

IEB 2016

53rd Workshop Inner Ear Biology Montpellier September 17-21

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SYMPOSIUM - September 18, 2016
New horizons in hearing rehabilitation



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For more information: ieb2016@ant-congres.com

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Welcome to the Inner Ear Biology 2016

For the 4th time after the successful meetings in 1981, 1994 and 2006, Montpellier has the privilege to organize the Inner Ear Biology (Sept 17th to 21st, 2016).



We aim to reflect the dialogue that exists between basic and applied research, by complementing the usual scientific program with an opening day focused on clinical aspects (last advances, future directions and challenges in innovative therapies and rehabilitation of the inner ear diseases).

Located on the Mediterranean seashore, Montpellier (pop. 270,000) stands as a recognized high-tech pole. However, the city keeps the spirit of the past, when doctors and scientists came from all around the world, to share their knowledge in medicine, law and philosophy: its Medical School runs since the 12th century and now its Universities amount 65,000 students.

Welcome to Montpellier!

Join us for the 53rd IEB workshop and its Symposium!

A photograph of a historic building in Montpellier, featuring stone walls, arched windows, and a prominent conical roof structure. The image is used as a background for the lower section of the page.

COMMITTEES

LOCAL COMMITTEE

Jean-Luc Puel, PhD (*President*)
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SUMMARY

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PROGRAM AT A GLANCE

SATURDAY, SEPT 17	19.00 20.30	<i>Welcome reception</i>	
SUNDAY, SEPT 18	8.30 18.30	SYMPOSIUM New horizons in hearing rehabilitation	
MONDAY, SEPT 19	8.30 10.30 10.55 13.00 13.00 14.30 16.30 17.00 18.45 19.00	OC SESSION Hair Cell Anatomy and function OC SESSION Protection and Regeneration <i>Working lunch Med-EI</i> OC SESSION Age and Noise-Induced hearing loss OC SESSION Hearing rehabilitation <i>Fabre Museum visit</i>	  
TUESDAY, SEPT 20	8.30 10.30 11.00 12.45 12.45 14.15 15.45 16.15 17.45 17.45 18.45 19.00	OC SESSION Genetic of deafness OC SESSION Membrane and fluids <i>Working lunch Cochlear</i> OC SESSION Auditory nerve OC SESSION Physiopathology and auditory pathway Workshop Business Meeting <i>Gala Dinner departure</i>	  
WEDNESDAY, SEPT 21	8.30 10.15 10.45 12.30	OC SESSION Ototoxicity, hair cell death, aminoglycosides OC SESSION Hair cell: genetics and development	

SUNDAY, SEPT 18

8.30 to 18.30

8.30 SYMPOSIUM: New horizons in hearing rehabilitation

8.30 Welcome

Jean-Luc PUEL, *Institute for Neurosciences of Montpellier – Inserm UMR 1051, France*

8.45 Sound coding in the early auditory system

Philip JORIS, *KU Leuven, Belgium*

9.25 Putting the «Neural» Back in Sensorineural Hearing Loss: Primary Degeneration of the Cochlear Nerve in Noise and Aging

Sharon G. KUJAWA, *Harvard Medical School, Boston, MA, USA*

10.05 Coffee break

10.35 Present and Future In Conventional Hearing Aid

Mark LAUREYNS, *Amplifon Center for Research and Studies, Milan, Italy*

11.15 Separate electrophonic and electroneural responses with cochlear implant stimulation of a hearing ear

Andrej KRAL, Mika Sato, Peter Baumhoff, *Medical University Hannover, Germany*

11.55 Hearing the light: Optogenetic Stimulation of the Auditory Nerve

Tobias MOSER, *University Medical Center Göttingen, Germany*

12.35 Lunch

14.00 Does Genetic Diagnosis Improve Cochlear Implant Indication ?

Sandrine MARLIN, *Necker – Enfants Malades Hospital, Paris, France*

14.40 Gene therapy restores auditory and vestibular function in a mouse model of Usher syndrome, type IC

Gwennaëlle GÉLÉOC, *Harvard Medical School, Boston, MA, USA*

15.20 Electroporation Gene Delivery Using The Cochlear Implant

Gary D. HOUSLEY, *University of New South Wales, Sydney, Australia*

16.00 Coffee break

16.30 New models to mimic and understand hereditary hearing loss

Brigitte MALGRANGE (*University of Liege, Belgium*)

17.10 Non-autonomous responses to hair cell stress and death

Lisa L. CUNNINGHAM, *National Institute on Deafness and Other Communication Disorders, Bethesda, MD, USA*

17.50 Prevention of Aminoglycoside Ototoxicity: Introducing ORC-13661

Edwin W RUBEL, David W. Raible, Julian Simon, *University of Washington, Seattle, USA*

MONDAY, SEPT 19

8.30 to 19.00

8.30 OC SESSION: Hair Cell Anatomy and Function

Chairs: A.Lyzakowski A, E.Reisinger

- 8.45 ○1 Activity dependent phosphorylation by CaMKII δ alters the Ca²⁺-affinity of otoferlin
Herget M, Meese S, Gahlen F, Adams CM, Ficner R, Ricci AJ, Heller S, Reisinger E (USA and Germany)
- 9.00 ○2 The compact active zone topography of mature mammalian auditory hair cell ribbon synapses promotes a fast proton-mediated block of Ca²⁺ current
Vincent PFY, Von Gersdorff H, Dulon D (France and USA)
- 9.15 ○3 Inner ear hair cell mitochondria characterized by EM tomography into distinct subpopulations
Lysakowski A, Sobkiv S, Patel A, Arias K, Patel M, Ghatala L, Lesus J, Babu V, Price S, Guy Perkins G (USA)
- 9.30 ○4 Microtubule meshwork remodeling accounts for the auditory neuropathy AUNA1
Surel C, Guillet M, Lenoir M, Bourien J, Sendin G, Joly W, Delprat B, Lesperance M, Puel JL, Nouvian R (France and USA)
- 9.45 ○5 Loss of phosphoinositol-4,5-bisphosphate reduces single channel conductance of mammalian inner hair cell MET-channels
Effertz T, Becker L, Ricci AJ (USA)
- 10.00 ○6 Localization of reverse-polarity mechano-sensitive channels in cochlear hair cells
Beurg M, Fettiplace R (USA)
- 10.15 ○7 GABAA regulation of hair cell HCN1/CNGA3/GABARAPL2 molecular pathways
Drescher MJ, Vong A, Selvakumar D, Ramakrishnan NA, Drescher DG (USA)
- 10.30 Coffee-break and poster visit on exhibition space

10.55 OC SESSION: Protection and Regeneration

Chairs: B.Malgrange, A.Edge

- 10.55 ○8 The clinical stage H4R antagonist SENS-111 outperforms clinical comparators for the treatment of spontaneous nystagmus in a rat model of acute unilateral vestibular loss
Gueguen C, Bressieux S, Challuau D, Petremann M, Saleur A, Dyhrfjeld-Johnsen J (France)
- 11.10 ○9 Dysferlin as a correlate of cochlear membrane fusion and repair
Drescher DG, Drescher MJ, Selvakumar D, Genene Holt A (USA)
- 11.25 ○10 The cellular stress response in the noise- and ototoxic drug-exposed cochlea
Anttonen T, Herranen A, Pirvola U (Finland)

MONDAY, SEPT 19

8.30 to 19.00

- 11.40 ○11 STAT3-induced otoprotection
Szczepek AJ, Gerschner E, Olze H, Mazurek B (Germany)
- 11.55 ○12 Regeneration of hair cells by Wnt signaling in Lgr5-positive cells in the newborn mouse cochlea
Edge A (USA)
- 12.10 ○13 Netrin 1 is a mediator of IGF-1 protective effects on cochlear hair cells
Nakagawa T, Yamahara K, Yamamoto N, Ito J, Omori K (Japan)
- 12.25 ○14 Neuronal differentiation of hair follicle bulge-derived stem cells co-cultured with mouse cochlear tissue explants
Schomann T, Mezzanotte L, Hendriks SH, Frijns JHM, Huisman M (The Netherlands)
- 12.40 ○15 Notch modulation impacts the differentiation of human induced pluripotent stem cell-derived otic progenitor cells
Lahlou H, Lopez A, Fontbonne A, Nivet E, Feron F, Zine A (France)
- 12.55 Presentation of IEB 2017
T. Lenarz (Germany)
- 13.00 *Working lunch Med-El* **MED•EL**
From Hearing Aid to Middle Ear and Cochlear Implant
Chairmen: J-L.Puel, M.Beliaeff (France)
VIBRANT SOUNDBRIDGE Vibroplasty Couplers and/or BONEBRIDGE: Indications and Surgical Techniques
J.Nevoux (France)
Electrodes Insertion Depth and Atraumaticity Factors
F.Venail (France)
Cochlear Implant in Single Sided Deafness
P.Lefebvre (Belgique)
Interphase Gaps and Cochlear Health
S.Klis (The Netherlands)
- 14.00 *Coffee-break and poster visit on exhibition space*

MONDAY, SEPT 19

8.30 to 19.00

14.30 OC SESSION: Age and Noise-Induced Hearing Loss

Chairs: S. Kujawa and J. Wang

- 14.30 ○16 Statocyst sensory epithelia ultrastructural analysis of cephalopods exposed to noise
Solé M, Lenoir M, Durfort M, López-Bejar M, Lombarte A, André M
(Spain and France)
- 14.45 ○17 Development of cochlear neuropathy in adenosine receptor knockout mice
Vlajkovic SM, Ambepitiy K, Barclay M, Thorne PR (New Zealand)
- 15.00 ○18 Age-related mouse IGF-1 haploinsufficiency increases cochlear injury by increasing the inflammatory response
Celaya-Puertolas AM, Rodriguez-de la Rosa L, Pulido S, Roma-Mateo C, Zubeldia JM, Pallardo F, Varela-Nieto I (Spain)
- 15.15 ○19 Oxidative stress-induced p66 expression: Key mechanism of age-related cochlear sensory hair cell loss
Benkafadar N, François F, Puel JL, Wang J (France)
- 15.30 ○20 The role of PI3K-AKT signaling in noise-induced hearing loss
Ryan AF, Pak K, Bodmer D, Ryals M, Brand Y, Kurabi A (USA)
- 15.45 ○21 Bandwidth of input loss is key for tinnitus characteristics after noise trauma
Nowotny M, Kiefer L, Gaese BH (Germany)
- 16.00 ○22 Hyperacusis and tinnitus development over age and the influence of noise exposure in the rat behavioral model
Möhrle D, Singaravelu SK, Ni K, Bing D, Varakina K, Zimmermann U, Knipper M, Rüttiger L (Germany and China)
- 16.15 ○23 Cognitive dysfunction and degeneration with enhanced p-Tau expression in the hippocampus of mice with noise-induced hearing loss
Park SN, Kim MJ, Kim HL, Kong JS, Kim DK, Park SY (Korea)
- 16.30 Coffee-break and poster visit on exhibition space

MONDAY, SEPT 19

8.30 to 19.00

17.00 OC SESSION: Hearing Rehabilitation**Chairs: A.Krall, F. Venail**

- 17.00 **O24** The role of peripheral processes in electrically evoked compound action potentials
Ramekers D, Buitenhuis PJ, Klis SFL, Versnel H (*The Netherlands*)
- 17.15 **O25** Cochlear implantation in an ototoxic model – A role for the ‘cochlear immune system’?
Abbas L, Cacciabue-Rivolta DI, Smyth D, Rivolta MN (*United Kingdom, Belgium*)
- 17.30 **O26** Amniotic membrane in cochlear implantation
Roemer A, Sato M, Lenarz T, Kral A, Warnecke A (*Germany*)
- 17.45 **O27** Effects of steroid application with a cochlear catheter on impedances and ECAPs after cochlear implantation
Prenzler N, Rolf Salcher R, Leifholz M, Gärtner L, Warnecke A, Lenarz T (*Germany*)
- 18.00 **O28** Detection of the electrode array translocation by the insertion forces variations: optimal axis vs. inaccurate axis
Torres R, Kazmitcheff G, De Seta D, Ferrary E, Sterkers O, Nguyen Y (*France*)
- 18.15 **O29** Optical stimulation of spiral ganglion neurons and laser-induced cochlear responses depend on different mechanisms
Rettenmaier A, Baumhoff P, Lenarz T, Kral A, Reuter G (*Germany*)
- 18.30 **O30** Characterizing optogenetic stimulation of the auditory pathway using multichannel optical CIs
Keppeler D, Wrobel C, Jeschke M, Hoch G, Goßler C, Töpperwien M, Ruther R, Schwaerzle M, Hessler R, Moser T (*Germany*)
- 19.00 **Fabre Museum visit**

TUESDAY, SEPT 20

8.30 to 18.45

8.30 OC SESSION: Genetic of Deafness

Chairs: P.Gasparini, B.Delprat

- 8.30 ○31 **Epidemiology, genetics and social impact of ARHL in elderly Portuguese**
Flook M, Lopes S, Alves L, Aparício M, Martins JH, Silva E, Murta A, Andrade S, Andrade C, O'Neill A, Matos T, Antunes M, Escada P, Alcântara P, O'Neill J, Villaverde Cabral M, Fialho G, Caria H (*Portugal*)
- 8.45 ○32 **Next generation sequencing (NGS) and in vivo/in vitro approaches for the molecular characterisation of Hereditary Hearing Loss in Italian and Qatari families**
Giroto G, Morgan A, Vozzi D, Rubinato E, AlKowari MK, Di Stazio M, Badii R, Bhaghat S, Abdulhadi K, La Bianca M, Gasparini P (*Italy, Qatar*)
- 9.00 ○33 **Genome wide association studies (GWAS), targeted re-sequencing (TRS) and animal studies: A powerful multi-step approach for the discovery of the genetics of age-related hearing loss (ARHL)**
Morgan A, Vozzi D, Vuckovic D, La Bianca M, D'Eustacchio A, Concas MP, Pirastu M, Gasparini P, Giroto G (*Italy, Qatar*)
- 9.15 ○34 **hfh14, a novel locus on mouse chromosome 12 affects early-onset, high frequency hearing loss**
Yasuda SP, Obara Y, Suzuki S, Wada K, Nishito Y, Takada T, Shiroishi T, Kikkawa Y (*Japan*)
- 9.30 ○35 **A novel variant in PRKCB segregates low-frequency hearing loss in familial Meniere's disease**
Requena T, Martin-Sierra C, Frejo L, Price SD, Batuecas A, Santos-Perez S, Soto-Varela A, Lysakowski A, Lopez-Escamez JA (*Spain, USA*)
- 9.45 ○36 **Gene therapy and cell therapy targeting cochlear gap junction formation for GJB2 associated hearing loss**
Fukunaga I, Iizuka T, Hatakeyama K, Fujimoto A, Minowa O, Ikeda K, Kamiya K (*Japan*)
- 10.00 ○37 **Heterozygous mutation of Ush1g/Sans in mice causes early-onset progressive hearing loss, which is rescued by reconstituting the strain-specific mutation in Cdh23**
Kikkawa Y, Miyasaka Y, Shitara H, Suzuki S, Shiroishi T, Kominami R, Yonekawa H (*Japan*)
- 10.15 ○38 **Eps8 regulates cochlear and vestibular hair cell development**
Tavazzani E, Spaiardi P, Zampini V, Contini D, Manca M, Russo G, Prigioni I, Marcotti W, Masetto S (*Italy, United Kingdom*)
- 10.30 *Coffee-break and poster visit on exhibition space*

TUESDAY, SEPT 20

8.30 to 18.45

11.00 OC SESSION: Membrane and Fluids**Chairs: R.Fettiplace, D.Dulon**

- 11.00 ○39 Sound-induced motility of outer hair cells explained by stochastic resonance in nanometric sensors
Shapira E, Pujol R, Plaksin M, Kimmel E (*Israel, France*)
- 11.15 ○40 Inner ear evaluation of a novel mutant mouse for the Wolfram syndrome
Brun E, Lenoir M, Bonnefont AL, Korchagina Y, Wang J, Bourrien J, Puel JL, Delprat B (*France*)
- 11.30 ○41 Evaluation of the dehydration effect of isosorbide on the hydropic guinea pig cochleae using optical coherence tomography in vivo
Kakigi A, Uehara N, Takubo Y, Egami N, Yamashita S, Yamasoba T, Nibu K (*Japan*)
- 11.45 ○42 Vestibular function change in vasopressin-induced hydrops model
Kim M (*Korea*)
- 12.00 ○43 Absence or loss of the distal endolymphatic sac portion and its aldosterone-regulated ion transport function in Meniere's disease
Eckhard AH, Nadol JB Jr, Adams JC (*USA, Switzerland*)
- 12.15 ○44 In vitro nanoparticle-based delivery of hydrophobic drugs
Valente F, Simoni E, Bysell H, Astolfi L, Martini A (*Italy, Sweden*)
- 12.30 ○45 Optimising steroid delivery to the inner ear, a comparison of route of administration and the utilisation of adjuvant agents
Creber NJ, Eastwood H, Hampson A, Tan J, Chambers S, O'Leary SJ (*Australia*)

12.45 *Working lunch Cochlear***Cochlear biological research and technology development****Chairmen: A.Uziel, S.Cabrol** (*France*)**D.Smyth** (*Belgium*), **L.Abbas** (*United Kingdom*), **G.Housley** (*Australia*)13.45 *Coffee-break and poster visit on exhibition space***14.15 OC SESSION: Auditory Nerve****Chairs: A.Ricci, J.Bourrien**

- 14.15 ○46 Deciphering spiral ganglion neurons heterogeneity by single-neuron transcriptome profiling
Pramanik G, Durruthy R, Leu R, Levichev S, Waldhaus J, Heller S, Mustapha M (*USA*)
- 14.30 ○47 Sound coding in the auditory nerve of gerbils
Huet A, Justal T, Desmadryl G, Puel JL, Bourrien J (*France*)

TUESDAY, SEPT 20

8.30 to 18.45

- 14.45 **O48** Ion channels in the human cochlea -Towards further understanding of electric activation of the human auditory nerve
Liu W, Scharf-Moren N, Benav R, Glueckert R, Garnham CW, Schrott-Fischer A, Rask-Andersen H (*Sweden and Austria*)
- 15.00 **O49** Influencing neural firing with neurotrophins
Cai H, Wright T, Gillespie L, O'Leary S, Needham K (*Australia*)
- 15.15 **O50** Prostaglandin-F-analogue bimatoprost reveals neuroprotective effects in spiral ganglion neurons in vitro
Warnecke A, Lenarz T, Roemer A (*Germany*)
- 15.30 **O51** Neuroprotective effect of monomethyl fumarate on spiral ganglion neurons
Ahrensmeier C, Kwiatkowska M, Ellrichman G, Gold R, Dazert S, Volkenstein S (*Germany*)
- 15.45 *Coffee-break and poster visit on exhibition space*

16.15 OC SESSION: Physiopathology and Auditory Pathway**Chairs: P Joris, R Nouvian**

- 16.15 **O52** Modelling effect of dehiscence in osseous spiral lamina
Ni G, Elliott S (*United Kingdom*)
- 16.30 **O53** Microglial subpopulations in rat DCN and their changes in tinnitus models
Vitale V, Sanchini G, Solinas S, Pizzala R, Perin P (*Italy*)
- 16.45 **O54** Circadian rhythm regulates differential responses of day and night noise exposure in the inferior colliculus
Park J, Cederroth CR, Basinou V, Meltser I, Lundkvist G, Canlon B (*Sweden, Korea*)
- 17.00 **O55** Effects of anodal transcranial direct current stimulation (tDCS) on auditory cortex structural plasticity in a model of noise-induced hearing loss
Paciello F, Podda MV, Rolesi R, Zanin F, Eramo SLM, Troiani D, Grassi C, Paludetti G, Fetoni AR (*Italy*)
- 17.15 **O56** Hearing loss and altered temporal resolution in knockout mice with the deleted brain-specific link protein *Bral2*
Popelář J, Díaz Gómez M, Lindovský J, Rybalko N, Syka J (*Czech Republic, Spain*)
- 17.30 **O57** Altered auditory perception processing and neural connectivity in normal hearing individuals with tinnitus
Hong SK, Park S, Park JH (*Korea*)

TUESDAY, SEPT 20

8.30 to 18.45

- 17.45 **O58 SPECIAL LECTURE:** Assessment of the quality of reporting of otorhinolaryngology articles involving animal models using the ARRIVE statement
Bezdjian A, Klis S, Peters J, Grolman W, Stegeman I (*The Netherlands*)

18.00 BUSINESS MEETING

IEB Business meeting discussions and forthcoming meetings

- 19.00 **Gala dinner - Bus departure at level 0 (Corum)**
Announcement of the Spoendling award



WEDNESDAY, SEPT 21

8.30 to 12.30

8.30 OC SESSION: Ototoxicity, hair cell death, aminoglycosides**Chairs: J Schacht, G Paludetti**

- 8.30 **○59** Cisplatin-based chemotherapy, ototoxicity and prevention ▯ can inner ear research make any difference to patients with head and neck cancer?
Engmér Berglin C, Videhult Pierre P, Fransson A, Linder B, Laurell G (Sweden)
- 8.45 **○60** Cisplatin chemotherapy and ototoxicity: Comparing the antitumor and antioxidant efficacy of two phenolic compounds, Curcumin and Ferulic Acid
Fetoni AR, Paciello F, Mezzogori D, Rolesi R, Eramo SLM, Di Pino A, Troiani D, Grassi C, Paludetti G (Italy)
- 9.00 **○61** Interaction between innate immune cells and auditory neurons is regulated by fractalkine signaling
Warchol ME, Kaur T, Strong M, Rubel EW, Hirose K (USA)
- 9.15 **○62** Pneumolysin-dependent ototoxicity of *Streptococcus pneumoniae* in organ of corti explant cultures
Perny M, Solyga M, Roccio M, Grandgirard D, Leib SL, Senn P (Switzerland)
- 9.30 **○63** G6PDH - a crucial enzyme in protection against aminoglycoside ototoxicity
Schrepper T, Luo L, Schacht J (USA)
- 9.45 **○64** Ablation of Sestrin2 enhances susceptibility to gentamicin-induced hair cell death
Levano S, Ebnoether E, Ramseier A, Cortada M, Bodmer D (Switzerland)
- 10.00 **○65** Loss of synapses and calyceal junctions between type I vestibular hair cells and afferent endings are early events during chronic ototoxicity in rats
Llorens J, Jedynak P, Greguske E, Prades S, Boadas-Vaello P, Sedó-Cabezón L (Spain)

10.15 *Coffee-break and poster visit on exhibition space***10.45 OC SESSION: Hair Cell: Genetics and Development****Chairs: M Mustapha, G Géléoc**

- 10.45 **○66** Sox2 deletion impairs the inner ear neurosensory development
Dvorakova M, Bohuslavova R, Fritzsche B, Jahan I, Chumak T, Syka J, Pavlinkova G (Czech Republic and USA)
- 11.00 **○67** Gata3 is required for the differentiation of mouse inner hair cells
Bardhan T, Marcotti W, Holley M (United Kingdom)
- 11.15 **○68** The role of Bmp6 in formation of the anterior and posterior semicircular canal ducts in the zebrafish inner ear
Baxendale S, Maier EC, Obholzer N, Burbridge S, van Hateren N, Montserrat Garcia Romero M, Yokoya K, Knight R, Megason SG, Whitfield TT (United Kingdom and USA)

WEDNESDAY, SEPT 21

8.30 to 12.30

- 11.30 **O69** FGFR1 regulates a transport of PCDH15 during inner ear hair cell specialization
Honda A, Kita T, Misaki K, Ahmed Z, Richardson G, Yonemura S, Ladher RK
(Japan, USA, United Kingdom and India)
- 11.45 **O70** Characterization of Lgr5+ progenitor cell transcriptomes in the apical and basal turns of the mouse cochlea
Waqas M, Guo L, Zhang S, Chen Y, Tang M, Li H, Chai R *(China)*
- 12.00 **O71** Vlgfr1-mediated signaling pathway is important for hearing
Du H, Chen P, Wang Y, Sun J, Xu Z *(China)*
- 12.15 **O72** Minding species gaps in the cochlea: non-human primate and patient derived-hiPSC models for the study of hereditary hearing loss
Hosoya M, Fujioka M, Suzuki N, Okano H, Ogawa K *(Japan)*
- 12.30 *End of IEB Workshop*



Abstracts available online:
www.IEB2016.com

Hair Cell Anatomy and Function

- P1** Automated cell counting in cochlear histological samples
Saleur A, Baecker V, Dyhrfeld-Johnsen J (France)
- P2** Three dimensional characterisation of hair cells in normal and pathological states
Bullen A, Whittaker M, Bakay W, Forge A (United Kingdom)
- P3** Cytoarchitectural constraints of repair in auditory supporting cells
Anttonen T, Ulla Pirvola U (Finland)
- P4** Plasma membrane bound otoferlin levels correlate with hearing loss in different otoferlin mutant mouse models
Al-Moyed H, Hoch G, Wichmann C, Strenzke N, Reisinger E (Germany)
- P5** Impaired sound encoding in PSD-95 knockout mice
Yamanbaeva G, Jung SY, Wong MH, Strenzke N (Germany)
- P6** RIM-BP2 as a regulator of synaptic transmission fidelity at inner hair cell ribbon synapses
Krinner S, Jung SY, Wichmann C, Moser T (Germany)
- P7** A trial of establishment for TRIOBP ko in vitro model using induced pluripotent stem cells
Ohnishi H, Kitajiri S, Taura A, Taniguchi M, Ebisu F, Sakamoto T, Yamamoto N, Ito J, Omori K, Takayuki Nakagawa T (Japan)
- P8** Non-canonical resurgent Na⁺ currents in Spiral Ganglion Neurons from hearing mice
Browne L, Smith K, Jagger D (United Kingdom)
- P9** P2X7-dependent purinergic signalling in cochlear glial cells
Heard H, Browne L, Smith K, Jagger D (United Kingdom)
- P10** Hearing dysfunction in otoferlin I515T mutant mice
Pelgrim M, Yamanbaeva G, Jeschke M, Reisinger E, Strenzke N (Germany)
- P11** The contribution of inward rectifier K⁺ currents to the membrane physiology of cochlear glial cells
Smith K, Murphy P, Browne L, Bullen A, Jagger D (United Kingdom)
- P12** IK,L properties of vestibular Type I hair cells are affected by the nerve calyx ending
Prigioni I, Tavazzani E, Spaiardi P, Manca M, Russo G, Masetto S (Italy)

Hair Cell: Genetics and Development

- P13** Single-cell transcriptome protocol of the cochlear development
Yamamoto N, Tona Y, Kita T, Ohnishi H, Nakagawa T, Omori K (Japan)
- P14** Autonomous calcium transients in interdental cells during the critical period of cochlear development
Schade-Mann T, Pirritano D, Engel J, Eckrich T (Germany)
- P15** Neurosensory development in the human foetal vestibular end organs
Chacko LJ, Pechriggl EJ, Fritsch H, Rask-Andersen H, Blumer MJF, Schrott-Fischer A, Glueckert R (Austria and Sweden)

Membrane and Fluids

- P16** Heat shock proteins in human perilymph
[Schmitt H](#), [Roemer A](#), [Zeilinger C](#), [Salcher R](#), [Pich A](#), [Lenarz T](#), [Staecker H](#), [Warnecke A](#) (Germany and USA)
- P17** Ion Channels in the Human Cochlea Towards further understanding of electric activation of the human auditory nerve
[Liu W](#), [Schart-Moren N](#), [Benav H](#), [Glueckert R](#), [Garnham CW](#), [Schrott-Fischer A](#), [Rask-Andersen H](#) (Sweden and Austria)
- P18** Various types of cochlear gap junctions and Connexin26-hemichannel localization reproduced in human cell lines
[Hatakeyama K](#), [Fukunaga I](#), [Fujimoto A](#), [Ikeda K](#), [Kamiya K](#) (Japan)
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Cassel R, Bordiga P, Watabé I, Tonetto A, Assie A, Gharbi A, Chabbert C (France)

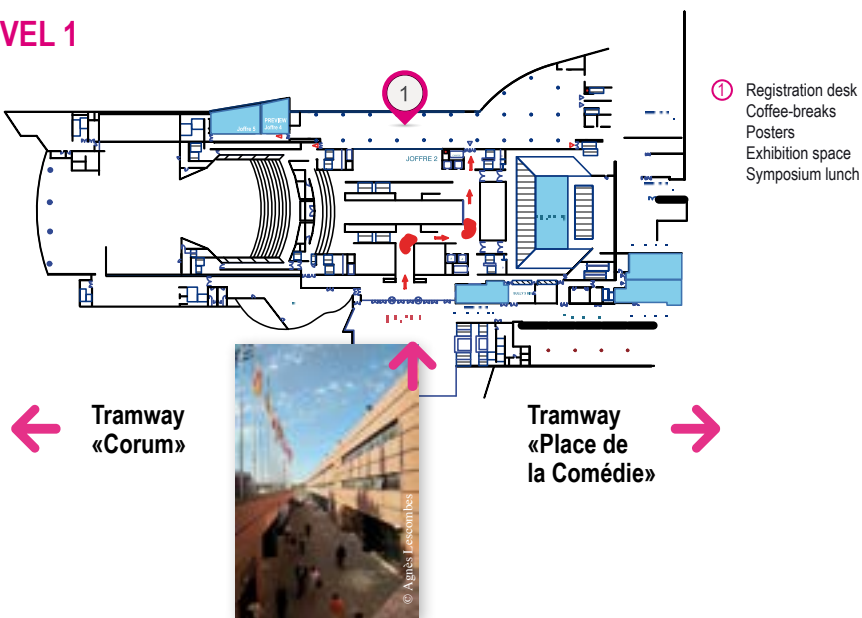
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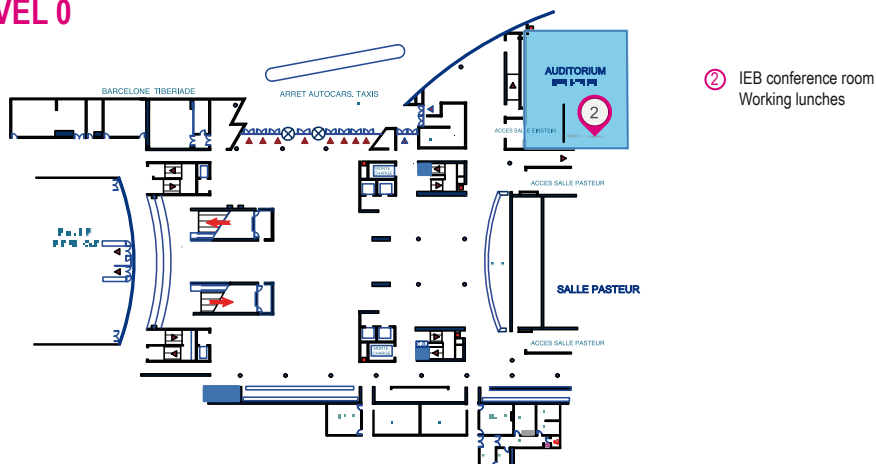
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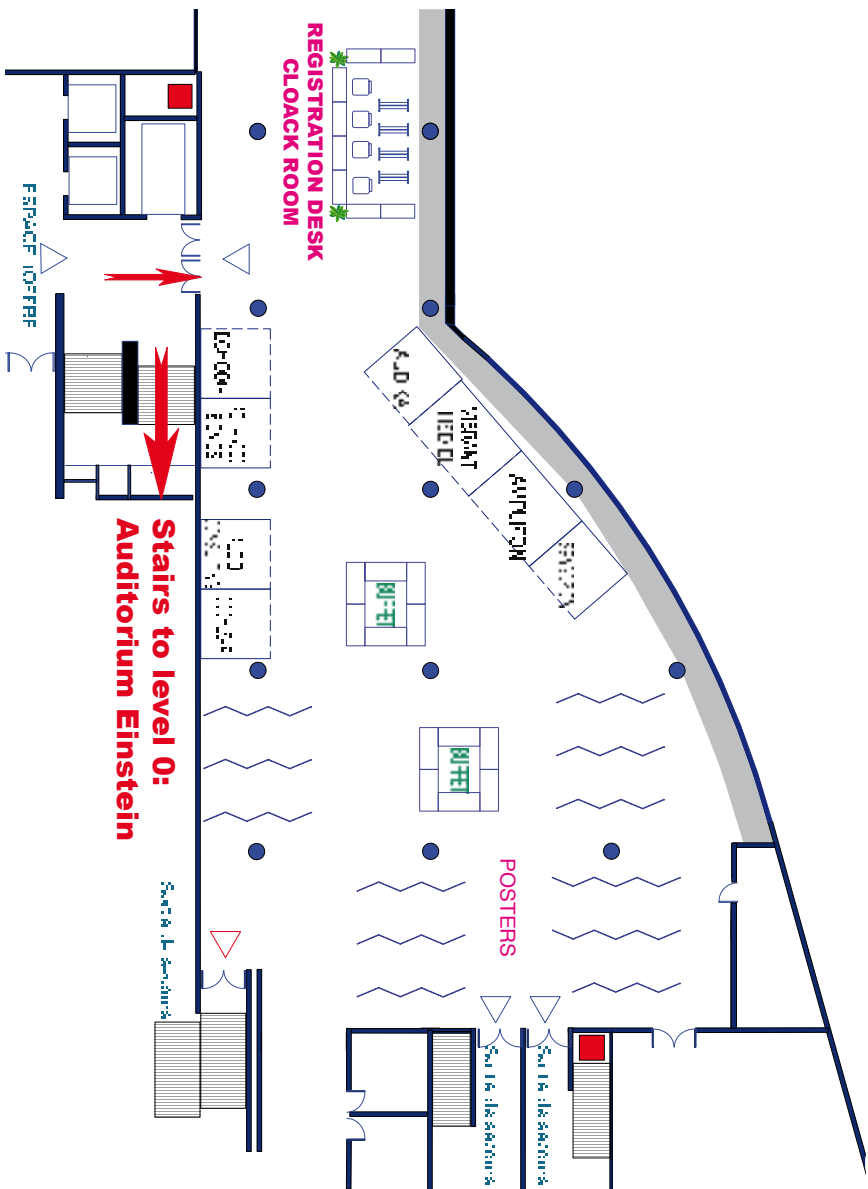
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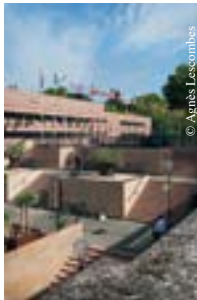
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EXHIBITION MAP



HOW TO REACH THE VENUE



Congress venue

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- By car -

Follow signs for Montpellier - Centre Historique - Le Corum
Satnav coordinates: Lat.: 43.62 - Long.: 3.89
Underground car park (charge): 2 entrances – 500 spaces -
Set-down on the west side near the bus stops

- By tram -

Lines 1 – 2 – 4 – Le Corum stop
Getting to the Corum from Montpellier - St-Roch TGV station
Tram lines 2 and 4 (2 stops)
10 minutes' walk

- From the airport -

Airport shuttle – line 120 – get off at the 'Place de l'Europe'
www.herault-transport.fr

- Taxi -

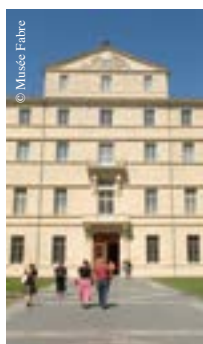
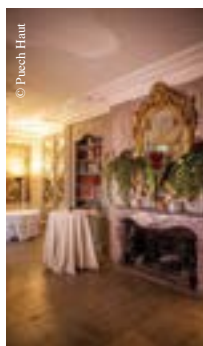
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Gala Dinner

Tuesday, september 20

Puech-Haut Castle, Saint-Drézéry



THE STONES OF CHÂTEAU PUECH-HAUT ARE RICH WITH HISTORY

Gérard Bru, owner of the Puech-Haut estate, is a man of great personal resources. An industrialist who decided to go into wine production, he returned to his oldest passion: working on the land.

His habit is to think big! For him, half-measures will never do. He knows where he's going and how to get there. From his years in industry, he has honed the skills of management and development. He is a man of the "land" and he has always been someone who insists on concrete results and not just talk. While some dream and plan, he is a doer. Starting with a land devoid of any grapes, he has built what is now one of the largest vineyards in the region, and at its heart was born the "Château Puech-Haut", built from the stones of the old Prefecture of Montpellier. This was originally a bourgeois residence built in Italian style between the 17th and 18th centuries, and it was slated for demolition.

Gérard Bru bought the structure and then transplanted it stone by stone in the middle of his new property. During this work, he discovered an ornamental stone ram's head from a 12th or 13th century chapel, and it became the emblem of Château Puech Haut. Given the things he has achieved, Bru is something of a magician.

Musee Fabre visit

Monday, september 19

Musée Fabre, Montpellier

Frédéric Bazille exhibition: «La jeunesse de l'impressionisme»

Discover one of the most beautiful fine-arts museums in France
Reopened in 2007 after four years of renovation, the Fabre Museum now offers 7,000 m2 of exhibition area for presenting the 900 works from the prestigious Museum collections. Located at the entrance to the Museum, La Portée by Daniel Buren invites visitors on a fabulous journey through art history. The pathway is interspersed with paintings, sculptures and graphic arts from the 16th to 21st centuries.

The major artistic movements and the great names of painting are found here: masterpieces by Rubens, Poussin, Fabre, Bazille, Courbet and Soulages are showcased in a singular, luminous setting. Each year, prestigious international exhibitions are shown along with the permanent collections. These include: Gustave Courbet; Caravaggio and the European Caravaggisti; The Golden Age of painting in Naples and Senufo unbounded.

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ABSTRACTS

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Philip X. Joris

Laboratory of Auditory Neurophysiology, University of Leuven, Belgium

Compared to other sensory systems, the auditory system is quite unusual in a number of respects. Whereas in vision and touch different objects tend to strike different parts of the receptor surface, sounds from different sources are summed acoustically into a single pressure wave striking the two eardrums. Moreover, compared to the optic and somatosensory nerves, the auditory nerve is relatively uniform in its properties. Thus, the central nervous system receives less differentiated input in the auditory modality than in other sensory systems. However, the auditory brainstem transforms this relative homogeneous input into a striking range of parallel channels which extract different features of sounds. A wide range of structural and functional specializations enable neurons to achieve this. Already at the first synaptic level, in the cochlear nucleus, extreme differences in response properties are found, and these differences are taken a step further in subsequent subcollicular nuclei. I will briefly illustrate how different sound features are coded at different brainstem sites, with an emphasis on the dimension in which the auditory system excels: the coding of the temporal stimulus features.

To extrapolate the fine-grained physiological knowledge obtained in animals to humans, a first important issue concerns the (dis)similarity of the input of the sensory organ to the central auditory system. I will discuss current views on fundamental properties of the human cochlea relative to those properties in commonly studied laboratory species. Although all mammals share a basic architectural plan of auditory brainstem connections and physiological properties, there is also an enormous variety between species, reflecting the widely different ecological niches occupied by mammals. Our tools to study central brainstem physiology in humans are currently limited: I will illustrate some limitations to be aware of in the interpretation of mass potentials obtainable from humans.

Sharon G Kujawa PhD

Massachusetts Eye and Ear Infirmary, Harvard Medical School

A longstanding view of acquired sensorineural hearing loss (SNHL) has been that cochlear hair cells are among the most vulnerable elements in the cochlea and that, in the vast majority of cases, cochlear nerve fibers degenerate if, and only long after, there is loss of their peripheral hair cell targets. This view arose, fundamentally, because of the temporal offset between post-insult degeneration of hair cells and loss of the spiral ganglion cell (SGC) bodies of the primary auditory neurons with which they communicate: In animal models exposed to noise or ototoxic drugs, hair cell loss can be widespread within hours, whereas the loss of SGCs is typically not detectable for weeks to months after insult.

Threshold elevations accompany hair cell damage and loss; for human assessments, the pure tone threshold audiogram is a key metric of this overt, sensory hearing loss, providing documentation of the magnitude of the audibility loss, its pattern as a function of frequency, and to some extent underlying site(s) of dysfunction (e.g. middle ear, inner ear). It has long been known, however, that audiometric thresholds do not always reflect reported or documented auditory perceptual difficulties and that thresholds and otopathology are not always well aligned.

Recent work in animal models has shed new light on this disconnect. It is now clear, at least in the noise-exposed and aging ear (Kujawa and Liberman 2009; Sergeyenko et al. 2013), 1) that cochlear neurons are a primary target, 2) that their peripheral synaptic connections are the most vulnerable elements and 3) that cochlear nerve synapses can be destroyed even when hair cells survive. This basic result has now been observed in multiple mammalian species, including compelling preliminary observations in human temporal bones. Beyond noise and aging, gentamicin-treated mice and temporal bones of humans who received aminoglycosides in life can display diffuse cochlear neuropathy for treatments not sufficient to cause hair cell loss.

Neurophysiological and morphologic studies suggest that the resulting neural loss is biased toward the subset of cochlear nerve fibers with high thresholds and low spontaneous rates (SR) of firing. Thus, cochlear synaptopathy and low-SR neuropathy can be widespread in ears with intact hair cell populations and normal audiograms, where it has been called “hidden” hearing loss. This observation further suggests that the synaptopathy should contribute to problems hearing in a noisy environment, since, in the normal ear, the low-SR fibers are particularly resistant to continuous noise masking.

Although the loss of hair cell synapses immediately silences the affected cochlear neurons, the slow death of the cell body and central projections provides a long therapeutic window within which hair cells might be reconnected to the brain via therapies designed to elicit spiral ganglion neurite extension and hair cell synaptogenesis. Thus, the need for better understanding of the prevalence and functional consequences of cochlear synaptopathy is clear.

Work supported by grants from the NIH/NIDCD, DoD and ONR.

Mark Laureyns

Amplifon Center for Research and Studies, Milan, Italy, Thomas More University College, Audiology Department, Antwerp, Belgium

Conventional hearing aids have improved significantly the last decades and will improve further the coming years. We will present and overview of relevant improvements we notice today and foresee in the future.

But more significant improvements are to be expected from professional hearing care.

When providing hearing care – the audiogram is a very limited source of information. It only evaluates when people start to hear pure tones at different frequencies ... we need to look at all dimensions of hearing “performance” and how the “person” perceived these dimensions.

Therefore we identified six dimensions we systematically want to assess when providing hearing care;

- Audibility (hearing soft sounds)
- Understanding Speech in Quiet
- Understanding Speech in Noise (SPIN)
- Noise Acceptance (ANL)
- Focus (working memory capacity – concentration)
- Central Auditory Processing (Localization, Binaural Masking Release, ...)

The last three aspects are rarely assessed in clinical practice today. Therefore we started multiple multicenter studies on the feasibility to use the following tests in clinical practice:

* An optimized version of the “ANL test” in multiple languages as a tool to assess Noise Acceptance.

* A new “Adaptive Reading Span Test” procedure in multiple languages to assess “Working Memory Capacity”

* A new “Binaural Masking Release Test” as a tool to assess Central Auditory Processing Performance, Understanding Speech in Noise and Understanding Speech in Quiet .

We will present the results on feasibility and reliability of these test procedures, the relation with self-perceived performance on these dimensions and the relation with advance hearing aid signal processing.

Some of the surprising results of these studies is, that the default setting of hearing instrument manufacturers are rarely appropriate for specific patients, so there certainly room for improvement by individualized “patient centered” professional hearing care. Separate electrophonic and electroneural responses with cochlear implant stimulation of the hearing ear.

Andrej Kral, Mika Sato, Peter Baumhoff

Institute of AudioNeuroTechnology, Hannover Medical University, Hannover, Germany

Introduction: Electroacoustic stimulation in subjects with residual hearing is becoming more widely used in clinical practice. However, little is known about the properties of electrically induced responses in the hearing cochlea.

Objective: The present study investigated the patterns of excitation in the inferior colliculus (IC) with electrical stimulation of hearing and deafened cochleae to identify the locations where electrophonic and electroneural responses are generated.

Method: Cochlear implantation was performed through a cochleostomy in normal hearing guinea pigs under general anesthesia. A Neuronexus double-shank 32-channel electrode array was stereotactically placed in the contralateral side of the inferior colliculus parallel to the tonotopic axis. The electric stimuli were charge-balanced biphasic electric pulses, 100µs/phase. Thresholds, firing rates and dynamic ranges were determined from unit activity recorded in the midbrain and was related to the acoustic characteristic frequency (CF) of the unit. The cochlea was subsequently deafened with the implant left in place and the stimulation was repeated in the deaf condition. The response patterns to electrical stimuli before and after deafening were compared.

Results: Cochlear implant was not acutely harmful for normal hearing. Acoustic stimulation revealed an ordered frequency representation along the shanks of the electrode arrays, covering CFs in the range of 1 – 32 kHz. In hearing cochleae, two spots of activity were observed: one at low CFs (~ 5 kHz) and one at high CFs (> 9 kHz). After deafening, the thresholds of electrical stimulation increased and the electrical dynamic range decreased significantly. Most extensive changes were observed in the low CF region. Moreover, with sinusoidal electrical stimuli, the apical excitation shifted with changing frequency of the electrical stimulus, the basal one corresponded to the place of the stimulating electrode in the cochlea.

Conclusion: The low threshold, the large dynamic range and the change with deafening suggest that the low CF response was predominantly hair-cell mediated (electrophonic). This electrophonic response appeared at the dominant frequency of the electrical stimulus. A direct neural response with higher thresholds, small dynamic range and less change after deafening was observed in the CF region >9kHz. Consequently, electrical stimulation of a hearing cochlea results in two spatially separate regions of electrophonic and electroneural activation. Bipolar stimulation revealed that the electrophonic response is more effectively generated if the stimulating electrodes are more apical. In monopolar stimulation differences in properties of the two stimulation sites were less pronounced than in bipolar stimulation.

Supported by Deutsche Forschungsgemeinschaft (Cluster of Excellence Hearing4all)

Tobias Moser (for the Göttingen Cochlear Optogenetics Program)

Institute for Auditory Neuroscience, University of Göttingen Medical Center, Göttingen, Germany, Auditory Neuroscience Group, German Primate Center, Göttingen, Germany, Auditory Neuroscience Group, Max-Planck-Institute 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. We aim to improve frequency and intensity resolution of cochlear implant coding by establishing spatially confined optical stimulation of spiral ganglion neurons (SGNs). We have established optogenetic stimulation of the auditory pathway in rodents using virus-mediated expression of channelrhodopsins to render SGNs light-sensitive. Optogenetic stimulation of spiral ganglion neurons activated the auditory pathway, as demonstrated by recordings of single neuron and neuronal population responses at various stages of the auditory system. Fast opsins enabled SGN firing at physiological rates (hundreds per second). We approximated the spatial spread of cochlear excitation by recording local field potentials in the inferior colliculus in response to suprathreshold optical and electrical stimuli, which suggested a better frequency resolution for optogenetic than for electrical stimulation. Moreover, we found activation of neurons in primary auditory cortex. In a collaborative effort we develop and characterize flexible μ LED-based multichannel intracochlear stimulators. Optogenetic stimulation of the auditory nerve is feasible and bears great potential for future application in research and hearing restoration.

Electrical versus optical stimulation of the cochlea

Top: in electrical CIs usually 12-24 electrodes are used to stimulate SGNs. Current spread leads to activation of a large population of neurons along the tonotopic axis, thereby limiting the frequency resolution and dynamic range of electrical coding.

Bottom: optical stimulation promises spatially confined activation of SGNs allowing for a higher number of independent stimulation channels and, thereby, improving frequency and intensity resolution.

Sandrine Marlin

Necker- Enfants Malades Hospital, Paris, France

Hearing loss and hearing impairment are mainly genetic. More than 500 syndromic forms of deafness have been described and more than 90 different genes were implicated in isolated deafness.

One of the objectives of the geneticist is to participate to the management of the patients regarding cochlear implant indication.

I will present an overview of the relation between genotypes and cochlear implant results.

Gwenaëlle G.S. Géléoc¹, Bifeng Pan^{1*}, Charles Askew^{1*}, Alice Galvin^{1*}, Selena Heman-Ackah¹, Yukako Asai¹, Artur A. Indzhukulian², Jennifer J. Lentz³, Luk H. Vandenberghe⁴, Jeffrey R. Holt¹
¹ Dpt of Otolaryngology, F.M. Kirby Center for Neurobiology; Boston Children's Hospital, Harvard Medical School, Boston, MA. ² Dpt of Neurobiology, Harvard Medical School, Boston, MA. ³ Dpt of Otorhinolaryngology & Bio-communications and Neuroscience Center, LSU Health Sciences Center, New Orleans, LA. ⁴ Schepens Eye Research Institute, Boston, MA. *These authors contributed equally

Ush1c c.216G>A is a knock-in mouse model with a cryptic splice site mutation found in French-Acadian USH1C patients. The mutation results in expression of truncated harmonin at the expense of the full-length protein. Homozygous Ush1c c.216G>A mice (c.216AA) suffer from severe hearing loss and vestibular dysfunction at one month of age. To explore the feasibility of gene augmentation therapy to treat USH1C, we assessed hair cell survival and function in postnatal c.216AA mice. We demonstrate that many hair cells retain hair bundles and mechanosensitivity during the first postnatal week. We show that truncated harmonin does not compete with the full-length harmonin for binding partners since overexpression in wild type and heterozygous (c.216GA) mice does not disrupt hair cell, auditory or vestibular function. To assess early gene therapy intervention, we developed adeno-associated viral vectors (AAV2/1 and AAV2/Anc80) expressing one of two harmonin splice forms. We used vectors that encoded fluorescently-tagged harmonin and demonstrated successful targeting of the proteins in vitro and in vivo after early postnatal round window membrane (RWM) injection. Finally, we show that postnatal day one RWM injection of AAV2/Anc80.CMV.harmonin-b preserves mechanotransduction as well as auditory and vestibular function in c.216AA mice. Scanning electron microscopy reveals decreased hair cell loss and preserved hair bundles with normal staircase morphology in rescued ears. We conclude that gene augmentation therapy is a promising therapeutic approach for restoration of hearing and balance in mouse models of human USH1C.

Supported by: Manton Center for Orphan Disease (BCH); Foundation Fighting Blindness; Bertarelli Foundation; Kidz-b-Kidz Foundation; Jeff and Kimberly Barber Gene Therapy Research Fund.



