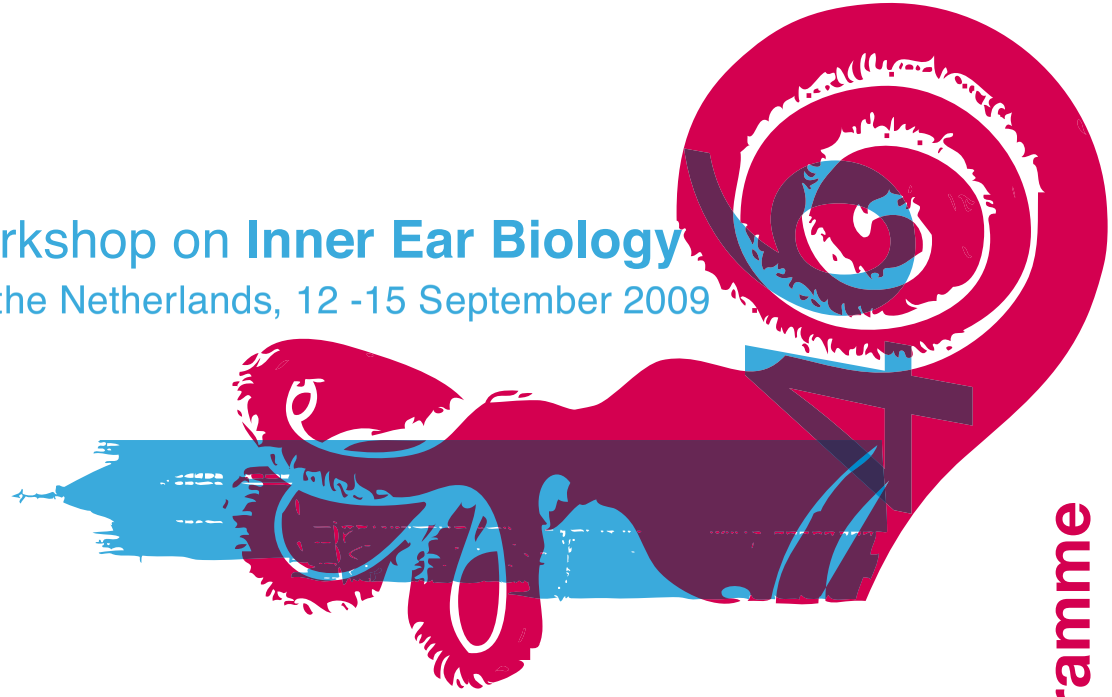
46th Workshop on **Inner Ear Biology**

Utrecht, the Netherlands, 12 -15 September 2009



Programme and Abstract Book

46th Workshop on **Inner Ear Biology**

Utrecht, the Netherlands, 12 -15 September 2009

Programme and Abstract Book

Design logo & layout: Esther Beekman (www.estherontwerpt.nl)

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PROGRAMME OVERVIEW

Conference venue
The conference will take place at the Railway Museum (“Spoorwegmuseum”) in Utrecht. The workshop will be held in the Auditorium (“Bedrijfsschool”) of the Museum. The registration desk will be staffed on Sunday and Monday in the lobby of the “Bedrijfsschool”.

Saturday 12th September

18:00 - 20:00	Registration & welcome reception at the Academy Building (“Academiegebouw”) and the adjoining Cloister Garden. The Academy Building is next to the Dom Cathedral and its Bell Tower (“Domtoren”) in the city centre.

Sunday 13th September

08:30 onwards	Registration
09:00 - 09:15	Welcoming remarks
09:15 - 10:30	Scientific session 1
10:30 - 11:15	Break and poster viewing
11:15 - 12:30	Scientific session 2
12:30 - 14:00	Lunch and poster viewing
14:00 - 15:15	Scientific session 2 (continued) and Scientific session 3
15:15 - 16:00	Break and poster viewing
16:00 - 17:30	Scientific session 4

Monday 14th September

08:30 onwards	Registration
09:00 - 10:30	Scientific session 5
10:30 - 11:15	Break and poster viewing
11:15 - 12:30	Scientific session 6
12:30 - 14:00	Lunch and poster viewing
14:00 - 15:15	Scientific session 7
15:15 - 16:00	Break and poster viewing
16:00 - 17:00	IEB Business Meeting
17:30 - 19:00	Boat trip through the canals of Utrecht; drinks are served on board
19:00 - 22:00	Conference dinner at the “Spoorwegmuseum”

Tuesday 15th September

09:00 - 10:30	Scientific session 8
10:30 - 11:15	Break
11:15 - 12:15	Scientific session 9
12:15 - 12:30	Closing remarks

Coffee and refreshments will be provided at the session breaks; lunches will be served in the Third-Class Waiting Room (“Wachtkamer 3e klasse”). Conference dinner is held in the Main Exhibition Hall of the “Spoorwegmuseum”.

Posters are on display for the duration of the Workshop in the Third-Class Waiting Room (“Wachtkamer 3e klasse”), from Sunday morning 08:00 onwards. Posters should be removed on Tuesday morning not later than 11:00. The maximum sizes of the poster display boards are 97 cm wide x 147 cm high (portrait format).

We should like to acknowledge the generous support of the following sponsors:

- Advanced Bionics
- Cochlear
- Med-El
- Wetenschappelijke Stichting Onderzoek Keel-, Neus- en Oorheelkunde Utrecht “ORLU”

Advanced Bionics, Cochlear and Med-EL financially supported the welcome reception and the lunches. The boat trip through the canals of Utrecht and the conference dinner at the “Spoorwegmuseum” were made possible by financial support from Cochlear.

SCIENTIFIC PROGRAMME

ORAL PRESENTATIONS

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

Sunday 13th September

08:30 onwards		Registration
09:00 - 09:15		Welcoming remarks
09:15 - 10:30		**SESSION 1: Transduction and neurotransmission (Part 1)** (moderators: P van Dijk and J Ashmore)
09:15 - 09:30	**O 01**	**Two populations of hair cell transducer channels enhance auditory sensitivity and dynamic range** Van Netten SM, Kros CJ
09:30 - 09:45	**O 02**	**Measurements with optical coherence tomography in the mammalian cochlea** De Boer E, Nuttall AL
09:45 - 10:00	**O 03**	**Mechanical tuning of the tectorial membrane in the basilar papilla of the frog** Van Dijk P, Schoffelen RLM, Segenhout JM
10:00 - 10:15	**O 04**	**The interplay beween electromotility and hair bundle motility in the cochlea** Ó Maoiléidigh D, Jülicher F
10:15 - 10:30	**O 05**	**Secondary cochlea vibrations** Offutt G
10:30 - 11:15		Break and poster viewing

11:15 - 12:30		**SESSION 2: Basic audiovestibular research** (moderators: RA Tange and PA Santi)
11:15 - 11:30	**O 06**	**3D Cytoarchitecture and morphometry of the cochlea of the CBA/J mouse** Santi PA, Johnson SB
11:30 - 11:45	**O 07**	**Historical studies on the vascular supply of the cochlea** Tange RA, Mudry A
11:45 - 12:00	**O 08**	**Vestibular function in a new animal model for Meniere's disease** Kakigi A, Yamasoba T, Takeda T
12:00 - 12:15	**O 09**	**Developmental changes in the frequency-tuning properties of rat vestibular ganglion neurons** Chihara Y, Iwasaki S, Yamasoba T, Sahara Y
12:15 - 12:30	**O 10**	**A re-examination of the striated organelle in vestibular end organs** Lysakowski A, Vranceanu F
12:30 - 14:00		Lunch and poster viewing
14:00 - 15:15		**SESSION 2: Basic audiovestibular research (continued)** (moderators: RA Tange and PA Santi)
14:00 - 14:15	**O 11**	**Differential passage of gadolinium through the mouse inner ear barriers evaluated with 4.7-T MRI** Zou J, Zhang W, Poe D, Zhang Y, Ramadan UA, Pyykkö I
14:15 - 14:30	**O 12**	**Diagnostic magnetic resonance images without gadolinium for inner ear diseases** Sakamoto T, Adachi T, Narazaki M, Matsuda T, Nakagawa T, Ito J

14:30 - 15:15 **SESSION 3: Genetics and deafness genes**
(moderators: GA van Zanten and L van Laer)

14:30 - 14:45 **O 13** **Molecular epidemiology of hearing loss in the Portuguese population: Two approaches**
Simões-Teixeira H, Matos TD, Moreno F, Fialho G, Del Castillo I, Caria H

14:45 - 15:00 **O 14** **Association of functional single-nucleotide polymorphisms (SNPs) of PTPN22 and FcγRIIIa (CD16a) human genes with Meniere's disease in a southeast Spanish population**
Lopez-Escamez JA, Acosta L, Moreno A, Saenz Lopez P, Gazquez I, Perez-Garrigues H, Lopez-Nevot A, Lopez-Nevot MA

15:00 - 15:15 **O 15** **Involvement of T-cell receptor beta alterations in the development of otosclerosis linked to OTSC2**
Schrauwen I, Venken K, Vanderstraeten K, Thys M, Hendrickx JJ, Fransen E, Van Laer L, Govaerts PJ, Verstreken M, Schatteman I, Stinissen P, Hellings N, Van Camp G

15:15 - 16:00 Break and poster viewing

16:00 - 17:30 **SESSION 4: Inner ear pathology**
(moderators: JCMJ de Groot and AF Ryan)

16:00 - 16:15 **O 16** **Mechanisms of aminoglycoside uptake into hair cells**
Ryan AF, Masatsugu M, Pak K

16:15 - 16:30 **O 17** **Special type of presbycusis in the Fischer 344 rat strain**
Syka J

16:30 - 16:45 **O 18** **Adenosine receptor signaling mitigates noise-induced cochlear injury**
Vlajkovic SM, Wong CY, Guo CX, Lee KH, Gupta R, Housley GD, Thorne PR

16:45 - 17:00 **O 19** **Mitochondrial dysfunction, pro-inflammatory cytokines and tauopathy sign aging cochlea and hearing impairment**
Wang J, Ladrech S, Menardo J, Casas F, Bourien J, Maurice T, Lenoir M, Puel JL

17:00 - 17:15 **O 20** **Infiltrating macrophages in the cochlear labyrinth barrier in response to loud sound stimulation mediated by iNOS signaling**
Shi X, Nuttall AL

17:15 - 17:30 **O 21** **c-Myc regulates mitochondrial peroxiredoxin in mouse cochlear hair cells**
Sha SH, Chen FQ, Schacht J

Monday 14th September

08:30 onwards Registration

09:00 - 10:30 **SESSION 5: Prevention of cochlear damage**
(moderators: SFL Klis and J Schacht)

09:00 - 09:15 **O 22** **Stress and survival pathways in the mammalian cochlea**
Bodmer D, Caelers A, Radojevic V, Brand Y, Traenkle J

09:15 - 09:30 **O 23** **Somatostatin and gentamicin-induced auditory hair cell loss**
Brand Y, Caelers A, Monge A, Radojevic V, Setz C, Bodmer D

09:30 - 09:45 **O 24** **The role of prostaglandin E receptor subtype EP2 and EP4 In the cochlea**
Hori R, Nakagawa T, Yamamoto N, Hamaguchi K, Ito J

09:45 - 10:00 **O 25** **Geldanamycin attenuates ototoxicity caused by gentamicin in the organ of Corti explants**
Szczepek AJ, Yu Y, Haupt H, Mazurek B

10:00 - 10:15 **O 26** **Protective effect of X-linked inhibitor of apoptosis protein against noise-induced cochlear lesions in C57 mice**
Wang J, Tymczyszyn N, Yu Z, Yin S, Bance M, Morris DP, Robertson GS, Korneluk RG

10:15 - 10:30 **O 27** **Clinical trial for local IGF-1 treatment for acute Sensorineural hearing loss: From the bench to the clinic**
Nakagawa T, Sakamoto T, Kikkawa YS, Hiraumi H, Yamamoto N, Tabata Y, Inui KI, Ito J

10:30 - 11:15 Break and poster viewing

11:15 - 12:30 **SESSION 6: Molecular biology**
(moderators: TA Peters and J Santos-Sacchi)

11:15 - 11:30 **O 28** **Notch signaling specifies prosensory regions in the inner ear**
Yamamoto N, Chang W, Ito J, Kelley MW

11:30 - 11.45 **O 29** **The molecular mechanism of DFNB28 deafness**
Kitajiri S, Sakamoto T, Belyantseva IA, Goodyear RJ, Stepanyan R, Fujiwara I, Bird JE, Riazuddin Sa, Riazuddin Sh, Ahmed ZM, Hinshaw JE, Sellers JR, Bartles JR, Hammer JA, Richardson GP, Griffith AJ, Frolenkov GI, Friedman TB

11:45 - 12:00 **O 30** **ATP8B1 is involved in the function of hearing**
Peters TA, Stapelbroek JM, Beynon AJ, Bull L, Oude Elferink RP, Van Zanten GA, Klomp LWJ, Houwen RHJ

12:00 - 12:15 **O 31** **Prestin as a bicarbonate-chloride transporter**
Mistrík P, Daudet N, Ashmore J

12:15 - 12:30 **O 32** **HSF1 is essential for the maintenance of inner ear function**
Yamashita H, Sugahara K, Mikuriya T, Hirose Y, Shimogori H, Nakai A

12:30 - 14:00 Lunch and poster viewing

14:00 - 15:15 **SESSION 7: Transduction and neurotransmission (Part 2)**
(moderators: H Versnel and J Syka)

14:00 - 14:15 **O 33** **Fate of mammalian hair cells and stereocilia after loss of the stereocilia**
Jia SP, He DZ

14:15 - 14.30 **O 34** **Regulation of outward K_v currents by extracellular chloride in the outer hair cell**
Li X, Surguchev A, Navaratnam D, Santos-Sacchi J

14:30 - 14:45 **O 35** **Rate-level functions at different temporal locations in the chick cochlear nerve adaptation curve**
Saunders JC, Lazaridis E, Avissar M

14:45 - 15:00 **O 36** **Otoferlin: A synaptotagmin-like calcium sensor?**
Reisinger E, Brigande J, Bulankina A, Koch M, Kügler S, Brose N, Moser T

15:00 - 15:15 **O 37** **Otoferlin interacts with myosin VI: Implications for the basolateral synaptic structure of the inner hair cell**
Heidrych P, Kuhn S, Zimmermann U, Franz C, Engel J, Dunker S, Pusch CM, Ruth P, Pfister M, Marcotti W, Blin N , Knipper M

15:15 - 16:00 Break and poster viewing
16:00 - 17:00 IEB Business Meeting
17:30 - 19:00 Boat trip through the canals of Utrecht
(drinks are served on board)
19:00 - 22:00 Conference dinner at the “Spoorwegmuseum”

Tuesday 15th September

09:00 - 10:30 **SESSION 8: Gene and stem cell therapy**
(moderators: MA Huisman and TR Van De Water)

09:00 - 09:15 **O 38** **Localized neurotrophin gene therapy for controlling auditory neurve regeneration after hearing loss**
Richardson RT, Wise AK, Hume C, Flynn BO, Jeelall Y, Suhr C, Sgro B, O'Leary SJ, Shepherd RK

09:15 - 09:30 **O 39** **Regeneration of nerve fibers into the deaf ear**
Shibata SB, Cortez SR, Beyer LA, Wiler JA, Raphael Y

09:30 - 09:45 **O 40** **Secretion of growth factors and chemokines by adipose tissue-derived stromal cells**
Yoshida A, Nakagawa T, Kitajiri S, Inaoka T, Kita T, Ito J

09:45 - 10:00 **O 41** **A novel stem cell for auditory neuron regeneration**
El Seady R, Huisman MA, Frijns JHM

10:00 - 10:15 **O 42** **Neural stem cells from the cochlear nucleus**
Rak K, Wasielewski N, Radeloff A, Hagen R, Jablonka S, Pühringer D, Sendtner M, Mlynski R

10:15 - 10:30 **O 43** **Differentiation of human embryonic and human induced pluripotent stem cells along the otic lineage**
Masaki K, Starlinger V, Byers B, Nguyen H, Heller S

10:30 - 11:15 Break

11:15 - 12:15 **SESSION 9: Cochlear protection after implantation**
(moderators: W Grolman and RT Richardson)

11:15 - 11:30 **O 44** **Inner ear protection after cochlear implantation**
Eastwood H, Chang A, O'Leary SJ

11:30 - 11:45 **O 45** **Dexamethasone therapy and methods to control the effects of trauma in cochlear implant design**
Jolly C, Mirzadeh H, Kiefer J, Martini A, Truy E, Ibrahim H, Garnham C

11:45 - 12:00 **O 46** **Cochlear implant electrode array-eluted dexamethasone conserves hearing: Genetic and molecular mechanisms**
Van De Water TR, Dinh CT, Haake S, Eshraghi AA, Angeli S, Telischi FF, Balkany TJ

12:00 - 12:15 **O 47** **Evaluation of the elution of dexamethasone from the silicone of the electrode array: Preliminary safety studies**
Magosso S, Astolfi L, Giordano P, Pannella M, Sathiyaseelan T, Simoni E, Cascella V, Giari L, Hatzopoulos S, Prosser S, Braun S, Tillein J, Martini A

12:15 - 12:30 Closing remarks

12:30 END OF IEB 2009

POSTER PRESENTATIONS

(in alphabetical order)

A possible involvement of dopamine in modulation of synaptic transmission in the frog semicircular canal (P 01)
Andrianov GN, Ryzhova IV, Tobias TV

Preventive effects of thymus graft on age-related hearing loss (P 12)
Baba S, Iwai H, Inaba M, Sakaguchi M, Lee S, Ikehara S, Tomoda K

Protection of neural elements and sensory hair cells in the guinea pig cochlea with chronic intrascalar administration of glia cell-derived neurotrophic factor (GDNF) (P 32)
Bitsche M, Glueckert R, Miller J, Altschuler R, Prieskorn D, Schrott-Fischer A

Cochlear targets of hyperbranched poly-L-lysine after application on the round window membrane in the mouse and rat (P 31)
Buckiova D, Popelář J, Chumak T, Kadlecova Z, Klok HA, Syka J

Spatiotemporal expression of glycine receptors in the murine cochlea (P 40)
Buerbank S, Brandt N, Engel J, Becker K, Becker CM, Schulze H, Schick B, Knipper M, Dlugaiczyk J

Noise exposure during early development modifies the auditory startle reflex in adult rats (P 42)
Burianová J, Rybalko N, Bureš Z, Syka J

Prevalence of GJB2 mutations in the Portuguese population (P 50)
Chora J, Rodrigues R, Trincão C, Simões-Teixeira H, Matos, T, Fialho G, Caria H

The efficiency of a single injection or continuous delivery of nanoparticles to the middle ear using an Alzet micro-osmotic pump (P 34)
Chumak T, Buckiova D, Popelář J, Syka J

Uptake and toxicity tests of hyperbranched poly-L-lysine on PC12 and OCk-3 cells (P 57)
Corbacella E, Astolfi L, Kadlecova Z, Klok HA, Martini A

Generation of a tamoxifen-inducible hair cell-specific TR-β1 knockout mouse model (P 38)
Dettling J, Franz C, Zimmermann U, Rüttiger L, Zuo J, Feil R, Flamant F, Knipper M

Novel interaction partners of otoferlin (P 18)
Duncker SV, Heidrych P, Zimmermann U, Bress A, Pfister M, Ruth P, Blin N, Knipper M

Salicylates downregulate AQP-6 expression in sensory epithelia of the inner ear (P 45)
Fontana JM, Laforenza U, Tritto S, Botta L, Perin P

New tools in hearing research: Inducible conditional hair cell-specific knockout and knockin mouse models and what we can learn from them (P 39)
Franz C, Dettling J, Kuhn S, Brandt N, Zimmermann U, Winter H, Rüttiger L, Engel J, Hirt B, Marcotti W, Flamant F, Zuo J, Blin N, Knipper M

Multifunctional nanoparticle vizualisation at the light and electron microscopic level: Methods for finding the needle in the haystack (P 29)
Glueckert R, Rieger G, Roy S, Bitsche M, Schrott-Fischer A

Pharmacokinetics of gentamicin entry into the cochlea following systemic applications (P 61)
Hahn H, Salt AN, Gill RM, Schuhmacher U, Plontke SK

Influence of interleukin-6 on cisplatin-induced cochlear hair cell loss (P 09)
Haupt H, Yu Y, Szczepek AJ, Mazurek B

Spiral ganglion cell survival after round window application of Gelfoam soaked with brain-derived neurotrophic factor (P 21)
Havenith S, Versnel H, Agterberg MJH, De Groot JCMJ, Sedee RJ, Klis SFL

Structure of the otoferlin C2A domain of *Rattus norvegicus* (P 47)
Helfmann SA, Neumann P, Fasshauer D, Dickmanns A, Brose N, Moser T, Ficner R, Reisinger E

Estrogen and hearing (P 63)
Hultcrantz M, Stenberg EA, Simonoska R

Inoculation of helper T cells as a strategy for the prevention of age-related hearing loss in SAMP1 mice (P 13)
Iwai H, Baba S , Inaba M, Sakaguchi M, Lee S, Ikehara S, Tomoda K

Activating cGMP signaling cascades by blocking phosphodiesterase-5 preserves cochlear hair cells and protects from noise-induced hearing loss (P 28)
Jaumann M, Gubelt M, Dettling J, Zimmermann U, Wolpert S, Brandt N, Engel J, Köpschall I, Rohbock K, Hütter J, Sandner P, Knipper M, Rüttiger L

The effect of memantine on experimentally gentamicin-induced vestibulotoxicity in guinea pig (P 04)
Kim EH, Park YH

Otolithic function and brain activation in humans (P 55)
Kumagami H, Kitaoka K, Terakado M, Sainoo Y, Fujiyama D, Kawata A, Takasaki K, Takahashi H

^{18}F-FDG small-animal PET study and neuroanatomical tracing on the vestibular system in rats (P 17)
Lange E, Best C, Stier U, Dellani PR, Buchholz HG, Bausbacher N, Schröder H, Dieterich M, Schreckenberger M, Reuss S

P2Y4 receptor-mediated regulation of amiloride-sensitive sodium transport in the Reissner's membrane (P 19)
Lee JH, Kim CH, Kim HY, Lee HS, Chang SO, Oh SH

Unique protein expression in the human spiral ganglion: Do they play a role for cell interaction and neuron survival in man? (P 06)
Liu W, Boström M, Kinnefors A, Rask-Andersen H

The ultrastructural distribution of prestin in rat cochlear outer hair cells (P 51)
Mahendrasingam S, Beurg M, Fettiplace R, Hackney CM

Effect of glycine (ant)agonist on auditory nerve fiber activity (P 43)
Matsumoto M, Dlugaiczyk J, Singer W, Knipper M, Rüttiger L

