materials and Methods. From Jan. 2012 to Dec. 2014, a total of 104 consecutive patients with Bell's palsy were enrolled. Patients were divided into two groups through the final recovery state; complete recovery group and an incomplete recovery group. oVEMP within 1week after onset, Electroneurography (ENoG), Electromyography (EMG), Ipsilesional pure tone audiometry (PTA) and scaling with initial and final House-Brackmann grades were retrospectively examined in 85 complete recovery patients and 19 incomplete recovery patients.
Results. Eighty-five patients recovered completely and nineteen patients recovered incompletely. The mean value of ocular vemp asymmetry was significantly higher in incomplete recovery group than complete recovery group. ($P < 0.05$). In the analysis for evaluating the factors acting on the prognosis of the Bell's palsy, there was no association between the rate of ipsilesional PTA threshold, caloric test and rate of abnormal EMG and recovery state. But, the initial state of ENoG, initial H-B grade and the rate of oVEMP were significantly correlated with the rate of recovery. ($P = 0.025$, $P = 0.013$, $P = 0.009$)
Conclusion. oVEMP could be a useful tool for predicting the prognosis of Bell's palsy comparable to ENoG and H-B grade.

P30
EFFECT OF MICROPRESSURE TREATMENT ON ENDOLYMPHATIC HYDROPS
M. Barbara, E.Covelli, L.Volpini, S.Monini
Department of NESMOS, University of Rome, Italy

Pressure treatment is regarded as one therapeutical option in case of Meniere's disease, an inner ear disorder thought to be characterized by endolymphatic hydrops.
Electrocochleography (EcochG) is regarded to be a sensible tool for detection of endolymphatic hydrops when the SP/AP ratio goes beyond 0.5. A micropressure treatment was administered to a cohort of definite Meniere's patients refractory to medical therapy for at least 1 year. EcochG was performed before and 1, 3 and 6 months after micropressure treatment delivered by the Meniett device. The results post-treatment condition was assessed via the DHI questionnaire that was filled at the same time interval.
A hydropic condition was found in all the treated subjects before starting the pressure treatment. One month after, most of the subjects felt relief from the disease despite nearly all of them still displayed an hydropic ECochG pattern. Three and 6 months after the treatment, a significant improvement at DHI corresponded to a remarkable reduction of the SP/AP ratio, i.e. absence of endolymphatic hydrops.
Pressure treatment as delivered by the Meniett device shows to improve patient's symptomatology. This finding seems to also correspond to an improvement of the hydropic condition, as assessed by electrocochleography.

POSTER SESSION III
MOLECULAR BIOLOGY

P31
FURTHER CHARACTERIZATION OF THE RECENTLY DESCRIBED *SLC26A4* C.918+2T>C MUTATION AND REPORTING OF A NOVEL VARIANT OF UNKNOWN CLINICAL SIGNIFICANCE
A.C. Gonçalves[1*], R. Santos[2], A. O'Neill[2,3], J. O'Neill[3,4], P. Escada[2], F. Fialho[1], H. Caria[1,5]
*[1]BioISI, Biosystems & Integrative Sciences Institute Faculty of Science of the University of Lisbon, Portugal; [2]ENT-CHLO, HEM, ENT Service, Centro Hospitalar de Lisboa Ocidental EPE, Hospital Egas Moniz, Portugal; [3]FCM/UNL, Department of Anatomy, Faculty of Medical Sciences, New University of Lisbon, Portugal; [4]CEFITEC, Faculty of Sciences and Technology, New University of Lisbon, Portugal; [5]ESS/IPS, School of Health, Polytechnic Institute of Setúbal, Portugal *Present address: UCL Ear Institute, 332 Gray's Inn Road, London WC1X 8EE, United Kingdom*

Pendred syndrome (PS) is the second most common type of autosomal recessive syndromic hearing loss (HL). It is characterised by sensorineural HL and goiter with occasional hypothyroidism. These features are generally accompanied by malformations of the inner ear, as enlarged vestibular aqueduct. In about 50% of the probands, mutations in the *SLC26A4* gene, coding for pendrin, are the cause of the disease. Here we report the case of a Portuguese female patient, aged 47, presenting severe to profound HL and hypothyroidism. Her mother and sister, already deceased, had also suffered from HL and goiter. After MRI and CT scanning, it could be observed manifested enlarged vestibular aqueduct and endolymphatic sac. Additional videonystagmography revealed bilateral hyporeflexia. Molecular study of the patient included screening of *GJB2* (Cx26) coding region, *GJB6* (Cx30) common deletions, and all *SLC26A4* exons, as well as intronic regions 8 and 14. A new mutation, c.918+2T>C, recently described in

heterozigosity, was found in homozygosity in the intronic region 7 of the *SLC26A4* gene. This mutation, occurring in the second nucleotide of the intronic region 7 of the *SLC26A4* gene, which corresponds to a splicing donor site, is predicted to eliminate the respective splicing event, leading to skipping of the exon 8. The Pendred phenotype presented by the patient is most probably due to the homozigosity observed for this mutation. Whilst sequencing control samples, a novel mutation c.821C>G was also found in heterozygosity in the exon 7 of *SLC26A4* gene and is predicted to be damaging. This study reports the finding of two novel *SLC26A4* genotypes and provides new insight on the phenotypic features associated with PS.

P32

DIFFERENTIAL ROLE OF NO-SENSITIVE GYANYLYL CYCLASE ISOFORMS NO-GC1 AND NO-GC2 IN AUDITORY FUNCTION IN ADULT MICE

Dorit Möhrle[1], Nicole Eichert[1], Steffen Wolter[1], Evanthia Mergia[2], Doris Koesling[2], Andreas Friebe[3], Marlies Knipper[1], Lukas Rüttiger[1]

[1]University of Tübingen, Department of Otolaryngology, Tübingen Hearing Research Centre (THRC), Molecular Physiology of Hearing, Tübingen, Germany; [2]University of Bochum, Department of Pharmacology and Toxicology, Bochum, Germany; [3]University of Würzburg, Department of Physiology, Würzburg, Germany

Objective. In the inner ear, elevated cyclic guanosine 3'-5'-monophospate (cGMP) levels were shown to have a sheltering role for cochlear hair cells and hearing function. However, the individual roles of the two nitric oxide-sensitive guanylyl cyclase isoforms (NO-GC1 and NO-GC2) as cGMP generators in this protective effect are still unclear.

The aim of this study was to investigate how the deletion of either one of the α-subunits of NO-GC (NO-GC1 KO or NO-GC2 KO) or deletion of the β_1-subunit of NO-GC (NO-GC KO), leading to global lack of NO-GC expression, affects hearing function, vulnerability to noise exposure and recovery from acoustic trauma in mice.

Methods. Hearing thresholds and supra-threshold auditory processing at sensation level of NO-GC knockout and wildtype mice were analyzed by measuring the auditory brainstem responses (ABRs). Outer hair cell function was assessed by the distortion product of the otoacoustic emissions (DPOAEs). ABRs and DPOAEs were recorded before and after exposure to intense noise, leading to auditory trauma. Immunohistochemistry was performed on cochlear sections.

Results. Comparison between the NO-GC1 KO, NO-GC2 KO, NO-GC KO and wildtype mice suggests a differential role of the two NO-GC isoforms in auditory function. NO-GC knockout mice strains showed similar hearing thresholds, but different vulnerability to acoustic noise exposure, presumably involving compensatory upregulation of distinct efferent feedback loops (lateral and medial olivocochlear system).

Conclusions. The results suggest non-redundant roles of the two NO-GC isoforms in auditory function. The results will be discussed regarding NO-GC as a proposed cGMP generator in functionally distinct parts of the auditory pathway and considering NO/cGMP-signaling as an otoprotective cascade after noise-induced damage of the ear.

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P33

REPRODUCTION OF VARIOUS TYPES OF COCHLEAR GAP JUNCTION PLAQUES IN HUMAN CELL LINE

Kaori Hatakeyama, Katsuhisa Ikeda, Kazusaku Kamiya

Department of Otorhinolaryngology, Juntendo University Faculty of Medicine, Tokyo, Japan

Objectives. The mutations in connexin26 (Cx26), a cochlear gap junction protein, represent a major cause of pre-lingual, non-syndromic deafness, as they are responsible for as many as 50% of such cases in certain population. Recently, we reported that Cx26-dependent gap junction plaque (GJP) disruption occured as the earliest change during embryonic development, results in a drastic reduction in the GJP area and the protein levels in Cx26 mutant mouse models (Kamiya et al., J Clin Invest, 2014;124(4)1598-1607) and Brn4 deficient mice, a model of DFN3 non-syndromic deafness. To elucidate the mechanism of this biochemical change, we developed the molecular live imaging system targeting GJP composed of Cx26 and Cx30. Our final goal is to screen the chemicals to stabilize the cochlear GJPs at the cell borders.

Methods. The cells with the transient expression and stable expression of human wild type Cx26, mutant Cx26 (R75W) and wild type Cx30 untagged and tagged with GFP or mCherry were generated with HEK293 and HeLa cell lines.

Results. With our newly generated connexin expressing cells, we observed various types of GJPs as well as Cx26-mutant mouse cochleae. Our system enabled us to analyze the formation, trafficking, membrane integration and degradation of GJPs composed of Cx26 and Cx30 in live cell monitoring.

Conclusions. These imaging systems will enable the large scale drag screening targeting GJP formation and stabilization for Cx26 associated deafness.

P34

CAVEOLINS ACCUMULATES AT THE ORGAN OF CORTI IN GJB2 ASSOCIATED DEAFNESS

Takashi Anzai, Kazusaku Kamiya, Katsuhisa Ikeda

Department of Otorhinolaryngology, Juntendo University Faculty of Medicine, Bunkyo-ku, Tokyo, Japan

Introduction and Purpose. The mutations in GJB2 which encodes Connexin (Cx) 26, a cochlear gap junction protein, represent a major cause of pre-lingual, non-syndromic deafness. Recently, we demonstrated that Cx26 mutation resulted in a drastic reduction in the gap junction plaque (GJP) and caveolins associated with the GJP disruption (Kamiya, J Clin Invest, 2014). Late onset degeneration of the organ of Corti observed in our two Cx26 mutant mice are thought to be a secondary pathology of the hearing loss which can be rescued by gene transfer of Gjb2 (Iizuka, Hum Mol Genet, 2015). In this study focused on caveolin protein which may be an important factor of this degeneration. Caveolins are integrated plasma membrane protein and structural component of caveolae membranes. Recent studies showed that the over expression or abnormal localization of caveolins associated with delayed wound healing or cellar aging in several organs. The purpose of this study is to investigate the association of caveolins in the pathology of Cx26 related hearing loss.

Method. We analysed the expression and localization Caveolin-2 in the Cx26-deficient mouse (Cx26f/f P0-Cre) with localized gene deletion in the inner ear under the control of the protein 0 (P0) promoter.

Results. We observed significantly increased protein level of Caveolin-2 in Cx26 mutant cochleae. In Cx26 mutant mice, the organ of Corti revealed compression and squeezing of the OHC by the surrounding supporting cells. Although, only diffused labelling of caveolins were observed in the control mice, there were accumulated caveolins in the organ of Corti in Cx26 mutant mice. Especially, these accumulations were notably observed in the outer hair cells (OHCs), Deiter's cells and pillar cells. The cells with abnormal accumulated caveolins were significantly increased in Cx26 mutant mouse. We focused on OHCs and find that the shape of OHCs changed into hourglass like shape and accumulated caveolins on the membrane.
Conclusion. In this study, we suggested that caveolins in cochlea may play a crucial role in the progress of GJB2 associated deafness.

P35
SAMPLING OF HUMAN PERILYMPH AND PROTEOME ANALYSIS OF PERILYMPH BY MASS SPECTROMETRY
Heike Schmitt[1], Andreas Pich[2], Giorgio Lilli[1], Günter Reuter[1], Thomas Lenarz[1]
[1]ENT Department, Hannover Medical School, Hanover, Germany; [2]Core Facility Proteomics, Hannover Medical School, Hanover, Germany

Objective. The background information about etiology and pathophysiology of the inner ear (IE) hearing loss is still very limited. As a consequence of the difficult access to structures and fluids of the IE, knowledge about numerous biochemical and physical parameters of perilymph is insufficient. Also testing of hypotheses derived from clinical observations is highly challenging. By advanced experience in cochlear implantation the sampling of human perilymph during surgeries is possible. Perilymph sampling is performed carefully by a developed sampling method. The analysis of sampled human perilymph by shot-gun proteomics enables the identification of protein composition of perilymph per se and possible changes of perilymph composition might be significant for sensorineural hearing loss.
Methods. Sampling of human perilymph was performed during IE surgeries with modified micro-glass-capillaries via the round window. It was crucial to avoid contamination of the samples and to gain a sufficient volume of perilymph for analysis. Protein content of the human perilymph samples was analyzed by the dotMETRIC™ Assay (G-Biosciences) and individual proteins were identified by a shot-gun proteomics approach and data-dependent analysis using orbitrap mass spectrometry (Thermo Fisher Scientific). Max Quant software was used for identification and quantification.
Results. In total 41 perilymph samples with volumes of 1.5-10 µl were obtained and analyzed by mass spectrometry revealing about 300 different proteins per sample and approximately 1000 different proteins in total. Protein composition of perilymph samples was compared to corresponding serum and liquor samples. All body fluids shared several similarities but at least 204 proteins were solely identified in perilymph. Samples were grouped according to age and type of surgery as well as audiogram data. Proteins with statistically significant changes between different sample groups were subjected to classification by GO annotations.
Conclusions. In-depth proteome analyses of perilymph samples identified about 1000 different proteins displaying a specific protein pattern for perilymph. Some indications for proteins specific to particular subgroups were found. However, further perilymph samples have to be analyzed for confirmation that these candidates might be marker proteins specific for certain diseases of the IE.
Supported by the DFG Cluster of Excellence EXC 1077/1 "Hearing4all".

P36
A TRANSGENIC MOUSE MODEL (BLEV) FOR VISUALIZATION OF THE DIFFERENTIAL USAGE OF BDNF EXON IV AND VI
Dario Campanelli[1], Wibke Singer[1], Eleonora Passeri[1], Hyun-Soon Geisler[1], Da Guo[2], Florian Mayer[2], Jing Hu[2], Verena Bautze[3], Jörg Strotmann[3], Ulrike Zimmermann[1], Lukas Rüttiger[1], Rama Panford-Walsh[1], Marlies Knipper[1]
[1]University of Tübingen, Department Of Otorhinolaryngology, Hearing Research Center Tübingen, Molecular Neurobiology, Tübingen, Germany; [2]Werner Reichardt Centre for Integrative Neuroscience, Sensory Mechanotransduction, Tübingen, Germany; [3]University of Hohenheim, Fg. Physiology, Stuttgart, Germany

Objective. Brain-derived neurotrophic factor (BDNF) is a key modulator of neuronal plasticity events (for reviews see: Bramham, 2005, Rauskolb et al.,2010). The transcription of the BDNF gene in rodents is mediated by eight alternative promoters linked to eight 5' untranslated exons that result in different stability, translatability and targeting of each of the transcripts (Pattabiraman, 2005, Timmusk et al., 1995). Among the untranslated transcripts BDNF exon IV and VI have previously got particular attention as they both have been shown to alter their expression in the hippocampus in an activity dependent manner (Chapman et al., 2012). The elucidation of the differential usage of the distinct BDNF promoters is one of the most intriguing and challenging current research fields in neuroscience as tools which allow the selective monitoring or deletion of the activity dependent usage of the transcripts are missing.
We here developed an alternative approach to achieve insight in the selective BDNF transcript function.
Methods. A transgenic mouse line was generated in which the transcription of BDNF exon IV and VI can be visualized in parallel via either a cyan (BDNF exon IV) or a yellow (BDNF exon VI) fluorescent protein without affecting the basic BDNF expression.
Results. With the designed construct the previously described differential expression pattern of BDNF exon IV CFP and exon VI YFP is observed in primary neuronal culture. The Blev mice are viable, fertile, and have normal life span and gain of weight. The BDNF expression levels are normal and not accidentally altered by the transgene. The activity-dependent usage of BDNF exon IV CFP and exon VI YFP is shown by an increased expression in the hippocampus after kainate treatment. Furthermore, a stimulus dependent expression pattern of BDNF exon IV CFP and exon VI YFP is seen in the hippocampus and the auditory cortex after different sound exposure protocols which is related to the number of inner hair cell ribbon synapses
Conclusions. The BLEV mouse line is a promising model organism to monitor the activity-dependent expression of BDNF exon IV and VI during sound/trauma-induced central plasticity changes.
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P37

ANALYSES OF THE DIFFERENTIAL USAGE OF BDNF EXON IV AND VI UNDER DIFFERENT SOUND EXPOSURE PARADIGMS USING A TRANSGENIC BDNF MOUSE LINE (BLEV)

Giulia Asola, Wibke Singer, Dario Campanelli, Hyun-Soon Geisler, Ulrike Zimmermann, Lukas Rüttiger, Rama Panford-Walsh, Marlies Knipper

University of Tübingen, Department Of Otorhinolaryngology, Hearing Research Center Tübingen, Molecular Neurobiology, Tübingen, Germany

Brain-derived neurotrophic factor (BDNF) is a key modulator of neuronal plasticity events (for reviews see: Bramham, 2005, Rauskolb et al.,2010). The transcription of the BDNF gene in rodents is mediated by eight alternative promoters linked to eight 5' untranslated exons that result in different stability, translatability and targeting of each of the transcripts (Pattabiraman, 2005, Timmusk et al., 1995). Among the untranslated transcripts BDNF exon IV and VI have previously got particular attention as they both have been shown to alter their expression in the hippocampus in an activity dependent manner (Chapman et al., 2012). The elucidation of the differential usage of the distinct BDNF promoters is one of the most intriguing and challenging current research fields in neuroscience as tools which allow the selective monitoring or deletion of the activity dependent usage of the transcripts are missing.

We here chose an alternative approach to achieve insight in the selective BDNF transcript function. A transgenic mouse line is generated in which the transcription of BDNF exon IV and VI can be visualized in parallel via either a cyan (BDNF exon IV) or a yellow (BDNF exon VI) fluorescent protein without affecting the basic BDNF expression.

Using defined enriched to traumatic auditory stimuli (Singer et al., 2013), we here observe a differential stimulus dependent YFP (BDNF exon VI) and CFP (BDNF exon IV) pattern in the ascending auditory pathway.

Hearing function, supra threshold ABR waves, IHC synapse morphology, and inhibitory/excitatory maker proteins are analysed in the ascending auditory pathway and the limbic system in young and aged mice.

We here describe interesting new insights for monitoring age-dependent plasticity changes related to BDNF.

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P38

DELETION OF EHD4 DISTURBS HEARING FUNCTION

Yan Zhu[1], Jens Schaller[2], Markus Plomann[2], Marlies Knipper[1], Lukas Rüttiger[1]

[1]University of Tübingen, Department of Otolaryngology, Tübingen, Germany; [2]University of Cologne, Institute for Biochemistry 2, Cologne, Germany

The recycling of membrane proteins is vital for multiple cellular processes including nutrient uptake, the regulation of surface receptors, adhesion molecules and ion channels, and synaptic vesicle retrieval in neurons. The mechanisms regulating these processes are mediated by multiple dynamic protein complexes. Previous studies identified the EHD proteins as components of these complexes that also play a role in tissue development and function, however, only a few studies emphasize the process of receptor internalization and recycling in hair cells and hearing function. Here, we investigated the expression of EHD4 in the cochlea and its role in the hearing function. The expression of EHD4 in the hair cells was investigated using high resolution deconvoluted flourescence microcscopy and immunostaining with specific antibodies on cochlea sections and whole mount preparations. Hearing measurements were conducted on EHD4 knock-out (KO) mice and matched wild-type (WT) littermates at different age. Stimulus-evoked auditory brainstem response (ABR), suprathreshold ABR and distortion product otoacoustic emission (DPOAE) were measured and analyzed in respect of ABR/DPOAE thresholds and DPOAE amplitudes/growth functions. The findings are discussed in the context of a possible novel role for EHD4 in linking endocytosis and/or recycling processes with hearing function.

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PEX5 DEFICIENCY RESULTS IN HEARING LOSS

Jae-Young Lim[1], Min-Soo Kim[1], Joon No Lee[1], Se-Jin Kim[1], Seong-Kyu Choe[1], Channy Park[2], Raekil Park[1*]

[1]Department of Microbiology, Center for Metabolic Function Regulation, Wonkwang University School of Medicine, Iksan, Jeonbuk, South Korea; [2]Department of Head and Neck Surgery David Geffen School of Medicine at UCLA, Los Angeles, USA

Peroxisomes are subcellular organelles that are involved in various metabolic reactions, including fatty acid oxidation, bile acid synthesis, plasmalogen biosynthesis, and detoxification of reactive oxygen species. Mutations in genes involved in peroxisome biogenesis or peroxisomal function cause inheritable genetic disorders. Notably, a sensory deafness has been known as one of the clinical symptoms associated with peroxisome defects, such as Zellweger syndrome, infantile Refsum disease, acyl-CoA Oxidase deficiency, and D-bifunctional protein deficiency. However, the mechanism of hearing loss caused by peroxisome dysfunction is unclear. Since PEX5 plays an important role for peroxisome biogenesis and its function, we generated cochlea-specific PEX5 knockout mice to test the role of peroxisomes in auditory system. The results show that PEX5 deficiency in cochlea causes hearing impairment, damage of stereocilia and spiral ganglion neuron degeneration. Our findings provide new insights for understanding the link between peroxisomes and hearing function.

P40

HOTSPOT-MUTATION ANALYSIS OF THE EGFR, KRAS, BRAF PATHWAY IN SPORADIC VESTIBULAR SCHWANNOMAS

Igor Stenin[1], Stefan Hansen[2], Karl Schäfer[3], Jörg Schipper[1]

[1]University Hospital Duesseldorf, Department of Otorhinolaryngology, Duesseldorf, Germany; [2]University Hospital Essen, Department of Otorhinolaryngology, Essen, Germany; [3]University Hospital Duesseldorf, Department of Pathology, Duesseldorf, Germany

Introduction. Vestibular schwannomas (VS) are benign tumors arising from the Schwann cells of the of the vestibular nerve. Established therapeutical options are „wait and scan" and invasive surgical tumor removal or radiosurgery. As various studies provided evidence of overexpression and activation of epidermal growth

factor receptor (EGFR) family in VS, the inhibition of the EGFR pathway emerged as a possible non-invasive therapeutical option. Indeed, tyrosin kinase inhibitors (TKI) such as lapatinib (dual inhibitor of EGFR and ErbB2) or erlotinib (inhibitor of EGFR) reduce VS growth and proliferation and the growth of VS xenografts. Mutations of EFGR and the downstream signaling proteins KRAS and BRAF are known to have major impact on clinical efficacy of EGFR TKI. In this study we characterized the mutation profile of EGFR, KRAS and BRAF in 20 sporadic VS tumor specimen.

Materials and Methods. 20 VS specimen were acquired during surgical removal of the tumor. The specimen were formalin-fixed and-embedded in paraffin blocks. DNA was extracted using the Qiagen BioRobot EZ1. After quantification and purification of the DNA, EGFR (Exon 18, 19, 21), KRAS (Exon 2) and BRAF (Exon 15) hotspot mutations were analyzed employing Sanger sequencing method.

Results. DNA extraction and mutation analysis from paraffin embedded blocks was successful. EGFR, KRAS, BRAF showed no mutation in the 25 sporadic VS tumor specimen, all samples revealed wild type status.

Conclusions. We conclude that EGFR, KRAS, BRAF mutations are not involved in the pathophysiology of VS. Potential treatment of VS with TRK is not affected by EGFR, KRAS, BRAF pathway mutation status. Further investigations are needed to identify additional targets and prognostic markers.

P41
EXPRESSION PATTERN OF GRHL2, AN AGE-RELATED HEARING IMPAIRMENT GENE, IN COMMON MARMOSET (CALLITHRIX JACCHUS) INNER EAR

Makoto Hosoya[1], Masato Fujioka[1], Takashi Inoue[2], Hideyuki Okano[3], Kaoru Ogawa[1]

[1]Dept. of Otolaryngology, Keio Univ. School of Medicine, Tokyo, Japan; [2]Central Institute for Experimental Animals, Kanagawa, Japan; [3]Dept. of Physiology, Keio Univ. School of Medicine, Tokyo, Japan

Introduction. GRHL2, also known as TFCP2L3, is a transcriptional member of grh/CP2 family, a mammalian homolog of the Drosophila gene grainyhead. The mutations of GRHL2 gene cause DFNA28 and the locus associates with progressive, down-sloping hearing loss (Peters LM, 2002). GRHL2 is also known as a novel transcription factor that binds to and regulates the activity of the human telomerase reverse transcriptase gene promoter and age-related hearing impairment-related genes (Van Laer et al., 2008). In the mouse model, targeted null of GRHL2 was embryonic lethal and thus no animal model has been available for the deafness study (Rifat et al., 2010). To elucidate the discrepancy of the phenotype among species, and to explore the pathophysiology of deafness associated with GRHL2 gene mutation, we examined expression of GRHL2 in the Common Marmoset (Callithrix jacchus), non-human primate, inner ear.

Material and Method. Young adult marmosets (n=4) were transcardially perfused with saline fixation with 4%PFA. The fixed temporal bone of marmoset was prepared in the cryosection after decalcification by EDTA. Immunohistochemistry for GRHL2 was performed with rabbit anti-GRHL2 antibody (1:200, HPA004820, SIGMA-ALDRICH).

Results. Our result clearly showed species-differences in the expression pattern of GRHL2. In mice, its expression in the cochlea was broadly detected in all types of epithelial cells (Linda M. Peters et al., 2002). In common marmoset, GRHL2 was observed epithelial cells but was absent from those in stria vascularis. Instead, there were additional expressions in the spiral ligament and spiral limbus.

Discussion and Conclusion. These results suggested that the phenotypical difference can be accounted by the species-differences in the expression pattern. Our detailed analyses of GRHL2 expression in the cochlea would be a key finding for understanding the disease mechanism of DFNA28 and age related hearing impairment. Common Marmoset would be a powerful and extensive tool for investigating pathophysiology of human auditory disorder that cannot be explained by rodent mutant models.

P42
ENDOPLASMIC RETICULUM STRESS MAY PARTICIPATE IN THE PATHOGENESIS OF AGE-RELATED HEARING LOSS AND CISPLATIN-INDUCED OTOTOXICITY

Wenwen Wang, Yu Sun, Weijia Kong

Department of Otorhinolaryngology, Union Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, China

Objective. To estimate the role of endoplasmic reticulum (ER) stress in the age-related hearing loss and cisplatin-induced ototoxicity.

Methods. Twenty C57BL/6 mice were involved in each experiment respectively. For the first study, 1-2 months old mice termed young group (n=10) and 12-14 months old mice termed aged group (n=10) were tested for auditory brainstem responses (ABR). The alternations of endoplasmic reticulum stress markers GRP78, CHOP, and caspase-12 were evaluated by western blot and immunochemistry during aging in the cochlea. For the second study, twenty mice were randomly divided into two groups, the control group and the cisplatin group. The mice from the cisplatin group were underwent a single intraperitoneal injection with cisplatin at a dose of 16mg/kg (body weight), while the saline were instead in the control group. ABR was recorded at 3 days after cisplatin treatment. The changes between the expressions of ER stress markers were also detected.

Results. A significant degeneration in the basal turn of the cochlea and hearing loss at the high frequency was found in the aged group. GRP78 was significantly decreased in the organ of Corti (OC) and spiral ganglion cells (SGCs) of the aged cochlea, while CHOP was remarkably increased in the OC, SGCs and stria vascularis (SV). Although caspase-12 was up-regulated, the cleavage of caspase-12 was not observed. In the study about cisplatin, an elevated hearing threshold at high frequency was recorded in the mice of the cisplatin group. GRP78, CHOP, and cleaved caspase-12 were increased after cisplatin treatment. CHOP was observed to be higher in the OC, SGCs, and particularly in the intermediate cell of the SV.

Conclusion. ER stress may be implicated in the pathogenesis of age-related and cisplatin-induced hearing loss. The difference between each kinds of hearing loss needs further study.

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EXPRESSION PATTERN OF WOLFLAMIN, THE WOLFRAM SYNDROME 1 GENE (WFS1) PRODUCT, IN COMMON MARMOSET (*CALLITHRIX JACCHUS*) INNER EAR

Noriomi Suzuki[1], Masato Fujioka[1], Makoto Hosoya[1], Naoki Oishi[1], Takashi Inoue[2], Hideyuki Okano[3], Kaoru Ogawa[1]
[1]Dept. of Otolaryngology, Keio Univ. School of Medicine, Tokyo, Japan; [2]Central Institute for Experimental Animals, Kanagawa, Japan; [3]Dept. of Physiology, Keio Univ. School of Medicine, Tokyo, Japan

Wolfram syndrome (OMIM: 222300) is an autosomal recessive disorder of the neuroendocrine system, known as DIDMOAD (Diabetes Insipidus, Diabetes Mellitus, Optic Atrophy, and Deafness) syndrome. Patients show mutations in the *WFS1 gene*. The *WFS1 gene* encodes an 890 amino acid protein, called Wolframin, that is predicted to have nine helical transmembrane segments in the endoplasmic reticulum (ER). Wolframin functions in ER calcium homeostasis and unfolded protein responses. Dysregulation of these cellular processes results in the development of ER stress, leading in apoptosis.

Hearing loss associated with Wolfram syndrome is typically a high frequency sensorineural hearing loss, although low frequencies may become affected as well. Limited literatures describing temporal bone pathology indicate both hair cell loss in the lower basal turn and the atrophy of stria vascularis in the apical turn. On the contrary, the expression of Wolframin protein in mice was observed widely and uniformly in the sensory epithelium but was absent in the stria vascularis. While WFS1 knockout mice suffer diabetes, the hearing level of the strain was completely normal.

In order to elucidate the discrepancy of the phenotype among species, and to explore the pathophysiology of deafness associated with *WFS1* gene mutation, we examined expression of Wolframin in the Common Marmoset (*Callithrix jacchus*), non-human primate, inner ear.

Young adult marmosets (n=2) were transcardially perfused with saline followed by fixative with 4%PFA. The fixed temporal bone of marmoset was prepared in the cryosection after decalcification by EDTA. Immunohistochemistry for WFS1 was performed with rabbit anti-WFS1 antibody 1:200 (HPA029128, SIGMA-ALDRICH). The result revealed strong immunoreactivity in outer hair cells, external sulcus cells and stria basal cells.

The expression pattern of Wolflamin in Common Marmoset inner ear was different from that of mouse, and the pattern may account for the hearing phenotypes in Wolfram syndrome patients. Common Marmoset would be a powerful and extensive tool for investigating pathophysiology of human auditory disorder that cannot be explained by rodent mutant models.

POSTER SESSION IV
COCHLEAR IMPLANTS

P44

SURGICAL OUTCOMES OF COCHLEAR IMPLANT IN PATIENTS WITH BONY COCHLEAR NERVE CANAL ATRESIA AND STENOSIS

Yun Suk An[1], Tae Su Kim[2], Kwang-Sun Lee[3]
[1]Department of Otorhinolaryngology - Head and Neck Surgery, Bundang Jesaeng Hospital, Daejin Medical Center, Seongnam, South Korea; [2]Department of Otolaryngology, Kangwon National University, Chuncheon, South Korea; [3]Department of Otolaryngology, Asan Medical Center, University of Ulsan College of Medicine, South Korea

Objectives. To determine the incidence and clinical characteristics of bony cochlear nerve canal (BCNC) anomalies and to compare speech perception after cochlear implantation (CI) between children with BCNC malformation and age-matched children with normal cochleae.