Gary D. Housley

Translational Neuroscience Facility & Department of Physiology, School of Medical Sciences, UNSW Australia, Sydney, NSW 2052, Australia

Patient recruitment criteria for cochlear implants (CIs) is broadening, with an increasing focus on preservation of residual (low frequency) hearing, and commensurate heightened hearing performance expectations. However, the neural interface of cochlear implants has changed little over time, and despite the considerable advances in the speech processor capabilities, hearing quality with CIs is still far from natural. A key challenge is to use the linear electrode array comprising just a few platinum electrodes to selectively drive the tens of thousands of spiral ganglion neurons normally tonotopically mapped along the length of the cochlea. Central to this is closing the 'neural gap' between the electrodes and the surviving spiral ganglion neurons. Sensori-neural hearing loss (SNHL) is reflected by loss of the organ of Corti hair cells and supporting cells, which reduces the critical neurotrophin support to the spiral ganglion neurons, with consequent loss of the peripheral neurites and atrophy of the soma within Rosenthal's canal. The current spread that occurs at the stimulus levels required to recruit these SGNs is such that position-based mapping is coarse, resulting in poor pitch perception. A range of neurotrophin delivery strategies stimulate peripheral neurite extension and promote survival of SGNs in cochleae of animal models of SNHL, but the challenge is to achieve this safely and direct and constrain the neurite extension. Our approach to this challenge has been to develop a cochlear implant array – driven gene electrotransfer platform for focal delivery of naked plasmid DNA with a brain-derived neurotrophic factor (BDNF) expression cassette incorporating a GFP reporter (Pinyon et al. *Sci. Transl. Med.*, 2014). Voltage pulses are delivered through the CI array to electroporate targeted mesenchymal cells close to the array. Using *ex vivo* and *in vivo* experiments with the guinea-pig cochleae, supported by studies with HEK293 cell monolayers, we identified that the wiring configuration of the CI implant array was a key factor in the efficiency of gene delivery. Mapping of the electric field around the array in different configurations showed that electric field focusing produced a compression of field strength which enables gene electrotransfer with applied voltages lower than that necessary for conventional 'open-field' electroporation (Browne et al., *Gene Ther.*, 2016). 'Dial-up' control of the shape and area of the region of GFP positive cells is possible by controlling the field focusing and voltage pulse parameters; although the required charge delivery is currently outside the practical limits of current CI systems. The GFP-positive mesenchymal cells express BDNF (evident from immunofluorescence of a flag-tag) and within 7 days SGN neurite extension beyond the osseous spiral lamina and into scala tympani occurs. Functional assessment using bipolar stimulation via the CI for eABR in a deafened guinea pig model indicated that this gene therapy treatment had the desired effects of lowering threshold current levels for SGN recruitment and increasing the dynamic range of the input – output function, indicative of progressive recruitment into the local SGN population as the current levels were increased. These promising developments around local naked DNA –based gene electrotransfer in the cochlea support utilization of this process for targeted neurotrophin gene therapy during CI surgery to enhance SGN survival and optimize the neural interface. Funding: Australian Research Council DP150104754 & LP0992098 with support from Cochlear Ltd. Declaration: Patents associated with this development are assigned to the New South Innovations, the commercialization arm of UNSW Australia.

Brigitte Malgrange

Alström Syndrome (AS) is a human, autosomal recessive, genetic disorder characterized by numerous clinical symptoms including deafness. AS is caused by mutations in the *ALMS1* gene encoding the ALMS1 protein localized in centrosomes and basal bodies of primary cilia. *ALMS1* has been implicated in ciliogenesis, intracellular trafficking, cell cycle control and cellular differentiation, among others. However, the molecular mechanisms that give rise to deafness are still unknown. Here, we used human induced pluripotent stem (hiPS) cells generated from fibroblasts of healthy and AS patients to decipher the pathophysiological role of mutated *ALMS1* in deafness.

We established hiPS cells from patient fibroblasts using non-integrative Sendai viral vectors. These cell lines were validated *in vitro* and *in vivo*. Using a stepwise protocol, we demonstrate that healthy hiPSCs can generate a population of otic progenitor cells (OSCs) that are then able to differentiate into hair cells (HCs) when co-cultured with mouse feeder cells. We then applied this differentiation protocol to AS hiPSCs generated from AS patients. We showed that, AS hiPS cells are able to generate OSCs and HCs similarly to wild type hiPS cells. While differentiation capabilities seem normal in AS hiPS cells, we found numerous abnormal features in AS-OSCs. In particular, AS-OSCs present cell proliferation defects and abnormal primary cilium development.



[Lisa Cunningham](#), Lindsey May, Elyssa Monzack, Andrew Breglio

National Institute on Deafness and other Communication Disorders, NIH, Bethesda MD USA

Mechanosensory hair cells are sensitive to death from a variety of stresses, including noise trauma, aging, and exposure to therapeutic drugs with ototoxic side effects. Cellular stress can activate signal transduction pathways that promote cell death, but it can also activate pathways that promote cell survival. It is often the balance (or imbalance) of these death vs. survival signals that determines whether the cell under stress ultimately lives or dies. Our data indicate that the glia-like supporting cells that surround sensory hair cells are critical mediators of life-vs.-death signaling in response to hair cell stress. Supporting cells respond to hair cell stress in ways that can promote survival of the stressed hair cell. After hair cell death, supporting cells engulf dead hair cells and remove them from the sensory epithelium, possibly preventing further damage. Our current studies are aimed at understanding the intercellular signaling that occurs between hair cells and supporting cells in response to stress caused by ototoxic drugs.

Edwin W Rubel, David W. Raible, Julian Simon

University of Washington, Fred Hutchinson Cancer Research Center, Seattle WA USA

We tested the hypothesis that ORC-13661, a new drug candidate derived from a unique small molecule screen, will provide robust protection of mammalian inner ear hair cells and prevent hearing loss associated with chronic aminoglycoside exposure.

Aminoglycoside antibiotics (AGs) remain mainstay treatments for life-threatening infections like sepsis, TB and CF. Common adverse conditions are permanent hearing and balance disorders, and kidney toxicity. Using mechanosensitive hair cells on the body of free-swimming larval zebrafish, we were able to screen for small drug-like molecules that could prevent AG ototoxicity without interfering with bactericidal efficacy.

Drug properties and efficacy of positive compounds were enhanced by medicinal chemistry and iterative testing.

Lead Compounds were tested in mature rats exposed to 10-12 days of Amikacin treatment. Hearing was tested by assessing ABR thresholds prior to drug treatment and 2 weeks following treatment with Amikacin alone or Amikacin plus BPN-13661.

Dose-response studies of rats given 10-day Amikacin exposure indicated that 20 dB hearing loss was significantly but incompletely prevented by co-administration of 1 mg/kg/day of BPN-13661 (PO) and completely protected by 5 mg/kg/day of BPN-13661 co-administration. Treatment of rats with Amikacin for 12 continuous days reliably produced hearing loss reaching >50 dB at the highest frequencies tested. Coadministration with BPN-13661 completely protected this hearing loss to within 10dB of the pretest at all frequencies tested. Studies of hair cell loss in the same rats revealed a close relationship between the ABR results and the loss/survival of outer hair cells in the organ of Corti.

BPN-13661 appears to be a likely candidate for clinical trials in the near future.



Meike Herget (1,2) Sandra Meese (3), Felix Gahlen (4), Christopher M. Adams (5), Ralf Ficner (3), Anthony J. Ricci (1), Stefan Heller (1), Ellen Reisinger (6)

(1) Department of Otolaryngology, Head and Neck Surgery, Stanford School of Medicine, Stanford, CA 94305, USA; (2) present address Avails Medical, Inc., Menlo Park, CA 94025, USA; (3) Department of Molecular Structural Biology, Institute for Microbiology and Genetics, and Collaborative Research Center 889, University of Göttingen, D-37075 Göttingen, Germany; (4) Department of Otorhinolaryngology, Head and Neck Surgery, Ruhr-University Bochum, D-44787 Bochum, Germany; (5) Stanford University Mass Spectrometry, Stanford University, Stanford, CA 94305, USA; (6) Molecular Biology of Cochlear Neurotransmission Group, Department of Otorhinolaryngology, University Medical Center Göttingen, Germany, and Collaborative Research Center 889, University of Göttingen, D-37075 Göttingen, Germany

Introduction: Otoferlin, a multi-C2 domain protein, is required for exocytosis from auditory inner hair cells. Despite its vital role for vesicle replenishment and likely involvement in vesicle fusion and endocytosis, a potential biochemical regulation of otoferlin has not been studied to date.

Methods: By immunoprecipitation from chicken utricles using the HCS-1 antibody, we identified Ca^{2+} /calmodulin dependent serine/threonine kinase II delta (CaMKIId) as a potential otoferlin interaction partner. The interaction was further tested with pull downs from HEK293 cells and an immunohistochemistry based proximity ligation assay in rat inner hair cells. Isolated C2 domains of otoferlin were expressed in *E. coli*, purified, and co-incubated with recombinant CaMKIId and cofactors to identify sites of phosphorylation by mass spectrometry. The effect of phosphosites on Ca^{2+} binding was studied by mutating phosphorylated serine or threonine residues to aspartate and a microscale thermophoresis assay.

Results: Otoferlin and CaMKII interact in HEK293 and in rat inner hair cells. CaMKIId phosphorylates otoferlin in vitro at 10 serine/threonine residues, five of which are located within C2 domains. Phosphorylation of otoferlin in inner hair cells was enhanced by stimulating explanted organs of Corti with high extracellular K^{+} saline and could be blocked by the CaMKII inhibitor KN-93. The phosphomimetic mutation in the C2C domain increased the Ca^{2+} affinity compared to the non-phosphorylated C2C domain, however the Ca^{2+} affinity was still rather low. For the C2F domain, the Ca^{2+} affinity was reduced by three phosphomimetic mutations by at least one order of magnitude.

Conclusion: Our data suggest that otoferlin is regulated by activity dependent phosphorylation via CaMKII.

Philippe FY Vincent¹, Henrike von Gersdorff², Didier Dulon¹

*1*Université de Bordeaux, Institut des Neurosciences de Bordeaux, Equipe Neurophysiologie de la Synapse Auditive, Inserm U1120, 33076 Bordeaux, France; *2*The Vollum Institute, Oregon Health and Science University, Portland, Oregon 97239

We show that a fast transient block of Ca^{2+} channels (ICaTB) by exocytosed protons occurs in mouse and gerbil inner hair cells (IHCs) when they are bathed in a physiological bicarbonate-based external solution. The mechanism of ICaTB is explained by the acidified content of the synaptic vesicles that is released concomitantly with glutamate in the synaptic cleft during vesicular release. Exocytosed H^{+} rapidly bind to the mouth of the nearby Ca^{2+} channels, containing glutamate residues, and instantaneously reduces the Ca^{2+} conductance. By manipulating intracellular Ca^{2+} rises with BAPTA in IHCs, we show that ICaTB operates only during synchronous multivesicular release, which requires otoferlin as a candidate Ca^{2+} sensor. Furthermore, we show that ICaTB is absent in the immature pre-hearing IHC synapses, where Ca^{2+} channels are loosely coupled to the docked vesicles at ribbon-type active zones. Moreover, ICaTB is also absent in the mature calyx of Held synapse where Ca^{2+} channel clusters are known to be relatively dispersed within the conventional active zone. We propose that ICaTB occurs only in synapses that operate via Ca^{2+} nanodomain-triggered multivesicular release orchestrated within a compact active zone. This H^{+} regulation of Ca^{2+} channels provides a fast feedback mechanism that efficiently reduces Ca^{2+} current and transmitter release. Finally, we propose that ICaTB constitutes a novel mechanism that contributes to the fast adaptation of spike rates at auditory nerve fibers.



Anna Lysakowski (1), Sofia Sobkiv (1), Aashutos Patel (1), Kevin Arias (1), Meet Patel (1), Laila Ghatala (1), Joseph Lesus (1), Vidya Babu (2), Steven D. Price (1), Guy Perkins (3)
 (1) Dept. of Anatomy and Cell Biology, Univ. of Illinois at Chicago, Chicago, IL, USA, (2) Illinois Math and Science Academy, Aurora, IL, USA, (3) National Center for Microscopy and Imaging Research (NCMIR), Univ. of California at San Diego, San Diego, CA, USA

Mitochondrial forms of deafness are well known, yet we know very little about inner ear mitochondrial structure. The central hypothesis of this study is mitochondria in hair cells and inner ear sensory epithelia are non-homogeneous and the structural and molecular differences they exhibit are related to their differential responses to ototoxic insults, such as aminoglycoside toxicity. It is likely that due to variations in physical structure and corresponding molecular composition, various sub-populations of hair-cell mitochondria are differentially affected by ototoxic insults, such as aminoglycoside antibiotics and chemotherapeutics. EM tomography and graphic reconstruction methods were used. Our results indicate there are 3 different-sized populations of mitochondria in the vestibular epithelium (based upon volume and surface area measurements): large (found in the subcuticular region of central zone type I vestibular hair cells); medium (in all hair cells and afferents); and small (in efferent boutons). We are also investigating cochlear mitochondria. By reconstructing several examples of each type, we are determining average volume and surface area and the number and type of cristae (tubular, lamellar or tubulo-lamellar) for each type. Hair cells have lamellar cristae (the high-energy form), while afferents and efferents have tubular cristae. In addition, we are investigating the number, size, and locations of crista junctions (intersections of the cristae with the inner mitochondrial membrane) in relation to other significant structures within the cell. As an example of our structural findings, we find many mitochondrial cristae adjacent to a ribbon synapse are perpendicular to (or polarized toward) the ribbon. Thus, these cristae align end-on with the ribbon and the crista junctions on that side of the mitochondrion open towards it. Crista junctions, which function as diffusion barriers to molecules inside mitochondria, thus concentrating the oxidative phosphorylation complexes that generate ATP, are also thought to be key regulators for apoptosis effector release. Finally, we are also attempting to characterize the three sub-populations by ratiometric measurements of mitochondrial potential using JC-1. We conclude that there are significant differences in morphology among these 3 mitochondrial sub-populations. Our next goal is to determine whether one population is more susceptible than others to aminoglycosides.

Clément Surel (1), Marie Guillet (1), Marc Lenoir (1), Jérôme Bourien (1), Gaston Sendin (1), Willy Joly (1), Benjamin Delprat (1), Marci Lesperance (2), Jean-Luc Puel (1), Régis Nouvian (1)

(1) Inserm U1051 - Institute for Neurosciences of Montpellier - University of Montpellier, France; (2) University of Michigan, Ann Arbor, MI, USA

We show that a fast transient block of Ca^{2+} channels (ICaTB) by exocytosed protons occurs in mouse and gerbil inner hair cells (IHCs) when they are bathed in a physiological bicarbonate-based external solution. The mechanism of ICaTB is explained by the acidified content of the synaptic vesicles that is released concomitantly with glutamate in the synaptic cleft during vesicular release. Exocytosed H^+ rapidly bind to the mouth of the nearby Ca^{2+} channels, containing glutamate residues, and instantaneously reduces the Ca^{2+} conductance. By manipulating intracellular Ca^{2+} rises with BAPTA in IHCs, we show that ICaTB operates only during synchronous multivesicular release, which requires otoferlin as a candidate Ca^{2+} sensor. Furthermore, we show that ICaTB is absent in the immature pre-hearing IHC synapses, where Ca^{2+} channels are loosely coupled to the docked vesicles at ribbon-type active zones. Moreover, ICaTB is also absent in the mature calyx of Held synapse where Ca^{2+} channel clusters are known to be relatively dispersed within the conventional active zone. We propose that ICaTB occurs only in synapses that operate via Ca^{2+} nanodomain-triggered multivesicular release orchestrated within a compact active zone. This H^+ regulation of Ca^{2+} channels provides a fast feedback mechanism that efficiently reduces Ca^{2+} current and transmitter release. Finally, we propose that ICaTB constitutes a novel mechanism that contributes to the fast adaptation of spike rates at auditory nerve fibers.



Thomas Effertz, Lars Becker, Anthony J Ricci

Department of Otolaryngology, Stanford University, 300 Pasteur Drive, Stanford CA 94305, United States

The mammalian cochlea utilizes specialized sensory cells, hair cells, to translate sound into chemical/electrical signals. The hair cells sensory organelle, the hair bundle, consists of actin filled stereocilia, arranged in a staircase pattern. The hair bundle is most sensitive to deflections towards the tallest row of stereocilia, during which mechano-electrical-transduction (MET) channels, residing at the top of the middle and shortest stereocilia row, open and allow for a cation inflow (MET-current). MET-currents of adequate size result in neurotransmitter release. How stereocilia deflections open the MET-channels remains unknown. The gating force resulting from stereocilia deflections could be transferred directly through a chain of proteins or indirectly through the cell membrane, or a combination of both.

Previous data shows a compartmentalization of the stereociliar lipid bilayer suggesting a biological relevance of lipid compositions for hair cell function (Zhao et al. 2012). Our immunohistochemical data supports this assumption and shows that phosphoinositol-4,5-bisphosphate (PIP2) is concentrated at the tips of stereocilia, close to the presumed location of the MET-channels in mammalian hair cells. In addition, depletion of PIP2 through inhibition of its enzymatic replenishment (utilizing phenylarsine-oxide, PAO; PIP2 rundown in about 10-15 minutes) or internal application of PIP2 scavenger molecules (i.e. poly-lysine) effects the MET-current. Most notably we found a reduction of peak MET-current, an increase of resting open probability, and a loss of fast adaptation in rat inner hair cells. We show that the reduction of MET-current in PAO treated cells, is due to a reduction of single channel conductance. Utilizing an excess amount of PIP2 in our intracellular patch clamp solutions protects against the effect of PIP2 depletion through PAO, indicating that the observed effects are directly PIP2 related. The data thus suggests that PIP2 functions as a cofactor for the MET-channel or closely adjacent proteins, which are able to effect the pore of the MET-channel.

Maryline Beurg, Robert Fettiplace

Department of Neuroscience, University of Wisconsin, Madison WI 53706, USA

INTRODUCTION Two types of mechanically-sensitive (MS) currents have been described in cochlear hair cells: conventional MS currents evoked by displacements of the hair bundle towards its tallest edge, and anomalous MS currents, elicited by bundle displacements in the opposite direction, referred to as reversed-polarity currents. Conventional MS transducer channels are located at the bottom end of the tip links, but reverse-polarity MS currents appear after severing tip links with BAPTA, suggesting a different site for this channel.

METHODS We investigated localization of reverse-polarity MS channels using cell-attached patch recordings on cochlear outer hair cells (OHC) in wild type mice after BAPTA destruction of tip links and during embryonic and neonatal development. Hair bundles were stimulated with a fluid jet. Single mechano-sensitive channels were recorded in cell-attached mode.

RESULTS MS channels were obtained on the apical surface around the base of the hair bundle and were activated by suction through the patch pipette. MS channels appeared about five minutes after breaking the tip links with BAPTA, and had a mean conductance of 61 ± 6 pS in Na-saline. The ensemble average displayed an adapting time course matching the kinetics of the reverse-polarity current. Reverse-polarity currents were also recorded in wild-type mice during embryonic and early neonatal development but they disappeared in the first few postnatal days contemporaneously with up-regulation of the conventional MS transducer current. These too were associated with MS channels on the OHC apical membrane with conductance of 54.0 ± 0.2 pS. The reverse-polarity current, however it was evoked, but not the normal current, was suppressed by raising the intracellular calcium concentration.

CONCLUSION Reverse-polarity currents reflect activation of stretch-sensitive channels on the hair-cell apical plasma membrane. Loss of the tip links, or other conditions that abolish normal transduction, reduces calcium influx so decreasing the cytoplasmic concentration of the divalent. We suggest lowering calcium promotes insertion of reverse-polarity channels. These anomalous channels resemble conventional MS transducer channels in conductance and pharmacology, indicating an inter-relationship between the two channel types. The susceptibility of reverse-polarity currents to various mutations will be discussed. Supported by NIH grant RO1 DC01362.

Marian Drescher, Angela Vong, Dakshnamurthy Selvakumar, Neeliyath Ramakrishnan,
Dennis Drescher
Wayne State University School of Medicine, Detroit, Michigan, USA

INTRODUCTION: Transcripts for subunits alpha1, alpha2, beta1, beta2, beta3 and gamma2 of the GABAA ionotropic chloride channel have been identified in adult mammalian organ of Corti (Vong et al., ARO Abstr, 2016) and GABAA alpha1 mRNA sequence elucidated in purified teleost saccular hair cells (Drescher et al., GenBank Accession No. KF644440). Our objective is to characterize the molecular input of GABAA via protein-protein interactions (PPI) to hair-cell exocytosis/endocytosis and mechanotransduction, and specifically, the co-regulation of hair-cell HCN1, CNGA3, and GABARAPL2.

METHODS: Yeast-two hybrid (Y2H) mating protocols for GABAA alpha1, CNGA3, HCN1, HCN2 and protocadherin 15 CD3 in both hair cell models revealed direct PPIs, confirmed by Y2H co-transformation. Surface plasmon resonance yielded KDs and Ca2+-dependence of PPIs predicting, for proteins with multiple binding partners, the likely outcomes of competitive binding in vivo, tested in vitro in pull-down assays.

RESULTS: In direct binding interactions for hair cells, GABAA alpha1 interacts with RACK1, which in turn interacts with HCN1, which switches between tip-link protocadherin 15 CD3 (with filamin A) and HCN2 (with Ih). GABAA alpha1 also interacts with low-density lipoprotein receptor-related protein 1 (LRP-1), a binding partner of the CNGA3 channel amino terminus, also the site for binding tip-link cadherin 23 or alternatively, myosin VIIa. LRP-1 co-functions with alpha2 macroglobulin, which directly interacts with protocadherin 15 CD3 establishing a link between HCN1 and CNGA3. Further, LRP-1 binds to beta1-integrin sandwiched between filamin A and EMILIN1, which itself directly binds the carboxy terminus of CNGA3. In forming the ionotropic chloride channel, GABAA alpha1 couples to GABAA gamma2 which binds GABARAPL2, a GABAA-associated protein. GABARAPL2 directly interacts with protocadherin 15CD3, and tmc1 can be immunoprecipitated with GABARAPL2. In Y2H, CNGA3 interacts with FKBP9/peptidyl-prolyl-cis/trans-isomerase binding to FK506/calcineurin/AC9, also targeted by FKBP8, a binding partner of tmc1 and CFTR.

CONCLUSIONS: The multiplicity of direct binding partners for “key” proteins in hair cells implies the possibility of their regulation in formation of alternate protein complexes (Ramakrishnan et al., J Biol Chem, 2012; Selvakumar et al., J Biol Chem, 2013), underlying a balanced hair-cell response to physical stimuli such as stretch, and changes in intracellular Ca2+ and phosphorylation.

Cindy Gueguen (1), Stéphanie Bressieux (1), Désiré Challuau (1), Mathieu Petremann (1), Aurélie Saleur (1), Jonas Dyhrfeld-Johnsen (1)
Sensorion SA, Montpellier, France

Introduction: Without efficient treatments options, patients suffering from vertigo of vestibular origin are severely impacted in their daily life. Current offlabel use of drugs like antihistamines and corticosteroids lack efficacy or carry significant side-effects, frequently forcing patients to remain bedridden. SENS-111 is a novel histamine H4R antagonist in clinical development for acute vestibular vertigo, having demonstrated good efficacy in preclinical models without side-effects. Present work evaluates the capacity of SENS-111 to reduce vertigo-associated symptoms in comparison to/combination with human equivalent doses (HED) of clinically used compounds meclizine and methylprednisolone.

Methods: Unilateral vestibular excitotoxic lesion was induced in 12 week-old female Long-Evans rats using intra-tympanic injection of kainate (40-45 mM, t=0). The time-course of treatment effects of SENS-111 (10mg/kg), meclizine (5.2mg/kg), methylprednisolone (6.2mg/kg) and their combinations was evaluated on spontaneous nystagmus after baseline recordings at t=1h.

Results: SENS-111 reduced spontaneous nystagmus frequency by 21% already 1h after administration compared to saline vehicle ($p=0.011$). After 3h and 5h, nystagmus frequency was reduced respectively by 13% and 20%. Meclizine and methylprednisolone showed little early treatment effect with tendency to increase nystagmus at later time-points (respectively t=1h: ~2% and ~5% reduction; t=3h: 8% and 23% increase; t=5h: 5% and 9% increase; none statistically significant) compared to DMSO. As DMSO effects differed from saline, comparisons of drugs and combinations were performed using treatment effect relative to vehicle. The combination of meclizine and SENS-111 abolished the treatment effect of SENS-111 alone at t=2h ($p=0.030$), t=4h ($p=0.231$) and t=6h ($p=0.021$). The combination of methylprednisolone and SENS-111 was not significantly different from SENS-111 alone at t=2h, however at t=4h ($p=0.019$) and t=6h ($p=0.042$) the addition of methylprednisolone abolished the positive treatment effect of SENS-111 alone.

Conclusions: SENS-111 alone significantly reduces vertigo symptoms in rats following vestibular excitotoxic insult whereas the clinical comparators meclizine and methylprednisolone have no effect on spontaneous nystagmus frequency in the acute phase at HED. Furthermore, the addition of meclizine or methylprednisolone to SENS-111 treatment abolished the positive effect of SENS-111 alone. This work demonstrates that SENS-111 is a potent drug candidate to treat patients with vestibular disorders.

Dennis G. Drescher, Marian J. Drescher, Dakshnamurthy Selvakumar, Avril Genene Holt
Wayne State University School of Medicine, Detroit, Michigan, USA

Introduction: Cochlear hair cells are vulnerable to injury by sound, and their membranes become porous after noise exposures that cause auditory threshold shifts (Mulroy et al., *Hear Res* 115: 93-100, 1998). After recovery, the membranes reseal. We sought molecular correlates of auditory recovery in the ferlins, proteins representing six vertebrate genes including those for otoferlin and dysferlin. Since dysferlin is known to be involved in muscle repair, we investigated its presence in the cochlea and studied dysferlin binding partners compatible with the formation of a cochlear repair complex.

Methods: Adult Black Agouti rats were exposed to 118 dB SPL noise for 1.5 hours, followed by a 24-hour recovery period. Dysferlin-specific Hamlet-1 antibody was used, with diaminobenzidine or immunofluorescence detection and confocal microscopy. Dysferlin N-terminal C2A domain, dysferlin C-terminal C2F domain, annexin-1A, syntaxin-4, affixin, calpain-3, mitsugumin-53, and AHNAK were inserted into pRSET vectors for expression, and the resulting fusion proteins purified. Yeast two-hybrid, surface plasmon resonance (SPR), and mass spectroscopic analysis were employed to ascertain binding partners of dysferlin.

Results: We detected dysferlin-like immunoreactivity in hair cells of the cochlea. Fluorescence for dysferlin overlapped phalloidin-labeled actin in stereocilia of cochlear outer hair cells and inner hair cells. Dysferlin immunofluorescence on outer hair cell stereocilia corresponded to the tops of individual stereocilia in stereociliary arrays. Dysferlin immunoreactivity was also found in cochlear Boettcher's cells of the outer sulcus and in root cells extending into the spiral ligament. By yeast two-hybrid analysis, dysferlin C2A and C2F domains bound annexin-A1 and syntaxin-4. SPR analysis yielded quantitative binding constants for dysferlin C2A and C2F with annexin-A1, syntaxin-4, calpain-3, affixin, mitsugumin-53, and AHNAK constructs. Dysferlin immunoprecipitation followed by mass spectroscopy identified previously-unknown putative dysferlin interactors.

Conclusions: Stimulation of repair proteins such as dysferlin in the inner ear may hasten recovery from auditory threshold shifts. Protein complexes of dysferlin and/or other ferlins may be key to an exocytotic/endocytotic membrane-repair model putatively underlying cochlear recovery from effects of loud sounds. A molecular correlate for recovery from noise-induced auditory threshold shifts is a missing link for understanding and possibly ameliorating temporary and permanent deafness.

Tommi Anttonen, Anni Herranen, Ulla Pirvola
Department of Biosciences, University of Helsinki, Finland

The cellular stress response network guides cells to initiate protective responses upon stressful stimuli. But if stress is too strong or prolonged, cellular stress signaling feeds into cell death pathways. Inhibition of detrimental processes or boosting protective processes of the stress response network offer protective therapeutic potential in many biological contexts. In the inner ear, inhibition of activation of the MAPK pathways has been studied as a pharmacotherapeutic approach to protect hair cells against traumas. Inhibition of the JNK pathway has been shown to confer protection of auditory hair cells against noise and ototoxic drugs, but the cellular mechanisms of JNK signaling in the inner ear are poorly understood. This understanding is required for the development of safe and efficient therapeutic interventions against hearing loss.

In the noise- and ototoxic drug-challenged cochlea, we have characterized the rapid and transient activation of the c-Jun transcription factor, the major downstream effector of stress-activated JNKs. Interestingly, rather than in the vulnerable outer hair cells, c-Jun is activated in several non-sensory populations of the cochlea. As hair cells are, however, partially protected against acoustic trauma in a mutant mouse model where JNK/c-Jun interaction is inhibited, a paracrine mechanism mediating the detrimental effects on outer hair cells is proposed.



Agnieszka J. Szczepek (1), Elisabeth Gerschner (2), Heidi Olze (1), Birgit Mazurek (2)

(1) Department of ENT, Charité-University Hospital Berlin, Germany ; (2) Tinnitus Center, Charité-University Hospital Berlin, Germany

STAT3 (signal transducer and activator of transcription 3) is a ubiquitous transcription factor. Upon activation, STAT3 translocates into the nucleus to mediate gene transcription or into mitochondria to support complex I of respiratory chain. The consequences of STAT3 activation include cell proliferation, differentiation, and production of cytokines, chemokines or neurokines. Here, we have investigated the ability of STAT3 to prevent in vitro auditory hair cell loss induced by cisplatin. Preventing predictable ototoxicity could be an effective way to avoid hearing loss.

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

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

Albert S.B. Edge

(1) *Harvard Medical School*

Wnt signaling is required for the differentiation of hair cells during embryogenesis. 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 in the embryo. Lgr5 continued to be expressed in the postnatal cochlea in a specific subset of supporting cells. In contrast to the limited ability to replace damaged cells in adult vestibular and auditory organs, cochlear cells from the neonatal mouse showed a capacity for hair cell replacement following ototoxic damage. Hair cell replacement 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. New hair cells were found predominantly in the area between inner and outer hair cells and had characteristics of outer hair cells. Lgr5-positive cells isolated from newborn mouse cochlea could be grown in vitro. The cells proliferated to large numbers after stimulation of Wnt signaling. 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. Lgr5-negative cells did not differentiate to hair cells. 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.



Takayuki Nakagawa, Kohei Yamahara, Norio Yamamoto, Juichi Ito, Koichi Omori

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

Introduction: Previous studies have demonstrated protective effects of IGF-1 on inner ear hair cells in vitro and in vivo. Our clinical trials have revealed the potential of IGF-1 as a therapeutic for the treatment of sudden sensorineural hearing loss. As for mechanisms of IGF-1 actions on cochlear hair cells, IGF-1 exerts protective effects via both MEK-ERK and IP3-Akt pathways, and induces up-regulation of several molecules including netrin 1. Here we examined whether netrin 1 is a mediator of IGF-1 effects on cochlear hair cells using cochlear explant cultures of neonatal mice.

Methods: Cochlear explant cultures of neonatal mice were used. Neomycin, an aminoglycoside, was used as a toxin for hair cells. We examined protective effects of netrin 1 against neomycin, expressions of netrin 1 receptors in cochleae and effects of antibodies for netrin 1 or an its receptor on hair cell protection by IGF-1.

Results: Netrin 1 showed protective effects on cochlear hair cells in a dose-dependent manner and inhibition of apoptosis of hair cells. Several receptors for netrin 1 were present in cochlear sensory epithelia. An antibody for netrin-1 or an its receptor attenuated protective effects of IGF-1 on hair cells.

Conclusions: Present findings have revealed that netrin-1 mediates IGF-1 actions for protection of cochlear hair cells, which can provide new insights for development of new therapeutics for sensorineural hearing loss.

Timo Schomann (1), Laura Mezzanotte (2), Sanne H. Hendriks (1), Johan H.M. Frijns (1), Margriet A. Huisman (1)

(1) Department of Otorhinolaryngology and Head & Neck Surgery, Leiden University Medical Centre, the Netherlands; (2) Optical Molecular Imaging, Department of Radiology, Erasmus Medical Centre, 3015 CE Rotterdam, the Netherlands

Introduction: Stem cell-based auditory neuron repair, in combination with a cochlear implant, may represent an attractive therapeutic option to restore sensory neuronal hearing loss. Neural crest stem cells are promising candidates for this type of therapy because they can differentiate into sensory neurons and glia and can easily be harvested from the hair follicle bulge. Despite these advantageous characteristics, neural crest stem cells have never been used in cell-based auditory neuron therapy. To establish the potential of this cell type, we focussed on two important aspects of successful auditory neuron regeneration: incorporation of hair follicle bulge-derived stem cells (HFBSCs) into cochlear tissue and their successive capability to differentiate into auditory neural phenotypes.

Methods: Cochleae of wild type mice were isolated whereafter modiolus and staircases were cut transversally, followed by dissection of the pieces. Next, the cochlear explants were cultured with lentivirally transduced HFBSCs. Transduced cells expressed copepod green fluorescent protein (copGFP) under control of the constitutively active elongation factor-1 alpha promoter (EF1-copGFP) or the promoter of the neuronal migration protein doublecortin (DCX-copGFP). EF1-copGFP cells were monitored during their migration across a cell-free zone towards the explant. After their arrival, the velocity of the cells was calculated. Co-cultures were fixed after two weeks with 1% formaldehyde in PBS for immunohistochemical staining.

Results: HFBSCs migrated with a velocity of 140 μm per hour towards cochlear explants and within five days after arrival, they formed a distinct fascicular pattern. While EF1-copGFP HFBSCs were constitutively expressing copGFP, DCX-copGFP cells only became green-fluorescent after integration into cochlear tissue. Immunohistochemical results showed that HFBSCs in the cochlear explants were positive for the neuronal marker neurofilament-medium and/or Atoh1, a marker for young hair cells and supporting cells. All HFBSCs present in the cochlear explants were negative for the glial markers S100 and EGR2.

Conclusion: These results suggest that HFBSC-derived neural progenitors integrate into cochlear explants and differentiate towards an auditory neuronal lineage. Thus, HFBSCs may be employed in cell-based therapies for auditory neuron regeneration.

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Hanae Lahlou¹, Alejandra Lopez¹, Arnaud Fontbonne¹, Emmanuel Nivet², François Feron², Azel Zine^{1,3}

¹ Université Aix-Marseille, LNI, CNRS-7260. Marseille, France; ² Université Aix-Marseille, NCIN, CNRS-7259. Marseille, France; ³ Université de Montpellier, Faculté de Pharmacie. Montpellier, France

Background: Human induced pluripotent stem cells (hiPSCs) technology holds great expectations for drug discovery and clinical applications such as cell transplantation. Along this line, progresses have been made in applying hiPSC technology to a variety of organ systems such as the retina, the cardiovascular system as well as the peripheral and central nervous systems. Noteworthy, the inner ear represents another system of interest for drug discovery and cell therapy (See Lopez et al. abstract) applications relying on the use of hiPSCs. The primary objective is attempted for efficient derivation of otic progenitors and hair cell-like cells from hiPSCs using a reliable in vitro induction protocol able to recapitulate in the vivo key developmental steps.

Methods: In a first step, we derived otic progenitor cells from hiPSCs using monolayer culture to expand these pluripotent cells on laminin coated-matrix in a medium, supplemented with FGF3/FGF10 during 12 days. In a second step, the otic progenitors were seeded on gelatin in medium containing-either retinoic acid and EGF or Notch pathway modulators for 2-4 weeks. At the end of the cultures, qPCR and immunostaining analyses were performed to assess the expression of otic and HC lineage markers.

Results: Our protocol leads to cells up regulating individual or combined expression pattern of comprehensive otic placode lineage markers (Pax2, Eya4..) at the end of the initial 12-day induction phase. Interestingly, a fraction of these FGF-induced otic progenitors upregulated a subset of HC markers under differentiating culture conditions. Our results revealed that otic progenitors differentiated under Notch pathway inhibition are more prone to up-regulate a subset of HC markers (Pou4F3, Myosin VIIa..), when compared either to untreated or RA/EGF-treated cultures.

Conclusion:

These data indicate that interference with Notch pathway during hiPSC derived otic progenitors differentiation is highly efficient for the generation of human hair cell-like cells in vitro. This work could be set the bases to a better comprehension of the mechanisms controlling human HC differentiation and could benefit to cell-based therapy for inner ear disorders.

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Marta Solé (1), Marc Lenoir (2), Mercè Durfort(3), Manel López-Bejar (4), Antoni Lombarte (5), Michel André (1)

(1) Laboratory of Applied Bioacoustics. Technical University of Catalonia. BarcelonaTech. Barcelona, Spain; (2) INSERM U.1051, Institute of Neurosciences of Montpellier, Montpellier, France; (3) Department of Cellular Biology, Faculty of Biology, University of Barcelona, Barcelona, Spain; (4) Department of Animal Health and Anatomy, Faculty of Veterinary Science, Universitat Autònoma de Barcelona, Cerdanyola del Vallés, Spain; (5) Renewable Marine Resources Department, Marine Sciences Institute (ICM-CMIMA-CSIC), Barcelona, Spain

Many anthropogenic noise sources are nowadays contributing to the general noise budget of the oceans. The extent to which sound in the sea impacts and affects marine life is a topic of considerable current interest both to the scientific community and to the general public. Cephalopods potentially represent a group of species whose ecology may be influenced by artificial noise that would have a direct consequence on the functionality and sensitivity of their sensory organs, the statocysts. These are responsible for their equilibrium and movements in the water column. Controlled Exposure Experiments, including the use of a 50-400Hz sweep (RL = 157 ± 5 dB re 1 μ Pa with peak levels up to SPL = 175 dB re 1 μ Pa) revealed lesions in the statocysts of four Mediterranean cephalopod species, when exposed to low frequency sounds: (n=76) *Sepia officinalis*, (n=4) *Octopus vulgaris*, (n=5) *Loligo vulgaris* and (n=2) *Illex condietii*. The analysis was performed through scanning (SEM) and transmission (TEM) electron microscopic techniques of the whole inner structure of the cephalopods' statocyst, especially on the macula and crista. All exposed individuals presented the same lesions and the same incremental effects over time, consistent with a massive acoustic trauma observed in other species: Immediately after exposure, the damage was observed in the macula and in the crista sensory epithelium. Kinocilia on hair cells were either missing or bent or flaccid. A number of hair cells showed protruding apical poles and ruptured lateral plasma membranes, most probably resulting from the extrusion of cytoplasmic material. Hair cells were also partially ejected from the sensory epithelium, and spherical holes corresponding to missing hair cells were visible in the epithelium. The cytoplasmic content of the damaged hair cells showed the presence of numerous vacuoles and electron dense inclusions not seen in the control animals. The lesions described here are new to cephalopod pathology. Given that low-frequency noise levels in the ocean are increasing (e.g. shipping, offshore industry, and naval maneuvers), and that reliable bioacoustics data on invertebrates are scarce, the present study and future investigations will bring an important contribution to the sustainable use of the marine environment.

Srdjan M. Vlajkovic (1), Kaushi Ambepitiya (1,2), Meagan Barclay (1), Peter R Thorne (1,2)
(1) Department of Physiology and (2) Discipline of Audiology, University of Auckland, Private Bag 92019, Auckland, New Zealand

Recent studies have shown that extensive loss of auditory afferent nerve synapses is the earliest sign of cochlear damage after exposure to noise. It can occur as the primary event, even without the loss of sensory hair cells, hence the name “cochlear neuropathy”. Currently, there is a paucity of treatment strategies for cochlear neuropathy and the associated hearing loss. Our previous studies have demonstrated that the stimulation of A1 adenosine receptors in the inner ear can mitigate hearing loss caused by exposure to traumatic noise or ototoxic drugs. Here, we investigate the role of adenosine receptors (AR) in the development of noise-induced cochlear neuropathy using genetically modified mice (C57BL/6 background) lacking genes for one of the two main types of AR in the inner ear (A1 and A2A). These mice were exposed to octave band noise (8-16 kHz, 100 dB SPL) for 2 hours to induce cochlear injury and hearing loss. Auditory thresholds and suprathreshold responses were assessed using auditory brainstem responses (ABR) before and two weeks post-exposure. The loss of afferent synapses and spiral ganglion neurons (SGN) were assessed by quantitative histology. Young A1RKO mice (6-8 weeks old) displayed a high frequency hearing loss (ABR threshold shift) prior to noise exposure, similar to hearing loss in ageing C57BL/6 wildtype mice. In addition, suprathreshold responses (ABR wave II amplitudes) were severely reduced in non-exposed A1RKO mice, reflecting poor functioning of auditory nerve fibres. This hearing loss was further aggravated by noise exposure. Noise-exposed A1RKO mice experienced a significantly greater loss of synaptic ribbons and SGN than wildtype mice. In contrast, the genetic deletion of the A2AR resulted in better preservation of afferent synapses and minimal loss of SGN after noise exposure compared to age-matched wildtype controls. Our results suggest that the A1R deficiency results in increased susceptibility to neural injury in the noise-exposed cochlea. In contrast, the A2AR deficiency increases the resistance of cochlear neural tissues to acoustic trauma. Distinct auditory phenotypes of A1RKO and A2ARKO mice suggest that the manipulation of adenosine receptors (A1R stimulation and A2AR inhibition) holds promise in the therapeutic management of cochlear neuropathy.

Celaya-Puertolas Adelaida M. (1,2), Rodríguez-de la Rosa Lourdes (1,2,3), Pulido Sara (1,2), Roma-Mateo Carlos (2,4), Zubeldia Jose Manuel (1,2,5), Pallardo Federico (2,4), Varela-Nieto Isabel (1,2,4)
 1 Institute for Biomedical Research "Alberto Sols" (IIBM), Spanish National Research Council–Autonomous University of Madrid (CSIC-UAM), Madrid, Spain; 2 Centre for Biomedical Network Research (CIBER); 3 Hospital La Paz Institute for Health Research (IdiPAZ), Madrid, Spain; 4 Department of Physiology, School of Medicine and Dentistry, University of Valencia, Valencia, Spain; 5 Gregorio Marañón Hospital, Madrid, Spain

Insulin growth factor type 1 (IGF-1) is a neurotrophic factor fundamental for the regulation of cochlear development, growth and differentiation. Human IGF-1 deficiency is associated with poor growth rates, mental retardation and syndromic hearing loss (OMIM608747). Equally, *Igf1*^{-/-} mice are dwarfs with poor survival rates, congenital profound deafness and age-related retinopathy. Low levels of IGF-1 are also associated with hearing loss and presbycusis in related human genetic syndromes. In this work we study the heterozygous *Igf1*^{+/-} mouse as a genetic model of these hearing disorders.

The auditory phenotype of young *Igf1*^{+/-} mice is similar to that of their wild type littermates. However, during ageing genetic haploinsufficiency adds to the physiological age-related decrease in the levels of circulating IGF-1 that fall below a pathological threshold earlier than the wild type mice. IGF-1 levels show an inverse correlation with auditory thresholds. IGF-1 haploinsufficiency accelerates progressive hearing loss and causes increased susceptibility to environmental stressors like noise. Noise exposure causes an irrecoverable increase of auditory thresholds in heterozygous mice, matched by an exacerbated cellular damage in the cochlea, infiltration of *Iba1*⁺ cells and apoptosis. At the molecular level, the chronic IGF-1 haploinsufficiency causes a pro-inflammatory state in the cochlea with higher expression of *Tgfb1* and *Il1b*. After noise exposure, the cochlear inflammatory response increases, accompanied by the hyperactivation of stress-related kinases (JNK) in heterozygous mice, which is maintained even a month after damage. Along these alterations, IGF-1 haploinsufficient mice show a defective activation of pro-survival (AKT), antioxidant and anti-inflammatory routes.

These data point to IGF1 as a genetic factor contributing to age-related hearing loss.

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Nesrine Benkafadar (1,2), Florence François (1,2), Jean-Luc Puel (1,2), Jing Wang (1,2)

(1) INSERM - UMR 1051, Institut des Neurosciences de Montpellier, 34295 Montpellier, France, (2) Université Montpellier, 34295 Montpellier, France

Background and Introduction: In our aging society, age-related hearing loss or presbycusis is increasingly important. Based on observations of temporal bones from patients with presbycusis, Schuknecht (Schuknecht and Gacek, 1993) proposed the classification into three major forms, namely sensory, neural, and strial presbycusis according to the location of damage (sensory epithelium, spiral ganglion neuron, or stria vascularis). To date, the mechanisms underlying the age-related hearing loss remain unclear. Based on our previous study (Menardo et al., 2013) showing that the premature age-related hearing loss observed in senescence-accelerated mouse prone 8 (SAMP8) mice was correlated with altered levels of anti-oxidant enzymes and decreased activity of mitochondrial functions, we hypothesize that the oxidative stress may play a key role in presbycusis.

Methods: To investigate the contribution of the oxidative stress in presbycusis, we exposed the p3 mouse cochlear explants to hydrogen peroxide (H₂O₂) in vitro. The cochlear cell senescence or degeneration was evaluated using the specific biomarkers. In addition, the role of endogenously-produced ROS in age-related hearing loss was assessed in adult p66KO mice which have a decreased ROS production.

Results: Our results provide the evidence that the oxidative stress plays a key role in age-related hearing loss and cochlear sensory hair cell apoptosis. We demonstrate that H₂O₂ exposure induced a premature occurrence of cochlear sensory hair cell senescence and apoptosis, illustrated by the massive increase of the cell senescence and apoptosis biomarkers such as SA-beta gal, p-H2AX, Annexin V and TUNEL, mainly in the cochlear sensory hair cells, but not in the spiral ganglion neurons. Interestingly, our in vivo results from p66 KO and WT mice provided the functional and morphological evidence that the targeting of oxidative stress by genetic interventions protect the cochleae against age-related sensory hair cell death and hearing loss.

Conclusion: Our results suggest that oxidative stress plays crucial role in age-related cochlear sensory hair cell degeneration and hearing loss. The use of anti-oxidants may be an attractive therapy to slowdown or stop the sensory presbycusis.

Allen F. Ryan, Kwang Pak, Daniel Bodmer, Matthew Ryals, Yves Brand, Arwa Kurabi
Department of Surgery / Otolaryngology, UCSD and VA San Diego Medical Center, La Jolla, CA 92037

Protein kinase B (AKT) signaling activated by phosphatidylinositol 3 kinase (PI3K), mediates diverse cellular processes. It often promotes survival and growth in response to extracellular signals. Several studies have shown that AKT inhibitors enhance damage to cochlear hair cells (HCs) due to a variety of causes. AKT has three isoforms, each encoded by a separate gene. However, relatively little is known about their role in the cochlea. In a prior study, we found that AKT2 and AKT3 play a protective role against aminoglycoside toxicity to HCs, while AKT1 does not. In addition, untreated akt1 gene knockout (KO) and akt2/3 double KO animals displayed progressive hearing loss, indicating a role in maintaining normal hearing.

We explored the role of AKT isoforms in noise-induced hearing loss. Mice 30-60 days old, with homozygous deletions of each of the akt genes or wild-type (WT) controls, were exposed to octave-band (8-16 kHz) noise at 105 dB SPL for 30 minutes, preceded and followed by ABR at 8, 16 and 32 kHz. In WT animals, noise exposure created ~40-60 dB of immediate threshold shift, depending upon frequency, which recovered to a permanent threshold shift (PTS) of 25-45 dB two weeks later. Akt3 null animals displayed enhanced PTS, especially at 8 and 32 kHz. Akt2 KOs were similar to WTs. Akt1 nulls showed severe hearing loss at 32 kHz prior to noise exposure, so noise effects could not be evaluated at this frequency. Pre-exposure thresholds at 8 and 16 kHz were in the normal range. At these frequencies, Akt1 nulls showed less PTS than WTs.

The results indicate that AKT3 plays a protective role in noise-induced hearing loss, while AKT 2 is less involved. Unexpectedly, AKT1 appears to contribute to noise-induced hearing loss. However, this is consistent with our recent observation that an AKT inhibitor protected HCs from aminoglycoside damage, as did an inhibitor of EGFRs which can act upstream of PI3K. Our data illustrate the potential complexity of HC damage signaling.

Manuela Nowotny, Lenneke Kiefer, Bernhard H. Gaese
Goethe University Frankfurt am Main, Institute of Cell Biology and Neuroscience

Introduction

Noise-induced hearing loss is a problem in developed countries and still a major cause of health problems such as hyperacusis and tinnitus. The diversity of tinnitus characteristics, ranging from complex sounds such as ringing and buzzing to single tones, is still poorly understood.

Methods

We addressed the question of potential causes for the observed diversity in tinnitus characteristics through experiments on adult Mongolian gerbils and investigated auditory brainstem responses (ABRs) measured at 2 to 20 kHz before, during and after intense noise stimulation (noise bands of 0.25, 0.5 and 1 oct around 8 kHz; 105 dB SPL). Measurements on the acoustic startle response were used to draw conclusions about the characteristics of the induced tinnitus.

Results

Permanent threshold shift in animals exposed to a narrow noise bandwidth (0.25 oct) were found several weeks after noise trauma, while broader noise bandwidths (0.5 oct, 1 oct) only induced temporary threshold shifts. Regardless of the applied bandwidth, animals from all groups, featured decreased amplitudes of the auditory nerve activity after noise trauma measured by ABRs. Interestingly, the risk for developing tinnitus was highest in the frequency range from 10 to 12 kHz after narrow-band noise, the frequency range around of highest hearing impairment. In the group with the broader noise band (0.5 oct), however we found two peaks for the tinnitus frequencies one at 8-10 kHz and a second at 16-18 kHz.

Conclusion

The frequency bandwidth of the overstimulating noise is a crucial factor for hearing loss and tinnitus induction in the tonotopical arrangement along the auditory pathway. Induced effects suggest even different mechanisms depending on frequency range. Narrow-band noise led to a tonal tinnitus perception related to overcompensation, while exposure to a broadband noise led to a ringing tinnitus perception, which could be explained by lateral input loss.