Prevention of cisplatin ototoxicity can be done by inhibition of DNA damage in cochlea cells (P 10)
Mendus D, Manz D, Oliver T, Jacks T, Thomale J

The collagen receptor DDR1 co-localizes with the non-muscle myosin IIA in mice inner ear (P 08)
Meyer zum Gottesberge AM, Gohla A

Reduced electromotility of outer hair cells as an additional mechanism underlying the deafness associated with mutations in connexin genes (P 16)
Mistrík P, Ashmore J

Steroid treatment of acute noise-induced hearing loss (P 54)
Müller M, Reimann K, Tisch M, Maier H, Löwenheim H

Noise-induced hearing loss mice models: Towards standardization of exposure conditions (P 35)
Murillo-Cuesta S, Lorenzo-García P, Martínez-Vega R, Cobo P, Varela-Nieto I, Cediel R

Idiopathic sudden sensorineural hearing loss in Sweden: Diagnostic protocol and treatment in relation to outcome (P 27)
Nosrati-Zarenoe R, Hultcrantz E

Regeneration of spiral ganglion neurons by transplating bone marrow stromal cell-derived neural progenitor cells (P 11)
Ogita H, Nakagawa T, Inaoka T, Sakamoto T, Ito J

Immunohistochemistry of SMI-32 neurofilament protein in the auditory cortex of the rat (P 41)
Ouda L, Druga R, Syka J

Morphological aspects of the semicircular canal of man (P 02)
Palma S, Meyer zum Gottesberge A, Boldrini P, Nucci R, Pareschi R, Martini A

Patterns of auditory and visual cortical activities in experimental deafness: An immunohistochemical study (P 03)
Park YH

Diffusion tensor imaging of central auditory pathway in children with congenital deafness (P 07)
Park KH, Chung KH, Chung WH

The role of the L-VDCC for activity-dependent BDNF transcription: A cell culture model (P 24)
Passeri E, Geisler HS, Pandford-Walsh R, Singer W, Knipper M

Contribution to the study of presbycusis in the Portuguese population (P 52)
Pereira L, Chora J, Teixeira H, Matos T, Fialho G, Caria, H

The role of the auxiliary Ca^{2+}-channel α2δ3 subunit for signal transmission in the auditory brainstem and acoustic startle reflex pathway (P 60)
Pirone A, Rüttiger L, Pilz P, Zuccotti A, Franz C, Friauf E, Knipper M, Engel J

Influence of brief noise exposure in juvenile rats on the response properties of inferior colliculus neurons in adult animals (P 33)
Popelář J, Bureš Z, Grecova J, Suta D, Syka J

Jagged-1 is essential for the boundary of mammalian prosensory patch probably via Notch3 (P 37)
Praetorius M, Hao J, Koesters R, Pfannenstiel S, Plinkert PK

The acoustic chiasm in pigmented and albino rats (P 49)
Reuss S, Closhen-Gabrisch S, Closhen C

Biochemistry of otoferlin C2F and its pachanga mutant form (P 48)
Reuter K, Pangrsic T, Schwander M, Fasshauer D, Brose N, Jahn R, Müller U, Moser T, Reisinger E

Dexamethasone release from cochlear implant silicone surfaces (P 26)
Rohm H, Sternberg K, Paasche G, Barcikowski S, Hahn A, Fadeeva E, Lenarz T, Schmitz KP, Stöver T

Cell-specific targeting to the inner ear by using nanoparticles with neurotrophin-derived peptides (P 25)
Roy S, Johnston AH, Newman TA, Glueckert R, Bitsche M, Dudas J, Rieger G, Schrott-Fischer A

Comparison of threshold, growth functions and peak-delay times by ABR and CAP measurements (P 53)
Rüttiger L, Jaumann M, Matsumoto M, Knipper M

Clinical outcomes of the MP3000™ sound-coding strategy optimization study in Freedom™ recipients (P 58)
Rypkema G, Killian M, Pesch J, Beynon A, Szyfter W, Allum J, Brokx J, Burdo S, Cuda D, Dhooge I, Dillier N, Estrada Leypón E, Eyles J, Falcón González JC, Festen J, Frachet B, Fürstenberg D, Gentine A, Gräbel S, Grolman W, Hey M, Hoppe U, Huarte Irujo A, Leone CA, Mazzoli M, Meyer B, Morera Pérez C, Müller-Deile J, Müller-Mazzotta J, Niemczyk K, Offeciers E, Paludetti G, Quaranta A, Roux-Vaillard S, Steffens T, Triglia J, Uziel A, VandeHeyning P, Wesarg T, Büchner A

Notch signaling regulates cochlear stem cells maintenance and sensory cell-fate determination (P 62)
Savary E, Smeti I, Hugnot J-P, Chabbert Ch, Zine A

Fibrosis, osteoneogenesis and immune reactions: A problem for cochlear implantation (P 30)
Schrott-Fischer A, Glueckert R, Burgess BJ, O'Malley J, Nadol JB

Tinnitus-specific features in the peripheral and central auditory system (P 22)
Singer W, Rüttiger L, Zuccotti A, Matsumoto M, Panford-Walsh R, Köpschall I, Rohbock K, Zimmermann U, Knipper M

Effects of electrical stimulation on the acoustically evoked compound action potential (P 46)
Stronks HC, Versnel H, Prijs VF, Grolman W, Klis SFL

Type-1 allergy-induced endolymphatic hydrops and the suppressive effect of leukotriene antagonist (P 05)
Takeda T, Takeda S, Kakigi A

Glucocortidoid receptors and 11β-hydroxysteroid dehydrogenase isoforms in the rat and human inner ear (P 56)
Terakado M, Sainoo Y, Takasaki K, Kumagami H, Takahashi H

Endolymphatic hydrops revealed by intravenous gadolinium injection in patients with Ménière's disease (P 23)
Teranishi M, Naganawa S, Tagaya M, Iwata T, Nakata S, Sone M, Nakashima T

Applicability of the phosphodiesterase inhibitor rolipram as neurotrophic factor for spiral ganglion cells (P 59)
Warnecke A, Scheper V, Berkingali N, Paasche G, Wissel K, Lenarz T, Stöver T

Auditory nerve degeneration in *pmn/pmn* mice (P 15)
Wasielewski N, Rak K, Radeloff A, Volkenstein S, Dazert S, Hagen R, Sendtner M, Mlynski R

Expression of nuclear factor kappa B (NF-κB) in the hydropic cochlea of guinea pigs after the direct injection of antigen into the endolympatic sac (P 20)
Watanabe KI, Tomiyama S, Yagi T

Protection of spiral ganglion neurons with neurotrophins and chronic electrical stimulation (P 14)
Wise AK, Fallon JB, Evans AJ, Andrew J, Pettingill LN, Geaney MS, Shepherd RK

3D-Modeling of the organ of Corti on the basis of laser scanning microscopic images (P 44)
Yarin YM, Fleischer M, Engelbrecht Th, Poznyakovskiy AA, Gärtner R, Hardtke HJ, Zahnert Th

Changes occurring in the central auditory system after sound exposure (P 36)
Zuccotti A, Singer W, Rüttiger L, Baur M, Köpschall I, Rohbock K, Zimmermann U, Knipper M

ABSTRACTS

ORAL PRESENTATIONS

O 01

Two populations of hair cell transducer channels enhance auditory sensitivity and dynamic range

Van Netten SM[1], Kros CJ[2]

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Hair cells in the inner ear transduce sound-induced displacements of their hair bundle into electrical currents over a wide dynamic range and with nanometer sensitivity. Quantitative gating-spring models of transduction so far assumed a single transducer channel engaged by a tip link. We have investigated gating-spring models in which a tip link connects to a pair of transducer channels (Van Netten et al. (2009) Pflug. Arch. 458: 273-281).
In line with previous observations, indicating transducer channels on either side of a tip link (Denk et al. (1995) Neuron 15: 1311-1321) we questioned whether a mechanical series engagement could account for measured transducer activation. We found that a series mode of activation accurately explains measured transduction if the two channel populations possess different sensitivity and set-points. Interaction, such as negative cooperativity, was found to be minimal.
This lack of interaction is also in line with a second alternative model considered. This model was motivated by the observation that a single tip link may split into two strands (Kachar et al. (2000) PNAS 97: 13336-13341), so that two channels are located at only one side of a tip link (Beurg et al. (2009) Nat Neurosci. 12: 12553-12558). This may effectively lead to summing the open probability of two channel populations activated in parallel, each with its specific set-point and sensitivity.
Both models accurately account for mechano-electric transduction, specifically the asymmetry as observed across several different hair-cell types, by one channel population that is specifically sensitive to small displacements while the other responds best to larger stimuli. Applying these model descriptions to measurements of depolarized hair cells leads to the conclusion that only the most sensitive channel population is affected by the fast calcium-dependent adaptation process.

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O 02

Measurements with optical coherence tomography in the mammalian cochlea

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With optical coherence tomography it is possible to make a scan through tissue and to display the optical reflectivity of the various layers. One step further is to measure vibrations, in our case of movements of several tissue layers inside the cochlea. We have been successful in simultaneously measuring vibrations of the basilar membrane (BM) and the reticular lamina (RL) in the cochlea of the guinea pig, in the region of high best frequencies (18 kHz). With sinusoidal stimulation the responses are frequency-selective and nonlinear. One striking difference between the two responses is that the RL response is consistently larger than the BM response. Furthermore, the “best frequency” of the RL is higher than that of the BM. In a provisional way we have considered the organ of Corti (OC) as an oscillating channel closed at both ends on which outer hair cells (OHCs) exert forces due to motility. In this type of model we can study in which way the fluid of the OC participates in the transportation of acoustical energy produced by the OHCs. It is now clear that the dynamics of the OC is an essential part of cochlear mechanics.

O 03

Mechanical tuning of the tectorial membrane in the basilar papilla of the frog

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The frog inner ear includes two sensory organs dedicated to the perception of airborne sound: the amphibian and the basilar papilla. The basilar papilla consists of a small patch of hair cells, embedded in a stiff support structure and partly covered by a tectorial membrane. Neurons connecting to the hair cells in this papilla are tuned to about 2.0 kHz. This paper investigates the possible mechanical basis of this frequency selectivity.
The inner ears of leopard frogs, *Rana pipiens*, were excised and placed in artificial perilymph. An inner ear was positioned under an optical sectioning microscope. Sinusoidal stimuli were applied by a piezo actuator, placed at the oval window. The tectorial membrane was visible via the round window. A digital camera was mounted on top of the microscope in order to obtain stroboscopically illuminated images of the tectorical membrane. By collecting images at a range of stimulus phases and a range of focal planes, a 3D movie of the membrane's movement was acquired.
The tectorial membrane showed a movement along the excitatory orientation of the hair cells. The membrane's mechanical response was frequency selective. The tuning frequency was near 2.0 kHz. The tuning quality was on average Q_{10dB}=2.0 (s.d. 0.8).
The mechanical frequency selectivity of the tectorial membrane corresponds to that of auditory neurons. Our data provide evidence for a mechanical basis of the frequency selectivity in the frog basilar papilla.

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O 04

The interplay beween electromotility and hair bundle motility in the cochlea

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The precise nature of the active process responsible for amplification within the mammalian inner ear is a topic of much current debate. In particular, the relative contributions of outer hair cell electromotility and hair bundle motility to this process remain controversial.
We present a physical description of cochlear micromechanics in order to address these issues. This description is based on measured geometrical and elastic properties of the cochlear partition and uses a model for hair bundle motility which can account for observations of isolated hair bundles. Furthermore, we add an empirically based description of the electromotile response of the outer hair cell and ion current flow within the cochlea. We utilize both analytical and numerical techniques to study the properties of this coupled system.
We find that this system can display high sensitivity to external pressure stimulation, high frequency discrimination and a compressive nonlinearity for physiologically relevant parameter values, in qualitative agreement with experimental observations. Moreover, this system can oscillate spontaneously, a property which may be related to the production of spontaneous otoacoustic emissions.
Individual hair bundles have been observed to be weak amplifiers. We show how outer hair cell electromotility can provide mechanical feedback to hair bundles embedded within the cochlear partition. This can enhance the performance of the system as a signal detector. Our work also reveals how the resonance frequency of the cochlear partition can be tuned by changing its physical properties, in agreement with experimental observations.
Overall our description indicates that outer hair cell electromotility and hair bundle motility can work together to provide the mammalian ear with its remarkable signal detection properties.

O 05

Secondary cochlea vibrations

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It is known that external sounds cause vibrations of the basilar membrane, but there are no accepted explanations for the source of otoacoustic emissions (OAE) or BM vibrations at frequencies not in the original stimulus (i.e. distortion products, DP).
I propose that the tectorial membrane (TM) is piezoelectric. There are no instruments that can physically measure piezoelectricity in a small sample, but the chemical nature of TM is acellular and mostly of water. It has long-chain collagen proteins that are surrounded by glycoproteins (tectorins) with an arrangement suggestive of actin-myosin. Piezoelectric structures are bidirectional with pressure stimuli evoking electrical potentials and electrical signals evoking vibrations that generate sounds in surrounding fluids.
Cochlear microphonics (CM) are electrical potentials known to be closely related to the otoacoustic emissions (OAE) and I propose that the CM excites the TM to vibrate and generate sounds that are recorded as OAE. Similarly when two tones are presented, they generate CMs and those potentials summate with a Fourier distribution. Those summed potentials (DP) will excite the TM to vibrate and the resulting sounds will stimulate the adjacent BM to vibrate (i.e. DP). Those secondary BM vibrations are present at locations with no externally generated vibrations and they will induce secondary CM at DP frequencies.

O 06

3D Cytoarchitecture and morphometry of the cochlea of the CBA/J mouse

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Although studied for over three centuries, the three-dimensional (3D) morphology of the cochlea has not been well characterized. Using a light-sheet microscope (TSLIM) five mouse cochleas from 4-week old animals were non-destructively, serially sectioned and the major cochlear components were segmented and 3D rendered.
The average volume (in µl, ±SEM) of following structures was estimated: scala vestibuli (0.36, 0.024), scala media (0.28, 0.014), scala tympani (0.32, 0.005), basilar membrane (0.009, 0.001), tectorial membrane (0.021, 0.002), round window membrane (0.004, 0.001), spiral ligament (0.182, 0.004), spiral limbus (0.032, 0.002), Rosenthal's canal (0.034, 0.002), stria vascularis (0.039, 0.002) and the organ of Corti (0.013, 0.001). Average volume of cochlear perilymph (0.681, 0.028) and endolymph (0.305, 0.014) was determined.
A 3D Cartesian coordinate system was developed to determine structure length using the basilar membrane as a reference axis. Length was determined along the centroid of the structure and average length of the basilar membrane 5.90 mm (±0.06), and Rosenthal's canal 1.88 mm (±0.04) were estimated. The basilar membrane extended beyond the length of Rosenthal's canal by 18.94% (±0.021) in the apex and 7.94% (±0.44) in the base.
Shape and morphometric changes of these structures along their length are difficult to understand due to their complex 3D anatomy. However, skeletonization and color mapping of changes in distance from the centroid is an effective way to reduce and visualize these data. Comparisons between the same structure in different animals can also be shown visually using the Procrustus method. We will also describe our attempt to autosegment and count spiral ganglion neurons within Rosenthal's canal.

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O 07

Historical studies on the vascular supply of the cochlea

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We present a literature study on the history of the vascularization of the cochlea and a description of the anatomy of the cochlea from the 16th century until now. Three different periods are recognizable in the development of knowledge concerning this subject: The macroscopic period with the description of the structure of the cochlea from the 16th to the 19th century, the microscopic period with the description of the part of the organ of Corti in the 19th century and the injection period with the description of the fine vascularization of the cochlea in the 20th century. Various techniques were used during these three periods, which will be presented here, using only original references. This historical study reveals the ingenuity of the researchers in using different technological progress to enhance their performance in research.

O 08

Vestibular function in a new animal model for Meniere's disease

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Surgical obstruction of the endolymphatic sac and duct results in endolymphatic hydrops. Although vertigo is a characteristic feature of Meniere's disease, episodes of imbalance are very rarely observed in models of endolymphatic hydrops. We have developed a more suitable animal model. This model is based on a combination of endolymphatic sac dysfunction and administration of a vasopressin type-2 receptor agonist (desmopressin). In this study, we investigated vestibular function by observing spontaneous nystagmus and the degree of endolymphatic hydrops. Postural control was also observed in two animals.
Sixteen pigmented guinea pigs were divided into control, surgical, desmopressin, and combined groups. In the surgical group, animals underwent electrocauterization of the endolymphatic sac and were then fed for 1 week (n=4). In the desmopressin group, animals were administered 100 µg/kg of desmopressin subcutaneously (n=4). In the combined group, animals underwent surgery, then fed for 1 week, before being administered desmopressin (n=4). In the control group, animals were administered saline subcutaneously (n=4). Nystagmus was recorded for one hour using an infrared system, after which the inner ear was studied morphologically. Sections were stained with hematoxylin and eosin and studied under a light microscope. Postural control was observed for one hour in two additional animals which underwent the same procedures as the combined group.
Incidences of spontaneous nystagmus were observed in 0, 0, 25, and 100% of the control, surgical, desmopressin, and combined groups, respectively. Morphological studies showed no, small degrees, small degrees, large degrees of hydrops in control, surgical, desmopressin, and combined groups, respectively. Intermittent imbalance was observed in the two additional animals which underwent the combined procedures.
These results indicate that the combined procedures induced extensive hydrops and resulted in spontaneous nystagmus and imbalance, and that this experimental animal seems to be a suitable vertigo model of Meniere's disease.

O 09

Developmental changes in the frequency-tuning properties of rat vestibular ganglion neurons

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Vestibular ganglion cells (VGCs) are primary afferent sensory neurons that transmit information about head movement to the central nervous system. In vivo studies have shown that the frequency of spontaneous activity of primary vestibular afferents increases during postnatal periods. It remains unknown whether the intrinsic firing properties of VGCs change in the frequency domain during postnatal periods. Here, we explored the developmental changes in the frequency-tuning properties of rat VGCs using the whole-cell patch-clamp technique.
Superior vestibular ganglia were isolated from neonatal (postnatal days (PD) 3 or PD 7) or juvenile (PD14) Sprague-Dawley rats. We injected sinusoidal currents with varying frequencies (1-100 Hz) and measured membrane potentials in the current-clamp mode.
During development, significantly different frequency-tuning properties of rat VGCs were found: the VGCs of PD3 and PD7 rats were tuned at around 40 Hz (best frequency), whereas the best frequency of PD14 rats shifted to lower frequencies around 10 Hz.
Our results suggest that rat VGCs change their tuning properties from high to low frequencies during the postnatal period. It is conceivable from our previous studies that the different compositions of potassium channels could be responsible for these different tuning properties. The developmental change in VGC may account in part for changing spontaneous activities of primary vestibular afferents in vivo.

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O 10

A re-examination of the striated organelle in vestibular end organs

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The striated organelle (SO) is a structure located in the subcuticular region of hair cells, consisting of alternating thick and thin filaments (Friedman et al., 1963; Ross, 1983). Although present in all cochlear and vestibular hair cells, the SO is particularly well-developed in type-I vestibular hair cells. It is shaped like an inverted, open-ended cone that contacts the cell membrane along its entire circumference and is separated from the cuticular plate by a layer of large mitochondria. In other hair cells, it is smaller and appears to be free-floating.
We studied its structure in rat and chinchilla hair cells with electron microscope (EM) tomography. In three-dimensional reconstructions, we have found so far that it is connected to at least some actin rootlets and is associated with microtubules, mitochondria and smooth endoplasmic reticulum. Actin rootlets contact the hair cell membrane near the SO, opposite the kinocilium. The subcuticular mitochondria are 5x larger in volume and 2x larger in surface area than those in the rest of the hair cell or those in type-II hair cells.
Our attempts to determine its protein composition have so far included immunohistochemical approaches. Confocal immunofluorescence places an actin-binding protein, α2-fodrin (brain spectrin), where the SO contacts the hair cell membrane and yotiao (AKAP-150) in the calyx membrane opposite the SO. EM immunogold places fodrin in the thick filaments.
Contact with the rootlets suggests that the SO might regulate hair-bundle stiffness, while its association with the cell membrane suggests the SO may help form the constricted neck characteristic of type-I hair cells.

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O 11

Differential passage of gadolinium through the mouse inner ear barriers evaluated with 4.7-T MRI

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The mouse is an excellent animal species for investigating human diseases including hearing loss for many reasons. With the help of contrast agents, MRI is a powerful tool to visualise the structure and function of the inner ear in vivo. However, such studies have not been done in the inner ears of mice.
The objective of the sudy was to demonstrate the limits of resolution of the fine inner ear structures in the mouse and to explore the function of intracochlear barriers by the administration of DOTAREM (gadoterate meglumine, Gd-DOTA) through intravenous injection (IV) or intratympanic (IT) approach.
Seventeen female FVB mice were included in the study. The inner ear MRI was performed with a 4.7-T MR scanner using both 2D and high-resolution 3D sequences. MRI region of interest signal intensity and perilymph volume in the inner ear were evaluated. 3D reconstruction was applied to demonstrate fine structures of the inner ear. Histological observation was performed with light microscopy.
Gd-DOTA highlighted the inner ear structures such as the scala tympani, scala vestibuli, cochlear lateral wall, cochlear aqueduct, vestibulum, and the semicircular canals. The endolymphatic compartment, scala media, was distinguished from the perilymphatic compartments because of poor uptake of Gd-DOTA. The dynamic uptake of Gd-DOTA in the perilymphatic compartments reached a platform between 90 min and 180 min with slight growth. The perilymph volume demonstrated by Gd-DOTA uptake was statistically significantly larger in the IV group (1.72 mm^3) than that in the IT group (1.28 mm^3) ($p<0.05$). The superior semicircular canal and cochlear aqueduct were mainly contributive to the increased perilymph volume in the IV group.
This study can be applied to future investigations of the pathological mechanisms of sensorineural hearing loss in mouse models with genetic, metabolic or other disorders.

O 12

Diagnostic magnetic resonance images without gadolinium for inner ear diseases

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Hearing impairment is often difficult to treat. One reason for this comes from the luck of recognition of different pathophysiologies in the inner ear in situ. Recent advances in magnetic resonance (MR) imaging technology should provide a tool to differentially recognize inner ear pathophysiology. There are reports of successful visualization of scalae using an intratympanically applied contrast agent containing gadolinium (Naganawa et al. (2009) Acta Oto-Laryngol Suppl. 560: 15-21; Zou et al. (2009) Acta Oto-Laryngol Suppl. 560: 22-31). However, sustained gadolinium ion in the inner ear can cause serious fibrosis. We tried to visualize inner ear structures by MR images without a contrast agent.
The objective of this study was to show the potential usefulness of inner ear MR images without a contrast agent for the diagnosis of inner ear disorders in mice.
MR images were taken from adult and embryonic mice using a 7-Tesla scanner. Inner ear structures such as scala tympani and scala vestibuli were successful visualized in 3-D images. Further visualizations will be demonstrated.
Conclusion: MR images without a contrast agent is potentially useful.