Design. This was a retrospective chart review of 851 patients who underwent either unilateral or bilateral CI at Asan Medical Center (Seoul, Korea) between January 2000 and December 2013. 44 patients (54 ears) with BCNC atresia or stenosis and 324 age-matched deaf children with non-syndromic normal inner ear who underwent CI were included.

Results. The mean age of BCNC atresia or stenosis at CI was 6.8 years. In the 54 BCNC anomaly ears, 44% had vestibular structural anomaly with normal cochlear structure. Internal auditory canal stenosis was found in 27 ears (50%). The average cross-sectional area (CSA) of the cochlear nerve (CN) was 50.2% smaller compared to that of the facial nerve (FN). The Categories of auditory performance (CAP), Meaningful auditory integration scale (MAIS), and open set mono- and bisyllable word tests of children with BCNC malformation were poor compared to those of age-matched normal controls ($p < 0.001$). The CAP, MAIS scores, and open set mono- and bisyllable word tests of children with CN deficiency were also poor compared to children with CN hypoplasia in BCNC anomalies ($p < 0.05$).

Conclusions. BCNC anomaly was found in 54 ears (6.3%) among 851 CI recipients. Most (44.4%) BCNC anomalies with CI showed vestibular structural anomaly with normal cochlear structure. Regardless of poor outcomes after CI, CI is a valid option for patients with BCNC anomalies with or without CND.

P45

THE DETERMINATION OF MOST COMFORTABLE LEVELS IN PATIENTS WITH A COCHLEAR IMPLANT WITH A HEARING LOSS AFTER MENINGITIS

Y.O. Radionova[1], N.N. Petrova[1], D.S. Klyachko[2]
[1]State Budgetary Educational Institution for Higher Professional Training «North-Western State Budgetary Medical University named after I.I. Mechnikov» of the Ministry of Health of the Russian Federation. ENT Department, Russia, Saint-Petersburg; [2]Saint Petersburg ENT and Speech Research Institute, Saint-Petersburg, Russia

Introduction. Fitting a speech processor (SP) in patients with a hearing loss after meningitis is particularly complex. The aim of

our investigation to identify the parameters of the fitting SP of patients with a meningitis deafness. This study used a computer with Maestro-4.2 software (MED-EL) connected to the DIB-2 programmer. For the registration of the stapedius reflex impedancemetry (AT-235h) in the decay mode was used.
Patients and Methods. The study group included 36 patients aged 1 to 60 years with a complete hearing loss after meningitis**.** The control group included 43 patients aged 1 to 60 years with a complete hearing loss not associated with meningitis or anomalies of the cochlea. In both groups, the average values of the impedances and the most comfortable levels (MCL) were calculated.
Discussion. The impedances of the basal and middle electrodes were significantly greater in patients with deafness after meneingitis than in the control group (p=0,0003). The average values of the MCL were significantly greater in patients.
with a hearing loss after meningitis than the control group (p=0.00001).
Correlation analyses showed that in patients with a complete hearing loss after meningitis the mean values of the impedances of each electrode and the average values of the MCL were correlated significantly (r = -0,85, p = 0,0004). In the control group the mean values of the impedances of each electrode and the average values of the MCL were not correlated significantly.
Regression analyses predicted the average values of MCL: y = -1,2552*x+30,615 (where "y" is calculated MCL, "x" is the mean value of the impedances of this electrode, "-1,2552" is the regression coefficient, "30,615" – free member) (p=0,0005, R2=0,71).
The data obtained can be used to configure the SPs of patients with sensorineural hearing loss after meningitis, however, for the purpose of increasing the accuracy of approximation of the initial data, and to identify additional correlations, further research is required.

P46
INTRAINDIVIDUAL COMPARISON OF PSYCHOPHYSICAL PARAMETERS BETWEEN PERIMODIOLAR AND LATERAL-TYPE ELECTRODE ARRAYS IN PATIENTS WITH BILATERAL COCHLEAR IMPLANTS

Junhui Jeong[1], Ji Hye Heo[2], Young Joon Seo[3], In Seok Moon[2], Jae Young Choi[2]
[1]Department of Otorhinolaryngology, Seoul Medical Center, Seoul, South Korea, [2]Department of Otorhinolaryngology, Yonsei University College of Medicine, Seoul, South Korea, and [3]Department of Otolaryngology-Head and Neck Surgery, Yonsei University Wonju College of Medicine, Wonju, South Korea

Objective. Perimodiolar electrode arrays were developed to improve stimulation of specific neuronal populations and to decrease power consumption; however, they can damage the cochlear structure. We examined and compared psychophysical parameters of perimodiolar and lateral-type electrode arrays in patients who received a different type of bilateral cochlear implant (CI) in each ear.
Study Design. Retrospective analysis.
Setting. Tertiary referral center.
Patients. Eight child patients (three males, five females) received a different CI in each ear (perimodiolar array and lateral array). They received the CIs sequentially (n = 7) or simultaneously (n = 1).
Interventions. Diagnostic, therapeutic, and rehabilitative.
Main Outcome Measures and Methods. Electrically evoked compound action potential, threshold level, comfort level, and dynamic range (DR) of the basal, mid, and apical electrodes were compared. We also surveyed battery consumption for each device.
Results. Electrically evoked compound action potential threshold, threshold level, and comfort level were lower for the perimodiolar-type electrode array than for the lateral-type electrode array in most patients. However, the DR for the perimodiolar array was narrower than for the lateral array. For most patients, there was little difference in battery life.
Conclusions. Although the level of electrical energy required for auditory stimulation seems to be lower for the perimodiolar electrode array than for the laterally placed array, the DR was wider and the amount of battery consumption was similar. The electrode array should be chosen by considering various patient factors, such as residual hearing.

P47
COCHLEAR IMPLANTS IN RECIPIENTS WITH SINGLE SIDED DEAFNESS: DIRECTIONAL HEARING, SPEECH INTELLIGIBILITY, LOUDNESS BALANCE AND PITCH MATCHING

Mark Praetorius[1], Maria Rösli-Khabas[2], Sebastian Hoth[2]
[1]Division of Otology and Neurotology; [2]Division of Audiology; Department of Otorhinolaryngology, Head and Neck Surgery, Heidelberg, Germany

Objective. To validate the benefit of cochlear implants (CI) in patients with single sided deafness (SSD).
Material and Methods. 20 patients with normal pure tone audiogram (n=8) or moderate hearing loss (n=12) in one ear, equipped with CI systems MED-EL SONATA/CONCERTO + OPUS2 (n=12), COCHLEAR CI24RE(ST) + CP810 (n=7) and Advanced Bionics HiRes90K + Harmony (n=1) on the contralateral ear and having at least 6 months of CI experience were tested with respect to directional hearing, speech perception in noise, binaural loudness matching (ABLB) and binaural pitch matching. 26 normal hearing controls were included for normative references.
Results. Addition of the CI significantly improves in directional hearing (hit rate improved from 14.9% to 15.6%, RMS error decreased from 125° to 93°) and in speech perception in noise (SRT improved from -2.3 to -6.0 dBsnr median), equivalent to a BILD = 3.7 dB). Alternate binaural loudness balancing showed that matching takes place at levels between 48 and 55 dB HL (group averages). In the pitch matching experiment, the standard deviation of the relative interaural frequency difference at 0.5, 1 and 2 kHz amounted to 24.5%, 22.8% and 24.0% respectively (compared to 11.7%, 14.4% and 12.3% in the control group).
Conclusions. In case of single sided deafness, the provision with CI considerably improves the audiological performance in terms of directional hearing, binaural signal equivalence and speech perception.

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OUTCOMES OF COCHLEAR IMPLANTATIONS IN PATIENTS WITH NARROW BONY COCHLEAR NERVE CANAL

Juyong Chung[1], Jeong Hun Jang[2], Jae-Jin Song[3], So Young Kim[3], Jun Ho Lee[3], Seung Ha Oh[3], Sun O Chang[4]
[1]Department of Otorhinolaryngology, Wonkwang University School of Medicine, Iksan, South Korea; [2]Department of Otorhinolaryngology, Kyungpook National University, College of Medicine, Taegu, South Korea; [3]Department of Otorhinolaryngology, Seoul National University College of Medicine, South Korea; [4]Department of Otolaryngology, Kangbuk Samsung Hospital, Sungkyunkwan University, School of Medicine, Jongno-Gu, Seoul, South Korea

Narrow bony cochlear nerve canal (BCNC) is a cause of sensorineural hearing loss (SNHL) that frequently necessitates cochlear implantation (CI), but its relevance on post-CI outcome is poorly understood. The aim of this study was to evaluate the implication of the width of BCNC on post-CI speech performance. A retrospective review of radiologic findings and post-CI speech performance was conducted on 452 pediatric patients that underwent CI from 2005 through 2012 at Seoul National University Children's Hospital. A BCNC was considered to be narrow when the diameter was less than 1.4mm on axial temporal bone computed tomography. Based on our previous article in 2012, the mean width of patients with normal BCNC is higher than 2.0 mm. The patients were divided into 3 groups according to the width of BCNC (Group1, 17ears: diameter < 1.4 mm, Group2, 14ears: 1.4 – 2.0 mm, Group3, 25ears: > 2.0 mm). Also, the presence and the diameter of the cochlear nerve were evaluated by reviewing internal auditory canal magnetic resonance imaging. A cochlear nerve deficiency (CND) was defined as a cochlear nerve that is smaller in diameter when compared with the adjacent facial nerve in the mid-portion of the internal auditory canal. Speech performance of the subjects was evaluated by category of auditory performance (CAP) score, open set score and picture vocabulary test.

CND was more frequently found in Group1. With regard to open set score and Picture Vocabulary test, Group1 and Group 2 patients showed significantly worse results than Group 3 patients ($P < 0.05$). The speech performance of patients with intact cochlear nerve was significantly better than those with CND ($P < 0.05$). Among patients with intact cochlear nerve and the BCNC width ≥ 1.4 mm, Group 2 showed significantly worse speech outcome as compared with Group 3. Speech performance at 24months after CIs was positively correlated with the widths of BCNC. Therefore, patients with narrow BCNC or CND seem to have poor outcome.

Narrow BCNC is an important prognostic factor indicating higher rates of CND and poor outcomes. The measurements of the BCNC can help to predict the outcomes of cochlear implantations.

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COCHLEAR IMPLANT DEVICE EXTRUSION SUCCESSFULLY MANAGED WITH ADVANCED LOCAL FLAP

Min Kim, Woo Jin Kim, Jong Won Lee, Shi Nae Park, Sang Won Yeo
Department of Otorhinolaryngology-Head and Neck Surgery, College of Medicine, The Catholic University of Korea, Seoul, South Korea

Complications after cochlear implantation are problematic for both device users and surgeons. Device extrusion immediately after the surgery or as a delayed manner is one of the most serious complications which have been managed with explantationand re-implantation for many years.

Here, we report two cases of cochlear implant device extrusion successful treated with advance local flap. One of the patients (62-year old women) who had the irradiated wound showed the postoperative infection and eventual extrusion of the internal device. Second patient (59-year-old woman) demonstrated the delayed device extrusion. Both cases were surgically managed with the advanced local flap and there was no recurrence of the extrusion of the device.

From the aspects of the economics as well as the clinical situationsof the cochlear implantee, these types of reconstructive surgical methods for the extruded cochlear implant should be considered to optimize the functional results of the cochlear implantee.

P50

ELECTRICALLY EVOKED COMPOUND ACTION POTANTIAL RECORDING AS A PREDICTIVE FACTOR FOR SPEECH PERCEPTION IN COCHLEAR IMPLANT PATIENTS: A SYSTEMATIC REVIEW

Ruben van Eijl[1,2], Patrick Buitenhuis[1], Inge Stegeman[1], Sjaak Klis[1,2], Wilko Grolman[1,2]
[1]University Medical Center Utrecht, Department of Otorhinolaryngology-Head and Neck Surgery, Utrecht, The Netherlands; [2]University Medical Center Utrecht, Brain Center Rudolf Magnus, Utrecht, The Netherlands

Objective. Speech perception after cochlear implantation is still largely variable among patients. Research suggests degeneration of the auditory nerve hinders CI outcome and evoked potential measurements are correlated to the state of the auditory nerve. In search for a possible predictive factor for performance after implantation this review evaluates the correlation between speech perception and characteristics of the electrically evoked compound action potential (eCAP) of the auditory nerve in cochlear implant patients.

Methods. A systematic search was performed in the PubMed and Embase databases for articles containing all three major themes: cochlear implants, evoked potentials and speech perception or any related terms. Remaining articles after subsequent title-abstract and full-text screening were critically appraised based on relevance and validity of the study.

Results. Seventeen of the 968 identified articles investigated the correlation between speech perception and attributes of the eCAP recordings. These studies could be subdivided in three main groups discussing the correlation with the attributes: presence of the eCAP, recovery time constant or slope of the amplitude-growth function. In some studies steeper slopes and faster recovery seemed to predispose better speech perception, however significance was not unambiguous. Several other studies did find a significant correlation between performance and presence of the eCAP. Outside the three main groups neural adaptation, forward masking, spread of excitation, threshold and change with varying IPG were also investigated, but none were significantly correlated.

Conclusions. Only some significant correlations between speech perception and any of the investigated characteristics of the eCAP have been reported in literature, however they were not unambiguous. More research is needed to further investigate this relation.

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VALUE OF COMPUTATIONAL IMAGINE ANALYSIS IN DIAGNOSIS OF COCHLEAR OSSIFICATION

Xinbo Xu, Hanbing Zhang, Xiaojie Ma, Xiao Han
Department of Otolaryngology, Qilu Hospital of Shandong University, Jinan, China

Objective. Cochlear ossification is a thorny situation during CI procedure, mild to severe ossification will lead to the obstacle during electrode advancement, incomplete electrode insertion or even failure to indentify the scala tympani. Unfortunately, there is no effective method to predict the ossification. CT scan still get some limitation in intra-cochlear imagination. We combined the CT scan and computational imagine analysis to facilitate the diagnosis of cochlear ossification.

Methods. 5 cases with cochlear ossification were retrospectively reviewed, meanwhile 10 corresponding cases of normal CI were included to the contrast. Every patient performed CT scan, and the original DICOM database was exported to OsiriX, to generate an axially reconstructed cochlear imagine from round window in every 0.1mm, totally 5. CT values of tympanal half of every imagine were sampled, and the variance was calculated. Linear regression was applied to both groups using these date and statistical check was performed. Still, combined with the intra-operative situation, the results were checked visually.

Results. (1)Variances of both groups were performed chi-square test and the two groups showed a significant deviation; (2)group of normal CI had more significant disposition to linear regression while the group of cochlear ossification got more discrete plots.

Conclusions. Difference of the two groups showed the effect of cochlear ossification in CT scan, and these impalpable changes can be amplified by our computational imagine analysis method. It might get some clinical contribution to the diagnosis of cochlear ossification.

P52

INNER EAR STRUCTURES DAMAGE DURING COCHLEAR IMPLANTATION. ANALYSIS OF INSERTION FORCES AND CONE BEAM CT IN TEMPORAL BONE SPECIMENS

Daniele De Seta[1-3], Guillaume Kazmitcheff[2], Renato Torres[2], Evelyne Ferrary[1,2,] Olivier Sterkers[1, 2], Yann Nguyen[1,2]
[1]AP-HP, Groupe Hospitalier Pitié-Salpêtrière, Unité Otologie, Implants Auditifs et Chirurgie de la Base du Crâne, Paris, France ; [2]UMR-S 1159 Inserm, Université Paris 6 Pierre et Marie Curie, France ; [3]Sensory Organs Department, Sapienza University of Rome, Italy

Objective. To determine if the cochlear anatomy influences the friction forces and scalar translocation during cochlear implant (CI) insertion.

Study Design. 12 fresh temporal bones were implanted at constant speed with the aid of a motorized insertion tool. The MedEl flex 28 array was used in this study. During the insertion the data of insertion force was recorded with a six-axes force sensor. The maximal peak of force, the force momentum, the sudden rise of the force and the smoothness of the curve were calculated and studied. Pre- and post-implantation Cone Beam CT scans were performed in order to study the cochlear anatomy. Anatomical characteristics, position of the array in the cochlear lumen and force metrics were compared to post implantation radiological images.

Results. The mean cochlear diameter was 9.08 mm (range 8.42-9.49), the cochlear height 3.2mm (range 2.9-9.6) the angle between the 1st and 2nd turn varied between 13° and 18°. A full insertion of the electrode array was achieved in 8 cochleae, a partial insertion in 4 (1 or 2 electrodes outsides). The maximal peak of force was in mean 72.55 mN (range 38-139). The radiological analysis showed a translocation rate of 25% (4/12) from the scala tympani to scala vestibuli located in the region between 160° and 180°. In 6 cases the location of the apical electrode and in 4 cases for the electrodes in the basal turn was not possible to be defined due to intermediate radiological localization.

Conclusion. A low peak of forces during the insertion of the MedEl flex 28 array was recorded. The area at the opposite of the round window (around 180°), where the straight electrode arrays impact the lateral wall, represents the site at major risk for translocation. No relationship was found between insertion forces and cochlear anatomy nor between insertion forces and basilar membrane perforation, further histological analysis will confirm these results and would better define the translocation rate for this array.

P53

TRANSDUCTION OF A COMPLEX SIGNAL THROUGH THE NORMAL COCHLEA AND THROUGH THE COCHLEAR IMPLANT

Mona Ibrahim Morad[1], Mohamed Eid Ibrahim[2], Mohamed Aziz Mohamed Talaat[1], Mirhan Khamis Eldeeb[1], [3]Hicham G. Elmongui
[1]Alexandria University, Faculty of Medicine, Otorhinolaryngology Department, Unit of Audiovestibular Medicine, Alexandria, Egypt; [2]Alexandria University, Faculty of Medicine, Radiology Department, Alexandria, Egypt; [3]Alexandria University, Faculty of Engineering, Computer and Systems Engineering Department, Alexandria, Egypt

Introduction. A complex signal with known spectral and temporal characteristics is transduced and transcribed with high reliability in the normal cochlea and subsequently to the brainstem auditory pathways. The complex/ speech ABR is a good vehicle to study such physiology. Processors of cochlear implants are designed to mimic the physiology of speech extraction features.

Hypothesis. Acoustic speech stimulation through normal cochleae provoke mirror image responses in the brainstem auditory pathway. CI provoke brainstem responses similar to the acoustic speech stimulus.

Subjects. Ten children implanted with Med-EL standard electrode array with full insertion depth Normal hearing subjects served as controls.

Methods. Speech syllable 40 msec /da/ was used to elicit speech ABR using a speaker and a contralateral vertical electrode montage. For speech feature extraction, FS4 temporal weighting was used. I/O intensity function was done. Non-contrast multislice CT of the petrous bones to affirm full electrode insertion depth.

Results. Intensity I/O function showed very close similarity between individual traces of the same intensity. Response threshold was reached at 30 dBHL. The fast /d/ consonant stimulation projected wave V, mean latency 3.75 msec at 70 dB. The sustained component of the stimulus/a/ presented as the known FFR. Latencies of the peaks and morphology of the traces in cochlear implantees are compared to the controls and presented in the poster.

Discussion. The close proximity between cABR responses in cochlear implantees and in controls as shown in I/O function

traces suggest that simple speech stimuli can be encoded in the brainstem with a similar morphology through a cochlear implant. The unaltered latency within the cABR dynamic range reflects absence of cochlear excitation pattern. The ratios of wave V to FFR is discussed in terms of nerve fiber integrity. This high fidelity transcription is expected to feed the auditory cortex and association areas with fundamental speech characteristics that would provide for developing a robust and high quality acoustic signature.

Conclusion. Cochlear implant transcribes the speech signal with comparable features to the normal cochlea. Brain plasticity triggered by this high fidelity brainstem transmission should enhance audition and recognition.

P54

COCHLEAR OPTOGENETICS AND µLED IMPLANT APPLICATION

Christian Wrobel[1,9], Marcus Jeschke[1], Daniel Keppeler[1], Victor H. Hernandez[1,2,3], Anna Gehrt[1], Gerhard Hoch[1], Christian Goßler[6], Ulrich T Schwarz[5,6], Patrick Ruther[6], Michael Schwaerzle[6], Abhishek Raj[6], Roland Hessler[7], Tim Salditt[8,10], Nicola Strenzke[4], Sebastian Kügler[11], Tobias Moser[1,2,9]

[1]Institute for Auditory Neuroscience, University of Goettingen Medical Center, Goettingen, Germany; [2]Bernstein Focus for Neurotechnology, University of Goettingen, Goettingen, Germany; [3]Department of Chemistry, Electronics and Biomedical Engineering, Division of Sciences and Engineering, University of Guanajuato, Guanajuato, Mexico.; [4]Auditory Systems Physiology Group, Department of Otolaryngology, University Medical Center Goettingen, Goettingen, Germany.; [5]Fraunhofer Institute for Applied Solid State Physics (IAF), Freiburg, Germany; [6]Department of Microsystems Engineering (IMTEK), University of Freiburg, Freiburg, Germany; [7]MED-EL, Innsbruck, Austria and MED-EL Germany, Starnberg, Germany.; [8]Department of Physics, University of Goettingen, Goettingen, Germany.; [9]Collaborative Research Center 889, University of Goettingen Medical Center, Goettingen, Germany; [10]Center for Nanoscale Microscopy and Molecular Physiology of the Brain, University of Goettingen, Goettingen, Germany; [11]Department of Neurology, University Medical Center Goettingen, Goettingen, Germany.

Our project on optogenetic cochlear stimulation aims to improve the performance of cochlear implants (CI). Current electrical implants provide open speech comprehension in the majority of patients but CI-users have difficulties to understand speech in background noise. This drawback likely originates from a wide current spread around the implants electrodes which limits the number of separable stimulation channels and consequently frequency resolution. Since light can be conveniently confined in space, optical stimulation – via increasing the number of independent channels – is expected to enhance frequency and intensity coding.

Optical stimulation of spiral ganglion neurons (SGNs) was achieved by expressing light gated ion channels (channelrhodopsins). To specifically target SGNs we employed adeno-associated virus-mediated gene transfer (AAV) with injections either into the embryonic otocyst (trans-uterine) or the adult cochlea of mice and gerbils, respectively. Injected AAVs contained the DNA of either CatCh (Kleinlogel et al. 2011, a channelrhodopsin variant with high light-sensitivity) or Chronos (Klapoetke et al. 2014, a channelrhodopsin variant with high light-sensitivity and the fastest kinetics described so far) under the control of the human Synapsin-1 gene promoter. The expression pattern of these blue light gated channelrhodopsin variants in the somata and fibers of SGNs was demonstrated with immunohistochemistry. Electrophysiological approaches, including auditory brainstem response recordings, recordings from the auditory nerve as well as from the auditory cortex, revealed optical activation of the auditory pathway (Hernandez et al. 2014 and preliminary data).

µLED multi-channel implants were developed in collaboration with semiconductor experts and could recently be inserted into the cochlea of transgenic ChR2 expressing rats. Auditory brainstem responses demonstrated functional devices in vivo with sufficient light power to activate the auditory pathway.

P55

PREOPERATIVE INTRATYMPANIC GLUCOCORTICOID HYDROGELS: EFFECTS IN A COCHLEAR IMPLANT MODEL

Clemens Honeder[1], Chengjing Zhu[1], Julia Clara Gausterer[2],Hanna Schöpper[3], Elisabeth Engleder[2], Lukas Landegger[1], Manuel Walter[1], Franz Gabor[2], Christoph Arnoldner[1]

1Department of Otorhinolaryngology, Medical University of Vienna, Vienna, Austria; 2Department of Pharmaceutical Technology and Biopharmaceutics, University of Vienna, Vienna, Austria; 3 Department of Pathobiology, Institute of Anatomy, Histology and Embryology, University of Veterinary Medicine Vienna, Vienna, Austria

Objective. Thermoreversible hydrogels for intratympanic injection, which can deliver glucocorticoids for a prolonged period of time, are available for experimental studies. The literature indicates that the preoperative use of glucocorticoids reduces hearing threshold shifts caused by cochlear implantation. Also, early glucocorticoid application showed higher otoprotective effects than an application directly before electrode-insertion. As no data on very early application was available, the aim of this study was to evaluate the application of a 6% dexamethasone/poloxamer407 hydrogel 1 and 7 days prior to surgery for effects on postoperative hearing threshold shifts.

Methods. 1 day or 7 days before the implantation of a cochlear implant electrode, 50 µl of 6% dexamethasone-hydrogel or control-hydrogel were applied to the round window niche of the experimental guinea pigs (n=10/group). Hearing was evaluated by the measurement of compound action potentials before the application, pre- and postoperatively as well as after 3, 7, 14, 21 and 28 days. Stimuli used included clicks and tone-burst from 1 to 32kHz. At the end of the study, temporal bones were harvested and histologically evaluated.

Results. At some frequencies the application of the 6% dexamethasone-hydrogel one day before cochlear implantation resulted in reduced compound action potential threshold-shifts as compared to the control group ($p<0.05$)

Conclusions. The application of a glucocorticoid hydrogel one day prior to cochlear implantation appears to protect residual hearing. Such an application protocol could easily be translated into the clinical setting and should therefore be evaluated in clinical studies.

P56

GUIDED AUDITORY NEURON GROWTH ON TOPOGRAPHICALLY MODIFIED NANOCRYSTALLINE DIAMOND

Yixiao Cai[1], Fredrik Edin[2], Wei Liu[2], Helge Rask-Andersen[2], Mikael Karlsson[1], Hao Li[2]

[1]Department of Engineering Sciences, Applied Materials Science, Uppsala University, Uppsala, Sweden; [2]Otolaryngology and Head & Neck Surgery, Department of Surgical Sciences, Uppsala University, Uppsala, Sweden

Cochlear implants (CI) have successfully been used for several decades in patients with profound hearing loss. Nevertheless, results vary between individuals and resolution of fine structures in the acoustic signal is generally poor. The bottleneck problem is the ability to deliver independent stimulation signals to the auditory neurons. Electric stimulation using phased channels could be a plausible alternative. However, this method requires significantly more and closer neuron - electrode contacts with ordered re-growth of the regenerated axons, which current CI technology cannot provide. Diamond is well known for its chemical inertness, high mechanical strength, wear resistance and extraordinary electrical properties. Recently, surface modification technologies made it possible to guide cellular adhesion and cell migration. Here, we demonstrate the potential application of specific textured nanocrystalline diamond (NCD) surface as one novel candidate in otological implants. Such textured NCD surfaces, consisting of micrometer-sized nail-head-shaped pillars, are fabricated by a sequence of micro/nano-fabrication processes including sputtering, photolithography and plasma etching. Murine spiral ganglion explants were attached to the patterned NCD surface without the need of extra-cellular matrix protein coating. Scanning electron microscopy and confocal laser scanning microscopy revealed explants adhesion and neural growth path specifically along the nail-head-shaped NCD pillars in an ordered manner, rather than those unmodified areas.

In conclusion, our data demonstrate that NCD pillars have strong affinity to auditory neurons and can be used to guide neurons growing into a defined network. In addition, NCD pillars also provide a stop signal and prevent further migration of neurons into non-structured areas. Together with its anti-bacterial and electrical properties, patterned NCD surface may provide an optimal neural – electrode interface, a fundamental basis for independent electric stimulation signals in CIs.

P57

TRANSLATIONAL TOOLS FOR ANIMAL STUDIES IN COCHLEAR IMPLANT RESEARCH

Jonathon Kirk[1], Daniel Smyth[2], Kristien Verhoeven[2], Claudiu Treaba[1]

[1]Cochlear Americas, Centennial, Colorado, USA; [2]Cochlear Technology Center, Mechelen, Belgium

Animal studies play a crucial role in establishing the safety and efficacy of cochlear implants, and their associated stimulation strategies. The availability of effective, reliable and representative tools and analytical techniques is imperative to enable quality translational research. This poster describes the suite of tools and techniques Cochlear™ has developed and made available to enable implant research.

These tools include:

- Cochlear implant electrode arrays designed specifically with the common animal models in implant research in mind. These arrays more accurately simulate the relative device volumes and insertion depths achieved clinically.
- Cochlear implant stimulators and animal housings to enable acute and chronic electrical stimulation more closely resembling clinical stimulation strategies.
- Drug delivery systems incorporating cannulated electrode arrays and refillable pumps for pharmacological studies.

These are combined with advanced and innovative imaging, histology, computational modelling and analytical techniques to provide a complete suite of world-leading research tools.

P58

EFFECTS OF LOCALLY APPLIED GERANYLGERANYLACETONE (GGA) ON ELECTRODE INSERTION TRAUMA IN COCHLEA IMPLANTED GUINEA PIGS

Jochen Tillein[1,2], Felix Schmidt[1], Claudia Settevendemie[1], Susanne Braun[2], Sebastian Strieth[1], Timo Stöver[1]

[1]Department of Otorhinolaryngology, Goethe University, Frankfurt, Germany; [2]Medel Starnberg, Germany

Objective. Cochlear implantation in patients with residual hearing can lead to hearing loss caused by e.g. inflammation. Preserving hearing is essential in patients who are candidates for combined electric and acoustic stimulation (EAS) which provides an additional benefit over electrical stimulation alone especially in speech understanding in noise and music listening. Anti-inflammatory drugs such as glucocorticoids are one possibility to counteract loss of residual hearing.

We chose the anti-ulcer drug geranylgeranylacetone (GGA) that has been successfully tested to alleviate noise induced hearing loss and age related hearing loss. GGA is a non-toxic inducer of the heat shock protein HSF1 which inhibits inflammatory cytokines without having the undesirable side-effects of glucocorticoids.

Methods. Two groups of normal hearing Guinea pigs were implanted with a custom-made electrode (MED-EL). 3µl of the drug GGA (GGA-group), or ringer solution (Ri-group, control) was infused through the cochleostomy before implantation using a micro-syringe pump. In each group the second ear was treated likewise omitting implantation and served as an additional control. Hearing loss (HL) was tested before and after drug/Ri application at day 0, 3 and 7 postoperatively by measuring click evoked compound action potentials (CAPs) and frequency specific CAP audiograms via electrodes implanted near the round window. Additionally electrode impedances were determined for the implant contacts at each time point.

Results. All differences found between GGA and control group were not statistically significant. However, there was one visible trend: CAP-thresholds were higher at all frequencies at day 0 in the GGA treated group but decreased to lower thresholds compared to controls at days 3 and 7. Similar results but with less HL were found at the non-implanted side. There was also a trend for lower impedances in the GGA group. HL at the implanted side of both groups showed a gradient from high (30-40dB) at the base (site of electrode location) to low frequencies (5-15 dB) at the apex.

Conclusions. Although not significant, the slight decrease of CAP thresholds and impedances suggests that GGA might have the potential to preserve hearing after implantation. Higher dosage and/or sustained drug application could enhance the efficacy.

P59

PMDS BIOCOMPATIBILITY IN PC12 NEURONAL CELL LINE

Edi Simoni[1], Filippo Valente[1], Alice Cani[1], LauraAstolfi[1], Alessandro Martini[1,3]

[1]Bioacoustics Research Laboratory, Department of Neurosciences, University of Padua, Padua, Italy; [2]ENT Surgery, Department of Neurosciences, University of Padua, Italy

The cochlear implant application may produce damage through the insertion of a silicone-embedded electrode in the cochlea, indeed it could cause pressure, shock, bleeding and tissue perforation. A further adverse effect is related to the reaction at the silicone materials causing apoptosis and necrosis in the spiral ganglion. In literature there are several studies dealing with the silicones biocompatibility, in which the authors analysed *in vitro* the ability of neuronal cells to adhere, grow and differentiate under silicone sheet.