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Dorit Möhrle (1), Sathish Kumar Singaravelu (1), Kun Ni (1, 2), Dan Bing (1), Ksenya Varakina (1), Ulrike Zimmermann (1), Marlies Knipper (1), Lukas Rüttiger (1)

(1) University of Tübingen, Department of Otolaryngology, Tübingen Hearing Research Centre (THRC), Molecular Physiology of Hearing, Elfriede-Aulhorn-Str. 5, 72076 Tübingen, Germany; (2) Department of Otolaryngology, Head & Neck Surgery, Shanghai Children's Hospital, Shanghai Jiao Tong University, Shanghai, China

Objective:

Hyperacusis and Tinnitus both often occur in conjunction with a loss of hearing sensitivity, but neither hearing threshold loss nor cochlear hair cell loss is essential to develop either condition [1]. In the present study we explore how the reduced sensory function of the cochlea and changes of central activity patterns in the ascending auditory pathway through noise-induced or age-related hearing loss (NIHL or ARHL) is involved in 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 in young, middle-aged and old rats [2]. 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 analyze the hair cell molecular phenotype.

Results:

Behavioral measurements of Hyperacusis and Tinnitus, before and after auditory overstimulation, were correlated to changes in peripheral and central hearing function.

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 auditory overstimulation.

Keywords:

Tinnitus, Hyperacusis, noise-induced hearing loss, age-related hearing loss, central compensation.

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[1] Dauman R., Bouscau-Faure F. (2005) "Assessment and amelioration of hyperacusis in tinnitus patients." *Acta Otolaryngol.* 125(5):503-9.

[2] Rüttiger, L., et al. (2003). «A behavioral paradigm to judge acute sodium salicylate-induced sound experience in rats: a new approach for an animal model on tinnitus.» *Hear. Res.* 180(1-2): 39-50.

Shi Nae Park 1, Min Jung Kim 1, Hong Lim Kim 2, Ji Sun Kong 1, Dong Kee Kim 1, So Young Park 1
(1) Department of Otorhinolaryngology-Head and Neck Surgery, College of Medicine, The Catholic University of Korea, Seoul, Republic of Korea; (2) Integrative research support center, College of Medicine, The Catholic University of Korea, Seoul, Republic of Korea

Objectives: Previous clinical studies demonstrated that hearing impairment is a potential risk factor, and even mild or moderate hearing loss as well as severe loss is associated with cognitive decline or dementia. This study was performed to investigate the effect of hearing loss on cognitive function during a long-term follow-up period and to evaluate neural degeneration and phosphorylated-Tau (p-Tau) expression in the hippocampus, the memory center, of mouse brain to show more direct evidence of the relationship between hearing and cognition. **Methods:** P1mo male C57BL/6 mice were used. Mice of the noise-induced hearing loss (NIHL) group were exposed to 110 dB SPL white noise for 60 min everyday for 20 days. ABR thresholds were serially measured up to 18mo. The cognitive function of the mice was measured by a radial arm maze (RAM) and novel object recognition (NOR) task. P-Tau expression in the hippocampus of the mouse brain was investigated by western blot assay, immunohistochemical staining, immune-transmission electron microscopic (TEM) study. Lipofuscin expression in the hippocampus was also evaluated by TEM. **Results:** During the 18mo follow-up period, hearing levels of the mice with noise-induced hearing loss remained, while those of the control mice slowly increased and the gap between two groups became closer from post-noise 12month. Most of the RAM performances improved gradually within each group across five trials, probably due to learning effect, but NIHL group showed lower performance scored than the control group at several different time points of the study period. NOR task also revealed the decreased function of cognition in NIHL group ($p < 0.05$). Enhanced p-Tau expression measured by western blot assay and immunohistochemical staining was observed in the hippocampus of the mice with NIHL compared to those of the control mice ($p < 0.05$). Intracellular lipofuscin was overexpressed in the hippocampus in mice with NIHL.

Conclusion: Moderate hearing loss induced by noise in mice resulted in cognitive impairment and enhanced expression of p-Tau as well as neural degeneration in the hippocampus of the mice, which provide the more direct causal relationship between hearing loss and cognitive dysfunction.

Dyan Ramekers (1,2), Patrick J. Buitenhuis (1), Sjaak F.L. Klis (1,2), Huib Versnel (1,2)

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

The condition of the auditory nerve, which is typically subject to degeneration after deafness, partly determines the hearing performance of cochlear implant (CI) patients. Electrically evoked compound action potentials (eCAPs) may be applied to assess the condition of the nerve. The eCAP depends on the number of surviving spiral ganglion cells. However, the contribution of peripheral processes (PPs) to the eCAP is debated, among others in relation to the N2 peak of the eCAP (Ramekers et al. 2015, *Hear Res* 321:12-24). We examine the role of the PPs in eCAPs recorded in deafened guinea pigs with a wide range of PP survival but constant SGC survival.

Female albino guinea pigs (n=12) were deafened with a co-administration of kanamycin (400 mg/kg s.c.) and furosemide (100 mg/kg i.v.). Three animals were sacrificed for histology two weeks after deafening. Nine animals were treated with brain-derived neurotrophic factor (BDNF) for a period of four weeks, starting two weeks after deafening (Ramekers et al. 2015, *J. Neurosci.* 35:12331-45). The period after cessation of treatment was varied between 0 and 8 weeks resulting in a wide range of PP packing densities between 35% and 100% of normal and a stable SGC packing density (about 80% of normal). eCAPs were recorded in terminal experiments.

Common eCAP variables as the latency and amplitude of the N1 and N2 peaks did not correlate with the PP packing density, nor did the N2/N1 ratio correlate with PP/SGC ratio. Visual inspection of the global eCAP waveforms did not reveal a trend with PP presence.

The role of PPs is very limited in their contributions to the eCAP. In electrical stimulation of the auditory nerve, the site of excitation seems to be mainly located near the cell body.

Leila Abbas (1), Daniela I. Cacciabue-Rivolta (1), Daniel Smyth (2) and Marcelo N. Rivolta (1)

(1) Centre for Stem Cell Biology, Department of Biomedical Science, University of Sheffield, Western Bank, Sheffield, United Kingdom S10 2TN; (2) Cochlear Technology Centre, Gebouw I, Schaliënhoefedreef 20, 2800 Mechelen, Belgium

Introduction: In cochlear implantation, the response of the host tissue can be a key determinant of the ultimate functioning of the device. If a large, fibrotic response is elicited, then this may impede signal transmission and lead to poor patient outcomes.

Methods: A gerbil model with a fully-implantable rodent stimulator (1) is used, in which the electrode is activated by a magnetic field. Using a three-dimensional field with waking animals allows us to establish a functional threshold for each animal – they show a characteristic behavioural response to cochlear stimulation, with increased watchfulness and the impression of ‘listening’. This is particularly evident in pre-deafened animals – an ototoxic lesion is induced prior to implantation by dosing with kanamycin and furosemide to efficiently ablate the cochlear hair cells, whilst sparing the vestibular systems. eABR measurements are also taken, whilst under anaesthesia.

Results: Cochlear implants have a similar functional range in both naïve and ototoxic-lesioned gerbils, reflected in both the waking and sedated measurements. Post mortem histology reveals that kanamycin/furosemide treatment does not seem to enhance the level of fibrosis or osteoneogenesis present in the cochlea of implanted animals – the scala tympani generally remains free from large amounts of extraneous tissue or inflammatory infiltrate. An interesting observation however is the appearance of β III-tubulin positive cells within the type II and type IV fibrocyte population of the cochlear lateral wall in pre-lesioned animals. These cells are reminiscent of dendritic cells in their morphology and may represent a community of lateral wall dwelling ‘cochlear immune system’ cells, based on their expression of other, cell-surface antigens.

Conclusion: Pre-lesioning the cochlear with kanamycin and furosemide may lead to the activation of immune surveillance within the lateral wall. However this increased reactivity does not appear to impinge on the success of subsequent cochlear implantation in terms of acute rejection of the implant. This is a key step forward into the next phase of this project, where alongside an implant, otic neural progenitors are being grafted into an ototoxic/neuropathy ‘double ablation’ model – it will be critical for these cells’ survival that they are transplanted into a sympathetic milieu: early indicators suggest that this is in fact the case. (1) Millard R. E. and Shepherd R. K., J. Neurosci Methods (2007) 166(2): 168-77.

Ariane Roemer, Mika Sato, Thomas Lenarz, Andrej Kral, Athanasia Warnecke
Hannover Medical School, Department Of Otolaryngology, Hannover, Germany

Why do we need stem cells in cochlear implant surgery? One of the main challenges is poor performance in ambient noise. Immunological processes upon cochlea implantation lead to a fibrotic and osteogenic alteration of the cochlea. To sufficiently activate the neurons, a larger electrical stimulation is needed and a higher threshold is recorded. This in turn leads to higher energy consumption - with more frequent battery changes – and to higher electricity spreading. This spreading leads to an imprecisely electrode-nerve fiber-interaction and thereby to vague speech perception. Due to degeneration of the peripheral process of the auditory nerve, the implant performance is additionally impaired. Amniotic stem cells are known to prevent fibrosis and to exert neuroprotection by the continuous delivery of growth factors. We therefore hypothesize that amniotic membrane will induce a dendrite growth towards the electrode as well as a reduced scarring during cochlear implantation.

Treating spiral ganglion neurons isolated from neonatal rats with amniotic membrane as well as supernatant derived from culture of amniotic membrane after 48 hours resulted in a significant increase of neuronal survival. This increased survival was highly significant when compared to cultures treated with BDNF. Based on these in vitro finding, animal studies in guinea pigs were performed. After electrode insertion via a cochleostomy, amniotic membrane was used for the coverage of the cochleostomy. In the control group, the cochleostomy was left untreated. All animals were implanted unilaterally and the contralateral side was left untouched and served as control. Auditory brainstem responses as well as radiological imaging were performed after surgery and during recovery time. The termination of the experiment was initiated after 3 weeks after operation. The cochleae treated with amniotic membrane showed an improved healing of the cochleaostomy without any adverse effects as confirmed by radiologic or audiologic evaluation.

In summary, amniotic membrane is a promising tool for future biological cochlear electrode implantation. It shows neuroprotective effects as well as advantages in wound healing.

Nils Prenzler, Rolf Salcher, Melanie Leifholz, Lutz Gärtner, Athanasia Warnecke, Thomas Lenarz
Hannover Medical School, Department Of Otolaryngology, Hannover, Germany

Introduction:

Systemic and local administered steroids can reduce insertion trauma and growth of fibrous tissue after cochlear implantation and thus increase the chance for hearing preservation and lower impedances. Several ways of administration have been described, e.g., systemic administration, diffusion via middle ear or drug releasing surface coatings. The motivation for the development of a cochlear catheter was to apply the drug to more apical regions without structural harm of the cochlear macro- and microstructure.

Methods:

The cochlear catheter (developed by Med-EL, Innsbruck) consists of a 20 mm long electrode silicone carrier with a hollow lumen and an opening at the tip for the delivery of fluids into apical regions of the cochlea. Five patients without relevant residual hearing (> 80dB hearing loss at 250Hz) received a cochlear flushing with diluted triamcinolone (4mg/ml) via the cochlear catheter before cochlear implantation with a Med-El Flex 28 electrode. Impedances and the slope of the electrically evoked compound action potential (ECAP) amplitude growth function (AGF) were measured directly after implantation in the operating theatre. Follow-up measurements were performed on days 3, 10, 17 and 24 and at first fitting. Results were compared to recipients of the same electrode type who did not receive any steroids.

Results:

Impedances were stable at a lower level until day 10 post-OP in patients who received a treatment with the cochlear catheter. However, the impedances rose between day 10 and day 24 post-OP. Additionally, first results of the ECAP AGF slopes will be presented.

Conclusions:

Flushing of the cochlea with the cochlear catheter before implant insertion seems to sufficiently deliver therapeutic concentrations of steroids as demonstrated by the temporary decrease of impedances. More clinical data are necessary in order to judge the impact of steroid application via the cochlear catheter on the spiral ganglion neurons as determined by the eCAP AGF.

Renato Torres (1,2,3), Guillaume Kazmitcheff (1,3,4), Daniele De Seta (1,2,3), Evelyn Ferrary (1,2,3), Olivier Sterkers (1,2,3), Yann Nguyen (1,2,3)

1. UMR-S 1159 "Minimally Invasive Robot-based Hearing Rehabilitation", Inserm, Paris 6 UPMC, Paris France; 2. Sorbonne Universities, UPMC Univ. Paris 6, Paris, France; 3. Otolaryngology Department, Unit of Otolology, Auditory Implants and Skull Base Surgery, AP-HP, Pitié-Salpêtrière Hospital, Paris, France; 4. Shacra Inria, Lille Nord-Europe, Université Lille-1, Lille, France

Introduction: Optimize the electrode array insertion will diminish the intracochlear trauma. During the insertion, the intracochlear structures are not visible from the cochlear opening, thus, the mental representation of the insertion axis is variable (around 15°) (Torres et al. 2015). We assess the insertion axis significance according to the intracochlear trauma during an automated robot-based system of alignment to the insertion axis.

Material and methods: Four fiducial marks were placed on the cortical bone and a cone beam CT were performed on eight temporal bones. Cochlear dimensions (length, width, height, 1st-2nd cochlear turn angle) were measured by OsiriX (Pixmeo, Geneva, Swiss). There were defined the position of the entry point to the cochlea and two insertion axis: an optimal insertion axis, and an inaccurate insertion axis (optimal+15°). A robot prototype (RobOtol) guided by a electromagnetic tracking system FasTrak (Polhemus, Vermont, USA) has been programmed to align a motorized insertion tool according to the planned insertion axis. The Digisonic SP EVO electrode array (Neurelec/Oticon Medical, Vallauris, France) has been inserted at 0.25 mm/s. The insertion forces were registered by the 6-axis sensor forces (Ati-Nano17, Apex, USA). The maximal force was analysed on the risk regions (a=140-210° et b=270-340°). A cone beam CT and the histology studies were performed to assess the position of each electrode.

Results: The translocation ratio was higher in an inaccurate insertion axis than an optimal insertion axis (75% vs. 25%). Four translocation were observed (144, 160, 196, 285°). A translocation was significantly associated to higher insertion forces than no translocation (zone a: 3136.3 vs. 2627.2 mN; p<0.05 and zone b: 12197.6 et 5140.6 mN; p<0.05, respectively)

Conclusion: The translocation ratio was higher in case of inaccurate axis insertion than the optimal axis insertion. At the first time, we show a real-time detection of the translocation. This work opens perspectives to predict and avoid this complication during cochlear implantation.

Alexander Rettenmaier, Peter Baumhoff, Thomas Lenarz, Andrej Kral, Günter Reuter
Department of Otolaryngology, Hannover Medical School, Hannover, Germany

It has been shown that the inner ear can be stimulated in vivo by laser pulses resulting in cochlear potentials corresponding to auditory evoked signals. Recent studies have suggested that the optical stimulation of the cochlea is based on the optoacoustic effect - the generation of a laser-induced pressure wave in the cochlea. In order to further investigate the stimulation mechanism we combined in vitro measurements of isolated spiral ganglion neurons with in vivo recordings of compound action potentials (CAP) during laser stimulation.

Spiral ganglion neurons, isolated from the cochleae of Sprague Dawley rats, were stimulated with laser pulses of 1860 nm wavelength. Their electrophysiological reactions to different laser parameters such as pulse duration and optical peak power were detected by means of the whole cell patch clamp technique. Pulse durations from 10 μ s up to 20 ms and optical peak powers from 50 mW up to 500 mW were investigated. In comparison, CAPs were recorded from Dunkin-Hartley guinea pigs during intra-cochlear laser stimulation with the same laser parameters. Additionally, the corresponding temperature change during laser stimulation was analyzed.

The irradiated cells show inward currents at resting potential, depending linearly on the peak power of the laser light. These reactions are clearly elicited by the laser beam and can be observed in voltage clamp measurements as current changes but are not big enough to elicit action potentials. Analysis of the temperature change during irradiation shows the dependency of the cellular reaction on temperature and its first time derivative. The laser-induced amplitude of the CAP signal also depends on the pulse peak power. However, the CAP is triggered by the second time derivative of the temperature which corresponds to the laser-induced pressure wave.

The results show that the stimulating effect of laser irradiation on the cochlea and on single cells depends on different mechanisms. While intra-cochlear stimulation relies on optoacoustic pressure generation, the cellular current responses depend on the laser-induced temperature change and its first time derivative.

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Daniel Keppeler¹, Christian Wrobel^{1,2}, Marcus Jeschke¹, Gerhard Hoch¹, Christian Goßler³, Mareike Töpperwien⁴, Patrick Ruther³, Michael Schwaerzle³, Roland Hessler⁵, Tobias Moser^{1,2}
1Institute for Auditory Neuroscience, University of Göttingen Medical Center, Göttingen, Germany; 2Collaborative Research Center 889, University of Göttingen Medical Center, Göttingen, Germany; 3Department of Microsystems Engineering (IMTEK), University of Freiburg, Freiburg, Germany; 4Department of Physics, University of Göttingen, Göttingen, Germany; 5MED-EL, Innsbruck, Austria and MED-EL Germany, Starnberg, Germany

Cochlear implant coding suffers from low frequency resolution due to wide current spread from stimulation contacts, which limits the number of independently usable channels and compromises speech comprehension in noise, music appreciation or prosody understanding of CI-users. Our goal is to overcome these drawbacks by pursuing an optogenetic approach: Optical stimulation can be spatially confined and thus promises lower spread of excitation in the cochlea. Accordingly, an increased number of independent stimulation channels is expected to enhance frequency resolution and intensity coding.

Recently we demonstrated the feasibility of optogenetic stimulation of the auditory pathway in rodents with neuronal expression of Channelrhodopsin-2 (ChR2) a blue light-gated ion channel (Hernandez et al. 2014). Immunohistological data showed expression of ChR2 in the somas and neurites of spiral ganglion neurons. Electrophysiological measurements including compound action potentials revealed specific activation along the auditory pathway upon blue light stimulation with a laser-coupled fiber in the cochlea.

In collaboration with semiconductor experts, we developed multichannel optical implants which accommodate 10 μ LEDs on a flexible substrate. These silicone encapsulated devices have been successfully implanted in rodent cochleae assessed in 3D models derived from x-ray tomography. Furthermore we could activate the auditory pathway with individual LEDs demonstrated by auditory brainstem responses.

Taken together, our experiments demonstrate the feasibility of optogenetic cochlea stimulation to activate the auditory pathway and lay the groundwork for future applications in auditory research and prosthetics.

Flook M1, Lopes S1, Alves L1, Aparício M3, Martins JH4, Silva E5, Murta A5, Andrade S6, Andrade C7, O'Neill A 8,9,10, Matos T1, Antunes M11, Escada P8,9,10 Alcântara P7; O'Neill J8,9,10, Villa-verde Cabral M 7, Fialho G 1, Caria H1,2

1Deafness Research group, BTR Unit, BioISI, Faculty of Sciences, University of Lisbon (FCUL), Portugal; 2ESS/IPS-School of Health, Polytechnic Institute of Setubal, Portugal; 3FCUL, Lisbon, Portugal; 4ENT Service, CHUC, Coimbra, Portugal, 5Widex, Lisbon, Portugal; 6ENT Service, Hospital José Luciano de Castro, Leiria, Portugal; 7ICS-IE-UL, Lisbon, Portugal; 8ENT Service, CHLO-HEM, Lisbon, Portugal; 9FCM/UNL, Lisbon, Portugal; 10CEFI-TEC/FCT/UNL- Lisbon, Portugal; 11 CEaul-FCUL Lisbon, Portugal

Age-related hearing loss (ARHL) is the most common sensory impairment in the elderly, affecting approximately 60% of all individuals over 65 years in the world. Due to the physiologic complexity of ARHL, its mechanisms are not fully understood as well as the relation between ARHL and health status.

The existence of genetic susceptibility predisposing for ARHL implies that this is not an inevitable condition, but instead a complex disease with possible treatment and prevention, what is particularly relevant as regards active aging. The genetic susceptibility for ARHL has been described through the association with different genes, some involved in hereditary deafness or oxidative metabolism, or with specific mitochondrial haplogroups.

It is reported that about 30% of the individuals with ARHL also have tinnitus, this leading to the increase of elderly frailty. Several studies referred the possibility of common metabolic pathways between ARHL and tinnitus.

In the present study, epidemiological and psychological data are presented for about 500 elderly individuals (over 65 years old) from the Portuguese population. Concerning genetic susceptibility, results from the study of genetic variants concerning NAT2 and GRM7 genes are presented. We also report a high variety of mtDNA haplogroups. Gender comparisons are discussed considering hearing loss and audiological patterns. Elderly individuals aged 70-80 or more have significantly an increased probability of having ARHL. Regarding emotional and social difficulties, the worst listeners present more difficulties and more depressive symptoms, being this more common in women. Of the total of the individuals, about 39% reported tinnitus, having high heterogeneity of epidemiologic factors.

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G. Giroto^{1,2}, A. Morgan¹, D. Vozzi², E. Rubinato¹, M. K. AlKowari², M. Di Stazio¹, R. Badii³, S. Bhaghat³, K. Abdulhadi³, M. La Bianca⁴, P. Gasparini^{1,2}

1University of Trieste, Trieste, Italy; 2Sidra medical research, Doha, Qatar; 3Hamad Medical Corporation, Doha, Qatar; 4IRCCS Burlo Garofolo, Trieste, Italy

Introduction

Hearing Loss (HL) is the most common sensorineural disorder characterised by clinical and genetic heterogeneity making much more complicated a molecular diagnosis. In the inherited forms GJB2, GJB6 and MTRNR1 mitochondrial genes play a major role worldwide although with huge differences in prevalence among populations. For more than 60% of cases the molecular diagnosis is not defined thus, there is a strong need to further explore the landscape of causative mutations/genes.

Methods

Thirty Italian and 25 Qatari HHL-families were screened for 113 HHL-genes by Targeted Re-Sequencing (TRS) followed, in negative cases, by Whole Exome Sequencing (WES) and by in vitro and in vivo validation of new HHL-candidate genes.

Ion Torrent PGM™ (4.356 amplicons ensuring approximately 92,6% coverage of the target region) and Ion Proton™ (293.903 amplicons ensuring approximately >99% coverage of the target region) were used for TRS and WES, respectively. Genomic variants were annotated by ANNOVAR and filtered according to: a) pedigree pattern of inheritance, b) allele frequency data, c) pathogenicity prediction. Functional studies were carried out in Zebrafish (expression and generation of K/O-K/I).

Results

TRS allowed us to characterize 12/30 Italian and 13/25 Qatari families (overall detection rate of 45%).

As regards to WES, two new genes, BDP1 and PSIP1 (Giroto et al. 2013 and Giroto et al. 2015), and 5 strong candidates, PIEZO1, LAMC1 (in 2 Qatari families with autosomal recessive inheritance), PLS1, SPATC1L (in 2 Italian families with autosomal dominant inheritance) and TBL1Y (in 1 Italian family with a Y-linked inheritance) were identified. Functional validations are now in progress. Preliminary data include: 1) expression studies of PLS1, PIEZO1 and LAMC1 in Zebrafish larvae revealing a strong gene-expression in the hair cells of the otic vesicle; 2) RT-PCR on TBL1Y showing cochlear expression in the Human cDNA and transfection of HEK cells displayed that the mutation alters the stability of the protein itself. Other candidates are now under final investigation.

Conclusions

These results clearly illustrate the relevance of a combined approach based on NGS technologies plus animal model validation in understanding the complexity of the genetics contribution of HHL. Updated data will be presented and discussed.

Anna Morgan¹, Diego Vozzi², Dragana Vuckovic², Martina La Bianca³, Angela D'Eustacchio³, Maria Pina Concas⁴, Mario Pirastu⁴, Paolo Gasparini^{1,2}, Giorgia Girotto^{1,2}

¹University of Trieste, Department of Medical Sciences, Trieste (Italy); ²Sidra Medical and Research Center, Doha (Qatar); ³IRCCS Burlo Garofolo, Trieste (Italy); ⁴IGM-CNR, Sassari (Italy)

Introduction

ARHL is the main sensory impairment of the elderly. It results from age-related degeneration of the cochlea due to genetic and environmental components. Despite the large number of people affected, little is known about ARHL genetic risk factors. To overcome this lack of knowledge we developed a multi-step approach based on GWAS on thousands of cases, followed by TRS of top GWAS hints to detected causative variants, and by functional in vitro and in vivo studies of these variants.

Methods

After GWAS (Girotto et al.2014, Wolber et al.2014, Vuckovic et al.2015) a TRS panel of 46 genes was developed and applied to sequence ~500 ARHL Italian patients coming from both inbred and outbred populations. TRS was performed using Ion Torrent PGM (LifeTechnologies): 1942 amplicons ensuring 96,55% coverage of the target region were analysed. Data were filtered according to quality value, pathogenicity prediction (Polyphen2, MutationTaster, SIFT, LRT) and conservation score (PhyloP). Variants were classified as ultra rare (MAF <0.001), rare (MAF <0.01) and common (MAF >0.01).

All mutations were confirmed by Sanger sequencing and tested in matched controls.

Results

Fifty-six mutations of interest were identified: 5 frameshift indels, 2 nonsense and 49 missense mutations, affecting 22 genes and 128 patients. According to the complex genetic structure of ARHL, 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.

To validate the pathogenicity of these genes/mutations, functional in vitro/in vivo studies are now in progress.

Data analysis revealed some interesting results. E.g. a homozygous missense mutation and a heterozygous missense mutation in SLC44A2 and SLC9A3R1 were identified. Both these variants are predicted to be pathogenic and are not present in matched controls. In situ hybridization experiments showed that both genes are expressed in Zebrafish inner ear and the generation of K/I model, followed by an accurate phenotypic characterization, will eventually prove their pathogenic role.

Conclusions

These findings demonstrate the usefulness of our combined approach (GWAS + TRS + functional studies) further supporting the potential role of these genes in causing ARHL. Updated data will be presented and discussed.

Shumpei P. Yasuda (1), Yo Obara (1, 2), Sari Suzuki (1), Kenta Wada (1, 3), Yasumasa Nishito (4), Toyoyuki Takada (5), Toshihiko Shiroishi (5), Yoshiaki Kikkawa (1)

(1) Mammalian Genetics Project, Tokyo Metropolitan Institute of Medical Science, Setagaya-ku, Tokyo 156-8506, Japan, (2) Graduate School of Life and Environmental Sciences, University of Tsukuba, Tsukuba, Ibaraki 305-8572, Japan, (3) Department of Bioproduction, Tokyo University of Agriculture, Abashiri, Hokkaido 099-2493, Japan, (4) Core Technology and Research Center, Tokyo Metropolitan Institute of Medical Science, Setagaya-ku, Tokyo 156-8506, Japan, (5) Mammalian Genetics Laboratory, National Institute of Genetics, Mishima, Shizuoka 411-8540, Japan

Age-related hearing loss (AHL) is a common disorder in older people. The prevalence of AHL increases with age and occurs in more than 50% of the older population (>70 years). However, the identification of genes associated with AHL is difficult in humans because the condition manifests through the interplay of the effects of environmental risk factors and several genetic modifiers. In this study, we identified a locus associated with AHL through genetic and phenotypic analyses of mouse consomic strain B6J-Chr12CMSM, which is an inbred strain with approximately half the centromere of chromosome 12 (Chr12) replaced with the homologous chromosome from the AHL-resistant MSM/Ms (MSM) in the AHL-susceptibility C57BL/6J (B6J) genetic background. B6J mice exhibit early-onset, age-related, high-frequency hearing loss (HFHL) because of the effect of homozygous *ahl* allele of the cadherin 23 gene (*Cdh23*) on Chr10. We demonstrated that B6J-Chr12CMSM mice showed resistance to HFHL despite having the *Cdh23^{ahl}* allele. Auditory Brain-stem Response thresholds for a 32 kHz stimulus significantly differed between B6J and B6J-Chr12CMSM/MSM homozygous mice, until at least 9 months of age, suggesting that a modifier(s) is associated with HFHL-resistance on Chr12. To identify this HFHL-resistant modifier, we performed a quantitative trait locus (QTL) linkage analysis on B6J-Chr12CMSM congenic mice, produced by consomic strain crossing with B6J mice. We mapped, to a QTL locus, an approximately 6 Mb region of Chr12 with highly significant LOD scores. There are currently no known genes and loci responsible for hearing loss in this genomic interval; therefore, we designated this as the *hfh14* locus, which is the fourth locus for HFHL in mice. Fourteen genes located on this locus bear non-synonymous substitutions. Moreover, microarray analysis comparing expression of MSM and B6J revealed three genes that showed ≥ 2 -fold change on this locus. These genes also showed ≥ 2 -fold change in B6J-Chr12CMSM mice compared to B6J. These data suggest that these non-synonymous substitutions and differentially expressed genes could be *hfh14* candidates.

Teresa Requena¹, Carmen Martin-Sierra Carmen¹, Lidia Frejo¹, Steve D. Price⁵, Angel Batuecas², Sofia Santos-Perez³, Andres Soto-Varela³, Anna Lysakowski^{5,6}, Jose A. Lopez-Escamez^{1,4}
¹Otology & Neurology Group CTS495, Department of Genomic Medicine, GENYO - Centre for Genomics and Oncological Research – Pfizer/University of Granada/ Junta de Andalucía, PTS, Granada, 18016 Spain;
²Department of Otolaryngology, Hospital Universitario Salamanca, Spain; ³Division of Otoneurology, Department of Otorhinolaryngology, Complejo Hospitalario Universitario, Santiago de Compostela, Spain; ⁴Department of Otolaryngology, Granada University Hospital, Granada, 18016, Spain; ⁵Dept. of Anatomy and Cell Biology, Univ. of Illinois at Chicago, Chicago, IL, 60612, USA; ⁶Dept. of Otolaryngology-Head and Neck Surgery, Univ. of Illinois at Chicago, Chicago IL

Introduction

Familial Meniere's disease (FMD) is a rare autosomal dominant (AD) disorder characterized by recurrent attacks of spontaneous vertigo, sensorineural hearing loss (SNHL), and tinnitus with incomplete penetrance. So far MD is associated with an accumulation of endolymph in the membranous labyrinth but the molecular mechanism is still unknown. The SNHL usually starts at low and medium frequencies with a variable progression to high frequencies.

Methods

We have performed whole-exome sequencing in an AD-MD family consisting of 2 patients with MD and a third one with SNHL without vestibular symptoms. DNA samples were sequenced in a SOLiD 5500xl platform. After alignment and annotation, variants were filter by our in-house control database and minor allelic frequency (MAF) <0.0001 according to dbSNP 142, Exome Aggregation Consortium (ExAC) and 1000 genome project. To reduce the list of possible candidate variants, we prioritized using different criteria. qPCR and immuno-labelling were performed to confirm the expression in human and rat tissue as well as to define which cell types express the candidate gene in the cochlea.

Results

We identified a novel missense variant in the PRKCB gene segregating low-to-middle frequency SNHL. Confocal imaging showed strong PKC II protein labelling in non-sensory cells, the tecta cells (TCs) and inner border cells (IBCs) of the rat organ of Corti with an expression gradient along the cochlea. PKC II signal was more pronounced in apical turn TCs of the cochlea compared with the middle and basal turns. In rat tissue we found that PKC II protein labelling was much higher in cochlear than in vestibular tissue.

Conclusions

PRKCB is a novel candidate gene for SNHL in FMD and the gradient of expression in TC along the cochlea may explain the onset of hearing loss as well as the progression from low to high frequencies SNHL in MD. In addition, the role of TCs in K⁺ recycling within the endolymph could explain endolymphatic hydrops development.

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Ichiro Fukunaga (1), Takashi Iizuka (1), Kaori Hatakeyama (1), Ayumi Fujimoto (1), Osamu Minowa (2), Katsuhisa Ikeda (1), Kazusaku Kamiya (1)

(1) Department of Otorhinolaryngology, Juntendo University Faculty of Medicine, Tokyo, Japan; (2) BioResource Center, RIKEN, Tsukuba, Japan

Background: Hereditary deafness affects about 1 in 2000 children and GJB2 gene mutation is most frequent cause for this disease. GJB2 encodes connexin (Cx) 26, a component in cochlear gap junction. We recently demonstrated that the drastic disruption of gap junction plaque (GJP) macromolecular complex composed of Cx26 and Cx30 are critical pathogenesis starting before hearing onset (Kamiya et al., 2014, J Clin Invest 124, 1598-1607). To develop the effective therapy for GJB2 associated hearing loss, restoration of gap junction plaque (GJP) macromolecular complex using virus vectors or multipotent stem cells such as induced pluripotent stem (iPS) cells and mesenchymal stem cell (MSC) are expected to rescue the hearing function of GJB2 related hearing loss.

Methods: Mouse induced pluripotent stem cells (iPS) were used for generation of Cx26-expressing cells with proper gap junction plaque between the cells. Adeno associate virus (AAV) were used for the GJB2 gene transfer and restoration of GJP. As animal model of GJB2 related hearing loss, we used 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, Cx26^{fl}/P0Cre as a model for insufficiency of gap junction protein.

Results and Conclusion:

By differentiation of iPS cells, we generated the Cx26-expressing cells with large gap junction plaque as cochlear cells. These cells should be useful for establishing inner-ear cell therapies that target GJB2-related hereditary deafness. In gene therapy, we demonstrated the restoration of GJP in cochlear tissue of Cx26^{fl}/P0Cre mice by Gjb2-AAV gene transfer. Furthermore, in vivo cochlear delivery of Gjb2 using AAV significantly improved the auditory responses and development of the cochlear structure of Cx26^{fl}/P0Cre mice (Iizuka, Hum Mol Genet, 2015, 24(13):3651-61). Using cell therapy or gene therapy to restore hearing in the mouse models of Gjb2-related deafness may lead to the development of therapies for human hereditary deafness.

Yoshiaki Kikkawa (1), Yuki Miyasaka (1), Hiroshi Shitara (1), Sari Suzuki (1), Toshihiko Shiroishi (2), Ryo Kominami (3), Hiromichi Yonekawa (1)

(1) Tokyo Metropolitan Institute of Medical Science-Tokyo-Japan, (2) National Institute of Genetics-Mishima-Japan, (3) Niigata University School of Medicine-Niigata-Japan

Usher syndrome (USH) is a condition that affects both hearing and vision and is characterized by a group of recessively inherited symptoms; however, some heterozygotes develop nonsyndromic, age-related hearing loss. Jackson shaker (*Ush1g-js*) is a mouse model of USH that exhibits abnormal behavior and congenital deafness caused by the homozygous mutation of *Ush1g/Sans* gene. We found that C57BL/6J-*Ush1g-js/+* heterozygous mice exhibit early-onset progressive hearing loss (ePHL) that accompanies the progressive degeneration of stereocilia in the cochlear outer hair cells. Interestingly, ePHL did not develop in mutant mice with the C3H/HeN background, suggesting that other genetic factors are required for ePHL development. Therefore, we performed classical genetic analyses and found that the occurrence of ePHL in *Ush1g-js/+* mice is associated with an interval in chromosome 10 that harbors the cadherin 23 gene (*Cdh23*), which is also responsible for USH in humans. To confirm this mutation effect, we generated C57BL/6J-*Ush1g-js/+*, *Cdh23-c.753A/G* double heterozygous mice using the CRISPR/Cas9-mediated *Cdh23-c.753A>G* knock-in method. The *Cdh23-c.753A/G* mice harbor a one-base substitution (A for G), and the homozygous A allele causes moderate hearing loss with aging. Analyses revealed complete recovery of ePHL and stereocilia degeneration in C57BL/6J-*Ush1g-js/+* mice. These results clearly show that the development of ePHL requires at least two mutant alleles of the *Ush1g* and *Cdh23*. Our results also suggest that because the USH proteins form a complex at the stereocilia, their interaction with each other may play a key role in the maintenance of stereocilia and in the prevention of ePHL.

Elisa Tavazzani (1), Paolo Spaiardi (1), Valeria Zampini (1), Donatella Contini (1), Marco Manca (1), Giancarlo Russo (1), Ivo Prigioni (1), Walter Marcotti (2), Sergio Masetto (1)

(1) Department of Brain and Behavior, University of Pavia, Pavia, Italy; (2) Department of Biomedical Science, University of Sheffield, Sheffield, UK

Deletion of Epidermal growth factor receptor pathway substrate 8 (Eps8), a gene involved in actin remodeling, causes deafness in mice. Cochlear inner hair cells from Eps8-knockout (KO) mice have abnormally short stereocilia and fail to acquire their mature array of basolateral voltage-gated K⁺ channels. Intriguingly, Eps8-KO mice show no vestibular deficits despite the fact that Eps8 is also expressed in vestibular hair cells. Since vestibular deficits are sometimes centrally compensated, we have investigated if vestibular Type I and Type II hair cells in Eps8-KO mice were also affected by Eps8 deletion. We found that, like cochlear hair cells, Eps8-KO vestibular hair cells have significantly shorter than normal stereocilia. However, KO Type I and Type II hair cells expressed a normal pattern of basolateral voltage-dependent ion channels. Consistent with this finding, the voltage response of KO vestibular hair cells to injected sinusoidal currents, which were used to mimic the mechanoelectrical transducer current, was analogous to that found in WT hair cells. We conclude that the absence of Eps8 has a weaker effect in vestibular hair cells compared to cochlear hair cells, since it affects the stereocilia length but not the voltage-dependent properties. This difference, together with the possible compensation for the shorter stereocilia by the cupula, may explain the absence of obvious vestibular deficits in Eps8-KO mice.



Einat Shapira¹, Rémy Pujol², Michael Plaksin¹, Eitan Kimmel¹

¹Russell Berrie Nanotechnology Institute and Technion-Israel Institute of Technology, Haifa 32000, Israel; ²INSERM (UMR 1051) - INM and Université de Montpellier, France

Here, we introduce a new model for the transduction of low SPLs into motion, in which the lateral wall (LW) of OHCs is the initial sensing site. The essential role of OHC's LW to mammalian audition is indicated by its unusual morphology and in OHCs' development during onset of hearing. We describe this unique structure as being composed of many nanometric acoustic motile sensors (NAMSS). Each sensor utilizes noise to detect low SPLs and responds by generating pulsating forces, which, when integrated into a synchronized mode of action, induce OHC motility. The newly described framework can function at relatively high frequencies, a characteristic of mammalian hearing. It provides hypersensitivity at low SPLs and compressive nonlinearity at high SPLs, ergo facilitating inner ear's wide dynamic range. Notably, we show that a sound pressure signal, masked by thermal noise several times higher in magnitude, can be amplified, filtered, and mechanically transduced to the cochlea. Potentially, the suggested system might inspire development of highly sensitive sensors, in particular to be incorporated in hearing aids.

Emilie Brun, Marc Lenoir, Anne-Laure Bonnefont, Yulia Korchagina, Jing Wang, Jérôme Bourrien, Jean-Luc Puel, Benjamin Delprat
Inserm U1051 - Institute for Neurosciences of Montpellier - University of Montpellier, France

Introduction: WFS1 encodes Wolframin, an endoplasmic reticulum (ER) transmembrane glycoprotein involved in calcium homeostasis. In addition, a deficiency in WFS1 induces disturbances in the adaptive ER stress response. Mutations in WFS1 cause the Wolfram syndrome (WS), an autosomal recessive disorder also known as DIDMOAD (Diabetes Insipidus, Diabetes Mellitus, Optic Atrophy and Deafness). To understand the role of WFS1 in inner ear physiology, the present study was designed to analyze inner ear physiology and morphology of a novel mice model presenting a DNA substitution in exon 8 inducing a missense mutation (864 E to K) in Wolframin.

Methods: To evaluate the hearing threshold at different frequencies in Wfs1E864K mutant mice, we measured their auditory brainstem responses (ABRs) at different ages. To assess the functionality of the outer hair cells (OHCs) we performed a measurement of distortion product otoacoustic emissions (DPOAEs). Vestibular functions have been assessed by different behavioral test. Morphological evaluation of inner ear has been realized by scanning and transmission electron microscopy.

Results: Observation of Wfs1E864K mutant mice showed a severe phenotype with a profound deafness at postnatal days 29 (P29). This deafness is associated to vestibular dysfunctions. The inner ear defects are due to a rapid hair cell loss in the organ of Corti and in vestibular epithelia between 23 and 31 postnatal days (P23-P31). In the cochlea, we have first observed a degeneration of the outer hair cells (OHC) at P23 followed by a fusion of the inner hair cells (IHC) stereocilia at P25. Notably, a complete loss of OHC and IHC is observed at P31.

Conclusions: Our novel mice model recapitulates the symptoms described in human WS patients. This preclinical model will be useful to test therapeutic strategies to preserve inner ear hair cells.



Akinobu Kakigi (1), Natsumi Uehara (1), Yuya Takubo (2), Naoya Egami (2), Shinji Yamashita (2), Tatsuya Yamasoba (2), Ken-chi Nibu (1)
(1) Kobe University, (2) The University of Tokyo

Background

Recently, we have evaluated the internal structure of the cochlea using optical coherence tomography (OCT) in vitro. OCT can deliver high resolution images and is similar to ultrasound imaging. So OCT should be used for in vivo imaging. We have developed in vivo imaging of the cochlea of guinea pig, recently. Using this procedure, in this paper, we will present evaluation of the dehydration effect of isosorbide on the hydropic guinea pig cochleae using optical coherence tomography in vivo.

Methods

Hartley guinea pigs were used. The animals were underwent the electro-cauterization of the left endolymphatic sac and 4-week feeding. To observe the cochleae with OCT in vivo, the bulla was opened via ventral approach. The animal was placed to obtain the mid-modiolar section of the cochlea, then we obtained images of the cochleae by using Santec OCT system (Santec Co., Aichi, Japan). Then, we administered intratympanically isosorbide that is osmotic expander for treatment of Meniere's disease.

Results

In this OCT study, we visualized the internal structures of the organ of Corti, Reissner's membrane, and lateral wall in the hydropic cochleae. The animals ablated the endolymphatic sac showed distention of the Reissner's membrane. Then, after the administration of isosorbide, the Reissner's membrane immediately reduced the bulge.

Conclusion

We could visualize the internal structures of the hydropic cochleae of guinea pigs in vivo. Intratympanic administration of isosorbide effects rapidly and strongly. These results indicate that middle ear instillation of isosorbide may become another option of treatment of Meniere's disease. We need further investigation before clinical use of intratympanic isosorbide instillation.

Minbum Kim

Department of Otorhinolaryngology, Catholic Kwandong University College of Medicine, Incheon, Korea

Objectives: To evaluate the efficacy of a new treatment for hydrops, the traditional hydrops model is not appropriate since it represents only the chronic stage of disease. Recently, a new dynamic model was introduced for acute aggravation of hydrops using the vasopressin type-2-receptor agonist, desmopressin. The purpose of this study was to evaluate vestibular function change in vasopressin-induced hydrops model.

Methods: In two to four weeks after surgical ablation of endolymphatic sacs in guinea pigs, acute aggravation of hydrops was induced by desmopressin (100ug/Kg, SC, VP). Auditory brainstem response (ABR) test and bidirectional sinusoidal harmonic acceleration (SHA) test with an animal rotator were performed before and after VP, respectively. Histologic sections parallel to the modiolar axis were made for observing changes in the Reissner's membrane and endolymphatic hydrops.

Results: In 60 to 90 min after VP, symmetry score on bidirectional SHA tests increased from 6.50 ± 7.60 to $25.23 \pm 9.84\%$ ($p=0.04$). While some animals showed larger response during rotation at the direction of hydrops ear (irritating response), the other showed smaller response (paretic response). Symmetric response was recovered in 120 to 180 min. In all the animals, endolymphatic hydrops with the distension of Reissner's membrane were histologically observed after VP. Interestingly, after multiple injection of VP, irritating response at 1st VP has changed into paretic response in some animals.

Conclusion: VP can transiently induce acute aggravation of hydrops and asymmetric vestibular dysfunction in guinea pigs. This model can be helpful to study a new treatment for the acute attack of hydrops. Furthermore, it might give some clues to explain the mechanism of bidirectional nystagmus in Meniere's disease.



Andreas H. Eckhard (1,2), Joe B. Jr. Nadol (1), Joe C. Adams (1)

(1) Massachusetts Eye and Ear Infirmary and Department of Otolaryngology, Harvard Medical School, Boston, MA, USA; (2) Department of Otorhinolaryngology-Head and Neck Surgery, University Hospital Zurich

Meniere's disease (MD) is a syndromal inner ear disease that is associated with a pathological accumulation of endolymphatic fluid in the inner ear – termed endolymphatic hydrops (EH). So far, EH is the only histopathological entity that was consistently found in cadaveric temporal bone (TB) specimens from patients with a clinical history of MD. The cellular and molecular pathomechanisms in the inner ear that underlie the generation of „idiopathic“ EH in MD still remain elusive. The present oto(immuno)histopathological study is the first that systematically analyzed the entire endolymphatic sac (ES), including its extraosseous portion (i.e. the ES portion in the dura mater of the posterior cranial fossa), in TB specimens from cases with idiopathic EH and a clinical history of MD (n = 44), as well as in cases with „secondary“ EH (n = 58) and in normal controls (n = 10). Our histological study revealed either severe degenerative or hypoplastic changes in the endolymph-lining epithelium of the extraosseous ES portion that were exclusively present in cases with idiopathic EH and clinical MD (93.2 % of the cases). Immunohistochemical analysis of the normal human extraosseous ES epithelium demonstrated the presence of mineralocorticoid hormone (aldosterone)-regulated ion channels and transporters (ENaC, NCC, ROMK, and Na⁺/K⁺-ATPase), which, in the kidney tubular system, are known to be crucial for transepithelial fluid transport. Notably, these mineralocorticoid hormone-regulated transport proteins were found to be absent in the degenerated/hypoplastic extraosseous ES epithelium in cases with idiopathic EH and clinical MD.

In summary, our results identify the most distal portion of the endolymphatic sac as an aldosterone-sensitive site of endolymphatic ion transport. In MD, the consistently associated absence/loss of this functionally distinct epithelium potentially causes disturbances of endolymphatic fluid homeostasis that ultimately lead to EH.

Filippo Valente (1), Edi Simoni (1), Helena Bysell (2), Laura Astolfi (1,3), Alessandro Martini (3,4)
(1) Bioacoustics Research Laboratory, Department of Neurosciences, University of Padua, Padua, Italy; (2) SP Technical Research Institute of Sweden, Chemistry, Materials and Surfaces, Stockholm, Sweden; (3) Foundation Onlus 'Staminali e Vita', Padua, Italy; (4) ENT surgery - Department of Neurosciences, University of Padua, Padua, Italy

Drug delivery systems based on nanoparticles represent an innovative approach for therapy of inner ear pathologies able to overcome the small size of the cochlea and its difficult access. Nanoparticles may deliver drugs within the inner ear with sustained release and increased the drug diffusion. Moreover they can reach all parts of the cochlea when applied near the round window. Our purpose was to develop a drug-delivery system using glycerol monooleate based nanoparticles (NPs) conjugated with hydrophobic drugs, useful to counteract ototoxicity and hearing loss. The NPs alone and conjugated with dexamethasone (a corticosteroid with limited solubility currently used for inner ear therapies) were first characterized from a physical-chemical point of view and in vitro tested for biocompatibility on a cell line derived from the organ of Corti of transgenic mice (OC-k3). The protective effect of NPs conjugated with dexamethasone (NPsD) was verified on OC-k3 against cytotoxicity induced by cisplatin, a widely used antitumor drug known to cause ototoxicity in vivo. No significant toxicity was detected using NPs and NPD under a concentration of 50 $\mu\text{g/ml}$ ($p < 0.05$). In OC-k3 the NPs were internalized 4 hours after treatment and the NPsD were able to significantly reduce the cisplatin cytotoxicity within 48 hours from treatment ($p < 0.05$). Based on these results, glycerol monooleate nanoparticles appear a suitable delivery system for dexamethasone in vitro. Further investigations will deal with NPsD tested in rat animal model.



Creber NJ., Eastwood H., Hampson A., Tan J., Chambers S., O'Leary SJ
Department of Otolaryngology, University of Melbourne, Australia

Introduction: Steroids are a widely accepted therapy to minimise hearing loss associated with inflammatory inner ear pathologies and hearing preservation following cochlear implant surgery. Many of these entities display characteristic pathologies at specific cochlear sub-sites. Despite this, little is known about cochlear steroid distribution following delivery. This collection of experiments compares perilymph and tissue distribution, and receptor activation following both local and systemic steroid administration methods.

Methods: Experiment 1 (adjuvant facilitating agents); 30 guinea pigs received administration of dexamethasone to the round window membrane (RWM) alone (n=6), or in combination with hyaluronic acid (n=6), histamine (n=6) or histamine and hyaluronic acid (n=6). Control animals received saline (n=6). After 30 minutes, perilymph was sampled from the cochlear apex and tissue harvested. Mass spectroscopy was employed to analyse perilymph dexamethasone distribution and immunohistochemistry performed to explore tissue penetrance and receptor activation. Experiment 2 (local Vs systemic administration). 16 guinea pigs received either local RWM dexamethasone (n=8) or intravenous administration (n=8). 60 minutes later perilymph sampling and tissue harvesting occurred and dexamethasone distribution and receptor activation recorded as per Experiment 1.

Results: Steroid delivery to the inner ear generated distinct and unique distribution patterns dependant on the mode of administration. Local delivery resulted in high perilymph concentrations, which correlated with strong tissue penetration. This was associated with a notable basal to apical gradient, with highest concentrations adjacent to the RWM. This effect was enhanced by the adjuvant agents histamine and hyaluronic acid, and increased time allowed for diffusion. Systemic delivery resulted in lower perilymph and tissue concentrations, but a uniform basal to apical gradient.

Conclusions: The route of administration greatly impacts upon steroid distribution within the cochlea. This knowledge should be employed when deciding the route of administration for cochlear pathologies with known manifestations at specific sub-sites.

Gopal Pramanik, Robert Durruthy Durruthy, Rose Leu, Stacy Levichev, Joerg Waldhaus, Stefan Heller, Mirna Mustapha¹

Department of Otolaryngology – Head and Neck Surgery, Stanford University, Stanford, CA, USA

In the mammalian cochlea, two major types of auditory neurons exist: Type I spiral ganglion neurons (SGNs) are myelinated and carry all the auditory information from organ of Corti inner hair cells to the auditory brainstem. More than 90% of all auditory neurons are SGNs. In sharp contrast, the remaining 10% unmyelinated type II SGNs (SGNII) innervate outer hair cells. In addition to the difference between the two types of neurons, SGNs are themselves morphologically and physiologically diverse; they consist of low and high threshold subtypes. Moreover, they differ in their susceptibility to noise insult. These differences between types and subtypes are likely driven by distinct gene expression profiles. Studies that define the molecular profiles of these different types and subtypes of neurons have been hampered by the inability to isolate pure populations of this group of neurons. We used a transgenic mouse with the unique feature of fluorescently labeled SGNI and SGNII and single cell gene expression profiling to distinguish between type I and type II SGNs. Moreover, to distinguish different SGNI subtypes that we hypothesize correlate with low and high threshold phenotype, we employed quantitative single cell RT-PCR for a preselected group of 192 candidate genes aimed to specifically distinguish among types and subtypes of SGNs. These studies will contribute to elucidate significant questions in the field such as molecular profiles that define the different types of SGNs (SGNI and SGNII) as well as different SGNI subtypes.