O 13

Molecular epidemiology of hearing loss in the Portuguese population: Two approaches

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In previous studies, mutations in DFNB1 were shown to account for ~19% of the cases of congenital deafness in the Portuguese population. The cause of deafness in the remaining cases, including the GJB2/GJB6 mono-allelic ones, has to be elucidated.
In order to investigate the spectrum of genes/mutations responsible for hearing impairment in the Portuguese population, two approaches were used. One consisted in studying 11 small families with apparent nonsyndromic congenital deafness. Haplotype analysis was performed for DFNB1, DFNB4, DFNB7/11, DFNB8/10, DFNB9, DFNB12, DFNB18, DFNB28, DFNB35, DFNB49, DFNB59 and DFNB67, using at least three microsatellite markers for each locus. Sequencing analysis of the related genes was carried out in the case of compatibility with linkage. The other approach consisted in studying a sample of ~185 unrelated probands with hearing impairment using a series of methods (Multiplex PCR, PCR-RFLP, sequencing, SNaPshot and dHPLC) to study a set of mutations in GJB2, GJB6, OTOF, TMPRSS3 and SLC26A4 and search for any mutation in the coding region of TMPRSS3 and TMC1.
In a consanguineous family we found compatibility with linkage to DFNB4 with autozygosity for this region; sequencing of the SLC26A4 gene revealed a novel mutation, c.1615 -2 A>G (IVS14 –2 A>G), in the homozygous state in the severely deaf siblings. In a non-consanguineous family, we found compatibility with linkage to DFNB8/10 in the heterozygous state. Sequencing of the TMPRSS3 gene revealed a known mutation, c.646C>T (p.R216C), in compound heterozygosity with a novel mutation, c.346G>A (p.V116M), in the profoundly deaf siblings. In both families, the genotype is likely to be the cause of deafness. These mutations were not found in 85 Portuguese controls with normal hearing.

O 14

Association of functional single-nucleotide polymorphisms (SNPs) of PTPN22 and FcγRIIIa (CD16a) human genes with Meniere´s disease in a southeast Spanish population

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Meniere's disease (MD) is a form of recurrent vertigo associated with sensorineural hearing loss, tinnitus and endolymphatic hydrops. Although the pathophysiology underlying the inner ear damage is unknown, autoimmunity and elevated circulating immunocomplexes have been related to MD. This study investigates the association of the SNPs FcγRIIa*A519G (rs1801274), FcγRIIIa*A559C (rs396991), PTPN22(rs2476601, 1858C/T) and CTLA-4 SNP (rs231775, 49A/G) to MD.
Ninety-two patients with MD from the southeast area of Spain fulfilling the 1995 AAO-HNS diagnostic criteria and 627 ethnically matched controls were studied. Blood samples from patients and controls were used to obtain DNA. The SNPs were genotyped using RT-PCR by a TaqMan 5' allelic discrimination assay with the fluorescent dyes VIC and FAM (Applied Biosystem, Foster City, CA). Amplifications were performed in an ABI Prism 7750 machine (AB) for continuous fluorescence monitoring. Genotype and allelic frequencies were compared in both groups by the χ^2 test with Fisher´s exact test and odds ratios (OR) with 95% confidence intervals (CI) were calculated.
Genotypes distribution of all the SNPs exhibited Hardy-Weinberg equilibrium in controls. The FcγRIIa SNP (rs1801274-G) frequencies did not differ between patients and controls (p=0.37). However, the frequency of SNP (rs396991-A) was significantly different between both groups (p=0.02), and the genotype AA, encoding the FcγRIIIa-F/F158 phenotype, was associated with MD (OR=1.83 (1.18-2.85), p= 0.005). No association was found between the +49A/G CTLA4 genotype and MD. Moreover, the heterozygote PTPN22 1858C/T genotype was found at a significantly higher frequency in patients than in controls (OR= 2.25, 95% CI:1.09-4.62; p=0.04).

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Conclusions: (1) The FcγRIIIa genotype AA, confering a Fc receptor with lower binding affinity for CIC, is associated with MD; and (2) The PTPN22 1858C/T genotype may confer differential susceptibility to MD in this population, supporting the role of the immune system in the pathophysiology of MD.

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O 15

Involvement of T-cell receptor beta alterations in the development of otosclerosis linked to OTSC2

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Otosclerosis is a common form of hearing loss, characterized by disordered bone remodeling within the otic capsule. In addition to conductive hearing loss, sensorineural hearing loss is present in 10% of the cases. Within otosclerotic foci, several immunocompetent T cells and immunomodulating factors can be found. Different etiological theories involving the immune system have been suggested, but these theories remain controversial. However, a genetic component is clearly present. Seven autosomal dominant loci have been published, but none of the disease-causing genes has been identified. This study focused on the exploration of the second otosclerosis locus on chromosome 7q34-36 (OTSC2), holding the T-cell receptor beta locus (TRB locus), a strong candidate for otosclerosis given its central function in the immune system.
Analysis of additional microsatellite markers could refine the region from 11.4 to 5.6 Mb, still containing the complete TRB locus. Subsequently, a number of immunological analyses were carried out to seek differences related to T-cell receptor beta (TCR-β). No altered TCRβ variable genes profile was present in OTSC2 patients, but a significantly lower TCR-β mRNA expression and percentage of blood circulating TCRαβ^{+} T cells was detected in OTSC2 patients compared to controls and sporadic patients. Further flow cytometric analysis illustrated more significant disturbances in specific T-cell subsets, including an increased CD28^{null} cell population.

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These disturbances could be associated with the abnormal bone remodeling present in otosclerosis, given the known effects of immunocompetent T cells on bone physiology. Although the precise mechanism is unclear, we propose that a genetic defect in the TRB locus leads to a disturbed T-cell development and ageing, potentially influencing T-cell reactivity towards unique structures within the otic capsule. In conclusion, these data implicate the TRB locus as the causative gene in the OTSC2 region and represent an important finding in the elucidation of the disease pathology.

O 16

Mechanisms of aminoglycoside uptake into hair cells

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The mechanism of aminoglycoside uptake into hair cells (HCs) is not well understood. It has been suggested, based on theoretical considerations and experimental data, that these antibiotics enter the HC rapidly via the mechanoeletrical transduction (MET) channels. Alternative hypotheses and data point toward more gradual uptake via endocytosis, perhaps mediated by binding of the aminoglycoside to membrane receptors and internalization during receptor inactivation or recycling. To address these two alternative mechanisms, we evaluated the influence of inhibitors of MET channel opening or endocytosis on the toxicity of gentamicin to neonatal mouse HCs in vitro. We used GFP-positive HCs so that cell damage could be evaluated throughout the experiment. To inactivate the MET channels of the HCs, we treated postnatal day 5 explants of mouse organ of Corti basal turn with BAPTA for 10 minutes each day to disrupt stereociliary tip links. This was followed by exposure to 50 μM gentamicin in media with normal levels of calcium. The procedure was repeated daily for three days. To inhibit clathrin-mediated endocytosis, we pretreated explants with chlorpromazine, which prevents the interaction of AP2 with clathrin, followed by co-administration of gentamicin and chlorpromazine for 3 days. To inhibit lipid-raft-mediated endocytosis we used filipin, which depletes lipid rafts from the cell membrane. The inhibitor dosages used produced no damage to HCs when applied alone. Both BAPTA and chlorpromazine significantly inhibited HC damage due to gentamicin. However, in both cases the protection was partial. Filipin had no effect. The results suggest that aminoglycosides enter HCs by more than one mechanism, utilizing both MET channels and clathrin-mediated endocytosis.

O 17

Special type of presbycusis in the Fischer 344 rat strain

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The auditory systems of most rat strains undergo a relatively slow process of aging with a characteristic deterioration of hair cell function accompanied by relatively small changes in the function of the lateral wall of the cochlea and other parts of the auditory system. The Fischer 344 strain represents a very different model of presbycusis: a rapid deterioration of hearing in the first months of life, manifested as increased hearing thresholds and decreased amplitudes of the distorsion product otoacoustic emissions (DPOAE; Popelář et al., 2003), is accompanied by limited hair cell loss, mostly in the basal and apical parts of the cochlea (Popelář et al., 2006). Pathological changes are also present in adult Fischer 344 rats in the stria vascularis and spiral ligament (Buckiová et al., 2006, 2007); however, the values of the endocochlear potential are relatively normal (Bielefeld et al., 2008). The discrepancy between the presence of relatively high numbers of preserved hair cells in the cochlea and missing DPOAE could be explained by the reduced levels of prestin immunolabeling in the hair cells of this strain (Chen et al., 2009). In the central parts of the auditory system (inferior colliculus and auditory cortex) of Fischer 344 rats decreased levels of glutamate decarboxylase were observed, the key enzyme in the synthesis of GABA (Burianová et al., 2009), and a pronounced decline in the number of parvalbumin-immunoreactive neurons (Ouda et al., 2008). Taken together, these results indicate that presbycusis in the Fischer 344 rat strain is distinct from the normal type of presbycusis observed in other rat strains.

O 18

Adenosine receptor signaling mitigates noise-induced cochlear injury

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Adenosine is a constitutive cell metabolite with a putative role in tissue protection and regeneration. Here, we report that adenosine receptor signalling can mitigate noise-induced cochlear injury in the rat. The potential for adenosine receptor activation to ameliorate noise-induced hearing loss was assessed using auditory brainstem responses (ABR). Wistar rats were exposed to broadband noise (110 dB SPL, 24 hours) to induce permanent hearing loss. Adenosine and selective adenosine receptor agonists (CCPA, CGS-21680 and Cl-IB-MECA) were applied to the round window membrane of the cochlea 6 hours after noise exposure. Follow-up ABRs, 48 hours after drug administration, demonstrated partial recovery of hearing thresholds in the cochlea treated with adenosine (non-selective adenosine receptor agonist) and CCPA (selective A_1 adenosine receptor agonist). In contrast, the selective A_{2A} adenosine receptor agonist CGS-21680 and the A_3 adenosine receptor agonist Cl-IB-MECA did not protect the cochlea from hearing loss. Free radical generation in the cochlea exposed to noise, demonstrated by nitrotyrosine immunoreactivity, was reduced by administration of adenosine and CCPA.
In the second study, selective adenosine A_1 receptor agonist adenosine amine congener (ADAC) lacking peripheral side effects was administered intraperitoneally (100 µg/kg/day) at time intervals after noise exposure (8-12 kHz, 110 dB SPL, 2-24 hours). Hearing thresholds were assessed using ABRs and the hair cell loss was evaluated by quantitative histology. The treatment with ADAC after noise exposure led to a substantial recovery of hearing thresholds. These results were upheld by increased survival of sensory hair cells and reduced nitrotyrosine immunoreactivity in ADAC-treated cochlea.

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We propose that ADAC could be a valuable treatment for noise-induced cochlear injury in instances of both acute and extended noise exposures. This study pinpoints A_1 adenosine receptors as attractive targets for pharmacological interventions to prevent or reduce noise-induced cochlear injury.

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O 19

Mitochondrial dysfunction, pro-inflammatory cytokines and tauopathy sign aging cochlea and hearing impairment

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Age-related hearing loss affects more than half of people over 60 years of age, making presbycusis a major health problem. Due the lack of reliable animal models, no efficient treatment is actually available (with the exception of hearing aids). Senescence-accelerated mice (SAM) had been produced to investigate the deleterious effects of ageing in the brain and cardiovascular system. In this study, we analyzed the time course, the morphological and the molecular correlates of age-related hearing loss in senescent-accelerated prone 8 (SAMP8) mice. Functional and ultrastructural data demonstrated that SAMP8 displayed same pathological features as reported in temporal bones from patient with presbycusis. Molecular analysis revealed mitochondrial dysfunction (decrease of cytochrome c oxidase and citrate synthase activity) leading to apoptosis in the organ of Corti, spiral ganglion and in the stria vascularis. Senescence also regulates macrophage activation in all the cochlear scalae and increased interleukin-1-beta (IL-1β) and tumor necrosis factor alpha (TNF-α). Selective autophagic vacuoles into the spiral ganglion neurons was confirmed by the increased expression of the autophagic marker microtubule light chain 3 (LC3). Accumulation of protein aggregates (lipofuscin) and Tau phosphorylated, demonstrates that spiral ganglion cell death shares common mechanisms with other degenerative pathologies, such Alzheimer and dementia.

O 20

Infiltrating macrophages in the cochlear labyrinth barrier in response to loud sound stimulation mediated by iNOS signaling

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Sound trauma impairs the cochlear blood-labyrinth barrier (BLB) and causes cochlear hypoxia and inflammation. Acoustic trauma promotes nitric oxide (NO) production through upregulation of iNOS in the ear. In this study we determined that NO effects bone marrow-derived cells (BMDCs) recruitment across the BLB after acoustic injury. Lethally irradiated $iNOS^{+/+}$ and $iNOS^{-/-}$ mice were transplanted with GFP^{+} BMDCs from C57Bl/6-Tg (UBC-GFP) mice. Four to ten weeks after transplantation, we assessed the population of GFP^{+} BMDCs in the region of BLB in both the $iNOS^{+/+}$ and $iNOS^{-/-}$ mice under non-sound stimulation and acoustic trauma conditions. Irradiated and bone marrow-transplanted control mice (not exposed to loud sound) had normal hearing thresholds, either immediately after irradiation or two months after irradiation. No GFP^{+} BMDCs were observed to migrate into the area of the BLB in the control mice one month following transplantation. Only small numbers of GFP^{+} BMDCs were found to infiltrate across the BLB after two months following transplantation in both the $iNOS^{+/+}$ and $iNOS^{-/-}$ mice. In contrast, when the animals were exposed to wide-band noise at a level of 120 dB for 3 hours per day for 2 consecutive days, robust BMDC migration was observed in the acoustic trauma cochlea. GFP^{+} BMDCs migration was most prominent during the first week after acoustic trauma, and accumulated significantly at two weeks in the stria vascularis. Most of the BMDCs expressed F4/80 and were identified as macrophages. Upregulation of iNOS was also observed in the stria vascularis immediately after acoustic trauma. GFP^{+} BMDCs recruitment was significantly reduced in the $iNOS^{-/-}$ mice in comparison with GFP^{+} BMDCs recruitment in the $iNOS^{+/+}$ mice. These data suggest that the circulating bone marrow cells were mobilized by acoustic trauma and migrated to the injured region. NO from iNOS may be one signal driving bone marrow cell recruitment during acoustic trauma.

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O 21

c-Myc regulates mitochondrial peroxiredoxin in mouse cochlear hair cells

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Peroxiredoxins are components of cellular antioxidant defenses and play a critical role in maintaining the intracellular redox balance and mitochondrial membrane potential. Peroxiredoxin 3 (Prx3) is a 2-Cys subclass, mitochondrion-specific enzyme required for normal mitochondrial function. Depletion of Prx3 results in increased intracellular levels of H_2O_2 and sensitizes cells to apoptotic signaling.
We have previously reported that Prx3 protein initially increases in the mouse cochlea in vivo in response to aging and aminoglycoside treatment but subsequently decreases, followed by hair cell death. We now investigate the regulation of expression of Prx3 in CBA/J mouse cochlear explants (postnatal day 3), challenged with 200 µM gentamicin which causes significant hair cell death after 20 hours. Expression of mRNA for Prx3 increased significantly after gentamicin treatment and protein levels of Prx3 were increased at 8 hours, but reduced by 16 hours in outer hair cells. Consistent with the mRNA expression, protein levels of c-Myc, a transcription factor that targets the Prx3 gene, and its phosphorylation at Thr58 and Ser62 was also increased after 8 hours of treatment. Supporting a connection between c-Myc and Prx3, 10058-F4, an inhibitor of c-Myc, prevented the early rise of Prx3 protein levels after gentamicin treatment.
These results demonstrate that an initial response of hair cells to oxidative stress is a homeostatic upregulation of Prx3 which, at least in part, depends on the transcription factor c-Myc. Subsequent downregulation or exhaustion of Prx3 in outer hair cells is followed by cell death.

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O 22

Stress and survival pathways in the mammalian cochlea

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Studies conducted over the last couple of years demonstrated that signalling pathways that operate in the organ of Corti (OC) play a central role in survival and death of hair cells. An important goal of molecular otology is to characterize these signalling pathways in normal inner ears and in inner ears exposed to different forms of stress, such as ototoxic substances and noise overexposure.
In this study, we used high-performance reverse-protein microarray technology and phosphospecific antibodies to examine the activation status of defined molecules involved in cellular signalling. We demonstrate that reverse protein microarrays based on the highly sensitive planar-wave guide technology provide an effective and high-throughput means to assess the activation state of key molecules involved in apoptotic and pro-survival signalling in microdissected OC explants over time.
In this study, we show that gentamicin and a specific NF-κB inhibitor increase the ratio of phospho-c-Jun/c-Jun in OC explants of postnatal rats soon after exposure to these drugs. In addition, we found a decrease in the phospho-Akt/Akt ratio in OC explants early after NF-κB inhibition. Finally, we observe an early decrease of the phospho-p38/p38 ratio in OC explants exposed to gentamicin or the NF-κB inhibitor, respectively.

O 23

Somatostatin and gentamicin-induced auditory hair cell loss

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Hair cells of the mammalian auditory system do not regenerate and, therefore, their loss leads to irreversible hearing loss. Aminoglycosides, among other substances, can irreversibly damage hair cells. Somatostatin, a peptide with hormone/neurotransmitter properties, has neuroprotective effects by binding to its receptor. In this study, we tested whether somatostatin can protect hair cells from gentamicin-induced damage in vitro. This study confirmed the expression of somatostatin receptor mRNA within the cochlea and analyzed the effect of somatostatin on gentamicin-induced hair cell damage and death in vitro.
Expression of somatostatin receptor mRNA in the rat cochlea was analyzed by the reverse transcriptase-polymerase chain reaction (RT-PCR). Protection of auditory hair cells from gentamicin was tested using two different concentrations (1 µM and 5 µM, respectively) of somatostatin.
We detected somatostatin receptor-1 and somatostatin receptor-2 mRNA in the organ of Corti, spiral ganglion, and stria vascularis by RT-PCR. Moreover, we could see significantly less hair cell loss in the organ of Corti that were pretreated with either 1 µM or 5 µM of somatostatin as compared with samples treated with gentamicin alone.
Decreased hair cell loss in somatostatin-treated samples that had been exposed to gentamicin provides evidence for a protective effect of somatostatin in aminoglycoside-induced hair cell death in vitro.

O 24

The role of prostaglandin E receptor subtype EP2 and EP4 in the cochlea

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Prostaglandin E1 (PGE1) is frequently used for the clinical treatment of acute sensorineural hearing loss (SNHL). However, the mechanisms for the clinical effects of PGE1 have not yet been elucidated. The physiological effects of PGE1 are mediated by PGE receptor subtypes EP1, EP2, EP3 and EP4, the respective agonists for which have been purified. PGE1 can increase vascular endothelial growth factor (VEGF) through particularly EP2 and EP4.
In this study, we examined the efficacy of local EP4 agonist application to the cochlea for the treatment of SNHL. The protective effects of local EP4 agonist treatment before or after noise exposure were tested in guinea pigs using measurements of auditory brainstem responses (ABRs) and histological analysis. Subsequently, we examined the efficacy of local EP2 and EP4 agonist application on the production of VEGF proteins and mRNA in mouse cochleae using ELISA and quantitative RT-PCR. We moreover investigated the localization of EP2, EP4, VEGF, and VEGF receptors, Flt-1 and Flk-1 in mouse cochleae by immunohistochemistry.
The results demonstrated EP2 and EP4 expression in the cochlea and showed that pre- and post-treatment with an EP4 agonist significantly attenuated threshold shifts of ABRs. Significant attenuation in the loss of outer hair cells was found in local EP4 agonist treatment before noise exposure. VEGF proteins and mRNA significantly increased and VEGF expression was strongly detected in the spiral ganglion cells, following local EP2 and EP4 agonist application. Flt-1 and Flk-1 expression were found in the cochlea.
These findings indicate local EP4 agonist treatment could attenuate acute SNHL, and local EP2 and EP4 agonist treatment could induce VEGF in particularly the spiral ganglion cells, which might act on cochlear structures expressing Flt-1 and Flk-1. VEGF increased through EP2 and EP4 receptors might be involved in mechanisms for the clinical efficacy of PGE1 on acute SNHL.

O 25

Geldanamycin attenuates ototoxicity caused by gentamicin in the organ of Corti explants

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Heat shock protein 70 (HSP70) protects inner ear cells from damage and death induced by e.g. heat or toxins. The benzoquinone ansamycin antibiotic geldanamycin (GA) was demonstrated to induce the expression of HSP70 in various animal cell types. The aim of our study was to investigate whether GA induces HSP70 in the organ of Corti (OC), which contains the auditory sensory cells, and whether GA can protect these cells from toxicity caused by a common aminoglycoside antibiotic, gentamicin
To address these questions, we used the OC explants isolated from p3-p5 rats. As a read-out, we used RT-PCR, ELISA and immunofluorescence.
We found that GA at the concentration of 2 µM efficiently induced HSP70 expression on mRNA and protein level in OC explants. Confocal microscopy revealed that HSP70 induced by GA is expressed by hair cells and interdental cells of spiral limbus. Pre-incubation of explants with 2 µM GA prior to adding gentamicin (500 µM) significantly reduced the loss of outer hair cells but not of inner hair cells, suggesting different mechanisms of otoprotection needed for these two cell types. GA-induced HSP70 in the auditory sensory cells and partially protected them from toxicity of gentamicin. Understanding the molecular mechanisms of GA otoprotection may provide insights for preventative therapy of the hearing loss caused by aminoglycoside antibiotics.

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O 26

Protective effect of X-linked inhibitor of apoptosis protein against noise-induced cochlear lesions in C57 mice

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Apoptosis has been reported to be one of the major reasons for noise-induced cochlear cell death. In the present study, we determined whether over-expression of the X-linked inhibitor of apoptosis protein (XIAP) protects the inner ear against noise-induced injury.
Transgenic C57BL/6J mice that ubiquitously over-express human XIAP via a ubiquitin promoter were compared with their wild-type littermates for hearing loss and cochlear lesions induced by exposure to two distinct noise regimes (125 dB SPL for 6 hours and 110 dB SPL for 300 hours). Hearing status was evaluated using the auditory brainstem response. Resulting cochlear lesions were assessed using surface preparations to measure hair cell (HC) and loss of spiral ganglion neurons (SGNs) and their fibers in the organ of Corti.
Significantly greater threshold shifts were found for both noise exposure regimes in wild-type mice when compared to the transgenic mice. Correspondingly, wild-type mice also showed a significantly greater degree of loss of HC, SGNs and their afferent fibers from HCs. However, the degree of HC loss was much less than expected based on the degree of threshold shift. Instead, the loss of nerve fibers and SGNs appears to better correlate with this functional measure of hearing sensitivity.
Western blot analysis failed to show significant changes in both endogenous XIAP and the 6Myc-XIAP product of the XIAP transgene induced by noise. The total levels of XIAP were 50-60% higher in transgenic mice due to presence of 6Myc-XIAP.
Although this increase in total XIAP seemed to impart a protective effect, ub-XIAP mice still displayed significant functional, HC, SGN and fiber losses compared to the control (pre-noise exposure) values, demonstrating limitations in the ability of this anti-apoptotic gene to completely protect against high-intensity noise.