Our aim was to evaluate whether four polydimethylsiloxane (PDMS) compounds (three fluid and one elastomere) are valuable for cochlear implants production, exposing an *in vitro* neuronal model (PC12 cells) to PDMS compounds (octadimethylsiloxane, hexadimethylsiloxane, decamethylcyclopentasiloxane and a silicon rod) at different dilutions and time of exposure. The toxicity was assessed testing the cell viability by MTS assay and analysing the cell death process by real time PCR.

Viability test demonstrate that after 24 hours both octadimethylsiloxane (at the dilution 1:10) and hexadimethylsiloxane (1:5) caused significant cell mortality, while decamethylcyclopentasiloxane became cytotoxic after 72 hours at the dilution of 1:100. The biomolecular investigation showed no change in the apoptotic pathway genes transcription. These results suggest that PMDS are biocompatible because cell death occurs by necrotic process caused by the formation of a PMDS surface film under the cell medium (preventing air exchange) and not releasing cytotoxic molecules on PC12 cells.

P60

THE CONTROLLED-RELEASE EFFECT OF HYALURONIC ACID ON DRUG DELIVERY TO THE COCHLEAR SPIRAL GANGLION

Yozo Inagaki[1,2], Sho Kanzaki[1], Masato Fujioka[1], Naoki Oisi[1], Kaoru Ogawa[1]

[1]KEIO University, Otolaryngology Head and Neck Surgery, Tokyo, Japan; [2]Hino Municipal Hospital, Otolaryngology, Tokyo, Japan

Objective. Spatiotemporal distribution of drugs in the inner ear could not precisely be evaluated due to its area's small size and its complicated compartments. In the present study, we investigated the effect of a carrier mucopolysaccharide, hyaluronic acid (HA), on the controlled drug delivery into the cochlea by using transgenic mouse-based in vivo imaging system previously reported by our group (Kanzaki & Fujioka et al, 2013).

Method. The drug delivery of HA dispersed- luciferin, a 280 MW substrate of luciferase, was investigated. Subcutaneous injection of HA-luciferin (HA-sc), saline dissolved luciferin (NS- sc) and intraperitoneal injection of saline dissolved luciferin (NS-ip) were performed to the transgenic mouse, *GFAP-luc*, which expresses luciferase in the cochlear spiral ganglion cells. Bioluminescence produced by a chemical reaction of luciferin-luciferase was monitored in vivo at real time by using the Xenogen-IVIS 100.

Result. Both peak time and half-life of the photon count were significantly prolonged in HA-sc groups compared to those in NS-sc and NS-ip groups. No significant difference was observed among their peak photon counts.

Conclusion. Our imaging system successfully detected differences in the pharmacokinetics of luciferin in the inner ear and the sustained-release effect of hyaluronic acid was observed. The result suggests the clinical significance of hyaluronic acid for controlling drug delivery to the cochlea.

POSTER SESSION V
NOISE INDUCED HEARING LOSS, OTOTOXICITY AND IMMUNOSYSTEM

P61

NRF2 ATTENUATES NOISE-INDUCED HEARING LOSS BY PREVENTING OXIDATIVE DAMAGE OF COCHLEA

Yohei Honkura[1,2], Shohei Murakami[2], Tetsuaki Kawase[1], Yukio Katori[1], Hozumi Motohashi[2]

[1]Tohoku University Graduate School of Medicine, Department of Otolaryngology-Head & Neck Surgery, Sendai, Japan; [2]Institute of Development, Aging and Cancer, Tohoku University, Department of Gene Expression Regulation, Sendai, Japan

One of the major mechanisms of high intensity noise-induced hearing loss has been considered as an increase of oxidative stress in cochlea based on the observation that the cochlear blood circulation is impaired during noise exposure. Thus, the noise exposure experiment is a model of cochlear ischemia-reperfusion injury.

A transcription factor NRF2 is a master regulator of numerous detoxifying and antioxidant genes in response to oxidative and electrophilic stresses. NRF2 is constantly ubiquitinated by KEAP1, resulting in the rapid degradation of NRF2. In the activated condition, stabilized NRF2 is translocated into the nucleus, binds to antioxidant/electorophile response elements, and activates the transcription of cytoprotective genes encoding detoxification enzymes (e.g., NAD(P)H dehydrogenase quinone 1 [Nqo1]), antioxidant enzymes (e.g., heme oxygenase-1 [Ho-1] and thioredoxin reductase 1 [Txnrd1]), drugtransporters, and enzymes for glutathione synthesis (e.g., glutamate-cysteine ligasecatalytic subunit [Gclc] and glutamine-cysteine ligase, modifier subunit [Gclm]). Although previous studies showed that NRF2 contributes to the protection from oxidative stress in various organs, NRF2 function in the cochlea has not been clarified. 2-cyano-3,12 dioxooleana-1,9 dien-28-imidazolide (CDDO-Im) is a potent inducer of NRF2. Pretreatment with CDDO-im has been shown protective from inflammatory insults and chemical toxicity in other organs. However, the protective effect of CDDO-Im in cochlea has not been tested.

To elucidate whether NRF2 protects inner ear against oxidative stress, we performed noise exposure experiments and CDDO-Im administration experiments, using *Nrf2*$^{+/+}$ mice and *Nrf2*$^{-/-}$ mice.

We demonstrated that CDDO-Im protects inner ear against oxidative stress induced by noise exposure in an NRF2-dependent manner, shown by ABR, RT-PCR, 4-HNE immunohistochemistry, and immunoblotting analysis.

This study suggests that pretreatment of NRF2 inducer is clinically useful to prevent hearing loss caused by increase of oxidative stress in cochlea.

P62

NOISE-INDUCED COCHLEAR F-ACTIN DEPOLYMERIZATION IS MEDIATED VIA ROCK2/P-ERM SIGNALING

Yu Han, Xian-Ren Wang, Jun Chen, Su-Hua Sha
Department of Pathology and Laboratory Medicine, Medical University of South Carolina, Charleston, SC, USA

Our previous work has suggested that traumatic noise activates Rho-GTPase pathways in cochlear outer hair cells (OHCs), resulting in cell death and noise-induced hearing loss (NIHL). In this study we investigated Rho effectors, Rho-associated kinases (ROCKs), and the targets of ROCKs, the ezrin-radixin-moesin (ERM) proteins, in the regulation of the cochlear actin cytoskeleton using adult CBA/J mice under conditions of noise-induced temporary threshold shift (TTS) and permanent threshold shift (PTS) hearing loss, which result in changes to the F/G-actin ratio. The levels of cochlear ROCK2 and p-ERM decreased 1 h after either TTS- or PTS-noise exposure. In contrast, ROCK2 and p-ERM in OHCs decreased only after PTS-, not after TTS-noise exposure. Treatment with lysophosphatidic acid, an activator of the Rho pathway, resulted in significant reversal of the F/G-actin ratio changes caused by noise exposure and attenuated OHC death and NIHL. Conversely, the down-regulation of ROCK2 by pretreatment with ROCK2 siRNA reduced the expression of ROCK2 and p-ERM in OHCs, exacerbated TTS to PTS, and worsened OHC loss. Additionally, pretreatment with siRNA against radixin, an ERM protein, aggravated TTS to PTS. Our results indicate that a ROCK2-mediated ERM-phosphorylation signaling cascade modulates noise-induced hair cell loss and NIHL by targeting the cytoskeleton.

P63

COCHLEAR RESPONSE TO ACUTE AND CHRONIC NOISE EXPOSURE IN ADENOSINE RECEPTOR-DEFICIENT MICE

Srdjan M Vlajkovic[1], Song Y Paek[1], Michelle Quinn[1], Detlev Boison[3], Gary D. Housley[4], Peter R. Thorne[1,2]
[1]Department of Physiology and [2]Discipline of Audiology, University of Auckland, Private Bag 92019, Auckland, New Zealand; [3]Legacy Research Institute, Portland, USA; [4]Department of Physiology and Translational Neuroscience Facility, School of Medical Sciences, University of New South Wales, Sydney, Australia

Exposure to noise and drugs toxic to the inner ear are major contributing factors to acquired sensorineural hearing loss at any age. We have previously shown that acquired hearing loss can be ameliorated in experimental animals by local or systemic administration of A_1 adenosine receptor agonists. However, we know little about how adenosine receptors (AR) affect injury processes in the cochlea and especially whether there is an interplay between the facilitatory and inhibitory AR responses that may define the extent and nature of reparative processes. To identify the broader role of AR in regulating the overall response of cochlear tissues to stress and injury, we investigated cochlear responses to acute and chronic noise stress in mice lacking genes for the two main types of AR found in the inner ear (A_1 and A_{2A} receptors). C57BL/6 mice (6-8 weeks) homozygous (+/+) and heterozygous (+/-) for the mutation (AR deletion) and wildtype mice (+/+) were exposed to acute traumatic noise (8-16 kHz, 105 dBSPL, 2 hours) or chronic (2 hrs/day for 20 days) octave band noise (8-16 kHz, 100 dBSPL). Auditory thresholds in response to tone pips and auditory clicks were assessed before and 14 days after the noise exposure using auditory brainstem responses (ABR). Our study demonstrates that the AR-deficient mice have normal ABR thresholds at ambient sound levels, but show increased vulnerability to acoustic injury compared to wildtype mice. ABR threshold shifts following exposure to acute noise were increased in A_1R-null mice compared to A_1R+/- and +/+ mice, whilst the A_{2A} receptor deletion had no effect. In contrast, hearing loss following exposure to chronic noise was aggravated in A_{2A}R null-mice, whilst the A_1 receptor deletion had no effect. We postulate that A_1 and A_{2A} receptors differentially regulate the main injury mechanisms in the cochlea (e.g. oxidative stress, glutamate excitotoxicity, inflammation), which may account for specific auditory phenotypes in AR-deficient mice. Improved understanding of the role of AR in cochlear response to acoustic injury is potentially a critical translational research area for the development of clinically relevant compounds for the therapeutic management of sensorineural hearing loss.

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P64

REDUCED CONNEXIN26 IN MATURE COCHLEA INCREASES SUSCEPTIBILITY WITH ACOUSTIC TRAUMA

Sen Chen, Xingxing Zhou, Yu Sun, Weijia Kong
Department of Otorhinolaryngology, Union Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, China

Objective. To investigate the role of Cx26 in noise induced hearing loss.

Methods. The P18 Cx26 knocked down mice were denoted by KD group(knock down group),and half of them were exposed to 110dB SPL continuous white band noise from P26,8 hours per day,for 5 days,which were denoted by KD+N group(knock down +noise group). Control group were the Cx26loxP/loxP mice injected by one dose of tamoxifen (IP) on postnatal day 18(P18), and half of them were exposed to the noise the same as KD+N group,and these were denoted by N group(noise group).Auditory brain response(ABR) was used to assess the hearing threshold of these mice.Whole-mount cochlear preparations were used to count the out hair cell number.Western blot was carried out to detect the expression level of Cx26 in these four groups of mice.The cochlear embedded in expoxy resin was axial sliced by 2μm continuously to observe the morphology.Transmission electronic microscope was performed to observe the ultra structure.

Results. Compared to the control group,there were no differences of hearing thresholds in the KD group on P30 and P45.The KD+N group and the N group displayed severe hearing loss of all frequencies on P30,and there was a 7.9dB SPL gap in the 8KHz between the two groups. On P45,they showed severe hearing loss in all frequencies,except the N group expressed moderate to severe hearing loss in 8KHz,and which was 13dB SPL lower than in the KD+N group.Western blot revealed that Cx26 expression level in N group was the same as C group on P30,but in the KD group and the KD+N group the Cx26 expression level were decreased.And the KD+N group expressed less Cx26 than the N group. Whole-mount cochlear preparations showed that on P30 and P45 out hair cells were intact in the C group and the K group,and out hair cells loss could be observed in the N group and the KD+N group,and aggravated from the apical turn to the basal turn.Epoxy slices indicated that the structure of cochlear in the C group and the K group were normal,while the organ of Corti crashed and the stria vascular swelled in the N group and the KD+N group.

Conclusion. Down regulated Cx26 at mature cochlea in mice could increase susceptibility with acoustic trauma and aggravate the cell degeneration in organ of Corti after noise exposure.

P65
NUTRITIONAL THERAPY AND OCCUPATIONAL HEARING LOSS

Natalya Petrova, Viia Panshina
North-Western State Medical University named after I.I.Mechnikov, St.Petersburg, Russia

Nutritional therapy is used to restore impaired body functions. Generally, it is able to reduce the stress response. Industrial noise factor with prolonged action triggers a stress response that leads to the development of sensorineural hearing loss. The early action of the noise is manifested by the increased level of anxiety of the employee.
Objective. The purpose of this study was to evaluate the effectiveness of medical nutrition in the complex of preventive measures of sensorineural hearing loss.
Methods. We examined 50 employees of one company with sensorineural hearing loss and its early manifestations. The remote control noise was above 80 dBA in the workplace each employee. Anxiety level was determined 3 times. The first study performed before prevention of sensorineural hearing loss, the second study – after prevention, including nutritional therapy, the third study - after prevention without nutritional therapy. The third study was conducted a year after the execution of the second study. Nutritional therapy was in the form of jelly, in which was botanicals, vitamins and minerals. Jelly was applied in a warm state, 1 per day, 200 ml, 1 month, before the working shift.
Results. The level of anxiety the second study was significantly lower than in the first study ($S2<S1$, $p<0.05$). The anxiety level of the third study was significantly higher than in the second study ($S3>S2$, $p<0.05$).
Conclusions. The level of anxiety among workers with occupational deafness following when you use therapeutic food in the complex of preventive measures. Selected nutritional therapy can reduce the stress response from exposure to occupational noise. Therefore, we can expect the lengthening of the period without progression of sensorineural hearing loss.

P66
OTOTOXICITY OF STREPTOCOCCUS SPECIES: AN *IN VITRO* MODEL

Magdalena Solyga[1,2,8], Michael Perny[1,2,4,6,7], Marta Roccio[1,4,6], Denis Grandgirard[2,6], Stephen L. Leib[2,3,6], Pascal Senn[1,4,5,6]
[1]Inner Ear Research Laboratory, Department of Clinical Research, University of Bern, Switzerland; [2]Neuroinfection Laboratory, Institute for Infectious Diseases, University of Bern, Switzerland; [3]Biology Division, Spiez Laboratory, Swiss Federal Office for Civil Protection, Spiez, Switzerland; [4]University Department of Otorhinolaryngology, Head and Neck Surgery, Inselspital, Bern, Switzerland; [5]Dept. of Otorhinolaryngology, Head & Neck Surgery, Hôpitaux Universitaires de Genève, France; [6]Laboratory for Regenerative Neuroscience, DCR, University of Bern, Switzerland; [7]Graduate School for Cellular and Biomedical Sciences, University of Bern, Switzerland; [8]Cranfield University, UK

Background. Up to 30% of patients surviving pneumococcal meningitis suffer from sensorineural hearing loss. The aim of this project is to set up an in vitro ototoxicity assay to study the interplay between bacteria (streptococcus species), bacterial toxins, antibiotics and the sensory epithelium of the Organ of Corti (OC) and to understand in more details, how meningitis caused hearing loss.
Methods. The Organ of Corti was isolated from 3-4 days old Wistar rats and cultivated on a polyester permeable membrane (Transwell-Clear Inserts, Corning), which lies on top of a fluid-filled chamber, where bacteria and other ototoxic substances were applied to. This set up resembles the in-vivo situation, in which bacteria infiltrate the cochlea almost exclusively through the scala tympani in the first place and are thus not in direct contact with the hair cells and spiral ganglion neurons. As an initial experiment, a dose-response curve with sisomicin was performed to act as a positive control for ototoxic hearing loss. Immunocytochemical staining for hair cells (Myosin-VIIa) was performed to assess the number of missing inner and outer hair cells upon treatment and to determine the characteristic pattern of hair cells loss for different regions of the OC (base, middle, apex). A semi-automatic ImageJ plugin was developed and used for rapid quantification of hair cell loss.
Results. Sisomicin-induced level of hair cells loss was dose-dependent. For high sisomicin concentrations (200µM, 500µM), survival rates of outer hair cells were approximately 2.5-folds higher at the apex than at the base. On the contrary, inner hair cells were predominantly damaged at the apex. Experiments with different streptococcus species/toxins are ongoing, and will be presented at the IEB meeting in September.
Conclusions. Our in vitro model allows for efficient ototoxicity screening in the paradigm of bacteria-associated hearing loss. Furthermore, this study will hopefully expand our knowledge about ototoxic mechanisms of Streptococcus infections and provide a platform for the development of otoprotective/otoregenerative therapies for meningitis-associated hearing loss.

P67
TRANSTYMPANIC MODEL OF CISPLATIN-INDUCED OTOTOXICITY: COMPARISON OF THE COCHLEA AND THE VESTIBULE

Angela Callejo[1,2], Amandine Durochat[3], Christian Chabbert[3,4], Ivan Domenech Juan[2,5], Jordi Llorens[1,5], Sophie Gaboyard-Niay[3]
1Departament de Ciències Fisiològiques II, Universitat de Barcelona, L'Hospitalet de Llobregat, Spain; [2]Departament d'Otorinolaringologia i Patologia Cérvico-Facial, Institut Universitari Dexeus, Barcelona,Spain; [3]Sensorion S.A., Montpellier, France; [4]Aix Marseille Université, UMR 7260, Marseille, France; [5]Institut d'Investigació Biomèdica de Bellvitge (IDIBELL), L'Hospitalet de Llobregat, Spain

Cisplatin is an efficient chemotherapeutic agent widely used to treat a variety of cancers but major side effects, such as ototoxicity, limit its use and dosage. Cisplatin induced ototoxicity has been extensively studied but no treatment exists. One main limiting factor is the availability of appropriate animal models. Parallel additional side effects, such as nephrotoxicity, results in a high rate of mortality that prevent from studying ototoxicity. Ototoxicity consists of hearing loss but it also includes vestibular dysfunction. Susceptibility of the vestibule to cisplatin toxicity was reported but no study specifically addressed the question extensively.
Aim of the present work was the development of an animal model to study cisplatin-induced inner ear toxicity by means of transtympanic injection. This induction method provides an

easy, short and reproducible paradigm causing low morbidity. Specifically addressed questions were 1) the dose-response relationship, allowing for the identification of doses suitable for subsequent otoprotective studies, and 2) the relationship between the cochlear and the vestibular lesions.
Inner ear sensory organs were investigated using immunohistochemistry and fluorescent microscopy to perform morphometric analysis of the cochlea and the vestibule. Quantification of hair cells and/or hair bundles was used to compare data from both organs in different rat strains, Long Evans and Wistar of both genders. Parallel evaluation of the vestibular behavior through the vestibular deficit score enables direct relationship between cochlear and vestibular lesions and functions.
Transtympanic cisplatin (0.5 to 2 mg/ml) induced dose dependent damage in both the cochlea and the vestibule. In male Long-Evans rats, a dose dependent loss of outer hair cells from the apical 1 ½ turns of the cochlea was obtained. This histopathological analysis of the auditory sensory organ was correlated with a dose dependent induction of vestibular sensory epithelium lesions and dysfunction. Comparison to Long Evans female and Wistar, male and female, showed a higher sensitivity of female than male to cisplatin induced ototoxicity but no difference between strains were observed.

P68
REDOX IMBALANCE IN STYRENE OTOTOXICITY AND ACOUSTIC TRAUMA: IN VIVO PROTECTIVE EFFECT OF QTER

R. Rolesi[1], F. Paciello[1], S.L.M. Eramo[2], A.R. Fetoni[1], G. Paludetti[1], D. Troiani[2]
[1]Department of Head and Neck Surgery and [2]Institute of Human Physiology, Università Cattolica, Medical School, Rome, Italy

Introduction. Styrene is an industrial solvent. Styrene ototoxicity has been reported in industrial workers. Styrene exposure causes a reduction of the GSH levels and an increase of lipid peroxidation. Because GSH is an important antioxidant, it has been speculated that reduction of GSH level would induce oxidative stress. As well as in styrene-induced ototoxicity, a progressive increase of ROS have been demonstrated to be involved in NIHL. Namely, several studies have suggested a synergistic interaction between noise and styrene, but the combined exposure to noise+styrene on redox imbalance in the cochlea is not been reported. The aim of this study is to investigate the mechanisms involved in styrene ototoxicity and the effect of the antioxidant Q-ter on the styrene-induced cochlear injuries and on the combined effect of noise+styrene.
Methods. Rats were exposed to styrene by gavage (400mg/Kg) and to chronic noise exposure (97 dB SPL, 10 kHz, 60 min/day, 3 weeks, 5 days/week). Two groups were simultaneously treated with the Q-ter (100 mg/Kg) over the same period. Functional evaluation was performed by ABR and DOPAE. We studied the immunostaining for redox imbalance in the cochlea.
Results. Our results demonstrate that hearing loss and cochlear damage by styrene exposure are increased by the concomitant exposure to noise and Q-ter treatment can reduce damage caused by chronic exposure to noise+styrene.
Conclusion. We speculate that the association between noise and styrene exposure represent a risk factor for the health workers and the antioxidant treatment provides a promising preventive approach.

P69
HYDROGEN PEROXIDE IN INNER EAR CELLS AS AN OXIDATIVE STRESS MODEL

Alice Cani[1], Edi Simoni[1], Filippo Valente[1], Laura Astolfi[1], Alessandro Martini[2]
[1]Bioacoustics Research Laboratory, Department of Neurosciences, University of Padua, Italy; [2]ENT surgery - Department of Neurosciences, University of Padua, Italy

Hearing loss caused by ototoxic drugs, infections and acoustic trauma lead to the generation of reactive oxygen species (ROS) in the cochlea. Oxidative stress arises when there is a marked imbalance between the production and removal of ROS. The cell injury caused by the interaction of ROS with different cellular components could direct to an inflammatory reaction. In particular, free radicals can cause damage by reacting with DNA, proteins, cytosolic molecules, cell surface receptors, and breaking down membrane lipids. ROS produced by the mitochondria induce lipid peroxidation in the cochlea and also leads to inflammation and production of the pro-inflammatory cytokines, interleukin-6 and tumor necrosis factor α. In auditory tissues, under physiological and pathological conditions, the oxidative stress play a key role in the inductions of cell death through both apoptosis or necrosis process. To study the oxidative stress in *in vitro* cell model we tested the effect of hydrogen peroxide (H_2O_2), as a reactive oxygen species, on OC-k3 cells, as inner ear epithelial cell line, by cell viability assay, morphology and apoptosis, protein pattern analysis and cell cycle. H_2O_2 was cytotoxic in OC-k3 cells in a dose-dependent manner. Cell morphology, Annexin V-FITC assays and cell cycle, which displayed an arrest in phases S-G2/M, showed that cell death is due to necrosis. Finally, Western blot analysis confirmed the oxidative damage.
Our findings demonstrate the vulnerability of inner ear cells to ROS: OC-k3 cell line treated with H_2O_2 is an effective model of oxidative stress for the development and improvement of therapeutic strategies of hearing loss prevention.

P70
WNT ACTIVATION PROTECTS AGAINST NEOMYCIN-INDUCED HAIR CELL DAMAGE IN THE MOUSE COCHLEA

Renjie Chai[1,2], Liman Liu[3,4], Yan Chen[3,4], Yanping Zhang[3,4], Yingzi He[3,4], Wenli Ni[3,4], Dan You[3,4], Shan Sun[3,4], Makoto M. Taketo[6], Lei Wang[5], Huawei Li[3,4,5]
[1]Key Laboratory for Developmental Genes and Human Disease, Ministry of Education, Institute of Life Sciences, Southeast University, Nanjing, China; [2]Co-innovation Center of Neuroregeneration, Nantong University, Nantong, China; [3]Department of Otorhinolaryngology, Hearing Research Institute, Affiliated Eye and ENT Hospital of Fudan University, Shanghai, China; [4]Central laboratory, Affiliated Eye and ENT Hospital of Fudan University, Shanghai, China; [5]Institutes of Biomedical Sciences, Fudan University, Shanghai, China; [6]Department of Pharmacology, Graduate School of Medicine, Kyoto University, Kyoto, Japan

Recent studies have reported the role of Wnt/β-catenin signaling in hair cell (HC) development, regeneration, and differentiation in the mouse cochlea; however the role of Wnt/β-catenin signaling in HC protection remains unknown. In this study, we took advantage of transgenic mice to specifically knock out or over-activate the

canonical Wnt signaling mediator β-catenin in HCs, which allowed us to investigate the role of Wnt/β-catenin signaling in protecting HCs against neomycin-induced damage. We first showed that loss of β-catenin in HCs made them more vulnerable to neomycin-induced injury, while constitutive activation of β-catenin in HCs reduced HC loss both *in vivo* and *in vitro*. We then showed that loss of β-catenin in HCs increased caspase-mediated apoptosis induced by neomycin injury, while β-catenin overexpression inhibited caspase-mediated apoptosis. Finally, we showed that loss of β-catenin in HCs increased Foxo3 expression and reactive oxygen species (ROS) levels after neomycin treatment, which might be responsible for the increased aminoglycoside sensitivity of HCs. In contrast, β-catenin overexpression reduced Foxo3 expression and ROS levels, suggesting that β-catenin is protective against neomycin-induced HC loss. Our findings demonstrate that Wnt/β-catenin signaling plays an important role in protecting HCs against neomycin-induced HC loss and thus might be a new therapeutic target for the prevention of HC death.

P71

CONNEXIN 43 ACTS AS A PRO-APOPTOTIC MODULATOR IN CISPLATIN-INDUCED AUDITORY CELL DEATH

Yeon Ju Kim, Young Sun Kim, Jong Joo Lee, Oak-Sung Choo, Yun-Hoon Choung

Department of Otolaryngology, Ajou University School of Medicine, Suwon, South Korea

Purpose. Gap junction (GJ) coupling may play a role for intercellular communication by the "Good Samaritan effect" or "bystander effect". Non-junctional connexins (Cxs) also may play some gap junction-independent roles in cell death or survival. The purposes of this study were to investigate the role of non-junctional Cxs in ototoxic drug-induced apoptosis of auditory cells and to evaluate the effect of GJ inhibitors on cisplatin-induced hearing loss using *in vivo* animal models.

Materials and Methods. Non-junctional Cx43 proteins in HEI-OC1 cells were prepared with three techniques. 1) Low confluence culture ($5X10^3/cm^2$). 2) Construction of short lengthened Cx43 - Cx43-NT (amino acid 1-256, membrane domain) and Cx43-CT (amino acid 257-382, cytoplasmic domain). 3) Brefeldin A (BFA), an ER-Golgi trafficking inhibitor. A live/dead cell viability assay and western blotting was done under knock-down conditions (siRNA-Cx43). Additionally, For *in vivo* animal studies, carbenoxolone (CBX, 50 mg/kg) and 18- alpha glycyrrhetinic acid (18α-GA, 100 mg/kg) was intraperitoneally injected to rats treated with cisplatin (16 mg/kg). Aauditory brainstem response and morphologic analysis was done with immunihistochemistry.

Results. Knock down of non-channel Cx43 (siRNA) inhibited cisplatin-induced cell death in MTT assay and Western blot. This finding was not changed by disruption of Cx43 trafficking with BFA. HeLa cells expressing the Cx43-FL (full length), Cx43-NT or Cx43-CT showed enhanced sensitivity to cisplatin compare to Mock cells. In animal studies, hearing thresholds of ABR in CBX+cisplatin-treated rats (were significantly better than those in the cisplatin- only treated rats. In SEM findings, loss of stereocilia in outer hair cells was much more in the cisplatin-treated rats than in the CBX+cisplatin-treated rats.

Conclusion. Cx43 plays a pro-apoptotic role in cisplatin-induced auditory cell death, which is either dependent or independent on GJ intercellular communications. Targeting Cx-mediated signaling control may be necessary for designing new therapeutic strategies for drug-induced ototoxicity.

P72

MATRIX METALLOPROTEINASE INHIBITOR ATTENUATES COCHLEAR LATERAL WALL DAMAGE INDUCED BY INTRATYMPANIC INSTILLATION OF ENDOTOXIN

Chul Ho Jang, Young Ho Choi, Yong Beom Cho

Chonnam National University, Chonnam University Hospital, Otolaryngology, Gwangju, South Korea

Objective. Oxytetracycline and ilomastat are inhibitors of matrix metalloproteinases (MMPs). Their efficacy in protecting against cochlear damage induced by the intratympanic instillation of lipopolysaccharide (LPS), as a means of inducing labyrinthitis, was investigated.

Materials and Methods. Experiments were performed in 21 young male guinea pigs. Intratympanic instillation of LPS was done in the control group (n=7). Intratympanic instillation of oxytetracycline or ilomastat was done after LPS instillation in the experimental group. Measurements of auditory brainstem response (ABR) and cochlear blood flow (CBF) were performed. The organ of Corti was evaluated by field emission scanning electron microscopy (FE-SEM). The blood-labyrinth barrier (BLB) integrity was evaluated with Evans blue uptake. Gelatin zymography was used to assess the expression of active MMP-2 and MMP-9.

Results. Ears treated with MMP inhibitors were significantly protected from hearing loss compared to the LPS group. In LPS group, there was a significant decrease of CBF. However, experimental group displayed a statistically significant recovery of CBF. FE-SEM revealed hair cell damage in the LPS-treated group, but hair cells presented a normal appearance in MMP inhibitors. The LPS group showed a marked increase of Evans blue extravasation in the cochlea. However, MMP inhibitors significantly reduced the BLB opening. Active MMP-9 was expressed in the LPS group. Treatment with MMP inhibitors attenuated active MMP-9 expression.

Conclusion. The MMP inhibitors oxytetracycline and ilomastat protect from cochlear lateral wall damage caused by LPS-induced labyrinthitis.

P73

FETAL THYMUS GRAFT ENABLES RECOVERY FROM AGE-RELATED HEARING LOSS AND EXPANSION OF CD4-POSITIVE T CELLS EXPRESSING IL-1 RECEPTOR TYPE 2 AND REGULATORY T CELLS

Hiroshi Iwai[1], Muneo Inaba[2]

[1]Kansai Medical University, Takii Hospital, Department of Otolaryngology, Moriguchi City, Osaka, Japan; [2]Kansai Medical University, Hirakata Hospital, Department of Medicine, Hirakata City, Osaka, Japan

Accumulating evidence has indicated the relationship between the systemic immune system and the central nervous system including the inner ear. We have found that age-related developments of T-cell dysfunction, hearing loss, and degeneration of cochlear spiral ganglion (SG) neurons in 6-month-old mice were recovered at 12 months old after fetal thymus transplants were given twice,

and that CD4+ T cells expressing interleukin 1 receptor type 2 (IL-1R2) and naturally occurring regulatory T cells (nTregs), which expanded in aged 12-month-old mice, were reduced in the thymus-grafted mice of the same age. Therefore, it is conceivable that the rejuvenation of systemic immune function by fetal thymus grafts contributes not only to the activation of cellular immunity but also to the decrease of IL-1R2+CD4+ T cells or nTregs, which cause age-related hearing loss (AHL) as a consequence of neurodegeneration of the cochlear neurons. Further studies on the interactions among IL-1R2 expression on CD4+ T cells, Tregs, and neuronal cells and also on the relationships between fetal thymus grafting and the rejuvenation of systemic immunity should be designed in order to advance towards therapeutic effects on neurosenescence, including AHL.