Antoine Huet (1,2), Thomas Justal (1,2), Gilles Desmadryl (1,2), Jean-Luc Puel (1,2), Jerome Bourien (1,2)

(1) INSERM - UMR 1051, Institute for Neurosciences of Montpellier, Montpellier, France (2) University of Montpellier, Montpellier, France

Gerbils possess a very specialized cochlea in which the low-frequency inner hair cells (IHCs) are contacted by auditory nerve fibers (ANFs) having a high spontaneous rate (SR), whereas high frequency IHCs are innervated by ANFs with a greater SR-based diversity. This specificity makes this animal a unique model to investigate, in the same cochlea, the functional role of different pools of ANFs. The distribution of the characteristic frequencies of fibers shows a clear bimodal shape (with a first mode around 1.5 kHz and a second around 12 kHz) and a notch in the histogram near 3.5 kHz. Whereas the mean thresholds did not significantly differ in the two frequency regions, the shape of the rate-intensity functions does vary significantly with the fiber characteristic frequency. Above 3.5 kHz, the sound-driven rate is greater and the slope of the rate-intensity function is steeper. Interestingly, high-SR fibers show a very good synchronized onset response in quiet (small first-spike latency jitter) but a weak response under noisy conditions. The low-SR fibers exhibit the opposite behavior, with poor onset synchronization in quiet but a robust response in noise. Finally, the greater vulnerability of low-SR fibers to various injuries including noise- and age-related hearing loss is discussed with regard to patients with poor speech intelligibility in noisy environments.

Wei Liu¹, Nadine Scharf-Moren¹, Heval Benav², Rudolf Glueckert³, Carolyn W. Garnham², Annelies Schrott-Fischer³, and Helge Rask-Andersen¹

¹Department of Surgical Sciences, section of Otolaryngology Head and Neck Surgery, Uppsala University Hospital,

²Research and Development, MED-EL GmbH, Innsbruck, Austria, ³Department of Otolaryngology, Medical University of Innsbruck, Austria

Introduction: The generation sites and mode of action potentials (APs) in the various portions of the human cochlear nerve (CN) is unknown but voltage-dependent sodium channels Na⁺ (Nav) assumingly play a significant role. Voltage-dependent K⁺ channels (Kv) and Ca⁺⁺ (VGCCs) may be essential for the modulation of APs. Known expression and localization is essential for better understanding effects of electric stimulation of the CN under various conditions. **Methods:** To analyze the expression of Na⁺ and K⁺ channels in human CN and organ of Corti (Nav1.1 Nav1.2 Nav1.3 Nav1.6, Nav1.7 Nav1.8, Nav1.9, Kca1.1, Kv1.1, Kv1.2, Kv1.3, Kv3.1b, Kv7.1, Kir4.1, as well as ion transporters) using immunohistochemistry in surgically obtained tissue. Confocal and super-resolution fluorescence structured illumination microscopy (SIM) were used. Image and data precision were evaluated from ZEN calibration on 40 nm beads. A lateral precision of approximately 80 nm and 250 nm axially were obtained. **Results:** We identified for the first time the sodium channel Nav1.6 in the node of Ranvier with associated Caspr in the juxtaparanode of the human spiral ganglion. In hemi-node (beneath the habenular perforata) and some axon hillocks, the channel protein and caspr could be identified. Apart from Nav 1.6, other Na⁺ channels were found in the cochlea. Kv1.1 and Kv1.2 were present in the juxtaparanodal region together with contactin associated protein (Caspr) around the node of Ranvier. Kv7.1 as well as the transporter NKCC1 in spiral ganglion neurons. Kca1.1 (BK channel)-immunoreactivity was found in spiral ganglion neurons as well as in hair cells stereocilia. Our results on Ca⁺⁺ channels are still preliminary. **Conclusions:** The study results showed the importance of Nav1.6, coordinating with other channels, for the generation of action potentials in the human CN. **Acknowledgements** The study was performed together with and partly funded by the MED-EL GMBH, Fuerstenweg 77a, 6020 Innsbruck, Austria. This study was also supported by ALF grants from Uppsala University Hospital and by the Foundation of "Tysta Skolan," the Swedish Deafness Foundation (HRF) and generous private donations from Börje Runögård and David Giertz, Sweden.

Helen Cai (1,2), Tess Wright (1), Lisa Gillespie (3,4), Stephen O'Leary (1), Karina Needham (1)
(1) Department of Otolaryngology, University of Melbourne, East Melbourne, Australia, (2) Neuroscience Research Australia, Sydney, Australia, (3) Bionics Institute, East Melbourne, Australia, (4) University of Melbourne, Parkville, Australia

Introduction: Neurotrophic factors such as brain-derived neurotrophic factor (BDNF) and neurotrophin-3 (NT3) play important roles in neural protection and synaptogenesis in the inner ear. However, neurotrophins also have the ability to alter ion channel expression and activity, and thereby influence neural firing, and thus therapeutic potential. To understand what implications neurotrophin delivery will have for auditory function, this study examines the activity of primary auditory neurons in response to exposure to combined BDNF and NT3.

Methods: Whole cell patch-clamp recordings were made from dissociated primary auditory neurons taken from early post-natal rats. Neurons were cultured either without neurotrophins (untreated; UT), or in the presence of both BDNF and NT3 (at either 10 ng/ml or 50 ng/ml; NT10 and NT50 respectively) for 1, 2 or 3 days in vitro.

Results: Recordings were made from 475 neurons. The influence of combined BDNF and NT3 over UT varied with time for resting membrane potential, capacitance and input resistance. Most notably, the input resistance of UT neurons was significantly larger than NT10 and NT50 neurons at 3 days. Firing rates increased across time for all groups, and whilst NT50 neurons displayed more activity than other groups at 1 day, these differences were absent at 2 and 3 days, suggesting time in culture is most influential over firing output. No clear changes in firing threshold were seen with time or treatment. Alternatively, firing latency tended to be longer in NT groups, particularly at 3 days, and action potential duration shorter in both NT10 and NT50 when compared to UT neurons.

Conclusion: Exposure to combined BDNF and NT3 influences input resistance, as well as action potential latency and duration. Neural activity was also dependent on time-in-culture, with many firing properties changing independently of the presence or concentration of neurotrophins present.

Athanasia Warnecke, Thomas Lenarz, Ariane Roemer
Hannover Medical School, Department Of Otolaryngology, Hannover, Germany

Introduction: Prostaglandin F_{2α} (PGF_{2α})- receptors are located in the stria vascularis, the inner and outer hair cells as well as in the spiral ganglion. However, their effects have not been investigated hitherto. Thus, we hypothesize that the prostaglandin F analogue bimatoprost has neuroprotective properties und opens therefore a novel aspect in biological cochlear implantation.

Methods: Dissociated spiral ganglion neurons (SGN) from neonatal rats were treated with different concentrations of bimatoprost. The survival rate was analysed. To determine a neuroprotective effect in whole organ cultures, microexplants of SGN were treated as well. Furthermore, the usefulness of bimatoprost in order to provide a matrix for cellular attachment and growth was investigated by generating a composition of heparansulfate and bimatoprost for SGN cultures. After 48 hours, cell cultures were fixed and analysed.

Results: Using bimatoprost, an increase in neuronal survival of up to 474.2% was achieved when compared to the negative control without any supplements. The SGN microexplants showed a better adhesion, an improved neuronal outgrowth as well as a higher concentration of neuronal supporting cells when they were offered a matrix composed of heparansulfate in combination to bimatoprost.

Conclusion: Bimatoprost reveals neuroprotective effects in the inner ear and supports neuronal adhesion in an artificial extracellular matrix. It therefore opens the door for new pharmacological therapies in otology especially for the modulation of the cochlear microenvironment.

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C. Ahrensmeier (1), M. Kwiatkowska (1*), G. Ellrichman (2), R. Gold (2), S. Dazert (1), S. Volkenstein (1)

(1)Klinik für Hals-Nasen-Ohrenheilkunde, Kopf- und Hals-Chirurgie der Ruhr Universität Bochum, St. Elisabeth-Hospital; (2)Neurologische Universitätsklinik der Ruhr-Universität Bochum, St. Josef Hospital; () present adress: Universitäts-HNO-Klinik Tübingen*

Introduction: Fumaric acids are well established drugs for Psoriasis treatment and due to their neuroprotective effects for treatment of Multiple sclerosis. In the auditory system, the loss of sensory hair cells induces a progressive degeneration of spiral ganglion neurons (SGN). The only therapeutical options for patients with a severe to profound hearing loss are Cochlear-Implants (CIs). In CIs, the hearing quality depends on the remaining SGN population which are the target cells for electrical stimulation. In this study we aim to investigate the neuroprotective effect of monomethyl fumarate (MMF) on auditory neurons.

Methods: Spiral ganglion neurons of postnatal mice were cultured as single cells, in organotypic explant cultures and in neurosphere assays. After 24h in organotypic culture cells were exposed to oxidative stress (50 μ M H₂O₂) for 2 h and treated additional 48 h with MMF. Survival and neurite length were measured under MMF towards control groups without MMF treatment by immunohistochemical staining.

Results & conclusion: MMF significantly increased the survival and neurite length in each setting towards the control groups. The neuroprotective effect of MMF on auditory neurons and the associated survival of SGN population may be used in combination with CIs and lead to an improvement of functional results in these patients.

Guangjian Ni, Stephen Elliott

Institute of Sound and Vibration Research, University of Southampton, Southampton, SO17 1BJ, UK

The osseous spiral lamina develops in the early stages of fetal life as the result of differentiation of scala tympani and scala vestibuli. Defects in the osseous spiral lamina may exist as part of a more extensive deformity, Mondini dysplasia for example, or as an isolated cochlear finding. Patient with defects of osseous spiral lamina often suffers from progressive hearing loss. The effect of the defect on the cochlear response to acoustic stimuli is, however, still unclear. This preliminary work is aiming to provide some insights into a specific defect in the osseous spiral lamina found in several patients. From histological images, it can be found that parts of the spiral lamina are missing. In this study, we focus on the mechanical effects of these missing parts on the basilar membrane response to the stapes excitation only. The predictions are calculated using two types of models: an one-dimensional elemental model, which provides theoretical insight, and a three-dimensional finite element model of the cochlea, which shows similar results to the elemental model and helps validate its assumptions. Although this work does not model every aspect of the defect, it does provide a way of predicting the possible mechanical effects of hearing defects.



Vincenzo Vitale (1), Gabriele Sanchini (1), Sergio Solinas (2), Roberto Pizzala (3), Paola Perin (1)
 (1) Dept. of Brain and Behavioral Sciences, University of Pavia, Italy; (2) Dept. of Biomedical Sciences, University of Sassari, Italy; (3) Dept. of Molecular Medicine, University of Pavia, Italy

Tinnitus has been associated to maladaptive plasticity in several brainstem nuclei, and in particular in the dorsal cochlear nucleus (DCN) (Shore et al. 2016). The onset of tinnitus after induction is modulated by several factors, one of which could be glia-associated metaplasticity, similarly to neuropathic pain (Grau et al. 2014).

Glial activation is a well-known response of nervous tissue to insult, and chronic tinnitus after noise trauma is associated to microglial activation in the rat DCN (Baizer et al. 2015). We have previously observed acute microglial responses in rat DCN after noise trauma, salicylate, and cochlear destruction (Perin et al. 2016). In all cases, microglial density significantly increased; however, local heterogeneities in microglial responses were seen. This could be important, since microglial cells are small enough to be confined to one of the three DCN layers, allowing their differential modulation; moreover, microglial cells apposed to neuronal somata, as found in cerebral cortex, could be involved in sensing neuronal output (Wogram et al. 2016).

In the present work we started looking for quantitative indicators for microglia morphology and association to other cells. For morphology, we performed Sholl analysis (Sholl 1953), skeleton reconstruction (measuring the order of branching) and measures of soma and process sizes (for each order found in skeletal analysis). Soma deviation from sphericity was also monitored as a sign for satellite microglia (Wogram et al. 2016), which (judging from this parameter) appeared to be present throughout the DCN. To look for spatial association of microglia with neurons, we calculated distances between nuclei centroids, and obtained average nearest-neighbour distances. Microglial morphological changes were observed after all treatments, but due to the large variability of control microglial branching patterns, differences encompassing the entire population were only significant after ipsilateral cochlear destruction. Significativity did not change when splitting the DCN in superficial and deep layer. The present results suggest that microglial morphology, although affected by tinnitus-inducing treatment, is not well described by single indicators. We are currently elaborating a multifactorial analysis as in (Gabbito et al. 2016) integrating weighted contributions from localization, soma shape, branching pattern and nearest neighbour.

Jung-sub Park (1,3), Christopher R. Cederroth (1), Vasiliki Basinou (1), Inna Meltser (1), Gabriella Lundkvist (1,2) and Barbara Canlon (1)

(1) Laboratory of Experimental Audiology, Department of Physiology and Pharmacology, Karolinska Institutet, 171 77 Stockholm, Sweden (2) Department of Neuroscience, Karolinska Institutet, 171 77 Stockholm, Sweden; (3) Department of Otolaryngology, Ajou University School of Medicine, 164, Worldcup-ro, Yeongtong-gu, Suwon, 16499, Korea

The circadian clock has been proved to coordinate physiological bodily functions in anticipation of the daily light-dark cycle. It has been shown that the cochlea possesses the peripheral circadian clock machinery and the vulnerability from noise damage varies with the time of day. However, the circadian clock in the central auditory pathway has not been investigated. In the present study, we therefore explored the circadian clock system of the inferior colliculus (IC), which plays an important role in noise-induced pathologies such as tinnitus, hyperacusis and audiogenic seizures. Using PER2::LUC transgenic mice and real-time bioluminescence recordings, we revealed circadian oscillations of Period 2 protein in IC explants for up to 1 week. Serial coronal sections of the IC showed that Period 2 is homogeneously expressed throughout the whole IC. Core clock genes and clock-related genes (Cry1, Bmal1, Per1, Per2, Rev-erba, and Dbp) displayed robust circadian molecular oscillations in the IC. Expression levels of early-induced genes and clock genes during 24 hours revealed a differential response to day or night noise exposure. Rev-erba, and Dbp genes were affected only by day noise exposure, whereas Per1 and Per2 were affected only by night noise exposure. However, the expression of Bdnf was affected by both day and night noise exposure, suggesting that plastic changes are unlikely involved in the differences in day or night noise sensitivity in the IC. These findings strongly support the importance of circadian responses in the IC and emphasize the importance of circadian mechanisms for understanding central auditory function and related disorders.



Paciello F1, Podda MV2, Rolesi R1, Zanin F1, Eramo SLM2, Troiani D2, Grassi C2, Paludetti G1, Fetoni AR1

(1) *Institute of Otolaryngology, (2) Institute of Human Physiology, Università Cattolica, School of Medicine, Rome, Italy*

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

Methods: Male adult rats were exposed to noise (100 dB, 60 min/day for 10 consecutive days). The day after the last session of acoustic trauma rats underwent sham stimulation (Sham Noise group) or anodal tDCS (Noise+tDCS) over the left auditory cortex (tDCS: 350 μ A for 20 min for two consecutive days). Control animals with normal hearing also underwent the same tDCS stimulation (Ctrl-tDCS). Auditory Brainstem Responses (ABR) were measured to assess auditory function. After the last ABR recording rats were sacrificed and their brains processed for: (1) Golgi-Cox staining to assess changes in dendrite morphology and spines in auditory cortices; (2) Western blot analyses in order to evaluate the expression of molecular markers of synaptic plasticity (BDNF and Synaptophysin) in the auditory cortices.

Results: Morphological analysis showed a decrease in the number of dendritic spines after noise exposure, whereas tDCS increased spine density both in Ctrl-tDCS and Noise+tDCS groups. This effect was observed in both apical and basal dendrites of layer II-III neurons of the left and right auditory cortices. Branching analysis revealed that noise induced a decrease of dendritic length in both apical and basal dendrites. tDCS affected differently neuronal morphology in the experimental conditions: in layer II-III an increase of dendritic length was observed in apical and basal dendrites in Ctrl-tDCS, whereas a reduction of dendritic nodes was observed in the unstimulated cortex in Noise+tDCS animals. Western blot analyses indicated an increase of synaptic plasticity-related proteins (BDNF and Synaptophysin) in the auditory cortices after tDCS.

Conclusion: We speculate that anodal tDCS can counteract the central damages caused by noise exposure by modulating synaptic plasticity. Targeting synaptic plasticity may provide a major breakthrough for therapeutic interventions.

Jiří Popelář (1), Miguel Díaz Gómez (2), Jiří Lindovský (1), Natalia Rybalko (1), Josef Syka (1)
(1) *Institute of Experimental Medicine, Czech Academy of Sciences, Prague, Czech Republic;* (2) *Medical Electronics (MED-EL), Madrid, Spain*

OBJECTIVE

The brain-specific link protein *Bral2* represents a substantial component of the perineuronal nets enwrapping neurons in the central nervous system. Perineuronal nets are formed in brain structures at the end of the critical development period.

METHODS

The hearing function in knockout *Bral2*^{-/-} (KO) mice was investigated during postnatal development and in young adult animals and the results were compared with wild type *Bral2*^{+/+} (WT) mice. Acoustic startle reflex (ASR), prepulse inhibition of ASR (PPI of ASR) and recording of auditory brainstem responses (ABRs) were used to elucidate the role of *Bral2* in auditory signal processing.

RESULTS

The amplitudes of the ASR and the efficiency of PPI of ASR, produced by prepulse noise stimulus or gap in continuous noise, was similar in 2-week-old WT and KO mice. Over the 2-month postnatal period the increase of ASR amplitudes was larger in WT mice than in KO mice. The efficiency of the PPI of ASR significantly increased during the 2-month postnatal period in WT mice, whereas in KO mice the PPI efficiency did not change after the end of the critical period. Hearing thresholds in 2-month-old WT mice, based on the ABR recordings, were significantly lower at high frequencies in comparison with KO mice. However, amplitudes of individual waves of click-evoked ABR did not differ between WT and KO mice, suggesting similarly reliable synaptic transmission. Temporal resolution (tested with two broad band noise stimuli separated by a gap of 1-50 ms duration) and neural adaptation (tested with ABR evoked by a series of 4 clicks with variable inter-click intervals) were better in 2-month-old WT mice than in the age-matched KO mice.

CONCLUSIONS

Evidently, the absence of *Bral2* protein in KO mice, that is accompanied by a defect in the formation of perineuronal nets at the end of the developmental period, results in KO mice, in comparison with WT mice, in increased hearing thresholds at high frequencies and weaker temporal resolution, probably due to the altered function of fast spiking neurons.

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Sung Kwang Hong, Sejik Park, Jung-Hye Park

Department of Otolaryngology, Hallym University Hospital, Anyang, South Korea

Introduction: Our main hypothesis is attention on neural response may be modified in the tinnitus brain. To test the hypothesis, we adapted trailblazing methods for minimizing auditory loss component and analyzing directional information flow of neural oscillation across different regions beyond simple mapping and localization of brain activity.

Materials and methods: 15 tinnitus patients without hearing threshold shift on conventional testing and paired 15 healthy subjects (all right handed, M:11, F:4, age 21-43yrs) were included in this study. All tinnitus subjects exhibited definite tinnitus at least on one ear on tinnitus matching testing and filled out a standard THI and VAS. The EEG was recorded using 32 channels with a sample frequency 1000Hz and a bandpass filter between 0.1-250 Hz. Experimental paradigm consisted of top down and bottom up condition, in which 400 stimuli were presented in a random series, once every 1.7s (± 0.2 s) consisting of target (tinnitus matched pitch frequency) and standard acoustic stimuli (500 Hz) at 75 dBnHL using binaural insert earphone with probability 0.2 and 0.8. Grand average ERP component including auditory N1, N2 and P3 were presented as total power filed of in each condition. MATLAB-based toolbox "eConnectome", which was modified by customized code in the MATLAB interface to fit into our research needs, were used for estimating over the directional interactions of brain functional network related perceptual process

Results: Grand average N1, N2 latency and amplitude did not show significant difference between two groups while more intense P3 revealed in healthy subjects. In top down condition, directional flow of neural oscillation from BA 41 to BA 8 and BA10 was observed in healthy subjects. However, altered flow pattern was observed in tinnitus brain on theta, beta and gamma band, which indicates alteration of auditory perception processing exist in tinnitus patients.

Conclusion: Overall, our results suggest isolated activity of auditory cortex may not sufficient to generate the tinnitus percept in normal hearing tinnitus patients. Altered directional information flow without functional connectivity to other consciousness supporting complex including prefrontal cortex was observed in tinnitus brain, which may provide a meaningful evidence of cognitive therapy for tinnitus patients.

Aren Bezdjian, Sjaak F.L. Klis, Jeroen P.M. Peters, Wilko Grolman, Inge Stegeman

Department of Otorhinolaryngology and Head & Neck Surgery, University Medical Center Utrecht, Utrecht, The Netherlands

Background

Experimental animal models are essential to the advancement of science, provided that experiments are well designed, performed, interpreted and reported. In order to optimize the overall quality of reporting scientific research using animal models, the ARRIVE (Animal Research: Reporting of In Vivo Experiments) guidelines were developed by the UK-based National Centre for the Replacement Refinement & Reduction of Animals in Research. These guidelines are endorsed by important scientific organizations and journals.

Objectives

The primary aim was to assess the quality of reporting of scientific publications using animal models in the field of otorhinolaryngology by using the ARRIVE checklist for scoring. Also, articles published in top otorhinolaryngology or "ENT" journals were compared to those in multidisciplinary journals with higher impact factors.

Methods

Based on ISI Web of Knowledge impact factors, the top 5 multidisciplinary and ENT journals were selected. A Pubmed database search was conducted using predefined filters for retrieval of animal research articles and articles in otorhinolaryngology with date and journal restrictions. To assess for the quality of reporting, articles were scored according to the ARRIVE guidelines.

Results

ENT journals (n = 35) reported a mean of 57.1% of adequately scored items (95% CI: 53.4 - 60.9%; Median: 58.3%), whereas articles published in multidisciplinary journals (n = 36) reported a mean of 49.1% of adequately scored items (95% CI 46.2 - 52.0%; Median: 50.0%). Thus, ENT journals adhered to the ARRIVE guidelines statistically significantly better than articles published in multidisciplinary journals ($p < .05$).

Conclusion

Although ENT journals had better quality of reporting scores, adherence to the ARRIVE guidelines is generally poor in the field of otorhinolaryngology. There is still a need for improving reporting standards in otorhinolaryngology papers using animal models. We suggest that editorial boards and funding agencies endorse the ARRIVE guidelines for optimal quality of reporting when using animals in scientific research.



Engmér Berglin C, Videhult Pierre P, Fransson A, Linder B, Laurell G

Department of Surgical Sciences, Otorhinolaryngology and Head and Neck Surgery, Uppsala University, Sweden

Introduction: Head and neck cancer (HNC) is a collective term for malignant tumors in the upper aero-digestive tract. Treatment options are single modality treatment (surgery or radiotherapy) or combined modality treatment. Cisplatin is an antineoplastic drug that can be used in chemoradiation in subgroups of patients with HNC. Treatment toxicities observed in patients with HNC play a major part of acute side-effects and long-term sequelae. Cisplatin-based chemotherapy can cause irreversible ototoxic effects. Cisplatin-induced hearing loss is most likely to present after a high single dose treatment. Experimental studies have shown that cisplatin-induced hearing loss involves oxidative stress and mitochondrial dysfunction.

It is reasonable to speculate that differences exist in ototoxic mechanisms induced by cisplatin between experimental animals without cancer and patients treated for HNC. The purpose of the study is to find strategies to prevent cisplatin-induced hearing loss that can be taken into clinical trials.

Methods: We will report three methods for prevention of cisplatin-induced hearing loss in experimental animals: otoprotectors given by intravenous administration, intratympanic administration and inhalation. Cisplatin was given to guinea pigs by a single intravenous injection.

Results: The results show that all three methods for prevention have the potential to protect the inner ear in guinea pigs.

Conclusions: There is rather robust evidence that cisplatin-induced hearing loss can be prevented, at least partially in experimental animals, while the results in humans have so far been poor. The findings of these studies will be discussed in light of the overall burden of treatment in patients with HNC. We will discuss which type of otoprotection would be reasonable to use in clinical settings.

Fetoni AR1, Paciello F1, Mezzogori D2, Rolesi R1, Eramo SLM2, Di Pino A1, Troiani D2, Grassi C2, Paludetti G1

1Institute of Otolaryngology, 2Institute of Human Physiology, Università Cattolica, School of Medicine, Rome, Italy

Introduction: The clinical usefulness of Cisplatin is limited by the incidence of chemoresistance and its adverse side-effects such as ototoxicity. In a previous study we showed the efficacy of Curcumin administration in potentiating the antitumoural activity of cisplatin in vitro in oral squamous cell carcinoma, and, at the same time, in protecting against ototoxicity in vivo. In this study we focused on Ferulic Acid (FA), a phenolic compound with free radical scavenging property, in order to compare the efficacy of the two molecules in protecting against cisplatin ototoxicity without interfering with its antitumoral activity.

Methods: In vitro studies were conducted in head and neck squamous cell carcinoma treated with cisplatin (1.56 μ M) and different doses of curcumin (0.5, 1, 3.67, 6.75 μ M) or FA (10, 25, 50, 100, 200, 400, 600 μ M). We performed cell counts at incubation times: 24, 48 and 72 hours and tumor progression analysis and immunofluorescence for Nrf2 and pStat3 activation. For in vivo studies, Curcumin (200 mg/kg) or FA (150 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), morphological analysis (Rh-Ph staining), and immunofluorescence for Nrf2/HO-1 expression in cochlear cryosections at days 3 and 5 post cisplatin injection.

Results: In vitro Curcumin exerted a dose-dependent pro-apoptotic effect, causing a decrease in cell viability and tumor growth. Similarly, FA induced a pro-apoptotic effect on cell viability at low doses. However, at FA high doses the anti-proliferative effect decreased as also confirmed by immunofluorescence analyses. In vivo, Curcumin otoprotection was more evident with respect to FA. However, both phenolic compounds attenuated hearing loss caused by cisplatin, as well as OHC loss and an increase of endogenous antioxidant enzymes (Nrf2/HO-1 activation pathway) was found.

Conclusion: Our preliminary results suggest that both Curcumin and FA can be used as adjuvant in cisplatin therapy by potentiating its antitumoral activity and by preventing the ototoxic side effects. Further studies need to be performed to assess which phenolic compound provides the best therapeutic intervention.

Mark E. Warchol (1), Tejbeer Kaur (1), Melissa Strong (2), Edwin W Rubel (2), Keiko Hirose (1)

1) Department of Otolaryngology, Washington University School of Medicine, St. Louis MO USA; (2) Department of Otolaryngology, University of Washington School of Medicine, Seattle WA USA

Introduction

Macrophages respond to cochlear injury but their precise role in subsequent pathology is not known. We are using transgenic mouse models to study the interactions between macrophages and auditory neurons.

Methods

The fractalkine receptor CX3CR1 is expressed on all macrophages and microglia. Experiments utilized CX3CR1-GFP/Pou4f3-huDTR mice, which express GFP in all macrophages/microglia, and also permit the selective ablation of hair cells by treatment with diphtheria toxin (DT). Additional experiments used kanamycin/furosemide or octave-band noise to lesion hair cells. Spiral ganglion neurons (SGNs) were immunolabeled for neurofilament and neurons of the ventral cochlear nucleus (VCN) were labeled with fluorescent Nissl stain.

Results

DT-induced hair cell death led to enhanced numbers of macrophages within the organ of Corti and the spiral ganglion. The numbers of macrophages associated with the cochlear sensory epithelium peaked at 14 days post-lesion and then declined. However, macrophage numbers within the ganglion remained elevated until >60 days after hair cell injury. Fractalkine (CX3CL1) is known to mediate signaling between neurons and macrophages. We observed immunoreactivity for CX3CL1 on spiral ganglion neurons, suggesting that fractalkine might play a role in macrophage recruitment and/or SGN survival. Genetic deletion of fractalkine receptor CX3CR1 resulted in diminished numbers of spiral ganglion macrophages after DT-mediated hair cell injury, but not after kanamycin/furosemide ototoxicity. Notably, however, the loss of CX3CR1 resulted in diminished SGN survival after hair cell loss caused by DT, ototoxicity or noise. Together, these results suggest that normal fractalkine signaling is critical for the survival of SGNs after hair cell injury, but that fractalkine is probably not the sole signal that recruits macrophages into the spiral ganglion. In additional studies, we have found that hair cell injury leads to increased numbers of microglia (the 'resident macrophages' of the CNS) in the VCN and that deletion of CX3CR1 enhances this microglial response.

Conclusions

Hair cell injury leads to increased numbers of macrophages within the spiral ganglion and enhanced numbers of microglia in the VCN. Fractalkine signaling appears to regulate both of these responses, but its role is complex and may depend on the nature of the cochlear injury.

Michael Perny (1,2,3,5,6), Magdalena Solyga (1,2), Marta Rocco (1,3,5), Denis Grandgirard (2,5), Stephen L. Leib (2,5), Pascal Senn (1,4,5,6)

(1) Inner Ear Research Laboratory, Department of Clinical Research, University of Bern; (2) Neuroinfection Laboratory, Institute for Infectious Diseases, University of Bern; (3) University Department of Otorhinolaryngology, Head and Neck Surgery, Inselspital, Bern; (4) Dept. of Otorhinolaryngology, Head & Neck Surgery, Hôpitaux Universitaires de Genève; (5) Laboratory for Regenerative Neuroscience, DCR, University of Bern; (6) Graduate School for Cellular and Biomedical Sciences, University of Bern

Introduction: Up to 30% of patients surviving pneumococcal meningitis suffers from sensorineural hearing loss. We have recently reported the ototoxic effect exerted on neurosensory cells by Pneumococcal meningitis infection in vivo. The aim of this project was to further analyse the mechanisms of *S. Pneumoniae* dependent ototoxicity, with specific focus on the bacterial toxin pneumolysin, using an in vitro culture system of the Organ of Corti (OC).

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). The membrane (0.4 µm) was placed on top of a fluid-filled chamber, where bacteria and other ototoxic substances were applied. This set up aimed at mimicking the in-vivo situation, in which bacteria infiltrate the cochlea almost exclusively through the scala tympani and are thus not in direct contact with the hair cells. Immunocytochemical staining for hair cells (Myosin-VIIa) and neurons (TUJ) was performed to assess the number of missing inner and outer hair cells and spiral ganglion neurons upon treatment, for different regions of the OC (base, middle, apex).

Results: OC culture exposure to the *S. Pneumoniae* P21 Serotype 3 strain showed a predominant damage of outer hair cells (OHCs), which was dose- and time-dependent. Inner hair cells (IHCs) were not significantly damaged. Spiral ganglion neurons were also not affected (unless higher doses of bacteria were used).

In order to study the contribution of the bacterial toxin pneumolysin to the observed cochlear damage, we compared hair cell loss in explants exposed to a pneumolysin deficient mutant strain of *S. Pneumoniae* (D39ΔPLY) and to its WT counterpart (D39). We observed a decreased survival of IHCs and OHCs after exposure to both strains, which was dose-dependent and most prominent in the basal turn. IHC survival was significantly higher with *S. Pneumoniae* D39ΔPLY compared to D39, especially in the basal and middle turn. Experiments with the recombinant pneumolysin confirmed the predominant effect on IHCs in the basal region.

Conclusions: The in vitro model we have developed allows studying in detail the mechanisms of neurosensory cell damage upon *S. Pneumoniae* infections.



Thomas Schrepfer, Linghui Luo, Jochen Schacht
Kresge Hearing Research Institute, University of Michigan, Ann Arbor, Michigan, USA

The generation of reactive oxygen species (ROS) is a cause of hair cell death by aminoglycoside antibiotics. Consequently, both endogenous and exogenous antioxidants can mitigate the ototoxicity of these antibiotics. The ultimate reductant for endogenous ROS removal systems is NADPH which is primarily generated by glucose 6-phosphate dehydrogenase (G6PDH), the enzyme catalyzing the first steps in the pentose phosphate pathway. We hypothesized that a decreased activity of G6PDH should aggravate the adverse effects of aminoglycosides by compromising the ability to counter oxidative stress.

Explants of the organ of Corti from CBA/J mice (postnatal day 2 – 3) were incubated with gentamicin for up to 72 h. The treatment led to the formation of ROS and progressive outer hair cell loss with a base-to-apex gradient and a half-maximal toxic concentration of 4.7 μ M gentamicin for a 72-h incubation. To mimic a G6PDH deficiency, we added the NADP analogue and G6PDH inhibitor 6-aminonicotinamide (6-AN). Addition of 6-AN increased ROS production and enhanced hair cell loss in a dose-dependent manner. In order to ascertain that the observed effects were indeed due to an NADPH deficiency, we stimulated the synthesis of NADPH via NADP⁺-linked malate enzyme, a secondary pathway of NADPH generation. In the presence of 6-AN, the addition of malate and simultaneous inhibition of fatty acid synthesis protected hair cells against gentamicin toxicity. Finally, we tested sensitivity to aminoglycosides in G6PDH-deficient mice. During the course of 600 mg kanamycin/kg body weight bid, 5 out of 13 mutant mice died while wild type littermates (and other strains of mice) tolerated the treatment well.

The results strongly support the involvement of ROS in aminoglycoside-induced ototoxicity and the crucial role of NADPH as endogenous protectant. Furthermore, G6PDH deficiency appears to sensitize the animals to systemic lethal effects of aminoglycosides. This result is particularly interesting because G6PDH-deficiency is the most common human enzymopathy rendering carriers sensitive to oxidative agents including common foods (e.g., fava beans; "Favism"). By extension, this population may also have an enhanced susceptibility to aminoglycoside antibiotics.

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Soledad Levano; Eliane Ebnoether; Alessia Ramseier; Maurizio Cortada; Daniel Bodmer

Department of Biomedicine and Clinic of Otolaryngology, Head and neck Surgery, University Hospital Basel, Basel, Switzerland

Introduction

Ototoxic drugs like gentamicin can damage sensory hair cells of the inner ear by production of reactive oxygen species (ROS). Sestrins, a family of stress-responsive proteins, exert its antioxidant function by two main pathways: activation of nuclear factor erythroid 2-related factor (Nrf2) pathway or inhibition of the mammalian target of rapamycin complex 1 (mTORC1) activity. Inhibition of mTORC1 induces autophagy by regulating the transcription of autophagy genes. We hypothesized that sestrin2 may protect hair cells from gentamicin-induced oxidative stress.

Methods

C57BL6/J wild type and sestrin2 knockout mice at P4-P5 were used for our in vitro experiments. Gene expression of sestrins was assessed via real-time PCR and western blot. Localization of sestrin2 in mouse cochlea was performed by immunostaining and confocal microscopy. Hair cell damage was performed using different concentrations of gentamicin.

Results

All three sestrins were detected in the inner ear compartments. Sestrin2 immunoreactivity was detected in sensory hair cells and spiral ganglion. After gentamicin exposure high rate of hair cell loss was observed in sestrin2 knockout mice as compared to wild type mice. Gentamicin downregulated phosphorylation of AMPK α and upregulated p70S6K in treated wild type explants. In contrast, protein phosphorylation remained unchanged in sestrin2 knockout explants after gentamicin treatment. In addition, sestrin2 expression was downregulated in gentamicin-treated organs from wild type mice.

Conclusion

Our data provide evidence that sestrin2 plays an important role in the protection of hair cells against gentamicin. Ablation of *Sesn2* increased susceptibility of hair cells to gentamicin. The mTOR signaling pathway appears to be modulated by gentamicin during the hair cell death.



Jordi Llorens (1,2,3), Paulina Jedynak (1), Erin Greguske (1), Sílvia Prades (1), Pere Boadas-Vaello (4), Lara Sedó-Cabezón (1,2)

(1) *Universitat de Barcelona, Barcelona, Catalonia, Spain*; (2) *Institute of Neurosciences University of Barcelona, Barcelona, Catalonia, Spain*; (3) *Institut d'Investigació Biomèdica de Bellvitge (IDIBELL), L'Hospitalet de Llobregat, Catalonia, Spain*; (4) *Universitat de Girona, Girona, Catalonia, Spain*

Human and animal data indicate that degeneration of the sensory hair cells (HCs) is the main consequence of exposure to ototoxic chemicals. However, other effects have been scarcely studied and HC degeneration cannot explain some clinical observations. One of these is the recovery in vestibular function reported at times during washout after chronic ototoxicity. We assessed the loss and subsequent recovery in vestibular function in rats exposed to the ototoxic compound 3,3'-iminodipropionitrile (IDPN) for four weeks, and studied in the vestibular epithelia the cellular and molecular events associated with these functional alterations. IDPN ototoxicity resembles aminoglycoside ototoxicity, but offers a more dependable model for chronic exposure in rats. The comparison of behavioral and ultrastructural data showed that loss of vestibular function appeared before the loss of HCs or stereociliary coalescence became evident. Nevertheless, a complete dismantlement of the calyceal junctions between type I HCs and calyx endings was observed by transmission electron microscopy at these early stages of functional loss in cristas and utricles. Immunohistochemical observations revealed loss of the junction proteins caspr1 and tenascin-C, and misplacement of KCNQ4. The decrease in caspr1 and tenascin-C was not associated with a decrease in expression of the corresponding mRNA sequences, as assessed by RT-PCR. If rats were allowed a 4 week washout period after exposure, a good recovery of vestibular function was recorded. This was associated with a recovery in the ultrastructural appearance of the calyceal junctions, and in the quantities and distributions of caspr1, tenascin-C and KCNQ4. We also examined the calyceal synapses by immunostaining cristas for ribeye and either PSD-95 or GluA2 puncta. Chronic IDPN ototoxicity caused loss of pre- and post-synaptic puncta, and limited recovery in this effect was recorded at the end of the washout period. The present data reveal new forms of damage and repair in the vestibular epithelium of adult mammals, including dismantlement of the calyceal junction and a robust capacity for its rebuilding. These findings contribute to a better understanding of the phenomena involved in progressive vestibular dysfunction and its potential recovery during and after ototoxic exposure.

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Martina Dvorakova (1,4), Romana Bohuslavova (1), Bernd Fritsch (2), Israt Jahan (2), Tetyana Chumak (3), Josef Syka (3), Gabriela Pavlinkova (1)

(1) Institute of Biotechnology, Czech Academy of Sciences, Prague, Czech Republic; (2) Department of Biology, University of Iowa, Iowa City, Iowa, USA; (3) Institute of Experimental Medicine, Academy of Sciences, Prague, Czech Republic; (4) Faculty of Science, Charles University, Prague, Czech Republic

Introduction

Main functional cells of the vertebrate inner ear are formed from a common neurosensory domain. Transcription factor Sox2 is expressed in the neurosensory precursors which will develop as hair cells, supporting cells or neurons. This expression pattern indicates the role of Sox2 in the specification of neurosensory cells and in the regulation of differentiating transcription factors, such as Neurog1 and Atoh1. The precise function and interactions of Sox2 with other factors during inner ear development are still incompletely understood. Our aim is to determine the function of Sox2 in the inner ear using the tissue specific deletion of Sox2.

Methods

We used a conditional knock-out mutation of Sox2 gene by *Isl1-cre* in mice. The inner ear was analyzed in different developmental stages by immunohistochemistry against specific cell types: Sox2 for supporting sensory cells, Myosin7a for hair cells, Tubulin for nerve fibers and *Isl1* for ganglion cells. The sensory epithelium was additionally examined by electron microscopy. The overall morphology of the inner ear was reconstructed as a 3D structure.

Results

Isl1-Cre^{+/+}; Sox2^{f/f} mutant mice (Sox2 CKO) died immediately after birth although their gross morphology was normal. In contrast, the morphology of the mutant inner ear was severely affected. The vestibular system had disconnected semicircular canals, the utricle was small and spherical, the saccule was small, all three ampullae were missing and the cochlear duct was 20% shorter compared to controls. All sensory epithelia were significantly smaller in Sox2 CKO and showed variable numbers of hair cells and supporting cells. The expression level of Sox2 was decreased in the whole ear and Sox2 was never expressed in the cochlear apex. Only a few isolated hair cells differentiated in the base of the cochlea. A severe reduction of radial neuronal fibres was detected in Sox2 CKO embryos.

Conclusion

Our results demonstrate that the premature deletion of Sox2 in inner ear neurosensory precursors alters the development and the overall morphology of the inner ear with sensory areas being the most affected. These data indicate that Sox2 is necessary for the formation and differentiation of both sensory cells and neurons in the inner ear.

Tanaya Bardhan, Walter Marcotti, Matthew Holley
Department of Biomedical Science, University of Sheffield, UK

The zinc finger transcription factor Gata3 is expressed in neuronal, epithelial and mesenchymal tissue throughout development of the auditory system, from the otic placode to the mature sensory epithelium. It is widely expressed outside of the ear and influences numerous different cell behaviours. Null mice die in embryonic development and early, targeted deletions prevent inner ear development beyond the formation of a stunted otocyst. More subtle phenotypes have been observed following targeted deletion from the developing spiral ganglion. Haploinsufficiency for gata3 in man causes hypoparathyroidism, sensorineural deafness and renal dysplasia (HDR) syndrome and heterozygous mice suffer hearing loss that is apparently associated with functional deficits in outer hair cells (OHCs). However, the cause of hearing loss is unknown and the specific functions of gata3 are not clear. We studied the physiological differentiation of embryonic, neonatal and young adult hair cells in heterozygous gata3 mice and compared it with mice in which gata3 was deleted selectively in inner hair cells (IHCs) during embryonic development. We achieved this by using otoferlin to drive Cre-mediated excision of the floxed Gata3 alleles in vivo. In heterozygous mice, some OHCs died at early postnatal stages but they developed normal basolateral membrane currents and mechano-electrical transduction currents. However, mature IHCs showed functional deficits in $I_{k,f}$, which is the large conductance calcium-activated outward potassium current carried by BK channels, and in $I_{k,n}$, which is the negatively-activated, delayed rectifier potassium current carried by KCNQ4 channels. IHCs from which both gata3 alleles were selectively deleted from around embryonic day E16, showed a similar but stronger phenotype to that seen in heterozygous animals. This is the first evidence that Gata3 has a cell-autonomous function in the physiological differentiation of hair cells and it suggests that functional deficits in IHCs account for much of the hearing loss suffered by people with HDR syndrome.

Sarah Baxendale (1), Esther C. Maier (1), Nikolaus Obholzer (2), Sarah Burbridge (1), Nicholas van Hateren (1), Maria Montserrat Garcia Romero (1), Kazutomo Yokoya (3), Robert Knight (3), Sean G. Megason (2), Tanya T. Whitfield (1)

(1) Bateson Centre and Department of Biomedical Science, University of Sheffield, Sheffield, S10 2TN, UK; (2) Department of Systems Biology, Harvard Medical School, 200 Longwood Avenue, Boston, MA 02115, USA; (3) Department of Craniofacial Development and Stem Cell Biology, King's College London, London, WC2R 2LS, UK

Introduction

The complex structures of the zebrafish inner ear all form from a simple ball of epithelial cells. This process requires a highly orchestrated interaction of different signalling pathways to specify the many different cell types and structures. Morphogenesis of the semicircular canal system begins when projections of tissue, driven by extracellular matrix production, move into the lumen of the otic vesicle. Here, they fuse to form pillars of epithelium that span the vesicle lumen and become the hub of the developing canal. We have analysed a mutant, *cloudy*, that has truncated anterior and posterior canals.

Methods

To identify the *cloudy* mutation, we used RNAmapper. We have used in situ hybridization to evaluate temporal and spatial distribution of gene expression patterns in the otic vesicle in both wild-type and *cloudy* mutant embryos. Using a new transgenic line expressing GFP in the cell membranes of the otic epithelium, we have analysed the structural changes occurring during development and compared these in *cloudy* mutants at the cellular level using light-sheet microscopy. Finally, we have used automated tracking of fish movement to quantify behavioural defects associated with balance disorders.

Results

cloudy mutants have a premature stop mutation in the *bmp*er (BMP-binding endothelial regulator) gene. *Bmp*er protein is known to both promote and inhibit BMP function in different contexts. *bmp*er is expressed in the dorsal region of the otic vesicle and also in the cristae. Both BMP signalling and cristae have an important role in formation of the semicircular canal ducts in all species. In *cloudy* mutants, *bmp*er expression is strongly down-regulated, as is expression of some otic markers, including *dlx5*; however, gene expression in the endolymphatic duct is not affected. Light-sheet imaging reveals a change in the dorsal structures from 48 hours post fertilisation, when the epithelial projections fuse to form the pillars. Analysis of adult homozygous *cloudy/bmp*er mutants has identified a behavioural signature consistent with the structural defects observed in the semicircular canals.

Conclusions

This work describes a new role for *Bmp*er in regulating morphogenesis of the anterior and posterior semicircular canal ducts in the zebrafish inner ear.

Akira Honda (1), Tomoko Kita (1), Kazuyo Misaki (2), Zamal Ahmed (3), Guy Richardson (4), Shigenobu Yonemura (2), Raj K. Ladher (1)(5)

(1) Laboratory for Sensory Development, RIKEN Center for Developmental Biology, Kobe 650-0047, Japan; (2) Electron Microscope Laboratory, RIKEN Center for Developmental Biology, Kobe 650-0047, Japan; (3) Department of Biochemistry and Molecular Biology and Center for Biomolecular Structure and Function, University of Texas MD Anderson Cancer Center, Houston, TX 77030, USA; (4) School of Life Sciences, University of Sussex, Brighton BN1 9QG, UK; (5) NCBS, GKVK PO, Bellary Road, Bangalore, KA, India

The mechanosensory cells of the inner ear, the hair cells (HCs), detect sound and balance information through its distinctive apical architecture, the hair bundle. The hair bundle consists of the kinocilium and stereocilia, and the kinocilium is critical for proper hair bundle formation. The kinocilium can be considered a modified primary cilium, a cell surface organelle found on most epithelia in vertebrates. While the mechanisms of kinocilia specialization are not well understood, ciliary localization of protocadherin-15 (Pcdh15) is a key step. Here, we uncover a new role for fibroblast growth factor receptor 1 (FGFR1) in establishing the morphology of hair bundle. We find that upon activation, FGFR1 binds and recruits protocadherin-15 (Pcdh15) into the hair cell kinociliary transport pathway. Our results identify a kinociliary transport pathway, coordinated by FGFR1 signaling and regulating kinocilia specialization and subsequent hair bundle morphogenesis.

Muhammad Waqas (1,2,3), Luo Guo (4,5), Shasha Zhang (1,2), Yan Chen (4,5), Mingliang Tang (1,2), Huawei Li (4,5), Renjie Chai (1,2,3)

(1) State Key Laboratory of Bioelectronics, Institute of Life Sciences, Southeast University, Nanjing 210096, China; (2) MOE Key Laboratory of Developmental Genes and Human Disease, Institute of Life Sciences, Southeast University, Nanjing 210096, China; (3) Co-Innovation Center of Neuroregeneration, Nantong University, Nantong, China; (4) Department of Otorhinolaryngology, Hearing Research Institute, Affiliated Eye and ENT Hospital of Fudan University, Shanghai, China; (5) Central Laboratory, Affiliated Eye and ENT Hospital of Fudan University, Shanghai, China

Lgr5⁺ supporting cells (SCs) are enriched hair cells (HC) progenitors in the cochlea. Multiple studies have shown a difference in the proliferation and HC regeneration ability of SCs between the apical and basal turns. However, the detailed differences between the transcriptomes of the apical and basal Lgr5⁺ SCs have not yet been investigated. We found that when isolated by FACS, Lgr5⁺ cells from the apex generated significantly more HCs and had significantly higher proliferation and mitotic HC regeneration ability compared to those from the base. Next, we used microarray analysis to determine the transcriptome expression profiles of Lgr5⁺ progenitors from the apex and the base. We first analyzed the genes enriched and differentially expressed in Lgr5⁺ progenitors from the apex and the base. Then we analyzed the cell cycle genes and the transcription factors that might regulate the proliferation and differentiation of Lgr5⁺ progenitors. Lastly, to further analyze the role of differentially expressed genes and to gain an overall view of the gene network in cochlear HC regeneration, we created a protein-protein interaction network. Our datasets suggest the possible genes that might regulate the proliferation and HC regeneration ability of Lgr5⁺ progenitors, and these genes might provide new therapeutic targets for HC regeneration in the future.

Haibo Du (1), Panpan Chen (1), Yanfei Wang (1), Jinpeng Sun (2), Zhigang Xu (1)

(1) Shandong University School of Life Sciences, Jinan, Shandong 250100, China; (2) Shandong University School of Medicine, Jinan, Shandong 250012, China

Introduction: 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. Previously we showed that Vlgr1 could regulate G-protein signaling pathway through Goi coupling, resulting in adenylate cyclase (AC) inhibition. Noticeably, the deafness-associated Vlgr1 Y6236fsX1 mutant showed increased Goi coupling and enhanced AC inhibition activity.

Methods: To further explore the role of Vlgr1-mediated signaling pathway in hearing, we developed Vlgr1 Y6236fsX1 knock-in mice. The integrity of ankle-links was examined by SEM, and the auditory function was evaluated by ABR measurement. In addition, downstream target genes of Vlgr1-mediated pathway were identified by Microarray and validated by Q-PCR.

Results: ABR measurement revealed that the Vlgr1 Y6236fsX1 knock-in mice are profoundly deaf. SEM analysis showed that the hair bundle of knock-in mice are disorganized, but the ankle-links seem unaffected. Microarray analysis revealed that several genes are up-regulated in Vlgr1 knockout mice, which was confirmed by Q-PCR. Further examination showed that the expression of these genes is down-regulated in Vlgr1 Y6236fsX1 knock-in mice, consistent with our previous finding that Vlgr1 Y6236fsX1 mutant has increased Goi coupling activity.