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O 27

Clinical trial for local IGF-1 treatment for acute sensorineural hearing loss: From the bench to the clinic

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The objective of the study was to test the efficacy of local IGF-1 application via biodegradable hydrogels for acute sensorineural hearing loss that did not respond to systemic glucocorticoid treatment.
We designed a cohort study of phase I-II clinical trial to examine the safety and efficacy of local IGF-1 treatment for corticosteroid-resistant acute sensorineural hearing loss. Primary outcomes were set to evaluate hearing levels in pure-tone audiometry 12 weeks after the treatment. Secondary outcomes were rates for the occurrence of adverse effects including middle ear inflammation and vertigo.
We report the preliminary results and typical two cases that showed significant hearing improvement. No severe adverse effects were found in registered patients. On day 21 after the onset, a 56-year-old man received local IGF-1 treatment. The average of hearing levels of 5 frequencies recovered 26 dB 12 weeks later. On day 25 after the onset, a 60-year-old female received local IGF-1 treatment. The average of hearing levels of 5 frequencies recovered 17 dB 4 weeks later. Notably, the thresholds at 250 and 500 Hz returned to 20 dB, within a normal range. No significant adverse effects were observed in these two cases.
Two cases presented here demonstrated significant recovery of hearing, although local IGF-1 application was performed over 20 days after the onset, suggesting the therapeutic potential of local IGF-1 treatment.

O 28

Notch signaling specifies prosensory regions in the inner ear

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Notch signaling plays a role in several aspects of inner ear development. However, an early role for Notch as an inducer of prosensory domains has remained unclear in part because of discrepancies between the phenotypes of mice with deletions of single Notch-related genes. One explanation for these discrepancies is functional compensation by other Notch receptors or ligands. Therefore, to determine the effects of complete elimination of Notch signaling, we generated an inner ear-specific knockout of the Rbpsuh gene. RBP-J protein, which is encoded by Rbpsuh gene, is a transcriptional co-activator for all Notch molecules and thus deletion of this protein inhibits all Notch signaling.
Foxg1Cre mice were crossed with Rbpsuhflox mice to delete the Rbpsuh gene in the otocyst. To examine the effects of differences in deletion efficiency, both Rbpsuh-/flox and Rbpsuhflox/flox mice were used. The inner ear phenotypes were determined at various developmental stages.
When deletion efficiency was low hair cells were present in the cochlea, however the number of inner hair cells increased while that of outer hair cells was decreased. This phenotype is similar to the phenotype reported previously for the Foxg1Cre line mediated knockout of the notch ligand Jag1. In contrast, when deletion efficiency was high, the cochlea contained a small patch of hair cells located at the extreme apex.
These results indicate that Notch signaling mediates several aspects of inner ear development. Early in inner ear formation, Notch signaling specifies the future sensory epithelia by regulating the formation of prosensory patches while at later time points, Notch signaling determines the number of cells that will develop as hair or supporting cells within the prosensory domains.

O 29

The molecular mechanism of DFNB28 deafness

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Mutations of TRIOBP were identified as the cause of human hereditary deafness DFNB28. Novel alternative splice isoforms of TRIOBP (TRIOBP4 and TRIOBP5) were identified and RT-PCR showed their expression in inner ear, retina and brain. All DFNB28 mutations are located in exon 6. Since transcripts of the initially identified and ubiquitously expressed isoform TRIOBP1 (Seipel et al., 2001) do not contain the amino acid sequence encoded by exon 6, TRIOBP1 is likely not to be affected by DFNB28 mutations. On the other hand, TRIOBP4 and TRIOBP5 isoforms are truncated by DFNB28 mutations and are likely to be necessary for hearing. We generated antibodies and confirmed that the TRIOBP4 and TRIOBP5 are localized in the stereocilia rootlets of hair cells. The densely packed bundle of actin filaments of

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a rootlet extends into the cell body and is embedded in the cuticular plate. A mouse that is null for both TRIOBP4 and TRIOBP5 was generated by gene targeting. This homozygote mutant mouse is profoundly deaf. At P7, before the onset of hearing in the mouse, stereocilia looked almost normal by scanning electron microscopy. But transmission electron microscopic images revealed that rootlet structure never develops in the homozygote mutant mouse. Mutant stereocilia without rootlets are abnormally flexible at the fulcrum, and eventually degenerate causing deafness. In vitro study using the purified full length TRIOBP4 with actin showed that TRIOBP4 does not affect actin polymerization, but it does bundle actin filaments to generate a rootlet-like structure. Both our in vivo and in vitro investigations indicate that TRIOBP4 is a potent key regulater to make actin bundles at stereocilia rootlets.

O 30

ATP8B1 is involved in the function of hearing

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ATP8B1 deficiency is caused by autosomal recessive mutations in ATP8B1, which encodes the putative phosphatidylserine flippase. The disease is primarily characterized by cholestasis, either as progressive (progressive familial intrahepatic cholestasis type 1, PFIC1) or intermittent (benign recurrent intrahepatic cholestasis type 1, BRIC1). Since part of the patients reported reduced hearing capability, the role of ATP8B1 in auditory function was investigated.
The hearing was tested in ATP8B1 deficient patients (BRIC1) and mice ($Atpb1^{G308V/G308V}$ mutants). Immunohistochemistry was used to investigate the expression and localization of ATP8B1 in the murine inner ear and morphological methods were used to examine the consequences of ATP8B1 deficiency for the inner ear structures.
Our results show that ATP8B1 deficiency, both in patients and in mutant mice, causes sensorineural hearing loss. Furthermore, we determined that ATP8B1 is specifically localized in the stereocilia of cochlear hair cells. These hair cells progressively degenerate in mutant mice.
The present data indicates that the mechanosensory function and integrity of the cochlear hair cells is critically dependent on ATP8B1 activity, possibly through maintaining lipid asymmetry in the cellular membranes of stereocilia.

O 31

Prestin as a bicarbonate-chloride transporter

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Prestin is expressed in the lateral membrane of outer hair cells (OHCs) and is essential for OHC somatic electromotility and cochlear amplification. On the basis of its amino-acid sequence, prestin (SLC26A5) belongs to the solute carrier 26 family of transporters which exchange halides for SO_4^{2-} or HCO_3^-. The original electrophysiological analysis of mammalian prestin (Oliver et al., 2001) suggested that such transport functions are minimal; more recent experiments with radioactively labeled substrates also failed to identify a significant HCO_3^- transport (Bai et al., 2009).
We have employed sensitive intracellular pH fluorescence probes as an alternative approach to asses the possibility that prestin transports HCO_3^-. A DNA-coding sequence of super-ecliptic pHluorin, a pH-sensitive variant of GFP, was attached to the C-terminus of prestin and the resulting DNA construct overexpressed in a CHO cell line. As a control for endogenous transport, pHluorin was targeted to the membrane intracellularly using a myristilation-targeting peptide. The experimental data indicate that in the presence of extracellular HCO_3^- the intracellular pH recovers from the CO_2-induced acidification 4 times faster in cells transfected with prestin. This acceleration requires low (4 mM) extracellular Cl^- consistent with prestin transporting HCO_3^- intracellularly in exchange for Cl^-. The process was significantly reduced by extracellular application of 10 mM salicylate. As a pHfluorin-independent assay, recovery (i.e. HCO_3^- loading) was also only found in those cells expressing prestin using BCECF as a cytoplasmic pH probe. Preliminary quantitative modelling of this system produce pH time courses that mirror the experimental data under reasonable assumptions about the appropriate rate constants.
These data therefore suggest that prestin can act as a weak HCO_3^-/Cl^- antiporter although the effects are anticipated to be greater in OHCs than in expression systems due the higher prestin copy number.

O 32

HSF1 is essential for the maintenance of inner ear function

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Heat shock response is known as the fundamental protection system of cells against stress. The expression of heat shock proteins is regulated by heat shock transcription factor-1 (HSF1). The purpose of the present study is to evaluate the role of HSF1 in the maintenance of inner ear.
In one of the experiments, we used HSF1-knockout mice. These mice, which were bred in normal condition, showed hearing loss after 36 weeks. The histological findings revealed the loss of hair cells in cochleae. In the next experiments, we used the DBA/2J mice, which showed age-related hearing loss. These mice were bred with feed containing the activator of HSF1: geranylgeranylacetone (GGA). The western blot analysis showed the expression of heat shock proteins in cochleae of the mice. In these DBA mice, the hearing loss were suppressed as compared with the DBA mice bred with the normal feed. The histological findings showed the protection of cochlear hair cells with the upregulation of heat shock proteins.
These results suggest that HSF1 is the important molecule for the maintenance of inner ear function. The hair cells could be protected against the stress with the heat shock response.

O 33

Fate of mammalian hair cells and stereocilia after loss of the stereocilia

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Cochlear hair cells transduce mechanical stimuli into electrical activity. The site of hair cell transduction is the hair bundle, an array of stereocilia with different height arranged in a staircase. Tip links connect the apex of each stereocilium to the side of its taller neighbor. The hair bundle and tip links of hair cells are susceptible to acoustic trauma and ototoxic drugs. It has been shown that hair cells in lower vertebrates and in the mammalian vestibular system may survive bundle loss and undergo self-repair of the stereocilia. We simulated the acoustic trauma-induced tip link damage or stereociliary loss by disrupting tip links or ablating the hair bundles in the cultured organ of Corti from neonatal gerbils. Our goals were to determine whether cochlear hair cells could survive the trauma and whether the tip link and/or the hair bundle could be regenerated. Hair-cell survival and development as well as stereociliary morphology and function were examined using confocal and scanning electron microscopies, and electrophysiology. Most bundleless hair cells could survive and develop for about 2 weeks. However, no spontaneous hair-bundle regeneration was observed. When tip links were ruptured, repair of tip links and restoration of mechanotransduction were observed in less than 24 hours. Our study suggests that the dynamic nature of the hair cell's transduction apparatus is retained despite that regeneration of the hair bundle is lost in mammalian cochlear hair cells.

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O 34

Regulation of outward K_v currents by extracellular chloride in the outer hair cell

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Two major potassium currents – termed I_K and $I_{K,n}$ – are found in outer hair cells (OHCs) of the guinea-pig cochlea. Interestingly, we now find that the 4-AP sensitive, outward K^+ conductance of the OHC is also sensitive to chloride, although, in contrast to prestin, extracellularly. At a holding potential of -40 mV, I_K is inhibited by changing extracellular Cl^- levels from 150 mM to 5 mM, with a K_d of 50 mM. Other K_v channel conductances in supporting cells, such as the Hensen and Deiters' cells, are not affected by reduced extracellular Cl^-. We also tested heterologously expressed Slick and Slack K^+ channel conductances, but found no extracellular Cl^- sensitivity.
In order to elucidate the mechanism of I_K sensitivity to Cl^- in OHCs, activation and inactivation kinetics of I_K were examined. Lowering extracellular Cl^- shifted $V_{1/2}$ of I_K inactivation from -40 mV to -50 mV, but had no effect on activation. Thus, Cl^- sensitivity of Ik might arise in part from a hyperpolarizing shift in inactivation. We are honing in on the molecular identity of this conductance.

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O 35

Rate-level functions at different temporal locations in the chick cochlear nerve adaptation curve

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The decay of cochlear nerve discharge rate during stimulation (neural adaptation), results from synaptic vesicle depletion at hair cell neurotransmitter release sites. The adaptation curve has three stages, an initial high rate of discharge, then a decaying response, and finally a relatively constant discharge rate. The present study examined the relation between discharge rate and stimulus intensity level (the RL function) at each stage of adaptation. The hypothesis tested was that the kinetics of synaptic vesicle depletion, as reflected in the RI curve, was the same at each stage of adaptation.
Anesthetized chicks were presented with time-locked 100 ms CF pure tones, 40 times per intensity, at progressively increasing intensities. Discharge activity was recorded with each stimulus trial, and a raster plot of this activity was constructed. This plot demonstrated response dynamics as a function of stimulus duration and intensity. The raster was divided into three segments, the first 15 msec, the middle 70 msec and the last 15 msec, and discharge rate was determined in S/sec over all intensities. In 146 units from control animals the RL function in each segment was categorized as a saturating, sloping, straight, or sloping-down type. Similar determinations were made for 111 units in chicks exposed to intense sound causing cochlear damage.
The RI types in each segment of the raster plot were the same in 76% of the control units. However, in exposed animals the RI functions were similar in 93% of units. The greater homogeneity of RI type in exposed units occurred in spite of a considerable threshold shift and spontaneous activity reduction.
The results are discussed with regard to the kinetics of hair cell synaptic vesicle release at different times in the adaptation function, the hair cell types on the basilar papilla, and the effects of damaging sound exposure.

O 36

Otoferlin: A synaptotagmin-like calcium sensor?

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Otoferlin, a multi-C2 domain shown to be essential for a late step in exocytosis of the auditory hair cells, is currently discussed to replace synaptotagmin (syt) at this excitatory synapse. This hypothesis is based on: (1) otoferlin having 6 or 7 C2 domains, of which 3 are predicted to bind Ca^{2+}; (2) the absence of syt 1, 2 and 3 at the first auditory synapse (Safieddine and Wenthold, 1999); (3) the interaction of otoferlin with syntaxin 1 and SNAP-25 in immunoprecipitation assays; and (4) the absence of fast vesicle release in $Otof^{-/-}$ hair cells (Roux et al., 2006).
In this study, we transduced auditory inner hair cells of $Otof^{-/-}$ mice with syt 1 and tested exocytosis by patch-clamp capacitance measurements, but could not restore Ca^{2+}-triggered exocytosis. Next, we transfected the developing otocysts of $Otof^{-/-}$ embryos at E12 with syt 1 and measured hearing by auditory brainstem response in 3-week-old animals. In comparison to the untransfected ear, no difference in hearing could be detected. Further, we analyzed exocytosis in autaptic cultures of syt1-deficient hippocampal neurons, but found no effect when overexpressing otoferlin.
Together, this study suggests that the mechanism of otoferlin function is different from syt action.

O 37

Otoferlin interacts with myosin VI: Implications for the basolateral synaptic structure of the inner hair cell

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One of the genes underlying hearing impairment in mice and humans is the sequence coding for otoferlin. Mutations within OTOF lead to a recessive disorder called DFNB9. Several studies have indicated otoferlin's association with ribbon synapses of cochlear sensory hair cells, as well as data showing the protein's presence in neurons, nerve fibers and hair cells, suggesting a more ubiquitous function. We recently notified otoferlin's absence despite exocytosis in hypothyroid animals questioning other Ca-sensing proteins to be upregulated under hypothyroid conditions. Molecular studies were therefore performed to identify possible candidates substituting for the supposed Ca^{2+}-sensor function of otoferlin under hypothyroidism. On the other side, search for the otoferlin binding partner may also help to clarify otoferlin's identity as calcium sensor under hypothyroid or normal conditions. Using yeast two-hybrid screen and mass spectroscopy, otoferlin interaction partners were identified and its interaction verified upon co-expression, co-localization and co-immunoprecipitation. Results will be discussed in the context of the undoubtful essential role of otoferlin for exocytosis of hair cells.

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O 38

Localized neurotrophin gene therapy for controlling auditory neurve regeneration after hearing loss

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The cochlear implant relies on spiral ganglion neurons (SGNs) to transmit electrical signals from the implant to the brain. However, SGNs progressively degenerate after deafness due to loss of neurotrophins normally supplied by sensory hair cells. Exogenous neurotrophins protect SGNs from degeneration but cause abnormal dendrite resprouting due to a lack of a target for the nerves to grow towards.
To create a target-derived neurotrophin source to control the direction of resprouting SGN dendrites following deafness. It was hypothesized that injection of viral gene transfer vectors into the scala media would result in more localized and more relevant neurotrophin gene expression patterns compared to injection into the scala tympani.
Adenoviral vectors were generated containing the gene for green fluorescent protein (GFP) alone or in combination with genes for brain-derived neurotrophic factor or neurotrophin-3. These were injected into the scala tympani or scala media of guinea pigs deafened for 1 week via aminoglycosides. After 3 weeks, cochleae were examined for gene expression, SGN survival and dendritic response to gene expression.
Scala media injections of adenoviral vectors resulted in more localized gene expression than scala tympani injections, with gene expression consistently observed within the partially degenerated organ of Corti and in the spiral limbus. There was a 1.8 and 1.85 fold greater SGN survival following neurotrophin gene transfer to the scala media compared to the scala tympani and compared to GFP gene transfer to the scala media, respectively ($p<0.05$). There was also evidence of localized dendritic responses to neurotrophin gene expression within the organ of Corti from scala media injections, a first step in controlling the direction of SGN dendrite regeneration via gene therapy. A localized source of neurotrophins can control the regeneration of SGN dendrites after deafness and hence help improve place-specific sound perception with a cochlear implant.

O 39

Regeneration of nerve fibers into the deaf ear

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Following elimination of cochlear hair cells, differentiated supporting cells are replaced by a flat epithelium, and the radial neurons are typically absent in the first and second turns. The flat epithelium is a target for stem cell therapy for hair cell replacement. For new hair cells to become functional, it is necessary for them to be innervated. Therefore, attracting neurons into this tissue is an important step towards such therapy. Our goal was to determine whether neurons can be attracted into the flat epithelium by elevated levels of BDNF.
We deafened the left ears of pigmented guinea pigs with an injection of 10% neomycin (10 µl) into the scala tympani via a cochleostomy in the basal turn. Seven days later, we inoculated the cochleae with 5 µl Ad.BDNF into the scala media via a cochleostomy in the second turn. Contralateral ears and ears inoculated with Ad.empty served as controls. Fourteen days after inoculation, the ears were assessed histologically using whole mounts or plastic sections. Tissues were labeled with antibodies to neurofilament and peripherin to determine the extent and type of nerve fibers in the flat epithelium. Epifluorescence of Ad.BDNF treated ears revealed an increase of neurofilament-positive fibers in the flat epithelium.
Positive staining of some neurites for peripherin suggest that type II afferents were present. Ad.empty inoculated ears contained no radially-extending neurons in the flat epithelium. The results show that Ad.BDNF inoculation into the flat epithelium can induce innervation. The data demonstrate the ability of neurons to grow in large numbers into the flat epithelium of the deaf cochlea. Innervation of the flat epithelium has implications for feasibility of several therapies including cochlear implantation and stem cells.

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O 40

Secretion of growth factors and chemokines by adipose tissue-derived stromal cells

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Autologous stem/progenitor cells transplantation is emerging as a novel therapeutic option for hearing loss. Recent reports indicated that adipose tissues could supply pluripotent cells, adipose tissue-derived stromal cells (ADSCs). ADSC transplantation was reported to show beneficial effect on ischemic diseases. It is thought that the effects of the therapy are due to not only the pluripotency of the cells but also to growth factors and chemokines the cells secrete. When we take such a paracrine effect into consideration, it is easier for us to use pluripotent cells derived from its own individual than ES cells in terms of carcinogenesis and immune response. We examined what kind of growth factors and chemokines ADSCs secrete.
ADSCs were harvested from C57BL/6 mice and cultured for 72 hours in serum free medium. After that, we measured the concentration of 16 kinds of growth factors and chemokines (TGF-β1, IGF-1, NGF, HGF, VEGF, MCP-1, M-CSF, EGF, MIP-α, GM-CSF, G-CSF, SDF-1, PDGF, BDNF, BMP2, FGF-basic) in the supernatant by ELISA.
As a result we found that ADSCs secreted TGF-β1, IGF-1, NGF, HGF, VEGF, M-CSF.
It has been reported that IGF and HGF have effects of protecting inner ear hair cells against its injury. It has also been reported that TGF-β1 controls chemotaxis of macrophages and microglias and can control inflammation and heal damaged tissues. The fact that ADSCs are able to secrete growth factors and chemokines related to protecting and healing inner ear cells suggests that transplantation of adipose tissue-derived cells into the inner ear can be applied to the therapy against inner ear injury.

O 41

A novel stem cell for auditory neuron regeneration

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To patients suffering from sensorineural hearing loss, a cochlear implant (CI) is the best applied therapy currently. Implant recipients experience benefits, nevertheless understanding speech in noisy circumstances and music perception remains challenging. It is suggested that stem cell-based therapy, by increasing the number of auditory neurons, will improve the performance of a CI.
A new type of stem cell has been identified, a multipotent precursor cell derived from the hair follicle from adult mammalian dermis, the epidermal neural crest stem cell (EPI-NCSC). For transplantation purposes the use of EPI-NCSCs has numerous advantages above that of other stem cells: (1) they are easily accessible; (2) they are good candidates for autologous transplantation, which will avoid graft-versus-host disease; and (3) there is no evidence of tumour formation.
The aim of this research is to explore the capacity of EPI-NCSCs to differentiate into neurons with hallmarks of auditory neurons. These neurons are bipolar and express sensory neuron characteristics such as TrkB/C co-expression and Glu2/3R presence.
Hair follicles from whiskers of mice and guinea pigs and from human scalp were cultured in proliferation medium. Subsequently, EPI-NCSCs were differentiated by supplying pertinent neurotrophic support. Quantitative immunohistochemistry was performed to count the number of neural stem cells (nestin) and neurons (TUJ1). Additionally, the expression of TrkB, TrkC and GluR2/3R was tested.
After proliferation, the majority of the cells (90%) were positive for nestin. The average yield per follicle was $2x10^5$, while for transplantation purposes $2\text{-}5x10^5$ cells are required. About 70% of the differentiated cells showed a bipolar morphology and were positive for the neural marker TUJ1. In the majority of these cells co-expression of TrkB, TrkC and GluR2/3R was found.
Concluding, auditory neuron-like cells from hair follicle stem cells were successfully generated, fuelling efforts to develop autologous stem cell-based transplantation therapies for deaf patients.