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P74

IFN/IFNAR1 SIGNALING IS CRUCIAL IN THE CONTROL OF VIRAL INFECTION IN THE COCHLEAR SENSORY EPITHELIUM

Yushi Hayashi[1,2], Koji Onomoto[3,4], Mitsutoshi Yoneyama[3,4], Takashi Fujita[4], Nobuyuki Tanaka[2]

[1]Institute of Clinical Research (Sensory Organs), National Hospital Organization Tokyo Medical Center, Tokyo, Japan; [2]Institute for Advanced Medical Sciences, Nippon Medical School, Kawasaki, Japan; [3]Medical Mycology Research Center, Chiba University, Chiba, Japan; [4]Institute for Virus Research, Kyoto University, Kyoto, Japan

Although there are some diseases of the cochlea which are possibly related to viral infection such as sudden hearing loss, Meniere's disease and congenital sensorineural hearing loss, there were no reports which clarify the relationship between viral infection and inner ear diseases. We have reported so far that not auditory hair cells but supporting cells produce type I interferon (IFN) that is an anti-viral cytokine through retinoic acid inducible gene-I (RIG-I) like receptor (RLR) signaling cascade against viral infection. The aim of this research is to investigate the influence of type I IFN produced by supporting cells on the cochlear sensory epithelium.

To understand the kinds of cells affected by type I IFN produced by supporting cells, we investigated the expression pattern of interferon alpha and beta receptor 1 (IFNAR1) which is a receptor of type I IFN using immunohistochemistry. Then we examined the change of viral infection pattern using IFNAR1 knock out mice. From these experiments, we concluded that IFN/IFNAR1 signaling prevents virus from infecting cochlear hair cells.

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P75

IMMUNE-RESPONSE OF GUINEA PIG COCHLEAE AFTER GRAFTING HUMAN CELLS AND ITS PREVENTION BY BONE MARROW DERIVED STROMAL CELLS

Masaaki Ishikawa, Hiroe Ohnishi, Desislava Skerleva, Tatsunori Sakamoto, Norio Yamamoto, Juichi Ito, Takayuki Nakagawa

Department of Otolaryngology, Head and Neck Surgery, Graduate School of Medicine, Kyoto University, Kyoto, Japan

In cell transplantation to experimental animals, immune responses due to allograft or xenograft could be an obstacle for transplant survival (LaRosa et al., 2007, Aggarwal et al., 2005). Although inner ear was previously considered as an immune-privileged organ, the presence of immune cells/inflammatory cells into cochlea has been recently reported (Okano et al., 2008, Zhang et al., 2012). Recent studies have indicated immunomodulatory effects of bone marrow derived stromal cells (BMSCs) (Le blank et al., 2008). In our study, we investigated cochlear immune responses due to xenograft, and immunomodulatory effects of BMSCs into cochleae.

Human iPS cell-derived neurons cultured on a three-dimensional collagen matrix were used as transplants, and guinea pigs were used as an experimental animal. We prepared guinea pig BMSCs for administration at densities of 1.0×107 cells/ml in PBS. We transplanted to the total of 14 animals, and divided them into 2 groups: Animals without BMSCs treatment and with BMSCs treatment. For animals without BMSCs treatment, the total of 7 animals was treated with immunosuppressant (FK-506). For animals with BMSCs treatment, the remaining animals were treated with FK-506 and BMSCs. Intravenous injection of BMSCs was performed immediately after transplantation. For animals untreated with BMSCs treatment, PBS injection was performed. Daily injection of FK-506 (1mg/kg) was performed during their survival term. All animals were sacrificed 7 days after transplantation.

In animals without BMSCs treatment, hematoxylin and eosin (HE) staining demonstrated severe infiltration of inflammatory cells in spite of FK-506 treatment. In immunohistochemistry, inflammatory cells were positive for CD45, a marker of leukocyte. These findings indicated the necessity of further treatment to control immune responses due to xenograft. In characterization of inflammatory cells, some CD45-positive cells expressed a marker of helper T cell, but not a marker of killer T cell. In animals with BMSCs treatment. infiltration of inflammatory cells was limited. In addition, the number of surviving transplanted cells was significantly increased by BMSCs treatment.

In transplantation of xenograft into cochleae, severe immune responses were induced, and FK-506 treatment was not adequate for controlling immune responses. Systemic application of BMSCs contributed for controlling immune responses, resulting in better survival of transplants.

POSTER SESSION VI
GENETICS OF DEAFNESS

P76

IN VITRO EVALUATION OF PATHOGENIC POTENTIAL OF THE NOVEL *TMPRSS3* MUTATIONS AND GENOTYPE-PHENOTYPE CORRELATION

Ah Reum Kim[1,4], So Ra Im[2], Sang Min Park[2], Woo Jin Park[2], Joo Hyun Park[3], Byung Yoon Choi[4]

[1]Department of Otorhinolaryngology, Seoul national University Hospital, Seoul, South Korea; [2]College of Life Sciences, Gwangju Institute of Science and Technology, Buk-gu, Gwangju, South Korea; [3]Department of Otorhinolaryngology-Head and Neck Surgery, Dongguk University International Hospital, Siksa-dong, Ilsandong-gu, Goyang-si, Gyeonggi-do, South Korea; [4]Department of Otorhinolaryngology, Seoul National University Bundang Hospital, Seongnam, South Korea

TMPRSS3, which encodes a transmembrane serine protease, is one of the main causative genes for hereditary hearing loss, alteration of which results in a discrete phenotype of autosomal recessive non-syndromic sensorineural hearing loss (NSHL) (DFNB8/10). Others and we previously reported an interesting correlation between the protease activity based upon the yeast-based protease assay and the severity of the hearing loss. In this study, I identified two missense TMPRSS3 variants c.G346A (p.V116M), c.G871C (p.V291L) with unknown pathogenic potential that were in trans with a probably pathogenic splice site variant (c.783-1G>A) of the gene in a 6 year-old girl (SNUH67-156) through targeted exome sequencing of 200 known deafness genes and subsequent bioinformatic analyses. She manifested severe to profound nonsyndromic hearing loss (NSHL) early in her 1st decade, indicating that her auditory phenotype is more likely to be DFNB10. Among the three variants, the novel splicing variant c.783-1G>A from the maternal allele was most likely to be pathogenic since this would clearly abolish the canonical splicing site. To better address the pathogenic potential of the remaining two missense variants, I employed an in vitro yeast based proteolysis activity assay as previously described. I constructed a wild type, p.V116M, p. V291L and a double mutant (p.V116M and p. V291L) TMPRSS3 cDNA by in vitro mutagenesis. Our in vitro assay has revealed that p.V291L and p.V116M was pathogenic and non-pathogenic, respectively. I was not able to find any synergistic pathogenic effect from the double mutant as compared with single mutants. These in vitro results were sharply contrasted with the in silico prediction. The null protease activity from the p.V291L in conjunction with the probably pathogenic splice site variant was compatible with the DFNB10 phenotype from SNUH67-156. In conclusion, distinction between polymorphisms and mutations of TMPRSS3 should rely on the in vitro biochemical assay. My confirmation on the genotype-phenotype correlation of this gene paves the way for establishment of a personalized auditory rehabilitation.

P77

GENE EXPRESSION STUDY OF THE VESTIBULAR SYSTEM OF THE *IGF1* DEFICIENT MOUSE

Lourdes Rodríguez-de la Rosa [1, 2, 3], Silvia Murillo-Cuesta[1, 2, 3], Julio Contreras[1, 2, 4], Joaquín Dopazo[2,5], Isabel Varela-Nieto[1, 2, 3], Marta Milo[6]

[1]Instituto de Investigaciones Biomédicas "Alberto Sols" (CSIC-UAM), Madrid, Spain; [2]CIBERER (ISCIII), Madrid, Spain; [3]IdiPAZ, Madrid, Spain; [4]Facultad de Veterinaria (UCM), Madrid, Spain; [5]Centro de Investigación Príncipe Felipe, Valencia, Spain; [6]University of Sheffield, UK

The auditory and vestibular organs form the inner ear and have a common developmental origin although different functions. The vestibular system is responsible of balance. Insulin like growth factor 1 (IGF-1) has a key role in the development of the cochlea and its deficiency causes sensorineural hearing loss in man and mice. During chicken early development, IGF-1 modulates neurogenesis and survival in the cochleovestibular ganglion but no further studies have been conducted to explore the role of IGF-1 in the vestibular system.

In this study we have compared the whole transcriptome of the vestibular organ from *Igf1*$^{-/-}$ and *Igf1*$^{+/+}$ mice at different developmental and postnatal times. RNA was prepared from E18.5, P15 and P90 vestibular organs of both genotypes and the transcriptome analyzed in triplicates using Affymetrix® Mouse Gene 1.1 ST Array Plates. An initial analysis of data was performed with packages available as part of Bioconductor and subsequently the data was analysed and then compared using VSN-RMA and mmBGX for detection of false positives and quantify uncertainty. The differential expression of genes and miRs selected from the study of these arrays were validated by RT-qPCR.

The morphology of the vestibular organ did not show differences between genotypes and no obvious alterations of vestibular function were observed in the *Igf1* deficient mouse. Functional analysis in silico was carried out on genes that were differentially expressed between genotypes and across time to define possible pathways that are involved in the development of the vestibular organ or affected by the lack of IGF-1.

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P78

IDENTIFICATION OF A NOVEL RECESSIVE MUTATION OF MYO7A IN PROFOUND HEARING LOSS BY TARGETED EXOME SEQUENCING

Jihye Rhee[1], Ah Reum Kim[2], Byung Yoon Choi[2,3] *(J. Rhee and A. R. Kim contributed equally to this work)*

[1]Department of Otorhinolaryngology-Head and Neck Surgery, Seoul National University Hospital, Seoul, South Korea; [2]Department of Otorhinolaryngology-Head and Neck Surgery, Seoul National University Bundang Hospital, College of Medicine, Seoul National University, Kyunggi, South Korea; [3]Sensory Organ Research Institute, Seoul National University Medical Research Center, Seoul, South Korea

The MYO7A spans a region of 86.98 kb of genomic DNA and encodes Myosin VIIA. Mutations of the MYO7A can cause both syndromic (USH1B) and non-syndromic (DFNB2, DFNA11) hearing loss in humans. Almost 700 variants in the MYO7A were reported and mutation of it is one of the common causative variants

in Korean cochlear implantees (3/93). In this study, we focused on the sibling with autosomal recessive hearing loss. They were detected by newborn hearing screening test and the thorough medial history history taking, physical examination and audiologic evaluation were performed to rule out any syndromic features. They showed profound degree sensorineural hearing loss. Neither visual impairment nor no vestibular dysfunction was observed among the two affected patients. The pattern of hearing loss itself is not different between syndromic and non-syndromic hearing loss. The subjective visual symptoms of the sibling were not appeared at 13 and 9 year-old, respectively. As the usual onset of retinitis pigmentosa is 9.5 year-old with standard deviation 8, the hearing loss can be considered as nonsyndromic. After GJB2 sequencing revealed no convincing mutation, screening of 134 known deafness genes was performed by targeted resequencing (TRS-134). After basic filtering of the variants, we identified that the affected patients carried p.H133D, a known pathogenic SNP (rs111033187) and p.Lys542Argfsx80, a novel frameshift mutation as a compound heterozygous state, Our study reports a novel frameshift mutation of MYO7A and again confirms a significant contribution of MYO7A to prelingual nonsyndromic autosomal recessive profound SNHL in Koreans.

P79

ROLE OF *SYNJ2* IN HIGH FREQUENCY PROGRESSIVE HEARING LOSS

Elisa Martelletti[1], Annalisa Buniello[1,2], Johanna C. Pass[1,2], Neil J. Ingham[1,2], Jacqueline K. White[2], Karen P. Steel[1,2]
[1]Wolfson Centre for Age-Related Diseases, King's College London, Guy's Campus, London, UK; [2]Wellcome Trust Sanger Institute, Hinxton, Cambridge, UK

Synaptojanin 2 (Synj2) is a phosphatidylinositol phosphatase which removes the 5-position phosphates from phosphoinositides, such as PIP_2 and PIP_3. It is a key enzyme in the phosphoinositide signalling cascade and in clathrin-mediated endocytosis. We are interested in exploring the effect of *Synj2* mutation on the development and function of inner hair cell synapses.
Synj2 mutant mice (*Synj2*$^{tm1b(EUCOMM)Wtsi}$) were derived from the *Synj2*^{tm1b} allele generated at the Sanger Institute. Auditory Brainstem Response (ABR) measurements were recorded from ketamine/xylazine anaesthetised mice at 2, 4, 6, 8, 12 and 14 weeks old in response to click stimuli and tone pips ranging from 6-42kHz. X-gal staining was performed on whole inner ears which were later embedded in paraffin and sectioned. *Synj2* mutant mice (*Synj2*$^{tm1b(EUCOMM)Wtsi}$) were derived from the *Synj2*^{tm1b} allele generated at the Sanger Institute. Auditory Brainstem Response (ABR) measurements were recorded from ketamine/xylazine anaesthetised mice at 2, 4, 6, 8, 12 and 14 weeks old in response to click stimuli and tone pips ranging from 6-42kHz. Cochlea whole mount preparations were labelled with anti-neurofilament antibody and CtBP2 antibody. Specimens (mutant n=4, control n=4) were imaged using confocal microscopy.
Synj2^{tm1b} mutant mice were tested at 2 weeks by ABR and they had normal thresholds, but ABR recordings from 4 weeks onwards showed progressive increase of thresholds for frequencies higher than 30 kHz in comparison to the littermate controls. X-gal staining on sections of inner ear revealed that *Synj2* is expressed in the spiral ganglion and Claudius' cells. Inner ear clearing revealed no abnormalities in the gross structure of the inner ear. We investigated the morphology of nerve terminals in 4 week old *Synj2*^{tm1b} mutants and observed swelling of nerve fibres under inner hair cells.
ABR measurements showed that *Synj2*^{tm1b} mutant mice have normal thresholds at 2 weeks, but they lose high frequency sensitivity from 4 weeks onwards, associated with swelling of inner hair cell nerve endings. This suggests that some defects occur during that window of time. The *Synj2*^{tm1b} mice are a useful tool to improve our knowledge of mechanisms underlying high frequency progressive hearing loss.

P80

POLYMORPHISMS IN GENES INVOLVED IN OXIDATIVE STRESS IN PATIENTS WITH MÉNIÈRE'S DISEASE

Masaaki Teranishi[1], Yasue Uchida[2], Naoki Nishio[1], Ken Kato[1], Hironao Otake[1], Tadao Yoshida[1], Michihiko Sone[1], Saiko Sugiura[3], Fujiko Ando[4], Hiroshi Shimokata[5], Tsutomu Nakashima[6]
[1]Department of Otorhinolaryngology, Nagoya University Graduate School of Medicine, Nagoya, Japan; [2]Department of Otolaryngology, Aichi Medical University, Aichi, Japan; [3]Department of Otorhinolaryngology, National Center for Geriatrics and Gerontology, Aichi, Japan; [4]Department of Health and Medical Sciences, Aichi Shukutoku University, Aichi, Japan; [5]Graduate School of Nutritional Sciences, Nagoya University of Arts and Sciences, Aichi, Japan; [6]Ichinomiya Medical Treatment & Habilitation Center, Aichi, Japan

Introduction. Although the etiologies of Ménière's disease (MD) remain unclear, genetic factors could contribute, at least in part. Recently, accumulating evidence has demonstrated that oxidative stress is related to the pathology of inner ear disease. We investigated the association between genetic polymorphisms located in genes related to oxidative stress and susceptibility to MD in the present study.
Methods. Patients affected by MD, who attended the Department of Otorhinolaryngology of the Nagoya University Hospital between November 2007 and March 2011, were enrolled in the study. The subjects of the control group were selected from the comprehensive Longitudinal Study of Aging (NILS-LSA), an ongoing population-based study with a two-year follow-up, conducted by the National Institute for Longevity Sciences. Polymorphisms in the genes: glutathione peroxidase 1 (*GPX1*; rs1050450); paraoxonase 1 (*PON1*; rs662 and rs854560); *PON2* (rs7493); superoxide dismutase 2 (*SOD2*; rs4880); methionine synthase (*MTR*; rs1805087); methionine-synthase reductase (*MTR*; rs1801394); nitric oxide synthase 3 (*NOS3*; rs1799983);caveolin 1 (*Cav1*; rs3840634); melatonin receptor 1B(*MTNR1B*; rs1387153); NAD(P)H oxidase p22(phox)subunit (*NADH/NADPHp22phox*; rs4673); and mitochondria5178 (*MT5178*; rs28357984) were investigated for statistical analysis.
Results. The *Cav1* polymorphism was significantly associated with a risk of MD; in addition, the OR for the *Cav1* polymorphism and MD risk was 1.849 (CI: 1.033–3.310) with adjustment for age and sex. The remaining polymorphisms failed to show any associations with the risk of MD.
Conclusion. In conclusion, the *Cav1* polymorphisms were significantly associated with the risk of MD.
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P81

A RETROSPECTIVE STUDY ON GJB2 ALLELES IN PATIENTS WITH NONSYNDROMIC SENSORINEURAL HEARING IMPAIRMENT: GENOTYPE/AUDITORY PHENOTYPE CORRELATION AND DESCRIPTION OF RARE VARIANTS

Ilaria Stanghellini[1], Silvia Palma[2], Cristina Falcinelli[1], Maria Consolatrice Guarnaccia[3], Elisabetta Genovese[3], Antonio Percesepe[1]

[1]Medical Genetics Unit, Department of Mother & Child, University Hospital of Modena, Italy; [2]Audiology, Primary Care Department, Asl Modena, Italy; [3]Otolaryngology Department, University Hospital of Modena, Italy

Nonsyndromic sensorineural hearing impairment (NSSHI) represents the most common sensory disorder in humans affecting 1 out of 650 newborns, with half of the cases showing a mendelian etiology.

More than 80 genes are involved in NSSHI, but biallelic mutations in the connexin 26 coding gene (*GJB2*) are responsible for up to 50% of genetic cases and, therefore, mutation analysis of *GJB2* is a highly prescribed test nationwide and worldwide.

The aim of this study is to retrospectively analyze the *GJB2* genetic tests performed in individuals with NSSHI referred to the Audiology center and Medical Genetics Unit of the Modena University Hospital, Italy, and to correlate the genotype with auditory phenotype. 23.1% (115) of the 498 patients enrolled in the study, showed at least one variation in *GJB2*, with 32 homozygotes, 15 compound heterozygotes, and 68 heterozygotes detected. More specifically, 71 subjects (61.7% of the total cases with mutations) showed at least one allele with 35delG, which was present in homozygosity in 28 patients, in compound heterozygosity in 11, and in simple heterozygosity in 32. Additionally, eight variants identified in patients with hearing impairment (HI) were considered rare for their frequency below 1% in the general population and in the HI databases. Four variants (I20T, V95M, N206S, c.-22-2A > C) were detected in compound heterozygosity with known mutations resulting in a range of phenotypes from mild to profound, whereas the W3R, C218Y, K221N, and c.-22-6T > C variants were found in simple heterozygosity. For these last a direct genotype/audiotype correlation was not done due to the lack of evidence of an effect of the single *GJB2* variation on the patient's phenotype and only *in silico* prediction of pathogenicity was possible.

In conclusion we report the data on *GJB2* mutation in the population under study and, based on patients' phenotype, reported frequency, and *in silico* prediction analysis, we suggest the prognostic value of eight rare *GJB2* alleles, which may be of help to the clinician in counseling patients who carry such variants.

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SYNDROMIC SENSORINEURAL HEARING LOSS AND MITOCHONDRIAL DNA MUTATIONS IN MELAS AND CPEO PATIENTS

Gabriella Cadoni[1], Serenella Servidei[2], Monica Giuliani[1], Guido Primiano2, Luca Liberati[1], Pasqualina Picciotti[1], Guido Conti[1]

[1]Department of Head and Neck Surgery and and [2]Department of Neuroscience of the Catholic University of the Sacred Heart (UCSC), Policlinico A. Gemelli, Rome, Italy

Background. Hearing loss has been reported to be common in patients with mitochondrial disorders, a group of diseases characterized by pleiomorphic clinical manifestations due to defects in mitochondrial oxidative phosphorylation. This study aimed to investigate audiological findings in a cohort of patients affected by mitochondrial disease.

Methods. Comprehensive audiological evaluations, including pure tone audiometry, tympanometry, speech audiometry, oto-acoustic emissions (TEOAEs) and auditory brainstem responses (ABR) were performed in 20 Italian Patients with confirmed mitochondrial DNA (mtDNA) defects: 12 affected by mitochondrial encephalomyopathy with lactic acidosis and stroke-like episodes (MELAS) and 8 by chronic progressive external ophtalmoplegia (CPEO).

Results. A3243G mtDNA was found in all MELAS cases, while single deletion in mtDNA was detected in all CPEO patients. Out of the 20 subjects, 18 showed bilateral sensorineural hearing loss (HL), while the reaming two patients were normal hearing. The degree of HL was mild in 8 patients (4 MELAS, 4 CPEO), moderate in 6 (3 MELAS and 3 CPEO), severe in 2 (2 MELAS) and profound in 2 (2 MELAS). High level of percentage of mutation (blood and muscle) resulted associated with higher degree of hearing loss. HL shape was flat in 9 cases (7 MELAS and 2 CPEO), down sloping in 8 (4 CPEO and 4 MELAS) and U-shaped in 1 (1 MELAS). The degree of hearing impairment was lesser in CPEO patients than in MELAS cases. Finally, most of our patients showed a predominantly cochlear type of hearing dysfunction, and only one patient appeared to be affected by central auditory pathway damage.

Conclusions. An involvement of the auditory system is a common finding in patients with mitochondrial diseases, and the site of damage can include cochlea, auditory nerve, central auditory pathway up to the cortex. Hearing loss may contribute to disability in these patients and is commonly associated with multisystem involvement. Genotype, mutant load of mtDNA and other unknown factors may contribute to heterogeneity of hearing impairment in mitochondrial disease.

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A HAYSTACK FULL OF NEEDLES: THE COMPLICATED INTERPRETATION OF RARE GENETIC VARIANTS IN A GERMAN FAMILY WITH AUTOSOMAL DOMINANT NON-SYNDROMIC HEARING LOSS

Michaela A. H. Hofrichter[1], Barbara Vona[1], Erdmute Kunstmann [1], Indrajit Nanda[1], Thomas Haaf [1]

[1]Institute of Human Genetics, University of Würzburg, Germany

Deafness is the most common human sensory deficit affecting approximately one per every 500 newborns. Non-syndromic hearing loss (NSHL) is an extremely genetic heterogeneous disorder with approximately 50% of all cases having an underlying genetic etiology. The majority of NSHL follows an autosomal recessive pattern of inheritance (75-80%), represented by deafness loci B (DFNB); however, autosomal dominant (15-20%) (DFNA), X-linked (2-5%) (DFNX) and mitochondrial (~1%) inheritance is also described. Approximately 30 genes are currently implicated in dominantly inherited NSHL.

We investigated a family with autosomal dominant NSHL segregating across three generations. Affected individuals in the first and second generations have postlingual, bilateral hearing loss (HL) progressing to moderate-to-severe in the high frequencies. The third generation with two affected individuals currently have mild-to-moderate HL, in which one has mid-frequency and the other having high-frequency HL. A parent-child trio including

one affected parent and child were sequenced in parallel using the next generation sequencing TruSight One panel (Illumina) using a MiSeq desktop sequencer which targeted 4813 genes including around 100 HL genes. Data filtering using GensearchNGS software detected the following *in silico* predicted pathogenic variants in HL genes: *TECTA* (DFNA12/DFNB21) c.4437C>G (p.Cys1479Trp); *MYO6* (DFNA22/DFNB37) c.224A>G (p.His75Arg); *MITF* c.1132G>A (p.Ala378Thr); *SLC26A5* (DFNB61) c.-53-2A>G; and *USH1C* (DFNB18A) c.2377C>T (p.His793Tyr). Variants in *TECTA* (autosomal dominant and recessive) and *USH1C* (autosomal recessive) each co-segregated in six individuals with HL in all three generations. However, the *TECTA* variant is most likely responsible for the HL in this family, based on the autosomal dominant inheritance in the family and the strong pathogenic prediction. Whether the *USH1C* or the other variants contribute to HL will be discussed.
In summary, our study highlights the power of screening trios for detection of the genetic complexity in HL cases including alternative possible pathogenic variants and emphasizes the necessity of familial segregation testing for a comprehensive understanding of the mutational picture.

P84
CHARACTERISTCS OF INNER EAR HEARING LOSS DEPENDING ON DIFFERENT MUTATIONS OF GJB2 GENE

Judith Arnolds[1], Sven Saleik[1], Patrick Munder[1], Bernd Wollnik[2], Dagmar Wieczorek[3], Alma Kuechler[3], Diana Arweiler-Harbeck[1]
[1]Department of Otorhinolaryngology, University Hospital of Essen, University Duisburg-Essen, Germany; [2]Institute of Human Genetics, University Hospital of Cologne, Germany; [3]Institute of human Genetics, University Hospital of Essen, University Duisburg-Essen, Germany

Objective. Four in 1000 newborns suffer from congenital inner ear hearing loss. 30% of these are of syndromal origin, 70% non-syndromal origin. 50% of autosomal recessive non-syndromal hearing loss is caused by different mutations of *GJB2* gene. In this study we analysed wether kind and localisation of the mutation influence the time of onset and the exted of hearing loss, and in which way there are differences berween affected family members due to i. e. different expressivity.
Methods. Data of 34 children with different mutations of *GJB2* gene was analysed regarding hearing loss, localization of the mutation, pre-, and postoperative hearing as well as family history. Genetical diagnostics were routinely made before cochlea implantation. Genetic findings of the parents were available in 30%.
Results. While the parental generation seldomly presented phenotypic inner ear hearing loss, almost half of the siblings suffered from high grade inner ear hearing loss. The threshold for click ABR was not measurable in 60% in homozygous childrenwhen diagnosed for the first time, while only in 46% of heterozygous children. Heterozygous children werde implanted later than homozygous children suggesting a later onset of deafness. Regarding the amount of heterozygous patients (38%), it appears likely that there may be more compound heterozygous cases with unknown second mutation. The development of hearing abilities of homozygous children (polysyllabic words 12 mon postop: 65%, 36 mon postop: 84,5%) seems to be a little less and slower than that of heterozygous children (polysyllabic words 12 mon postop: 83%, 36 mon postop: 91,3%), though other influences as implants or method of surgery still have to be reappraised.
Conclusion. Early genetic diagnostics and advice are important for children with inner ear hearing loss of unknown genesis also in less severe cases to assure adequate therapeutic and familial counselling.

P85
DIAGNOSTIC YIELD OF A TARGETED GENE SEQUENCING APPROACH WITHIN A REGIONAL UNIVERSAL NEWBORN HEARING SCREENING AND CHILDHOOD SURVEILLANCE PROGRAM: A 2 YEAR EXPERIENCE IN FRIULI VENEZIA GIULIA, ITALY

Eva Orzan[1], Giorgia Girotto[2], Marco Gregori[1], Raffaella Marchi[1], Caterina Marchese[1], Lorenzo Monasta[3], Anna Morgan[2], Martina La Bianca[4], Diego Vozzi[4], Paolo Gasparini[2,4]
[1]Audiology and ENT, Institute for Maternal and Child Health IRCCS "Burlo Garofolo", Trieste, Italy; [2]Dep. Medical Sciences, University of Trieste, Trieste, Italy; [3]Clinical Epidemiology, Institute for Maternal and Child Health IRCCS "Burlo Garofolo", Trieste, Italy; [4]Maternal and Child Health-IRCCS "Burlo Garofolo", Trieste, Italy

Because most universal newborn hearing screening (UNHS) programs lack an etiologic focus, the reported percentage of permanent hearing loss (PHL) with uncertain etiology can range from 30 to 60%. Recent developments in the molecular diagnosis of hereditary HL comprise the potential to clarify the large number of subjects in whom the putative genetic aetiology has not been characterized, yet the utility and efficiency of these approaches are still to be defined in terms of planning of services. Published data have indeed focused on family-based or selected populations recruited from ENT or genetic consultations. This circumstances have driven us toward a more quantitative understanding of the potential diagnostic yield of a next generation sequencing (NGS) approach linked to a systematic etiologic approach, within the activity of a UNHS and childhood hearing surveillance (CHS) program.
In Friuli Venezia Giulia region, an early intervention program has been successfully implemented by the regional health care agency. From July2012 to June 2014, the program involved 124 UNHS and 812 CHS referrals. The subsequent comprehensive audiologic assessment confirmed the presence of PHL in 47 cases (1,5‰ of newborns and 2,4% of CHS referrals), that were addressed to the single tertiary center to determine the pathogenesis, identify related medical conditions and provide treatment recommendations. Pathogenetic assessment results were discussed by a multidisciplinary team and cases were further classified into 4 etiologic grouping, depending on type of PHL, ENT examination, GJB2/GJB6 mutation analysis, family history, risk factors, associated features and complementary examination findings: (1) PHL of exogenous origin (2) defined PHL syndrome (3) DFNB1 PHL; (4) un-characterized non-syndromic PHL, for which we employed targeted re-sequencing by Ion Torrent PGM™ (LifeTechnologies) to analyze coding and UTR regions of 96 genes related to PHL and hearing function. Although the diagnostic process could not be completed in 4 subjects, we were able to find a causative gene in 40%. In this light, NGS techniques have the potential to achieve a sensitive and cost-effective manner to test causative genes, when combined with an efficiently managed diagnostic protocol. The yield is higher for progressive or late-onset PHL, conditions that drift towards negative UNHS results.

P86

A NEW TARGETED RE-SEQUENCING PANEL FOR UNVEILING THE GENETIC CAUSES OF AGE RELATED HEARING LOSS (ARHL)

Anna Morgan[1], Diego Vozzi[2], Dragana Vuckovic[2], Martina La Bianca[2], Angela D'Eustacchio[2], Maria Pina Concas[3], Mario Pirastu[3], Paolo Gasparini[1,2], Giorgia Girotto[1]

[1]Dept. Medical Sciences, University of Trieste, Trieste, Italy; [2]IRCCS Burlo Garofolo, Trieste, Italy; [3]Institute of Population Genetics, National Research Council of Italy, Sassari, Italy

To date, very little is known about the genetics of ARHL which is the most common sensory disorder affecting millions of people worldwide. In this study, 464 Italian patients coming from both inbred and outbred populations were sequenced using a targeted re-sequencing (TS) panel of 46 ARHL candidates genes including 13 from published GWAS data (Girotto et al. 2014, Wolber et al. 2014) and 33 from animal studies (in collaboration with M.Bowl-MRC, S.Dawson-UCL). TS was based on Ion Torrent PGM™ (Life Technologies) (1942 amplicons ensuring approximately 96,55 % coverage of the target region). Sequencing data were filtered according to frequency values (e.g. ExAC, 1000G databases), pathogenicity prediction (e.g. SIFT, Polyphen2) and conservation score (e.g. PhyloP). All mutations absent or present with a minor allele frequency<10^{-3}, were confirmed by Sanger sequencing and tested in matched controls. Preliminary results report 5 frameshift indels, 2 nonsense and 56 missense mutations; some of them affect genes showing a similar function. Interestingly, 7 mutations lie in genes encoding solute carriers (*SLC28A3*, *SLC16A6*, *SLC9A3R1 SLC44A2)*, 4 mutations are located in genes involved in Ca^{2+} signaling (*STRN, PCDH20, DCLK1*) and 25 affect genes belonging to cytoskeleton and cell motility families (*XIRP2*, *FN1, CEP104*, *PTPRD, EPS8, ANK2*). Three scenarios are present: 1) different mutations in the same gene, 2) the same mutation in different patients and 3) different mutations in the same patient. As regards to 1) three frameshift deletions in *XIRP2* gene were found in 7 patients. A genotype-phenotype correlation was conducted and, interestingly, all these individuals showed a high-frequency hearing loss strongly resembling the phenotype recently described in animal models (Francis et al. 2015). In the second scenario (2) a new nonsense mutation in *SLC28A3* gene was detected in 19 patients belonging to a large Sardinian pedigree from an isolated population. Finally, in agreement with the complex genetic structure of ARHL, 3) up to 3 mutations in different genes were detected in some patients. In this light, pathway analysis can highlight some key interactions. These findings clearly demonstrate the usefulness of our approach (GWAS+animal studies+TS) further supporting the potential role of these genes in causing ARHL.