Conclusions: Vlgr1 Y6236fsX1 knock-in mice are developed and are shown to be profoundly deaf. The ankle-links are unaffected in Vlgr1 Y6236fsX1 knock-in mice, whereas the expression of several Vlgr1-target genes is down-regulated, suggesting that Vlgr1-mediated signaling pathway might play an important role in hearing.

Makoto Hosoya (1,2), Masato Fujioka (1), Noriomi Suzuki (1), Hideyuki Okano (3), Kaoru Ogawa (1)
(1) Department of Otorhinolaryngology/HNS, Keio University School of Medicine; (2) The Laboratory of Auditory Disorders and Division of Hearing and Balance Research, National Institute of Sensory Organs, National Tokyo Medical Center; (3) Department of Physiology, Keio University School of Medicine

The rodent model has been contributing to the study of hereditary hearing loss. Particularly, gene modification (e.g. knock-out, knock-in, transgenic) is a powerful tool for investigating phenotypes of genetic diseases in detail to understand the pathophysiology. However, there are diseases of which mouse models cannot recapitulate human phenotypes, for example Pendred syndrome/DFNB4 H723R mutation in SLC26A4 gene. To circumvent such a pitfall and to further understand intractable cochlear sensorineural hearing loss, we have been investigating the physiology/ pathophysiology of monogenic, bilateral, gradual but fluctuating deafness using common marmoset (*Callithrix jacchus*), a non-human primate model, and human iPSC technology. In the talk I will first introduce our data showing differential expression patterns of deafness genes in the cochlea of marmoset in comparison with those in mouse. Second, I will present an example revealing human phenotypes of disease-state cochlear cells derived from patient derived-iPSCs harboring mutations in the SLC26A4 gene. I will provide data explaining why SLC26A4 mutant mouse models do not work, display a novel “degenerative phenotype” in the cochlear cells of Pendred syndrome that may account for the fluctuation in hearing, and moreover, candidate “rational” drugs preventing progressions. We anticipate that the combination of non-human primate experiments and iPSC-based in vitro model may be useful for translational studies investigating the pathogenesis of other forms of hereditary deafness, and idiopathic hearing loss, to identify new treatments for these conditions.



Aur lie Saleur (1), Volker Baecker (2), Jonas Dyhrfeld-Johnsen (1)

(1) Sensorion SA, Montpellier, France, (2) MRI - Biocampus Montpellier (US9-INSERM), Montpellier, France

The cochlea is the auditory organ of the inner ear, containing inner and outer sensory hair cells, organized in respectively one and three rows inside the organ of Corti. The cochlear spiral structure with tonotopic, sensorineural organization makes precise histological studies demanding, however the information provided supports and facilitates interpretation of functional audiometric studies.

We aim to develop automated algorithms for quantifying cochlear hair cells in sections of 200 μm from the cochlear apex to base to facilitate higher throughput histology for preclinical drug development. Similar approaches exist for other tissues, but none are applicable to the particular organization of the cochlea.

Temporal bones of adult Wistar rats were extracted following PFA fixation and the organ of Corti dissected after decalcification (EDTA 10%). Hair cells were immunolabelled with Myosin VIIA (1:1000)/Alexa Fluor 594 (1:700) conjugated donkey anti rabbit. 3D image stacks of the organ of Corti were obtained using a Zeiss Apotome Fluorescence microscope and the entire structure was reconstructed as a mosaic of 4 stacks.

From the stitched 3D stack a binary mask of the inner and outer hair cells was created using the spot detection algorithm of Imaris. The mask image stack was imported into ImageJ. A maximum intensity projection was applied. The user selected cochlea was then straightened. On the straightened cochlea the cells are automatically counted for each segment of 200 μm using an ImageJ macro (available from http://dev.mri.cnrs.fr/projects/imagej-macro/wiki/Cochlea_Hair_Cell_Counting).

When comparing manual versus automatic counts on 3 cochleae, we have obtained a good reproducibility for median and base parts as well as the major part of the apex. At the very top of the apex, comparison was complicated by some deformation of the hair cells rows, rendering 2D visual identification of hair cells difficult for manual counting. However the Imaris algorithm efficiently identified hair cells in 3D, permitting better cell counts. Further manual counting using 3D stacks by 2 separate operators will facilitate additional comparisons.

In summary, preliminary results show, that using this automated algorithm for cochlear hair cell counting reliably reproduces the manual counts in the majority of the cochlear tissue, with significant gains in time.

Bullen, A., Whittaker, M., Bakay, W., Forge, A.
UCL Ear Institute, 332 Gray's Inn Road, London, WC1X 8EE

Introduction

Cellular structures are adapted specifically to support a cell's function, and damage to such structures is an important factor in disease. In order to correctly characterise such structures it is important that they are examined in three dimensions, and with sufficient resolution to understand the relationships between intracellular components. We have previously used 3D electron microscopy techniques to examine the cellular architecture of outer hair cells, and in this work we combine such techniques to examine two features in different hair cell types, the mitochondrial arrangements of outer hair cells, and the effects of noise on inner hair cells in a mouse model of 'hidden hearing loss'.

Methods

Cochleae from normal gerbils and mice, and from mice that had undergone noise exposure were fixed and prepared for electron microscopy. Samples were examined by TEM, Electron Tomography (ET), Serial Block Face Scanning Electron Microscopy (SBF-SEM) or array tomography SEM methods. Data was analysed by semi-manual reconstruction and stereological methods.

Results

In outer hair cells, our examination of mitochondrial size, shape and distribution revealed relationships between mitochondrial shape and location and indicated that a connection between spatial arrangements and varying morphologies of mitochondria are present across species.

For inner hair cells (IHCs), comparing our previous work on IHC structural arrangements with cells that had undergone a damaging noise exposure showed changes that encompassed a large number of neurons and severely affected the shape of the inner hair cell. Structural changes were also shown at IHC synaptic regions.

Conclusions

For both inner and outer hair cells, the use of 3D electron microscopy techniques allows the examination of ultrastructure at high levels of resolution, across several cells and in context with other organelles and intracellular structures. In outer hair cells, this has identified a relationship between mitochondrial morphology and spatial arrangement, preserved between species, that may indicate arrangements crucial to the normal functioning of OHCs. In IHCs, significant impacts to IHCs and surrounding structures were shown, more severe than was expected from previous work. In the synaptic regions, concomitant changes occur within IHCs when neurons are damaged.

Tommi Anttonen(1), Ulla Pirvola(1)

(1) Department of Biosciences, University of Helsinki, Finland

INTRODUCTION

Auditory supporting cells of lower vertebrates are able to generate new hair cells to replace lost ones. Mammalian supporting cells have lost this regenerative capacity and concurrently gained rigid apical cytoskeletal specializations. Closure of the surface of the organ of Corti by supporting cells upon hair cell loss is vital for the maintenance of surviving cells, as failure to do so leads to additional damage. Understanding how this repair process is performed by supporting cells is important, as this process should not be impaired by the possible hair cell regeneration or protection interventions.

METHODS

To characterize the initial steps of epithelial wound healing in the organ of Corti, acute and fast loss of outer hair cells was induced by injections of kanamycin and furosemide. Ototoxicologically challenged cochleas were analyzed with serial block-face electron microscopy and light microscopy 36–48 hours post-lesion.

RESULTS AND CONCLUSIONS

By studying the acute stages of wound healing in the organ of Corti *in vivo*, we have observed that the apical junctional domains of supporting cells, including cell adhesion proteins, lag behind the membrane extensions of the same cells that close the epithelial surface. Therefore, the leading edge of the closing membrane extensions does not originate solely from the apical junctional domains. This is supported by the findings that supporting cells that lack junctional contacts with dying hair cells contribute to the surface repair process: They send long processes of their basolateral membrane to the epithelial surface. On the other hand, inner pillar cells, that have apical junctions with dying hair cells, do not contribute to wound healing. These observations show that junctional domains are not sufficient for the capacity of supporting cells to contribute to the epithelial surface repair. Instead, the proximity of the basolateral membrane of supporting cells to dying hairs cell seems to be more important. This is supported by the observations that Deiters' cells recruit their basolateral membrane to surface closure sites in an asymmetric fashion. Finally, these observations suggest that the signal for epithelial repair is, in part, diffusible and not exclusively contact-dependent.

Hanan Al-Moyed (1), Gerhard Hoch (2), Carolin Wichmann (3), Nicola Strenzke (4), Ellen Reisinger (1)

(1) *Molecular Biology of Cochlear Neurotransmission Group*, (2) *Institute for Auditory Neurosciences*, (3) *Molecular Architecture of Synapses Group*, (4) *Auditory Systems Physiology Group*, (1-4) *Inne-rEarLab, University of Goettingen, Germany*

Intro: Otoferlin, a protein that plays an essential role in inner hair cell exocytosis, is known to be located at synaptic vesicles, intracellular membranes, and the plasma membrane. The human Ile515Thr mutation found in the OTOF gene has been associated with auditory fatigue, speech comprehension deficits, and temperature sensitive hearing loss. Here, we investigated the correlation between the phenotype caused by this mutation and both the subcellular localization and protein levels of otoferlin, using the Otof1515T/I515T mouse model.

Methods: Otoferlin protein levels and subcellular localization were compared via immunohistochemistry between wild type and two otoferlin mouse models: Otof1515T/I515T, and pachanga. To study the effects of temperature, wild type and Otof1515T/I515T cultured inner hair cells were incubated at 35.5°C, 37°C, and 38.5°C and analyzed with fluorescence immunohistochemistry and confocal microscopy. The ultrastructural localization of otoferlin and its distribution among different cellular compartments in both genotypes were studied through pre embedding immunogold electron microscopy.

Results: Both Otof1515T/I515T and pachanga mutant inner hair cells exhibit similar reduced overall otoferlin protein levels, but differ in the amount of plasma membrane bound otoferlin. Whereas almost no otoferlin was bound to the plasma membrane in pachanga, otoferlin's distribution in Otof1515T/I515T was similar to wild type. Notably, in Otof1515T/I515T inner hair cells, otoferlin overall and plasma membrane levels decreased further when subjected to elevated temperature. The immunogold EM data show prominent otoferlin localization at the plasma membrane as well as at endosomal structures, but not at synaptic vesicles in both wild type and Otof1515T/I515T.

Conclusion: The different amounts of plasma membrane bound otoferlin seem to correlate with the sustained exocytosis levels and hearing phenotypes found in all three genotypes, that range from normal hearing (wildtype) to mild (Otof1515T/I515T) and profound hearing loss (pachanga). Furthermore, temperature elevation lowered the levels of plasma membrane bound otoferlin in the Otof1515T/I515T mouse model, providing a potential explanation for the temperature sensitive hearing loss in humans.

Gulnara Yamanbaeva, Sang Yong Jung, Man Ho Wong, Nicola Strenzke

(1) InnerEarLab, University Medical Center Göttingen, Göttingen, Germany

(2) Institute for Auditory Neuroscience and InnerEarLab, University Medical Center Göttingen, Göttingen, Germany

(3) European Neuroscience Institute Göttingen, Göttingen, Germany

(4) InnerEarLab, University Medical Center Göttingen, Göttingen, Germany

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

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

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

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

Stefanie Krinner (1,2,4), SangYong Jung (1,3), Carolin Wichmann (1,2,5), Tobias Moser (1,2,3,4)

(1) InnerEarLab, Institute for Auditory Neuroscience, University Medical Center Göttingen (UMG)

(2) Collaborative research Center 889, UMG

(3) Nanoscale Microscopy and Molecular Physiology of the Brain (CNMPB), Göttingen

(4) IMPRS MSc/PhD Program Molecular Biology & Neuroscience, International Max-Planck research School & GGNB Graduate School, Göttingen,

(5) Molecular Architecture of Synapses, Institute for Auditory Neuroscience, UMG

Introduction

The ribbon synapse of inner hair cells (IHCs) mediates very rapid exocytosis and sustains high rates of synaptic vesicle fusion (SV) to indefatigably encode sound. This is achieved through the tight coupling of voltage-gated calcium channels (VGCC) to the SV release machinery and through a highly efficient SV pool organization and SV replenishment. The molecular machinery in IHC active zones (AZs) is highly specialized and differs remarkably from that in neurons. Many proteins that are essential for the regulation of conventional neuronal SV fusion are apparently absent from IHCs. Moreover, several proteins were identified to be uniquely expressed or enriched in IHCs and were shown to be essential components for IHC SV fusion. Disruption of e.g. Otoferlin can cause deafness in mouse models and humans. One synaptic protein common to both IHC and neuronal synapses is the evolutionary conserved RIM-binding protein (RIM-BP) that interacts with VGCC and RIM. Here, we tested the high significance of RIM-BP2 at regulating the fidelity of synaptic transmission at IHC AZs.

Methods

We took a multidisciplinary approach to elucidate the function of RIM-BP2 in IHC SV fusion. IHCs of freshly dissected organs of Corti (P14-21) from RIM-BP2 knock-out mice (RIM-BP2^{-/-}) and littermates (RIM-BP2^{+/+}) were used for capacitance and ICa measurements by perforated patch-clamp recordings, confocal life-cell calcium imaging, immunohistochemistry combined with confocal and STED microscopy and transmission electron microscopy.

Results & Conclusion

A first glimpse at the morphology of RIM-BP2^{-/-} IHC ribbon synapses indicates a preserved AZ structure and synapse number. However, whole-cell patch-clamp recordings revealed a moderately reduced calcium influx and significant reduction of rapid and sustained SV exocytosis, most prominent during 50 ms depolarization. Paired pulse and train stimulation experiments revealed a replenishment phenotype for short recovery intervals and thus suggest that RIM-BP2 might be a molecular determinant responsible for organizing a fast-recycling SV pool. It could mobilize and position SVs in close vicinity to VGCC (positional priming) in order to replace rapidly released SVs (RRP). This mechanism might enable IHCs to respond to small, graded changes in membrane potential and to recover RRP SVs efficiently and quick.

Hiroe Ohnishi (1), Shin-ichiro Kitajiri (1), Akiko Taura (2), Mirei Taniguchi (1), Fumi Ebisu (3), Tatsunori Sakamoto (4), Norio Yamamoto (1), Juichi Ito (1)(5), Koichi Omori (1), Takayuki Nakagawa (1)

(1) *Department of Otolaryngology, Kyoto University - Kyoto - Japan*

(2) *National Hospital Organization Kyoto Medical Center - Kyoto - Japan*

(3) *Osaka University - Osaka - Japan*

(4) *Kitano Hospital - Osaka - Japan*

(5) *Shiga Medical Center Research Institute - Shiga - Japan*

Introduction: Many responsible genes for hereditary hearing loss have been identified. We have been focusing on TRIOBP, which is one of these deafness genes. Stereocilia are composed of actin bundles which form rootlets extending into the cytoplasm. TRIOBP is localized at the rootlets of stereocilia and loss of TRIOBP causes defect of rootlets and hearing loss after birth. Until now, Triobp KO mice were used for functional analysis. However, cochlea explants culture is inefficient in gene transfer. To facilitate the gene transfer experiments in Triobp KO mice, we aimed to establish Triobp KO in vitro model using induced pluripotent stem (iPS) cells.

Method: We generated iPS cells from Triobp KO mouse embryonic fibroblasts (MEFs) using retrovirus gene transfer system. Then we characterized the obtained embryonic stem (ES) cell-like cell. We examined the expression of pluripotent stem cell marker, differentiation potency into three germ layer cells, genotype and transgene silencing of these ES cell-like cells. Then, we examined the hair cell induction of Triobp KO mouse-derived iPS cells.

Results: We obtained five lines of ES cell-like cells. We confirmed pluripotent stem cell marker expression and differentiation potency, transgene silencing and Triobp KO mouse genotype of these ES cell-like cells. Based on these results, we determined that these ES cell-like cells are Triobp KO mouse MEF-derived iPS cells (TRIOBP KO iPS cells). After examination of hair cell induction from TRIOBP KO iPS cells, we obtained PAX2 positive otic progenitor cells.

Conclusion: We generated TRIOBP KO iPS cells and confirmed differentiation potency of these iPS cells into otic progenitors. These results suggest that TRIOBP KO iPS cells could be differentiation potency into hair cell.

Lorcan Browne (1), Katie Smith (1), Dan Jagger (1)
(1) UCL Ear Institute, University College London, UK

Type I Spiral Ganglion Neurons (SGNs) transmit acoustic information from inner hair cells to the brainstem. In vivo, these neurons can have high spontaneous firing rates (~50 spikes/s), and are capable of firing up to 300 spikes/s in response to sound (Winter et al, *Hear Res* 1990). Action potentials are generated at the initial segment of the SGN peripheral neurites, and are then propagated between nodes of Ranvier by classical saltatory conduction (Hossain et al, *J Neurosci* 2005). Voltage-gated Na^+ (Nav) and K^+ (Kv) channels are targeted differentially at the spike initiation site and nodes (Smith et al, *J Neurosci* 2015), in a spatial pattern that optimises temporal precision of action potentials. Here we re-examined Na^+ currents (INa) in cultured SGNs from hearing mice, in order to elucidate how they might maintain high firing rates.

Whole-cell patch clamp recordings were made from SGNs from juvenile C57BL/6 mice (P12-P14), cultured for 1-3 days (Smith et al, *J Neurosci* 2015). Initial recordings were carried out in normal Na^+ -containing bath solution, and Cs^+ -based pipette solution was used to minimise IK. In some experiments the bath solution had a lowered Na^+ concentration (ensuring adequate voltage clamping of INa) and it was supplemented with IK and ICa blockers.

Stepwise depolarisations from hyperpolarised holding potentials activated large, voltage-dependent transient inward currents. Sensitivity to 300 nM Tetrodotoxin (TTX) identified these as INa. INa displayed activation and inactivation in the canonical manner, but on repolarisation to potentials between -60mV and -20mV in some SGNs there was a slowly rising and slowly decaying current. This current was also sensitive to TTX, and bore hallmarks of the “resurgent” INa that supports spontaneous and persistent fast-spiking in cerebellar Purkinje neurons (Lewis & Raman, *J Physiol* 2014).

A resurgent INa in SGNs provides a mechanism for fast spiking by making Na^+ channels available at depolarised sub-threshold membrane potentials. We are presently assessing the distribution of resurgent INa in SGNs, and how it might contribute to heterogeneous firing behaviour in SGNs.

Gregory Heard (1), Lorcan Browne (1), Katie Smith (1), Dan Jagger (1)
(1) *UCL Ear Institute, University College London, UK*

Spiral ganglion neurons (SGNs) are essential during normal acoustic hearing, and when electrical hearing is provided by cochlear implants. Elsewhere in the nervous system there is a growing appreciation of complex neuro-glial communication which regulates action potential propagation and supports neuronal longevity. Here we examined the potential for purinergic neuro-glial communication in the cochlea.

Dissociated cochlear cultures were prepared from neonatal or juvenile rats (P6 or P14). Whole-cell voltage clamp recordings were performed on dissociated glia cultured for 1-3 days in vitro. Purinergic agonists were applied locally via a pico-spritzer, and antagonists were applied in the bath. Confocal immunofluorescence was performed on formaldehyde-fixed cochlear cultures or vibratome slices to determine the localisation of purinergic receptors.

Patch recordings from putative glial cells revealed voltage-gated currents consistent with those described elsewhere (see Smith et al, this meeting). Brief exposure to the P2X7 agonist BzATP (10 μ M, 1-2s) activated non-desensitising inward currents which grew in magnitude with successive applications. These currents were blocked approximately 90% by the P2X7-specific antagonists A-740003 (100nM) or JNJ-47965567 (100nM). Prolonged exposure to BzATP (>10s) activated a distinct secondary conductance. In both dissociated cultures and cochlear slices, P2X7 immunofluorescence was localised to glial cells but not to SGNs.

In summary, cochlear glial cells express functional P2X7 channels which may play roles in physiological neuro-glial signalling. These ionotropic receptors have been implicated in the regulation of cell death pathways and the release of large molecules under normal and pathological conditions, and so we are currently examining their contribution to auditory nerve function during normal hearing and in conditions associated with hearing loss.

Maike Pelgrim (1), Gulnara Yamanbaeva (1), Marcus Jeschke (2), Ellen Reisinger (3), Nicola Strenzke (1)

(1) Auditory Systems Physiology Group, Department for Otolaryngology and InnerEarLab, University Medical Center Göttingen

(2) Institute for Auditory Neuroscience, University Medical Center Göttingen; Auditory Neuroscience Group, German Primate Center, Göttingen

(3) Molecular Biology of Cochlear Neurotransmission Group, Department for Otolaryngology and InnerEarLab, University Medical Center Göttingen

Introduction: Mutations in the hair cell specific protein otoferlin lead to hearing impairment or deafness. In human patients the I515T point mutation causes only a mild increase in hearing thresholds but a severe speech perception deficit and auditory fatigue. Here, we analyzed hearing function in Otof I515T/I515T mice, in which inner hair cell exocytosis is impaired during prolonged stimulation.

Methods: To analyze sound encoding in Otof I515T/I515T mice, we use recordings of auditory brainstem responses, single unit recordings from the auditory nerve and the central nucleus of the inferior colliculus and behavioral studies. Specifically, we analyze sound thresholds, frequency tuning, rate-intensity-functions, phase locking to amplitude modulated tones and responses to paired stimuli.

Results: The Otof I515T/I515T mice have elevated ABR thresholds and a reduced amplitude of ABR wave I, while subsequent peaks are better preserved. Single units from the auditory nerve fibers show normal spontaneous spiking, frequency tuning and thresholds, but reduced spike rates and increased jitter with a striking dependence on the repetition rate and duration of the stimulus. In response to paired stimuli, the response to the second pulse is reduced and longer silent intervals are required for recovery. Preliminary results show similar changes in single neurons of the inferior colliculus. Consistently, experiments using prepulse inhibition of the startle response and operant conditioning show an impairment of the perception of silent gaps in background noise.

Conclusion: Single unit recordings from Otof I515T/I515T mice indicate an unusual sound encoding deficit with a use-dependent reduction of spike rates which we believe to reflect impaired vesicle reformation at the inner hair cell ribbon synapse. The peripheral deficit in the encoding of amplitude modulated and paired tones cannot be compensated at least up to the level of the inferior colliculus. The resulting gap detection deficit likely contributes to the communication problems of human patients with otoferlin mutations.



Katie Smith, Phoebe Murphy, Lorcan Browne, Anwen Bullen, Dan Jagger
UCL Ear Institute, University College London, London, UK

The auditory nerve transmits acoustic information from cochlear hair cells to the brainstem. Whilst the electrophysiological properties of spiral ganglion neurons have been well characterised, very little is known about the properties of their closely associated glial cells. At least three types of glial cells are associated with spiral ganglion neurons: satellite glial cells (SGCs) which wrap around the neuronal somata, and myelinating or non-myelinating Schwann cells which ensheath afferent neurites. Here we examined the membrane properties of these glial cells with a particular focus on SGCs.

Dissociated cultures were prepared from the cochleae of hearing mice (C57Bl/6; postnatal day P14-P15). Whole-cell voltage clamp recordings were performed on glial cells cultured for 1-3 days *in vitro*. Immunofluorescence was performed on paraformaldehyde-fixed dissociated cochlear cultures and vibratome sectioned cochlear slices. Polyclonal antibodies were used to determine the localisation of inwardly-rectifying K⁺ (Kir) channel subunits.

Three broad electrophysiological profiles were identified amongst the population of glia: cells with a large weakly inward-rectifying K⁺ current (IKir), cells with a delayed outward-rectifying K⁺ current and cells with both inward and outward-rectifying K⁺ currents. Glial cells wrapped around neuronal somata in acutely dissociated cochlear preparations also displayed large IKir suggesting that this current was associated with SGCs. The weakly rectifying IKir was blocked by 100 μ M Ba²⁺, a non-specific Kir channel blocker, and 100 μ M desipramine which blocks Kir3 and Kir4 channels. Kir4.1 immunofluorescence was localised to SGCs in both dissociated cultures and cochlear sections suggesting that Kir4.1 channels likely mediated the weakly rectifying IKir.

Cochlear glial cells have distinct biophysical profiles which may reflect their physiological roles *in vivo*. SGCs have a large weakly inward-rectifying K⁺ current likely mediated by Kir4.1 channels. Further studies are required to determine the precise role of Kir4.1 in SGCs which may act to preserve neuronal excitability by buffering K⁺ in the extracellular space and/or ensuring a negative resting membrane potential to optimise uptake of neurotransmitters.

I. Prigioni, E. Tavazzani, P. Spaiardi, M. Manca, G. Russo, S. Masetto
Department of Brain and Behavioral Sciences, University of Pavia, Pavia, Italy

Mammalian vestibular epithelia have a distinctive sensory cell, called Type I hair cell, that is contacted by an afferent calyx enveloping the entire cell basolateral membrane. Type I cells express a signature low-voltage-activated outward rectifying K⁺ current, IK,L, which is responsible for their low input resistance at rest. Despite its functional importance, however, IK,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 IK,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, IK,L properties in adult animals were in fact consistent among cells and did not change during the recording. IK,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 IK,L, and suggests that in vivo IK,L properties are dependent on K⁺ accumulation into the synaptic cleft. Intercellular K⁺ accumulation might represent a direct way to change both the hair cell and the calyx membrane potential, thus allowing an additional form of communication that cooperates with the conventional glutamatergic synaptic transmission.

Norio Yamamoto, Yosuke Tona, Tomoko Kita, Hiroe Ohnishi, Takayuki Nakagawa, Koichi Omori
Dep. Otolaryngology, Head and Neck Surgery, Graduate School of Medicine, Kyoto University

Introduction

Since the cochlear development takes a complex process and it is composed of various types of cells with different characteristics, comprehensive analysis of gene expression on a single-cell basis is important. For this purpose, most of studies obtain a single target cell using a cell sorter from transgenic mice expressing fluorescent protein. Although this method is quite efficient, two factors can change the physiological gene expression patterns. One is the usage of transgenic system. Insertion of transgene of fluorescent protein in the genome can affect the enhancer or promoter of some genes. Even knock-in of fluorescent protein gene may cause the haploinsufficiency of the targeted gene. The other factor is the cell sorter. When a single cell runs through the sorting tube, the quite fast run-through and narrow diameter of the tube distort the morphology of the single cell, causing the gene expression pattern change.

The amplification of cDNA also raises a problem of non-linearity. In other words, genes whose expression level is low are not amplified sufficiently.

To overcome these problems, we established a technique to pick up a single cell from dissociated cochlear and to amplify all genes linearly.

Methods

We dissect out the embryonic cochlea and divide the epithelia and mesenchyme using Thermolysin. After dissociation of the cochlear epithelia, we pick up a single cell using glass pipette with diameter of 40 μm . The picked-up cells are subject to the extraction of mRNA and the established cDNA amplification protocol (Kurimoto et al. 2006). The method directionally amplifies cDNAs highly representatively from single cells using relatively few PCR cycles and achieve the linear amplification of cDNA.

Results

We picked up 144 single cells. Two different house keeping genes were detected in the cDNA of 102 samples. Quality of the cDNA was confirmed by checking the RNA degradation plot using microarray results. Several well-known genes related to the cochlear sensory region were detected in cDNA from Sox2 positive single cells on E13.5.

Conclusion

Our method for the single cell analysis of the cochlear development will be beneficial to detect unknown factors that affect the cochlear development.

Thore Schade-Mann, Denise Pirritano, Jutta Engel, Tobias Eckrich
Center for Integrative Physiology and Molecular Medicine (CIPMM), Homburg, Germany

The tectorial membrane (TM) is essential for a normal hearing function. It stretches across the sensory epithelium of the inner ear from the interdental cells (IDCs) towards the outer hair cells making contacts with their stereocilia. It is spiralling along the longitudinal axis of the cochlea and consists of collagens and glycoproteins. Mutations of these proteins lead to aberrant TM formation and deafness. During the critical period of cochlear development, IDCs are thought to secrete TM proteins into the endolymph, but little is known about the physiology of IDCs and TM formation. Thus, we stained neonatal cochlear cryosections and performed Ca^{2+} imaging of acutely dissected organs of Corti at postnatal day 1, 4-5 and after the onset of hearing, using the Ca^{2+} indicator Fluo-8 AM (Fluo-4 derivative). Neonatal IDCs generated autonomous Ca^{2+} transients at a rate of ~ 1 event/10 min. Those Ca^{2+} signals were variable in shape and duration. Applying $10 \mu\text{M}$ ATP or UTP evoked Ca^{2+} oscillations in all IDCs at ~ 0.1 Hz, whereas $1 \mu\text{M}$ led to oscillations in only part of the IDCs at ~ 0.05 Hz. Furthermore, the developmental age had a significant impact on spontaneous events and the response to ATP/UTP. Ca^{2+} transients never spread to neighbouring cells. Our results emphasize the role of IDCs during the critical phase of cochlear differentiation.



Lejo J Chacko 1, Elisabeth J. Pechriggl, 2 Helga Fritsch, 2 Helge Rask-Andersen, 4 Michael J.F. Blumer, 2 Anneliese Schrott-Fischer, 1 Rudolf Glueckert 1,3

(1) Department of Otolaryngology, Medical University of Innsbruck, Anichstr. 35, 6020 Innsbruck, Austria

(2) Department of Anatomy, Histology, and Embryology, Division of Clinical and Functional Anatomy, Medical University of Innsbruck, Muellerstrasse 59, 6020 Innsbruck, Austria

(3) University Clinics Innsbruck, Tirol Kliniken, Anichstr. 35, Innsbruck, Austria

(4) Department of Otolaryngology, Uppsala University Hospital, 751 85, Uppsala, Sweden

Balance orientation depends on the precise operation of the vestibular end organs and the vestibular ganglion neurons. Previous research on the assemblage of the neuronal network in the developing foetal vestibular organ has been limited to data from animal models. Insights into the molecular expression profiles and signalling moieties involved in embryological development of the human foetal inner have been limited. We present an investigation of the vestibular system with specific focus on the hair cell differentiation and innervation pattern using an uninterrupted series of unique specimens from gestational weeks 8 to 12. Peripherin positive nerve fibres innervate the entire crista and utricle rather than innervating only the peripheral regions of the cristae and the extra-striolar region of the statolithic organs in chinchillas, rat and mice. At week 9 transcription factors PAX2 & PAX8 were observed in the hair cells whereas PAX6 was observed for the first time among the supporting cells of the cristae and the satellite glial cells of the vestibular ganglia. Glutamine synthetase, a regulator of the neurotransmitter glutamate is strongly expressed among the transitional zones of the utricle and supporting cells in the sensory epithelium indicating early onset of glutamatergic activity.

Our study provides first-hand insight into the foetal development of the vestibular end organs as well as their innervation patterning by means of immunohistochemical and electron microscopic techniques contributing towards our understanding of balance development.

Heike Schmitt (1,2), Ariane Roemer (1,2), Carsten Zeilinger (3), Rolf Salcher (1,2), Andreas Pich (3), Thomas Lenarz (1,2), Hinrich Staecker (4), Athanasia Warnecke (1,2)

(1) Department of Otolaryngology, Hannover Medical School, Carl-Neuberg-Str. 1, 30625 Hannover, Germany

(2) Cluster of Excellence of the German Research Foundation (DFG; "Deutsche Forschungsgemeinschaft") "Hearing4all"

(3) Biomolecular Research Centre Hannover (BMWZ)

(4) Department of Otorhinolaryngology, University of Kansas

Objective: Data about the etiology and pathophysiology of inner ear (IE) diseases leading to hearing loss and especially changes of the composition of the IE fluid perilymph are still very limited. This is mainly due to the difficult access to structures and fluids of the IE. Heat shock proteins (HSP) belong to a superfamily of stress proteins and promote refolding of denatured proteins. Interestingly, HSP may either prevent or promote cell injury. The aim of the study was to analyze the presence of HSP in human perilymph (HP) derived from cochlear implant patients and to correlate their presence with audiological and etiologic data.

Methods: HP sampling was performed during IE surgeries like CI implantations and vestibular schwannoma surgeries with translabyrinthine approach via the round window or the semicircular canal. Individual proteins were identified by a shot-gun proteomics approach and data-dependent analysis using orbitrap mass spectrometry (Thermo Fisher Scientific) and Max Quant software for identification. The residual hearing of patients was determined by prae- and postoperative data and compared with different HSP identified in HP. Also, differences in HSP occurrence of children and adults and vestibular schwannoma patients were analyzed.

Results: 10 subgroups of HSP were identified in HP samples. Only 33% of the patients with protected residual hearing showed an expression of HSP90. Whereas in two of three that lost their hearing, HSP90 (alpha and the beta subtype) were identified. In perilymph of all patients with preserved residual hearing, HSP70 (subtypes 1 and 6) was identified, whereas subtype 4 was identified in only 17%.

Conclusions: In-depth proteome analyses of HP samples in correlation to patients' audiogram data leads to the hypothesis that HSP70 is associated with preservation of residual hearing after cochlear implantation, whereas HSP90 is associated with loss of residual hearing. An increase in HSP due to tumour progression was not visible.

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Wei Liu¹, Nadine Scharf-Moren¹, Heval Benav², Rudolf Glueckert³, Carolyn W. Garnham², Annelies Schrott-Fischer³, and Helge Rask-Andersen¹

¹Department of Surgical Sciences, section of Otolaryngology Head and Neck Surgery, Uppsala University Hospital, ²Research and Development, MED-EL GmbH, Innsbruck, Austria, ³Department of Otolaryngology, Medical University of Innsbruck, Austria

Introduction: The generation sites and mode of action potentials (APs) in the various portions of the human cochlear nerve (CN) is unknown but voltage-dependent sodium channels Na⁺ (Nav) assumingly play a significant role. Voltage-dependent K⁺ channels (Kv) and Ca⁺⁺ (VGCCs) may be essential for the modulation of APs. Known expression and localization is essential for better understanding effects of electric stimulation of the CN under various conditions. **Methods:** To analyze the expression of Na⁺ and K⁺ channels in human CN and organ of Corti (Nav1.1 Nav1.2 Nav1.3 Nav1.6, Nav1.7 Nav1.8, Nav1.9, Kca1.1, Kv1.1, Kv1.2, Kv1.3, Kv3.1b, Kv7.1, Kir4.1, as well as ion transporters) using immunohistochemistry in surgically obtained tissue. Confocal and super-resolution fluorescence structured illumination microscopy (SIM) were used. Image and data precision were evaluated from from ZEN calibration on 40 nm beads. A lateral precision of approximately 80 nm and 250 nm axially were obtained. **Results:** We identified for the first time the sodium channel Nav1.6 in the node of Ranvier with associated Caspr in the juxtaparanode of the human spiral ganglion. In hemi-node (beneath the habenular perforata) and some axon hillocks, the channel protein and caspr could be identified. Apart from Nav 1.6, other Na⁺ channels were found in the cochlea. Kv1.1 and Kv1.2 were present in the juxtaparanodal region together with contactin associated protein (Caspr) around the node of Ranvier. Kv7.1 as well as the transporter NKCC1 in spiral ganglion neurons. Kca1.1 (BK channel)-immunoreactivity was found in spiral ganglion neurons as well as in hair cells stereocilia. Our results on Ca⁺⁺ channels are still preliminary. **Conclusions:** The study results showed the importance of Nav1.6, coordinating with other channels, for the generation of action potentials in the human CN. **Acknowledgements** The study was performed together with and partly funded by the MED-EL GMBH, Fuerstenweg 77a, 6020 Innsbruck, Austria. This study was also supported by ALF grants from Uppsala University Hospital and by the Foundation of "Tysta Skolan," the Swedish Deafness Foundation (HRF) and generous private donations from Börje Runögård and David Giertz, Sweden.

Kaori Hatakeyama, Ichiro Fukunaga, Ayumi Fujimoto, 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 occurred 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 were generated with HeLa cells or HEK293 cells. Gap junction plaques were immunolabeled by the antibodies for Cx26 and Cx30. Cx26-Hemichannels were detected by the specific antibody against Cx26 extracellular loop (EL).

Results: With these connexin expressing cell lines, we observed various genotypes of GJPs as well as Cx26-mutant mouse cochleae. Our cell lines enabled us to analyze the GJP assembly, trafficking, membrane integration and degradation of GJPs composed of Cx26 and Cx30. In this system, we observed the difference of incorporated gap junction plaque according to the types of GJPs. The antibody against Cx26-EL enabled us to visualize the Cx26 hemichannel together with gap junction plaque formations.

Conclusions: These in vitro gap junction formation will enable the large scale drug screening targeting GJP formation for Cx26 associated deafness.

Young Sang Cho (1), Ki Eun Lee (1), Ji Eun Choi (1), Hyun Woo Park (1), Yi-Kyung Kim (2), Hyung-Jin Kim (2), Won-Ho Chung (1)

(1) Department of Otorhinolaryngology-Head & Neck Surgery, Samsung Medical Center, Sungkyunkwan University School of Medicine, Seoul, Korea

(2) Department of Radiology, Samsung Medical Center, Sungkyunkwan University School of Medicine, Seoul, Korea

Introductions:

This study aimed at investigating the usefulness of IV Gd enhanced MRI in diagnosis of Meniere's and comparing disease the degree of endolymphatic hydrops with audiogram.

Material and methods:

Fifteen patients diagnosed with definite Meniere's disease according to the AAO-HNS criteria were enrolled in the study. All patients underwent heavily T2-weighted MRC, hT2W 3D FLAIR with inversion time of 2250ms (positive perilymph image), and hT2W-3D-IR with inversion time 2050ms (positive endolymph image) 4 hours after intravenous administration of single-dose gadolinium-based contrast material. Two different radiologists semi-quantitatively measured the size of the endolymph for the cochlear and vestibule by setting a threshold pixel value.

Results:

The cochlear and vestibular hydrops was significantly observed in affected ear than unaffected ear. The mean ratios of endolymph in cochlea were 0.31 for affected ear and 0.19 for unaffected ear (p

Conclusion:

This study presents that Gadolinium enhanced MRI scans via intravenous route is a very useful diagnostic method for endolymphatic hydrops in Meniere's disease. The cochlear and vestibular hydrops was significantly correlated with average pure-tone thresholds.

Naoyuki Kanoh

Ear Nose Throat Doctor, Kobe, Japan

Introduction: Recently, gene discovery approaches have led to understand the human circadian behaviors, including some diseases. The author previously reported that idiopathic facial palsy (Bell's Palsy) occurred during sleep.

Today, in order to clarify why the vertigo attack in most of the patients with Meniere's disease occurred late in the morning (Prof. Manabi Hinoki, Kyoto University), the author reviewed my reported data, and together with some bibliographical investigations, considering from the point of the hormonal circadian rhythm.

Methods and Results: The author reported that adrenergic hormonal control or regulation is essential for the maintenance of the inner ear homeostasis, especially for the inner ear electrolytes and/or stria Na-K ATPase activity using inner ear fluid collection, Na and K concentration determination, and ultracytochemistry.

Recent literatures reported the circadian adrenergic hormones rhythm was determined, namely, the norepinephrine and epinephrine level decreased during sleep, and increased in the morning and day. My previous data showed the adrenergic agents increased the stria Na-K ATPase activity and considered to increase endolymph production.

Conclusion: Increased endolymph production late in the morning is thought to be the one of the reasons why the vertigo attack in Meniere's disease occurred late in the morning.

In the near future, circadian gene approach to the patients with Meniere's disease will require through the point of the humoral (hormonal) regulation.



Ayumi Fujimoto (1), Ichiro Fukunaga (1), Kaori Hatakeyama (1), Atena Nishikawa (1), Toru Aoki (1), Katsuhisa Ikeda (1), Kazusaku Kamiya (1)

(1) Department of Otorhinolaryngology, Juntendo University Faculty of Medicine

Introduction

Hereditary deafness affects about 1 in 1600 children and GJB2 gene mutation is most frequent cause for this disease. GJB2 encodes connexin26 (Cx26), a component in cochlear gap junction. Cx26 is mainly expressed in cochlear supporting cells and fibrocytes, and forms large gap junction plaque (GJP) macromolecular complex. To date the differentiation of induce pluripotent stem (iPS) cells into hair cell-like cell have been reported, although the differentiation into cochlear supporting cells and fibrocytes expressing Cx26 is not accomplished yet. To differentiate these cells into cochlear cells which is non-neural ectoderm, neural cells are needed to be excluded in the ectodermal differentiation. Our goal is to establish method to differentiation of iPS cells into Cx26 positive cells with proper GJP formation as cochlear cells.

Methods

The cochlear feeder cells were developed from adult cochlear tissue to support cochlear differentiation. Undifferentiated mouse iPS cells were cultured in mediums which contain several reagent cocktails on the cochlear feeder cells. Cx26 expression was analyzed by immunohistochemistry. GFP signals controlled by Nanog promotor Nanog-GFP were monitored as an undifferentiated state marker. To investigate the characterization of cochlear feeder cells, surface antigens were identified by flow cytometry.

Results

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

Conclusions

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

Ichiro Fukunaga 1, 2, Ayumi Fujimoto 1, Kaori Hatakeyama 1, Toru Aoki 1, Atena Nishikawa 1, Osamu Minowa 3, Katsuhisa Ikeda 1, Kazusaku Kamiya 1

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

2: Research Institute for Diseases of Old Age, Juntendo University Graduate School of Medicine, Tokyo, Japan

3: BioResource Center, RIKEN, Tsukuba, Japan

Introduction: 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 significantly improved GJP formation and the auditory function (Iizuka et al., Hum Mol Genet. 2015). Cochlear cells are not readily accessible for biopsy or direct drug administration because of anatomical limitations. Therefore, embryonic stem (ES)/induced pluripotent stem (iPS) cells are an important tool for studying the molecular mechanisms underlying inner-ear pathology as well as for generating cells for replacement therapies. Differentiation of ES/iPS cells into Cx26 expressing cells and the Cx26-GJP forming cells have not been reported yet. In this study, we developed a new strategy for differentiation of mouse iPS cells into Cx26 expressing cells.

Method: 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, Cx26 and Cx30 mRNA levels increased in several condition compared with undifferentiated iPS cells. In a part of the aggregates, Cx26 positive cells were observed and these cells showed GJP formations as cochlear cells. Aggregates were subcultured in adherent culture, and proliferation of Cx26-GJP forming cells was observed.

Conclusion: In this study, we 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.

João Vicente Dorgam (1), Adriana Andrade Batista Murashima (1), Maria Rossato (1), Elizabete Rosa (2), Miguel Angelo Hyppolito (1)

(1) *Laboratory of Neurobiology of Hearing, Department of Ophthalmology, Otorhinolaryngology and Head and Neck Surgery, Ribeirão Preto Medical School, University of São Paulo*

(2) *Department of Cell and Molecular Biology and Pathogenic Bioagents, Ribeirão Preto Medical School, University of São Paulo*

Tympanic membrane may have its anatomical structure compromised, evolving with drilling that may be permanent or temporary in the middle ear diseases and trauma. There is an interest to simplify the microsurgical procedures for the reconstruction of the tympanic membrane using growth factors, hyaluronic acid, keratinocytes culture, insulin and the mesenchymal stem cells. The adipose tissue is a rich source and easy access as pluripotent stem cells. The stromal vascular fraction of adipose tissue can also generate endothelial progenitor cells or muscle.

PROPOSE: To study the effect of the application of adipose Stem Cells(CTA) in the regeneration of the tympanic membrane(TM) injured in a model of traumatic perforations in a control group; CTA+Fibrin Glue group using CTA supported by fibrin glue, in different observation times, with 3 days, 5 days and 7 days. The animals(wistar, *Rattus norvegicus*) were assessed for the time of the auditory threshold and closing of perforation. Tympanic membrane will be evaluated by conventional histology and immunohistochemistry confocal.

RESULTS: The means value of electrophysiological auditory thresholds was not differed among the study groups. CTA+Fibrin Glue TMs showed smaller perforations after three ($p<0.01$) and five ($p<0.01$) days of treatment. After seven days 100% of the perforations were healed in the CTA+Fibrin Glue TMs and 30% of the control group had not healed. Cytokeratin expression in the epithelial layer (ANTI-KGF-FGF7) were observed after 5 ($p = 0.02$) and seven ($p = 0.01$) days on CTA+Fibrin Glue group. The ANTI-VEGF and ANTI-FGF10 expression was not significant among the two groups. The human Anti-Mitochondria antibody was expressive in the epithelial layer after three ($p<0.01$) and five ($p<0.01$) days of treatment, on the same keratinocyte positive to ANTI-KGF-FGF7.

CONCLUSION: The adipose tissue is a rich source and easy access as pluripotent stem cells, with precursor cells of adipocytes in varied degree of differentiation, vascular cells and stromal cells of support that can differentiate in vitro, in cells, chondrogenics, adipogenesis, osteogenic and myogenic. CTA+Fibrin glue could be functioning as a xenograft from human to rat (*Rattus norvegicus*) and could be considered its use for the reconstruction of the tympanic membrane.

Ji Sun Kong, Sung Hun Lee, Woo Jin Kim, Sang Won Yeo, Shi Nae Park

Department of Otorhinolaryngology-Head and Neck Surgery, College of Medicine, The Catholic University of Korea, Seoul, Republic of Korea

Objective: Patients with PET experience troublesome symptoms such as autophony, ear fullness, humming tinnitus and tympanophonia. This study was performed to investigate the surgical results of trans-tympanic angiocather insertion for intractable Eustachian tube.

Materials and methods: All data of 18 patients (21 cases) who underwent transtympanic tripod-tipped-bone wax-filled angiocatheter (TTBA) insertion between June 2013 and Sep 2015 were retrospectively analyzed. Surgical results and the level of satisfaction of the patients about the surgical therapy were evaluated with VAS scores about 5 symptoms of PET.

Results: The mean age was 46.4 years. Male to female ratio was 11 to 7 and right to left ear ratio was 8 to 7 and both were in 3 cases. The period of follow-up was from 1 to 22 months (mean 10.2 months). Full insertion of TTBA (20~27mm in length, 22~16F) was achieved and immediate disappearance of the troublesome symptoms were observed. The average of VAS score was decreased significantly. In 4 ears, the catheter was replaced with a larger one because of the recurrence of aural symptoms during the follow up period. In 2 cases, revision operation was performed because of spontaneous extrusion of TTBA via nasopharyngeal orifice.

Conclusion: Surgical results of trans-tympanic angiocatheter insertion for intractable patulous Eustachian tube were relatively good, although long-term follow up to observe recurrence of the symptoms and extrusion of catheter is still necessary.

Fetoni AR 1, Rolesi R 1, Paciello F 1, Sergi B 1, Troiani D 2, Polimeni R 1, Paludetti G 1

1 Institute of Otolaryngology, 2 Institute of Human Physiology, Università Cattolica, School of Medicine, Rome, Italy

Introduction: Cholesteatoma is a well-demarcated non-cancerous lesion derived from an abnormal growth of keratinizing squamous epithelium in the temporal bone. Currently, no viable non-surgical therapies are available and although this pathology is very common, mechanisms underlying its pathogenesis is still unclear. Differences between congenital cholesteatoma, which is specific to childhood, and acquired cholesteatoma, which affects children as well as adults are well established clinically. However few studies addressed the interactions between matrix keratinocytes and perimatrix fibroblasts which play an important role in the cholesteatoma tissue homeostasis. Differentiation, proliferation and migration of the matrix keratinocytes require both paracrine and autocrine signaling involving angiogenetic and inflammatory pathways that have been demonstrated in the pathogenesis of disease. The aim of this work was to provide evidences on the role of angiogenetic factors and inflammatory mediators in cholesteatoma and evaluate whether the different expression could be related to clinical features in children and adults patients.

Methods: Cholesteatoma matrices and retro auricular skin were harvested during surgery in patients underwent to surgery. The Hematoxylin Eosin staining was performed in all samples in order to identify the cholesteatoma matrix. We investigated both angiogenetic and proliferative factors expression: VEGF, PDGFr, TGF-beta, IL-1alpha and pSTAT3 were upregulated as compared to normal skin using western blotting and immunohistochemical stainings.

Results: The expression of VEGF and PDGFr, TGF beta, IL-1 alpha and pSTAT3 protein were stronger in cholesteatoma tissue than in the skin. There was a significant difference on their expression between cholesteatoma samples in children and adults. Increased expression of angiogenetic and pro-apoptotic factors correlates with the cholesteatoma invasiveness in the adults patients, but not in children.

Conclusions: Significant relationship has been observed between the expression of the mediators above mentioned and the extend of cholesteatoma in adult cases. The identification of different proliferation and invasiveness biomarkers in adults and children affected by cholesteatoma could represent an useful tool for diagnosis and surgical strategies planning.

Julia Clara Gausterer (1), Elisabeth Engleder (1), Clemens Honeder (2), Chengjing Zhu (2), Michael Wirth (1), Christoph Arnoldner (2), Franz Gabor (1)

(1) Department of Pharmaceutical Technology and Biopharmaceutics, University of Vienna, Vienna, Austria

(2) Department of Otorhinolaryngology, Medical University of Vienna, Vienna, Austria

Introduction:

Bioadhesive drug delivery systems might help to improve the treatment of middle ear diseases by prolonging the residence time of the drug delivery system in the tympanic cavity leading to an extended contact time between the drug and the middle ear mucosa (MEM), resulting in increased therapeutic efficacy.

To estimate the utility of carbohydrate-binding proteins (so-called lectins) as potential bioadhesive ligands, the glycosylation pattern of the MEM of guinea pigs, an approved model for middle ear research, was characterised.

Methods:

Five fluorescent-labeled plant lectins with different carbohydrate specificities were incubated with viable guinea pig MEM specimens at 4°C and the binding capacities of each lectin were calculated based on the respective MEM-associated relative fluorescence intensity values. Additionally, the interaction specificity between the lectin and its corresponding carbohydrate was investigated by competitive inhibition of carbohydrate binding sites on the MEM.

Results:

The accessibility of glycocalyx-bound carbohydrate moieties of the MEM decreased in the following order: sialic acid + N-Acetyl- β -glucosamine > α -mannose + galactosamine > N-Acetyl- β -glucosamine > α -L-fucose >> mannose.

Among all lectins investigated, fluorescein-labeled wheat germ agglutinin (F-WGA) showed the strongest bioadhesive properties. Competitive binding studies using the complementary carbohydrate N,N',N''-Triacetyl-chitotriose led to a decrease in MEM-associated F-WGA of up to 90%, depending on the amount of carbohydrate. This finding confirms the high specificity of the F-WGA-MEM interaction. Fluorescence microscopic images and co-localisation experiments revealed that F-WGA bound to acidic mucopolysaccharides of the glycocalyx that cover the cilia, therefore identifying the cilia as binding sites of F-WGA within the MEM.