O 42

Neural stem cells from the cochlear nucleus

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Stem cells and their role in hearing development as well as their potential therapeutic target are of increasing interest. Multipotential stem cells have been identified in the mammalian central nervous system, as well as in the inner. Until now, there has been no description of potential neural stem cells in the cochlear nucleus. The aim of this study was to proof the existence of this type of cells in the cochlear nucleus.
Therefore, the cochlear nucleus of p4 rats (Sprague-Dawley) was dissected and triturated enzymatically. Cells were cultured in Neural Stem Cell (NSC) medium for generation of primary neurospheres. The spheres were then dissociated and plated on poly-D-lysin/laminin coated coverslips in differentiation medium or in NSC medium for generation of secondary neurospheres, respectively. Cells were incubated with BrdU prior to fixation for cell division analysis. Fixation and staining was done using different specific primary antibodies and consecutively with secondary antibody for fluorescence and confocal laser scanning microscopy after several intervals of time.
Primary and secondary neurosphere formation could be detected in all cochlear nucleus stem cell cultures of postnatal day 4 pubs. Cells in spheres showed distinct labeling for Nestin, a marker for neural stem and progenitor cells of the central nervous system. Differentiation into neurons, astrocytes and oligodendrocytes as shown by the markers β-III tubulin, GFAP and MBP was observed. Cell division could be detected by BrdU incorporation in undifferentiated neurospheres and single cell cultures after differentiation.
The present study with neural stem cells culture from the cochlear nucleus represents all features of neural stem cells: cell division, existence of progenitor cells and differentiation into cells of the neuronal lineage. These findings help to better understand developmental features of the cochlear nucleus and a possible use of these cells by stimulation with specially designed ABI electrodes.

O 43

Differentiation of human embryonic and human induced pluripotent stem cells along the otic lineage

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Degeneration and death of cochlear hair cells and their associated spiral ganglion neurons is causal in more than 80% of individuals with hearing loss. In mammals, cochlear hair cells do not regenerate which results in permanent hearing loss. Stem cell technology provides a potential therapeutic approach for cochlear hair cell regeneration. Toward the development of such therapy, we developed a stepwise protocol for differentiating human embryonic stem cells (hESCs) and human induced pluripotent stem (iPS) cells along the otic lineage.
H9 human embryonic stem cells were obtained from WiCell Research Institute and maintained on mouse embryonic fibroblast in hESC media. Human induced pluripotent stem cells were developed from a Parkinson patient. Differentiation involved initial exposure to Wnt and TGF-β inhibitors to promote ectodermal formation followed by IGF for rostralization. These cells were then exposed to FGFs to promote differentiation into otic progenitors. To test whether these otic progenitors had the capacity to differentiate into hair cells, the otic progenitors were co-cultured with chicken utricular stroma cells.
The stepwise differentiation protocol described above led to approximately 70% Pax2-positive cells. To further confirm that these cells were otic progenitors, immunocytochemistry and RT-PCR for other otic markers were utilized. Following further co-culture of these cell populations with chicken utricular stroma cells, upregulation of hair cell markers in both hESCs and human iPS cells were seen both with RT-PCR and immunocytochemistry. SEM images of these cells revealed stereociliary structures on the apical surface. We are in the process of narrowing the combination of defined media that are capable of more efficiently differentiating otic progenitors into hair cells.
We have developed a stepwise protocol for differentiating both hESCs and human iPS cells into hair cells in vitro.

O 44

Inner ear protection after cochlear implantation

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Local delivery of protective agents to the cochlea offers a number of clinical advantages over systemic delivery, particularly in terms of reduced side effects and increased local concentrations. The objective of our study was to investigate whether anti-oxidants and anti-inflammatories delivered locally to the cochlea via the round window before surgery can result in an increased level of residual hearing after cochlear implantation.
Both a basal and second turn guinea pig cochlear implant model was used to measure the level and extent of hearing protection after cochlear implantation. Drug delivery was achieved via a bead of seprapak (with the drug absorbed), which was placed on the round window at various time points before implantation. Pure tone hearing thresholds were measure before and at a number of time point post surgery and cochleae were examined histochemically.
Both anti-oxidants and anti-inflammatories are able to protect hearing after cochlear implantation. In particular, the anti-inflammatory, dexamethasone is able to protect from direct implant trauma at the site of cochleostomy as well as indirect trauma at remote locations within the cochlea. As well as protecting hearing both classes of drugs appeared to reduce the level of the on-going inflammatory reaction after implantation.
It appears that the level of hearing protection achieved via the local application of protective agents is controlled by a number of parameters including; application time, initial concentration, inter-cochlea drug distribution and hair cell sensitivity. A number of these parameters can be modelled and manipulated in order to produce clinically significant levels of inner ear protection.

O 45

Dexamethasone therapy and methods to control the effects of trauma in cochlear implant design

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The use of superflexible and soft electrodes, inserted through the round window membrane, does not completely suppress the risk of residual hearing loss in cochlear implant patients. Complete or partial loss of residual hearing can decrease the finite neural reserve of the inner ear, a fact which is particularly unacceptable for young children and infants facing the possibility of a number of re-implantations during their lifetimes. It is postulated that an acute or semi-chronic pharmacological approach at the time of electrode insertion has the potential to further reduce the risk of hearing loss. Indirect mechanisms of hearing loss after electrode insertion are in part associated with the release of inflammatory mediators, such as the TNF-α cytokine, from lateral wall fibrocytes, due to either cochleostomy and puncturing of the spiral ligament or friction of the electrode against the lateral wall. Dexamethasone is an anti-inflammatory and anti-apoptotic drug which can protect hair cells against TNF-α ototoxic effects in animal models (Haake et al., 2009). Techniques to incorporate and deliver dexamethasone to the inner ear intra- and/or post-operatively are being developed and tested. The most promising technique uses the natural elution of dexamethasone crystal from the electrode silicone after mixing 2% to 10% of the corticosteroid with the elastomer. Sub-microgram/day elution along the length of the electrode increases hair cell survival during electrode trauma in animal models, as measured with CAP or ABR. Another approach is being tested to evaluate long-term hair cell protection when a single bolus of dexamethasone is slowly and pecisely injected in a specific location of the scala tympani prior to electrode insertion. Method and devices will be presented and results of animal studies will be discussed.

Reference: Haake SM, Dinh CT, Chen S, Eshraghi AA, Van De Water TR (2009) Dexamethasone protects auditory hair cells against TNFα-initiated apoptosis via activation of PI3K/Akt and NFκB signaling. Hearing Research, in press.

O 46

Cochlear implant electrode array-eluted dexamethasone conserves hearing: Genetic and molecular mechanisms

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The objective of this study was to determine if polymer-eluted dexamethasone can conserve hearing against trauma-induced loss and begin to identify and characterize mechanisms of dexamethasone's otoprotection on both molecular and gene expression levels.
Both an animal model of electrode trauma-induced loss of auditory function/hair cells and an in vitro model of inflammatory cytokine-induced apoptosis of hair cells tested the otoprotective efficacy of biopolymer (SIBS)-eluted dexamethasone. Inhibitor and gene expression (RT^2-PCR) studies probed the mechanisms of otoprotection by dexamethasone against cytokine-induced apoptosis of organ of Corti explant hair cells. ANOVA and post-hoc tests determined significance ($p<0.05$). Dexamethasone eluted from SIBS-coated electrode arrays conserves hearing in an animal model of trauma-induced hearing/hair cell loss and protects auditory hair cells within TNF-α challenged organ of Corti explants. Inhibitor studies demonstrate that activation of NF-kB is required for this otoprotective action of dexamethasone against TNF-α ototoxicity. Gene expression studies identify Bax, Bcl-2, Bcl-xl and TNFR1 as targets of NF-kB within dexamethasone treated/TNF-α challenged explants. TNF-α exposure upregulates pro-apoptosis related genes, e.g. Bax, and downregulates anti-apoptosis genes, e.g. Bcl-xl, while treatment of TNF-α exposed cultures with eluted-dexamethasone reverses these cytokine-induced changes in gene activity causing a down regulation of Bax expression and an up regulation of both Bcl-2 and Bcl-xl. This results in a dramatic shift in the Bax/Bcl-2 ratio favoring hair cell survival within the TNF-α/dexamethasone treated explants. Dexamethasone treatment also reverses TNF-α induced elevation of TNFR1 expression (an inflammation-related gene), thereby protecting both hair cells and hearing.
Polymer-eluted dexamethasone retains its otoprotective efficacy activating NF-kB signaling which downregulates pro-apoptosis and upregulates anti-apoptosis related genes. Development of a dexamethasone-eluting electrode array has the potential to conserve a patient's residual hearing allowing for improved electro-acoustic based stimulation (EAS) of both auditory neurons/nerves and hair cells.

O 47

Evaluation of the elution of dexamethasone from the silicone of the electrode array: Preliminary safety studies

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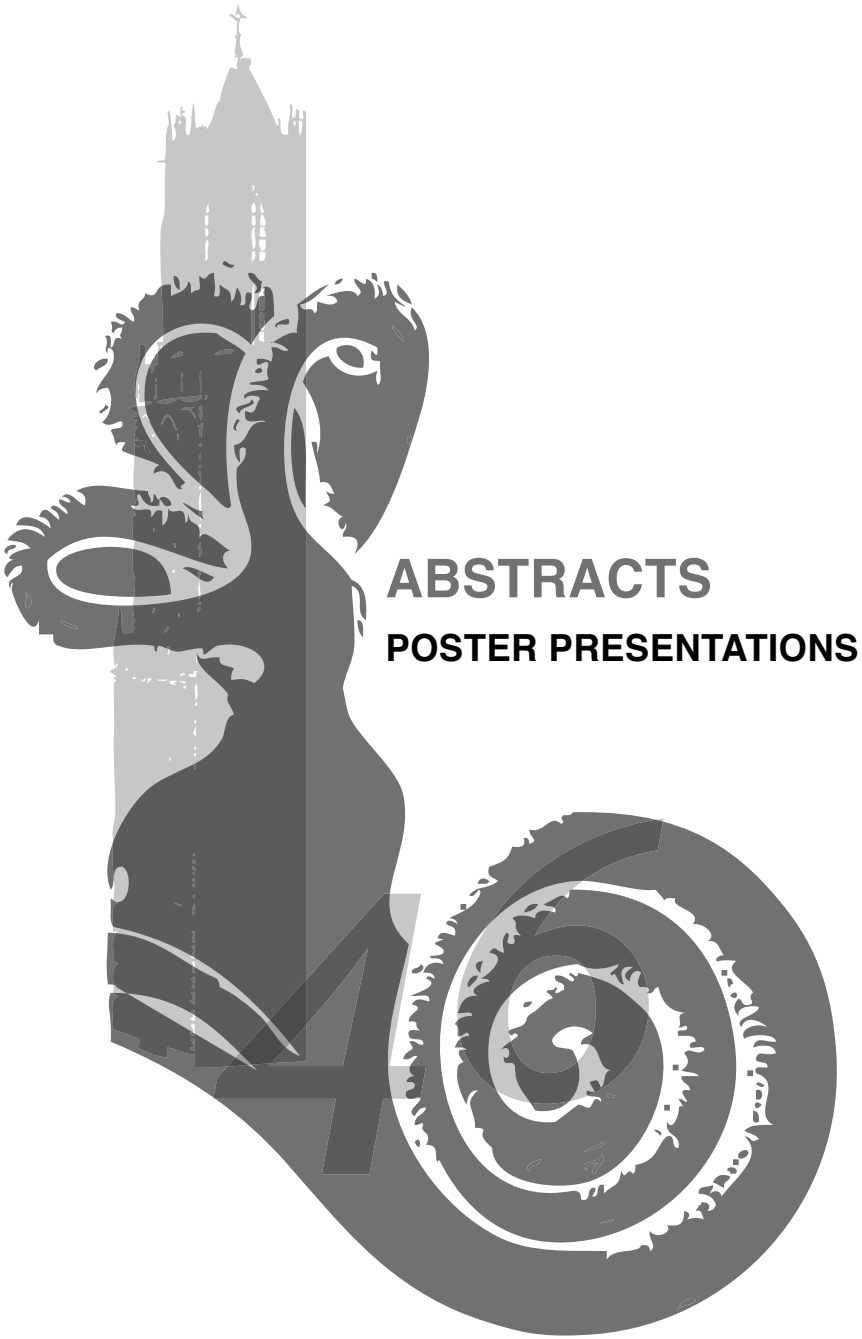
Slow release of dexamethasone added in low amounts to cochlear implant silicone rods is able to reduce hearing loss secondary to insertion trauma. The effects of 10% dexamethasone added to silicone rods were evaluated in an animal model. Six guinea pigs were unilaterally implanted with tapered rods of eluting silicone and six controls with non-eluting ones. Implantation was performed through a 0.7-mm cochleostomy, followed by 3-mm deep rod insertion. Hearing threshold audiograms were acquired prior to implantation and during the next two weeks by recording compound action potentials with electrodes near the round window. The mean threshold shifts in ears with dexamethasone eluting rods two weeks after implantation were 2dB ± 2dB, while in ears with control rods they were 7dB ± 2dB. After two weeks bullae of each animal were filled with 10% horseradish peroxidase (HRP) immediately before sacrifice to verify cochleostomy sealing. Each bulla including the cochlea was removed, decalcified, embedded in paraffin and longitudinally cut into 5-µm thick sections. Sections were examined for HRP-positive particles inside the scala tympani at the cochleostomy site, macrophages, percentage of tissue growth in scala tympani and complete tissue sealing around cochleostomy. Also, rods samples were explanted and tested for bacterial contamination. Preliminary results show no bacterial contamination and no enhanced number of macrophages. A slight tissue growth was present in scala tympani which was, however not significantly different between the two groups. Apparently, dexamethasone eluting from silicone rods does not suppress cochleostomy sealing and reduces the residual hearing loss after implantation, supporting its use as slow-release as well as anti-inflammatory additive in cochlear implants.



ABSTRACTS

POSTER PRESENTATIONS

P 01

A possible involvement of dopamine in modulation of synaptic transmission in the frog semicircular canal

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The present study was undertaken to determine the role of dopamine (DOP) in the excitability of the peripheral vestibular system in the frog. For this purpose, using multi-unit recording of action potentials from the semicircular ampulla posterior whole nerve in the isolated preparation with the aid of external perfusion, we investigated the effects of DOP agonists that are involved in modulatory actions on synaptic transmission in the frog semicircular canals.
External application of DOP (0.1-1 mM), D1 agonist chloro-APB hydrobromide (CAPB; 50-100 μM) and D2 agonist quinerolane (QUI; 50-100 μM) induced a dose-dependent and reversible decline in the resting discharge frequency. 100 μM CAPB and 100 μM QUI induced a decrease in firing frequency (mean 3.5±1.2 and 61.5±6.2% of the control level, respectively, n=6). Firing evoked by 1 μM AMPA, 50 μM NMDA and 300 μM ACPD could be depressed by administration of 50 μM CAPB by 67.1±9.4% (n=5, *P<0.05), 39.1±16.8% (n=5, *P<0.05) and 49.5±10.2% (n=5, *P<0.05), respectively. In similar conditions, firing evoked by AMPA, NMDA and ACPD could be depressed by administration of 50 μM QUI by 25.7±8.4% (n=4, *P<0.05), 38.8±14.2% (n=5, *P<0.05) and 34.2±9.8% (n=4, *P<0.05), respectively. The inhibitory action of DOP agonists on L-glutamate responses persisted in high Mg2+ solution in conditions of selective activation of postsynaptic membrane.
The results obtained suggest that DOP may interact with both D1 and D2 receptor subtypes. The inhibition of NMDA, AMPA, ACPD responses by DOP agonists suggests that DOP exerts inhibitory control over both ionotropic and metabotropic types of L-glutamate receptors, and that one possible site for DOP action is on the postsynaptic membrane of the synapse. This mechanism may result in the reduction of the activated firing rate, thus, preventing over-excitation and excitotoxic injury of the afferent dendrites after external application of L-glutamate and excessive receptor stimulation.

P 02

Morphological aspects of the semicircular canal of man

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The purpose of this work is the study of the human vestibular labyrinth morphology in ultrastructure in order to identify useful elements for a better understanding of the basic mechanisms of the dark cell area functions. Dark cells are located in the utricle and in the ampulla of semicircular canals, marginally to sensorial ephitelium, they are involved in transport of potassium in the endolymph. Potassium (K^+) is the main cation and it is essential for mechanotransduction.
Preparations coming from the labyrinth of 22 patients undergone excision of neurinoma through translabyrinthine access at the Department of Otorhinolaryngology of the Civil Hospital of Legnano were included. The vestibular organs that had been just removed were fixed in 2.5% glutaraldehyde in a phosphate tampon, dehydrated in ethanol and embedded in Araldite. After this procedure semithin sections (0.5-1.0 μm) were prepared, coloured with toluidine blue and observed under an optical microscope. Ultrathin sections, contrasted with uranyl acetate and lead citrate were observed at the Center of Electron Microscopy of Ferrara.
The dark cell area, in all the vestibular organs we observed, presents some constant characteristics: the density of the dark cells near the ampulla, the tight relationship with the melanocytes and with the capillaries, the relationship with the light cells by means of occluding apical junctions. The function of melanin in the inner ear is still not completely clear. It is well known that the lack of melanocytes is crucial and it is associated with sensorineural hearing loss. A correlation between age, dimension of the neurinoma and the morphological aspect of melanosomes was made. It is suggested that melanin plays an important role under certain pathological conditions of the inner ear.

P 03

Patterns of auditory and visual cortical activities in experimental deafness: An immunohistochemical study

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Recently, functional studies for auditory cortex are being watched with interest in accordance with development of many radiologic equipments and surgical devices for sensorineural hearing loss. And It is well known that the function of the central auditory pathway is essential for hearing rehabilitation. There are some papers about the functional or metabolic changes of auditory cortex in deafness and in patients with cochlear implantation. The aim of this study was to investigate indirectly the metabolic changes of primary auditory cortex and visual cortex by c-fos immunoreactivities in experimentally induced permanent threshold shift animal model.
Ototoxic drugs (kanamycin and furosemide) and noise were used for induction of permanent threshold shift. Cochlear damages were evaluated with auditory brainstem responses (ABR) and morphologic studies, and c-fos immunoreactivities were observed with the lapse of time after deafening.
After administration of ototoxic drugs and noise exposure, ABR threshold shifts were not recovered until three months. Cochlear damage was observed in broad areas of the cochlea. c-fos immunoreactivities in the primary auditory cortex were increased in acute period, but it was decreased after one month. It was recovered again with the level of control in three months later. In the visual cortex, increased and sustained immunoreactivities were observed after drugs and noise exposure. This result shows the plasticity of auditory cortex and possibility of some kinds of auditory-visual cross modal plasticity.

P 04

The effect of memantine on experimentally gentamicin-induced vestibulotoxicity in guinea pig

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The *N*-methyl-D-aspartate (NMDA) related glutamate receptor plays a pivotal role in aminoglycoside-induced ototoxicity. Memantine is known as a safe NMDA antagonist. In this study, we intend to observe effect of menmantine in a gentamicin-induced vestibulotoxicity animal model.
Vestibulotoxicity was induced with local administration of gentamicin and memantine was injected intraperitoneally in the study group. Histomorphological studies for vestibule were performed via light and electron microscopy, and immunohistochemistry was performed for iNOS, nitrotyrosine, and TUNEL.
The numbers of hair cells were decreased significantly in the gentamicin group than in the gentamicin-memantine group after 7 days. iNOS expression was detected only in the gentamicin group. Nitrotyrosine expression and TUNEL-positive cells were more detected in the gentamicin group than in the gentamicin-memantine group.
Memantine has a protection effect on gentamicin-induced vestibulotoxicity in animal model.

P 05

Type-1 allergy-induced endolymphatic hydrops and the suppressive effect of leukotriene antagonist

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The objective of this study was to investigate the allergic endolymphatic hydrops and inhibition effect of leukotriene antagonist.
In Experiment 1, 50 guinea pigs were actively sensitized with DNP-Ascaris twice every month, and were provoked with an injection of DNP-BSA 1 week after the second sensitization. The alterations in the inner ear were investigated histologically at 1, 12, 24, 36 and 48 hours following the provocation. Remaining 10 animals were the control group which received no sensitization but only distilled water. In Experiment 2, 40 guinea pigs were actively sensitized in the same manner. One week after the second sensitization, animals received oral administration of leukotriene antagonist (pranlukast), and were provoked in the same manner 1 hour after then. In Experiments 1 and 2, the alterations in the inner ear were investigated histologically at 12, 24, 36 and 48 hours following provocation, and quantitatively assessed the changes of the endolymphatic space. In Experiment 3, 6 out of 10 guinea pigs were actively sensitized and provoked in the same manner. Four animals of the control group were only injected distilled water subcutaneously. One hour after these procedures**,** the changes in p-AVP were levels investigated.
In Experiment 1, endolymphatic hydrops was observed 12, 24, 36 and 48 hours after the last sensitization. In all sensitization groups, the increase ratios of the cross-sectional area of the scala media were different significantly from that of the control group ($p < 0.05$). In the endolymphatic sac were observed the degranulation of mast cells. In Experiment 2, in the animal groups with leukotriene antagonist, endolymphatic hydrops was not observed at all. In Experiment 3, p-AVP levels were significantly elevated in animals with the sensitization ($p < 0.01$).
The sensitization with DNP-Ascaris produced allergic endolymphatic hydrops and the elevation of p-AVP, and allergic endolymphatic hydrops were inhibited by leukotriene antagonist.

P 06

Unique protein expression in the human spiral ganglion: Do they play a role for cell interaction and neuron survival in man?

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Cochlear implantation (CI) depends on surviving spiral ganglion (SG) neurons. In patients with loss of peripheral neurites these cells may survive as unipolar excitable neurons. We studied the micro-anatomy and protein expression to explore underlying mechanisms of this survival capability in humans.
The study was based on six human cochleae taken out at surgery with transotic approach to remove life-threatening giant petroclival meningioma. The cochleae were from ears with normal audiogram. The study was approved by the local ethics committee (no. C254/4, no. C45/7 2007) and patient consent was obtained. The cochleae were fixed with 4% paraformaldehyde and decalcified in 0.1 M Na-EDTA. Sections were embedded in Tissue-Tek (OCT; Polysciences), rapidly frozen and cryostat sectioned at 8-10 µm. Antibodies against gap-junction proteins Cx26, Cx30, Cx43, Cx36, myelin basic protein MBP, S-100, nerve growth factor receptors GFR-α1, c-Ret, TrkA, TrkB, TrkC, GDNF, NTN, PSP, ART, BDNF, TUJ-1, NF-160 and parvalbumin were used, mostly in double-staining fashion. Sections were imaged in an inverted fluorescent microscope (Nikon TE2000) equipped with a Nikon D-eclipse C1 confocal system.
Human SG type-I cells surprisingly expressed Cx30. At confocal microscopy these deposits were found to be mostly associated with the cell periphery, but also the cell interior. Nerve perikarya also expressed Cx36 to some degree. Cx43 was expressed in the satellite cells in its un-phosphorylated form. Type-I cells expressed c-Ret, TrkB and NTN.
Lack of MBP in the satellite cell support earlier data that the SG perikarya are unmyelinated and indicates fundamental differences in function of the human SG compared to animals. Connecting proteins and cell-cell interaction may provide means for synchronization between SG cells and could be related to the coding of speech signals but also for preservation of neurons in patients with long-term sensorineural hearing loss. Presence of GDNF receptor c-Ret as well as tyrosine kinase receptor TrkB in human SG may suggest that corresponding ligands play a role as survival factor for these neurons.