P87

DFNB1 LOCUS ANALYSIS IN SÃO TOMÉ AND PRÍNCIPE POPULATION

Cristina Caroça[1,2,3], Tiago Morim de Matos[4,5], Graça Fialho[4], João Paço[1,2], Helena Caria[3,4]

[1]University Center of Otorhinolaryngology - Hospital CUF Infante Santo, Lisbon, Portugal; [2]Nova Medical School, Faculty of Medical Sciences of Lisbon, Lisbon, Portugal; [3]Higher School of Health, Polytechnic Institute of Setúbal, Setúbal, Portugal; [4]Biosystems & Integrative Sciences Institute (BioISI), Faculty of Sciences of the University of Lisbon, Lisbon, Portugal; [5]Higher School of Technology of Barreiro, Polytechnic Institute of Setúbal, Setúbal, Portugal

During humanitarian Otorhinolaryngology medical missions of in São Tomé and Príncipe, it was found a prevalence of sensorineural hearing loss (SNHL) of 29.1% in a sample of 316 individuals (2-35 year-old) from the São Tomé and Príncipe population. After conducting a clinical investigation to identify risk factors associated with SNHL, otoscopy and diagnostic tests (pure tone audiogram or brainstem evoked potentials) to quantify the hearing loss, we began the study of possible etiologies.

The genetic etiology associated with DFNB1 has been assessed by analysing a sample of 92 bilateral SNHL patients and 179 normal-hearing individuals, both by sequencing the coding region of *GJB2* gene and by investigating the presence of the two large deletions described for *GJB6* (del1830 and del1854). The remaining individuals (n=45) from the total sample, who present unilateral hearing loss, have also been assessed as regards DFNB1.

The results obtained suggest the genetic influence of Euro-Asiatic populations in São Tomé and Príncipe population, evidenced by the presence of p.Met34Thr, p.Val37Ile and p.Val153Ile mutations, all previously identified in the Portuguese population. Variants already described in African populations (p.Arg75= and p.Val167Met) were as well observed, as it would be expected. Having into account the number of patients analysed, our data suggest that DFNB1-related SNHL is not a major cause for SNHL in the population of São Tomé and Príncipe.

This is the first study aiming at identifying genetic causes for deafness in the population of São Tomé and Príncipe.

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PRESBYCUSIS AND MTDNA

J. Chora[1], M. Flook[1], T.D. Matos[1], G Fialho[1], H. Caria[1,2]

[1]BioISI, Biosystems & Integrative Sciences Institute Faculty of Science of the University of Lisbon, Portugal; [2]ESS/IPS, School of Health, Polytechnic Institute of Setúbal, Portugal

Presbycusis or age-related hearing loss (ARHL) is a multifaceted common phenomenon, with a broad spectrum of causes, environmental and/or genetic, having cognitive and psychosocial consequences. The existence of susceptibility genetic factors predisposing for presbycusis implies that ARHL is not an inevitable condition, but a complex disease with possible treatment and prevention.

We present, in this study, results on genotype-phenotype correlation between mtDNA haplogroups and the degree of presbycusis in an elderly group (n=400) from the Portuguese population. Other epidemiological and etiological factors are considered. The ancestry of the Portuguese population and its connections with Africa and European countries is also discussed, since not only haplogroups common in European countries are found in our sample but also L and U6.

We aim to contribute to better counseling and to the definition of the best approaches for prevention and therapeutics, thus contributing for a hearing healthcare culture.

P89

CLASSIFICATION, PATHOPHYSIOLOGY AND AUDIOLOGICAL PATTERNS OF AGE-RELATED HEARING LOSS

R. Santos[1], A.R. Lameiras[1], A. O'Neill[1,2,3], P. Escada[1,2,3], J. O'Neill[2,3], G. Fialho[4], H. Caria[4,5]

[1]ENT-CHLO, HEM, ENT Service, Centro Hospitalar de Lisboa Ocidental EPE, Hospital Egas Moniz, Portugal; [2]FCM/UNL, Department of Anatomy, Faculty of Medical Sciences, New University of Lisbon, Portugal; [3]CEFITEC, Faculty of Sciences and Technology, New University of Lisbon, Portugal; [4]BioISI, Biosystems & Integrative Sciences Institute Faculty of Science of the University of Lisbon, Portugal; [5]ESS/IPS, School of Health, Polytechnic Institute of Setúbal, Portugal

Presbycusis (age-related hearing loss-ARHL) is a major health problem affecting nearly 70% of people aged ≥ 65 years old. It is responsible for communication disabilities (social deafness), isolation and reduced cognitive auditory input.

Due to its physiology complexity, the mechanisms of ARHL are not fully understood. They involve circulatory disturbances, apoptosis, genetic susceptibility and environmental causes.

Schuknecht (1969) developed a classification for ARHL which establishes an association between pathophysiological mechanisms and alterations of cochlear morphology underlying ARHL and a specific pattern of audiometric evaluation with clinical relevance. Sensorial ARHL is associated with degenerative changes in the organ of Corti and outer hair cells at the basal turn of the cochlea and is characterized by an audiometric pattern with loss in the high frequencies. Neural ARHL is characterized by neuronal loss, in particular of the neuronal afferents, with audiometric representation by a gradually downward curve and important negative impact in auditory discrimination. Metabolic ARHL results from atrophy of the stria vascularis with consequent alterations in the endolymph potential and changes in spiral ligament morphology, which is represented by a flattened curve with loss in all frequencies in the audiometric evaluation. Cochlear conductive ARHL is associated with alterations in cochlear conduction as result of stiffness of the basilar membrane at the basal turn of the cochlea and it is characterized by loss in the low frequencies. Mixed ARHL is characterized by loss in high frequencies as result of degenerative changes in the outer hair cells mainly in the basal turn of the cochlea; impaired discrimination due to severe loss of cochlear neuronal population; loss in all frequencies as effect of decreased stria vascularis cells and alterations in spiral ligament; loss in low frequencies due to injury in the apical turns of the cochlea. Considerations on these clinical data integrating the patient evaluation are crucial for genetic susceptibility evaluation. Some examples are presented.

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AGE-RELATED HEARING LOSS: IS THIS A PREVENTABLE CONDITION?

M. Aparício[1], T.M. Matos[2], P. Arguello[3], M. Antunes[4], A. O'Neill[5,6,7], P. Escada[5,6,7], J. O'Neill[6,7], F. Fialho[2], H. Caria[2,3]

[1]Faculty of Sciences, University of Lisbon, Portugal; [2]BioISI, Faculty of Sciences, University of Lisbon, Lisbon, Portugal; [3]School of Health, Polytechnic Institute of Setúbal, Setúbal, Portugal; [4]CEAUL, Faculty of Sciences, University of Lisbon, Portugal; [5]ENT-CHLO, HEM, ENT Service, Centro Hospitalar de Lisboa Ocidental EPE, Hospital Egas Moniz, Portugal; [6]FCM/UNL, Department of Anatomy, Faculty of Medical Sciences, New University of Lisbon, Portugal; [7] CEFITEC, Faculty of Sciences and Technology, New University of Lisbon, Portugal

The better articulation between social sciences and biomedical sciences, due to the increased knowledge on the pathophysiological mechanisms of cellular aging and the genetic susceptibility factors underlying some age–related disorders, is particularly relevant to the field of active aging. We have been studying age-related hearing loss (ARHL), by attempting to identify predisposing genetic factors and by assessing the impact of ARHL in the quality of life of the elderly citizens. The study here presented focuses on ≈ 450 individuals from the Portuguese population, aged >65, and includes audiological evaluation, assessment of sociological parameters, and genetic analysisinvolving genotyping of variants in genes previously associated with ARHL in other populations.

Socio-demographic parameters of the elderly individuals of the sample are also considered. The statistical analysis is towards the identification of natural patterns of hearing decrease considering both the shape and the magnitude of the audiological curve and the characterization of such patterns through statistical models. In conclusion, we discuss biological and clinical aspects, taking into consideration both the genetic results from the ARHL Portuguese population and the social dimensions of ageing. Thus, this study expects to contribute to the prognosis of ARHL in the future.

POSTER SESSION VII
STEM CELLS AND GENE THERAPY

P91

HYALURONIC ACID PRETREATMENT FOR SENDAI VIRUS-MEDIATED COCHLEAR GENE TRANSFER

Takaomi Kurioka[1], Kunio Mizutari[1], Katsuki Niwa[1], Makoto Inoue[2], Yasuji Ueda[2], Akihiro Shiotani[1]

[1]Department of Otolaryngology, Head and Neck Surgery, National Defense Medical College, Saitama, Japan; [2]DNAVEC Corporation, Ibaraki, Japan

Gene therapy with viral vectors is one of the most promising strategies for sensorineural hearing loss. However, safe and effective administration of the viral vector into cochlear tissue is difficult because of the anatomical isolation of the cochlea. We investigated the efficiency and safety of round window membrane application of Sendai virus, one of the most promising non-genotoxic vectors, after pretreatment with hyaluronic acid (HA) to promote efficient viral translocation into the cochlea. Sendai virus expressing the green fluorescent protein reporter gene was detected throughout cochlear

tissues in the presence of HA. Quantitative analysis revealed that maximum expression was reached 3 days after treatment. The efficiency of transgene expression was several hundred-fold greater with HA pretreatment than without it. Moreover, this approach did not cause hearing loss. These findings reveal the potential utility of gene therapy with the Sendai virus and HA to treat human deafness.

P92

A SIMPLE STEPWISE METHOD FOR HAIR CELL INDUCTION FROM HUMAN INDUCED PLURIPOTENT STEM CELLS

Hiroe Ohnishi, Desislava Skerleva, Shin-ichiro Kitajiri, Tatsunori Sakamoto, Norio Yamamoto, Juichi Ito, Takayuki Nakagawa
Kyoto University, Graduate School of Medicine, Department of Otolaryngology, Head and Neck Surgery, Kyoto, Japan

Objective. Disease-specific induced pluripotent stem (iPS) cells generated from patient somatic cells are considered as useful tools for studying the pathophysiology of inner ear disease and for drug discovery to treat inner ear diseases. We have to establish the stable induction methods for the differentiation of human iPS cells into inner ear hair cells for this purpose. Therefore, in the current study, we investigated the effect of a simple induction method for hair cell induction from human iPS cells.
Materials and methods. We used a stepwise method mimicking inner ear development for induction of inner ear hair cell-like cells. Human iPS cells were differentiated into preplacodal ectoderm, otic placode, and hair cell-like cells sequentially. The first step was preplacodal ectoderm induction. According to a previous study that showed spontaneous differentiation of human ES cells into the preplacodal ectoderm, we cultured human iPS cells on a Matrigel-coated plate in a serum free N2/B27 medium for 8 days. In the second step, we treated the cells after preplacodal ectoderm induction with basic fibroblast growth factor (bFGF) for induction of differentiation into otic placode-like cells for 15 days. In the final step, we incubated the cells after induction of differentiation into otic placode-like cells in a serum-free medium containing Matrigel for hair cell induction for 48 days. We performed immunocytochemical analyses of each induction step. And after hair cell induction, the surface morphology of cultured cells was examined by scanning electron microscopy.
Results. After preplacodal ectoderm induction, we observed the expression of the genes that are expressed in preplacodal ectoderm in over 90% of cultured cells. In the next step, we obtained otic placode marker-positive cells by culture with bFGF. Finally, we induced the hair cell-like cells by culture in serum free medium for 48 days. The hair cell-like cells which we induced show the expression of a hair cell marker and stereocilia bundle-like constructions on their apical surface.
Conclusion. Our simple stepwise method with only bFGF, without the use of xenogeneic cells induced the hair cell-like cells from human iPS cells.

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EVALUATION OF THE EFFECT OF HUMAN ADIPOSE-DERIVED STEM CELLS (ASCS) ON SPIRAL GANGLION NEURONS TO IMPROVE COCHLEAR IMPLANTATION

Philipp Schendzielorz, Yingjun Zhi, Anna Lundershausen, Kristen Rak, Johannes Völker, Agmal Scherzad, Katrin Frölich, Rudolf Hagen and Andreas Radeloff
University Hospital Würzburg, ENT Department, Würzburg, Germany

Introduction. Cochlea implants (CI) are the state-of-the-art therapy in profoundly hearing impaired or deaf patients. Adipose-derived stem cells (ASCs) secret diverse growth and neurotrophic factors and can be transplanted autologously. In a series of in vitro experiments, the effect of human adipose-derived stem cells (ASCs) on
rat spiral ganglion neurons (SGN) was assessed to determine if ASC can potentially be applied in cochlear implantation.
Methods. Human ASC have been cultivated and supernatants were analyzed by ELISA.
Enzymatically dissociated spiral ganglions from rats were co-cultured with or without human ASCs in transwell-systems. After fixation and staining of the cells they were counted and the length of the neurites were measured.
Furthermore human ASC in different concentrations were co-cultivated with spiral ganglions from rats to determine the dose-related efficacy.
Results. ASC produce neuroactive substances like BDNF, GDNF or laminin. Co-culture trials showed that ASC promote enhanced survival of SGN and increased neurite outgrowth after 24h and 48h. Dose-effect relation revealed that lower concentrations of ASC resulted in decreased spiral ganglion cell counts and neurite lengths.
Discussion and Conclusion. These results suggest that ASC may improve the survival of spiral ganglion cells and the neuritogenesis of auditory neurons. In future this may enhance the bioelectric interface in cochlear implantation.

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COMPARISON OF THE DIFFERENTIATION OF NEURAL STEM CELLS AND PRIMARY NEURONS FROM THE RAT COCHLEAR NUCLEUS BY SPONTANEOUS CALCIUM ACTIVITY

C. Voelker, K. Rak, J. Voelker, P. Schendzielorz, R. Hagen, A. Radeloff
Department of Oto-Rhino-Laryngology, Plastic, Aesthetic and Reconstructive Head and Neck Surgery and the Comprehensive Hearing Center, University of Wuerzburg, Germany

The cochlear nucleus is the first relay station for acoustic derived neuronal input from the inner ear. In the cochlear nucleus the second auditory neurons arise, which transport the auditory information to higher levels in the brainstem. Neural stem cells have been described in the post-natal cochlear nucleus recently (Rak et al. 2011). The identified cells display all features of NSC, particularly the capacity to perform mitosis and differentiation in neuronal progenitor cells as well as all cells of the neuronal lineage. The aim of the present study was to determine the differentiation capacity by calcium imaging techniques.
Cochlear nuclei of P5 rats were dissected microscopically. Cells were cultured for 4 weeks in stem cell medium (Neurobasal, GlutaMAX,

B27, EGF, FGF-2) and expanded by formation of neurospheres. After dissociation of the spheres single cells were plated in stem cell medium and changed into differentiation medium (Neurobasal, GlutaMAX, B27, retinoic-acid (10 µM)) for measurements one day after plating. To compare the activity of neural stem cells with primary neurons, dissociated cochlear nucleus neurons were also plated directly in differentiation medium. Both types of cells were loaded with the calcium-sensitive fluorophore Oregon-Green (OG) subsequently and measurements were performed on day 0 and 4 of differentiation. Afterwards the measured in-vitro cultures were fixed with paraformaldehyde. Consecutively neuronal and glial markers were identified immunocytochemically in these cells and analysed by fluorescence microscopy.
In the undifferentiated state 0.5 +/- 0.6 peaks per minute could be measured in the neuronal stem cells. After differentiation there were significantly more peaks per minute (3.2 +/- 1.2). The primary cochlear nucleus neurons displayed an average of 1 +/- 0.5 peaks per minute at day 1 and 5 +/- 2 peaks per minute after the differentiation.
The present study showed that the differentiation of cochlear nucleus derived stem cells can be measured by calcium imaging and are comparable with the activity of primary neurons of the cochlear nucleus. These insights possibly can help to provide a better insight into the development of the auditory system and could be used for new approaches in the treatment of auditory disorders in the future.

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HUMAN DERMAL FIBROBLASTS DEMONSTRATE POSITIVE IMMUNOSTAINING FOR NEURON- AND GLIA- SPECIFIC PROTEINS

Cynthia J. Janmaat[1], Karien E de Rooij[2], Simon C. de Groot[1], Heko Locher[1], John C.M.J. de Groot[1], Johan H.M. Frijns[1], Margriet A.Huisman[1]
[1]Department of Otorhinolaryngology, Leiden University Medical Center, Leiden, the Netherlands; [2]Percuros B.V., Enschede and Department of Radiology, Leiden University Medical Center, Leiden, the Netherlands

In stem cell cultures from adult human tissue, undesirable contamination with fibroblasts is frequently present. The presence of fibroblasts obscures the actual number of stem cells and may result in extracellular matrix production after transplantation. Identification of fibroblasts is difficult because of the lack of specific fibroblast markers. In our laboratory, we aim to establish a cell-based therapy for regeneration of spiral ganglion cells. For that purpose, we isolate and expand neural-crest-derived stem cells from human hair follicle bulges and investigate their potential to differentiate into glial cells and neurons. To establish cellular identities, we perform immunohistochemistry with antibodies specific for glial and neuronal markers, and use fibroblasts as negative control. We frequently observe that human adult dermal fibroblasts also express some glial and neuronal markers. In this study, we have sought to determine whether our observations represent actual expression of these markers or result from cross-reactivity. Immunohistochemistry was performed on human adult dermal fibroblasts using acknowledged neuronal and glial antibodies followed by verification of the data using real-time qRT-PCR. Human adult dermal fibroblasts showed expression of the glia-specific markers SOX9, glial fibrillary acidic protein and KROX20 as well as for the neuron-specific marker class III β-tubulin, both at the protein and mRNA level. Furthermore, human adult dermal fibroblasts showed false-positive immunostaining for S100β and to a lower extent for OCT6. Our results indicate that immunophenotyping as a tool to determine cellular identity is not as reliable as generally assumed, especially since human adult dermal fibroblasts may be mistaken for neuroglial cells, indicating that the ultimate proof of neuronal or glial identity can only be provided by their functionality.

P96
HYPERBARIC OXYGEN TREATMENT OF BONE MARROW DERIVED HUMAN MESENCHYMAL STEM CELLS

Jennifer Schulze[1], Gerrit Paasche[1], Hans Lamm[2], Thomas Lenarz[1], Athanasia Warnecke[1]
[1]Department of Otorhinolaryngology, Head and Neck Surgery, Hannover Medical School, Hannover, Germany; [2]Otolaryngology (HBO), Lister Krankenhaus, Hannover, Germany

Objectives. Severe hearing loss is characterized by loss of hair cells and degeneration of spiral ganglion neurons (SGN). It is treated with a cochlear implant (CI). However, neuronal degeneration and inflammation after insertion of a CI electrode may hamper the performance. Human mesenchymal stem cells (MSC) secrete a variety of different factors, e.g., brain-derived neurotrophic factor (BDNF), which could act neuroprotective and anti-inflammatory. This is important for the development of a biohybrid electrode, i.e., a cell (MSC) coated electrode. Neuroprotective factors could protect residual SGN and lead to a guided outgrowth of dendrites in the direction of the CI electrode. Hyperbaric oxygen therapy (HBOT) utilizes the administration of 100% oxygen at greater pressures than normal atmospheric level in compression chambers and has proven to regenerate the inner ear from damage. Thus, the aim of the study was to investigate the effect of HBOT on the proliferation, the genome and the proteome of MSC.
Methods. The MSC were grown in 48-well plates and were repeatedly exposed to 100% oxygen at pressures of 1.0, 1.5 and 2.0 bar for 90 minutes in an experimental compression chamber. The effects of HBOT on cell proliferation were investigated in relation to normoxic and normobaric control cells (NOR). Moreover, the neuroprotective and neuroregenerative effect of HBO-treated MSC was analysed by cultivation of spiral ganglion neurons (SGN) in collected supernatants. The composition of the secretome was analysed and gene arrays were performed.
Results. The number of MSC started to decrease in HBO-treated samples after four consecutive HBO treatments compared to NOR. After ten HBO treatments, MSC treated with 2.0 bar oxygen showed the lowest cell number. Treating SGN cultures with supernatants of MSC significantly increased the survival rate of SGN and the neurite length when compared to the negative control (serum deprived). Even though HBOT influenced protein secretion as well as gene expression, it did not further increase the neuroprotective and neuroregenerative effect on SGN.
Conclusion. In order to utilize HBOT as adjuvant therapy for biohybrid electrodes, further evaluation of the necessary conditions - *in vitro* and *in vivo* - is needed.
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P97

DIFFERENTIATION OF MOUSE IPS CELL INTO CX26-POSITIVE CELL AND FORMATION OF INTER CELLULAR CX26-GAP JUNCTION PLAQUE

Ichiro Fukunaga[1], Kaori Hatakeyama[1],Toru Aoki[1], Atena Nishikawa[1], Ayumi Fujimoto[1], Osamu Minowa[2], Katsuhisa Ikeda[1], Kazusaku Kamiya[1]

[1]Juntendo University Faculty of Medicine Department of Otorhinolaryngology; [2]BioResource Center, RIKEN, Tsukuba, Japan

Objectives. Congenital deafness affects about 1 in 1000 children and more than half of them have a genetic background such as Gap Junction Beta 2 (*GJB2*) gene mutation, the gene encoding the Connexin 26 (Cx26) protein. Recently, we reported that disruption of the Cx26-dependent gap junction plaque (GJP) is associated with the pathogenesis of *GJB2*-related deafness (Kamiya *et al*., *J Clin Invest.* 2014) and the cochlear gene transfer of *Gjb2* using an adeno-associated virus (AAV) significantly improved GJP formation and the auditory function (Iizuka *et al., Hum Mol Genet*. 2015). On the other hand, we developed a novel strategy for inner ear cell therapy with bone marrow mesenchymal stem cells (Kamiya *et al., Am J Pathol.* 2007). Induced pluripotent stem (iPS) cells can be produced by reprogramming of somatic cells and are capable of self-renewal and differentiation into various types of cells as embryonic stem (ES) cells. Although, many studies have shown the differentiation of ES/iPS cells into Cx37/40/43/45 expressing cells, the differentiation into Cx26 expressing cells and the Cx26-gap junction formation has not been reported yet. In this study, we developed a new strategy for differentiation of mouse iPS cells into Cx26 expressing cells.

Methods. We examined the strategy to induce Cx26 expressing cells with GJP formation from mouse iPS cells using modified methods of previous studies for inner ear differentiations with aggregate formation.

Results. After the aggregate formation in the several differentiation conditions, epithelial like cells were observed at the surface of these aggregates. Notable differences in epithelial formation and thickness were observed among these conditions. In a part of the aggregates, Cx26 positive cells were observed and these cells showed GJP-like formations as cochlear cells. Myosin 7a positive cells were also observed in the other part of these aggregates.

Conclusions. This is a first report to demonstrate the differentiation of mouse iPS cells into Cx26 positive cells followed by Cx26-GJP formation. By using these Cx26-positive cells, it is expected to establish the inner ear cell therapy for hearing recovery in *GJB2*-related hereditary deafness.

P98

CHEMICAL INDUCTION OF SENSORY CELL DIFFERENTIATION IN AN OTIC STEM CELL-BASED *IN VITRO* ASSAY

Aurélie Dos Santos[1], Mirko Jaumann[1], Andrea Müller[1], Fiona Speichinger[1], Marcus Müller[2], Hubert Löwenheim[2]

[1]University of Tübingen Medical Center, Department of Otorhinolaryngology, Head Neck Surgery, Hearing Research Center, Tübingen, Germany; [2]University of Oldenburg, Department of Otorhinolaryngology, Head Neck Surgery, European Medical School, Oldenburg, Germany

The mammalian auditory epithelium, the organ of Corti, has only limited regenerative capacity. This causes irreversible sensorineural hearing loss, for example caused by acoustic trauma or ototoxic drugs. Towards a therapy for sensorineural hearing loss, the development of in vitro cultures of mammalian sensory cells enable pharmacological drug screening. We set out to develop an in vitro assay for drug screening based on stem- or progenitor cells derived from the murine postnatal organ of Corti. These cells spontaneously differentiate into hair cell- (HC) and supporting cell-like cells (SC), however with low efficiency in vitro (Oshima et al., JARO, 2007).

To gain further insight in differentiation processes towards hair- and supporting cells, we modulated different signaling pathways in our in vitro otic stem cell-based assay in a low-to-medium throughput screening. Numerous signaling pathways are involved during the inner ear development inter alia the Notch and the canonical Wnt/b-catenin signaling pathways (Lanford et al., Nat Gen., 1999; Jacques et al., Dev., 2012).

A small library of Notch signaling inhibitors (i.e. gamma-secretase, pan-Notch inhibitors), Wnt/b-catenin signaling pathway activators (i.e. GSK3 inhibitors) and growth factors (i.e. EGF) was tested in a 96-well plate format. The ability of the compounds to influence sensory cell fate in vitro was analyzed and evaluated using the ImageXpress Micro XLS High-Content Screening microscope (Molecular Devices).

We observed a significant increase of hair cell (Myosin 7a) and supporting cell-like cells (SOX2) in vitro after treatment of the otic spheres with the selective Notch inhibitor (BMS906024, 1 µM, 24 h) and with EGF (5 and 20 ng/ml, 14 days). Our results show that the mouse in vitro assay based on cochlear stem- or progenitor cells is a valuable tool for low-to-medium throughput screening. The technology allows improvement of a functional ototoxic/ otoprotective drug-screening model for high content / throughput screening in future.

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P99

INDUCTION OF SENSORY NEURONS BY SMALL MOLECULES

Rouknuddin Ali[1], Evelina Blomberg[2], Anna Falk[1], Lars Ährlund-Richter[2], Mats Ulfendahl[1]

[1]Department of Neuroscience, Karolinska Institutet, Stockholm, Sweden; [2]Department of Women's and Children's health, Karolinska Institutet, Stockholm, Sweden

Background. The loss of hair cells and auditory neurons is permanent in humans and cell therapy approaches have been suggested for replacing the missing cells. Different methods have been used to differentiate stem cells into particular cell lineages. Chemical approaches with small molecules with specificity for genes or gene product have been shown to be valuable tools in stem cell research. Compared to genetic modifications chemical approaches have a number of advantages; being more convenient to use, a higher degree of temporal control (rapid and reversible effects), and large possibilities to be used at different concentrations and combinations. Small molecules have been used to trigger a rapid conversion of embryonic stem cells to a neural cell fate. Isoxazole 9 (ISX9 a neurogenic agent), SB431542 (TGFβ pathway inhibitor) and metformin (a first line drug for diabetes) have been shown to induce neural differentiation in adult neural stem cells and

embryonic stem cells respectively. Isoxazole mediates a neuroD gene induction via activation of Ca^{2+} influx, which increases the expression of NeuroD1 transcription factor, important in spiral ganglion development. In this study, these small molecules were tested for their ability to differentiate neuroepithelial stem cells into sensory neural progenitors.

Methods. Neuroepithelial stem (NES) cells were transferred to polyornithine/laminin-coated coverslips and pre-cultured in differentiation medium for an initial two days, followed by the addition of titrated doses of small molecules for 4 days. This culture condition was then replaced with medium containing BDNF/NT3I for an additional 7 days. Half of the medium volume was changed every second day. Cells were collected for qPCR and immunocytochemistry.

Result. Addition of SB431542 or metformin revealed no measurable effects. Quantitative mRNA analysis of ISX9 treated cells for sensory neural markers Brn3a, GATA3, and peripherin showed a 6/26/27-fold increase, respectively ($p<0.05$). Preliminary data from immunocytochemistry also show higher numbers of Brn3a/tuj1 positive neurons in ISX9 treated cells at the tested time points.

Conclusion. The results suggest that small molecule targeting of ISX9 could enhance the induction of sensory neurons from stem cells and of potential use in protocols aiming for the replacement of sensory neurons in the cochlea.

P100
PURIFICATION AND NEUROGLIAL DIFFERENTIATION OF MULTIPOTENT HAIR-FOLLICLE-BULGE-DERIVED STEM CELLS

Timo Schomann, Fleur ten Tije, Shirley Man, Carlijn N.E. Peerboom, Johan H.M. Frijns, Margriet A. Huisman
Department of Otorhinolaryngology and Head & Neck Surgery, Leiden University Medical Center, Leiden, the Netherlands

A prerequisite for stem-cell-based therapy is the unlimited availability of adult tissue as a source for autologous stem cells. However, stem cell cultures from adult mouse and human tissues are often contaminated by fibroblasts. After engraftment these contaminating cells may induce inadvertent formation of extracellular matrix, which can have potentially dangerous consequences, and therefore minimally-perturbative purification strategies are needed.

We have developed a protocol to obtain a pure population of multipotent hair-follicle-bulge-derived stem cells (HFBSCs) by means of adhesion to PuraMatrix (PM), a hydrogel-based self-assembling scaffold forming an effective fibrous micro-environment for neural cells. In addition, we used the innate capacity of neural stem cells to form neurospheres as a second approach to separate HFBSCs, which are derived from the neural crest, from contaminating fibroblasts. Subsequently, isolated stem cells were differentiated and the expression patterns for a selected panel of neuronal, glial and fibroblast markers were investigated by means of immunohistochemistry.

HFBSCs cultured in PM stained positive for the glial markers Krox-20 and S100B. They also expressed the neuronal stem cell markers Gap-43 and nestin as well as laminin, but TUBB3 – expressed abundantly in fibroblasts – could not be demonstrated. HFBSCs cultured under serum-free conditions were able to form free-floating neurosphere-like structures within four days. Cells isolated from these spheres stained positive for the glial markers S100B and Gap-43 as well as for the neuronal marker TUBB3. In contrast, human adult dermal fibroblasts do not have the capacity to form free-floating spheres.

We present two novel protocols for stem cells purification, both preserving the capacity of HFBSCs to differentiate into a neuroglial lineage. Since both protocols yield a slightly-different neuronal cell types after differentiation, we will in a future study investigate the performance of both, the PM-derived and neurosphere-derived cell types using in an *in vivo* model of inner ear regeneration. In conclusion, we think that purification of stem cells prior to transplantation is crucial and both protocols will contribute to the safety of autologous cell-based therapy, especially with regard to transplantation of cells in delicate areas, such as the brain and the inner ear.

P101
INTENSIVE SUPPORTING CELL PROLIFERATION AND MITOTIC HAIR CELL GENERATION BY GENETIC REPROGRAMMING IN NEONATAL MOUSE COCHLEA

Wenli Ni[1], Chen Lin[1], Luo Guoa[2], Wenyan Li[1], Huawei Li[1,2,3,4]
[1]Department of Otorhinolaryngology, Affiliated Eye and ENT Hospital of Fudan University, Shanghai, China; [2]Key Laboratory of Hearing Medicine, Ministry of Health, Affiliated Eye and ENT Hospital of Fudan University, Shanghai, China; [3]Institutes of Stem Cell and Regeneration Medicine, Institution of Biomedical Sciences, Fudan University, Shanghai, China; [4]State Key Laboratory of Medical Neurobiology, Fudan University, Shanghai, China

Hair cell loss is the predominant cause for hearing and balance disorder. In mammals only limited hair cell regeneration has been identified in neonatal cochlea in vivo. Notch and Wnt are two fundamental pathways, which regulate the proliferation of progenitor cells and the cell fate determination of inner ear. β-catenin overexpression led the proliferation of supporting cells, while Notch inhibition initiated the proliferation of supporting cells and mitotic regeneration of hair cells via β-catenin. We observed intensive proliferation of supporting cells by up-regulation of Wnt and inhibition of Notch signal pathway in $sox2^+$ cells in vivo. However, only a small amount of proliferative supporting cells trans-differentiated into hair cells. Furthermore, more mitotic hair cells were generated through the overexpression of Atoh1 in proliferative cells in vivo. Our study reprogrammed the progenitor cells in vivo and provided a new way to stimulate hair cell mitotic regeneration.