Conclusions:

Lectin-mediated bioadhesion to the MEM represents a new, promising concept to prolong the residence time of the drug delivery system in the tympanic cavity and to increase the therapeutic efficacy in the treatment of middle ear diseases, e.g. Otitis media.

Jae Hyung Hwang, Dong-Kee Kim, Hyu Eun Park, Sang-A Back, Hyang Rim Park, Soo Whan Kim, Yooyeon Park, Shi Nae Park

Department of Otorhinolaryngology-Head and Neck Surgery, College of Medicine, The Catholic University of Korea, Seoul, Republic of Korea

Objectives: Allergy is considered as one of important etiologic factors of otitis media with effusion (OME). In the present study, we evaluated the causal effect of allergy on OME in an animal model, and investigated the secondary effect of bacterial infection.

Methods: Allergy and control animals were subdivided into groups with and without intratympanic injection of lipopolysaccharide (IT-LPS). Allergic otitis media was induced via intraperitoneal ovalbumin injection with intranasal challenge. We assessed the occurrence of OME in allergic animals and the effect of IT-LPS on allergic otitis media. We also investigated the Th1 and Th2 responses in the middle-ear mucosa. Hearing of the animals was measured by ABR and DPOAE.

Results: OME was observed in 75% of the allergic animals. After IT-LPS, 100% of the control and allergy groups showed otitis media. Light microscopy revealed that the middle-ear mucosa of animals of both groups was also significantly increased after IT-LPS, and the Th1 response (IL-2 and IFN- γ) and Th2 response (IL-5 and IL-13) cytokines were expressed at higher levels in the allergy group with IT-LPS than in the control group with IT-LPS. Hearing tests between the allergy and control group with IT-LPS did not reveal any differences.

Conclusion: Our findings may be direct evidence of an allergic causal effect on OME. Th2 response cytokines were strongly expressed in allergic OME, and the inflammatory reaction to LPS was more intense in the allergic group, which indicates that otitis media related to allergy can be severely aggravated by an inflammatory reaction to bacterial infection.

Michaela A.H. Hofrichter (1), Barbara Vona (1), Reza Maroofian (2), Indrajit Nanda (1), Barry A. Chioza (2), Wafaa Shehata-Dieler (3), Erdmute Kunstmann (1), Jörg Schröder (1), Andrew H. Crosby (2), Thomas Haaf (1)

(1) *Institute of Human Genetics, Julius Maximilians University, Würzburg, Germany*

(2) *Molecular Genetics, RILD Institute, University of Exeter, Royal Devon and Exeter NHS Hospital, Wonford, Exeter, United Kingdom*

(3) *Comprehensive Hearing Center, Department of Otorhinolaryngology, University Hospitals, Würzburg, Germany*

Hearing loss (HL) is a heterogeneous disease, inherited in 50% of all cases, which affects people at any time throughout life. Depending on the inherited variant/gene, the severity, progression and onset of HL can be predicted according to case studies in addition to traditional elucidation of risk/mode of inheritance. Different strategies are required for detection of pathogenic variants and investigation of HL in detail. These reasons are the large number of presently identified HL genes (over 100) and the challenges of mutation identification within small families or those with ambiguous clinical histories.

In our study, we investigated 20 parent-child or parent-sibling trios and 20 probands from consanguineous Iranian families using a next generation sequencing approach. All subjects were prescreened and negative for frequent GJB2 mutations and advanced to next generation sequencing using the Illumina TruSight One panel (4813 genes including approximately 100 HL genes). Sequencing was performed with MiSeq/NextSeq 500 desktop sequencers and analysis followed using GensearchNGS and included *in silico* prediction and segregation analysis.

Trio analyses are powerful for investigation of sporadic and dominant, and are supportive in unusual cases including unilateral and possible syndromic HL. Herein, we identified a likely pathogenic variant in around 30% of our unresolved cases. Trio analysis allowed rapid identification of a familial TECTA mutation which showed concordant co-segregation, uncovered a combination of biallelic compound heterozygous USH2A and homozygous USH1G mutations in a subject and effectively detected a *de novo* CEACAM16 mutation.

As opposed to non-consanguineous individuals, probands from consanguineous unions have not benefited from trio-analysis because homozygous variants are easily detected for further segregation validation. We identified the most likely pathogenic variant in half (50%) of all affected Iranian families, with the recessive genes MYO15A and SLC26A4 most frequently involved.

Genetic diagnosis of HL has a valuable multifactorial impact. However, this must be approached on a patient-by-patient basis that is effectively maximized through our strategy that can include trio sequencing. This strategy, when combined with clinical data, has finally provided a solution to the genetics puzzle in a significant proportion of cases to date.

Jae Joon Han (1), Pham Nguyen (2), Jin Hee Han (1), Ah Reum Kim (3), Byung Yoon Choi (1)
 (1) Department of Otorhinolaryngology, Seoul National University Bundang Hospital, Seongnam, Korea
 (2) Department of Otorhinolaryngology, Children's Hospital, Ho Chi Minh City, Vietnam
 (3) Biomedical Research Institute, Seoul National University Hospital, Seoul, Korea

More than 100 different pathogenic mutations and 24 polymorphisms of GJB2 have been identified. Among them, p.V37I is one of variants that are frequently found, and the prevalence of that variant in normal hearing control group was known as about 1% in Korea. This study aimed to evaluate the prevalence of p.V37I variant in Vietnam, and to find the pathogenic role of this variant in Vietnam's hearing loss patients.

We included 117 normal hearing control participants (NH group) and 90 autosomal recessive severe to profound hearing loss patients (HL group) to perform screening for most frequent eleven variants of 5 genes (GJB2, SLC26A4, CDH23, 12S rRNA, and TMPRSS3) using The U-TOP™ HL Genotyping Kit.

In HL group, causative genes were found with this screening in eight patients and diagnostic yield of this screening kit was 8.9%. Homozygote of GJB2:V37I (N=5, 5.6%), GJB2:235delC (N=1, 1.1%), 12SrRNA:1555A>G (N=1, 1.1%) and compound heterozygote of GJB2:V37I and GJB2:235delC (N=1, 1.1%) were found. The prevalence of single Heterozygote mutations of GJB2:V37I was 27.8%. Minor allele frequency of GJB2:V37I was 20% (36/180 alleles) in HL group. In NH group, GJB2:V37I was most frequent variant with this screening and its allele frequency was 9.8% (23/234 alleles). In addition to nineteen GJB2:V37I heterozygote mutation (16.2%), two homozygote of GJB2:V37I variant was also found (1.7%). the allele frequency was twice higher in HL group than NH group ($P<0.01$). The prevalence of homozygote or compound heterozygote of GJB2:V37I in HL group (6.7%) was higher than that of homozygote of GJB2:V37I in NH group (1.7%), although the difference could not reach to statistical significance ($P=0.08$).

In Vietnam, minor allele frequency of GJB2:V37I variant was relatively high, compared with other populations. Therefore, this population may be good model for elucidate clinical phenotypes for GJB2:V37I variant. Higher prevalence of homozygote or compound heterozygote of GJB2:V37I in HL group may imply the pathogenic role of GJB2:V37I variant in severe to profound hearing loss population of Vietnam. In the future, Next Generation Sequencing will be followed to identify other pathogenic genes.

Teresa Requena (1), Carmen Martin-Sierra (1), Alvaro Gallego-Martinez (1), Anna Lysakowski (2,3), Jose A. Lopez-Escamez (1,4)

(1) Otology & Neurotology Group CTS495, Department of Genomic Medicine, GENYO - Centre for Genomics and Oncological Research – Pfizer/University of Granada/ Junta de Andalucía, PTS, Granada, 18016 Spain.

(2) Dept. of Anatomy and Cell Biology, Univ. of Illinois at Chicago, Chicago, IL, 60612, USA

(3) Dept. of Otolaryngology-Head and Neck Surgery, Univ. of Illinois at Chicago, Chicago IL

(4) Department of Otolaryngology, Granada University Hospital, Granada, 18016, Spain

Introduction

Meniere's disease (MD) is a complex disorder characterized by recurrent attacks of spontaneous vertigo associated with sensorineural hearing loss (SNHL) and tinnitus, with a strong familial aggregation (prevalence of 8-10% of familial cases (FMD). We have analyzed two multiplex families with autosomal dominant (AD) inheritance pattern of MD, to study the genetic causes of this disorder, identifying the candidate variants segregating the MD phenotype.

Methods

WES data were processed in a SOLiD 5500xl platform. Single Nucleotide Variants (SNVs) were filtered by our in-house control database. Remaining variants were annotated with Annovar software and filtered using a minor allelic frequency MAF > 0.0001 according dbSNP 142, ExAC and 1000 genomes databases. To prioritize candidate variants we used the following bioinformatics tools: Pathogenic Variant –PAVAR score, an in house scoring system based upon seven tools estimating the effect in protein structure and phylogenetic conservation; and bioinformatics tools that include phenotype information such as Exomiser v2 and Variant Annotation Analysis and Search Tool (VAAST) + Phevor. All variants were validated by Sanger sequencing technology in a 3130 Genetic Analyzer. RNA from human inner ear tissue (cochlea and semi-circular canals) was obtained from schwannoma surgery patients.

Results

After the filter and prioritization process we obtain a list of candidate variants. Two candidate variants in SEMA3D and DPT genes, segregating the MD and the hearing loss phenotype, respectively, were validated by Sanger sequencing. Through RT-PCR we validated that both genes are expressed in the human inner ear cochlea and semi-circular canals.

Conclusions

We have identified by WES two potentially pathogenic variants in SEMA3D and DPT genes in two families with AD FMD. In addition we confirm the expression of these genes in human cochlea and semi-circular canals.

Our findings add new candidate genes in FMD, and they anticipate genetic heterogeneity for the disorder.

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Licia Lugli (1), Walter Just (2), Elisabetta Genovese (3), Silvia Palma (4), Daniele Monzani (3), Fabrizio Ferrari(1) and Antonio Percesepe (5)

(1) Neonatal Intensive Care Unit, Department of Mother and Child, (5) Medical Genetics Unit, Department of Medical and Surgical Sciences, (3) Audiology Unit, Department of Diagnostic Medicine, Clinical and Public Health, University of Modena, Modena, (4) Otolaryngology Department, Community Healthcare Services, Modena, Italy and (2) Human Genetics, University of Ulm, Ulm, Germany

Branchio-oculo-facial syndrome (BOFS; MIM#113620) is a rare autosomal dominant developmental disorder primarily affecting the derivatives of the first and second pharyngeal arches. Three major systems are involved: the branchial derivatives, presenting with pits or fistulae in the cervical or auricular region; the eye, with anomalies including micro/anophthalmia, cataract, coloboma, strabismus, ptosis, and nasolacrimal duct obstruction; and the craniofacial region, with malformations and dysmorphisms represented by dolichocephaly, malformed pinnae, thick nasal tip, upslanted palpebral fissures, and cleft lip with or without cleft palate.

Additional common findings include conductive, sensorineural or mixed hearing loss, ectodermal anomalies (small teeth, dysplastic nails, and sparse and prematurely gray hair), hemangioma, ectopic thymus, and scalp cysts; in contrast, renal and cardiac anomalies, growth restriction, and mental retardation have been less frequently described.

Diagnostic criteria requiring the involvement of all three regions or two of the main features plus an affected first degree parent or ectopic thymus have been proposed; however, because of the variable expression, the clinical diagnosis can remain elusive, especially in the first years of life. Molecular confirmation can be achieved through the mutation analysis of the TFAP2A gene, the only causative gene presently known.

We describe the case of a newborn with a suggestive branchial and facial phenotype, but very mild ocular features, in whom the diagnosis of BOFS was confirmed by the finding of a de-novo heterozygous mutation in the TFAP2A gene.

Lukas Varga (1,2), Daniel Danis (2), Martina Skopkova (2), Ivica Masindova (2), Zuzana Kabatova (1), Iwar Klimes (2), Milan Profant (1), Daniela Gasperikova (2)

(1) Department of Otorhinolaryngology – Head and Neck Surgery, Faculty of Medicine and University Hospital, Comenius University, Bratislava, Slovakia; (2) Institute of Experimental Endocrinology, Biomedical Research Center, Slovak Academy of Sciences, Bratislava, Slovakia

Introduction: Tectorial membrane is a gelatinous acellular inner ear structure important for hearing. It consists of several collagenous and non-collagenous proteins which provide its unique mechanic features. Mutations in genes encoding these proteins may lead to sensorineural hearing loss.

Methods: We selected 20 unrelated subjects with familial nonsyndromic hearing loss with autosomal dominant or autosomal recessive inheritance, who were negative for GJB2 etiology. Two probands were analyzed by gene panel (66 deafness genes) and 18 probands by whole exome sequencing (WES) (BGI, Hong Kong). Ninety-five known genes associated with NSNHL were screened in the WES group. Variants were prioritized in favor of the novel or rare variants (1000 Genomes phase 3, ESP6500, ExAC, dbSNP141, in-house) in highly conserved positions (GERP) and with high predicted in-silico pathogenicity (PolyPhen, Sift, CADD). Severity of hearing loss was assessed by pure tone audiometry in all participating subjects.

Results: We found 5 families (25 %) carrying mutations in genes encoding tectorial membrane proteins. Three different previously described mutations in TECTA gene were identified in 4 families. All of them had autosomal dominant inheritance. One family harbored yet unknown homozygous mutation in OTOG gene. Hearing loss was moderate to moderately severe in most of the affected individuals, regardless of the mutation. Patients who required hearing rehabilitation benefited from conventional hearing aids.

Conclusions: Tectorial membrane defects are common cause of familial hearing loss in the Slovak population. However, testing larger cohorts is required to provide their exact epidemiological significance.

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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 Graduate School, Nagoya, 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 immune-inflammatory responses are related to the pathology of inner ear disease. We investigated the association between genetic polymorphisms located in genes related to the immuno-inflammatory process and susceptibility to MD in the present study.

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

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

Conclusion: In conclusion, the GPIa polymorphisms were significantly associated with the risk of MD. **Keywords:** Ménière's disease, case-control study, polymorphism

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References: [1] Calabrese V, Cornelius C, Maiolino L, Luca M, Chiaramonte R, Toscano M A, Serra A. Oxidative stress, redox homeostasis and cellular stress response in Meniere's disease: role of vitagenes. *Neurochem Res* 2010;35: 2208-2217. [2] Huang Y, Teranishi M, Uchida Y, Nishio N, Kato K, Otake H, Yoshida T, Sone M, Sugiura S, Ando F, Shimokata H, Nakashima T. Association between polymorphisms in genes encoding methylenetetrahydrofolate reductase and the risk of Ménière's disease. *J Neurogenet.* 2013; 27: 5-10. [3] Teranishi M, Uchida Y, Nishio N, Kato K, Otake H, Yoshida T, Suzuki H, Sone M, Sugiura S, Ando F, Shimokata H, Nakashima T. Polymorphisms in genes involved in the free-radical process in patients with sudden sensorineural hearing loss and Ménière's disease. *Free Radic Res.* 2013; 47: 498-506.

Dalia Alvarez-Morujó (1), Daniel López-López(1), M.Javier Herrero-Turrión(1), David Sánchez Benito (1,2), Sonia Hernández-Noriega (1), Ricardo Gómez-Nieto(1,2), Antonio J. Alvarez-Morujó (1,3), Dolores E. López (1,2)

(1) INCYL-University of Salamanca/IBSAL, Salamanca, Spain

(2) Dep. Biology and Patology, University of Salamanca, Spain

(3) Dep. Anatomy and Histology, University of Salamanca, Spain

The ADGRV1 Gene, also known as GPR98, VLGR1b or Mass1, encodes a member of the G-protein coupled receptor superfamily. The encoded protein contains a 7-transmembrane receptor domain, binds calcium and is expressed in the nervous system. Mutations in this gene are associated with Usher syndrome 2 and familial febrile seizures. We study the expression of this gene in the *Mesocricetus auratus* strain GASH:Sal, a genetic audiogenic seizure hamster, inbred at the University of Salamanca.

Ictal events in strains susceptible to audiogenic seizures, cause gene deregulation in the inferior colliculus, the nucleus that triggers the seizures. Because of this, we study the expression of ADGRV1 gene with real-time RT-PCR, comparing stimulated Syrian control hamster with the stimulated GASH:Sal. We also perform the ADGRV1 gene expression studies in basal conditions.

Always, the GASH:Sal exhibited the ADGRV1 gene down regulated compared with the control in the same conditions.

The protein encoded by the ADGRV1 gene was proposed as molecular components of fibrous ankle links between adjacent stereocilia of mechanosensitive hair cells.

We also study the morphology of the Corti's organ was evaluated by scanning electron microscopy, compared with control Syrian hamsters.

We found morphological damage, mainly in the spatial organization of the stereocilia of the outer hair cells. The inner hair cells also exhibit changes in the stereocilia number and disorganization in the stereocilia links

The under-expression of this gene in the GASH:Sal could be related with the origin of the seizures, and also with the observed hearing alterations and morphological changes in the stereocilia of the organ of Corti.

Stephanie Eckrich (1), Dietmar Hecker (2), Barbara Fell (1), Kerstin Blum (1), Jennifer Ihl (1), Kerstin Fischer (1), Bernhard Schick (2), Dusan Bartsch (3), Jutta Engel (1)

(1) Dept. of Biophysics, Saarland University, Homburg, Germany; (2) Dept. of Otorhinolaryngology, Saarland University, Homburg, Germany; (3) Dept. of Molecular Biology, Central Institute of Mental Health, Mannheim, Germany

Throughout life inner hair cells (IHCs) require Cav1.3 voltage-gated Ca^{2+} channels (VGCCs). In mature IHCs, Ca^{2+} currents elicit synaptic vesicle release and thereby signal transmission to the auditory pathway. Before the onset of hearing at postnatal day 12 (P12) in mice, IHCs produce Ca^{2+} action potentials thought to drive terminal maturation of IHCs and auditory pathway. Systemic Cav1.3 $^{-/-}$ mice are deaf due to lack of transmitter release from the IHCs. Additionally, lack of Ca^{2+} action potentials before the onset of hearing disrupts terminal differentiation resulting in immature-like IHCs with deformed synapses. Presence of Cav1.3 in several nuclei of the auditory pathway might further add to the phenotype in Cav1.3 $^{-/-}$ mice [Hirtz (2011) JNeurosci 31:8280]. To separate the multiple roles of Cav1.3 in IHCs and auditory pathway, new mouse models with cell- and age-specific deletion are required.

Here, we used Cav1.3 $^{\text{flex}}$ mice with Cre-dependent ablation of Cav1.3 coupled to eGFP expression via flip insertion of loxP sites [Satheesh (2012) HumMolGen 21:3896]. These mice were crossbred with i) Pax2Cre mice with embryonic Cre expression in the cochlea [Ohyama (2004) genesis 38:195] or ii) PrestinCre mice with hair cell specific Cre expression starting from around P10 [Tian (2004) DevDyn 231:199]. We investigated Ba^{2+} currents (IBa) through Cav1.3 channels using whole-cell patch clamp recordings of IHCs and expression of IHC proteins with whole-mount immunolabelling. Auditory brainstem responses (ABR) and distortion product otoacoustic emissions (DPOAE) were used to assess hearing function.

Due to mosaic deletion of Cav1.3 in homozygous Cav1.3 $^{\text{flex/flex}}$ mice, we used Cav1.3 $^{-/-}$ flex mice. Embryonic cochlea-specific deletion of Cav1.3 (Cav1.3 $^{-/-}$ flex:Pax2Cre mice), eliminating effects of central Cav1.3 deletion, caused a phenotype very similar to systemic Cav1.3 $^{-/-}$ mice. Cre expressed from around P10 in Cav1.3 $^{-/-}$ flex:PrestinCre mice did not simultaneously cut Cav1.3 in all IHCs resulting in individual IHC phenotypes ranging from Cav1.3 $^{-/-}$ -like to nearly wildtype-like within 3 weeks. A slow turnover rate of Cav1.3 might further add to this heterogeneity. The Cav1.3 $^{-/-}$ -like phenotype in part of these IHCs shows a requirement of Cav1.3 for maintenance of the IHC phenotype even after the onset of hearing.

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Mina Park 1, Hyun Bin Cho 2, Min-Young Kim 3, Ah-Reum Kim 3, Tong Mook Kang 2, Byung-Yoon Choi 3

1 Department of Otorhinolaryngology-Head and Neck Surgery and Healthcare Research Institute, Healthcare System Gangnam Center, Seoul National University Hospital

2 Department of Physiology, Sungkyunkwan University School of Medicine

3 Department of Otorhinolaryngology-Head and Neck Surgery, Seoul National University Bundang Hospital

Objective:

We tried to describe two novel KCNQ4 mutations and elucidate the pathologic mechanisms underlying the different phenotype and extend the phenotypic spectrum of KCNQ4 mutations.

Materials and Methods:

Two ADNSH families (SB62-110, SB155-271), which show different audiologic configurations, were recruited. SB62-110 and SB155-271 showed ADNSH prominent in low- and high-frequency hearing loss, respectively. Targeted exome sequencing was performed to reveal a molecular etiology of ADNSH of the two families. The KCNQ4 mutations detected from the families were cloned in expression vectors and expressed in mammalian cells. We compared the subcellular localization, K-current, and protein expression amounts of mutant KCNQ4 with those of the wild type. Additionally, we performed protein modeling for the missense mutation of KCNQ4 to pursue the pathogenic role.

Results:

Molecular genetic testing revealed two novel mutations, p.R331Q and c.811_816del from SB62-110 and SB155-272, respectively. We could not identify any difference of subcellular localization of two mutant KCNQ4 protein compared with that of the wildtype. However, the 2 aa deletion mutant (c.811_816del) protein affecting p-loop showed significantly decreased expression compared with wildtype and p.R331Q mutant. Amounts of potassium currents were significantly lowered both in the p.R331Q and c.811_816del compared with wildtype, indicating the pathogenic potential of two variants. The pathogenic mechanism of p.R331Q was predicted to affect interaction of p.R331 residue in the proximal C-tail of KCNQ4 with phosphatidyl inositol 4,5-bisphosphate (PIP2) based on the 3D protein modeling. Different pathogenic mechanism proposed for p.R331Q may account for the different audiologic configuration from that related to other KCNQ4 variants

Conclusion:

Our study extends the audiologic phenotypic spectrum from high frequency SNHL to include low to mid frequency SNHL. The different pathologic mechanism seems to underlie the contrasting phenotypes of KCNQ4 mutations.

Shiryazdanov R.U., Yashin S.S.

Scientific Head, Professor E.L. Ovchinnikov, PhD., Samara State Medical University, Samara, Russia

The nature of human's hearing is extremely complicated and contradictory according to attempts of its own explanation. Biophysical mechanisms of sound transduction in the human ear are determined by anatomical structures, which form the organ of hearing, and are implemented by physical laws, that define sound as a wave process. Morphological substratum, which provides function of converting sound's energy into the receptor's potential, represents acoustic receptors - the inner and outer hair cells. They are distant relative to sound sources and are localized in the organ of Corti of the cochlear duct.

Considering that all of the energy transformations in the ear occur on its barrier formations – on the membranes and membrane-like structures, that separate the various organ's departments, we conclude that the acoustic energy is converted under the joint action of the tectorial and basilar membranes on auditory receptors. The coordinates, according to acoustic-wave model, are determined by formulas: for vestibular membrane: $xvs(f) = L_o(1 - \delta)$, for the tympanic: $xts(f) = L_o(1 + \delta)$, where L_o – the length of the cochlear duct, $\delta = (L_o - x)/L_o$ – dimensionless coordinate of the energy maximum of the standing wave, and at equal temporal decisions for both stairs: $tmax(f) = L_o/v(1 + \delta) + n/(2f)$, where the parameter $n = 0, 1, 2, \dots$ determines the order of the temporal maximum of the standing wave with frequency f .

As a result of research, we came to a conclusion:

- 1) The projection of the oscillations' peaks of vestibular and basilar membranes on the organ of Corti is determined by a single point x
- 2) Coordinates of vestibular and basilar membranes for the relevant receptors perform oscillations synchronously
- 3) Coordinates of vestibular and basilar membranes for the relevant receptors perform oscillations in antiphase.

Shiryazdanov R.U., Yashin S.S.

Scientific Head, Professor E.L. Ovchinnikov, PhD., Samara State Medical University, Samara, Russia

Biophysical mechanisms of sound transduction in the human ear are determined by anatomical structures, which form the organ of hearing, and are implemented by physical laws, that define sound as a wave process. We consider that all energy transformations in the ear take place on its barrier formations – on the membranes and membrane-like structures. The acoustic energy of sources must experience many transformations, before it will turn into the energy of nerve impulse, which leads to the perception of the audio signal.

The first barrier is the eardrum. Its presence is evolutionally determined by the necessity of compensation losses of energy of sound waves with the possible absence of it. It converts energy of sources' waves into the energy of the mechanical vibrations of the auditory ossicles of the middle ear followed by conversion by the membrane of the oval window as a second barrier again to the energy of the wave process of the inner ear's fluids.

Created in perilymph, running sound waves, reaching the third barrier - the round window membrane, are reflected. Reflected waves together with straight are subjected interference with the establishment of longitudinal standing waves. The perilymph's deformation leads to vibrations of vestibular and basilar membranes of the cochleas. The fourth barrier – is the vestibular membrane, thanks to which the energy of the standing longitudinal wave of the perilymph is transformed into the energy of the transverse oscillations. As a result, in the medium staircase a new running wave arises, whose energy is absorbed by the tectorial membrane as the fifth barrier. From the side of the scala tympani the energy of standing waves of the perilymph is converted into energy of vibrations of the basilar membrane as a sixth barrier. The joint action of the tectorial and of the basilar membranes on the auditory receptors leads to the generation an electrical potential by them, which leads to auditory sensations.



Charlène Batrel, Antoine Huet, Jing Wang, Gilles Desmadryl, Régis Nouvian, Jean-Luc Puel, Jérôme Bourien

(1) INSERM - UMR 1051, Institute for Neurosciences of Montpellier, Montpellier, France

(2) University Montpellier, Montpellier, France

Auditory nerve fibers (ANFs) transmit acoustic information from the sensory hair cells to the cochlear nuclei. Probing in experimental and clinical audiology the whole ANFs population remains a difficult task as the ANFs notably differ in their threshold and synchronization index. Thus, the low-spontaneous rate (SR) fibers, which have a high threshold, a delayed and a large jitter in their first spike latency, are not detectable in the far-field compound action potential. Here, we develop a novel method to track the activity of the ANFs along the tonotopic axis. Using neural noise recording at the round window of the gerbil's cochlea, the spectral component of the ANFs, corresponding to the power spectrum density (PSD) can be monitored. In response to low sound-level stimulation, PSD predominates in the low-frequency cochlear regions, while a second component of responses centered on higher cochlear frequency regions appeared beyond 30 dB SPL. At 60 dB SPL, PSD show a bimodal distribution with a cut off frequency around 5.6 kHz. In addition to correlate with the ANFs mapping along the tonotopic axis, the loss of low-SR fibers leads to a reduction in the high frequency PSD response, where the low-SR fibers are preferentially located. Thus, round window PSD responses may provide a useful tool to probe the distribution of ANF in humans or other species for which direct single unit recordings are not feasible.

Charles Petitpre (1), Anil Sharma (1), Saida Hadjab (1), Francois Lallemand (1)
(1) Karolinska Institutet - Stockholm - Sweden

Our ability to encode acoustic signals depends on neurons found in our peripheral auditory organs, the cochlea. The mammalian cochlea contains two distinct types of spiral ganglion neurons (SGNs). Type I neurons have been well characterized and are the primary carrier of the auditory signal, whereas type II SGNs function is still poorly understood. We aim to understand the difference between the two types of neurons at molecular level, in order to access the function of type II SGNs.

Genetically labelled SGNs at both postnatal day 3 (P3) and adult (P30) were dissociated, individually sorted by FACS and sequenced using Smart-Seq2. The single-cell transcriptome revealed specific biomarkers for either type I or type II SGNs. Several genes involved in canonical pathways were selected and their expression were further confirmed by in situ hybridization from embryonic stage to adult. This will give us insight into the specification of type II SGNs.

Taking advantage of the Cre recombinase driver lines of our newly identified markers, we characterised the central innervation patterns of type I and II neurons. This lays the groundwork for fully defining the neuronal circuits and function of type II SGNs.

Luciana Macedo de Resende (1), Fabrice Giraudet (2), Sirley Alves da Silva Carvalho (1), Denise Utsch Gonçalves (3), Aurélien Mulliez (4), Thomas Benichou (5), Elodie Gentes (5), Paul Avan (2), Igor Tauveron (5)

(1) *Speech language pathology and audiology department - Federal University of Minas Gerais- Belo Horizonte – Brazil*

(2) *Laboratory of Neurosensory Biophysics, UMR INSERM 1107, University of Auvergne, Clermont-Ferrand, France*

(3) *ENT department- Federal University of Minas Gerais- Belo Horizonte – Brazil*

(4) *Department of biostatistics, University Hospital, Clermont-Ferrand, France*

(5) *Endocrinology department, University Hospital, Clermont-Ferrand, France*

Introduction: Diabetes Mellitus (DM) is a highly prevalent metabolic disease. In France, 7.5% of the national population is diabetic, and estimates for the year 2035 show an increase up to 8.2%. Many aspects in overall health are affected by DM and the disease is also an important risk factor for the development of hearing loss. The nature, extension and pathophysiology of this disease in relation to the auditory system still raise questions. The purpose of this study was to describe the auditory function of diabetic patients and search for correlations among clinical status and hearing outcomes.

Methods: Auditory function was measured in a first stage through audiometry (a 4-frequency average was calculated for each ear, pure tone average - PTA), transient evoked otoacoustic emissions (TEOAE), tympanometry and acoustic reflexes. If the patient "failed" this screening, measures of auditory function were completed with distortion product otoacoustic emissions (DPOAE) and auditory brainstem responses (ABR). Results were compared to normative data and correlated regarding type of diabetes, age, presence of nephropathy, retinopathy, peripheral neuropathy scores, number of hypoglycemies and glycosylated hemoglobin.

Results: 80 patients completed the screening protocol and among these, 70 (87.5%) presented some abnormality. Audiometric analysis showed that diabetic patients present an increased prevalence of hearing loss in relation to normative values according to age. Diabetic patients also presented increased absence of TEOAE in the presence of normal audiometric thresholds (50%). ABR findings include increased absolute latency values for waves I, III and V; absence and/or reduced amplitude of wave I, even in patients whose thresholds were within normal values. Another interesting finding was the absence of acoustical reflexes in the patients with normal PTA(33.3%). Abnormal audiological results were present regardless of sex, age and type of diabetes. Nephropathy presented strong correlation with overall auditory outcomes. Results indicate that probable auditory neuropathy and cochlear pathology may coexist in this population.

Steffen Wolter (1), Dorit Möhrle (1), Dennis Zelle (1), Marlies Knipper (1), Hannes Schmidt (2), Lukas Rüttiger (1)

(1) *University of Tübingen, Department of Otolaryngology, Tübingen, Germany*

(2) *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 type 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 [1]. Also in the cochlear nucleus (CN), distinct auditory nerve fiber (ANF) types that differ in their discharge rate and sound sensitivity bifurcate, sending collaterals to the anteroventral, posteroventral, and dorsal subdivisions. The lack of GC-B has been shown to lead to a loss of bifurcation in the CN without obvious functional deficits [2]. Here, we describe the hearing function of adult GB-B knock-out mice and their wild-type littermates in detail, using evoked auditory brainstem response (ABR), distortion product otoacoustic emission (DPOAE) and auditory steady state response (ASSR). Histological correlates of the auditory phenotype were verified by applying immunohistochemistry on fixed sections of cochlea and brain tissue and high-resolution fluorescence microscopy. We discuss the results in the context of previous findings.

This work was supported by grants from the Deutsche Forschungsgemeinschaft (FOR 2060 project FE 438/5-1)

[1] G Ter-Avetisyan, FG Rathjen, H Schmidt: Bifurcation of axons from cranial sensory neurons is disabled in the absence of Npr2-induced cGMP signaling. *J Neurosci* 2014, 34(3): 737–747; [2] CC Lu et al.: Mutation of Npr2 leads to blurred tonotopic organization of central auditory circuits in mice. *PLoS Genet* 10(12): e1004823

Brahim Tighilet (1), Sophie Duthiel (2), Isabelle Watabe (1), Karina Sadlaoud (1), Alain Tonetto (3)
(1) *2Laboratoire de Neurosciences Intégratives et Adaptatives, UMR 7260 Aix-Marseille Université-
CNRS, Fédération de Recherche 3C, 13331 Marseille Cedex 03, France.*

(2) *Department of Psychiatry, Yale University School of Medicine, New Haven, Connecticut 06519*

(3) *Fédération de Recherche Sciences Chimiques Marseille FR 1739, Pôle PRATIM, 13331 Marseille
Cedex 03, France*

Reactive cell proliferation rapidly occurs in the cat vestibular nuclei after unilateral vestibular neurectomy (UVN) and has been reported to facilitate the recovery of posturo-locomotor functions. Interestingly, while animals experience impairments for several weeks, extraordinary plasticity mechanisms take place in the local microenvironment of the vestibular nuclei: newborn cells survive and acquire different phenotypes, such as microglia, astrocytes, or GABAergic neurons, while animals eventually recover completely from their lesion-induced deficits. Because brain-derived neurotrophic factor (BDNF) can modulate vestibular functional recovery and neurogenesis in mammals, in this study we aimed to examine the effect of BDNF chronic i.c.v. infusion versus K252a (a Trk receptor antagonist) in our UVN model. Results showed that long-term i.c.v. infusion of BDNF accelerated the restoration of vestibular functions and significantly increased UVN-induced neurogenesis, whereas K252a blocked that effect and drastically delayed and prevented the complete restoration of vestibular functions. Further, since the level of excitability in the deafferented vestibular nuclei is correlated with behavioral recovery, we examined the state of neuronal excitability using two specific markers: the cation-chloride cotransporter KCC2 (which determine the hyperpolarizing action of GABA) and GABAA receptors. We report for the first time that during an early time window following UVN, significant BDNF-dependent remodeling of excitability markers occurs in the brainstem. These data suggest that GABA acquires a transient depolarizing action during recovery from UVN, which potentiates the observed reactive neurogenesis and accelerates vestibular functional recovery.

Kensuke Kiyomizu (1,2), Ryuichiro Takeda (3), Keiji Matsuda(2), Koji Torihara (2), Yasushi Ishida (4), Kensei Yoshida (1), Tetsuya Tono (2)

(1) Department of Psychiatry, Yoshida Hospital, Nobeoka, Japan (2) Department of Otorhinolaryngology, Head & Neck Surgery, Faculty of Medicine, University of Miyazaki, Miyazaki, Japan (3) Department of Healthcare And Security Center, University of Miyazaki, Miyazaki, Japan (4) Department of Clinical Neuroscience, Faculty of Medicine, University of Miyazaki, Miyazaki, Japan

[Introduction] Japan is a hyperaging society. The number of patients with dementia are increasing and might be 4.62 million people in 2012. We reported hearing tests in patients with dementia.

[Subjects] The subjects were 77 patients (20 men, 57 women, age range, 61-93; mean age 80.0 years) with dementia. The 77 patients were classified as follows: Alzheimer's in 51 (66.2%), vascular in 8 (11.8%), mixed in 2 (2.9%), Lewy Body (DLB) in 5 (6.5%), frontotemporal (FTD) in 6 (11.3%), others in 2 (2.6%). We used revised Hasegawa's dementia scale (HDS-R) as a cognitive function test. The results were classified into 4 groups: normal: ≥ 21 ; mild: $16 \leq 20$; moderate: $11 \leq 15$; severe: ≤ 10 . The audiometric tests results for each ear were classified into 4 groups: normal: pure tone average at 0.5, 1, 2kHz (PTA) ≤ 25 dB; mild: $26 \leq 40$ dB; moderate: $41 \leq 70$ dB; severe: ≥ 71 dB. The bilateral PTA results were classified into 4 groups: normal, right hearing loss (HL), left HL, bilateral HL.

[RESULTS] In HDS-R, 25 (32.5%) cases gave normal results, 21 (27.3%) showed mild, 18 (23.4%) showed moderate and 13 (16.7%) showed severe. In the audiometric tests on the right ear, 3 (3.9%) cases gave normal results, 16 (20.8%) showed mild, 48(62.3%) showed moderate and 10 (13%) showed severe. On the left ear, 4 (5.2%) cases gave normal results, 19 (24.7%) showed mild, 36(46.8%) showed moderate and 18 (23.4%) showed severe. Bilateral audiometric test results showed normal hearing in 1 (1.3%) case, right HL in 2 (2.5%), left HL in 3 (3.9%) and bilateral HL in 71 (91.2%). In total therefore, 76/77 (98.7%) had HL.

[Discussion] Patients with dementia may have hearing loss or balance disorders but they sometimes did not recognize. We are trying to treat them using collaborative approach in both otolaryngologic and psychiatric methods.

Kensuke Kiyomizu (1,2), Ryuichiro Takeda (3), Koji Torihara (2)
 (1) *Department of Psychiatry, Yoshida Hospital, Nobeoka, Japan,* (2) *Department of Otorhinolaryngology, Head & Neck Surgery, Faculty of Medicine, University of Miyazaki, Miyazaki, Japan,* (3) *Department of Healthcare And Security Center, University of Miyazaki, Miyazaki, Japan*

[Introduction] Japan is a hyperaging society. The number of patients with dementia are increasing and might be 4.62 million people in 2012. We reported patients with dizziness and dementia.

[Subjects] Out of 951 patients (304 men, 647 women, age range, 4-95; mean age 60.3 ± 18.1 years), 50 patients (12 men, 38 women) had dementia.

[Case reports] Case 1 was 86-year-old woman. She had forgetfulness, irritability and delusion of theft in 2001. She came our hospital in 2005. She had vertigo, hearing loss and left-beating nystagmus in 2006, and received the therapy of anti-vertigous drugs and hearing aid. Her revised Hasegawa's dementia scale (HDS-R) was 16 / 30. Case 2 was 71-year-old woman. She had severe dizziness and anxiety and came our hospital in 2010. She wanted to receive physical therapy for her diabetes, but her blood glucose level was 449. Her HDS-R was 14 / 30.

[Discussion] Patients with dementia may have balance disorders or hearing loss but they sometimes did not recognize. We are trying to treat them using collaborative approach in both otolaryngologic and psychiatric methods.

Angelika Kübler 1, Wibke Singer 1, Chengfang Chen 1, Hyon-Soon Geisler 1, Lukas Rüttiger 1, Marlies Knipper 1

1 University of Tübingen, Department of Otolaryngology, Tübingen Hearing Research Centre (THRC), Molecular Physiology of Hearing, Elfriede-Aulhorn-Str. 5, 72076 Tübingen, Germany

Age related hearing loss is one of the most common disabilities in humans. In rodents we could show that age related hearing loss occurs only in the last third of the lifespan. Before disturbed OHC function becomes obvious, an elevation of hearing thresholds for higher frequencies is already detectable. These changes were found in old and middle aged animals.

We recently reported, that, as long as sufficient neuronal gain is preserved, suprathreshold temporal coding of amplitude modulated stimuli was maintained, independent of age. In old animals central neuronal gain was reduced and temporal coding failed (Möhrle et al. 2016). The decline in neuronal gain was associated with a reduced level of plasticity markers, e.g. brain derived neurotrophic factor (BDNF). This finding corresponds to a significant reduction of BDNF activity-dependend transcripts observed in cochlear high frequency turns over age (Rüttiger et al. 2007). BDNF orchestrates survival and outgrowth of neurons mapping low frequency sound information and drives refinement of auditory circuitries during the onset of sensory experience and in the adult brain (Singer et al. 2014).

Here we present a mouse model expressing a reporter for a plasticity marker to investigate presbycusis in the cochlea and the brain.

Möhrle et al. Loss of auditory sensitivity from inner hair cell synaptopathy can be centrally compensated in the young but not old brain. *Neurobiol Aging*. 2016 May; 44:173-184.
Singer et al. The function of BDNF in the adult auditory system. *Neuropharm* 2014 Jan; 76: 719-728.

Rüttiger et al. BDNF mRNA expression and protein localization are changed in age-related hearing loss. *Neurobiol Aging* 2007 Apr; 28(4):586-601.

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Yukiko Hanada (1)(2), Yasumitsu Takimoto (1)(2), Takao Imai (1), Yukiko Nakamura (2), Hidenori Inohara (1), Shoichi Shimada (2)

(1) Department of Otorhinolaryngology - Head and Neck Surgery, Osaka University Graduate School of Medicine, Osaka, Japan

(2) Department of Neuroscience and Cell Biology, Osaka University Graduate School of Medicine, Osaka, Japan

Introduction:

Although previous studies have shown age-related change of vestibular function in human, the decrement of vestibulo-ocular reflex (VOR) has been controversial. Moreover, so far little is known about age-related vestibular dysfunction in animal models. We examined age-related change of VOR in mice using high-speed video-oculography (VOG) analyzing system (PLoS One, Imai et al., 2016).

Methods:

C57BL/6J female mice at 7 weeks and 13 months were used for this study. The mouse was fixed at the center of a turntable and eye movements were recorded using a high resolution infrared 240-Hz camera. Eye and turntable (head) movements were recorded using a 240 Hz high resolution infrared CCD camera recording system. The turntable (head) was rotated sinusoidally at 0.5Hz, 1.0Hz, and 2.5Hz using a metronome by hand in darkness. Movements of the turntable and eye were recorded by two cameras. These Images were synchronized. VOR gain and phase shifting were analyzed according to information from recorded eye and turntable images.

Results:

The VOR gain was significantly different between the 13 months and 7 weeks groups.

Conclusions:

This VOR analyzing system and these results may be useful for studying age-related change of vestibular function and its treatments.

Ah-Ra Lyu (1), Yong-Ho Park (2)

(1) Department of Medical Science, Chungnam National University, Daejeon, Republic of Korea

(2) Department of Otolaryngology-Head and Neck Surgery, College of Medicine, Chungnam National University, Daejeon, Republic of Korea

Objective: The purpose of this study was to investigate the effect of early postnatal NT-3 (NT3) support on hearing acquisition.

Study design: A prospective experimental animal study.

Subjects and Methods: Adenoviral (Ad) vectors expressing green fluorescence protein (GFP) alone or in combination with NT3 were injected into the scala tympani (ST) through the round window of 5 postnatal day old (P5) rats. Changes in NT3 mRNA level, hearing thresholds and morphological studies were done after the viral vector injection.

Results: NT3 mRNA was significantly increased in the Ad-GFP-NT3 group compared to the normal developmental group and Ad-GFP alone group. GFP was widely expressed in the cochlea such as in the hair cells, supporting cell area, and spiral ganglion neurons. Auditory brainstem response (ABR) thresholds were significantly lower in the Ad-GFP-NT3 group compared to the normal developmental group and Ad-GFP alone group at 15 postnatal days (P15).

Conclusion: These results show that early postnatal NT3 overexpression may accelerate the acquisition of hearing in rats.

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Toru Miwa 1, Ryosei Minoda 1, Fan-yan Wei 2, Kazuhito Tomizawa 2

1 Department of Otolaryngology-Head and Neck Surgery, Kumamoto University, Kumamoto, Japan

2 Department of Molecular Physiology, Kumamoto University, Kumamoto, Japan

Introduction

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

Transfer RNAs (tRNAs) contain a wide variety of posttranscriptional modifications that are important for accurate decoding. Mammalian mitochondrial tRNAs (mt-tRNAs) are modified by nuclear-encoded tRNA-modifying enzymes (Wei, 2013). Wei et al. have reported that cdk5 regulatory subunit-associated protein 1 (cdk5rap1) is responsible for 2-methylthio (ms2) modifications of mt-tRNAs. And deficiency in ms2 modification markedly impaired mitochondrial protein synthesis. This resulted in respiratory defects in cdk5rap1 knockout (KO) mice.

However, cdk5rap1 ms2 modification deficiency is still unknown to affect with hearing function. We herein investigated on influence of a mitochondrial dysfunction caused by the ms2 modifications of mt-tRNAs on hearing utilizing cdk5rap1KO cells in vitro and cdk5rap1 KO mice in vivo.

Materials and Methods

Embryonic fibroblast (MEF) cells of homozygous and heterozygous cdk5rap1 KO mice were grown in opti-MEM high-glucose medium supplemented with 10% fetal bovine serum at 37 °C and 5% CO₂. Senescent cells were detected by senescence-associated beta-galactosidase (SA-βgal). Apoptotic hair cells were detected by Terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) assay according to the manufacturer's protocol. The numbers of SA-βgal-positive and total cells, the numbers of TUNEL-positive and Hoechst-positive cells were counted from three randomly selected microscopic views respectively.

Auditory brainstem Response (ABR) was measured in cdk5rap1 KO mice and wild type mice. The stage of those mice was postnatal 4 weeks and postnatal 60 weeks.

Results

The number of senescent positive cells significantly increased as passaging cdk5rap1 KO cells. The number of TUNEL positive cells were significantly larger in Cdk5rap1 KO cells than hetero cells. ABR thresholds in cdk5rap1 KO mice (postnatal 60 weeks) showed significantly higher any frequency than those in wild type mice in same stage. However, ABR thresholds in cdk5rap1 KO mice (postnatal 4 weeks) showed no difference from wild type mice in same stage.

Conclusions

Our results suggest that ms2 modifications of mt-tRNAs may induce an apoptotic program in the inner ears and accelerated their aging, thereby causing aging-hearing loss.

Miriam Vogt, Wibke Singer, Marie Manthey, Michela Dotta, Marlies Knipper, Lukas Rüttiger
*University of Tübingen, Department of Otolaryngology, Tübingen Hearing Research Centre (THRC),
 RG Molecular Physiology of Hearing, Elfriede-Aulhorn-Str. 5, 72076 Tuebingen, Germany*

Stress affects the whole organism with differential impact dependent on type, intensity, duration, epigenetic status and previous basal condition. Once perceived, the primary response is the release of glucocorticoids (cortisol in humans, corticosterone in rodents) from the adrenal glands which affects tissues throughout the body, including brain and inner ear, which facilitates coping behavior and reinstatement of homeostasis. The balance between corticosteroid actions induced via activation of mineralocorticoid receptor and glucocorticoid receptor determines the differential response to stress. While both glucocorticoid receptors are known for their delayed genomic role they can also act as mediators of non-genomic signaling for rapid adaptive responses. Concerning noise-induced hearing loss, recent studies in rats have revealed an influence of glucocorticoid signaling in the protection of and remission to acoustic trauma. This is of great clinical importance since there is a common and increasing practice to use glucocorticoids for hearing preservation. Besides counteracting inflammation, glucocorticoid receptors modulate central plasticity and, as shown also by our group (Singer et al., 2013), alter the inner hair cell vulnerability to noise.

The main focus of the current study was to investigate the differential impact of pharmacologically altered stress responsiveness on IHCs synapse vulnerability.

We analyzed hearing function and central brain responses (suprathreshold ABR waves) as well as the changes of cochlear and central biomarkers in animals with endogenous or applied stress variations pre and post acoustic trauma. The stress responsiveness were altered with mineralocorticoid and glucocorticoid agonists and/or antagonists. Stress was quantified by urine measures of cortisol.

The findings are discussed in the context of multisided influences of acute or chronic stress on peripheral and central auditory processing.

Singer et al. Noise-induced inner hair cell ribbon loss disturbs central arc mobilization: a novel molecular paradigm for understanding tinnitus. *Mol Neurobiol.* 2013 Feb;47(1):261-79



Marie Manthey (1), Dario Campanelli (1), Wibke Singer (1), Lukas Rüttiger (1), Marlies Knipper (1)
 (1) University of Tübingen, Department of Otolaryngology, Tübingen Hearing Research Centre (THRC), Molecular Physiology of Hearing, Elfriede-Aulhorn-Str. 5, 72076 Tübingen, Germany

We demonstrated previously that BDNF (studied in the BDNF Pax2 Cre mouse) in the cochlea or lower brainstem regions but not in the higher frontal or cortical brain regions improves auditory fidelity with sensory experience [1]. This auditory fidelity includes the improved sensitivity of auditory fibers, the lowering of hearing thresholds, the enlarged dynamic range, shortening of latency and altered inhibitory strength. The changes of markers for inhibitory neuronal connections (GAD 67, PV) spread along the entire auditory pathway as well as hippocampal circuits [1]. Here we asked, to what extent the failure in BDNF Pax2 Cre ko mice to develop appropriate auditory acuity and fidelity would influence the capability of the central auditory pathway to compensate for injury (noise trauma) induced cochlear deprivations [2,3]. We exposed BDNF Pax2 Cre ko and wt mice to enriching (80dB SPL), mildly traumatic (100dB SPL), and traumatic (120dB SPL) sound and measured various functional (ABR) and molecular markers (plasticity genes, markers for inhibition and excitation) along the auditory pathway. The findings are presented and discussed in the context of sensory experience-induced maturation of auditory fidelity as potential prerequisite for adaptive homeostatic plasticity.

[1]Chumak et al. BDNF in lower brain parts modifies auditory fiber activity to gain fidelity but increases the risk for generation of central noise after injury. *Mol. Neurobiol.* 2015; [2] Singer et al. Noise-induced inner hair cell ribbon loss disturbs central arc mobilization: a novel molecular paradigm for understanding tinnitus. *Mol Neurobiol.* 2013 Feb;47(1):261-79 ; [3] Rüttiger et al. The reduced cochlear output and the failure to adapt the central auditory response causes tinnitus in noise exposed rats. *PLoS One.* 2013;8(3):e572472013

Hong-Wei Zheng, Kayla Hill, Su-Hua Sha

Department of Pathology and Laboratory Medicine, Medical University of South Carolina, Charleston, SC 29425

Inhibition of caspase activation prevents noise-induced outer hair cell (OHC) death and hearing loss (NIHL). However, inhibition of caspases promotes the activation of receptor-interacting protein (RIP) kinases, which may foster necrotic-like OHC death. Here, we investigated the interaction of activated caspases and RIP kinases in noise-induced apoptotic and necrotic OHC death using adult CBA/J mice exposed to broadband noise from 2–20 kHz at 106 dB SPL for 2 hours inducing permanent threshold shifts (PTS). Both apoptotic and necrotic OHC nuclei were observed in the lower basal region of the cochlea examined 1 hour after noise exposure. Treatment with pan-caspase inhibitor ZVAD blocked noise-induced activation of caspase-8 and reduced the number of apoptotic nuclei. In contrast, such treatment increased the levels of RIP1 and RIP3, resulting in necrotic OHCs. Conversely, blocking noise-induced over-expression of RIP1 and RIP3 by treatment with necrosis inhibitor necrostatin-1 (Nec-1) or RIP3 siRNA (siRIP3) decreased the number of necrotic OHC nuclei, but increased the number of apoptotic nuclei without increasing activation of caspase-8, while increasing activation of caspase-9 and promoting EndoG translocation into OHC nuclei. Finally, ABR functional measurements and morphological assessment of OHCs showed that only ZVAD treatment marginally reduced noise-induced deficits, while combination of ZVAD and siRIP3 treatment potentiated protective effects against NIHL. In conclusion, noise-induced OHC apoptosis and necrosis are modulated by caspases and RIP kinases, respectively. Inhibition of either pathway shifts the prevalence of OHC death to the alternative pathway. Thus, effective protection from NIHL may require a multi-pronged approach. The research project described was supported by R01 DC009222 from the National Institute on Deafness and Other Communication Disorders, National Institutes of Health.