P 07

Diffusion tensor imaging of central auditory pathway in children with congenital deafness

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Diffusion tensor imaging (DTI) is a useful tool to evaluate the orientation of white matter tracts in vivo and yields an index of microstructural integrity. In humans, white matter tracts from brainstem to subcortical projections have rarely been investigated, especially in congenitally deaf children. The purpose of this study is to investigate the development of central auditory pathway using DTI in congenitally deaf children as compared with normal-hearing ones.
DTI was performed in 15 congenitally deaf and 15 age-matched normal-hearing subjects. Measured were the fractional anisotropy (FA) values at cochlear nucleus, superior olivary complex, lateral lemniscus, inferior colliculus, medial geniculate body and acoustic radiation using a region of interest analysis by two observers.
Agreement of FA values between two observers was high only at inferior colliculus. FA values at every region of interest showed no correlations with age in both deaf and control group. The comparison between deaf and normal-hearing subjects did not reveal significant differences in FA values at all regions of interest.
Congenital deafness might make no alteration on the microstructural integrity of central auditory pathway from cochlear nucleus to acoustic radiation.

P 08

The collagen receptor DDR1 co-localizes with the non-muscle myosin IIa in mice inner ear

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Discoidin domain receptor-1 (DDR1) is a tyrosine kinase receptor that is activated by native collagen. DDRs regulate cell adhesion and a broad range of cell behavior. Similarly, NMHC-IIa, a nonmuscle myosin heavy chain isoform encoded by MYH9, is implicated in the regulation of cell spreading and directional migration in response to various stimuli.
The dysfunction of MYH9 protein is associated with syndromic and nonsyndromic hearing loss (Lalwani et al., 2000). Deletion of the DDR1 gene in mouse is associated with a severe decrease in auditory function and substantial structural alterations, especially of the cells in which contractile elements were detected (Meyer zum Gottesberge et al., 2008). The most striking were the alteration seen in the organ of Corti. Supporting cells of the organ of Corti showed reduced anchorage to the basilar membrane and degeneration of the supporting cells and alteration of outer hair cells occurred, changes that may directly contribute to the threshold shift of the auditory function seen in knockout animals.
Using confocal microscopy we identified co-localization of DDR1 and MYH9 in the outer hair cells and in the spiral ligament in particular region adjacent to bony wall, which is populated by myofibrocytic cells (type-III fibrocytes), cells containing various contractile elements including actin, α-actinin, non-muscle myosins and tropomyosin. We propose that DDR1 and related collagen receptors as well as proteins in the actomyosin complex may act in concert to maintain the tension of the inner ear and to allow proper auditory signal transduction.

P 09

Influence of interleukin-6 on cisplatin-induced cochlear hair cell loss

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One of the unwanted side effects of chemotherapy with the use of cisplatin is a damage to the auditory epithelium (ototoxicity) resulting in irreversible hearing loss. Recent report indicated a crucial role of three pro-inflammatory cytokines (IL-1β, IL-6 and TNF-α) in the cisplatin-induced ototoxicity. Here, we wanted to scrutinize the influence of IL-6 on the survival of cochlear hair cells in the absence or presence of cisplatin.
We used the organs of Corti (OC) dissected from Wistar rats (p3-p5). The expression of IL-6 receptor in the OC was determined using RT-PCR and agarose gel electrophoresis. To assess the properties of IL-6 and cisplatin, we cultured the OC explants with recombinant IL-6 (0.3, 3, 30 and 90 ng/ml), cisplatin (15 µM) or simultaneously with IL-6 (30 ng/ml) and cisplatin (15 µM). One day later, the number and morphology of the hair cells was assessed using fluorescent phalloidin staining and microscopy.
We demonstrated that the mRNA encoding both chains of IL-6 receptor (IL-6Rα and gp130) is expressed in the OC. We showed that IL-6 has no negative effect on the morphology or number of hair cells. Lastly, we found that the exogenously added IL6 does not contribute to, but moderately inhibits the cisplatin-induced hair cell loss.
Our results suggest that IL-6 does not augment the cisplatin-induced cochlear hair cell loss. In contrast, IL-6 may have otoprotective qualities when used together with cisplatin in vitro.

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P 10

Prevention of cisplatin ototoxicity can be done by inhibition of DNA damage in cochlea cells

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Cisplatin is a well-known and effective chemotherapeutic drug, which is highly toxic to cancer cells, but also has a number of accompanying side effects. The cellular processing of cisplatin involves numerous events, which may play a role in its efficiency: uptake and efflux, formation, recognition and repair of DNA lesions, transduction of DNA damage signals, and induction of cell death. Our study focuses on prevention of ototoxic and nephrotoxic side effects by cisplatin.
We used a mouse model to study the distribution of cisplatin-DNA adducts in tissues of inner ear and kidney. In both organs we found cells that accumulate cisplatin in 10 times more than surrounding cells. These cells are proximal tubule cells in the kidney cortex and hair cells and marginal cells in the inner ear (cochlea part). Furthermore, we examined numbers of small molecules, which are cationic drug and have been reported as putative inhibitors of cation and cisplatin transport in cell line developed from kidney proximal tubules (Müller et al., 2005, Ciarimboli et al., 2005).
We picked one (we named DIPH) from 12 molecules that showed significant inhibition of cisplatin-DNA adducts in cochlea and kidney cortex cells, when it was applied 30 minutes before cisplatin.
Next, we treated mice repetitively 4 times by cisplatin or with DIPH pre-treatment. Auditory brainstem responses test shows prevention of hearing loss in mice treated with modulator (DIPH). Tumor cells that are sensitive to cisplatin do not show reduction in adducts after such treatment (in vivo and in vitro). Regression of tumor grow in primary lung tumor mouse model was observed in both cisplatin and DIPH plus cisplatin treatments.
To check if platination of nuclear DNA is a crucial toxic event for non-dividing cells, we used DNA repair-deficient XPA-transgenic mice. We showed that toxicity of cisplatin for normal tissues such mice increase dramatically. Mice with XPA-repair deficiency developed hearing problems with 7 times fewer doses than wild-type mice.

P 11

Regeneration of spiral ganglion neurons by transplating bone marrow stromal cell-derived neural progenitor cells

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Previous studies have indicated the potential of cell therapy for the regeneration of the spiral ganglion neurons (SGNs). We investigated the potential of bone marrow stromal cells (BMSCs) as a source of transplants. BMSCs have the potential to differentiate into various kinds of cell types including neurons. We previously investigated BMSC transplantation into the cochleae without neural induction before transplantation. The results showed a limited number of transplanted cells expressing neural markers. In the present study, we applied BMSCs after differentiating them into neural progenitor cells. The aim of this study was set to examine the potential of BMSC-derived neural progenitors for SGN regeneration.
BMSCs were harvested from the femurs and the tibias of guinea pigs. BMSCs were cultured in serum-free medium containing basic fibroblast growth factor and epidermal growth factor. After 7 days, we collected sphere-forming cells, which are positive for nestin. To investigate the potential of BMSC-derived spheres in vivo, we injected BMSC-derived spheres into the cochlear modiolus of guinea pigs that had been damaged by local ouabain application. Histological analyses were performed 4 weeks after transplantation.
The survival of transplants in the cochlear modiolus. A number of transplants exhibited expression of a mature neuron marker.
The present findings indicate that BMSC-derived neural progenitors are potent transplants for regeneration of neurons in the cochlea.

P 12

Preventive effects of thymus graft on age-related hearing loss

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Age-related hearing loss, known as presbycusis, is characterized by progressive deterioration of auditory sensitivity. Many factors have been proposed as contributing to this sensorineural hearing loss, including genetics as well as diet, socio-economics, and environmental variables. Some reports suggested that thymus atrophy may be a cause of age-related disease since an age-related decline of various physical functions is generally preceded by a decline in T-cell immune functions. We have previously shown that age-related immune dysfunction affects the development of presbycusis in an animal model using bone marrow transplantation (Brain Res 2006, 2008) and suggested that some types of accelerated presbycusis result not from defects in the cochlea but from defects in T cells, which change to mature T cells in the thymus from precursor T cells differentiated from the hemopoietic stem cells.
In the present study, we transplanted the fetal thymus under the renal capsule of the SAMP1 mouse strain, which shows an early occurrence of thymus atrophy and accelerated dysfunctions of immunocompetent cells, particularly T cells, followed by various signs of senescence including accelerated presbycusis with degeneration of spiral ganglion cells in an inherited pattern. Results indicated that the presbycusis and T-cell dysfunction in the grafted mice were significantly prevented. Because there was no lymphocyte infiltration in the spiral ganglion in the grafted mice, it is conceivable that the systemic immune system, including T cells, indirectly influences the inner ear. Further studies into the relationship between age-related systemic immune functions and neurodegeneration mechanisms may provide additional information pertinent to the treatment of age-related diseases.

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P 13

Inoculation of helper T cells as a strategy for the prevention of age-related hearing loss in SAMP1 mice

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Aging is accompanied by dysfunctions of immunity (immunosenescence) in diverse species, including humans and rodents. This decline is particularly characterized by perturbation of the T-cell system, whereby involution of the thymus reduces the output of mature T cells derived from hematopoietic stem cells. It has been reported that the inner ear mounts an immune response that connects to the systemic immune system. We previously reported that the manipulations of systemic immune functions prevent or treat the cochlear disorders (Exp. Gerontol. 2003; J. Neuroimmunol. 2005; Brain Res. 2008). The SAMP1 mouse strain shows an early occurrence of accelerated dysfunctions of immunocompetent cells, particularly helper T (Th) cells, followed by acceleration of senescence including hearing impairment with the degeneration of spiral ganglion cells.
In this study, we examined whether the supply of Th cells overcomes the senescence-related decline of hearing in the mice. We injected with splenocytes (lymphocytes), T cells, Th ($CD4^+$) cells, killer T ($CD8^+$) cells, or B cells from syngeneic donors (SAMP1) of different ages intravenously into host SAMP1 twice. Only mice inoculated with the lymphocytes containing Th cells (i.e. whole splenocytes, T cells, and Th cells) of young donors showed delay of accelerated hearing loss. There was no local infiltration of lymphocytes in the spiral ganglion. These findings may indicate that maintenance of the systemic Th-cell functions prevents age-related cochlear dysfunctions, and that the repeated inoculation of autologous Th cells which had been gathered, expanded, and preserved in the host of the juvenile age prevents its presbycusis.

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P 14

Protection of spiral ganglion neurons with neurotrophins and chronic electrical stimulation

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In the deaf cochlea spiral ganglion neurons (SGNs) undergo continual degeneration that ultimately leads to neuron death. The exogenous application of neurotrophins (NTs) can prevent SGN degeneration and even promote regrowth. Furthermore, combining chronic intracochlear electrical stimulation (ICES) with NTs can enhance the survival effects of NTs and lower electrical thresholds. However, following the cessation of NT delivery SGNs continue to degenerate. Therefore techniques that deliver NTs over a long period of time are required to maintain the therapeutic benefit of NT treatment.
We have used cell-based therapy to provide NTs in combination with an intracochlear electrode array in a long-term deafened cat model. Cats were neonatally deafened with neomycin, and at two months of age were implanted with encapsulated porcine choroid plexus cells (NTCell, LCT Inc.) and the stimulating electrode array. The choroid plexus cells produce NTs and were encased in alginate capsules that enabled the diffusion of NTs into the cochlear fluids. Environmentally derived ICES was delivered chronically via a clinical stimulator (Nucleus CI24M, Cochlear™) and processor (Esprit 3G, Cochlear™). Five cats received chronic ICES only. Six cats received NTs without chronic ICES and six cats received NTs in combination with chronic ICES. Control animals (n=7) were normal hearing and were not implanted.
The results indicate that chronic ICES alone (without NTs) did not provide greater SGN survival compared to the contralateral untreated cochlea. Importantly, chronic ICES in combination with NTs provided greater SGN protection than NTs alone or chronic ICES alone (ANOVA $P<0.003$). Treatment with NTs alone led to an improvement in thresholds from electrically evoked brainstem responses (ANOVA $P<0.003$). These results indicate that cell-based NT delivery in combination with ICES can promote SGN survival. These findings have important implications for future strategies that will combine cochlear implantation with systems that deliver drugs safely to the cochlea.

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P 15

Auditory nerve degeneration in *pmn*/*pmn* mice

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The progressive motoneuronopathy (pmn) mouse represents a recessively inherited animal model for a motoneuron disease. This syndrome is characterized by a progressive axonal degeneration of peripheral nerves. Mice with homozygous mutation (*pmn*/*pmn*) of the TBCE gene show first signs of progressive neuronopathy by starting with a weakness of the hind limbs at postnatal week 3. At the same time, the animals develop a progressive hearing loss. The aim of this work was an analysis of the cochlea and the auditory nerve to study the origin of this hearing loss.
The cochlea and the auditory nerve of postnatal day 28 *pmn*/*pmn* and control mice were retrieved. Slides were prepared in transverse and longitudinal direction to the modiolus. The organ of Corti was stained by Azan immunhistochemistry. The spiral ganglia and the auditory nerve were labelled by an antibody against β-III tubulin. Additionally, whole mount preparations were performed and stained by phalloidin toxin, DAPI and antibodies against β-tubulin and β-III tubulin. Subsequently histomorphology of the organ of Corti, spiral ganglia and the auditory nerve was analysed.
No difference could be detected between the morphology of hair cells, the organ of Corti and the soma of the spiral ganglia neurons in *pmn*/*pmn* and control mice respectively. The numbers of hair cells and spiral ganglia neurons were unaltered. In contrast to normal hearing control animals, the auditory nerve in the *pmn*/*pmn* mice showed an excessive degeneration and a significantly reduced number of axons.
Initially normal-hearing *pmn*/*pmn* mice suffer primary auditory nerve degeneration despite the presence of a morphological intact cochlea. Detailed electrophysiological analysis is necessary to test whether *pmn*/*pmn* mice could be a useful animal model in clinical relevant auditory diseases.

P 16

Reduced electromotility of outer hair cells as an additional mechanism underlying the deafness associated with mutations in connexin genes

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Mutations in the GJB2 gene encoding for the connexin 26 (Cx26) protein are the most common sources of nonsyndromic deafness. Cx26 is a building block of gap junctions (GJ), establishing connectivity between cells in distinct cochlear compartments. It has been suggested that circulation of K^+ and metabolites is essential for normal development of hearing for, in animal models of Cx26 deficiency, sensory hair cell death occurs. However, failed K^+ homeostasis, although implied, may not be the only responsible mechanism for hearing loss.
In search of GJ-dependent mechanisms we have used a large scale three-dimensional model of mechano-electrical transduction in the cochlea (Mistrik et al., 2009). This computational model allows evaluation of the effect of Cx26 mutations on OHC potentials which control the prestin-driven amplification mechanism. Two types of Cx26 mutations were considered: (1) those (e.g. F83T, M34T or V37I) which reduce the GJ conductivity; and (2) those (e.g. R57W) which also reduce the extracellular conductivity in the organ of Corti as a result of a maturational defect of the organ of Corti.
The simulations indicate: (1) that GJ coupling within the organ of Corti cells enhances OHC function, decreasing receptor potential attenuation; and (2) that GJ mutations can affect both amplitude and frequency selectivity of the OHC receptor potential over a broad frequency range. The largest effect was observed for the R75W mutation. In this case the attenuation of OHC potential amplitude at high frequencies increased from -6 to -21.5 dB/decade. The quality factor of tuning Q_{10dB} decreased by 15%.
The OHC electromotility is crucial for sound amplification, granting the cochlea its high sensitivity and frequency selectivity. These in-silico considerations suggest that the reduction of OHC somatic electromotility could represent an additional critical factor in the Cx26-related forms of deafness.

P 17

^{18}F-FDG small-animal PET study and neuroanatomical tracing on the vestibular system in rats

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The purpose of this study was to identify vestibular processing of cerebrocortical brain regions in rats by functional brain imaging, using small-animal positron emission tomography (PET). In addition, a neuroanatomical tracing was performed to examine connection between selected regions.
The glucose metabolism during vestibular stimulation was investigated by using ^{18}F-fluorodesoxyglucose (FDG) tracer. Brain activity of twelve rats was measured under two conditions in Micro-PET Focus 120: (1) with vestibular galvanic stimulation (t=50', 0.2 mA, 1 Hz) right or left; and (2) with sham stimulation during FDG uptake. Vestibular stimulation was performed by electrical stimuli applied via electrodes above the external auditory meatal cartilage and the parietal subperiost. PET imaging started 60 min after i.p. injection of 25-32 MBq FDG for 30 min. By using the image pre-processing routines of Statistical Parametric Mapping (SPM), the images were realigned to a MRI image of the rat brain, and a FDG template was generated. After spatial normalisation to FDG template, voxelwise analyses (paired t-tests) with SPM were made.
An anterograde neuronal tracing with *Phaseolus vulgaris* leuco-agglutinin into the vestibular processing thalamic nucleus parafascicularis was performed.
The FDG-PET showed a significantly increased glucose metabolism in specific brain regions including the left temporo-parietal cortex and the amygdala during right and left galvanic vestibular stimulation. Using neuronal tracing, we identified anterogradely labelled terminal fields in the parietotemporal cortex and amygdala.
The results indicate the left parietotemporal cortex and amygdala as vestibular processing cerebrocortical brain regions.

P 18

Novel interaction partners of otoferlin

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Otoferlin, a protein with several C2 domains present in cochlear sensory cells, termed inner hair cells (IHC) and outer hair cells (OHC), was found to be mutated at various positions in an autosomal recessive deafness form, DFNB9 (Yasunaga et al., 1999; Mirghomizadeh et al., 2002; Varga et al., 2003). Otoferlin was suggested to be the Ca^{2+} sensor for IHC exocytosis (Roux et al., 2006), strengthened by the finding of Ca^{2+}-dependent interaction of otoferlin with syntaxin-1, SNAP-25 and CaV1.3 (Roux et al., 2006; Ramakrishnan et al., 2009).
In previous studies mass spectrometry and a yeast two-hybrid assay were used to screen for novel otoferlin interaction partners. Hereby, among others, the proteins Rab8b (Heydrich et al., 2008) and myosin VI (Heydrich et al., 2009) were identified, both having a role in intracellular transport. Moreover, data strongly support a role of otoferlin for endocytosis and replenishment of vesicles (Heidrych et al., 2009).
The present study identifies further novel otoferlin interaction partners, substantiated by co-localization in mouse inner hair cells as well as co-immunoprecipitation of the complex using in vitro and in vivo studies.

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P 19

P2Y4 receptor-mediated regulation of amiloride-sensitive sodium transport in the Reissner's membrane

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The epithelial cells of Reissner's membrane (RM) form much of the boundary separating endolymph from perilymph in the cochlea and are capable of transporting Na^+ out of endolymph via epithelial Na^+ channel (ENaC). However, much remains to be known as to the mechanism of regulation of Na^+ absorption in RM. We investigated P2Y signaling as a possible regulatory mechanism of ENaC in gerbil RM using voltage-sensitive vibrating probe technique and immunohistochemistry.
Results showed that uridine triphosphate (UTP) induced partial inhibition of the amiloride-sensitive short-circuit current (I_{sc}), but not with pre-treatment of amiloride. The response to UTP was inhibited by reactive blue-2, but not by suramin or pyridoxalphosphate-6-azophenyl-2',4'-disulfonic acid, which indicates this P2Y receptor as the P2Y4 subtype. These functional results were supported by immunolocalization of P2Y4 in RM. The phospholipase C inhibitor U-73122 markedly inhibited the action of UTP on ENaC. In contrast, neither modulation of protein kinase C signaling using phorbol 12-myristate 13-acetate (PMA) and GF 109203X nor application of 2-aminoehoxydiphenyl borate (2-APB) affected P2Y4-mediated regulation of ENaC.
P2Y4-mediated inhibition of ENaC activity might be explained by decreased open probability of ENaC due to depletion of phosphatidylinositol 4,5-biphosphate ($PI(4,5)P_2$) concentration in the plasma membrane.

P 20

Expression of nuclear factor kappa B (NF-κB) in the hydropic cochlea of guinea pigs after the direct injection of antigen into the endolympatic sac

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We immunohistochemically examined the expression of nuclear factor kappa B (NF-κB) in the hydropic cochlea of guinea pigs.
Keyhole limpet hemocyanin was directly injected into the endolymphatic sac. Morphological changes of the endolymphatic hydrops were observed in the cochlea of all animals after the injection of keyhole limpet hemocyanin.
NF-κB was detected in the lateral wall, the organ of Corti and the spiral ganglion cells.
It is known that various enzymes are activated under inflammatory conditions. Inducible nitric oxide synthase gene is regulated by NF-κB. We have reported that inducible nitric oxide synthase was involved in the same experimental model. High levels of nitric oxide can lead to inner ear dysfunction.
Our results suggest that NF-κB may mediate the pathogenesis of endolymphatic hydrops and play an important role under inflammation.

P 21

Spiral ganglion cell survival after round window application of Gelfoam soaked with brain-derived neurotrophic factor

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Several studies have shown that treatment with neurotrophins protects spiral ganglion cells (SGCs) in hair-cell deprived cochleae. In most of these studies the neurotrophins are applied by means of a cannula which is attached to a mini-osmotic pump and inserted into the cochlea. Other application methods that might be more suited for clinical use have been developed. We have examined whether round window application of Gelfoam infiltrated with neurotrophins results in survival of SGCs in deafened guinea pigs.
Guinea pigs were deafened by means of co-administration of kanamycin and furosemide.The functional effect of the deafening procedure was confirmed by recording auditory brainstem responses (ABRs) to acoustic click stimuli. Two weeks after deafening, Gelfoam cubes (1x1x1 mm) were soaked in 6 µl of a 1 mg/ml solution of brain-derived neurotrophic factor (BDNF) and deposited on the round window of the right cochlea. Subsequently, a gold-ball electrode was placed on the round window, to be used as stimulus electrode. Alternating monophasic pulses were delivered through this electrode to electrically evoke ABRs (eABRs). Two or four weeks after deposition of the Gelfoam, both left (untreated) and right (BDNF-treated) cochleae were fixed and processed for histological examination.
We found that the local BDNF treatment was effective in the basal turn, but not in the middle or apical turn. Two weeks and four weeks after deposition of the Gelfoam, the SGC packing densities were significantly larger in the basal turn of BDNF-treated cochleae than in the untreated cochleae. The treatment had no effect on the size of SGCs. In animals treated with BDNF, eABR amplitudes were relatively stable during four weeks, although lower than in normal-hearing animals.
We conclude that Gelfoam-based delivery of BDNF can be applied to preserve SGCs in the basal turn of the cochlea.