P102
ISOLATION AND CHARACTERIZATION OF NEURAL STEM CELLS FROM THE INFERIOR COLLICULUS

Johannes Voelker, Christine Voelker, Kristen Rak, Philipp Schendzielorz, Rudolf Hagen, Andreas Radeloff
Department of Oto-Rhino-Laryngology, Plastic, Aesthetic and Reconstructive Head and Neck Surgery, Comprehensive Hearing Center, University of Wuerzburg, Germany

The inferior colliculus (I.C.), following the cochlear nucleus und lateral lemniscus, is the third relay station of the auditory pathway. Auditory midbrain implants can be used in this area to recover damage to the peripheral auditory pathway. Neural stem cells could be identified in several parts of the central nervous system and in the cochlear nucleus during the past years (Rak et al., 2013). The I.C. has not yet been studied in regard to its stem cell potential. The aim of this study was to investigate the potential of this brainstem

nucleus for self-renewal and differentiation into progenitor cells and cells of the neuroectodermal line.
I.C. of p6 CD rats were dissected microscopically and cultured free-floating in stem cell medium (Neurobasal, B27, GlutaMAX, EGF, bGF) for 4 weeks after dissociation. Single cells from these cultures were plated on glass coverslips and cultured for 8 days in differentiation-medium under deprivation of growth factors. After fixation with PFA, immunofluorescence analysis was done. In addition whole-mount organ-cultures of the I.C. were analysed. After cryofixation, the histological specimens were examined immunohistocemically.
This study could prove a potential for progenitor cell expansion in free floating cultures and whole-mount organ-cultures of the I.C. of rats. In histological preparations neural stem cell markers of the auditory system could be identified. These progenitor cells showed the potential to differentiate into all lines of the neuroectodermal line.
These results display that the I.C. has an neurogenic niche, like other brain areas. The detected neural stem cells could be helpful in the future for the development of new treatment options for hearing disorders of the auditory pathway and might be used for improved coupling of neuroelectrodes.

P103
SCREENING FOR SURFACE MARKERS THAT WOULD ALLOW FOR THE PROSPECTIVE IDENTIFICATION AND PURIFICATION OF HESC-DERIVED OTIC PROGENITORS

Sarah L. Boddy, Darrell M. Barrott, Paul J. Gokhale, Marcelo N. Rivolta
Centre for Stem Cell Biology, Biomedical Sciences Department, University of Sheffield, UK

Stem and progenitor cells hold great promise for treating auditory conditions through direct replacement of damaged components of the inner ear. However, current differentiation protocols yield heterogeneous populations, which may include cells with the capacity to affect the success of such therapies both positively and negatively. Protocols have been developed to induce differentiation of human embryonic stem cells (hESCs) along otic lineages and have been shown in animal models to be capable of rescuing auditory function. Whilst no evidence of side-effects such as tumour formation was seen in these animals, their relatively short lifespan may mask such problems. Hence, to improve current differentiation and transplantation protocols, it is essential to gain a more comprehensive understanding of the cell types found in cultures induced towards otic progenitor fates and to develop methods that would allow their prospective identification and isolation.
We present data from an antibody-based screen undertaken to probe the cell surface proteome of individual cells and colonies within otic progenitor cultures derived from a hESC reporter line where GFP is driven by an otic enhancer. In order to maintain morphological information alongside single-cell data, we chose a bioimaging platform combining a human cell surface antibody panel with an automated microscopy imaging system. Pilot studies have identified a potential surface antibody candidate for separating otic neuronal progenitors from heterogeneous differentiating cultures. The surface antibody colocalizes with GFP reporter expression in the morphologically distinctive ONPs, and also segregates with other otic markers such as FOXG1 and PAX2.
Characterisation of the cell surface of otic progenitors will not only improve understanding of the biology of such cells generated *in vitro*, but will also facilitate direct comparisons with cells obtained *in vivo*. Such information may offer avenues to further manipulate their differentiation into more phenotypically equivalent inner ear hair and neuronal cells, thereby improving their therapeutic potential. From a practical perspective, identification of markers with functional antibodies suitable for use in fluorescence-activated cell sorting will enable specific sub populations to be isolated prior to implantation, reducing the health risks of carrying over additional proliferative cells that may give rise to tumours.
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POSTER SESSION VIII
CELL PHYSIOLOGY

P104
THE HAIR CELL SYNAPTIC COMPLEX AND NEUROJUNCTIONAL CONNECTIVITY

Dennis G. Drescher[1,2], Neeliyath A. Ramakrishnan[1], Marian J. Drescher[1]
[1]*Laboratory of Bio-otology, Department of Otolaryngology and* [2]*Department of Biochemistry and Molecular Biology, Wayne State University School of Medicine, Detroit, MI, USA*

Objective. To provide evidence for hair-cell interaction of syntaxin-1 with the calcium sensor otoferlin presynaptically and with junctional proteins PTPRU (protein tyrosine phosphatase type U) and neuroligin-3 postsynaptically.
Methods. Interactions of individually-cloned otoferlin C2 domains with syntaxin-1 and self-interactions of otoferlin C2 domains were determined by yeast two-hybrid, pull-down, and surface plasmon resonance analysis. For junctional protein interaction studies, a yeast two-hybrid assay using trout syntaxin-1(B) was employed as bait with a hair-cell cDNA library as prey. Mammalian-equivalent regions of rat sequences corresponding to candidate prey sequences were further tested in yeast two-hybrid co-transformation experiments. Pull-down experiments were performed involving GST-syntaxin-1 fusion protein and total rat brain protein.
Results. The C2A and C2F domains of otoferlin interacted robustly with syntaxin-1 or its SNARE domain. C2F binding was calcium-dependent and C2A binding was calcium-independent. Self-binding of otoferlin C2F domain with C2C, D, E, or F showed an inverse calcium dependence, with increased binding as calcium concentration was *decreased*, most pronounced for C2F-C2E and least pronounced for C2F-C2F. Also, a binding partner for syntaxin-1 in trout saccular hair cells was identified by yeast two-hybrid with sequence identity to PTPRU, with interaction confirmed using rat sequences. We observed interaction between PTPRU and neuroligin-3 by yeast two-hybrid analysis and GST pull-down studies with specific antibodies.
Conclusions. Syntaxin-1 is an important SNARE synaptic complex component that interacts functionally with other proteins, such as the calcium sensor, otoferlin, in synaptic release. As opposed to an increase in binding with increased calcium, interactions between otoferlin C2F domain and its intramolecular C2 domains occur at

zero-to-low calcium, consistent with intra-C2 domain interactions forming a closed tertiary structure at low calcium that opens as calcium increases, suggesting a direct role for otoferlin in exocytosis and binding to SNAREs. Since PTPRs play crucial roles in cell signalling and interact with many proteins, including cell-junction and synaptic-junction proteins in active zone assembly, our results also point to a molecular complex formed between syntaxin-1 and PTPRU in the hair cell on the pre-synaptic side and neuroligin-3 on the post-synaptic side connecting the hair cell and primary afferent neuron.

P105

VLGR1-MEDIATED SIGNALING PATHWAY IS IMPORTANT FOR HEARING

Haibo Du[1], Qiaoxia Hu[2], Yanfei Wang[1], Jinpeng Sun[2], Zhigang Xu[1]
[1] Shandong University, School of Life Sciences, Jinan, China; [2] Shandong University, School of Medicine, Jinan, China

The very large G protein-coupled receptor 1 (Vlgr1) forms the core component of ankle-links of inner ear hair cells. Mutations of Vlgr1 gene have been shown to affect ankle-links and cause hearing loss. However, the mechanism of Vlgr1-mediated intracellular signaling and its role in hearing remain elusive. We found that Vlgr1 could regulate G-protein signaling pathway through Gαi coupling, resulting in adenylate cyclase (AC) inhibition. Interestingly, the deafness-associated Vlgr1 Y6236fsx1 mutant showed increased Gαi coupling and enhanced AC inhibition, suggesting that disregulation of Vlgr1-mediated signaling pathway might contribute to hearing loss. To further explore the role of Vlgr1-mediated signaling pathway in hearing, we developed knock-in mice with Vlgr1 Y6236fsx1 mutation. Preliminary examination showed that Vlgr1 mutant mice are profoundly deaf. Taken together, our data suggested that Vlgr1-mediated signaling pathway play an important role in hearing and disregulation of this pathway could cause hearing loss.

P106

HYDROGEN SULFIDE INDUCES STORE-OPERATED CA^{2+} ENTRY THROUGH ACTIVATION OF TRPV4 IN OUTER HAIR CELLS

Narinobu Harada[1], Yukari Ito[1], Ippei Takashima[2], Akio Ojida[2], Fumiko Sekiguchi[3], Atsufumi Kawabata[3]
[1]Hearing Research Laboratory, Harada ENT Clinic, Osaka, Japan; [2]Graduate School of Pharmaceutical Sciences, Kyushu University, Fukuoka, Japan; [3]Division of Pharmacology and Pathophysiology, School of Pharmacy, Kinki University, Osaka, Japan

There is increasing evidence that endogenous hydrogen sulfide (H_2S) acts as an important class of gaseous signal transmitter similar to NO and CO. It is also suggested that H_2S plays an important role in the nervous system. However the role of H_2S in the auditory signal transduction still remains unknown.

Here, we investigated whether H_2S can induce a Ca^{2+} response in outer hair cells (OHCs) of the guinea pig cochlea using the Ca^{2+}-sensitive dye fura-2. In the present study, we used NaHS as an H_2S donor. Production of H_2S by NaHS was detected in OHCs using the H_2S-sensitive fluorescent probe 2a-Cd^{II}. NaHS induced an increase in intracellular Ca^{2+} concentrations ($[Ca^{2+}]_i$) in OHCs with an initial rise followed by a sustained elevation even after washout of the agonist. In the absence of extracellular Ca^{2+}, NaHS-induced increase of $[Ca^{2+}]_i$ was abolished. HC-067047, a specific TRPV4 inhibitor inhibited the NaHS-induced increase of $[Ca^{2+}]_i$ in OHCs whereas nifedipine (an L-type Ca^{2+} channel blocker), NNC55-0396 (a T-type Ca^{2+} channel blocker) and AP18 (a specific TRPA1 inhibitor) did not inhibit it. Fura-2 fluorescence-quenching experiments with Mn^{2+} showed that NaHS triggered a Mn^{2+} influx. HC-067047 suppressed the NaHS-induced Mn^{2+} influx. The NaHS-induced increase of $[Ca^{2+}]_i$ in OHCs was inhibited by the pretreatment with caffeine, an activator of a Ca^{2+} release from ryanodine-sensitive Ca^{2+} stores, and by ryanodine, its inhibitor. 2APB, an inhibitor of store operated calcium entry (SOCE) suppressed thapsigargin-induced Mn^{2+} quenching in fura-2 fluorescence in OHCs. 2APB also inhibited the NaHS-induced increase of $[Ca^{2+}]_i$ in OHCs.

These results suggest that NaHS can induce a Ca^{2+} influx, which is presumably mediated by the activation of TRPV4 in OHCs. Thereafter the NaHS-induced Ca^{2+} influx can induce a Ca^{2+} release from ryanodine-sensitive Ca^{2+} stores, which results in the additional Ca^{2+} influx by SOCE in OHCs. H_2S may function as a neurotransmitter or a neuromodulator of the auditory signal transduction pathway.

P107

THE BIOPHYSICAL PROPERTIES OF $I_{K,L}$ IN MAMMALIAN VESTIBULAR TYPE I HAIR CELLS AND HOW THEY ARE AFFECTED BY THE NERVE CALYX

Elisa Tavazzani[1], Paolo Spaiardi[1], Marco Manca[1], Jacopo Magistretti[2], Giancarlo Russo[1], Ivo Prigioni[1], Sergio Masetto[1]
[1]Department of Brain and Behavioral Sciences, University of Pavia, Italy; [2] Department of Biology and Biotechnologies, University of Pavia, Italy

Mammalian vestibular epithelia have two different hair cells, Type I and II. While Type II cells are innervated by several bouton terminals, Type I cells are contacted by a single afferent calyx that envelopes the entire cell basolateral membrane. In addition, Type I cells express a distinctive low-voltage-activated outward rectifying K^+ current, $I_{K,L}$, which is responsible for their low input resistance at rest compared to Type II cells. Despite its functional importance, however, $I_{K,L}$ biophysical properties and molecular profile have not yet been defined. Its voltage- and time-dependent properties have been reported to vary at different developmental stages, among cells at a same age, and also over time in the same cell. By using patch-clamp recording from *in situ* and dissociated mouse *crista* Type I cells, we found that the observed variability in $I_{K,L}$ properties may be accounted for by different degrees of K^+ accumulation in the narrow space of the synaptic cleft between the hair cell and the residual nerve calyx. After complete calyx removal, $I_{K,L}$ properties in adult animals were in fact consistent among cells and did not change during the recording. $I_{K,L}$ in these cells showed a quite slow deactivation kinetics (time constant ~ 1 s at –80 mV), a complex activation kinetics best described by a three exponential function, a half-activation voltage of –69 mV, and a steep voltage dependence (S = 3.68). This study provides the first complete biophysical description of the genuine properties of $I_{K,L}$, while confirming that the calyx strongly limits K^+ diffusion out of the synaptic cleft. Intercellular K^+ accumulation might provide a direct way to depolarize both the hair cell and its calyx, thus allowing an additional form of communication that cooperates with the conventional glutamatergic synaptic transmission.

P108

SODIUM-ACTIVATED POTASSIUM CURRENT IS ACTIVATED BY THE ACID SENSING IONIC CHANNELS MEDIATED NA^+ INFLUX IN AFFERENT VESTIBULAR NEURONS

Blanca Cervantes[1], Rosario Vega[2], Enrique Soto[2]
[1]Instituto de Investigaciones Biomédicas "Alberto Sols", Consejo Superior de Investigaciones Científicas, Universidad Autónoma de Madrid (CSIC-UAM), Madrid, Spain; [2]Instituto de Fisiología, Universidad Autónoma de Puebla, Puebla, México

Vestibular afferent neurons (VANs) dynamics encode linear and angular acceleration within their firing patterns which are heavily influenced by the K^+ conductance expressed by vestibular neurons. In previous work we shown that the two sodium activated potassium channels (K_{Na}) subunits (Slack and Slick) are expressed in the VANs (Cervantes et al., 2013). Activation of K_{Na} depends of the intracellular Na^+. It was shown that Na^+ increase caused by activation of sodium channels during the action potential is sufficient to activate the K_{Na}. Thence, we asked whether the large Na^+ influx caused by acid sensing ion channels (ASIC) will also activate the K_{Na}. To answer this question ionic currents in isolated VANs were recorded by whole cell voltage clamp technique. Confocal microscopy analysis of colocalization using anti ASIC1 and anti Slack or Slick antibodies were also performed. In the voltage clamp to activate the ASIC current an acid pulse of pH 6.1 (5 s) was used, according to Mercado et al., 2006. We found that in 19 % of the cells (n = 26) the typical inward ASIC current was followed by an outward current at the end of the acidic pulse. Given that it has been shown that Li^+ permeate through the ASICs but does not activates the K_{Na} current. In our experiments the substitution of Li^+ for Na^+ in the extracellular solution (pH 7.4 and 6.1) did not modify the peak, sustained current, time course or desensitization of the acid gated inward current ($P > 0.05$, n = 5), however Li^+ significantly decreased the outward current which follows the ASIC activation 39 ± 12 % ($P < 0.05$, n = 5). The use of fluorescence antibodies shown that ASIC1 and Slick or Slack signals colocalize in the vestibular ganglion neurons and in the vestibular sensory epithelium. These results suggest that Na^+ influx through the ASICs may activate the K_{Na} current producing an outward current at the end of the acid gated current.

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P109

STAGGERING ALONG THE COCHLEA: DOES THE SHAPE OF AN INNER HAIR CELL MATTER?

Anwen Bullen, Antonio M Garcia de Diego, Andrew Forge, Jonathan Ashmore
UCL Ear Institute, London, UK

Objective. The IHCs lined along the cochlea provide sensory input for the auditory nerve fibres; these outnumber the IHCs by 10-20 times. It is thought that different subpopulations of fibres contacting an IHC encode different ranges of normal sound intensities but the cellular mechanisms responsible for the implied synapse heterogeneity are unresolved. Synapse position on the IHC may be a factor, with predominantly high threshold fibres forming contacts on one side of the cell. To identify cell polarity markers related to synapse heterogeneities we have used both 3D electron microscopic reconstruction of IHCs and vital marking of cells from the adult mouse cochlea during functional recording in the intact temporal bone.

Methods and Results. By 3D electron microscopy we have found that a large continuous endoplasmic reticular membrane is concentrated on the most 'flattened' surface of the IHC, a surface which can be identified unambiguously on reconstructing the cell. To eliminate the possibility that such morphology was a fixation artefact, we have used high resolution multiphoton confocal imaging of unfixed IHCs in situ. Applying the membrane label FM1-43, taken up by from the apical surface, cells towards the mid cochlear region (1-2.5 mm from the apex) often showed a staggered alignment, with the intracellular label preferentially distributed along the flattened surfaces and consistent with the electron microscopy observations. In separate experiments, IHCs were loaded with the calcium dye OGB5N during whole cell patch clamp recording. In such cases a pronounced flattened surface was usually apparent on the pillar side of the cell. In the cases where IHCs were dye coupled together, the degree of cell staggering was minimal, suggesting that such coupled cells originate from the more apical cochlear region (6-12 kHz).

Conclusion. Taken together, the data are consistent with IHC internal membrane structures, determined by 3D electron microscopy, being essential organising and functional components of the IHC synapses.

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P110

ACTIN FILAMENT NETWORK REGULATES EXOCYTOSIS AT THE HAIR CELL RIBBON SYNAPSE

Marie Guillet, Gaston Sendin, Jérôme Bourien, Jean-Luc Puel, Régis Nouvian
Institute for Neurosciences of Montpellier - Inserm U1051, Montpellier France

In this study, we probed whether the actin filament network regulates the synaptic vesicle exocytosis at the inner hair cell ribbon synapse. To do so, we measured calcium-triggered exocytosis using patch-clamp recordings. Our results suggest that actin network disruption increases exocytosis and that actin may spatially organize a sub-fraction of synaptic vesicle with respect to the calcium channel.

P111

HISTAMINE TYPE 4 RECEPTOR ANTAGONIST JNJ7777120 INHIBITS SODIUM CURRENT OF VESTIBULAR AFFERENT NEURONS OF THE RAT

Rosario Vega[1], Enrique Soto[1], Emilio Salceda[1], Eduardo Salinas[1], Emmanuel Seseña[2]
[1]Instituto de Fisiología, Benemérita Universidad Autónoma de Puebla, México; [2]Facultad de Medicina de laa Benemérita Universidad Autónoma de Puebla, México

The histaminergic drugs are some of the most commonly used in the treatment of vestibular disorders. Drugs JNJ7777120 and JNJ10191584 thought to be selective type 4 histamine receptor (H4R) were shown to potently inhibit action potential discharge of the vestibular afferent neurons (VAN) (Desmadryl et al., 2012). However, its mechanism of action on the VANs has not yet been determined, with this aim we studied the voltage dependent ionic currents modulated by JNJ7777120 and the participation of G-protein mediated signaling involved in the JNJ77777120 effect.

The action of JNJ7777120 on the VAN ionic currents from the rat were studied using the current and voltage clamp technique. In voltage clamp experiments JNJ7777120 decreased inward and outward current components, effect that remained in cells pretreated with pertussis toxin for 20 hours. The isolated voltage gated sodium current (INa) showed two components: a transient (INat) and a persistent (INap), the application of JNJ7777120 produced a concentration dependent inhibition of both components of the INa with an IC_{50} of 41 nM for the INat and 16 nM for the INap. The JNJ7777120 produced non-significant changes in both INat and INap current activation or inactivation parameters. The effects of JNJ7777120 remained even after the use of the G-protein blocker GDP-β-S and zero GTP in the pipette solution. The use of H4R agonists VUF4430 did not significantly modified the INa. In current clamp experiments JNJ7777120 decreased the action potential discharge produced by depolarizing current injection, and modified the action potential waveform decreasing the amplitude, the maximum depolarization rate, the maximum repolarization rate and increased the action potential duration. Our results show that JNJ7777120 inhibited the VAN electric activity through the inhibition of the INat, INap, through a mechanism independent of the H4R, and independent of G protein coupled receptors suggesting that JNJ7777120 directly interacts with the voltage gated sodium channels.

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P112

DETECTION OF "HIDDEN" AUDITORY NERVE FIBER LOSS

Charlène Batrel, Antoine Huet, Gilles Desmadryl, Jean-Luc Puel, Jérôme Bourien

Institute for Neurosciences of Montpellier, Inserm U1051, Montpellier France

Sound-evoked compound action potential (CAP), which captures the synchronous activation of the auditory nerve fibers (ANFs), is commonly used to probe deafness in experimental and clinical settings. Recent studies have shown that substantial ANF loss can coexist with normal hearing threshold and even unchanged CAP amplitude, making the detection of auditory neuropathies difficult. In this study, we took advantage of the round window neural noise (RWNN) to probe ANF loss in the ouabain-induced neuropathy model. ANF loss was induced by the application of ouabain onto the round window niche. CAP and RWNN of the gerbil's cochlea were recorded through an electrode placed onto the round window niche, 6 days after the ouabain application. Afferent synapses count and single-unit recordings were carried-out to determine the degree and the nature of ANF loss, respectively. Application of a low ouabain-dose into the gerbil RW niche elicits a specific degeneration of low spontaneous rate (SR) fibers, as shown by single-unit recordings. Simultaneous recordings (CAP/single-unit) demonstrate that low-SR fibers have a weak contribution to the CAP amplitude because of their delayed and broad first spike latency distribution. However, the RWMN amplitude decreases with the degree of synaptic loss. The RWNN method is therefore more sensitive than CAP to detect low-SR fiber loss most probably because it reflects the sustained discharge rate of ANFs. The round window neural noise is a faithful proxy to probe the degree and the SR-based nature of fiber loss. This method could be translated into the clinic to probe hidden hearing loss.

P113

INTRACELLULAR VESICLE TRAFFIC IN THE OUTER HAIR CELL OF THE GUINEA-PIG COCHLEA

Anthony W. Gummer, Susanne Badum, Csaba Harasztosi

Section of Physiological Acoustics and Communication, University of Tübingen, Germany

Rapid endocytic activity has already been investigated at the apical pole of the outer hair cell (OHC). Using the fluorescent membrane probe FM1-43 it was shown that vesicles are formed at the cuticular plate and transported intracellularly to the basolateral membrane and in the direction of the infranuclear pole. Different types of vesicles have been also revealed in the infranuclear pole using electron microscopy and horse peroxidase staining techniques.

Labelling OHCs with FM1-43 and using confocal laser scanning microscopy we have already demonstrated in freshly isolated OHCs that OHCs endocytose vesicles at the infranuclear pole with dynamics similar to the activity detected at the apex of the cell.

To investigate the intracellular vesicle traffic from the infranuclear pole towards the apex (basoapical traffic) or from the apex to the base (apicobasal traffic) of OHCs, a double barrel local perfusion was applied to stain exclusively one or both the apical and infranuclear poles independently using FM1-43 and FM4-64 as membrane markers of OHCs isolated from the mature guinea-pig cochlea.

In the first type of experiment, the synaptic pole of the OHC was labelled exclusively. The local speed of the basoapical vesicle traffic at distances of 5, 19, 37 and 67 μm from the basal pole was 0.44±0.02, 0.40±0.03, 0.26±0.07 and 0.08±0.04 μm/s, respectively (n=7). In the second type of experiment, the infranuclear and the apical poles of OHCs were stained independently (n=5). The calculated speed of basoapical traffic at the locations of 6, 23, 32 and 61 μm from the basal pole was 0.54±0.07, 0.39±0.08, 0.31±0.13 and 0.07±0.04 μm/s, respectively. At the same time in these cells at the locations of 5, 14, 23, and 31 μm from the apical pole of the cell the speed of the apicobasal traffic was 0.18±0.02, 0.13±0.02, 0.07±0.03 and 0.03±0.01 μm/s, respectively.

These data imply that although the dynamics of vesicle formation is similar at the opposite poles of the OHCs, the speed of vesicle traffic in the cytoplasm towards the apical pole of the cell is significantly larger than towards the infranuclear pole of the OHC.

P114

***EPS8* REGULATES K^+ CHANNELS EXPRESSION IN MOUSE COCHLEAR BUT NOT VESTIBULAR HAIR CELLS**

Paolo Spaiardi[1], Elisa Tavazzani[1], Valeria Zampini[1], Marco Manca[1], Jacopo Magistretti[2], Giancarlo Russo[1], Sergio Masetto[1], Ivo Prigioni[1]

[1]Department of Brain and Behavioral Sciences, University of Pavia, Italy; [2]Department of Biology and Biotechnologies, University of Pavia, Italy

Suppression of *Eps8*, a gene involved in actin remodeling, hampers normal stereocilia growth in cochlear and vestibular hair cells. However, this results in deafness but no obvious vestibular deficits. We had shown previously that, in the absence of Eps8, cochlear inner hair cells (IHCs) fail to express the mature array of voltage-dependent ion channels. Since it is not known if ion channel expression in *Eps8*-KO vestibular hair cells is also altered, in this study we compared the electrophysiological properties of Type

I and Type II vestibular hair cells recorded from wild type (WT) and Eps8-KO mice. We found that the normal pattern of voltage-dependent ion channels is preserved in vestibular hair cells from Eps8-KO mice, including the low-voltage activated K^+ current ($I_{K,L}$) specifically expressed by Type I vestibular hair cells. Consistent with this finding, the voltage response to injected sinusoidal currents mimicking the transducer current was normal in vestibular hair cells.
In conclusion, suppression of *Eps8* does not affect the electrophysiological properties of vestibular hair cells, which might explain the absence of obvious vestibular deficits despite their shorter stereocilia. Eps8, therefore, is a specific regulator of K^+ channel expression in mammalian IHCs, while other intracellular molecules must be responsible for regulating ion channels expression in vestibular hair cells.

P115
THE MYELIN PROTEIN ZERO-DEFICIENT MOUSE AS A MODEL FOR AUDITORY NEUROPATHY
K. Rak[1], J. Völker[1], S. Schendzielorz[1], J. Groh[2], R. Martini[2], R. Hagen[1], A. Radeloff[1]
[1]Department of Oto-Rhino-Laryngology, Plastic, Aesthetic and Reconstructive Head and Neck Surgery and the Comprehensive Hearing Center, University of Wuerzburg, Germany; [2]Department of Neurology, Developmental Neurobiology, University of Wuerzburg, Germany

Auditory neuropathy (AN) is characterised by normal function of the cochlear outer hair cells, but pathological transmission capacity of the auditory nerve to the brainstem. Individuals suffering from this disorder exhibit various degrees of hearing loss and generally have disproportionally poor speech understanding. In contrast to individuals with non-AN hearing loss, hearing aids including cochlear implants provide unsatisfying help due to the disturbed transmission of the signal by the auditory nerve. To understand more about the pathophysiology of the disease and to develop improved therapeutical strategies, new suitable animal models are needed.
The myelin protein zero (P0)-deficient mouse is an established animal model for inherited peripheral neuropathies, such as the Charcot-Marie-Tooth disease, in which hearing loss has also been described. Animals display hypomyelination and axonal loss in peripheral nerves. The question arose, if the hearing pathway in this animal model might also be disturbed.
Homozygous P0 knockout mice (P0-/-) and healthy wildtype animals were investigated at three different ages (3, 6 and 9 months p.P.). Cochleae were prepared for immunohistochemical and electron microscopic investigations. Numbers of outer hair cells and spiral ganglion neurons were counted and myelin as well axonal integrity in the auditory nerve were analysed.
There was no significant difference in the numbers of outer hair cells and spiral ganglion neurons between the knockout and the control animals. In the peripheral part of the auditory nerve there was progressive dysmyelination of spiral ganglion cell axons by P0-deficient Schwann cells. This was accompanied by reduced axonal diameter, decreased phosphorylation of neurofilaments and occasional formation of axonal spheroids as an indication of axonal perturbation. In conclusion, the results support the assumption that the PO-deficient mouse is a suitable animal model that exhibits some morphological similarities with AN. Due to the fact that mutations in the same gene can also lead to AN in humans, the model offers a chance to further study the development of the pathophysiology of this disease. This may lead to a better understanding of AN and might help to develop new therapeutic strategies.

P116
MICROTUBULE MESHWORK REMODELING IN THE AUDITORY NEUROPATHY AUNA1
Cément Surel[1], Marie Guillet[1], Marc Lenoir[1], Jérôme Bourien[1], Benjamin Delprat[1], Marci Lesperance[2], Jean-Luc Puel[1], Régis Nouvian[1]
[1]Institute for Neurosciences of Montpellier, Inserm U1051, Montpellier France; [2]Kresge Hearing Research Institute, Michigan University, Ann Arbor, USA

The *Diaphanous homolog 3* gene is responsible for the auditory neuropathy, AUNA1. Here, we examined the molecular mechanisms of AUNA1 using transgenic mouse lines, which have shown to replicate AUNA1. We found out that the microtubule meshwork undergoes an aberrant targeting within the cuticular plate of the inner hair cells, making them unable to transduce incoming sound stimulation.

P117
NEURONAL ENCODING OF SOUND IN NOISE
Antoine Huet, Gilles Desmadryl, Jean-Luc Puel, Jérôme Bourien
Institute for Neurosciences of Montpellier, Inserm U1051, Montpellier France

Sound encoding is achieved by the inner hair cells (IHCs) localized within the cochlear epithelium. Each IHC is innervated by 10-20 afferent auditory nerve fibers (ANFs) with different sensitivity to sound level: low level activates the high-spontaneous rate (SR) fibers, and increasing level gradually recruits medium- and then low-SR fibers. Whereas the sound coding was extensively investigated in quiet background, little is known about sound encoding in presence of an additional background noise. In this study, we performed single-fiber recordings from gerbil auditory nerve. Each fiber was stimulated by a tone burst presented at the fiber characteristic frequency in quiet or in presence of an additional broadband noise. Results show that the addition of a continuous background noise induced a fiber threshold shift associated to a sub-threshold firing elevation. The rate-versus-intensity function of high-SR fibers was drastically affected by background noise compared to low-SR fibers. For an additional noise presented at 60 dB SPL, the firing of high-SR fibers was completely saturated (flat rate-versus-level function) whereas low-SR fibers continued to encode the auditory cues. These results show that the low-SR fibers from the auditory nerve are essential for sound coding in noisy environments.