Anni Herranen (1), Tommi Anttonen (1), Ulla Pirvola (1)

(1) Division of Physiology and Neuroscience, University of Helsinki, Finland

Since the environment never remains unchanged, maintenance of homeostasis is a critically important function for all living organisms, and coping with stress is a challenge faced at different levels: organs, individual cells and subcellular structures. However, above a certain threshold of stressors, tissue and cell damage is ensued. Noise and toxic drugs are environmental stressors that damage and kill cochlear sensory cells. Several stress signaling pathways are known to be activated in traumatized cochlea and have been suggested to mediate the detrimental effects of stressors on cochlear sensory cells. One of these pathways is the JNK (c-Jun N-terminal kinase)/c-Jun pathway. However, the cell biological mechanisms of JNK/c-Jun signaling in the cochlea remain poorly understood. We have studied the dynamics of N-terminal phosphorylation of c-Jun, termed as the c-Jun stress response, in the noise- and ototoxic drug-damaged cochlea. c-Jun is rapidly phosphorylated upon trauma in many cell populations of the cochlea. The response is rapidly downregulated thereafter. Ototoxic lesion triggers a similar response pattern, but the response is stronger and broader. The vulnerable outer hair cells lack the c-Jun stress response before, during and after the exposure to the environmental stressors. Our results suggest that c-Jun N-terminal phosphorylation is a biomarker for stress in the cochlea, and not a marker for dying cells. Our results also suggest that c-Jun phosphorylation is part of a larger stress response in the cochlea, comprising both beneficial and detrimental components. Understanding the beneficial and detrimental components of stress signaling and how they are interconnected allows development of safe and efficient therapeutic interventions.

Gleice Cristina Colombari*, Adriana Andrade Batista Murashima*, Maria Rossato*, Elizabete Rosa**, Miguel Angelo Hyppolito*

**Laboratory of Neurobiology of Hearing, Department of Ophthalmology, Otorhinolaryngology and Head and Neck Surgery, Ribeirão Preto Medical School, University of São Paulo*

***Department of Cell and Molecular Biology and Pathogenic Bioagents, Ribeirão Preto Medical School, University of São Paulo*

There are so many questions about the molecular mechanisms involved in ciliary recovery to be answered, both, in normal situations and in exposure to intense noise. The mechanisms of the ciliary spontaneous recovery are not well established, if there is intrinsic mechanisms of ciliary turnover or the regeneration processes involved culminating in the auditory functional recovery, it is of huge importance.

PROPOSE: The aim of this research was to study the expression of F-actin, β -actin and myosin VIIa in outer hair cells of the cochlea before (G1), after exposure to an intense noise (G2) and at the fifth day of rest after such exposure (G3) and correlate this findings with the electrophysiological auditory thresholds.

RESULTS: The means value of electrophysiological auditory thresholds differed ($p \leq 0.01$) between the three different moments of hearing tests (pre-noise < post-rest < post-noise). The means value of the expression of F-actin and Myosin VIIa differed ($p \leq 0.01$) among the three groups, for the F actin expressions the means value were $G1 > G3 > G2$ and for the Myosin VIIa were G1.

Fetoni AR 1, Di Pino A 1, Eramo SLM 2, Paciello F 1, Rolesi R 1, Troiani D 2, Paludetti G 1
 1 *Institute of Otolaryngology*, 2 *Institute of Human Physiology, Università Cattolica, School of Medicine, Rome, Italy*

Introduction: It has been demonstrated that the increase of ROS generation and of lipid peroxidation, together with a concurrent decrease of antioxidant defenses, play a significant role in noise-induced hearing loss (NIHL). 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 Caffeic acid (CA), a common phenolic acid, which is abundant in plant products such as fruits, vegetables, coffee, tea and propolis (honeybee resin). CA has been described as an anti-inflammatory, anti-viral, anti-bacterial, anti-neoplastic and antioxidant compound. Furthermore, studies have shown that CA suppresses lipid peroxidation and inhibits the activation of nuclear transcription factor NF- κ B on oxidative stress and reduces nitric oxide (NO) formation in the brain, kidney, and liver. The aim of this study was to evaluate the protective effect of CA against NIHL.

Methods: Wistar rats (200-250 g) were used in this study. Animals were exposed for 60 minutes to a pure tone of 120 dB SPL, 10 kHz. Of these, a group was treated with CA (i.p. 30 mg/Kg 1h pre trauma and for 3 consecutive days). We evaluated auditory function by Auditory Brainstem Responses (ABR) recording, the extent of damage with rhodamine-phalloidin (Rh-Ph) staining, the superoxide amount with Dihydroethidium (DHE) assay, the magnitude of lipid peroxidation by 4HNE expression and of reactive nitrogen species production by NT-3 evaluation.

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

Conclusion: Our results demonstrate that CA treatment can reduce the oxidative cochlear damage caused by noise. The phenolic acid CA shows antioxidant properties as ferulic acid and curcumin against NIHL in the organ of Corti. Consequently CA can provide a promising approach against the oxidative stress induced by NIHL.

Natalia Rybalko 1, Dijana Mitrovic 2, Tetyana Chumak 1, Daniel Šuta 1, Josef Syka 1

(1) Department of Auditory Neuroscience, Institute of Experimental Medicine, ASCR, Prague, Czech Republic, (2) 2MED-EL Medical Electronics, Innsbruck, Austria

OBJECTIVE

Brief acoustic trauma in early, critical, developmental periods, may lead to altered auditory processing in adulthood. In the present study the auditory temporal resolution was examined in adult rats that have normal hearing thresholds despite noise exposure in the early postnatal period. We measured the acoustic startle response (ASR) and the efficiency of startle prepulse inhibition induced by gap in noise to assess the ability to detect the gap and to screen for hyperacusis and tinnitus.

METHODS

Rat pups (strain Long-Evans) were exposed to 125 dB SPL broad band noise for 8 min, on the 14th postnatal day. ASRs and their prepulse inhibition induced by gap in noise of different levels and different frequency ranges (gap-PPI) were examined in exposed and control rats at the age of 3 months. Hearing thresholds in all rats were assessed by auditory brainstem responses (ABRs).

RESULTS

A significant deficit was observed in the efficiency of the startle prepulse inhibition but not in the startle reactivity in exposed rats compared to controls. A decrease in the background noise level from 75 dB to 55 dB SPL led to a significant weakening of the gap-PPI in exposed rats in contrast to controls. A large deficit in the gap-PPI was observed in exposed rats under more difficult listening conditions (at lower level of background noise: 55dB SPL). Analyzing the gap-PPI for noises of different intensities and frequency ranges revealed signs of hyperacusis (pronounced suppression of ASR at lower noise intensities compared to controls) and tinnitus (deficit of gap-PPI in 10 kHz background noise) in some exposed rats. Cochlear histological analysis showed a significant decrease of the inner hair cell ribbon synapses in exposed rats.

CONCLUSIONS

Early acoustic trauma in rats that did not produce permanent threshold shift led to abnormalities in supra-threshold auditory sensitivity. Diminution of gap-PPI in exposed rats compared to controls reflects a gap detection deficit that indicates disorders in auditory temporal resolution. Behavioral evidence for a possible induction of hyperacusis and tinnitus following early acoustic trauma was observed in some adult exposed rats with a normal audiogram.

Francis Rousset (1), Marta Coelho (1), Vivianne Kokje (2), Pascal Senn (2)*, Karl-Heinz Krause(1)*
 * contributed equally

1. *Department of Pathology and Immunology, Faculty of Medicine, University of Geneva, Switzerland*

2. *Department of ENT, Head & Neck Surgery, University Hospital of Geneva, Switzerland*

Research over the last decades has identified a major role for reactive oxygen species (ROS) in hearing loss, including overexposure to noise, ototoxic drugs (e.g. cisplatin) and age-related hearing loss. NADPH oxidases (NOX) are a family of seven isoenzymes expressed in mammals dedicated to the production of ROS. While the physiology of NOX enzyme is at least partially understood (it includes bacteria killing, otoconia formation or thyroid hormone synthesis), there is increasing evidence that dysregulation of NOX activity contributes to pathological states. High levels and specific expression in the inner ear identifies NOX3 as a potential drug target to combat hearing loss.

Using mice with loss of function mutations in the NOX3 complex (NOX3- or p22phox-deficient), this study aims to address the role of NOX3 in noise-induced hearing loss.

Mice were anesthetized and exposed to 116 Db (SPL) for 2h to an octave band noise (8-16 KHz). Hearing thresholds, as assessed by the Auditory Brainstem Response (ABR) were determined before and 1 and 7 days after the noise exposure. At the end of the experiment, morphology of the cochlea, including hair cells and spiral ganglion neurons, was studied.

In the absence of ototoxic exposure, hearing threshold did not reveal differences between NOX3 mutant mice and their WT littermates. However, NOX3 mutant mice showed markedly improved hearing recovery, as assessed 7 days after noise exposure ($p < 0.01$ at 45kHz). We also studied p22phox mutant mice, which, in addition to lacking functional NOX3, also lack functional NOX1, 2, and 4. Unexpectedly, basal hearing of p22phox mutant mice was already significantly better than WT ($p < 0.0001$ at 16kHz). These differences extended to neighboring frequencies after the noise overexposure (11-32kHz). Histological studies to address the molecular and cellular pathways involved are ongoing.

Based on loss of function mutant mice, this study confirms NOX3 as a prime target for hearing loss. A contribution of other NOX isoforms, in particular the p22phox-dependent NOX1, NOX2 and NOX4, would also be compatible with our present results. The long term goal will be to limit over activity of NOX3 (and potentially other NOX isoforms) either by molecular or by pharmacological approaches.

Katrin Reimann 1, 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, Elfriede-Aulhorn-Str. 5, 72076 Tübingen, Germany

2 University of Bochum, Department of Pharmacology and Toxicology, Universitätsstr. 150, 44780 Bochum, Germany

3 University of Würzburg, Department of Physiology, Röntgenring 9, 97070 Würzburg, Germany

Objective: In the inner ear, the NO-cGMP signaling pathway has been described to facilitate protecting [1] but also damaging processes in response to traumatic events. However, the individual roles of the two nitric oxide-sensitive guanylyl cyclase isoforms (NO-GC1 and NO-GC2) as cGMP generators in these processes 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) [2], 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 isoform specific 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 we also included analysis of the cochlear microvasculature, see [3] for methods.

Results: Comparison between the NO-GC1 KO, NO-GC2 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. The details of the differential Phenotype will be presented.

Conclusions: The results will be discussed in the context of 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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[1]. M Jaumann et al.: cGMP-Prkg1 signaling and Pde5 inhibition shelter cochlear hair cells and hearing function. *Nat Med.* 2012, 18.2: 252-259

[2]. E Mergia et al.: Spare guanylyl cyclase NO receptors ensure high NO sensitivity in the vascular system. *J clin Invest* 2006, 116: 1731-1737

[3]. Chumak T et al.: BDNF in Lower Brain Parts Modifies Auditory Fiber Activity to Gain Fidelity but Increases the Risk for Generation of Central Noise after Injury. *Mol Neurobiol* 2015

Laura Astolfi (1)(2), Edi Simoni (1), Filippo Valente (1), Alice Cani (1), Milvia Chicca (3), Alessandro Martini (2)(4)

1) *Bioacoustics Research Laboratory, Department of Neurosciences, University of Padua, Padua, Italy*

2) *Foundation Onlus 'Staminali e Vita', Padua, Italy*

3) *Department of Life Sciences and Biotechnology, University of Ferrara, Ferrara, Italy*

4) *ENT surgery - Department of Neurosciences, University of Padua, Padua, Italy*

Coenzyme Q10 (2, 3-dimethoxy-5-methyl-6-decaprenyl-1, 4 benzoquinone, CoQ10) is known as an antioxidant able to counteract reactive oxygen species and lipoperoxides, preventing their cytotoxicity. However, this compound is hardly bioavailable because it is not soluble in water or lipids. A patented terclatrate version of CoQ10 with improved water solubility was recently developed (Q-Ter). We previously verified the efficacy of Q-Ter associated to Acuvul 400®, a multivitamin supplement, in preventing noise-induced hearing loss in a rat model by auditory brainstem responses (ABR). Deafening noise is known to cause blood flow alterations and oxidative stress in the inner ear. We therefore tested the antioxidant activity of Q-Ter associated to Acuvul 400® (respectively 500 mg/kg and 100 mg/kg, Q-Ter-A) on a rat model. A total of 50 male Sprague-Dawley rats was divided into 4 groups: noise-exposed (6 kHz white noise, 115±3 dB SPL, for 2 hours), noise-exposed and orally pretreated with Q-Ter-A, Q-Ter-A positive controls and untreated controls. The vascular and oxidative stress was assessed by immunohistochemistry and mRNA expression of specific markers (thrombomodulin, tissue factor, HIF-1α, JNK). The results showed that Q-Ter-A positive controls did not induce vascular or oxidative alterations in comparison to noise-exposed rats. The oral pretreatment with Q-Ter-A was not able to compensate noise-induced vascular alterations but could completely prevent noise-induced oxidative stress. Since previous ABR data showed that Q-Ter-A was able to completely prevent noise-induced hearing loss, probably the effects of the compounds are exerted only through antioxidant activity and not through vascular interactions.

Alfred L. Nuttall, Qing Yu, Teresa Wilson

Oregon Health & Science University, Oregon Hearing Research Center, 3181 SW Sam Jackson Park Road, NRC04, Portland, OR 97239

Introduction: The capillaries of the cochlear lateral wall are part of the blood-labyrinth-barrier (BLB) that separates inner ear fluids from systemic circulation. The BLB controls the exchange of solutes, protein, and water, and plays an essential role in maintaining inner ear homeostasis and the endocochlear potential. BLB function relies on the integrity of stria endothelial cell-cell contacts that is regulated in part by adherens junction (AJ) complexes. The mechanisms that regulate AJ function in cochlear lateral wall remain largely unknown. Here, we examined the role of focal adhesion kinase (FAK), a stress-responsive signaling factor that is a key mediator of both inflammation and vascular permeability, in modulating the response of the cochlear lateral wall to loud sound.

Methods and Results: The cochlear spiral ligament (SL) and stria vascularis (SV) were isolated from 10 week old CBA/CaJ mice and immunolabeled for VE-cadherin, β -catenin, and FAK. Confocal imaging revealed overlapping fluorescent signals for VE-cadherin and β -catenin at endothelial cell-cell contacts. FAK signal was present in the nucleus, cytoplasm, and at the plasma membrane. Loud sound exposure (105 dB SPL, 8-16 kHz, 2 hours) resulted in increased levels of FAK Y397 phosphorylation in SV ECs and type I fibrocytes of the SL which overlapped with VE-Cadherin at EC junctions. Next, we found that VS-4718, a small molecular FAK kinase activity inhibitor, attenuated both VEGF and H₂O₂-induced increases in pFAK Y397 phosphorylation levels in whole mount preparations of SV and SL tissues. To determine whether FAK kinase inhibition would attenuate noise-induced loss of hearing sensitivity, mice were treated with VS-4718 (30mg/kg, IP) or vehicle (PEG 400/2.25% DMSO/saline) at 1hr prior and 24 hrs post-loud sound exposure. Auditory brainstem response analysis revealed that VS-4718 treatment reduced loss of hearing sensitivity as compared to controls. Quantitative RT-PCR analysis showed that FAK kinase inhibition also reduced transcript levels of several pro-inflammatory factors following noise exposure.

Conclusions: Pharmacological inhibition of FAK activity proved protective against loud sound-induced increase of inflammatory factor expression and hearing threshold shifts, establishing a role for FAK activity in loud sound-induced hearing sensitivity loss.

Kensuke Kiyomizu (1,2), Keiji Matsuda(2), Koji Torihara (2), Ryuichiro Takeda (3) Yasushi Ishida (4), Kensei Yoshida (1), Tetsuya Tono (2)

(1) Department of Psychiatry, Yoshida Hospital, Nobeoka, Japan, (2) Department of Otorhinolaryngology, Head & Neck Surgery, Faculty of Medicine, University of Miyazaki, Miyazaki, Japan, (3) Department of Healthcare And Security Center, University of Miyazaki, Miyazaki, Japan, (4) Department of Clinical Neuroscience, Faculty of Medicine, University of Miyazaki, Miyazaki, Japan

[OBJECTIVE] We reported psychiatric (Psy) comorbidity in patients with dizziness. In this study, we investigated about tinnitus.

[METHODS] The subjects were 194 patients (80 men, 114 women, age range, 17-93; mean age \pm SD 61.7 \pm 18.0 years) with tinnitus. Patients were diagnosed using ICD-10.

[RESULTS] Psy comorbidity was revealed in 150 (77.3%). Of 150 patients, various types of Psy disorders (D) were found, such as anxiety or panic D (F41) in 77 (51.3%), mood D (F3) in 34 (22.7%), adjustment D or post-traumatic stress D (F43) in 4 (2.7%), other neurotic D (F48) in 5 (3.3%), organic mental D (F0) in 16 (10.7%) and schizophrenia (F2) in 5 (3.3%). Two case studies were a 56 year-old male case with a suicide attempt and a 67 year-old female case with schizophrenia.

[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.

Keywords: psychiatric comorbidity, collaboration

Lukas Rüttiger, Marlies Knipper

University of Tübingen, Clinic for Otolaryngology, Hearing Research Centre, Molecular Physiology of Hearing, Tübingen, Germany

Many disorders related to the loss of hearing function can be accompanied by the percept of Tinnitus. Due to the diversity in human etiology of disease the origin of this often strongly affecting phantom sound perception is not well understood. Animal models offer the opportunity to study tinnitus with standardized procedures. Common ways to induce tinnitus in animal models is the use of auditory noise exposure (to induce a persistent acoustic trauma) or high-dose salicylate treatment reversibly inducing tinnitus in mouse and man. Both paradigms are reported to impair the hearing in all treated animals. However, only a minor proportion of animals with documented threshold loss develops tinnitus (see e.g. Rüttiger et al 2013).

To study the relationship of peripheral hearing loss, central auditory function, and generation of tinnitus in more detail, rats were treated with salicylate and/or exposed with mild to strongly traumatizing sound. Tinnitus involvement was determined by a behavioral paradigm (conditioning technique). The loss of hearing function was measured by the auditory brainstem response (ABR) and distortion products of the otoacoustic emissions (DPOAE). To get insight into central auditory processing disorders after the tinnitus inducing treatment, ABRs were analyzed for amplitude and latency changes of the early ABR waves (ABR wave I, reflecting auditory nerve activity) and later ABR waves (ABR wave IV, in the rat reflecting activity in the lemniscus and inferior colliculus).

Data for Tinnitus development and loss of auditory responses are discussed in the context of compensatory changes of central auditory gain in the auditory brainstem for above-threshold responses. The results underline the importance of central gain for the generation of Tinnitus in situations of various amounts of auditory deprivation.

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Cosnier-Pucheu S.(1), Larroze-Chicot P.(1), Marie A.(1), Baudoux V (1), Cazals Y.(2), Norena A.(2), Cardoso M.(3), Laboulais A.(3), Goze-Bac C.(3)
 (1) Cilcare, Montpellier, France, (2) Aix-marseille University and CNRS, Marseille, France, (3) BioNanoMRI, CNRS, University of Montpellier, France

Tinnitus, the perception of a “phantom” sound in the absence of external stimulation, is a common consequence of damage to the auditory periphery. It affects around 15% of the population and may induce intolerable discomfort. Today, no treatment exists to cure tinnitus. Some candidate drugs are in the process of being developed and several have to be tested in animal models but it remains very difficult to detect tinnitus objectively and quantitatively in animals.

Objectives

The objective of this study is to search for reliable and valid experimental measures of the presence of tinnitus in order to promote the development of treatments against tinnitus. Aspirin consistently induces tinnitus at high doses. Using this salicylate model, we explored three different techniques to detect salicylate-induced alterations. Salicylate was administered in Long Evans rats by i.p. administration at the dose of 350 mg/kg during 4 days.

Preliminary results

The silent gap inhibition of acoustic startle reflex (GIAS) was measured. In control animals, a silent gap in a constant acoustic background inhibited the subsequent startle response to a very loud sound burst. After salicylate treatment we observed less silent gap induced inhibition of the acoustic startle.

Tinnitus must be associated with alterations in brain functioning. In this perspective we examined electrophysiological alterations at inferior colliculus and auditory cortex using recordings of spontaneous activity and evoked responses from microelectrodes. Our results showed an increase in amplitude of cortical responses evoked by tone bursts over a wide range of frequencies and intensities.

Finally, using MEMRI (Manganese Enhanced MRI) together with local Mn administration at the cochlear round window, we observed that salicylate increased MRI signals in different brain structures as compared with controls.

Conclusion

We obtained reproducible results with each technique. We intend to further develop our research and to finally validate our measures by comparison with operant conditioning experiments in the same animals. Psychophysical equivalents of GIAS may be used in humans. New chemical developments of Manganese compounds for administration in MRI suggest a putative translation to humans.

So Young Park 1, Min Jung Kim 1, Ilyong Park 2, Shi Nae Park 1

1 Department of Otorhinolaryngology-Head and Neck Surgery, College of Medicine, The Catholic University of Korea, Seoul, Republic of Korea

2 Department of Biomedical Engineering, College of Medicine, Dankook University, Cheonan, Republic of Korea

Objectives: Gap-prepulse inhibition of the acoustic startle reflex (GPIAS) has been used in rats and mice for tinnitus screening and assessment. Tinnitus animal models previously demonstrated the development of deficits in GPIAS. In this study, noise-induced tinnitus (NIT) in mice was serially evaluated by GPIAS after different types of noise exposure to determine the most appropriate method for tinnitus development. To investigate the relationship between NIT and stress, we firstly evaluated the stress hormones in the plasma as a pilot study.

Methods: Male C57BL/6 mice aged 1 mo were exposed to three different noise stimuli: 110 dB SPL white noise for 1 hr once, 4 hrs once, and 4 hrs everyday for 5 days. Auditory brainstem response (ABR) thresholds and distortion product otoacoustic emissions (DPOAE) were serially recorded up to 3 months. Tinnitus was also assessed serially by GPIAS to obtain GPIAS ratios. All the audiological and GPIAS data of three NIT groups were compared with those of the control group. Plasma levels of norepinephrine (NE) and cortisol were compared among the four groups.

Results: During the 3 mo follow-up period, hearing levels of three different NIT groups showed significantly elevated ABR thresholds with a mild increasing trend with time compared to the control group. DPOAE levels of the NIT groups were significantly lower than those of the controls without any inter-NIT-group differences. GPIAS ratios decreased in NIT groups up to 1 month, and subsequently, individual variations seemed to increase. NE level in the plasma of 1 hr NIT group and cortisol levels of all NIT groups were significantly higher than those of the controls.

Conclusion: NIT mouse models developed by different types of noise exposure and confirmed by GPIAS seem to be well established in this study. Elevated plasma level of stress hormone in NIT groups indicates its possible role as a biomarker in tinnitus-related stress condition, which still needs further investigation.



Woongsang Sunwoo (1), Mun Young Chang (2), So Young Kim (3), Young Ho Kim (1)

(1) Department of Otorhinolaryngology, Seoul National University College of Medicine, Seoul National University Boramae Medical Center, (2) Department of Otorhinolaryngology, Chung-Ang University Hospital, (3) Department of Otorhinolaryngology, Cha Medical Center

BACKGROUND:

Over-dosage of sodium salicylate (SS) is often used to make the tinnitus animal model. The protective effect of Korean Red Ginseng (KRG) against the salicylate-induced ototoxicity has not been studied previously. The present study was performed to investigate changes of expression levels of N-methyl D-aspartate receptor subunit 2B (NR2B) protein in inferior colliculus (IC) after administration of SS and/or KRG in rats.

METHODS:

Male Sprague-Dawley rats were divided into 4 groups and treated as follows: vehicle control group; SS group (sodium salicylate, 300 mg/kg, 5 days, IP); KRG group (KRG gel extract, 200 mg/kg, 12 days, PO); KRG-SS group. Auditory brainstem responses (ABRs) were recorded at pretreatment and 12 days posttreatment. Whole brains and inferior colliculus were harvested for immunohistochemical staining and Western blot assay using anti-NR2B antibody.

RESULTS:

There was no significant difference of mean ABR thresholds among groups before and after treatment. Western blot assay for anti-NR2B antibody of IC in SS group and KRG-SS group demonstrated the higher expression compared to in vehicle control group. However, the expression level in SS group did not show the significant difference compared to that in KRG-SS group. Immunohistochemical staining for anti-NR2B antibody in IC showed a higher expression level in SS group compared to vehicle control group, whereas weaker in KRG group and KRG-SS group.

CONCLUSION:

Salicylate-induced ototoxic rat model demonstrated over-expression of NR2B protein in IC. However, the administration of KRG did not show the significant reduction of protein expression level of NR2B in IC in response to administration of salicylate. These findings suggest that KRG may not enable the prevention of ototoxicity such as tinnitus by modulation of NMDA receptor expression.

S. Cosnier-Pucheu (1), P. Larroze-Chicot (1), A. Marie (1), Y. Cazals(2), A. Norena (2), J. Alam (3)
CILcare (1), University of Aix-Marseille (2), EIP Pharma, LLC (3)

Several publications suggested that Mitogen-activated protein kinase (MAPK) is involved in damage of cochlear OHC, and a recent study showed that the early effects of acoustic trauma leads to a transient activation of BDNF and MAPK in the inferior colliculus (IC). The effects of acoustic trauma in the IC is of particular interest because of its possible involvement in the generation of hyperacusis and tinnitus. Another study showed that these inhibitors decreased dose-dependently the auditory threshold shift and outer hair cell loss induced after acoustic trauma.

Based on these results, the objective of this study was to assess the effect in rats of a selective small molecule inhibitor of the alpha isoform of p38 MAPK, when administered by oral route, on auditory nerve (CAP and inferior colliculus (IC)) evoked potentials. Adult Long Evans rats were used, electrodes were implanted chronically. Measures were recorded at various frequencies from 2 to 32 kHz from 90 to 10 dB SPL. Rats anesthetized were exposed to a bilateral acoustic trauma of 1 hour, at 120dB (SPL), with a two-octave noise band (4-16kHz) for a vehicle group (n=9), two groups treated at 1.5mg/kg and 4.5mg/kg (n=10/group) and a non-exposed group (sham, n=11). Post acoustic trauma evoked responses were measured after 3, 7, 10, 14 and 16 days. The investigated compound was administered twice daily from day 8 post-trauma and during 7 days.

The noise exposure produced approximately a 20 to 30 dB transient CAP threshold shift measured 24 hours after the noise exposure in all exposed groups. An increase of the IC evoked potential amplitude was observed at 6kHz and was significant at day 3 and day 14 compared to the values observed in sham rats. Both doses of the p38 MAP kinase inhibitor reduced non significantly the amplitude increase of the IC response at 6kHz.

In conclusion, this first reported study of a selective p38 MAP kinase inhibitor after acoustic trauma shown some protection against inferior colliculus alterations induced by such trauma. However determination of its therapeutic potential requires further studies. The observed effects might be significant for hyperacusis and tinnitus treatment.

T. Schomann (1), J.C.M.J. de Groot (1), C.H. van der Ploeg (1,2), E.G.J. Hendriksen (2), D. Ramekers (2,3), S.F.L. Klis (2,3), J.H.M. Frijns (1), M.A. Huisman (1)

(1) Department of Otorhinolaryngology and Head & Neck Surgery, Leiden University Medical Center, Leiden, the Netherlands

(2) Department of Otorhinolaryngology and Head & Neck Surgery, University Medical Center Utrecht, Utrecht, the Netherlands

(3) Brain Center Rudolf Magnus, University Medical Center Utrecht, Utrecht, the Netherlands

Introduction: Round window membrane (RWM) application of the specific Na⁺/K⁺-ATPase inhibitor ouabain is known to selectively destroy type-I spiral ganglion neurons (SGNs) in the cochlea of several rodent species. This protocol has been used in various cell-based regeneration studies, mostly in Mongolian gerbils and rats, to induce partial ablation of the cochlear nerve. Since only conflicting data are available in the guinea pig, we investigated if RWM application of ouabain induces selective degeneration of SGNs in the guinea pig cochlea.

Methods: The auditory bulla of the right ear of albino guinea pigs (N=20) was opened via a retroauricular approach and a cube of gelfoam soaked in 10 µl of ouabain solution (10 µM, 100 µM, 1 mM and 10 mM) or PBS was placed upon the RWM. The left cochleas served as controls. Auditory function was assessed using click-evoked auditory brainstem responses (ABRs) before surgery and at 2, 4, and 7 days after surgery. After the final ABR recordings, animals were euthanized and the cochleas were fixed by intralabyrinthine perfusion and processed for histological examination.

Results: Animals treated with 1 mM or 10 mM ouabain showed a dramatic and progressive shift in hearing thresholds. The corresponding histological changes varied considerably and the animals can be divided into three main groups: (1) animals with complete loss of SGNs and outer hair cells (OHCs) in the basal and middle cochlear turns as well as partial OHC loss in the apical turn; (2) animals without loss of SGNs, but with OHC loss in the basal and middle turns; and (3) animals without any loss of SGNs and OHCs. In none of the animals did we observe loss of inner hair cells or histological changes in the stria vascularis. Animals receiving lower ouabain concentrations (10 µM and 100 µM) or PBS alone did not express decreased ABR responses, or changes in cochlear histology.

Conclusion: Our preliminary results show that RWM application of ouabain does not induce selective degeneration of type-I SGNs in the guinea pig cochlea.

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Erin Greguske (1), Maria Carreres-Pons (1), Meritxell Deulofeu (1), Blanca Cutillas (1), Judit Homs (2), Christian Chabbert (3), Pere Boadas-Vaello (2), Jordi Llorens (1,4,5)

(1) *Universitat de Barcelona, Barcelona, Catalonia, Spain*

(2) *Universitat de Girona, Girona, Catalonia, Spain*

(3) *Aix-Provence Université, Marseille, France*

(4) *Institute of Neurosciences University of Barcelona, Barcelona, Catalonia, Spain*

(5) *Institut d'Investigació Biomèdica de Bellvitge (IDIBELL), L'Hospitalet de Llobregat, Catalonia, Spain*

Vestibular toxicity in humans commonly results from chronic exposure, yet animal models to study this toxicity are usually based on acute or short-term repeated exposure paradigms. Thus, chronic ototoxicity models have been scarcely studied and, although the cellular and molecular mechanisms involved in chronic versus shorter exposure ototoxicity may be similar, they may also differ considerably. A recent study using rats exposed chronically to 3,3'-iminodipropionitrile (IDPN) has identified the dismantlement of the calyceal junctions between type IHCs and calyx endings as an early and reversible step in damage progression in the vestibular epithelium. This study examined whether these plasticity phenomena can be studied in a second species, the mouse. In several strain/sex combinations we recorded either limited vestibular toxicity or excessive systemic toxicity; however, exposure of male 129S1/SvImJ mice to 30 mM IDPN in the drinking water offered the desired model. As previously found in male Long-Evans rats, vestibular dysfunction, assessed weekly by a test battery, appeared progressively and reverted after the intoxication was terminated at 5 or 8 weeks of exposure. Confocal microscopy analysis of immunolabeled cristas from animals exposed for 5 or 8 weeks revealed a striking loss of proteins that characterize the calyceal junction, including the adhesion protein caspr1 and the extracellular matrix protein tenascin-C. Mislocalization of labeling for KCNQ4, a potassium channel also enriched in the calyceal junction area was also recorded. Recovery in immunolabeling for these proteins was observed after washout periods of 5 or 12 weeks following the 5 or 8 weeks of exposure, respectively. Therefore, this mouse model mimics the previously established rat model. Thus, the mouse can also be used to study the damage and repair phenomena occurring during chronic ototoxicity and recovery.

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Marion Souchal, Fabrice Giraudet, Paul Avan

Laboratoire de Biophysique Neurosensorielle, UMR INSERM 1107, Faculté de Médecine, Université d'Auvergne, Clermont Ferrand, France

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

Maurizio Cortada (1), Soledad Levano (1), Daniel Bodmer (1)
(1) Department of Biomedicine, University Hospital Basel, Basel, Switzerland

Introduction: Brimonidine, an α_2 -adrenergic receptor agonist (α_2 -AR), is used to treat open-angle glaucoma, ocular hypertension and facial erythema in rosacea. Several studies have shown neuroprotective effects of Brimonidine in the optic nerve, retinal neurons and spiral ganglion. This neuronal survival is mediated through the α_2 -AR. The presence of α_2 -ARs in outer and inner auditory hair-cells has been shown. However, the effects of Brimonidine on auditory hair-cells have never been investigated so far. The purpose of the study was to investigate the effects of Brimonidine on auditory hair-cells exposed to gentamicin.

Methods: Organ of Corti (OC) explants were pre-treated with Brimonidine and/or Yohimbine. Yohimbine is a specific α_2 -AR antagonist. Hair-cell damage was induced using Gentamicin. Hair-cell survival was determined counting surviving hair-cells with a fluorescence microscope. The Protein levels of p-ERK1/2 and p-Akt were examined by Western blot.

Results: Brimonidine and Yohimbine have both no toxic effect on hair-cells. Brimonidine showed to have a dose-dependent protective effect on auditory hair-cells from gentamicin-induced toxicity. The effect of Brimonidine is reverted, when hair-cells are pre-treated with Yohimbine. This suggests that the protective effect on hair-cells is transmitted through the α_2 -AR. Furthermore, Brimonidine seems to have a protective effect by upregulating p-Akt and p-ERK1/2 in vitro. However, this effect is not statistically significant.

Conclusions: For the first time, the effect of Brimonidine on auditory hair-cells was investigated. Brimonidine, acting directly via its α_2 -adrenergic receptor, seems to protect hair-cells from gentamicin-induced toxicity. Therefore, Brimonidine can be potentially used for prevention or treatment of sensorineural hearing loss in the future.

Yeon Ju Kim, Young Sun Kim, Beomyong Shin, Oaksung Choo, Jeong Hun Jang, Yun-Hoon Choung
Department of Otolaryngology, Ajou University School of Medicine, Suwon, South Korea

Purpose: Autophagy is a major intracellular degradation process, by which cytoplasmic material is degraded via the fusion of double-membrane compartments, autophagosomes with lysosomes. This process is important for the maintenance of cell homeostasis. Previous our data showed that enhanced autophagic flux can delay the aminoglycoside-induced ototoxicity, but the specific genes that are related with autophagy-dependent protective properties have not been identified in hair cells. To investigate the possible link between the autophagy process and hair cell protection against aminoglycoside-induced ototoxicity, we performed next generation sequencing (NGS) using ex vivo system.

Methods and Materials: Organ of Corti explants of Sprague-Dawley rats were cultured on tissue culture plates. The explants were exposed to: (a) Distilled water, (b) 50 μ M gentamicin and (c) 50 μ M gentamicin + 50 pM rapamycin (as autophagy inducer) for 2 days. Transcriptome of organ of Corti cells were examined using RNA-sequencing. Differentially expressed genes were further verified for with quantitative RT-PCR.

Results: First, we evaluated hair cell loss in the apical, middle and basal turns of the organ of Corti in each group. Immunohistochemical findings showed that the loss of stereocilia was observed much more numerously in the gentamicin-treated explants than in the gentamicin + rapamycin-treated explants. Then the RNA samples were extracted from each group and RNA-seq was performed. We have shown the lists of differently expressed genes between samples. RNA-seq results showed that 672 genes were up-regulated and 255 genes were down-regulated under the gentamicin-treated condition compared to the control group; gentamicin + rapamycin-treated group reversed some of these gene expression levels (344 genes were up-regulated and 163 genes were down-regulated). The roles of these genes are further investigated now.

Conclusions: Autophagy may be closely connected with the aminoglycoside-mediated hair cell death and survival through the expression of specific genes.

Sarah L. Boddy (1), Paul J. Gokhale (1), Darrell M. Barrott (1), Marcelo N. Rivolta (1).

(1) Centre for Stem Cell Biology, Department of Biomedical Science, University of Sheffield, Sheffield, UK

Various protocols now exist to differentiate stem cells towards otic cell fates. However, invariably they yield mixed populations containing the desired cell type as a sub-population. For stem cell-based therapies to progress towards the clinic, it is essential that cell populations for transplants are purified to remove potentially harmful contaminants, such as proliferative residual stem cells, which could lead to tumour formation or the failure of any cell graft. An established method to separate out component cell populations from heterogeneous cultures is Fluorescence-Activated Cell Sorting (FACS), but to use this method, specific cell-surface markers must be identified that bind to the key cell-type e.g. otic progenitors.

We have undertaken a large antibody screen against cell surface epitopes on otic progenitor cultures derived from two independent human embryonic stem cell (hESC) lines, H14s9 and Shf3.2. To further enhance the robustness of this screen, each line was differentiated using two distinct protocols developed in our lab. In this way we hoped to discard false-positive hits specific to a particular cell line, or reflecting the idiosyncrasies of a single protocol.

Screen plates were imaged using an IN Cell Analyser suite (GE Healthcare), preserving morphological information alongside fluorescence intensity levels. Data was first analysed objectively in terms of the comparative proportion of positive cells in each antibody condition relative to negative control wells. The hits obtained were then scrutinised subjectively, visually assessing the associated images to identify antibodies that bound specifically to cells with otic progenitor morphologies. The five top- ranked candidates for sorting otic progenitors were selected for further analysis. We have also identified a potential exclusion marker i.e. an antibody that binds to all non-progenitor cells within the differentiated cultures. Co-labelling and flow cytometry experiments indicate these markers identify different/ overlapping subsets of otic progenitors, which may signify different stages of development or distinct substates of physiological relevance.

Now we have identified cell surface antibodies that recognise hESC-derived otic progenitors, we hope to use these to purify progenitor populations in an effort to improve both transplant outcomes and downstream differentiation efficiencies in vitro.

Lopez-Juarez A (1)*, Lahlou H (1), Fontbonne A (1), Tonetto A (2), Brezun JM (1), Cazals Y (1), Zine A (1,3)

1 *Université Aix-Marseille, LRIA, CNRS-UMR 7260. Marseille, France*

2 *Université Aix Marseille, Fédération de chimie de Marseille. Marseille, France*

3 *Université de Montpellier, Faculté de Pharmacie. Montpellier, France*

In mammals, auditory hair cells (HCs) and supporting cells are only generated during embryonic development and loss of HCs due to environmental stresses, ototoxicity, genetic factors, or aging is irreversible. Cell based therapy approach for inner ear damage has received considerable attention over the past decade; however, two major challenges remain to be addressed 1) to obtain characterized human otic progenitors in vitro and 2) to promote their survival and migration into the damaged organ of Corti in vivo. Recently, our group was able to obtain human otic progenitors cells (hOPCs) from human induced pluripotent stem cells (hiPSCs). The in vitro differentiation of hOPCs gives rise to differentiated cells upregulating a subset of HC associated markers (Atoh1, Pou4f3, Myo7a) as revealed by qPCR and immunostaining analyses (See Lahlou et al., abstract).

Our final goal is to analyze the capacity of hOPCs to survive, migrate and differentiate within damaged organ of Corti in vivo. To this end, we developed a partial hearing loss model in the guinea pig using amikacin ototoxic drug. The level of hearing loss was evaluated both histologically by HC count and functionally by ABR. We observed that amikacin exposure at 400 mg/kg for a period of 11 days was sufficient to induce a partial hearing loss. The ABR thresholds of amikacin-treated animals increased by about 20-30 dB at 8-32 kHz as compared to ABR measured before amikacin exposure. Furthermore, HC count along the cochlear epithelium showed a partial and significant loss of OHCs within the corresponding tonopic areas.

For engraftment purpose, the hOPCs were labeled with Vybrant dye to track their fate in our hearing loss model. After transplantation through cochleostomy in amikacin-damaged cochleas, Vybrant positive-hOPCs were observed to migrate at different turns and tissues along the entire cochlear duct at least until 14 days post-engraftment.

These data suggest the amikacin-induced hearing loss as one valuable model to track the in vivo migration, integration and differentiation of hOPCs for extended periods post-engraftment within damaged cochleas.

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Christina Ahrensmeier (1), Stefan Dazert (1), Stefan Volkenstein (1)
(1) Ear, Nose and Throat Clinic, Head and Throat Surgery, Ruhr University of Bochum

Introduction: The auditory system consists of central and peripheral nervous system (CNS/PNS) components. By now, there is little knowledge about differences and common cell types of both parts in the auditory system published. In this project, we aim to compare the in vivo situation between cochlear nucleus (CNS) and spiral ganglion (PNS) at different postnatal stages and additionally we compare the in vitro situation towards the in vivo situation of the cochlear nucleus (CN) and the spiral ganglion (SG).

Methods: Postnatal mice (P3) were suspended into a neurospheres assay. Spheres were cultivated under the same conditions until passage 3/4 (including dissociation and passaging) and immunohistochemically stained. The SG and CN of postnatal mice (P3) were sectioned and also immunohistochemically stained. Additional differentiation cell culture experiments of plated neurospheres (passage 2) were performed for 7 d and immunohistochemically analysed as well.

Results & conclusion: We found an expected distribution of PNS and CNS glial cells and neurons in the in vivo and in vitro situation. Surprisingly, we also found MOG (Myelin oligodendrocyte glycoprotein), S100A6 (S100 calcium-binding protein A6 - astrocytes precursor cells) positive cells in spiral ganglion spheres and MPZ (Myelin protein zero - absent in CNS) positive cells in cochlear nucleus spheres, whereas none of these cells were detectable in the in vivo situation at P3 so far. GFAP (Glial fibrillary acidic protein) and S100 protein were distributed in higher amount in CN and SG spheres and as in comparison to in the spiral ganglion and cochlear nucleus. Our results suggest that the auditory system may be an exception in nervous system organization and that its neural stem and precursor cells may have the opportunity to generate several cell types - without the guidance of tissue cytokines, growth and neurotrophic factors - and are not limited to the PNS or CNS. Still, more research is necessary to clarify our recent results, immunohistochemical investigations of later time points (P14/adult) are already planned.

Jennifer Schulze (1,2), Michael Stolle (1), Ariane Römer (1,2), Thomas Lenarz (1,2), Martin Durisin (1,2), Athanasia Warnecke (1,2)

(1) *Department of Otorhinolaryngology, Head and Neck surgery, Hannover Medical School, Carl-Neuberg-Str. 1, 30625 Hannover, Germany*

(2) *Cluster of Excellence of the German Research Foundation (DFG; "Deutsche Forschungsgemeinschaft") "Hearing4all"*

Introduction: Platelet-rich plasma (PRP) is a small volume of plasma with a high concentration of platelets whereas platelet-poor plasma (PPP) consists only of pure plasma. PRP contains several growth factors and cytokines, e.g., insulin-like growth factor-1 and vascular endothelial growth factor, which are involved in tissue and central nervous system repair processes. Although the neuroprotective and regenerative properties of PRP have been already described, regenerative effects in the inner ear have not been reported thus far. Therefore, the effects of PRP and PPP on spiral ganglion neurons (SGN) and spiral ganglion (SG) explants were examined in vitro.

Methods: PRP and PPP were obtained from human venous blood by centrifugation of whole blood aliquots with a thixotropic gel in a closed centrifugation system (RegenKit®-BCT3, RegenLab, Switzerland). SGN and SG explants were dissected from neonatal Sprague-Dawley rats of both sexes (postnatal day 3-5). The neuroprotective effect of PRP was investigated by cultivation of SGN in serum-free medium supplemented with PRP in four different dilutions. PPP was mixed with calcium gluconate to coat the cell culture plates for cultivation of SG explants.

Results: PRP treatment significantly increased the survival rate of SGN when compared to the BDNF control. Gels consisting of PPP and calcium gluconate enhanced the adhesion of SG explants, mimicking an extracellular matrix. Moreover, the neuroregenerative process of SG explants was increased through cultivation on PPP calcium gluconate gels.

Conclusions: The present study demonstrated that PRP and PPP have neuroprotective and neuroregenerative abilities. Due to its autologous generation, PRP and PPP are readily available from the patients requiring therapy. In addition, the safety of this treatment is well known for other organ systems. Thus, the clinical usage of blood components such as PRP and PPP is more feasible than the direct administration of recombinant growth factors to the inner ear.

Acknowledgements: This work was supported by the Cluster of Excellence Hearing4all.

Gerrit Paasche (1,2), Piera Ceschi (1), Mareike Hütten (1), Maciej Wilk (1), Roland Hessler (3), Thomas Lenarz (1,2), Verena Scheper (1,2)

(1) Hannover Medical School - Hannover - Germany

(2) Cluster of Excellence «Hearing4all» - Hannover - Germany

(3) MedEl - Vienna - Austria

Dexamethasone is known to reduce tissue growth and to protect residual hearing after cochlear implantation. Despite this little is known about the effect of dexamethasone on spiral ganglion neurons (SGN). Therefore, results from different studies all applying dexamethasone were re-evaluated in order to investigate its effect on SGN.

First, in in vitro tests, dexamethasone was tested at concentrations of up to 10⁻⁴ mol/L (40ng/mL). No negative effect on SGN survival was detected. Moreover at a concentration of 10⁻⁹ mol/L (0.4pg/mL), a slight increase ($p < 0.05$) in survival was detected.

In four different in vivo models of dexamethasone delivery into scala tympani (A: 50mg/mL in a PEG-based hydrogel reservoir; B: incorporation into the silicone carrier of CI electrode arrays at 1% and 10% (w/w) concentration; C: delivery of 100 ng/mL via an electrode-micro-pump-system; D: incorporation of 15% and 30% (w/w) in a polymeric coating of cochlear implant electrode models), animals were sacrificed 4 or 12 weeks after implantation. SGN density was evaluated in all groups. Electrical hearing thresholds were analysed in chronically electrically stimulated animals.

SGN density was not affected by dexamethasone compared to respective controls. Simultaneously applied with electrical stimulation (ES), dexamethasone did increase the protective effect of ES on SGN density. Moreover, there is also a tendency of decreasing EABR thresholds compared to ES-only treated individuals.

Based on the results of these studies, dexamethasone concentrations as applied in these models are safe for inner ear delivery in terms of their effect on SGN density. Moreover, dexamethasone increased the neuron-protective effect of ES.

Blanca Cervantes (1,2) Isabel Sanchez-Perez (4), Carmen Herrero (5), Isabel Varela Nieto (1,2,3)

(1) Centro de Investigación Biomédica en Red de Enfermedades Raras, Madrid, Spain

(2) Instituto de Investigaciones Biomédicas 'Alberto Sols' CSIC-UAM, Madrid, Spain

(3) IdiPAZ, Instituto de Investigación Hospital Universitario La Paz, Madrid, Spain

(4) Dpto. Bioquímica. Fac. Medicina. Instituto de Investigaciones Biomédicas 'Alberto Sols' CSIC-UAM, Madrid, Spain

(5) Spiral Therapeutics, San Francisco, USA

HEI-OC1 (House Ear Institute – organ of Corti 1) is an epithelial otic cell line that was derived by Kalinec et al. (2003) from the cochlea of a transgenic mouse called Immortomouse™, which harbors a temperature-sensitive mutant of the SV40 large T antigen. The modulation of culture conditions allows the progression of the progenitor cells to a differentiated hair cell-like with a phenotype similar to that of the adult organ of Corti. One the most interesting characteristic of these cells is their sensitivity to ototoxic drugs such as aminoglycoside antibiotics or antineoplastic agents as cisplatin, which can cause sensorineural hearing loss. Cisplatin is a highly effective chemotherapeutic agent, but it has significant ototoxic side effects. Apoptosis is an important mechanism of cochlear hair cell loss following exposure to cisplatin. The present study examined the effects of LPT99, a second generation of APAF-1 inhibitors on cisplatin-induced apoptosis. Viability and growth rate of HEI-OC1 cells were determined using a crystal violet based staining method and the activation of Caspase-3, a biochemical marker for apoptosis cell death was evaluated with immunocytochemistry. We observed a dose-dependent decrease in cell viability after challenge with cisplatin (0-5 µg/ml); however survival rate increased in the presence of 1 µM LPT99. According, cells treated with cisplatin showed an IC50 of 4.47 ± 1.94 µg/ml, which increased dramatically to 10.51 ± 3 µg/ml in the presence of LPT99. In addition, immunofluorescence studies suggest that the activity of Caspase-3 after cisplatin treatment decreased in the presence of LPT99. These results suggest that LPT99 protected the cells HEI-OC1 against cisplatin-induced apoptosis by inhibiting of APAF-1. Research funded by the European Commission, under the FP7-PEOPLE-2013-IAPP-TARGEAR projects.

Silvia Murillo-Cuesta (1,2), Julio Contreras (1,2,3), Adelaida M. Celaya (1,2), Tania Jareño (1,2), Sandra Marchán (4), Javier Sanagustín (4), Estefanía Traver (4), Carmen Herrero (5), Isabel Varela-Nieto (1,2)

(1) Centro de Investigación Biomédica en Red de Enfermedades Raras, Madrid, Spain

(2) Instituto de Investigaciones Biomédicas 'Alberto Sols' CSIC-UAM, Madrid, Spain

(3) Facultad De Veterinaria, UCM, Madrid, Spain

(4) Laboratorios SALVAT, Esplugues de Llobregat, Spain

(5) Spiral Therapeutics, San Francisco, USA

Hearing loss is a common severe adverse effect of chemotherapy with platinum agents, especially cisplatin. Cisplatin induces the depletion of glutathione and antioxidant enzymes, leading to accumulation of reactive oxygen species in the cochlea, which trigger the intrinsic apoptotic pathway in hair cells. The apoptosis protease-activating factor-1 (APAF-1) binds to cytochrome c to form the apoptosome, which recruit procaspase-9 and activates effector caspases. APAF-1 inhibition has been previously validated as therapeutic strategy for the prevention of unwanted apoptosis. Here we evaluate the in vivo therapeutic effect of LPT99, a second generation APAF-1 inhibitor with good cochlear permeability, in the prevention of cisplatin induced-hearing loss in rats.