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P 22

Tinnitus-specific features in the peripheral and central auditory system

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Aberrant neuronal activity, occurring during tinnitus, is known to lead to changes in neuronal plasticity. However, molecular changes following sensory trauma and the subsequent response of the central nervous system are only poorly understood. In our rodent animal model, perception of tinnitus was studied by a behaviour test (Rüttiger et al., 2003; Knipper, 2003) thereby allowing to differentiate between animals, which experience tinnitus and those, which do not experience tinnitus. At different time points after tinnitus induction, the expression of several genes was analysed in the cochlea and the central auditory system.
Here, we present a summary of recent findings comparing and correlating the expression of different activity-dependent genes with functional and physiological data after different trauma paradigms and time points. Analysis was performed in the cochlea, the limbic system and the auditory cortex. Moreover, initial trials are done to pharmacologically reverse/influence changes in tinnitus behaviour and gene expression that occur after trauma, using local round window or systemic drug application.

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P 23

Endolymphatic hydrops revealed by intravenous gadolinium injection in patients with Ménière's disease

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Recently, we succeeded to visualize endolymphatic hydrops in patients with Ménière's disease after intratympanic injection of gadolinium (Gd) using a 3-Tesla MRI. However, Gd is generally administered intravenously for contrast enhancement of MRI. If endolymphatic imaging by intravenous Gd injection becomes readily available, the intravenous method will be used widely. Gd concentration in the perilymph reaches its maximum level four hours after the intravenous administration of the drug. In the present study, we attempted to visualize the endolymphatic space using 3D-FLAIR and 3D-real IR MRI taken 4 hours after intravenous injection of a double dose Gd in patients with Ménière's disease.
Four patients with Ménière's disease were enrolled in this study. Gadoteridol (ProHance®) was injected intravenously. The injected amount was 0.4 ml/kg (0.2 mmol/kg). Although the standard amount of gadoteridol is 0.2 ml/kg, a concentration of 0.4 ml/kg is permitted by the Japanese governmental health insurance system if the aim is to visualize metastatic brain tumors. 3D-FLAIR and 3D-real IR MRI were taken 4 hours after the intravenous gadoteridol injection. MRI scans were performed with a 3-Tesla MRI unit using a 32-channel array coil. The present study was approved by the Ethics Review Committee of the Nagoya University School of Medicine.
Endolymphatic hydrops was observed both in the cochlea and vestibule on the affected ears of the four patients with Ménière's disease. However, Gd concentration in the perilymph was lower than that obtained after intratympanic injection of Gd diluted eight-fold with saline.
Visualization of endolymphatic hydrops became possible after intravenous Gd injection in patients with Ménière's disease.

P 24

The role of the L-VDCC for activity-dependent BDNF transcription: A cell culture model

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Brain-derived neurotrophic factor (BDNF) plays a crucial role for activity-dependent plasticity, alteration of synaptic efficacy and balance of inhibition and excitation (Korte et al., 1995). A role of calcium in BDNF-mediated LTP and LTD responses are discussed in various studies (Ghosh et al., 1994; West et al., 2001; Tao et al., 2002; Arundine et al., 2003; Tippens et al., 2008). Recent studies point to a crucial role of BDNF/L-type calcium channels involvement for neuronal injury, as phantom pain (Shulga et al., 2008). Altered BDNF levels in the periphery of the cochlea post-trauma (Rüttiger et al., 2007, Panford-Walsh et al, 2008), may be the trigger for pathological imbalances of neuronal activity in the central auditory system. Interested in the role of L-type calcium channels for BDNF-mediated changes in the auditory system post-trauma, we generated a cell culture system in which we transfected YFP- and CFP-tagged BDNF transcripts (exon IV and exon VI), including their appropriate promoter regions. BDNF expression can be induced upon pilocarpine and kainate, indicating an endogenous appropriate signalling cascade that may mimic glutamatergic and cholinergic usage of BDNF promoter. Cotransfection studies with $Ca_v1.2$/$Ca_v1.3$ plasmids, Ca^{2+}-dependent transacting elements and siRNA studies will elucidate the usage of this in vitro cell system to analyse Ca^{2+}-dependent signalling cascade upstream of the BDNF promoter.

P 25

Cell-specific targeting to the inner ear by using nanoparticles with neurotrophin-derived peptides

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Neurotrophins are very important in sensory cell signalling and to its development. Four mammalian neurotrophins – NGF, BDNF, NT-3 and NT-4 – bind or activate one or more of the Trk family of tyrosine kinase receptors. Neurotrophin-derived peptides, which we used, have high targeting and more specific activity. We hypothesized that by conjugating spherical block-copolymer nanoparticle's (NPs) surface with two different peptides derived from NGF-β, we can obtain cell-specific uptake in the inner ear.
It permits cell-specific targeting to Trk and p75, in organotypic explant culture of the mouse inner ear. The cellular uptake of the NPs and cell specific targeting were evaluated by fluorescence microscopy, confocal laser microscopy and transmission electron microscopy (TEM).
We found targeting by ligand-coated NPs to spiral ganglion cells (SGNs), Schwann cells and nerve fibers. The efficiency of specific binding in between the two peptides are evaluated. Unspecific uptake of NPs were found with the scrambled peptides.
This novel finding indicates that in future it will be possible to reach the inner ear in a non-invasive way for drug delivery.

P 26

Dexamethasone release from cochlear implant silicone surfaces

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Spiral ganglion cell degeneration after hair cell loss and growth of fibrous tissue around the cochlear implant electrode array after implantation are currently in focus of research to optimize the interface between stimulation electrodes in the inner ear and nerve cells. Corticosteroids are proven to reduce the electrical impedances of electrode contacts, which are typically explained by a reduction of tissue growth around the electrode array.
In order to avoid implantation of a pump additionally to a cochlear implant for fluid based drug delivery, we started to investigate the dexamethasone (DX) delivery of degradable polymers either stored in small reservoirs created by laser in the silicone material of cochlear implants or from coatings on the surface of these materials. DX delivery kinetics was measured by means of HPLC.
Loading of the reservoirs strongly depends on the solvent. Best loading was received by using ethanol. However, DX delivery was nearly finished after 10 hours, independent on the solvent. In contrast, incorporating DX in poly(L-lactide) (PLLA) or poly(4-hydroxybutyrate) (P(4HB)) coatings, the release could be controlled by the polymer and the percentage of DX in the coating. Whereas the release from P(4HB) coatings was finished completely after few hours, using PLLA only 40 % of the incorporated DX were released after 72 hours.
The current investigations demonstrated that coating of cochlear implant materials with biodegradable polymers is a promising approach. Especially by using PLLA, the slow drug release can be ensured for several days thus covering the period of the largest increase in impedances after cochlear implantation.

P 27

Idiopathic sudden sensorineural hearing loss in Sweden: Diagnostic protocol and treatment in relation to outcome

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Idiopathic sudden sensorineural hearing loss (ISSNHL) is a rapid loss in hearing without known cause. Different theories about the etiology have resulted in different treatment policies. Spontaneous recovery is common. Therefore, the effect of therapy is difficult to evaluate at a single clinic where only a few patients are seen annually.
The aim of the present study was to analyze the management and treatment of ISSNHL patients in Sweden with regard to outcome.
A national database was developed with half of the ENT clinics in Sweden participating. They submitted a questionnaire for each patient with SSNHL covering the patient's background, current disorder, family history, examinations and treatment. Audiograms taken at the onset of SSNHL and after 3 months were requested.
Data from 400 patients were analyzed using ordinal logistic regression looking for interactions with hearing recovery and remaining hearing loss. Independent of treatment, heredity for hearing loss, age and vertigo were negatively correlated with outcome. Hearing loss in the mid-frequency region had best odds for hearing improvement and patients who were prescribed rest had higher odds for hearing improvement than those not prescribed rest.
Forty percent had a MRI or CT, with 4% acoustic neuromas diagnosed. Blood screening varied from simple routine tests to a complete analysis with HSP70, anti-neutrophilic cytoplasmic antibodies and Borrelia tests. 24% of patients with ISSNHL and hematological tests taken had one or more pathological findings. No difference between these patients and those with normal findings with respect to hearing improvement or remaining hearing loss. 60% of patients with ISSNHL were treated medically, primarily with corticosteroids. These patients did not recover better than those not medicated.
Conclusion: No standard program for diagnostics or treatment of ISSNHL exists in Sweden. Regardless of pathological findings, treatment is either corticosteroids or no medication with no difference in outcome regardless of treatment.

P 28

Activating cGMP signaling cascades by blocking phosphodiesterase-5 preserves cochlear hair cells and protects from noise-induced hearing loss

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Excessive noise is a global occupational health hazard with considerable physiological and social consequences, leading to noise-induced hearing loss and presbycusis. The development of novel prevention strategies for a disease that currently affects ±26 million people in the USA (NIDCD) is therefore of great socio-economic importance. Acoustic overstimulation damages outer hair cells (OHC), but also inner hair cells (IHC). Here, we show for the first time a significant reduction in the number of release sites of the IHC synapse. Treatment with vardenafil, a potent cyclic nucleotide phosphodiesterase-5 (PDE5) inhibitor, almost completely prevented noise-induced hearing loss and OHC damage, but also damage of IHC synapses. The beneficial effect of vardenafil on noise-induced damage was observed when administered before, but even up to one day after, trauma induction. Substantial PDE5 expression was found in both inner and outer hair cells, suggesting the existence of a protective cGMP-signaling cascade activated by noise trauma. PDEs are pharmacological targets in a variety of disorders, and can be inhibited or modulated by well-tolerated oral drugs. These results demonstrate a high potential of PDE5 inhibitors for protecting hearing function and hair cells, and inspire studying a novel class of drugs for the therapy of inner ear disorders.

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P 29

Multifunctional nanoparticle vizualisation at the light and electron microscopic level: Methods for finding the needle in the haystack

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There is a great need for the discovery of new drugs for pharmacotherapies of the inner ear. The specific targeting of certain cell types is assumed to improve drug delivery and bio-efficiacy and minimizing side effects to neighbouring tissue. Approaches using nanoparticle with targeted and controlable drug release have been used to develop new strategies for treating cancer and appear to evolve to a promising biotechnique for drug and non viral gene transfer.
The European Community Project NANOEAR aims to elucidate the use of nanoparticle-mediated drug administration and gene transfer in the inner ear. To chase the fate of nanoparticles in cochlear tissue we used different marker systems and imaging strategies for light and electron microscopy on mouse explant cultures. Hyperbranched polylysines, block co-ploymeres, silica, lipid core, lipocomplex, chitosan and polysterol nanoparticles with fluorescent dyes, quantum dots, iron oxide or biotin as a tag could be vizualized.
Conventional transmission electron microcopy is not sufficient to identify nanoparticles in a organotypic culture system and each visualisation systems has advantages and limitations. The different systems and vizualisation methods are compared and discussed.

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P 30

Fibrosis, osteoneogenesis and immune reactions: A problem for cochlear implantation

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At certain time periods after cochlear implantation, the performance of an implant can decrease and complete failure of the device can occur. This may be caused by fibrosis, reactions from cells of the immune system, foreign-body reactions or new bone formation around the electrode, especially in the vicinity of the cochleostomy. Here we present the case history of a 32-year-old woman with progressive bilateral sensorineural hearing loss starting at the age of 14 with profound deafness by age 29. She was implanted in the right ear one year prior to death and in the left ear 3 months prior to death. The cause of death was chronic renal disease and cardiac failure. The patient was able to use the telephone several weeks after implantation. The left implanted ear performed poorly with no ability for speech recognition. The left ear was 3D reconstructed after synchrotron radiation-based microtomography and next embedded in epoxy resin and sectioned for histological staining. The right ear was embedded in Araldite resin and sectioned with the implant in situ. There was a considerable amount of new tissue formation around the electrode. The electrode penetrated the scala vestibuli in both ears. Peripheral nerve fibers were detectable in the osseous spiral lamina.
Data are available on the possible impact of new tissue formation around an implant electrode or hypersensitivity reactions against electrode material. Immune responses to electrode components, such as silicon, or associated substances, or a delayed hypersensitivity response are the most probable causes for the tissue reactions present in this case. Other possible causes are discussed.

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P 31

Cochlear targets of hyperbranched poly-L-lysine after application on the round window membrane in the mouse and rat

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Dendritic and hyperbranched polymers may serve as a nonviral method of delivering a variety of macromolecules into the cochlea. The penetration of hyperbranched poly-L-lysine (HBPL) through the round window membrane and distribution in the inner ear were studied with the aim of discovering the targeting of specific intracochlear structures in the mouse and rat.
HBPL conjugated with fluorescein (FITC) via amide bond was applied on the surface of the round window membrane (RWM) in C3H mice and Long Evans rats. Bullas from mice exposed to 1.5 µl and rats exposed to 3-4 µl of HBPL (0.001 M) for 1, 3, or 7 days were fixed in paraformaldehyde (4%) and decalcified. Paraffin-embedded sections of the cochlea (10 µm) were stained with DAPI. A confocal microscope (Zeiss 510 DUO) was used for analysis.
Polymers were identified after 24 hours in the cochlea. They formed inclusions in the basilar membrane as well as in the cells of the organ of Corti (hair cells and supporting cells), neurons of the spiral ganglion and cells of the lateral wall. HBPL labeled with FITC did not cause any distinct morphological damage in the inner ear of the tested animals after their application on the RWM. HBPL polymers were localized in the cytoplasm, and some of them touched the nuclear membrane. A fluorescent signal was also seen in the nuclei of the spiral ganglion neurons. The nuclear localization was confirmed at in a series of optical sections. At seven days after application, fluorescence was still detected in the organ of Corti.
HBPL passed through the RWM in mice and rats.They entered the nuclei of neuronal cells in the rat spiral ganglion. Thus, HBPL may represent a suitable system for the delivery of genetic information into the inner ear.

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P 32

Protection of neural elements and sensory hair cells in the guinea pig cochlea with chronic intrascalar administration of glia cell-derived neurotrophic factor (GDNF)

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Deafferentation of the auditory nerve from loss of sensory cells is associated with degeneration of nerve fibers and spiral ganglion neurons (SGNs). SGN survival and peripheral nerve fiber regrowth following deafferentation can be enhanced by application of neurotrophic factors.
The present study analyzed distribution of afferent and efferent nerve fibers following deafness in guinea pigs using specific markers (parvalbumin for afferent fibers, synaptophysin for efferent fibers) and the effect of glial cell line derived neurotrophic factor (GDNF). Immediate treatment following deafness was compared with 3-week-delayed GDNF treatment. SGN counts were performed on deafened, GDNF-treated and normal-hearing animals.
Immediate treatment with GDNF results in some immunopositive fibers in the habenula perforata and within the scar tissue of the former sensory epithelium. Delayed administration of GDNF shows the neurotrophic effect on the afferent fiber system with plenty of immunoreactive fibers, partly extremely swollen. Inner hair cells and hair cell remnants in both treatment groups especially in the high-tone frequency area and apical turn suggests a delayed degeneration of sensory cells and some protective effect for inner hair cells. Both GDNF treatment groups show remarkably well preservation of SGNs without any changes in morphological appearance.

P 33

Influence of brief noise exposure in juvenile rats on the response properties of inferior colliculus neurons in adult animals

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During early postnatal development in rats, the maturation of the central auditory nuclei relies on the natural character of the incoming neural activity. Even a temporary intervention to the auditory system during the critical period results in a permanent deterioration of neuronal responsiveness in adult animals. In the current study, juvenile rats (strain Long-Evans) were briefly exposed to a broad-band noise (125dB SPL, 8 min, postnatal day 14) to produce a temporary hearing threshold shift. At the age of 3-6 months, the sound-evoked responses of individual inferior colliculus (IC) neurons were recorded in ketamine-xylazine anesthetized animals. We found that the neuronal representation of both frequency and intensity is severely affected in the IC of exposed animals, the anomalies being confined exclusively to the high-frequency IC regions (characteristic frequency, CF>8 kHz). The hearing thresholds and the neuronal excitatory thresholds did not differ between the exposed and control rats; however, the frequency tuning curves of neurons with a CF above 8 kHz had significantly wider excitatory response areas in the exposed animals, indicating the disrupted development of the high-frequency IC circuitry. In the exposed rats, the inhibitory sidebands had similar bandwiths as in the controls, but they were shifted to the side, thus allowing the expansion of the excitatory response areas. Furthermore, the affected neurons of exposed animals exhibited a significantly narrower dynamic range and a steeper slope of the rate-level function compared with the controls. The results indicate that a short-lasting exposure to intense sound during the sensitive period of postnatal development leads to the permament impairment of frequency tuning and sound-level coding in the IC neurons of adult rats. The altered response properties can be attributed to the incomplete development of the inhibitory circuitry as a consequence of the temporarily reduced input to the developing auditory centers during the critical period.

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P 34

The efficiency of a single injection or continuous delivery of nanoparticles to the middle ear using an Alzet® micro-osmotic pump

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The efficiency of various methods of nanoparticle (NP) delivery to the middle ear was tested in mice and rats. In ketamine (35 mg/kg) and xylazine (6 mg/kg) anesthetised animals, an opening in the auditory bulla was made via a postauricular approach. After a single injection of a NP suspension by a Hamilton syringe on a small piece of gelfoam placed in the round window niche of both mice and rats, NPs were detected in various cochlear partitions. A single injection of NPs loaded with the neurotoxic drug disulfiram in mice resulted in an increase of hearing thresholds two weeks after NP injection, assessed on the basis of auditory brainstem responses (reaching a maximal value of 25 dB at 16 kHz), and in marked cochlear morphological alterations. However, a single injection of disulfiram-loaded NPs in rats did not produce any change in hearing function and did not damage the cochlear tissues. To ensure that a larger number of disulfiram-loaded NPs would be transported to the cochlea, NPs were continuously applied to the middle ear using an Alzet® micro-osmotic pump. A catheter was fixed into the bulla, ending in the round window niche covered with a piece of gelfoam. The opposite end of the catheter was connected to a micro-osmotic pump filled with 100 ml of NP suspension and implanted subcutaneously on the rat's back. Seven-day continuous delivery of disulfiram-loaded NPs to the middle ear of rats resulted in apoptotic and necrotic changes of the spiral ganglion cells, manifested by a 20-40 dB threshold shift in the frequency range 8-25 kHz. The results of these experiments demonstrate that the efficient application of drugs to the cochlea of rats can be achieved by the delivery of drug-loaded NPs to the middle ear using an Alzet micro-osmotic pump.

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P 35

Noise-induced hearing loss mice models: Towards standardization of exposure conditions

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Animal models of noise-induced hearing loss (NIHL) require the use of standard noise exposure protocols to ensure experimental reproducibility. Here we present the design and start-up of an acoustic reverberant chamber and new sound stimuli for noise exposure in mice.
The dimensions of the chamber were optimized to confer the highest sound level with the minimum mean-squared deviation averaged in a given area inside the chamber. It was equipped with five tweeters in the ceiling, a metallic mesh cage to locate the animals and an infrared camera to monitor them. Two stimuli were designed with a high-pass filtering and linear with frequency gain to adapt the characteristics of the exposing noise to the rodent hearing. The “violet noise” (V) was synthesized from a white noise in the frequency range (0, $f_s/2$), f_s being the sampling frequency. The “violet swept” (VSS) consisted of a swept sine in the frequency range (f_1,f_2), in the time T, also with a linear with frequency gain of slope m_f. The initial frequency of the swept, f_1, was the cut off frequency for high-pass filtering.
Five experimental groups were established according to different intensities (100 or 120 dB SPL), duration of exposure (30 or 60 minutes) and type of noise (V or VSS). Noise of 120 dB SPL induced an irreversible hearing impairment regardless the type of stimuli, with permanent threshold shifts, suggesting a severe cochlear damage. In contrast, exposure to 100 dB SPL noise caused a moderate increase of ABR thresholds that progressively returned to baseline values one week after damage. Violet noise caused a slighter cochlear damage compared to violet swept-sine.
The standardization of noise exposure conditions contributed to improve data reproducibility and to increase the reliability of NIHL mice models.

The results of this work have been published in Applied Acoustics 70 (2009).

P 36

Changes occurring in the central auditory system after sound exposure

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Noise-induced hearing loss (NIHL) is often followed by the rise of auditory phantom sensation, tinnitus. Tinnitus is associated with altered neuronal activity in the central auditory nuclei shown in humans and mice (Kaltenbach et al., 2000; Eggermont and Roberts, 2004; Mahlke and Wallhäuser-Franke, 2004). Our objective was to investigate changes in activity-dependent gene expression in the periphery and in the central auditory system of animals exposed to different acoustic stimuli presented at 10 kHz.
In exposed animals behaviour tests were made (Rüttiger et al., 2003; Knipper, 2003) and hearing measurements were performed. Two weeks post-exposure the central auditory system was analysed in detail for molecular changes occurring during tinnitus. In particular the expression pattern of different genes was analysed in the auditory cortex. Coronal sections were made along the tonotopic axis of the auditory cortex. Gene expression patterns in regions representing different frequencies (4 kHz, 10 kHz, 25 kHz, 50 kHz) were compared, according to Doron et al. (2005). Here we describe in detail the characteristics of central auditory regions but also non-auditory regions (limbic system) affected by a peripheral trauma.
The data are discussed in the context of using gene expression patterns to monitor trauma-induced plasticity changes in the central system.

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P 37

Jagged-1 is essential for the boundary of mammalian prosensory patch probably via Notch3

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The notch-signaling pathway is well known for the “Jag2/Dl1-Notch-RBPJ-Hes1/Hes5-Math1“ cascade named “lateral inhibition”. Instead, Jagged-1, a ligand of Notch pathway, participates in the early stage of inner ear development through “lateral induction”. What is the exact function and its mechanism?
A conditional knockout model Pax8cre$^{+/-}$;Jag1flox/flox (Jag1-cko) was generated on C57BL/6J background. PCR was utilized for genotyping. As for the phenotype, general behaviour was investigated. ABR and swimming test were undertaken on both Jag1-cko and littermate controls. Hematoxylin and eosin staining and immunofluorescence were utilized to identify the morphological change in the inner ear.
It is firstly reported that Jag1-cko can survive with normal feeding and drinking. Overactivity, springing, high-speed circling and balance disability in water were observed. Comparing to the littermate control (n=12), the hearing threshold of Jag1-cko (n=9) was elevated. ABR was induced at very low and high frequencies in Jag1-cko. With hematoxylin and eosin staining, no obvious deficiency of the inner ear from Jag1-cko with gross view under light microscope was found. Immunostaining indicated that all the vestibular ending organs of Jag1-cko were with normal morphology. In the cochlea, the hair cells in the apical turn and the middle turn appeared to be normal, whereas the outer hair cells were missing in the basal turn.
Our conclusions: (1) Jagged-1 conditional knockout can survive, however, with poor hearing and balance disorders; (2) Morphologically, Jag1-cko develops normal inner ear structures except the absence of outer hair cells in the basal turn; and (3) Jagged-1 may interact with Notch3 to antagonize the inhibition effect on the Notch1 side, so that the boundary between prosensory patches and non-sensory epithelium can be defined.