P118

VASCULAR DEGENERATION IN THE COCHLEA DURING MCMV INFECTION IN A MOUSE MODEL

Mattia Carraro[1, 2], Ali Almishaal[4], Elaine Hillas[5], Matthew Firpo[5], Albert Park[5, 6], Robert Harrison[1,3]

[1]Institute of Biomaterials and Biomedical Engineering, University of Toronto, Toronto, ON, Canada; [2]Auditory Science Laboratory, Neuroscience and Mental Health, The Hospital for Sick Children, Toronto, ON, Canada; [3]Department of Otolaryngology-Head and Neck Surgery, University of Toronto, Toronto, ON, Canada; [4]Department of Communication Sciences and Disorders, University of Utah, Salt Lake City, UT, USA; [5]Department of Surgery, University of Utah, Salt Lake City, UT, USA; [6]Department of Surgery and Pediatrics, University of Utah, Salt Lake City, UT, USA

Introduction. Cytomegalovirus (CMV) is an important cause of congenital hearing loss. This virus is estimated to account for at least 20% of sensorineural hearing loss (SNHL) in young children and it is perhaps the only causal agent of SNHL that can be treated. Since there are no characteristic audiological/otological features unique to CMV, diagnosis is very challenging and more than 60% of these children will have passed their newborn hearing screening. It is clearly important to determine the pathologic changes resulting from CMV infection. A greater understanding of the underlying mechanisms of CMV induced hearing loss will provide insight into treatment and diagnosis. With this in mind we can use an animal model to investigate the effects of CMV on cochlear vasculature and neural pathways of hearing and potentially identify improved approaches to prevention and treatment.

Methods. We used Balb/C mice inoculated 3 days after birth with murine cytomegalovirus (mCMV-GFP) via intra-cerebral injection of 2000 pfu. At 4 weeks we tested their auditory function using distortion products otoacoustic emissions and auditory brainstem responses and, in order to evaluate vascular damage after 8-10 weeks, we perfused subjects with the Mercox II polymer, dissected the cochleas, partial corroded and imaged them with scanning electron microscopy.

From these corrosion casts we made detailed assessments of the pathologic effects of CMV infection on blood vessels and capillary networks in different regions of the cochlea.

Results. Our measures of auditory function point to a severe to profound hearing loss at 4 weeks and our corrosion casts reveal a clear involvement of the stria vascularis, organ of Corti vasculature and spiral ligament vessels.

There is not a clear cochlear region where the damage occurs first but the apical turn is affected more frequently than the other ones.

Conclusion. The micro-vasculature of the cochlea is clearly damaged by mCMV infection. Regarding possible progression of pathology, we did not detect any early mCMV-GFP expression near to the organ of Corti however we do observe haircell loss over time. This suggests that the vascular damage is a precursor to the subsequent degeneration of sensorineural elements leading to cochlear hearing loss.

POSTER SESSION IX
PHYSIOPATHOLOGY OF AUDITORY PATHWAYS AND TINNITUS

P119

POSSIBLE ROLE OF HERPESVIRIDAE FAMILY VIRUSES IN THE PATHOGENESIS AND EVOLUTION OF SENSORINEURAL HEARING LOSS

Walter Di Nardo[1], Roberta Anzivino[1], Eugenio De Corso[1], Paola Cattani[2], Rosaria Santangelo[2], Gaetano Paludetti[1]

[1]Institute of Otolaryngology, Catholic University of The Sacred Heart, Rome, Italy; [2]Institute of Microbiology, Catholic University of The Sacred Heart, Rome, Italy

Objective. The viral etiology has been the best studied hypothesis for idiopathic sensorineural hearing loss and has been so far supported indirectly by medical, clinical and serological history. The discovery of the virus within the inner ear can add information about the possible etiology and allow to investigate the pathophysiological basis of hearing loss. The purpose of this study was to identify the genome of Herpesviridae family viruses in the endolymphatic fluid of hearing impaired patients undergoing cochlear implant surgery.

Methods. 31 patients, aged between 1 and 69 years, suffering from sensorineural hearing loss, were subjected, during the cochlear implant placement, to a withdrawal of inner ear fluid. In each sample we looked for several viruses (HSV-1, HSV-2, CMV, VZV, EBV, Enteroviruses, Adenoviruses) through the use of two molecular methods and specific primer sets. Radiological exams, serology (specific IgG and IgM antibodies) and PCR of peripheral blood were also performed on the patients.

Results. While the search of the viral genome in peripheral blood was negative in all patients, in 3 patients it was found in the cochlea: 2 patients were positive for CMV and 1 for HSV-1 DNA.

Conclusions. The detection of the viral genome of CMV and HSV-1 in the endolabyrinthic fluid, collected from patients with hearing loss, in the absence of acute viral infection or congenital one, seems to show the ability of these viruses to remain in latent phase in the spiral ganglion and cause a persistent infection. This study supports the hypothesis that Herpesvirus (HSV-1 and CMV) may have a reactivation of a past and latent infection, suggesting a possible etiological role of virus (when acquired in post-natal age) in the genesis of idiopathic hearing loss.

P120

TRANSIENT INDUCTION OF DEAFNESS BY OPTOGENESIS TARGETING THE ENDOCOCHLEAR POTENTIAL IN THE INNER EAR

Mitsuo P Sato[1,2], Taiga Higuchi[1], Fumiaki Nin[1,3], Takamasa Yoshida[1,3,4], Karin Hori[1], Satoru Uetsuka[1,5], Samuel Choi[6], Shuichi Sakamoto[7], Hirohide Takebayashi[8], Katsumi Doi[2], Kenji Tanaka[9], Hiroshi Hibino[1,3]

[1]Department of Molecular Physiology, Niigata University School of Medicine, Japan; [2]Department of Otolaryngology-Head and Neck Surgery, Graduate School of Medicine, Kinki University, Osaka, Japan; [3]Center for Transdisciplinary Research, Niigata University, Japan; [4]Department of Otorhinolaryngology, Graduate School of Medical Sciences, Kyushu University, Japan; [5]Department of Otorhinolaryngology-Head and Neck Surgery, Graduate School of Medicine, Osaka University, Japan; [6]Department of Electrical and Electronic Engineering, Niigata University, Japan; [7]Department of Mechanical and Production Engineering, Niigata University, Japan; [8]Department of Neurobiology and Anatomy, Niigata University School of Medicine, Japan; [9]Department of Neuropsychiatry, School of Medicine, Keio University, Tokyo, Japan

Numerous people suffer from reversible or transient hearing disorders caused by damages in the inner ear. These disorders, which severely affect the quality of life, become irreversible in some occasions. Clearly, it is necessary to develop effective therapeutic strategies and drugs for the early stage of the diseases. To perform such crucial tasks, in this study we have designed novel model mice for sensorineural hearing loss with innovative technologies.

Cochlear hair cells, the sensory receptors for hearing, expose their membranes to an unusual body fluid, endolymph. The endolymph exhibits a highly positive potential of +80 ~ +120 mV. This called endocochlear potential (EP) sensitizes the hair cells. The loss of the EP induces deafness. The EP is maintained by ion transport in an epithelial tissue, stria vascularis. Little is known about the relationship between the impairment of the EP and the reversible or transient hearing disorders in human, although dysfunction of the stria vascularis by ischemia and ototoxic drugs seems to be involved in these diseases. Therefore, we have targeted this tissue.

Methods. We applied the optogenetic approach. In the cochlea of young rodents, the proteolipid protein (PLP) occurs in stria vascularis. We engineered PLP promoter to drive the expression of a light-gated cation channel, channelrhodopsin-2 (ChR2) in mice.

Results. Measurement of auditory brainstem response revealed that hearing threshold of the transgenic mice was increased by ~20 dB when their cochleae were exposed to blue light. Histological assays detected that, out of multiple cell types in the stria, ChR2-expression had been induced exclusively in intermediate cells. Electrophysiological experiments demonstrated that the illumination sharply reduced the EP from +110 mV to +96.8 mV in a few seconds. The potential continued to decline, reaching a plateau at +84.3 mV in 3 minutes. Upon cessation of the light exposure, the EP was completely recovered within 5 minutes. The extent of the EP reduction depended on duration and intensity of the illumination.

Conclusions. We have described temporal control of deafness accompanied by the EP reduction in the ChR2-expressing mice. The mice may serve useful tools to study reversible or transient hearing disorders.

P121

AUDITORY CORTEX PLASTICITY IN A RAT MODEL OF NOISE-INDUCED HEARING LOSS: EFFECT OF ANODAL TRANSCRANIAL DIRECT CURRENT STIMULATION

F. Paciello[1], F. Zanin[1], R. Rolesi[1], A.R. Fetoni[1], G. Paludetti[1], C. Grassi[2], D. Troiani[2], M.V. Podda[2]

[1]Department of Head and Neck Surgery, [2]Institute of Human Physiology, Università Cattolica, Medical School, Rome, Italy

Transcranial direct current stimulation (tDCS) is emerging as non-invasive tool capable of modulating cortical function by affecting neuronal excitability and synaptic plasticity. Our previous study demonstrated that chronic noise exposure causes morphological modifications in auditory cortices consisting in the reduction of apical and basal spine density in pyramidal neurons of layers II-III and V-VI (Fetoni et al., J. Neurosci. 2013). Here we investigated whether anodal tDCS could counteract these cortical damage by enhancing structural plasticity.

Male adult rats underwent chronic noise exposure (100 dB, 60 min/day for 10 consecutive days). The day after the last session of acoustic trauma rats underwent sham stimulation (*n=3*) or anodal tDCS (*n=3*) over the left auditory cortex (tDCS: 350 μA for 20 min for two consecutive days). Twenty days after the end of noise-exposure paradigm rats were sacrificed and their brains processed for Golgi-Cox staining to assess changes in dendrite morphology and spines. Morphological analysis revealed that anodal tDCS affected mainly pyramidal neurons of layer II-III by increasing the number of spines in both apical and basal dendrites and dendritic length in apical dendrites of the neurons of auditory cortices. In line with morphological analysis a parallel set of experiments showed an increase of phospho-CREB immunoreactivity in neurons of layer II-III three hours after tDCS in the left (stimulated cortex) and, though to a lesser extent, in the unstimulated one compared to what observed in sham-exposed rats.

Based on these preliminary results, we speculate that anodal tDCS could counteract the central damages caused by noise exposure by enhancing synaptic plasticity.

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P122

COCHLEAR MICROPHONIC: A GREAT COMEBACK AS NON INVASIVE TOOL FOR INTRACRANIAL PRESSURE MONITORING

Blandine Lourenço[1], Fabrice Giraudet[1], François Longeras[2], Xavier Durando[4], Jean Gabrillargues[3], Laurent Sakka[5], Paul Avan[1]

[1]Laboratory of Neurosensory Biophysics, INSERM UMR 1107, School of Medicine, University of Auvergne, Clermont-Ferrand, France; [2]Department of Neuro Intensive Care Unit, University Hospital of Clermont-Ferrand, France; [3]Department of Neuroradiology, University Hospital of Clermont-Ferrand, France; [4]Department of Oncology, Jean Perrin Comprehensive Cancer Centre, Clermont-Ferrand, France; [5]Department of Neurosurgery, University Hospital of Clermont-Ferrand, France

Over the last decade, we developed audiological methods to monitor noninvasively the intracranial pressure (ICP), based on the principle that through the cochlear aqueduct, cerebrospinal fluid (CSF) pressure variations are transmitted to the intralabyrinthine space and modify the impedance of the ear. The present work reports how a small increase in ICP relative to a reference situation

induces a phase shift in the cochlear microphonic potential (CM), in proportion to the ICP change.

Material and Methods. CM reflects mechanotransduction currents through basal cochlear outer hair cells. It can be detected by an extratympanic electrode through electrically conductive tissues. Here, sound stimuli were repeated 1-kHz tone-bursts at 80-90 dB SPL and the hand-held measuring device (Echodia Elios®) automatically plotted CM spectrum, phase and amplitude online.

Results. The first experiments and clinical applications show a good agreement between CM phase shifts and a number of parameters, level shifts induced by body tilt in man (Buki et al., 2009); ICP changes in gerbils brought about via an intracranial catheter (Buki et al., 2009); ICP variations produced by infusion tests for the diagnosis of chronic hydrocephalus in adults (Sakka et al., 2012); ICP variations measured invasively in patients with severe head trauma (Giraudet et al., submitted). In this presentation, we also present results from current studies conducted in patients expected to develop increased ICP in relation to tumor growth or vascular damage.

Conclusions. CM provides a reliable noninvasive means of monitoring changes of intralabyrinthine pressure and indirectly of intracranial pressure, robust in noisy places and in hearing-impaired subjects. Its monitoring is manageable in numerous clinical situations when invasive ICP measurements are impossible.

P123
CORRELATION BETWEEN MORPHOLOGY OF COCHLEAR EFFERENTS AND MEDIAL OLIVOCOCHLEAR REFLEX ACTIVITY IN MICE

Shi Nae Park, So Young Park, Sang A Bak, Jung Mee Park, Woo Jin Kim, Sang Won Yeo

Department of Otorhinolaryngology-Head and Neck Surgery, College of Medicine, The Catholic University of Korea, Seoul, Republic of South Korea

Objectives. The cochlear efferent system has been postulated to play a role in signal detection in noise and protection from noise-induced cochlear damage. Contralateral suppression (CS) of distortion product otoacoustic emission (DPOAE) can reflect medial olivocochlear reflex (MOCR) strength. In the present study, the authors investigated the relationship between the size of MOC efferent terminals (ETs) and the MOC efferent activity measured by CS of DPOAE.

Methods. Sixteen ears from 8 normal CBA mice aged 1 month were used. Auditory brainstem response thresholds to click and tone bursts (8, 16, and 32 kHz) were assessed. DPOAEs were recorded with or without contralateral acoustic stimulation (CAS) using 55 dB broadband noise. Signal to noise amplitude was considered as the actual DPOAE value. Suppression amplitude (SA) represented the DPOAE change after CAS. To reduce the blunting of mean SA caused by simply averaging the 16 'peaks or dips' at a fixed frequency, a maximum SA was selected among the grouped frequencies from the apex, middle, and base, respectively. In whole-mount cochleae, the ETs at the outer hair cell base were visualized with immunofluorescent labeling with anti-α-synuclein, an efferent synaptic vesicular protein. The ETs were examined using confocal laser-scanning microscopy and the captured images were imported into NIH ImageJ software for quantitative analysis. The diameters of all ETs shown were measured and averaged from each turn in a cochlea. The mean ET diameters/maximum SAs were statistically compared across the apex, middle and base at a significance level of 0.05.

Results. MOC efferent terminals were the largest in the middle, and the smallest in the base: 1.70, 1.84, and 1.53 μm in the apex, middle, and base, respectively ($p<0.001$). In CS of DPOAE test, the SA was found to be the largest in the middle frequency range: 3.44, 5.63, and 2.25 dB SPL in the apex, middle, and base, respectively ($p=0.016$).

Conclusion. Our results demonstrate that the size of the MOC ETs can be correlated with MOCR strength. Small ETs and weak MOCR of the cochlear base may partly explain the vulnerability of this region to noise trauma.

P124
ACOUSTIC TRAUMA AT THE ONSET OF HEARING AFFECTS AUDITORY TEMPORAL RESOLUTION IN ADULT RAT

Natalia Rybalko[1], Daniel Šuta[1,2], Jiří Popelář[1], Josef Syka[1]

[1]Department of Auditory Neuroscience, Institute of Experimental Medicine, ASCR, Prague, Czech Republic; [2]Department of Medical Biophysics and Informatics, Third Faculty of Medicine, Charles University, Prague, Czech Republic

Objective. Acoustic trauma during the critical period of development that results in transient or permanent hearing loss alters auditory processing in adulthood. In the present study we assessed the consequences of early acoustic trauma of different severity on auditory temporal resolution in rats.

Methods. Rat pups (strain Long-Evans) were exposed to 125 dB SPL broad band noise for 8 min (NE8), 12min (NE12) or 25 min (NE25) on the 14th postnatal day (hearing onset). The ability to detect gaps in noise was measured using prepulse inhibition (PPI) of the acoustic startle response (ASR) induced by gaps of 5-50ms in exposed and control rats at the age of 1-24 months. Hearing sensitivity was examined by auditory brainstem response (ABR) recording.

Results. One-month-old NE8 rats had auditory thresholds and gap-PPI performances similar to control animals. NE12 and NE25 rats at this age showed permanent hearing loss and a deficit in gap detection ability. During maturation a significant improvement in the gap-PPI performance was observed by the second month of life in all exposed and control rats. However, the deficit in gap-PPI performance in young adult NE12 and NE25 rats remained the same. By the age of 24 month exposed animals showed significant age related deterioration of gap-PPI performance that was not observed in control animals. In contrast to elder control animals, noise exposed rats also exhibited slightly elevated hearing thresholds at high frequencies (16-40 kHz) by the age of 24 months.

Conclusions. Acute acoustical trauma at the onset of hearing (NE12 and NE25 rats), resulting in a permanent hearing threshold shift, was accompanied by significant impairment of temporal resolution. Less severe acoustical trauma (NE8) led only to a transient hearing threshold shift with no deficit in gap detection ability. All groups of noise exposed rats exhibited age related deterioration of hearing by the age on 24 month: slightly elevated hearing thresholds and a significant deficit in gap-PPI performance, which was not observed in age-matched control animals.

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P125

ACTIVIN SIGNALING DISRUPTION IN THE COCHLEA DOES NOT INFLUENCE HEARING IN ADULT MICE

Lukas Horvath[1], Daniel Bodmer[2], [2]Vesna Radojevic, [1]Arianne Monge Naldi

[1]Inner Ear Research, Clinic for Otolaryngology, Head & Neck Surgery, University Hospital Zurich, University of Zurich, Switzerland; [2]Inner Ear Research, Clinic for Otolaryngology, University Hospital Basel, Switzerland

Introduction. Activin, a member of the TGF-alpha superfamily, plays an important role in the development, repair and apoptosis of different tissues and organs and in the development of the cochlea. Activin binds to its receptor ActRII, then dimerizes with ActRI and induces a signaling pathway resulting in gene expression. A study reported a case of fibrodysplasia ossificans progressiva (FOP) with an unusual mutation in the ActRI gene leading to sensineural hearing loss. This draws attention to the role of activin and its receptors in the developed cochlea. To date, only the expression of ActRII is known in the adult mammalian cochlea.

Methods. Transgenic mice with postnatal dominant negative ActRIB expression causing disruption of activin signaling *in vivo* were used for assessing hearing ability through auditory brainstem response (ABR) threshold and cochlear morphology.

Results. We present for the first time the presence of activin A and ActRIB in the adult mammalian cochlea.

Non-functioning ActRIB did not affect the ABR thresholds and did not alter the microscopic anatomy of the cochlea.

Conclusion. So we conclude that activin signaling is not necessary for hearing in adult mice under physiological conditions but may be important during and after damaging events in the inner ear.

P126

A NEW PATHOLOGICAL COCHLEAR PROFILE

Marion Souchal, Fabrice Giraudet, Paul Avan

University of Auvergne, School of Medicine, Laboratory of Neurosensory Biophysics - INSERM UMR 1107, Clermont-Ferrand, France

Hearing loss associated with outer hair cell (OHC) degeneration is typically described by elevated hearing thresholds. Auditory function in mice is assessed by the combined use of auditory brainstem responses (ABR) and distortion product otoacoustic emissions (DPOAEs), with hearing threshold elevation relating to reduced or absent DPOAEs, evidence of OHC function loss. Moreover, in this pathological context, Masking Tuning Curves (MTC) show changes in shape included elevations or disappearance of the tip associated with the loss of frequency selectivity. In our study, we characterized the evolution of hearing profiles of two common laboratory mice (C57BL/6JRj and CD1 – RjOrl:SWISS) In this longitudinal study, we observed the progressivity of hearing impairment (at different rates in the two strains). However, a surprising discrepancy was found. ABR thresholds at high frequencies remained close to normal values but associated with absent DPOAEs. The MTCs show shifted tips centered on the low frequencies. These data indicate that basal OHC are no longer functional and the perception of high frequencies is disturbed. Histological observations with scanning electronic microscopy revealed abnormal stereocilia bundle at the cochlea base, with stereocilia persisting imprints on the lower face of the tectorial membrane. These data suggest cochleo-tonotopic disorganization.

P127

APPLYING A VERBOTONAL METHOD TO REHABILITATION OF THE HEARING IMPAIRED CHILDREN

Ivanka Jurjević- Grkinić, Boška Munivrana, Vesna Mijić

Policlinic SUVAG, Department for Medical Rehabilitation of School Age Children, Department for Medical Diagnostics and Rehabilitation of Listening and Speech, Zagreb, Croatia

The rehabilitation of listening in the principles of verbotonal method (VT) implies expansion of optimal hearing field of impaired ear. That means achieved hearing transfer i.e., transpose of listening from impaired frequency range to undemaged part. For example, ear which is strong impaired in the high frequency range obtain a capability for listening of words and sentences which belong to high frequency range. In the first case there is patient whose hearing impaired progressively because of many ear abscess (the main disease is Mucopolisaharidose tip II), this patient listens with hearing aids. While in the second case patient with the same history of the hearing impairment (progressively) at the age of nine received cochlear implant because of the strong hearing impairment. A scale was used as a test for the purpose of assessing listening of speech (EHS), (Jurjević, 2002), which is the result of many years of work with hearing impaired children, using the VT method and its basic principles about listening. The EHS scale is the easy and simple way of examining the listening of speech in the conditions of everyday spontaneous communication, in order to gain insight into the real possibility of listening of speech in all its components. The EHS scale examines listening of speech through ten levels from basic auditory recognition to precise listening and discernment of pairs of similar words. When choosing words, the frequency of speech material in the speech of children of certain chronological age was taken into account, as well as representation of congruent pitch words according to their appearance in the Croatian language. Tasks could be repeated up to three times, making sure that the repetition of a task does not undermine values of spoken language. The system of errors was analyzed as well as the number of repetitions in which the correct answer was reached. The results of this test give us the valuable data in further procedures of speech and hearing rehabilitation.

P128

HYPERACUSIS AND TINNITUS DEVELOPMENT OVER AGE AND THE INFLUENCE OF NOISE EXPOSURE IN THE RAT BEHAVIORAL MODEL

Sathish Kumar Singaravelu[1], Dorit Möhrle[1], Kun Ni[2], Dan Bing[3], Ksenia Varakina[1], Mirjam Rieger[1], Marlies Knipper[1], Lukas Rüttiger[1]

[1]University of Tübingen, Department of Otolaryngology, Tübingen Hearing Research Centre (THRC), Molecular Physiology of Hearing, Tübingen, Germany; [2]Shanghai Jiao Tong University, Department of Otolaryngology-Head & Neck Surgery, Shanghai Children's Hospital, Shanghai, China; [3]Wuhan Jiefang Avenue No. 1095, Jong Ji Hospital, ENT Departemnt, Wuhan, China

Aim. The prevalence of Tinnitus and Hyperacusis increases steadily due to changing leisure behavior and demographic trends. Tinnitus and Hyperacusis often occur after sudden hearing loss, e.g. through noise-induced hearing loss (NIHL), but also aging processes may lead to progressive hearing loss (age related hearing loss, ARHL). Previous studies demonstrated the degeneration of auditory fibers

following "non-traumatic" loud sound and over age, even when audiometric thresholds are normal. In the present study we explore how the reduced sensory function of the hearing organ (cochlea, inner ear) is involved in changes of central activity patterns in the ascending auditory pathway of the CNS for the development of Tinnitus or Hyperacusis.

Methods. To investigate the effects of ARHL and NIHL on loudness perception (Hyperacusis) and Tinnitus, an animal model based on operant conditioning was applied for Tinnitus and extended to measure the behavioral correlates of (loudness perception) in middle-aged and old rats. Changes in hearing threshold, supra-threshold auditory processing at sensation level, and outer hair cell function were measured by auditory brainstem response (ABR) and distortion product otoacoustic emission (DPOAE). Immunohistochemistry was performed on cochlear sections to detect hair cell ribbon loss.

Results. Behavioral measurements of heightened loudness perception (Hyperacusis) and Tinnitus in middle-aged and old rats, before and after "non-traumatic" overstimulation involving temporary ABR threshold shift (TTS), were correlated to changes in hearing function and hair cell molecular phenotype.

Conclusion. The results suggest that the development of Tinnitus and/or Hyperacusis involves a distinct failure to adapt the central responsiveness and an insufficient compensation of the reduced cochlear output after TTS.

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P129

I KNOW WHAT YOU HEAR: OBJECTIFICATION AND DIFFERENTIAL DIAGNOSIS OF VASCULAR PULSATILE TINNITUS BY TRANSCANAL SOUND RECORDING AND SPECTRO-TEMPORAL ANALYSIS

Jae-Jin Song[1], Gwang Seok An[2], Inyong Choi[3], Dirk De Ridder[4, 5], So Young Kim[1], Hyun Seok Choi[1], Joo Hyun Park[6], Byung Yoon Choi[1], Ja-Won Koo[1], Kyogu Lee[2]

[1]Department of Otorhinolaryngology-Head and Neck Surgery, Seoul National University Bundang Hospital, Seongnam, South Korea; [2]Music and Audio Research Group, Graduate School of Convergence Science and Technology, Seoul National University, Seoul, South Korea; [3]Center for Computational Neuroscience and Neural Technology, Boston University, Boston, MA, USA; [4]Section of Neurosurgery, Department of Surgical Sciences, Dunedin School of Medicine, University of Otago, Dunedin, New Zealand; [5]BRAI2N, Sint Augustinus Hospital, Antwerp, Belgium; [6]Department of Otorhinolaryngology-Head and Neck Surgery, Dongguk University Ilsan Hospital, Goyang, South Korea

Although the symptoms are frequently classified as "objective tinnitus", in most cases vascular pulsatile tinnitus (VPT) is not equal to objective tinnitus because it is typically not easy to objectively document VPT. Thus, the present study developed a novel transcanal sound recording and spectro-temporal analysis method for the objective and differential diagnosis of VPT. This method was tested using six VPT subjects and six normal controls based on recordings obtained from the ipsilateral external auditory canal using an insert microphone with the subject's head in four different positions: 1) neutral head position, 2) head rotated to the tinnitus side, 3) head rotated to the non-tinnitus side, and 4) neutral position with manual compression of the ipsilateral carotid artery. The recorded signals were first analyzed in the time domain, and short-time Fourier transform was performed to analyze the data in the time-frequency domain. On temporal analysis, the ear canal signals recorded from the VPT subjects exhibited large peak amplitudes and periodic structures, whereas the signals recorded from the control subjects had smaller peak amplitudes and weaker periodicity. On spectro-temporal analysis represented by three-dimensional waterfall diagrams, all of the VPT subjects demonstrated pulse-synchronous, mutually exclusive acoustic characteristics that were representative of their respective presumptive vascular pathologies, whereas the control subjects did not display such characteristics. The present diagnostic approach may provide additional information regarding the origins of particular VPT cases as well as an efficient and objective diagnostic method. Furthermore, this approach may aid in the determination of appropriate imaging modalities, treatment planning, and evaluation of treatment outcomes. Future studies with a larger sample size, diverse etiologies, and more refined recording techniques are warranted.

P130

IS TINNITUS RETRAINING THERAPY USING VIDEO EDUCATION ON SMARTPAD AND SOUND SELF-THERAPY ALSO EFFECTIVE AS CONVENTIONAL ONE?

Yoonjoong Kim[1], Moo Kyun Park[1], Jun Ho Lee[1], Seung Ha Oh[1], Myung-Whan Suh[1], Young Ho Kim[2]

[1]Seoul National University College of Medicine, Seoul National University Hospital, Department of Otorhinolaryngology-Head and Neck Surgery, Seoul, South Korea; [2]Seoul National University College of Medicine, Boramae Medical Center, Department of Otorhinolaryngology-Head and Neck Surgery, Seoul, South Korea

Objective. To evaluate the efficacy of tinnitus retraining therapy (TRT) using video file, and to prove the efficacy of the TRT using video is not inferior to that using conventional manner.

Methods. We retrospectively reviewed the medical chart of total sixty-two patients who enrolled TRT program at Seoul National University Hospital and Boramae Medical Center. Nineteen of them have taken the TRT with video file, using tablet PC. Other fourty-three of them have taken the TRT with conventional manner, by trained otologist. We assessed VAS scores of awareness, annoying, loudness, effect on life by tinnitus, and tinnitus handicapped index (THI) score in each groups. All the item was checked before and after the TRT.

Results. The mean THI score in video-TRT group was initially 55.79 and it was decreased to 30.87 after the TRT. Whereas the mean THI score in conventional-TRT group was initially 50.7 and it was decreased to 29.12 after the TRT. There was no statistically significant difference in pre-TRT and post-TRT THI scores in both groups. Other parameters such as VAS scores of awareness, annoying, loudness, effect on daily life also showed no meaningful difference in both groups.

Conclusions. The TRT using video file is convenient, time-saving, economical treatment to the patients with tinnitus. And it is as effective as conventional TRT.

P131
RELATIONSHIP BETWEEN TINNITUS AND SUICIDAL BEHAVIOR IN KOREAN MEN AND WOMEN: A CROSS-SECTIONAL STUDY
Jae-Hyun Seo
College of Medicine, The Catholic University of Korea, Bucheon St. Mary's Hospital, Department of Otolaryngology-Head and Neck Surgery, Bucheon-si, South Korea

Objectives. The aims of this study were to investigate the prevalence of suicidal behavior in a representative sample of South Koreans with or without tinnitus and association between tinnitus and suicidal ideation and attempts.
Methods. Data were collected from the 2010–2012 Korean National Health and Nutrition Examination Survey (KNHANES, N=17,446). Participants provided demographic, socioeconomic and behavioral information and answer to the questionnaires for the presence and severity of tinnitus, mental health status about stress, depression, and suicidal thoughts and attempts.
Results. A total of 20.9% and 1.2% of participants with tinnitus, and 12.2% and 0.6% of those without, reported suicidal ideation and attempts, respectively ($p < 0.0001$ and $p = 0.001$). Participants reporting suicide attempts showed a higher proportion of severe annoying (6.0%) and irritating (11.8%) tinnitus than those with suicidal ideation (1.4% and 10.2%, respectively). Risks for experiencing tinnitus were significantly associated with suicidal ideation and attempts after adjusting for confounding variables.
Conclusion. This large population study regarding the relationship between tinnitus and suicide behavior have important implications of the needs for screening and evaluating the mental health status and suicidal behavior in tinnitus patients.

P132
CLINICAL CHARACTERISTICS AND THERAPEUTIC EFFICACY OF PALATAL MYOCLONIC TINNITUS
Woo Jin Kim, Jung Min Kim, So Young Park, Sang-Won Yeo, Shi Nae Park
Department of Otolaryngology-Head & Neck Surgery, College of Medicine, The Catholic University of Korea, Seoul, South Korea

Objectives. Palatal myoclonic(PM) tinnitus is a rare symptom of the ear classifying as objective tinnitus of muscle origin. Few studies about its clinical characteristics have been reported because of its rareness. We performed this study for further understanding of the clinical characteristics of palatal myoclonic tinnitus, and to evaluate the effectiveness of the medical and surgical intervention in our clinical setting.
Materials and Methods. Fifty two patients diagnosed as PM tinnitus at tertiary referral center of tinnitus clinic during the period of October 2003 to March 2015 have been included in the study. Their clinical characteristics, audiologic findings, psychological status and the responses of medical treatment, reassuarance, tinnitus retraining therapy and botox injection therapy have been retrospectively analyzed.
Results. Twenty two patients were diagnosed as only PM tinnitus (PM group) and twenty three patients have both PM and middle ear myoclonic (MEM) tinnitus (PM+MEM group). The mean age was 29.9 years (SD 17.01) and female was predominant than male. Majority of the PM tinnitus patients showed clicky tinnitus, whereas those who were combined of PM with MEM tinnitus had different types of sound not only clicky but also crackling, buzzing, tapping. Comparing PM group with PM+MEM group, PM+MEM group showed higher rate of sleep disturbance, annoyance score, BEPSI stress score ($p<0.05$). Reassurance and medical therapy seems to be quite effective in PM tinnitus, which presented significant lower status of tinnitus VAS scores during follow up period($p<0.05$). For the patients with intractable PM tinnitus (N= 8), botox injection was performed under EMG guidance or not. All of the patients with botox injection showed dramatic disappearance of their clicky tinnitus. Inevitable side effects such as hypernasal voice and velopharyngeal insufficiency were developed and lasted for about 1month. Recurrence of annoying PM tinnitus which needs repeated injection was not common in our case series (2/8 patients).
Conclusion. Understanding clinical, audiologic characteristics of the palatal myoclonus(PM) tinnitus will be helpful for early diagnosis and a treatment of the patients with this rare type of tinnitus. Conservative therapy followed by botox injection seems to be a reasonable management protocol for PM tinnitus.