LPT99 (50-200 mM in vehicle) was administered by transtympanic surgery in young male Wistar rats. Ototoxicity was induced immediately after by intraperitoneal slow infusion of cisplatin (10 mg/kg). Hearing was evaluated by registering the auditory brainstem response (ABR) to click and pure tones, before and 3 days after cisplatin administration. Finally, rats were euthanized and the cochleae dissected for total RNA extraction and gene expression or histology.

LPT99 treatment of rats attenuated the threshold shifts induced by cisplatin after 3 days of its administration when compared to the vehicle-treated rats. Thresholds of tone frequencies of 20, 28 and 40 kHz were significantly lower than in the cisplatin group. This protective effect was dose-dependent, showing 100 μ M dose the best profile. In addition, LPT99 diminished the alterations in ABR peak latencies and amplitudes induced by cisplatin administration. The expression of p53, Il1 α and Kim-1 was reduced in LPT99-treated cochleae, suggesting that LPT99 prevents inflammation and apoptosis.

In conclusion, transtympanic administration of LPT99 protects from cisplatin-induced hearing loss in rats, confirming APAF-1 as a valid pharmacological target and postulating LPT99 as a potential first in class drug candidate.

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Jana Schwieger (1,2), Karl-Heinz Esser (2), Thomas Lenarz (1,3), Verena Scheper (1,3)

(1) Department of Otolaryngology, Hannover Medical School, Hannover, Germany

(2) Department of Zoology, University of Veterinary Medicine Hannover, Foundation, Hannover, Germany

(3) Cluster of Excellence Hearing4all, German Research Foundation

Sensorineural deafness is mainly caused by damage of hair cells and subsequent degeneration of spiral ganglion neurons (SGN). Cochlear implants (CI), which stimulate the SGN electrically, are the therapy of choice. The benefit of the CI depends on number and excitability of the SGN. To identify treatment strategies for SGN survival, in vitro tests are performed on spiral ganglion cells (SGC), including all kinds of cell types located in the spiral ganglion. The non-neuronal cells influence neuroprotection and neuritogenesis of the SGN in culture and affect the evaluation of a direct effect of neuronal treatments. Also an overgrowth of the culture by dividing cells can be observed with increasing time of cultivation. A mitotic inhibitor (Cytarabine (AraC)) may decrease the proliferation of the non-neuronal cells to address these issues and is therefore tested in the SGC culture.

Neonatal rat SGC were dissociated and cultured for 4 and 7 days with and without AraC and addition of serum or neurotrophic growth factors (NTF; brain-derived neurotrophic factor and neurotrophin 3). Different cell types of the culture were identified by immunocytochemistry and the number of total cells and SGN were analyzed. Out of these results, the percentage of neurons was calculated. Additionally, neurite outgrowth and soma diameter were observed.

After 7 days, the total number of cells was significantly reduced in all inhibited conditions while the proportion of neurons was significantly increased, especially by NTF-addition. There was no significant difference in the number of neurons under mitotic inhibition and the effect of the NTF was not significantly reduced. Neither neurite length nor soma diameter of the analyzed neurons was affected by AraC. The immunocytochemistry verified a nearly complete removal of fibroblasts and an effective reduction of glial cells.

The addition of AraC is an effective tool to reduce the non-neuronal cell population and raise the number of SGN in culture without negatively affecting the neurons. AraC inhibits an overgrowth of the culture and allows for investigation of treatment strategies on SGN with reduced glial support.

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Kunio Mizutani (1)(2), Kyoko Kitao (2)(3), Susumu Nakagawa (2), Tatsuo Matsunaga (2), Satoshi Fukuda (3), Masato Fujii (2)

(1) Department of Otolaryngology-Head and Neck Surgery, National Defense Medical College, Saitama 359-8513, Japan

(2) Division of Research for Hearing and Balance Disorders, National Institute for Sensory Organs, National Tokyo Medical Center, Tokyo 152-8902, Japan

(3) Department of Otolaryngology-Head and Neck Surgery, Hokkaido University Graduate School of Medicine, Hokkaido 060-8638, Japan

Reduction of endocochlear potential (EP) is one of the main causes of sensorineural hearing loss. Previously, we described an animal model of severe cochlear lateral wall damage generated by the local administration of the mitochondrial toxin 3-nitropropionic acid (3-NP) into the round window niche. This model induces a decrease in the EP and causes a severe and selective damage to the cochlear fibrocytes in the cochlea. However, histological analyses indicate that after 3-NP-treated ear damage, the lateral wall fibrocytes spontaneously recover and the re-expression of Na/K-ATPase occurs in these cells in the cochlea at 2 months after 3-NP treatment. In this study, we investigated changes in the EP using a mouse model of acute cochlear energy failure, which comprised severe cochlear lateral wall damage induced by the local administration of 3-NP to the inner ear. We also analyzed the correlation between EP changes and histological findings in the cochlear lateral wall. We detected the recovery of the EP and hearing function at lower frequencies after severe damage of the cochlear lateral wall fibrocytes at the corresponding region. Remodeling of the cochlear lateral wall was associated with EP recovery, including the re-expression of ion transporters or gap junctions (i.e., Na⁺/K⁺-ATPase-β1 and connexin 26). These results reveal a mechanism for late phase hearing recovery after severe deafness, which is frequently seen in clinical settings.



Jae Young CHoi
Yonsei University

MicroRNA (miRNA)s are non-coding RNA composed by 20 to 22 nucleotides which regulate development and differentiation in various organs by silencing of specific RNA and regulation of gene expression. In present study, we show that miR-183 is essential for neuromast regeneration after neomycin-induced hair cell damage in zebrafish model.

Expression of mir183 was checked by in situ-hybridization. Microarray analysis for microRNA was performed. Haircell regeneration were observed after neomycin (10 μ M for 2hr) in zebrafish (72h after fertilization) neuromast by using fluorescent staining.

Microarray analysis clearly showed that miR 183 family were upregulated after neomycin treatment. We also confirmed the miR 183 was clearly expressed in neuromast and highly expressed during hair cell regeneration by in situ-hybridization. Furthermore morpholino 183 injection inhibited the hair cell regeneration. DAPT also inhibited the hair cell regeneration. Our study shows that miR183 family is essential for haircell regeneration after neomycin treatment. And notch signal pathway is involved in this regeneration mechanism.

Pierrick Bordiga (1), Raphaëlle Cassel (1), Yves Cazals (1), Rebecca Arfaras (1), Alain Tonetto (2), Axel Assie (2), Isabelle Watabé (1), Sophie Gaboyard-Niay (3), Ali Gharbi (1), Christian Chabbert (1)
 (1) Aix Marseille University, CNRS, LNIA, FR3C - Marseille - France
 (2) PRATIM Aix Marseille University - Marseille - France
 (3) Sensorion - Montpellier - France

Hearing and balance disorders constitute a growing health problem and an unmet medical need. In France, hearing loss affects more than 10% of the adult population, while vertigo is the third motive for consultation to the general practitioner doctor and represents 5 % of hospital emergencies. Hearing and balance pathologies therefore constitute a significant burden to our health care system. A large proportion of these pathologies is attributed to direct damage to the inner ear sensory endorgans. Acute cochlear or vestibular deafferentations are believed to play a role in auditory and vestibular syndromes such as noise-induced and sudden hearing loss, or labyrinthitis, vestibular neuritis, vertigo of ischemic origin and Menière disease. There is currently no targeted pharmacological therapy to efficiently repair the inner ear primary synapses. Several studies, performed in vitro or in vivo with rodent models of excitotoxically-induced inner ear damage, demonstrated that an endogenous and spontaneous process of synaptic repair occurs in the mammal inner ear after injury. This process is believed to support at least in part the functional restoration of hearing and balance.

Present project intends to explore the repair processes at inner ear primary synapses, to decipher their different phases and to identify the cellular pathways and effectors involved. For this purpose, we are currently developing in vitro and in vivo models of inner ear deafferentation. We set up models of excitotoxically-induced inner ear damages through sound overexposure or trans-tympanic application of glutamate agonist, and co-cultures of inner ear sensory organs and primary neurons.

Thanks to these models, we intend to study the sequence of tissue damage within the inner ear sensory organs, the functional progression of hearing and balance alteration, together with the modulation of genes involved in the regeneration of functional synapses. This will be achieved through combination of immunohistochemistry, behavioural monitoring, electrophysiological recordings and qRT-PCR methods. Presentation at the IEB meeting will detail the experimental models we developed and the first results we obtained.

Raphaëlle Cassel 1, Pierrick Bordiga 1, Isabelle Watabé 1, Alain Tonetto 2, Axel Assie 2, Ali Gharbi 1, Christian Chabbert 1

1. Aix Marseille Univ, CNRS, LNIA, FR3C, Marseille, France ; 2. Aix Marseille Univ, Plate-forme PRATIM, FR 1739 FR de Chimie

Vestibular pathologies are characterized by unpredictable episodes of vertigo accompanied by postural imbalances and loss of gaze fixation during movement. Current therapeutic solutions lack specificity and efficiency. This unsatisfactory situation can be attributed to a deficit of knowledge of the pathophysiological mechanisms underlying vestibular troubles. At the cellular level, excitotoxicity is believed to be a common consequence of several pathogenic conditions (ischemia, traumatism, infection, overstimulation, ototoxic compounds) leading to synaptic damages or even hair cells death. Such damages may support number of vestibular pathologies.

The present work is based on the development of a mouse model of excitotoxically-induced vestibular disorders and on the full exploration of the sequence of functional alterations that occur over weeks after the insult initiation. Induction of the vestibular disorder was achieved through unilateral transtympanic infusion of a glutamate-receptor agonist. Vestibular disorder was evaluated using a set of behavioral assessments that allowed us to track the sequence of postural and balance changes over time. Based on these behavioral analyses, we report that there are at least two distinct phases observed following an excitotoxic injury. An acute phase of behavioral dysfunction which is characterized by the almost stop of voluntary movements as well as by the presence of specific balance alteration signs also found in human clinical studies (head tilt, muscle dystonia inter alia). In a second phase lasting several days, balance disorders are less marked over time. Finally, after a certain period no more disorder was measurable. Based on previous histological of synapses in similar animal model, we anticipate that the acute phase may result from significant synaptic disconnection (as a consequence of the glutamate aggression on the postsynaptic glutamate receptors), while synaptic reconnection and plasticity mechanisms may support spontaneous recovery observed during the second phase.

The development and application to human of a correlation between vestibular disorders and tissue damages ambitions to assist the medical community to efficiently diagnose the different type and stage of vestibular impairments and ultimately enabling the prescription of appropriate medication.

Ryosei Minoda, Toru Miwa, Hiroki Takeda

Department of Otolaryngology-Head and Neck Surgery, Kumamoto University, Kumamoto, Japan

Introduction

The developing inner ears of mouse are an attractive experimental target in developing treatment modalities for congenital inner ear diseases and to study inner ear development. We have reported on successful gene transfer into the otocysts of E11.5 embryo in the uterine (Miwa & Minoda, Neuroreport 2011, Molecular therapy, 2013). Mouse embryonic inner ears at the later stages are also an interesting experimental target. However, there have been no established strategies to access the embryonic inner ears at latter stages in vivo because both the uterus wall became thick and turbid, and the embryos per se. become turbid at these stages.

In this study, we sought to access the feasibility to access the embryonic inner ears.

Materials and Methods

There are two requirements for approaching embryonic inner ears: known landmarks for speculating the location of the embryonic inner ear and being able to identify the body surface of the embryos through the uterine wall. Regarding the first requirement, we assessed the location of the embryonic inner ears in frozen tissue sections of E12.5 to E15.5 embryos. Regarding the second requirement, by partially removing the uterine wall, we could observe the embryo's body surfaces through the transparent yolk sac.

Results

The morphological study revealed that the distance from the skin to the vestibular space was generally shorter than that from the skin to the cochlea, and the lumen of the vestibules was the widest in E15.5. Considering these finding, we decided to target the vestibular spaces of E15.5 embryos. After ventral incision of CD-1 timed-pregnant mouse (E15.5), the uteri were exposed. Then after removing the uterine walls, 9 poly-arginine-fused EGFP (EGFP-9R) was injected into the inner ears. The treated embryos were harvested at 12 h after the treatments EGFP signals were robustly detected in the inner ear, including vestibular organs and cochlea epitheliums.

Conclusions

We clarified the feasibility for approaching the embryonic mouse inner ears at E15.5 in vivo. This method should be useful for various embryonic inner ear studies.

Caron Nicolas (1), Blanca Cervantes (2), Philippe de Medina (1), Lide Arana (3), Itziar Alkorta (3), Isabel Varela-Nieto (2)

(1) *Affichem SA, Toulouse, France*

(2) *Institute of Biomedical Research «Alberto Sols» (IIBM), Spanish National Research Council-Autonomous University of Madrid (CSIC-UAM) & Center for Cooperative Biomedical Research in Rare Diseases (CIBERER) Madrid, Spain*

(3) *Unidad de Biofísica (CSIC, UPV/EHU), and Departamento de Bioquímica y Biología Molecular, Universidad del País Vasco (UPV/EHU), P.O. Box 644, 48080Bilbao, Spain*

The House Ear Institute-Organ of Corti 1 (HEI-OC1) is one of the few mouse auditory cell line available for research purposes. This cells line has been used to investigate, among topics, screening of ototoxic drugs, apoptotic pathways, autophagy, senescence, mechanism of cell protection... Solid lipid nanoparticles (SLN) represent a new alternative carrier system to traditional carriers. SLN combine advantages of the traditional systems but avoid some of their major disadvantages. They can be used to deliver a drug or plasmid in the cells. Here, we have now performed studies to evaluate the toxicity of SLN in HEI-OC1 cells, and the timing for the SLN to enter in these cells. Our results indicate that the SLN is not toxic for HEI-OC1 cells and they enter in these cells very faster. In conclusion, the SLN represent a new safe carrier to deliverer a drug to the hair cells.

Anne GM Schilder, Helmuth van Es, Shakeel Saeed, Helen Blackshaw, Thanos Bibas, Dimitris Kikidis, Stephan Wolpert, Marcus Mueller, on behalf of the REGAIN consortium
The REGAIN consortium (www.REGAINyourhearing.eu)

Introduction: Hearing loss affects over 328 million adults and 32 million children worldwide. Sensorineural hearing loss due to loss of auditory hair cells is implicated in a large number of hearing loss cases and is considered irreversible. Preclinical studies have demonstrated that pharmacological inhibition of Notch signalling through inhibition of gamma-secretase can regenerate outer hair cells and partially restore hearing capacity. The international REGAIN (REgeneration of inner ear hair cells with GAMma-secretase INhibitors) consortium has been awarded a €5,8 million Horizon 2020 grant to translate these preclinical findings into a human proof of concept clinical study using a locally administered small molecule. The consortium considers this translation as the next crucial step in the development of a regenerative therapy for hearing loss.

Method: The project involves the preclinical development of a suitable formulation of the candidate Notch inhibiting molecule; inner ear PK studies with this formulation; GMP production and characterization of the molecule; the generation of a preclinical safety data package that will allow a first in man study and addresses systemic and ototoxicity properties of the molecule. A clinical protocol in patients with sensorineural hearing loss is under development for a multiple ascending dose safety study carried out in a controlled setting in a clinical research facility in the UK. This safety study will be followed up by a Proof of Concept study that will include patients in the UK, Germany and Greece.

The REGAIN consortium consists of 7 dedicated partners, coordinated by Audion Therapeutics and involving the UCL Ear Institute, Eberhard Karls University of Tübingen, The National and Kapodistrian University of Athens, Eli Lilly, Accelovance and topstart.

Conclusion: Small molecule drugs targeting the underlying biological causes of hearing loss in a safe way may meet a medical need for millions of patients, who currently rely on the limited benefits provided by hearing aids or cochlear implants. REGAIN aims to break through that current state-of-the-art, and to advance the first highly promising pharmaceutical treatment of hearing loss through clinical testing.

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Julie Bestel, Stéphane Garnier
Groupe Entendre

Introduction:

A bi-CROS configuration is sometimes proposed to patients with a cophotic ear while the other ear is aided by a conventional hearing aid. Its principle is to send the signal picked by the microphone of a hearing aid placed on the cophotic ear to the contra lateral hearing aid. In this study, we proposed a bi-CROS amplification to patients whose worse ear was not cophotic, and still aided by a conventional hearing aid. Unlike the bi-CROS, we kept both hearing aids active, meaning that the better ear was processing the signals from both sides, and that the worse ear was still aided. We called this configuration "Stereo-CROS", which could potentially improve localisation and speech intelligibility in noise.

Material and Methods:

This study was conducted in 10 subjects showing a dissymmetric hearing loss, with a difference in PTA of at least 20 dB HL between both ears, currently using two conventional hearing aids. We proposed patients to wear a pair of new hearing aids with 2 programs: "Normal" and "Stereo-CROS". Fitting was done in vivo, targeting an insertion gain as close as possible as the one patients were used to. Patients were sent back home during two weeks, with the instruction of switching between the two programs every day. After 2 weeks, intelligibility in noise was assessed using VCV words at several SNR, bracketing the SRT, in dichotic and reverse dichotic conditions, with the two programs.

Results:

Our preliminary results show that there is no difference in speech intelligibility in noise, between the standard program and Stereo-CROS, with an average SRT of around - 5 dB for both programs, in both conditions. Moreover, it seems that, in the condition where patients are assessed with noise sent on their bad ear, Stereo-CROS saturates the good ear, leading to discomfort.

Discussion and conclusion:

Our preliminary results suggest that an adaption of the insertion gain is necessary for the Stereo-CROS condition, to maintain comfort. We are currently assessing the last patients with this slight modification, which led to immediate positive feedback regarding comfort, and for which efficacy is currently being assessed.

I. Herisanu (1), S. Hoth (2), M. Praetorius (2)

(1) *University Clinic- ENT-Aachen- Germany*, (2) *University Clinic-ENT-Heidelberg- Germany*

Introduction: In deaf children, intraoperative anatomy may be surprising, even for an experienced surgeon after years of cochlear implantation practice. It has been suggested that CT scans postoperatively should be renounced on, due to radiation exposure and higher risk of developing a brain tumor in the adulthood. **Material and Methods** We present two children with bilateral congenital deafness with cochlear malformations in the preoperative CT and MRI. Both of them have single-cavity inner ear and cochlear agenesis on both sides. The intraoperative findings were surprising, posing difficulties to cochleostomy and electrode insertion. The postoperative CT showed malinsertion and misplacement in the tuba auditiva. A revision surgery right away in one case and the next day in the other case was necessary with electrode array correct positioning. None of the two children profited from the implant. After one year of CI-use, the parents of one child demanded a reimplantation, the parents of the other child wanted an explantation.

Conclusions: When intraoperative measurements show no response or are not reliable, CT position control of the electrode array in the cochlea is mandatory in children, especially when cochlear malformation is present. An intraoperative CT or DVT or even CT navigation guiding system can be considered. The cochlear implantation in children with inner ear anomalies is not a guarantee for hearing and speech development or understanding.



Laura Astolfi (1,2), Serena Danti (3,4), Edi Simoni (1), Giuseppe Gallone (3), Stefano Berrettini (4), Alessandro Martini (2,5)

1) *Bioacoustics Research Laboratory, Department of Neurosciences, University of Padua, Padua, Italy*

2) *Foundation Onlus 'Staminali e Vita', Padua, Italy*

3) *Department of Civil and Industrial Engineering, University of Pisa, Pisa, Italy*

4) *OtoLab, Azienda Ospedaliero-Universitaria Pisana (AOUP), Pisa, Italy*

5) *ENT surgery - Department of Neurosciences, University of Padua, Padua, Italy*

Cochlear implant (CI) is the only therapeutic option for patients with severe or deep hypacusis to restore hearing. However, CI surgery may cause an electrode insertion trauma, followed by inflammatory reaction, oxidative stress and electrode impedance leading to malfunction and patient frustration. We planned to improve the CI performance by developing an innovative electrode based on conductive and piezoelectric nanomaterials, which, upon a direct application in the scala tympani, will be able to self-generate electric stimuli. Nanomaterials were produced by solvent casting followed by compression molding. The piezoelectric copolymer polyvinylidene fluoride-co-trifluoroethylene, P(VDF-TrFE), used as a matrix, was doped with conductive carbon nanotubes (CNT) with different weight percentages (0.5%, 1%, and 1% w/w plus 1% Tween 20 surfactant). The chemical-physical analyses of P(VDF-TrFE)/CNT composites showed good piezoelectric properties. The biocompatibility of these composites was tested on cells derived from the organ of Corti (OC-k3). When cultured on P(VDF-TrFE)/CNT composites up to 48 hours, the OC-k3 did not show significant cell deaths ($p > 0.05$) nor morphological alterations such as cytoskeleton reorganization or nuclear shrinkage. In conclusion, P(VDF-TrFE)/CNT were found able to generate electricity when mechanically stressed and showed high biocompatibility. Based on these in vitro properties, these composites appear suitable as improvements of CI electrodes and further investigations of these materials on in vivo models may be planned.

Marcus Müller, Masaaki Ishikawa, Monika Kwiatkowska, Peter Bako, Ute Wank, Claudia Frick, Karl-Heinz Wießmüller, Pascal Senn, Hubert Löwenheim

(1) Department of Otolaryngology, Head and Neck Surgery, Tübingen Hearing Research Centre, Eberhard Karls University Tübingen, Tübingen, Germany

(2) Medical Campus University of Oldenburg, School of Medicine and Health Sciences - European Medical School, Research Center of Neurosensory Science, Cluster of Excellence Hearing4all, University of Oldenburg, Oldenburg, Germany

(3) EMC microcollections GmbH, Tübingen, Germany

(4) Department of Clinical Neurosciences, Service of Otorhinolaryngology, Head & Neck Surgery, HUG, University Hospital of Geneva, Switzerland

Deafness can be cured by surgically implanting a cochlear implant. Electrical current is used to stimulate auditory neurons bypassing the function of cochlear hair cells. A critical part of the system is the cochlear implant electrode array in the scala tympani, spatially distant from the peripheral processes of the auditory nerve. This anatomical gap between the electrode array and the nerve fibres results in high current consumption and a low spatial stimulation specificity, resulting in variability of sound quality and speech understanding. Recent research findings indicate that peripheral processes can grow under neurotrophin stimulation towards the electrodes. The project aims at developing a neuroprosthesis with a gapless interface to auditory nerve fibres. Gel-like matrix candidates with adequate physico-chemical and biological properties were selected from in vitro experiments testing compounds and matrices for neurite outgrowth and physiological properties. The concept was tested in a series of in vivo experiments using guinea pigs deafened using a local application of a kanamycin/furosemide mixture. One week later a cochleostomy was performed and the scala tympani was filled with a hydrogel mixed with BDNF. Then a 4 channel cochlea implant provided by MedEl was inserted. Six weeks after implantation eCAP was measured and ears were fixed for histology. Application of the BDNF-gel resulted in improved physiological responses and, in some cases, neurite outgrowth onto the cochlear implant. We observed neurite outgrowth to the position of the former organ of Corti, beyond that position neurites turned into the scala tympani and grew onto the cochlea implant. Our result that auditory neurons can be guided into the scala tympani onto the cochlear implant validates the concept to bridge the gap between the electrode array and the nerve fibres. Lowered eCAP threshold furthermore validates the concept of a potential decrease in energy consumption.

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Hideaki Ogita (1), Koji Nishimura (1), Yosuke Tona (2), Takayuki Nakagawa (2), Juichi Ito (1)

(1) *Shiga Medical Center Research Institute - Shiga - Japan*

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

Introduction

The cochlear implant is the most successful prosthesis that provides hearing for people with severe-to-profound hearing loss who do not receive adequate benefit from hearing aids (Thomas J, et al., 2002). Cochlear implants bypass damaged portions of the ear and directly stimulate the auditory nerve. But the power consumption of cochlear implants is generally higher than hearing aids. Theoretically, the closer placement of the electrode to the modiolus can decrease power consumption and stimulate spiral ganglia more specifically (Jeong J, et al., 2015, Shepherd RK et al., 1993, Cohen LT et al., 2002, Saunders E et al., 2002). For this purpose, we have developed new electrodes. The conventional cochlear implants are placed with in the scala tympani. The new electrodes have needles which are designed to penetrate the bony wall of the Rosenthal's canal and to stimulate spiral ganglion neurons directly. We evaluated the effect of decreasing the distance between electrodes and spiral ganglion neurons by measuring electrically evoked auditory brainstem response (EABR).

Methods

Guinea pigs which have normal hearing were used in the present study. The original electrode which have two needles were used. A cochleostomy was made in the basal turn of the cochlear. Then the electrodes were placed into the scala tympani. The bony wall of the Rosenthal's canal was penetrated with needles to stimulate spiral ganglion neurons directly. With the needle electrodes, we performed EABR. The current intensity was increased from 50 microamperes and varied in steps of 50 microamperes. Threshold was taken as the lowest current level in which the response was elicited. After the EABR measurement, the cochleae were collected and histological analysis was performed.

Results

Needles of the electrodes were inserted into the Rosenthal's canal at the basal turn of the cochlear. With the needle electrodes, EABR were successfully elicited. In the histological analysis, there is no apparent inflammation in the spiral ganglion neurons.

Conclusion

The penetrating electrode may contribute to reduce the power consumption of cochlear implants.

Masaaki Ishikawa 1), Monika Kwiatkowska 1), Hubert Löwenheim 1), Marcus Müller 1)
1.Univ.-HNO Klinik Tübingen, Otolaryngology, Head & Neck Surgery, Tübingen, Germany

Introduction: The electrically evoked auditory brainstem response (eABR) and compound action potential (eCAP) reflect responses of the auditory nerve (AN). The interpretation of these potentials for assessment of the AN function is still unclear. We compare eABR and eCAP outcome variables calculated from input-output functions in normal hearing animals implanted with a 4 channel cochlear implant (provided by MedEl).

Methods: Five guinea pigs were used for the experiments. Measurements were performed on day 7, 14, 28 and 42 after implantation. For the electrical stimulation, biphasic pulses with phase duration of 50 μ s were applied. Six bipolar electrode configurations were used for analysis, and grouped according to the width of inter-electrode contact separation: wide and narrow. The eCAP was recorded by a gold wire electrode placed near the round window niche, for eABR scalp and retro auricular electrodes (subcutaneous silver wire) were used. Threshold, maximum amplitude, slope, dynamic range and noise were calculated from input-output functions obtained at 1 dB (eCAP) or 3 dB (eABR) steps. Time course after implantation and inter-electrode contact separation were evaluated.

Results: In eABR, a reduction in maximum amplitude, increase in slope and reduction of dynamic range were found during the observation time. Such alterations were absent in eCAP. When inter-electrode contact separation was changed from wide to narrow, an increase in threshold, reduction in maximum amplitude, increase in slope and narrowing of dynamic range were found in eABR, while only threshold increased in eCAP. The correlation of thresholds between eCAP and eABR was strong ($R=0.69$, $P<0.05$).

Conclusions: The findings indicated that eCAP response is less influenced by post-operative time course and inter-electrode contact separation than eABR. The post-operative effects on eCAP outcome variables were less pronounced than those on eABR, indicating plasticity of the eABR response. Both, eCAP and eABR, can provide the identical information regarding the threshold of the AN. As a conclusion of these findings, eABR might be more sensitive for predicting the extent of functionalized auditory nerve in deafened cochleae.

The research has received funding from the European Community's Seventh Framework Programme under grant agreement No. 281056 (Project NANOCI).

Ursula M Paredes, Lisa S. Nolan, Ruth Taylor

UCL Ear Institute, University College London, 332 Gray's Inn Road, London, UK

Cochlear Implants (CI) are one of the most successful sensory prosthetic devices and can restore hearing perception to patients with significant sensorineural hearing loss. A tissue growth or 'fibrosis' has been reported to develop around the electrode array resulting in reduction in efficacy of CI. In spite of this, little is known about the origin or nature of this material, which cellular types contribute to its growth, or the molecular changes, which underpin this process.

To address this, we first used quantitative PCR to test for changes in levels of gene expression in molecular pathways involved in fibrotic or inflammatory responses soon after implantation using a mouse model for cochlear implantation. Second, we characterized the entire transcriptome of fibrotic sheet samples from around the lead to the electrode array recovered from human implant patients and used RNA-seq to understand possible cellular contribution to this growth.

For the mouse model we used whole cochleae from mice (n=4; C57BL/6J) at 4 months of age, which had been implanted with a fluorocarbon thread and sacrificed 5 days (n=3) post-implantation or after a 'sham' operation (n=1). Genes selected are involved in: production of collagen fibres during scarring, promoting fibrosis, osteoblastic cell differentiation, macrophage differentiation, cell proliferation and differentiation, hair cell markers and cell migration and cytoskeletal maintenance. Analysis showed that expression of FN1, PTPRC, VIM, TGFB1, involved in fibrosis and cell proliferation/differentiation were significantly up-regulated compared to control. COL1A2 and COL3A1 were also up-regulated in the implanted ear but this up-regulation did not reach statistical significance. However, when compared to sham operated cochleae, we found no difference in expression levels in the selected genes. RNA-seq study of showed substantial but expected individual variation in transcript abundance in most gene pathways due to variability in human samples, and also similarities in overrepresentation of fibrosis and fibrogenesis related pathways across all samples.

In conclusion, expression of genes involved in collagen fibre production during scarring, cell proliferation/differentiation is up-regulated in implanted cochleae from mice. However, this effect is induced by the surgery. RNA-seq analysis of human tissue showed general up-regulation of transcription of genes involved in fibrosis.

Youssef Adel (1), Jochen Tillein (1, 2), Uwe Baumann (1)

(1) Audiological Acoustics, Department of Otorhinolaryngology, University Hospital Frankfurt, Germany

(2) Life Science Research, MED-EL, Innsbruck, Austria

Introduction: Patients with severely impaired high-frequency hearing and residual low-frequency hearing cannot be sufficiently accommodated with conventional hearing aids. Using hearing preservation electrode designs and surgical techniques, these cases can be provided with cochlear implants (CIs), thereby facilitating ipsilateral electric and acoustic stimulation (EAS). Still, hearing preservation is partial and long-term degradation was observed. Possibilities of surgical monitoring and clinical follow-up using electrocochleography (ECoChG) in CI patients have been recently studied. However, interpreting the complex ECoChG signal with respect to cochlear health status has proven to be a difficult task. We thus propose an experimental setup to further investigate acoustically evoked potentials in a guinea pig model of cochlear implantation.

Methods: Intraoperative monitoring is typically achieved using extracochlear ECoChG recording at the round window. During implantation, the CI electrode contacts further provide the possibility of intracochlear recording and postoperative clinical follow-up in implanted patients. The MED-EL (Innsbruck, Austria) CI telemetry system is capable of measuring electrically evoked compound action potentials (eCAP). However, low-frequency ECoChG requires long measurement windows, particularly to obtain the ongoing response. An algorithm was developed using a research interface (University of Innsbruck, Austria) to expand the recording window. This was integrated into a research setup featuring intracochlear ECoChG recording via the CI hardware evoked by triggered acoustic stimuli.

Results: The new method was validated in a guinea pig model (Dunkin Hartley) implanted with a MED-EL custom-built four-contact CI electrode carrier, which caused moderate hearing loss. Intracochlear recordings from the CI electrode contacts and hardware were in accordance with extracochlear recordings from a gold-wire electrode at the round window niche via our laboratory system. Acoustically evoked ECoChG summation and difference responses were recorded for a 100 μ s click stimulus and 8 ms sinusoidal tone bursts in the frequency range 250 Hz to 4 kHz. Response magnitudes were different between basal and more apical electrode contacts. Post-mortem reference recordings further confirmed the results.

Conclusion: Recording intracochlear low-frequency ECoChG using the MED-EL CI telemetry system in an implanted guinea pig model was validated. Our method allows further investigation of acoustically evoked potentials with respect to cochlear health status.

Hao Li (1), Fredrik Edin (1), Hisamitsu Hayashi (2), Olafur Gudjonsson (3), Niklas Danckwardt-Lillieström (1), Håkan Engqvist (4), Helge Rask-Andersen (1), Wei Xia (4)

(1) Department of Surgical Sciences, Uppsala University Sweden, (2) Department of Otolaryngology, Gifu University, Japan, (3) Department of Neuroscience, Uppsala University, Sweden, (4) Department of Engineering Sciences, Uppsala University, Sweden

Using cochlear implants (CI) it is possible to restore a sense of hearing in congenitally deaf children and severely hearing-impaired adults. Despite its great success in the past 30 years there is still room for improvement, in particular regarding the frequency resolution along with music and speech perception. Increasing the number of stimulation points may provide a better stimulation mode for the auditory nerve but risks causing signal overlap and crosstalk. By closing the anatomical gap between spiral ganglion neurons and the CI, the stimulation thresholds may be lowered which could reduce current spread. This may enable a more discrete stimulation mode for individual neurons. This strategy depends on the regenerative capacity of auditory neurons, and the ability to attract and guide them towards the electrode to bridge the gap.

Calcium phosphate sphere nanoparticles (CPS-NP) were produced and applied to CI electrode surfaces. Their capacity for acting as reservoirs for neural attractants was investigated using 125I-labeled glia cell line-derived neurotrophic factor (GDNF). A 3-D in vitro model using human and murine neurons from primary inner ear tissue was used to assess the attractive properties of the growth factor containing nanoparticles.

The CPS-NP were found to retain growth factors with high affinity using real time measurements of uptake and release. The inner ear neurons were attracted by the growth factor loaded CPS-NP surfaces in the 3-D in vitro model. Cells migrating from explants were also more attracted to growth factor loaded CPS-NP surfaces than an unloaded CPS-NP surface.

By combining the nanoparticle-based reservoirs with nerve-stimulating gels and micro-sized stimulation points a high resolution CI may be created. This strategy could potentially enable the use of hundreds of stimulation points compared to the 12 – 22 used today. This could greatly improve the hearing sensation for many CI recipients.

Hassan A. Zabalawi (1), Mohamed Jomaa (2), Wenhui Song (3), Jonathan E. Gale (4)
(1) UCL Ear Institute/ Royal Free Hospital -London-UK, (2) UCL Centre for Nanotechnology and
Regenerative Medicine/ Royal Free Hospital-London-UK, (3) UCL Centre for Nanotechnology and
Regenerative Medicine/ Royal Free Hospital-London-UK, (4) UCL Ear Institute - London - UK

Sensorineural hearing loss (SNHL) is caused by hair cell loss and by the degeneration of spiral ganglion neurons (SGNs). Cochlear implants (CIs) restore hearing to profoundly deaf people by electrical stimulation of preserved SGNs. Thus, the extent of SGN degeneration dictates the efficacy of CIs. Progressive degeneration of SGN peripheral processes raises the thresholds needed for electrical stimulation, necessitating higher currents reducing battery life and the precision of frequency representation since a larger number of adjacent SGNs are stimulated. The replacement and/or directed regrowth of auditory neurons would be an important step in any attempt to restore auditory function in patients with damaged SGNs or hair cells.

We examined the viability of oligosilsesquioxane-poly(ϵ -caprolactone) (POSS-PCL), polyhedral oligomeric silsesquioxane poly(carbonate-urea) urethane (POSS-PCU) and polyvinylidene fluoride (PVDF) for SGN adhesion and survival. Due to its biocompatibility and piezoelectric properties, PVDF was chosen to add directional growth cues through electrospinning aligned nanofibre scaffolds. We are testing the ability of these scaffolds to control and potentially enhance SGN neurite regrowth. We aim to control alignment of SGN neurites in order to maintain SGN tonotopy while bridging the gap between SGNs and CI electrodes.

The effects of alignment on the length and orientation of re-growing SGN neurites and glia were tested in vitro using primary SGNs from C57BL/6 P5 mice. Firstly, two methods of SGN preparation were compared: SG explants and dissociated SGN cultures. After fixation and immunostaining, neural and glial cells were counted, the length and direction of the regrown neurites were measured. The angles at which SGN neurites extended significantly correlated with the angles of the nanofibres.

Preliminary data show that electrospun aligned nanofibrous PVDF scaffolds can modulate glial and SGN organization in vitro. We are optimising the mechanical and electrical properties of the nanofibres through incorporation of carbon nanotubes and graphene mini-sheets. The piezoelectric aligned electrospun nanofibrous scaffolds provide high mechanical and electrical versatility and can incorporate signaling molecules and additional structural cues to guide and promote SGN growth. The following properties signify that our scaffolds may be a promising material for future CI electrodes/devices.

Verena Scheper (1,2), Heike Janssen (1,3), Peper Hubka (3), Jens Tornøe (4), Pavel Mistrik (5), Lars Wahlberg (4), Andrej Kral (2,3), Thomas Lenarz (1,2,3), Wiebke Konecny (1,3)

(1) Department of Otolaryngology, Hannover Medical School, Hannover, Germany

(2) Cluster of Excellence Hearing4all, German Research Foundation, Hannover, Germany

(3) Institute of AudioNeuroTechnology and Department of Experimental Otolology, Hannover Medical School, Hannover, Germany

(4) NsGene A/S, Ballerup, Denmark

(5) MED-EL, Innsbruck, Austria

Profound hearing impairment can be overcome by direct electrical stimulation (ES) of the spiral ganglion neurons (SGN) of the auditory nerve via a cochlear implant (CI). Thus, survival of SGN is critical for CI efficacy. Exogenous application of glial cell line-derived neurotrophic factor (GDNF) has been shown to reduce SGN degeneration. A novel method for chronic neurotrophic delivery, which has already been used successfully in human patients with neurodegenerative diseases, is delivery by a hollow-fiber membrane capsule containing encapsulated, neurotrophin-producing cells (EC device).

The aim of this study was to reveal if EC devices are a feasible local drug delivery system in cochlear implanted ears. Additionally, we investigated if GDNF-releasing EC devices can decrease SGN degeneration. Neonatally deafened cats were unilaterally implanted with a CI and an EC device at 3 months of age. Potential treatment effects were assessed after 6 months, in comparison to the contralateral ear which served as internal control.

A control group that received EC devices not producing GDNF showed slightly elevated SGN densities in the implanted ear. The level of GDNF released from EC devices did not protect SGN from degeneration. However, when combining GDNF treatment with an initial ES (≤ 1 month), the treatment had a significant neuroprotective effect which persisted for months after cessation of treatment. Histological analysis revealed tight fibrotic encapsulation of the implanted EC device, which was reduced in ES-treated ears and highest in cochleae treated with GDNF-releasing EC devices without additional ES. This fibrosis is proposed to be responsible for the cessation of GDNF release from the EC device within 6 months after implantation, as revealed by ELISA, and subsequently resulted in a lack of SGN preservation.

We conclude that the EC device implantation is a safe method for drug delivery to the cat inner ear. In case of GDNF-release, caution has to be taken with regard to the extent of fibrous tissue formation. Furthermore, early ES of auditory neurons promotes a stable and successful long-term neurotrophic treatment of the SGN.

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Steven Kroon (1), Dyan Ramekers (1,2), Emma M. Smeets (1), Ferry G.J. Hendriksen (1), Sjaak F.L. Klis (1,2), Huib Versnel (1,2)

(1) Department of Otorhinolaryngology and Head & Neck Surgery, University Medical Center Utrecht, the Netherlands

(2) Brain Center Rudolf Magnus, University Medical Center Utrecht, the Netherlands

Damage to and loss of the organ of Corti leads to degeneration of the spiral ganglion cells (SGCs). Extensively examined in animal models, this degeneration process and the prevention thereof by neurotrophin treatment is well known with respect to the cell bodies. However, the effect of deafening and/or subsequent neurotrophin treatment on the SGC axons is largely unknown. The consequences of degeneration of the axons are relevant for cochlear implantation which is applied to a deafened system but depends on the condition of the auditory nerve.

We used six groups of female albino guinea pigs: normal-hearing (n=6); 2 weeks deaf (n=4), 6 weeks deaf (n=4), and 14 weeks deaf (n=4), all untreated; 6 weeks deaf (n=4) and 14 weeks deaf (n=4), all treated with brain-derived neurotrophic factor (BDNF). Deafening was executed with a co-administration of kanamycin (400 mg/kg s.c.) and furosemide (100 mg/kg i.v.). BDNF treatment was performed for a period of four weeks, starting two weeks after deafening (Ramekers et al. 2015, J. Neurosci. 35:12331-45). The axons were analyzed near the internal auditory meatus using light microscopy.

We observed a substantial loss of auditory nerve area (29%), reduction in axon number (59%) and decrease in axoplasm area (41%) fourteen weeks after deafening compared to normal-hearing controls. The correlation between axonal degeneration and that of the SGC somata in the same cochleas, was high, although axons appeared to degenerate more slowly. In the first two weeks after induction of deafness, the axonal cross-sectional area decreased but the axon number did not. After BDNF treatment axonal survival was similarly high as SGC survival. Compared to survival at 2 weeks after deafening the SGCs and axons did not significantly degenerate up to 8 weeks after treatment cessation (one-way ANOVA, $P > 0.1$).

After deafness each surviving SGC, and after BDNF treatment each rescued SGC possesses an axon. These findings confirm that neurotrophin treatment of the auditory nerve may be clinically relevant, for instance for cochlear implantation.

Jeremy L. Pinyon (1), Cherylea J. Browne (1,2), Nigel H. Lovell (3), Matthias Klugmann (1), Gary D. Housley (1)

(1) *Translational Neuroscience Facility, School of Medical Sciences, UNSW Australia, Sydney Australia*, (2) *School of Science and Health, Western Sydney University, Sydney, Australia* and (3) *Graduate School of Biomedical Engineering, UNSW Australia, Sydney, Australia*

Introduction: Focusing of electric fields using cochlear implant electrode arrays enables efficient gene electrotransfer of naked plasmid DNA (Pinyon et al. *Sci. Transl. Med.*, 2014; Browne et al., *Gene Ther.*, 2016). Transduction of mesenchymal cells with a brain-derived neurotrophic factor gene cassette in the guinea pig cochlea led to spiral ganglion neurite regeneration, improving cochlear implant performance. Here we assessed episomal plasmid loading and stability.

Methods: As a reference, HEK293 cell monolayers were transfected with DNA solution containing two plasmids differing only in their reporter (nuclear localized GFP or cytosolic mCherry). Transfection was via close-field gene electrotransfer using the cochlear implant array placed over the HEK293 cell monolayer in the presence of the plasmid cocktail. Cells were placed in an incubator and periodically imaged up to 24 days post-transfection. This strategy was then applied to transfection of the mesenchymal cells in adult guinea pigs in the basal turn of scala tympani. Cochlear tissue was harvested and fixed in 4% paraformaldehyde at days 3, 7, 14 and 28 post transfection.

Results: Initially all transfected HEK293 cells expressed both green and red reporters, with separation of reporter expression developing from ~ day 3. This indicated initial electrotransfer of multiple copies of the plasmids, with subsequent cell divisions effectively halving the copy number. By post transfection day 6, equal numbers of cells were green, red or yellow, indicating reduction of plasmid copy number towards single copy. Expression of either red or green reporter plasmids was maintained to day 24. In contrast, in the guinea pig cochleae, mesenchymal cells maintained the proportion of green : red : yellow signal indicating a lower initial plasmid copy number and absence of cell division. The number of mesenchymal cells expressing the reporters declined over time, with no expression evident by day 28.

Conclusions: These data are consistent with a lack of mitotic division of the mesenchymal cells, which are the target for this gene therapy strategy. Reduction in the number of cells expressing the episomal plasmid reporters over time may reflect either gene silencing or cell death. Funding: ARC DP150104754 & LP0992098 with support from Cochlear Ltd.

C.Ahrensmeier (1), M.Kwiatkowska (1*), G.Ellrichman (2), R. Gold (2), S.Dazert (1), S.Volkenstein (1)
1) *Klinik für Hals-Nasen-Ohrenheilkunde, Kopf- und Hals-Chirurgie der Ruhr Universität Bochum, St. Elisabeth-Hospital*
2) *Neurologische Universitätsklinik der Ruhr-Universität Bochum, St. Josef Hospital*
(*) *present adress: Universitäts-HNO-Klinik Tübingen*

Introduction: Fumaric acids are well established drugs for Psoriasis treatment and due to their neuroprotective effects for treatment of Multiple sclerosis. In the auditory system, the loss of sensory hair cells induces a progressive degeneration of spiral ganglion neurons (SGN). The only therapeutical options for patients with a severe to profound hearing loss are Cochlear-Implants (CIs). In CIs, the hearing quality depends on the remaining SGN population which are the target cells for electrical stimulation. In this study we aim to investigate the neuroprotective effect of monomethyl fumarate (MMF) on auditory neurons.

Methods: Spiral ganglion neurons of postnatal mice were cultured as single cells, in organotypic explant cultures and in neurosphere assays. After 24h in organotypic culture cells were exposed to oxidative stress (50 μ M H₂O₂) for 2 h and treated additional 48 h with MMF. Survival and neurite length were measured under MMF towards control groups without MMF treatment by immunohistochemical staining.

Results & conclusion: MMF significantly increased the survival and neurite length in each setting towards the control groups. The neuroprotective effect of MMF on auditory neurons and the associated survival of SGN population may be used in combination with CIs and lead to an improvement of functional results in these patients.



Carlos J Duque-Afonso 1,2 , Kai Bodensiek 1 , Tobias Moser, 1,2

(1) *Institute for Auditory Neuroscience & InnerEarLab, University Medical Center Göttingen, 37099 Göttingen, Germany*

(2) *International Max Planck Research School for Neurosciences, University of Göttingen, Germany*

The most successful neuroprosthetic device, the cochlear implant, has allowed patients with severe-profound sensorineural hearing impairment to accomplish pretty fair speech comprehension, but has failed in providing speech comprehension in noisy environments, good frequency discrimination and music appreciation. The main aim of the ERC project “OptoHear” is to develop and characterize the optogenetic activation of the cochlear spiral ganglion neurons in the inner ear as a research tool and as an improved therapeutic alternative to the current electrical cochlear implant, promising better frequency and intensity resolution. However, little is known about propagation of light in the cochlea. In particular, the impact of multiple scattering events upon the interaction of light at the wavelengths of interest for optogenetic stimulation with the various cochlear tissues needs to be considered. Scattering is expected to generate a complex light distribution profile and to diminish the spatial confinement and available irradiance. Given the critical impact that this could have on the spatial resolution of the optical stimulation, our main motivation for studying the spread of light in the cochlea is to estimate the three dimensional distribution of irradiance and the volume of excited neuronal tissue. To assess the spatial spread, we are combining ex vivo determination of optical parameters of fresh cochleae to feed into a geometrically realistic Monte Carlo simulation of the spread of light in the cochlea. In addition, we are studying the spread of the neuronal activation, as readout of the changes in the immunofluorescence levels of the immediate-early gene c-fos. Together, simulations and experiments will help to characterize better our stimulation strategy and could suggest improvements for both the spatio-temporal control of the illumination and the design of the light delivery devices.

David López de la Morena (1,2), Christian Wrobel (1,4,5), Sangyong Jung (1,4), Thomas Mager (3), Ernst Bamberg (3), Tobias Moser (1,4,6)

(1) Institute for Auditory Neuroscience, University of Göttingen Medical Center, Göttingen, Germany

(2) MSc/PhD Molecular Biology - International Max Planck Research School & GGNB Graduate School, Göttingen, Germany

(3) Max Planck Institute for Biophysics, Frankfurt am Main, Germany

(4) Collaborative Research Center 889, University of Göttingen Medical Center, Göttingen, Germany

(5) Department of Otorhinolaryngology, Head and Neck Surgery, Ruhr-University of Bochum, St. Elisabeth-Hospital Bochum, Bochum, Germany

(6) Bernstein Focus for Neurotechnology, Georg-August-University Göttingen, Göttingen, Germany

Optogenetic stimulation of the auditory nerve represents a promising alternative strategy to restore hearing. Unlike electrical current, light can be conveniently focused, and thus, auditory prostheses such as the optical cochlear implant (oCI) are expected to improve the frequency resolution of state-of-the-art CIs.

Here we report that a rapidly gating Chrimson variant (Chrimson XY), helped to overcome the limitations of current optogenetic tools regarding the fast speed of auditory signal transduction. Besides conferring better temporal fidelity its red-shifted action spectrum promises decreased scattering and risk of phototoxicity while enhancing light penetration in the tissue.

Adeno-associated virus (AAV)-mediated specific transduction of spiral ganglion neurons (SGNs) with Chrimson XY, under control of the human synapsin promoter (hSyn), renders them sensitive to light peaking around 590 nm. We injected AAV2/6-hSyn-Chrimson XY-YFP through the round window into the scala tympani in p3-p5 mice and analyzed expression and function 7-11 weeks after injection.

Immunohistochemistry and confocal imaging of YFP and calretinin immunofluorescence of the injected and non-injected ears revealed opsin expression levels and SGN viability. Every injected animal showed high Chrimson XY expression, averaging over 80% of the SGNs in the injected ear in all cochlear turns and below 5% in the non-injected ear. SGN viability was not significantly affected.

To examine opsin functionality we performed a posterior tympanotomy and inserted a 50 μ m optical fiber through the round window to project the light of a 594 nm continuous wave laser on the basal spiral ganglion and record optically-evoked auditory brainstem responses. We were able to elicit oABR with light intensities as low as 0.5 mW and short stimuli down to 80 μ s. oABR showed increasing amplitude and shorter latency with rising light intensity, while amplitudes decreased when shortening stimulus duration or enhancing frequency up to 200 Hz.

Both expression and functionality seem to be higher and more consistent than with previously used blue light-gated opsins. We have thus established postnatal transduction of SGNs in mice and conclude that red-shifted optogenetic stimulation of the auditory nerve mediated by Chrimson XY drives SGN firing with low light intensities and at rates similar to physiological SGN firing.

Maximising Hearing Preservation

For more than 25 years, preserving delicate cochlear structures has been at the core of MED-EL design philosophy. To maximise the potential for hearing and structure preservation, we're developing flexible CI arrays designed to steadily release anti-inflammatory agents evenly throughout the full length of the cochlea.

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