POSTER SESSION X
OTOPROTECTION AND DRUG DELIVERY SYSTEMS

P133
PROTECTION OF INNER HAIR CELLS FROM LETHAL INJURY: PREVENTING PROGRESSION TO PROFOUND HEARING LOSS
Fitim Fetahu, Andrew Forge, Ruth Taylor
UCL Ear Institute, University College London, UK

The organ of Corti is susceptible to damage under numerous conditions such as excessive noise, ageing and ototoxic drugs leading to hearing loss. In the mature mammalian cochlea, inner hair cells (IHCs) are less susceptible to damage than outer hair cells (OHCs), in contrast to the immature organ of Corti in vitro where IHCs susceptibility is similar to OHCs. The prolonged survival of IHCs in mature tissue in the absence of OHCs suggests that the cell survival/death pathways activated in IHCs may be different from those operating in OHCs.
Hypothesis. IHC resistance develops during later stage of cochlear development, and the crosstalk between autophagy, a cell survival mechanism, and apoptosis could play a critical role in the fate of hair cells under increased stress.
Aims. Here we investigate whether the inner hair cell resistance develops at a particular stage of cochlear development; whether autophagy plays a role in hair cell survival; and if this can be modulated using pharmacological reagents.
Methods. To investigate the difference in gentamicin induced hair cell loss, immature neonatal cultures of P3 mice were compared with P8 cochleae. The expression of an autophagy marker, microtubule-associated light chain protein, LC3B was used to investigate whether autophagy was upregulated under conditions of stress.
Results. We show that inner hair cell resistance develops with maturation of cochleae. The loss of IHCs in immature P3 organ of Corti was more extensive than that seen at P8.
In unstressed tissue, low levels of LC3B were noted in both hair cell

types. With increased oxidative stress, the level of LC3B expression was significantly up-regulated in sensory hair cells in both *in vitro* and *in vivo* studies. Co-treatment of organ of Corti with gentamicin and an autophagy enhancer, rapamycin promoted 'autophagic flux', which in turn enhanced hair cell survival.
These results suggest that autophagy plays a critical role in the fate of hair cells following treatment with ototoxic agents and once induced,can promote hair cell survival presumably by removing the damaged cytoplasmic organelles and protein aggregates.
Supported by Action on Hearing Loss.

P134
MOLECULAR TARGETS FOR ANTICANCER REDOX CHEMOTHERAPY AND SIDE EFFECTS: THE ROLE OF CURCUMIN ON PSTAT3 AND NRF2 SIGNALLING
A.R. Fetoni[1], F. Paciello[1], D. Mezzogori[2], S.L.M. Eramo[2], R. Rolesi[1], G. Paludetti[1], D. Troiani[2]
[1]Department of Head and Neck Surgery and [2]Institute of Human Physiology, Università Cattolica, Medical School, Rome, Italy

Cisplatin represents by far the most prominent therapeutic option against a wide spectrum of solid neoplasms. However, the clinical usefulness of cisplatin is limited by the incidence of chemoresistance and its adverse side-effects such as ototoxicity. Cellular resistance to cisplatin may involve several factors and, among them, the modulation of Nrf2 and Stat3 signalling play a critical role. Understand the molecular mechanisms underlying chemoresistance and, at the same time, limit cisplatin side effects is the major goal. A rationale approach should consider drugs acting as chemoprotector for normal cells and chemosensitizer for the tumor cells and, in this study, we focused on Curcumin, the yellow pigment in Indian saffron with antioxidant, antiflammatory and antitumoral properties. Therefore, we evaluated whether Curcumin administration can be used as an adjuvant in cisplatin therapy, potentiating the antitumoural activity of cisplatin *in vitro* in oral squamous cell carcinoma, and, at the same time, exerting protective properties against ototoxicity *in vivo*.
In vitro studies were conducted in head and neck squamous cell carcinoma treated with cisplatin (1.56 μM) and curcumin at different doses (0.5, 1, 3.67, 6.75 μM). We performed cell count and tumour progression analysis, TUNEL assay for apoptosis evaluation and immunofluorescence for Nrf2 and pStat3 activation. For i*n vivo* studies, curcumin dose of 100, 200 and 400 mg/kg, was injected 1 hour before cisplatin administration(16 mg/kg, i.p) and once daily for the following 3 days. We performed functional evaluation (ABR and DPOAEs), morphological analysis (Rh-Ph staining), and immunofluorescence for HO-1/Nrf2 and pStat3 expression in cochlear cryosections.
In vitro Curcumin exerts a dose-dependent pro-apoptotic effect, causing a decrease in cell viability and tumour growth. When administered in conjunction whit cisplatin, curcumin inhibits nuclear activation of Nrf2 and pStat3. *In vivo* curcumin attenuates hearing loss caused by cisplatin, as well as OHCs loss, and it potentiates the expression of endogenous antioxidant enzymes by inducing Nrf2/HO-1 activation pathway.
In conclusion, our results suggests that curcumin can be used as adjuvant in cisplatin therapy by potentiating the antitumoral activity of the drug and by preventing the ototoxic side effect.

P135
ACTIONS OF DENDROGENIN B IN THE MOUSE COCHLEA
Silvia Murillo-Cuesta[1,2,3], Néstor Vallecillo[1,2], Julio Contreras[1,2,4], Adelaida Celaya[1,2], Rafael Cediel[1,2,4], Brigitte Malgrange[5], Michaël R. Paillasse[6], Isabel Varela-Nieto[1,2,3]
[1]Institute of Biomedical Research "Alberto Sols" (IIBM), Spanish National Research Council-Autonomous University of Madrid (CSIC-UAM) Madrid, Spain; [2]Centre for Biomedical Network Research on Rare Diseases (CIBERER), Madrid, Spain; [3]Institute of Health Carlos III (ISCIII) Madrid, Spain; [4]Hospital La Paz Institute for Health Research (IdiPAZ) Madrid, Spain, Anatomy and Embriology Department, Veterinary Facuty, Complutense University of Madrid, Madrid, Spain; [5]Giga Neurosciences, University of Liège, Liège, Belgium; [6]Affichem SA, Toulouse, France

Cholesterol plays a key role in neural tissues and the alterations in its levels or trafficking have been related to neurodegenerative diseases and hearing loss. 5α-hydroxy-6β-[3-(4-aminobutylamino) propylamino] cholest-7-en-3β-ol, called dendrogenin B (DDB) is a cholesterol derivative that induces neurite outgrowth, neuronal differentiation and survival of normal neurons in vitro.
To evaluate the safety of DDB and its efficacy in the treatment of noise-induced hearing loss and associated inflammatory response, two month old male C57BL/6J mice were exposed to noise and treated with DDB administered surgically in the middle ear. Hearing was evaluated with auditory brainstem response (ABR) before noise and 2,7,14 and 28 days after exposure. Cochlear samples were taken to study morphology and inflammatory markers by RT-qPCR.
The safety study confirmed that DDB did not induce alterations neither in ABR thresholds nor in cochlear cytoarchitecture in mice. In addition, mice treated with DDB immediately after noise exposure showed significantly higher threshold shifts, especially for click and low frequencies, but also a better recovery of ABR thresholds, compared to mice treated with vehicle. Accordingly, the expression levels of the pro-inflammatory cytokines *Il1b, Il6* and *Tnfα,* and of anti-inflammatory *Il10* and *Il4* were simultaneously increased in the cochlea of mice treated with DDB.
These data pointed to a dual DDB effect, i) increasing cochlear inflammation and hearing loss after noise exposure and ii) promoting ABR threshold recovery and inflammation resolution.
Formulation work is now being extensively run to unravel the full therapeutic potential of the drug.
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NEUROPROTECTIVE AND NEUROREGENERATIVE POTENTIAL OF DENDROGENIN B IN *IN VITRO* MODELS OF HEARING LOSS

Elodie Bacquié[1], Nicolas Caron[1], Debadutta Deb[2], Néstor Vallecillo[3,4], Bénédicte Franco[2], Philippe Lefebvre[5], Philippe de Medina[1], Brigitte Malgrange[2], Isabel Varela-Nieto[3,4,6], Michaël R. Paillasse[1]

[1]Affichem SA, Toulouse, France, [2]Giga Neurosciences, University of Liège, Liège, Belgium; [3]Institute of Biomedical Research "Alberto Sols" (IIBM), Spanish National Research Council-Autonomous University of Madrid (CSIC-UAM) Madrid, Spain; [4]Centre for Biomedical Network Research on Rare Diseases (CIBERER), Spain, [5]ENT Service, University Hospital of Liège, Liège, Belgium, [6]Institute of Health Carlos III (ISCIII) Madrid, Spain

Background. Dendrogenin B, a new aminoalkyl oxysterol, has been shown to induce neurite outgrowth, expression of neuronal differentiation markers and survival of motoneurons *in vitro*. In this work, we wanted to further evaluate its neuroprotective/ neuroregenerative potency, in the peripheral nervous system including the cochlea, using different models from murine and human origin.

Results. Studies on neuroblastoma cells showed differentiation into neuron-like cells, protection from different ototoxic stresses (Cisplatin, hydrogen peroxide, glutamate). Studies on murine dorsal root neurons and spiral ganglion neurons showed an effect on the ramifications of dendrites, that was confirmed using human embryonic stem cell-derived cell line. Interestingly, it appears that Dendrogenin B does not modify differentiation process in the organ of Corti, exerting a neuron-specific effect. From a mechanistic point of view, we have been able to evidence involvement of nuclear transcription factors LXR, that are key players in cholesterol homeostasis in brain in the differentiating and anti-apoptotic effects of the drug, and unravel part of the cascades of signalization.

Perspectives. The present study shows a new class of molecules acting through an original pathway to induce protection/ regeneration of spiral ganglion neurons that could complement the present and upcoming therapies to meet patients' needs. Encouraging preliminary results of *in vivo* evaluation of the drug in models of hearing loss (noise, ototoxic drug, presbycusis) are presented by Murillo-Cuesta et al.

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P137

REDUCTION OF NOISE-INDUCED COCHLEAR SENSITIVITY LOSS BY INHIBITION OF MITOCHONDRIAL FISSION

Alfred Nuttall, Sarah Foster, Beth Kempton, Teresa Wilson

Oregon Health & Science University, Oregon Hearing Research Center, Portland, Oregon, USA

Background. The generation of reactive oxygen species (ROS) is one of the underlying mechanisms of noise-induced damage to the inner ear that leads to noise-induced hearing loss (NIHL). Following noise exposure, excessive ROS production by mitochondria can negatively impact auditory hair cell function and viability leading to permanent loss of hearing sensitivity. Mitochondria are highly dynamic organelles that are maintained by the opposing processes of fusion and fission, events which alter mitochondrial morphology, biogenesis, and energy metabolism. In this study, we tested the novel hypothesis that mdivi-1, a specific inhibitor of mitochondrial fission, will mitigate the deleterious effects of loud sound on hearing sensitivity.

Methods. CBA/CaJ mice were used in this study. Two different mdivi-1 administration routes were examined: 1) Intraperitoneal injection (IP group) of 5 mg/kg at 1 hour prior to noise exposure and again at 6 hours post-noise exposure; and 2) Outer ear canal application (OEC group) (25 µl of 50 µM solution) to one ear immediately following noise exposure. Control treatments consisted of an equal volume of vehicle. A moderately damaging sound exposure protocol of 103 dB (SPL)/8-16 kHz/2 hours was used. Auditory brainstem and distortion product optoacoustic emission thresholds were measured at 48 hours (IP) and 14 days (IP and OEC) following loud sound exposure. Outer hair cell counts were performed after hearing threshold measurements. Analysis of activated caspase 3 was performed at 24 hours post-noise exposure.

Results. At 48 hours and 2 weeks post-noise exposure, the IP group had significantly recovered hearing thresholds relative to vehicle treated mice. In the OEC group, the mdivi-1 treated ears had significantly reduced loss of hearing sensitivity relative to the vehicle treated contralateral ear. Morphological and immunological analysis revealed protection against loss of outer hair cells as well as reduced activation of caspase 3 in the lateral wall.

Conclusion. This study demonstrated the efficacy of using mdivi-1 as a therapeutic agent for protection against NIHL. Importantly, we have shown that OEC application of mdivi-1 after loud sound exposure significantly reduces noise-induced hearing loss and loss of OHCs.

P138

EFFICACY OF DIFFERENT ROUTES OF ADMINISTRATION FOR OTOPROTECTIVE AGENTS IN NOISE-INDUCED HEARING LOSS: SYSTEMIC VERSUS TRANS-TYMPANIC MODALITY

S.L.M. Eramo[1], R. Rolesi[2], A. Di Pino[2], F. Paciello[2], G. Paludetti[2], A.R. Fetoni[2]

[1]Institute of Human Physiology and [2]Department of Head and Neck Surgery Medical School, Università Cattolica, Rome, Italy

It has been demonstrated that the increase of reactive oxygen species (ROS) generation and lipid peroxidation, together with a concurrent decrease of antioxidant defenses, plays a significant role in noise-induced hearing loss (NIHL). This redox imbalance is largely responsible for cellular mechanisms that underlies hair cell death after noise exposure. Several molecules with antioxidant and scavenging properties have been proved to restore redox balance and to prevent oxidative stress-induced hair cells death. In this study, we focused on Rosmarinic Acid (RA) a natural antioxidant found in many herbs of the Lamiaceae family with antioxidant, anti-inflammatory and antiviral properties. Our study was designed to: 1- investigate the protective effect of RA against noise-induced oxidative stress in the cochlea; 2- evaluate the effectiveness of a RA, for different schedules of drug administration (systemic vs trans-tympanic modality) in order to establish the "best modality" for treatment.

Wistar rats (200-250 g) were used in this study. Animals were exposed to acoustic trauma (10 kHz at 120 dB for 60 min). Of these, a group was treated with RA (i.p. 10 mg/Kg 1h pre trauma and

for 3 consecutive days) and another groups was treated by trans-tympanic modality (RA 2% concentration 1 hour before noise exposure). Auditory function was evaluated in all animals by ABR (6-32 kHz) and DPOAEs (6-24 kHz) at several time points (1, 3 and 7 days after noise exposure). The hair cell damage was detected by rhodamine-phalloidin staining, the magnitude of lipid peroxidation by 4-hydroxynonenal(4-HNE) /8-isoprostane expression, the superoxide amount with DHE assay, apoptosis with tunnel assay
Functional analyses (ABR and DPOAEs) show a decrease of threshold shift after RA administration. This improvement of auditory function by RA was paralleled by a significant reduction in oxidative stress. The trans-tympanic modality of drug administration showed a similar degree of protection
Conclusion. The effectiveness of RA given via trans-tympanic injection is interesting for the future application of this minimally-invasive procedure in the treatment of ROS induced hearing loss.

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ROSMARINIC ACID UP-REGULATES ENDOGENOUS ANTIOXIDANT DEFENCES THROUGH THE ACTIVATION OF NRF2/HO-1 PATHWAY AND PROTECTS AGAINST NOISE-INDUCED HEARING LOSS

F. Paciello[1], A.R. Fetoni[1], R. Rolesi[1], S.L.M. Eramo[2], C. Mancuso[3], D. Troiani[2], G. Paludetti[1]
[1]Department of Head and Neck Surgery, [2]Institute of Human Physiology, [3]Institute of Pharmacology, Università Cattolica, School of Medicine, Rome, Italy

Noise induced hearing loss depends on the progressive increase of reactive oxygen species and lipoperoxidative damage in conjunction with the imbalance of antioxidant defenses. The redox-sensitive transcription factor nuclear factor erythroid 2-related factor 2 (Nrf2) plays a critical role in the regulation of cellular defense against oxidative stress including heme oxygenase-1 (HO-1) activation. In this work we describe a link between cochlear oxidative stress damage, induced by noise exposure, and the activation of Nrf2/HO-1 pathway. In our model, noise induces superoxide production and overexpression of the lipid peroxidation marker, 4-hydroxy-nonenals (4-HNE). To face the oxidative stress, the endogenous defense system is as well activated as shown by the slight activation of superoxide dismutases (SODs). In addition, we also observed the activation of the Nrf2/HO-1 pathway after noise exposure. In doing so, Nrf2 appears to promote the maintenance of cellular homeostasis under stress conditions. However, in this model the endogenous antioxidant system fails to counteract noise-induced cell damage and its activation is not enough effective in preventing the cochlear damage. The herb-derived phenol rosmarinic acid (RA) attenuates noise-induced hearing loss reducing threshold shift and promotes hair cell survival. In fact, RA enhances the endogenous antioxidant defences as shown by the decreased superoxide production, the reduced expression of 4-HNE and the up-regulation of SODs. Interestingly, RA potentiates the Nrf2/HO-1 signalling pathway as shown by immunohistochemical and western blot analyses. Thus, protective effects of RA are associated with the induction/activation of Nrf2-ARE signalling pathway in addition to RA direct scavenging capability.

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GLYCEROL MONOOLEATE NANOPARTICLES *IN VITRO* BIOCOMPATIBILITY FOR INNER EAR DRUG DELIVERY

Filippo Valente[1], Edi Simoni[1], Bysell Helena[2], Alice Cani[1], Andrea Fornara[2], Laura Astolfi[1], Alessandro Martini[3]
[1]Bioacoustics Research Laboratory, Department of Neurosciences, University of Padua, Italy; [2]SP Technical Research Institute of Sweden, Chemistry, Materials and Surfaces, Stockholm, Sweden; [3]ENT Surgery, Department of Neurosciences, University of Padua, Italy

An innovative drug delivery system for inner ear pathologies is based on the use of nanoparticles: they are considered good carries for drugs within the inner ear, improving the drug diffusion and being detected in all parts of the cochlea when applied near the round window.
In collaboration with the Institute for Surface Chemistry (YKI, Stockholm, Sweden) we tested glycerol monooleate nanoparticles (GNPs), which have been demonstrated to be permeable to the round window membrane.
In this study we present a complete analysis of the biocompatibility of the GNPs on an *in vitro* model, alongside with a chemical-physical characterization of this new nanoparticles.
GNPs have been also conjugated with Coenzyme Q10 (CoQ10): it is a powerful antioxidant able to lower damages caused by deafening noise or ototoxic drugs. However it has a very poor solubility and bioavailability.
In order to verify the CoQ10-GNPs efficacy we tested the drug protective effect against the Cisplatin (Cpt). This compound is a cytotoxic agent widely used for the treatment of solid tumors, its use comes with numerous side effects, especially as neurotoxicity, nephrotoxicity and ototoxicity. In particular Cpt could damage the auditory nerve, stria vascularis and outer hair cells.
GNPs and cisplatin effects were detected trough vitality assays, western blot, cell cycle analysis and morphological analysis.
The in vitro cell models were OC-k3 and PC12. The first is an immortalized cell line derived from the organ of Corti of transgenic mice, thus they are an inner ear epithelial cell line model. PC12 derived from rat pheochromocytoma and represent a model of neuronal differentiation.
Results suggest that GNPs are highly biocompatible with cells and the conjunction with CoQ10 leads to lower the Cisplatin cytotoxicity.

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EFFECT OF LOCALLY APPLIED DEXAMETHASONE ON SPIRAL GANGLION NEURON SURVIVAL AND SIZE IN VIVO

Verena Scheper[1], Mareike Hütten[1], Maciek Wilk[1], Claude Jolly[2], Jürgen Groll[3], Thomas Lenarz[1], Roland Hessler[2]
[1]Department of Otolaryngology, Hannover Medical School, Hannover, Germany; [2]MED-EL Innsbruck, Research & Development, Innsbruck, Austria; [3] University of Würzburg, Department of Functional Materials in Medicine and Dentistry, Würzburg, Germany

Objective. Fibrous tissue growth around the electrode array may occur after cochlear implantation. Research showed that the glucocorticoid dexamethasone (DEX) can reduce tissue growth as well as loss of residual hearing. The peripheral auditory neurons,

the spiral ganglion neurons (SGN), are the target of electrical stimulation with a cochlear implant (CI). Up to now little is known about the effect of local DEX treatment on those neurons. Therefore the effect of locally applied DEX on SGN survival and size was investigated in two sets of animal studies.

Methods. Two normal hearing guinea pig models and two DEX application techniques were used. A): Animals were implanted via cochleostomy with a PEG-based hydrogel reservoir containing 50mg/ml DEX. A control group received the same implant filled with hydrogel only.

B): DEX was incorporated into the silicone of CI electrode arrays at 1% and 10% (w/w) concentration. Electrodes prepared by the same process without DEX served as control. Electrodes were implanted through the round window membrane causing an electrode insertion trauma by withdrawing the electrode twice before leaving it in place.

All animals were sacrificed 4 weeks after implantation. SGN density and soma diameter were evaluated on ground and stained plastic embedded specimens.

Results. Using two different animal models and drug application strategies DEX did not affect the SGC density compared to the relevant control group. When delivering DEX via a hydrogel reservoir the SGN soma diameter was not changed compared to control, whereas DEX released from silicone of the electrode array increased the cell diameter compared to control (10%DEX: $p<0.05$; 1%DEX: $p<0.001$).

Conclusion. The diversity of results relating to the soma diameter may be due to the different animal models and application techniques used. If the different soma sizes have an effect on their functionality has to be evaluated in future studies. DEX concentrations as applied in these animal models are safe for inner ear delivery in terms of their effect on SGN density. This is an important finding in regard to clinical applications of DEX for local treatment of the inner ear.

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DEVELOPMENT OF A NEUROTROPHIC IMPLANT FOR SEVERE HEARING LOSS

Heike Janssen[1], Wiebke Konerding[1], Pavel Mistrik[2], Anandhan Dhanasingh[2], Jana Schwieger[1], Jens Tornøe[3], Lars U. Wahlberg[3], Thomas Lenarz[1], Andrej Kral[1], Verena Scheper[1]

[1]Department of Experimental Otology, Institute of Audioneurotechnology, Hanover Medical School, Germany; [2]MedEl, Innsbruck, Austria; [3]NsGene A/S, Ballerup, Denmark

Objective. In line with demographic changes, the percentage of patients who suffer from hearing loss is increasing. A large portion of this population is affected by sensorineural hearing loss (SNHL). In this case, a progressive degeneration of the organ of Corti may lead to a subsequent degeneration of the spiral ganglion neurons (SGN) which form the auditory nerve. People suffering from SNHL can be treated with a cochlear implant (CI) which directly stimulates the SGN. Therefore, the ongoing degeneration of the SGN is a limiting factor for CI efficacy. The exogenous application of neurotrophic factors (NTF) can stop these degenerative changes. The aim of this study was to develop an encapsulated cell (EC) therapy device capable of long-term intracochlear NTF production in combination with a CI. Therefore, we tested the long-term safety and functionality in an animal model of deafness: the domestic cat.

Methods. For this, we deafened cats neonatally with the aminoglycoside antibiotic Neomycin and co-implanted the EC with the CI in the scala tympani. Animals were implanted two to three month after deafening to allow for initial degeneration of the SGN. A subgroup received electrical stimulation through the CI 5 days/week, 4 hours/day. The control group received a control cell line not producing NTF. All animals were treated for six month. We performed electrically evoked auditory brainstem responses (eABR) with biphasic current pulses and harvested the cochlea for histological analysis of the SGN. The data were compared with data of normal hearing, acute deafened cats.

Results. We assume a correlation of eABR responses with cochlear status i.e. SGN survival. Compared to the negative control group without NTF production, the other groups may show better SGN survival. The best results in SGN survival may be achieved by a summating effect of neurotrophic support and electrical stimulation.

Conclusions. This project has an important contribution to the development for a new neurotrophic implant for severe hearing loss for human patients.

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DEVELOPMENT OF A RESEARCH SYSTEM FOR DRUG DELIVERY TO THE INNER EAR

Daniel Smyth[1], Alec Salt[2], Jonathon Kirk[3], Jared Hartsock[2]

[1]Cochlear Technology Centre, Mechelen, Belgium; [2]Cochlear Fluids Lab, Washington University, St. Louis, USA; [3]Cochlear Research & Technology Labs, Denver, USA

Our objective is to establish a pump and cannula based drug delivery research tool which is combined with an active cochlear electrode array for the guinea pig model.

Delivery of compounds to the inner ear is difficult and upredictable through oral or intravenous routes. In order to research the effect of pharmaceuticals and ototoxins on the inner ear biology a means of chronic delivery with established and modelled parameters was required. When considering drug delivery in conjunction with cochlear implantation it is important to know how the presence of an electrode array, cochleostomy and electrical stimulation will affect drug delivery.

Cochlear Ltd have developed electrode arrays, with 8 electrodes, that are designed for atraumatic insertion in the guinea pig cochlea. In the present work the guinea pig electrode array is combined with a cannula. The cannula is connected to a pump which provides reliable delivery of the marker compound dextran. In acute experiments the factors affecting delivery rate to the cochlea were explored and compared against modelled predictions. The effect of the electrode and compound delivery on auditory response is quantified. Longer term experiments in which the pump and electrode are in situ for 7 days will explore the effect of early fibrotic response on the delivery rate and the concentrations achieved in the cochlea.

In conclusion a reliable research platform has been developed which will allow the accurate and chronic delivery of compounds to the inner ear in a manner that is representative of drug delivery in conjunction with a cochlear implant electrode array.

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General information

Venue
Campus of the Catholic University of Rome, Italy.

- The **Satellite Symposium** *"From lab to clinic:The opportunities and challenges for hearing rehabilitation and inner ear therapies"* on 12 September will be held at the Auditorium of the University Campus

- The **52nd Inner Ear Biology Workshop** on 13-15 September will be held at the Giovanni XXIII Congress Center located inside the Gemelli Hospital of the Catholic University. It is possible to reach the Congress Center either through the main entrance (Largo A. Gemelli, 8) or, preferably, from Largo Francesco Vito 1, 00168 Roma.

Conference language
The official conference language is English.

Access to the conference site
Participants should wear the identification badge collected at the Registration Desk during all conference sessions and events.

Certificate of Attendance
All registered participants will receive a certificate of attendance.

Presenter guidelines

Oral Communications
The duration of each oral presentation for the Workshop is 10 minutes plus 3 minutes for questions and answers. Presenters are requested to adhere strictly to the allocated speaking time.
All presentations should be based on Microsoft PowerPoint 2003 or later versions. Speakers must bring their presentations to the "Slide Center" in one of the following formats:
- USB Flash drive or mobile hard disk
- CD-Rom or DVD
If you have movies/audio files in your presentation, please check in advance very carefully that it runs smoothly. Note that movie and audio files are typically not embedded in your PowerPoint file. Therefore be sure to bring with you all the original movie/audio files (.mpeg,.avi,.mpg,.wav, etc.) and report to the "Slide Center" well in advance.
Speakers should contact the technicians in the "Slide Center" to upload their presentations at least two hours before the beginning of their session or in the late afternoon of the day before, in case of early morning presentations. Those with Apple-based presentations should make sure they are saved in a PC-compatible format. Speakers will not be allowed to use their own laptop.

Posters
Each poster will be illustrated orally (**3 minutes** for main result presentation, with **3 Power Point slides**, one of which is to represent the actual poster) by the presenting Author during the respective poster session. Posters are to be put up on the poster board corresponding to the assigned poster number, on the morning of Sunday 13 September from 8.00 onwards – anyway, at least half an hour in advance of the respective Poster Session, and will remain on display until the end of the IEB Workshop.

Presenting authors are requested to hand in their slides at least 1 hour before the beginning of Poster sessions, as during the poster sessions the technicians at the slide centre will not receive any slides as busy with assisting in the poster rooms.
The IEB Workshop is not responsible for posters and materials left after the end of the congress. Poster presenters should be available for the short oral presentation and discussion with Chairpeople and Delegates during their Poster Session. Poster board dimensions are 100 cm wide (39 inch), 170 cm (67 inch) high (portrait orientation).

Adhesives for fastening the poster will be provided by the Organizing Secretariat. The official language is English. No translation will be provided.

European Accreditation (EACCME)
"MEET AND WORK SRL" is accredited by the European Accreditation Council for Continuing Medical Education (EACCME) to provide the following CME activity for medical specialists. The EACCME is an institution of the European Union of Medical Specialists (UEMS), www.uems.net.

The **'52nd Inner Ear Biology Workshop and Symposium - IEB 2015'** (event no. 12746) is designated for a maximum of **21 hours of European external CME credits**. Each medical specialist should claim only those hours of credit that he/she actually spent in the educational activity.
The EACCME credit system is based on 1 ECMEC per hour with a maximum of 3 ECMECs for half a day and 6 ECMECs for a full-day event.

Through an agreement between the European Union of Medical Specialists and the American Medical Association, physicians may convert EACCME credits to an equivalent number of AMA PRA Category 1 Credits™. Information on the process to convert EACCME credit to AMA credit can be found at:
www.ama-assn.org/go/internationalcme
Live educational activities, occurring outside of Canada, recognized by the UEMS-EACCME for ECMEC credits are deemed to be Accredited Group Learning Activities (Section 1) as defined by the Maintenance of Certification Program of The Royal College of Physicians and Surgeons of Canada.
Please note that UEMS requires a feedback on the educational activity offered by the congress organiser: delegates are therefore kindly requested to complete the assessment test and evaluation form received at the Registration Desk and return it to the CME Desk at the congress counter on the last day of attendance.
The CME credit certificate will be handed out to participants at the end of the conference.
It will be up to the participants to contact their National Accreditation Authority (NAA) to have their ECMECs recognised and/or converted into national credits according to the regulations being in force in their country. (The National Board of Health will have to receive both the Certificate of Attendance and the EACCME credit certificate collected at the Congress Secretariat Desk).

ECM Italy - Educazione Continua in Medicina
CME accreditation with the Italian Ministry of Health (ECM, for Italian participants) has been made as follows:
- Satellite Symposium "From lab to clinic:The opportunities and challenges for hearing rehabilitation and inner ear therapies" – 12 September 2015.

Categories: Biologist; Physician (disciplines of Audiology and Phoniatrics, Otolaryngology, Neurology and Pediatric Neuropsychiatrist); and for Audiometrists, Audio Prosthetists and Speech Therapists.
Event no. 211-133803. **4 ECM credits assigned.**
Event accredited for 300 participants.

52nd Inner Ear Biology Workshop – 13-15 September 2015
Categories: Biologist; Physician (disciplines of Audiology and Phoniatrics, Otolaryngology, Neurology and Pediatric Neuropsychiatrist); and for Audiometrists, Audio Prosthetists and Speech Therapists.
Event no. 211-133819. **11 ECM credits assigned**.
Event accredited for 300 participants.

Disclaimer/Liability
The Organisers are not liable for injuries to participants and/or accompanying persons, nor for any loss of, or damage to, luggage and/or personal belongings of participants and/or accompanying persons. No claim is made as to the accuracy and reliability of what reported in the website.

The Organizers gratefully acknowledge for their unconditioned support
to the Symposium & 52nd Inner Ear Biology Workshop:

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