P 38

Generation of a tamoxifen-inducible hair cell-specific TR-β1 knockout mouse model

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Thyroid hormone receptor β1 (TR-β1) dysfunction leads to deafness in humans and mice (Refetoff et al., 1993; Forrest et al., 1996). Deafness in TR-β1 mutant mice has been suggested to result from TR-β1-mediated control of fast-activating BK currents in inner hair cells (Rüsch et al., 1998). New results, however, suggest that deafness is not a result of delayed BK currents, but may have an origin outside the hair cells (Winter et al., 2009).
To further verify this presumption, we aimed to delete the TR-β1 receptor restrictively in hair cells, within a critical time period prior to the onset of hearing. To obtain a hair cell-specific deletion of TR-β1, we use a well-established CreLoxP system and a transgenic mouse model (Math1CreER^{TM}), in which the expression of the Cre-recombinase is under control of the Math1 promotor and its activation is inducible by tamoxifen (Chow et al., 2006). In the cochlea, Math1 is only expressed in hair cells, from E13 to P7, therefore Cre expression and gene deletion is presumed to be active during that time. This makes it possible to generate mice with a hair cell-specific deletion of TR-β1 prior to the onset of hearing. By crossing Math1CreER^{TM} mice with floxed TR-β1 mice, the obtained mouse model is ready for functional and cellular phenotyping.
First data will be presented that describe the hearing function of these inducible hair cell-specific TR-β1 knockout animals as well as the phenotype of hair cells.

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P 39

New tools in hearing research: Inducible conditional hair cell-specific knockout and knockin mouse models and what we can learn from them

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Thyroid hormone is essential for the development of the mammalian auditory system. Thyroid hormone receptor β1 (TR-β1) dysfunction leads to deafness in humans and mice. Deafness in TR-β1 mutant mice has been ascribed to TR-β1-mediated control of fast-activating BK currents in inner hair cells (Rüsch et al., 1998). However, normal hearing in BK$\alpha^{-/-}$ mutant mice in the first weeks contradicts this assumption. Recent studies point to a crucial role of TR-β1 for tectorial membrane development rather than final hair cell differentiation. Alternative functions are suggested by studies in our laboratory. New results implicate a critical role of TR-α1 in the regulation of the expression of ion channels in hair cells during final differentiation (Winter et al., 2006, 2007). Using conditional mouse models, we work on elucidating the role of thyroid hormone. In particular we are interested in final outer hair cell but also inner hair cell differentiation and maturation of normal exocytosis in inner hair cells.
We will present novel outcome of our work.

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P 40

Spatiotemporal expression of glycine receptors in the murine cochlea

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The glycine receptor (GlyR), a ligand-gated chloride channel, is one of the most important inhibitory receptors in the mammalian CNS. Four ligand-binding α-subunits (α1-4) and one structurally homologous β-polypeptide are known to date. Gephyrin anchors the pentameric receptor channel to the cytoskeleton. Our previous work implied a role for GlyRs in efferent innervation of the rat cochlea, mediated by the medial (MOC) and lateral (LOC) olivocochlear bundle. On this basis, we investigated the spatio-temporal expression of GlyR subunits and gephyrin in the murine cochlea with special focus on the localization of GlyRs at efferent synapses.
Quantitative and hair cell-specific RT-PCR, and immunofluorescence staining of cochlear cryosections were performed.
Absolute quantification of GlyR-α1-3, GlyR-β and gephyrin mRNA in the murine cochlea from P0-P21 revealed a preponderance of GlyR-α1 and α2 subunits before the onset of hearing, which is superseded by GlyR-α3 thereafter. While the number of GlyR-β transcripts increases from P0 to P21, high levels of gephyrin mRNA were found at all stages analyzed. Using hair cell-specific RT-PCR, a shift of GlyR and gephyrin transcripts from IHCs to OHCs was observed around the onset of hearing. These findings were verified on protein level by immunofluorescence staining of GlyRs in the organ of Corti. Double labelling of GlyRs and the presynaptic efferent marker synaptotagmin revealed a postsynaptic localization of GlyRs at LOC and MOC efferent synapses (P21).
Here, we provide evidence for an expression of GlyRs at efferent synapses of the murine cochlea. The developmental regulation of GlyR and gephyrin transcripts parallels the changes in efferent innervation around the onset of hearing. Taken together, the spatio-temporal expression pattern of GlyR mRNA and protein in the murine cochlea suggests a role of glycinergic neurotransmission in inner ear efferent innervation.

P 41

Immunohistochemistry of SMI-32 neurofilament protein in the auditory cortex of the rat

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SMI–32 is a neurofilament protein expressed predominantly in type-I excitatory neurons. The pattern of SMI-32 expression has been succesfully used for the parcellation of cortical areas in, e.g., hamsters and marmosets. The goal of this study was to characterize the populations of SMI-32-immunoreactive (SMI-32-ir) neurons in the rat auditory cortex. Analysis of SMI–32 immunoreactivity patterns revealed differences in the distribution and features of SMI-32-ir neurons across the auditory cortical fields and between auditory areas and the surrounding non-auditory cortex. Striking differences were evident in particular between the auditory cortex and the ventrally situated ectorhinal and perirhinal non-auditory areas. The numerical density and average volumes of SMI-32 immunoreactive neurons and the optical density of the neuropil were significantly higher in the auditory area (Te3) compared to the ventrally situated cortex. The differences were most pronounced in the population of layer V SMI-32-ir pyramidal neurons. Within the auditory cortex, the boundaries between the Te1, Te3 and Te2 areas (Zilles, 1989) could be established on the basis of SMI-32 immunohistochemistry. The highest numerical density and largest somas of SMI-32-ir neurons were found in the ventral associative Te3 area, followed by the caudal associative Te2 area and the auditory core (Te1). In summary, SMI-32 can serve as a useful marker for delineation of the auditory cortex in rats and for differentiating between the central auditory core and the associative auditory areas.

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P 42

Noise exposure during early development modifies the auditory startle reflex in adult rats

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The maturation of the auditory system is influenced by the character of the incoming neural activity during the early postnatal period. To study the changes in the auditory system induced by noise exposure in the early postnatal period, we used a behavioral paradigm consisting of the acoustic startle reflex (ASR) and the pre-pulse inhibition (PPI) of the startle response. The ASR and PPI were examined in 3-6 month-old rats exposed to noise (broad-band noise, 125 dB SPL, for 8 min) in early postnatal life (day 14). The ASR evoked by tone pips (2, 4, 8, 12 and 16 kHz) and white noise and the efficacy of prepulse inhibition of ASR (tone pips: 2, 4, 8, 12 and 16 kHz; acoustic startle pulse: white noise, 110 dB) were measured at various intensities in noise-exposed and control animals. Although the ASR thresholds in the exposed and control groups were similar, the amplitudes of ASR evoked by suprathreshold intensities at frequencies above 4 kHz were considerably lower in exposed animals than in controls. We used PPI as an index of the suprathreshold auditory function.The increase in the prepulse intensity was accompanied by a decrease of the ASR amplitude in both groups; however, the slope of the PPI function significantly differed between the groups. On the basis of our results we conclude that the observed differences in noise exposed animals in contrast to controls may arise as a consequence of underdeveloped inhibitory connections that retain characteristics of the immature auditory system.

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P 43

Effect of glycine (ant)agonist on auditory nerve fiber activity

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The olivocochlear efferent feedback system exerts direct impact on cochlear nerve activity and balances interaural sensitivity. So far, acetylcholine, GABA and dopamine are known to be transmitters of the inhibitory efferent system. Despite the wealth of information about glycinergic neurotransmission in the central auditory system, the inhibitory glycine receptor (GlyR) has not yet been regarded as an effector of efferent transmission in the cochlea. Recently, we described the expression of GlyR-α3, GlyR-β and gephyrin below inner hair cells and in outer hair cells of the adult cochlea suggesting that these inhibitory receptors may serve as target molecules of the efferent olivocochlear bundle (Dlugaiczyk et al., 2009). Aiming to get a first insight in the role of glycine on auditory nerve function we used compound action potential (CAP) measurements in rats upon in vivo application of taurine and strychnine as presumptive agonists and antagonists of glycine receptors, respectively.
CAP measurements were performed using the active electrode positioned on the round window. The amplitude of the CAP (N1-P1 peak-to-peak amplitude) at a range of sound pressures from 0-90 dB SPL was measured. The averaged amplitude of the CAP from 40-60 dB SPL was defined as a peak amplitude, derived from computer analysis. For local drug application, gel foam pellets were placed into the round window niche and supplied with 5 µl of artificial perilymph or taurine or strychnine. And the CAP was measured after 10 minutes, 1 hour and 3 hours.
The peak amplitude of the CAP was increased after strychnine application as compared to controls. We found no difference of CAP amplitude after local application of taurine and artificial perilymph. These findings are consistent with the hypothesis that glycine receptors serve as target molecules of the efferent olivocochlear bundle.

P 44

3D-Modeling of the organ of Corti on the basis of laser scanning microscopic images

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To get a better understanding about the function of the organ of Corti (OC), the knowledge of the frequency dependence of the size of different cell types plays a crucial role. The aim of this study was to provide a three-dimensional construction of the whole organ including the subcellular parts by using confocal laser scanning microscopy (CLSM).
After the fixation (4% formalin), we used the hemicochlea preparation and for staining of the cells we used Phalloidin Alexa 488, Hoechst and anti-α-tubulin antibodies. The images were recorded with a Leica SP5. The image processing, that means, filtering and segmentation, was performed using „IPTools“ (http://www.tu-dresden.de/medkhno/sites/wissenschaft/mittelohr/index.htm), whereas for 3D-reconstruction a commercial software package (AMIRA) was used.
As a result, all mechanically relevant structures were identified and quantified in size and shape. Moreover, we were able to estimate the frequency dependence of these values.
Based on this investigation, we built and refined a three-dimensional Finite-Element-Model of a part of the OC including the surrounding fluids, to deliver insight into the complex motion of the OC at a certain frequency point. Results from simulation are in good agreement with experimental observations according the motion of structures within the OC caused by fluid-structure-interaction.

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P 45

Salicylates downregulate AQP-6 expression in sensory epithelia of the inner ear

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Salicylates are well known for their reversible ototoxic effect, which include tinnitus and hearing loss. While the latter effect has been correlated to a direct inhibition of the OHC molecular motor prestin, the onset of tinnitus appears more complex to explain. The main targets for salicylates, i.e. cyclo-oxygenases (COX), are expressed in the ear, and prostaglandins, whose synthesis is COX-dependent, affect several targets ranging from NMDA receptors to membrane transport systems, all potentially able to impact cochlear transduction. In other systems, among the targets of prostaglandins are also aquaporins, which form water channels in lipid membranes. Several aquaporins are expressed in the inner ear, but their role is still largely unclear. In the present work we have studied the expression of AQP6 in the mouse ear in control conditions and after salicylate treatment.
C57BL6 mice were treated with aspirin (200 mg/kg) or with saline i.p. AQP6 expression was investigated by RT-PCR and qRT-PCR with specific primers designed on the published sequence after three days of treatment. Immunohistochemistry studies were done in the same conditions by using custom-made AQP6 antibodies on paraffin-embedded sections of decalcified temporal bone.
AQP6 mRNA expression in the inner ear was reduced by about 70% after treatment with aspirin. In control conditions AQP6 was observed in the spiral ganglion, in vestibular epithelia and in Deiters' cells in the organ of Corti. After treatment with salicylates, AQP6 labeling was dramatically reduced, especially in the sensory epithelia.
In this study we show that salicylates decrease the expression of AQP6 in the mouse cochlea. This result could suggest that AQP6 plays a role in the control of OHC action, since this aquaporin isoform displays both water and Cl^- permeability, and could affect both mechanical coupling and ionic homeostasis of OHCs through changes in Cl^- concentrations and/or Deiters' cell compliance.

P 46

Effects of electrical stimulation on the acoustically evoked compound action potential

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The criteria for implantation with a cochlear prosthesis have been broadened and people with considerable residual low-frequency hearing receive an implant nowadays. Preservation of residual hearing in the implanted ear improves speech intelligibility in noise and increases the esthetic perception of complex sounds. We assume that residual low-frequency hearing has optimal beneficial effects when interactions of electrical stimulation on acoustically evoked responses are minimal. We have investigated the effects of ipsilateral intracochlear electrical stimulation on the amplitude of the acoustically evoked compound action potential (CAP) in normal-hearing and partially deafened guinea pigs.
Electric stimuli were delivered via a 1-mm platinum wire electrode inserted in the basal turn of the cochlea and consisted of single biphasic pulses (40 μs/phase) or pulse trains approximately 10 ms in duration. CAPs were evoked with acoustic tone bursts of variable frequency and level. Effects of electrical stimulation on CAP amplitude were tested using a forward masking paradigm. Effects of the interval between the electrical and acoustical stimulus (EAI, 0-10 ms), pulse rate (500-4000 pulses/s) and pulse width (80-400 μs) of the electrical stimulus were tested.
Electrical stimulation suppressed CAPs evoked with high-frequency tones, especially at low sound levels. Suppression of low-frequency evoked CAPs was less pronounced. Recovery was complete at EAIs as short as 2 milliseconds indicative of direct neural stimulation. Pulse rate had no large effects. Pulse width affected CAP suppression indicative of electromechanical transduction playing a role in suppression.
These data indicate that basal (high-frequency) cochlear regions can be stimulated electrically without affecting low-frequency acoustical hearing. CAP suppression probably depended on direct electrical neural stimulation and electrophonic stimulation. These findings may be relevant for hybrid implant stimulation strategies.

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P 47

Structure of the otoferlin C2A domain of *Rattus norvegicus*

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Otoferlin, a protein of 220-kDa size, is expressed in the inner hair cells of the cochlea in vertebrates. Genetic mutations lead to deafness in man (DNFB9) and mouse (Roux et al., 2006). As auditory hair cells of otoferlin knockout mice show almost abolished exocytosis, Otoferlin is assumed to play an important role in the exocytosis machinery in inner hair cells. Otoferlin contains six (or seven) C2-domains (C2A-C2F) and one transmembrane domain. C2 domains of synaptotagmin or phospholipases have been studied extensively for their calcium- and phospholipid-binding properties, but ferlin C2 domains are less well characterized.
To elucidate the role of the first C2-domain of otoferlin, C2A, we explored its biochemical and structural characteristics. Therefore, the C2A domain was overexpressed in *E. coli*, affinity purified using a His_6-Tag and crystallized by sitting drop vapour diffusion.
The structure of C2A was resolved with 1.95-Å resolution. It shows a full C2-domain with eight β-strands, which is in opposition to previous predictions. As the otoferlin C2A domain folds with type-II homology, the putative Ca^{2+} binding regions are located between β-strands I and II and in the loop between β-strands V and VI. The surface of these loops, however, was found to be positively charged, and aspartate residues that are required to coordinate Ca^{2+} were not found at the appropriate positions. Next, we tested C2A's calcium- and phospholipid-binding by means of Isothermal Titration Calorimetry (ITC), floatation assays and lipid-overlay assays. In agreement with the predictions from the structure, we found that otoferlin C2A does neither bind Ca^{2+} nor does it show Ca^{2+}-dependent phospholipid binding.
In summary, the first C2-domain of otoferlin is a complete C2-domain, but does not bind Ca^{2+} ions or phospholipids.

P 48

Biochemistry of otoferlin C2F and its pachanga mutant form

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Otoferlin is expressed in auditory hair cells, and presumably targeted to synaptic vesicles. It contains six or seven C2 domains, which are widely known to bind phospholipids in a Ca^{2+}-dependent manner. A chemical mouse mutagenesis experiment yielded a deaf mouse line with a missense mutation in Otof. The so-called pachanga mice have a single nucleotide exchange in the C-terminal C2 domain (C2F) replacing one aspartate with a glycine in the primary structure. Using CD spectroscopy, we found a slight but significant structural alteration caused by this mutation. As aspartates usually coordinate Ca^{2+} ions in the Ca^{2+} binding loops of C2 domains we investigated the Ca^{2+} binding of both variants of the C2F domain. Next, we analysed phospholipid binding and found that this particular C2 domain does not bind phospholipids, neither in presence nor in absence of Ca^{2+} or PIP_2, which was also the case for the mutant C2F domain. However, the mutation led to lower protein levels and a slightly different subcellular distribution of otoferlin in auditory hair cells.
In addition to the biochemical analysis of the isolated C2F domain we tested the characteristics of inner hair cell exocytosis in pachanga mice and found a significant reduction in sustained exocytosis, but not in the fast component of vesicle release. Whether this is an effect of the lower protein abundance or a specific effect of the aspartate to glycine exchange needs further elucidation.

P 49

The acoustic chiasm in pigmented and albino rats

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Functional studies demonstrated differences in auditory perception between pigmented and albino animals. In the visual system, an enhanced contralateral projection in the optic chiasm was found to be related to hypopigmentation. We therefore studied the olivocochlear system to test for possible differences between albino and pigmented rats with regard to the ratio of ipsilateral and contralateral olivocochlear neurons.
The retrograde neuronal tracer Fluorogold was injected into the left cochlea in 8 Wistar and 8 Brown Norway rats. After 5 days, the animals were killed by ether overdose and fixed by intracardiac perfusion. Frozen sections of the brainstem were prepared and retrogradly labelled cells in the superior olivary complex were counted from both sides.
There was no difference in the projection pattern of intrinsic lateral olivocochlear neurons, located in the lateral superior olivary nucleus (LSO) between pigmented animals and albinos. Shell neurons, located in the dorsal periolivary region (DPO), the lateral nucleus of the trapezoid body (LNTB) and the caudal periolivary region (CPO), projected predominantely ipsilaterally in both groups.
A difference was observed in the medial olivocochlear system (rostral periolivary region (RPO), ventral nucleus of the trapezoid body (VNTB)). While in pigmented animals the amounts of retrogradely labelled neurons were similar on both sides, the contralateral projection outbalanced the ipsilateral projection in albinos.
We provide evidence for morphological differences in the laterality of the olivocochlear pathway (concerning the MOC system) between pigmented and albino rats. These resemble the differences observed in the visual system, and may account for the alterations in auditory perception present in albinos.

P 50

Prevalence of GJB2 mutations in the Portuguese population

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A high heterogeneity of GJB2 variants has been observed around the world in different populations. Moreover, the prevalence of some variants was shown to be population-specific. Therefore, in order to facilitate molecular diagnosis of congenital deafness and improve genetic counseling in each country/region, it is important to determine the prevalence of the different GJB2 alleles among the population.
In this study, aiming at idenfying regional differences, we extended our previous studies on the carrier frequency of GJB2 mutations by screening random neonates from different Portuguese regions.
We have considered a total of about 230 samples of neonates representing the major regions of Portugal mainland. Screening of GJB2 gene was performed by sequencing the entire coding region.
The analysis of the 230 samples included in this study led to the identification of different carriers of common GJB2 mutations and polymorphisms, some of them found for the first time in our population. These results, together with those obtained in the previous study on 300 hearing individuals, are jointly analyzed (n=530) and compared, considering the geographic specificities in relation to GJB2 variants.
A great variability of GJB2 variants was observed. Some interesting regional differences were found. The geographic distribution of these variants in the Portuguese mainland population is here discussed.

P 51

The ultrastructural distribution of prestin in rat cochlear outer hair cells

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Outer hair cells (OHCs) of the mammalian cochlea besides being sensory receptors also generate force to amplify sound-induced displacements of the basilar membrane thus enhancing auditory sensitivity and frequency selectivity. This force generation is attributable to voltage-dependent contractility of the OHCs underpinned by the motile protein, prestin. Prestin is located in the basolateral plasma membrane of OHCs and is thought to alter its conformation in response to changes in membrane potential. We have previously demonstrated the precise ultrastructural distribution of prestin by post-embedding immunogold labeling. The labeling was confined to the basolateral plasma membrane in hearing rats, but declined towards the base of the cells below the nucleus. In pre-hearing animals, prestin labeling was lower in the membrane and also occurred in the cytoplasm, presumably reflecting its production during development.
Here, the density of the labeling has been compared in low- and high-frequency regions of the cochlea with the nonlinear capacitance measured in similar regions in hearing rats. Non-linear capacitance is thought to reflect charge movements during conformational changes in prestin.
The densities of labeling in low- and high-frequency regions of the cochlea were similar. The OHC non-linear capacitance in the same regions as those assayed in the immunolabeling was also similar to within a factor of two, with charge densities of 9,000 to 16,000/μm^2 in the apex and base respectively.The results suggest that prestin density, and, by implication, force production, are similar in low- and high-frequency OHCs despite a large difference in basilar membrane stiffness at the two locations.

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P 52

Contribution to the study of presbycusis in the Portuguese population

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Presbycusis or age-related hearing loss (ARHL) is one of the major chronic diseases affecting the elderly population and may become a major health problem, considering the increase in life expectancy. Though no ARHL susceptibility genes have yet been identified, increased susceptibility to oxidative stress has been described in the general European population in association with the polymorphism NAT2*6A in the NAT2 (*N*-acetyltransferase 2) gene, suggesting a role of this gene for the development of ARHL. Human populations are divided into rapid, intermediate, and slow acetylator phenotypes, depending on the NAT2 genotype. The present work aims to screen NAT2 gene variants in a sample of individuals from the Portuguese population with presbycusis.
Following a detailed set of inclusion criteria internationally accepted, we have collected on FTA cards blood samples of elderly individuals from the Portuguese population, originated from all over the country, of both sexes, aged above 65 years and with clinical indication of ARHL. DNA extraction and PCR followed standard methodologies, and the amplified fragments were automatically sequenced in order to determine the genotypes for the chosen NAT2 polymorphisms. All individuals have signed written informed consent.
We present the different haplotypes found in the sample under study for selected NAT2 gene variants. We have observed that haplotypes NAT*5B and NAT*6A are well represented in our ARHL sample. The haplotype NAT*4 is also common, usually associated to NAT* 5B.
We here present the first study on presbycusis in the Portuguese population. The patterns of NAT2 variants found in the sample under study seem to be consistent with those previously observed in the general European population.

P 53

Comparison of threshold, growth functions and peak-delay times by ABR and CAP measurements

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Recordings of Auditory Brainstem Responses (ABR) by non-invasive, surface or subcutaneous electrodes and electrocochleography by invasive recordings of auditory evoked signals from the round window niche are standard methods to determine the hearing thresholds of humans and laboratory animals. From ABR recordings mainly the responses of the brainstem nuclei (nucleus cochlearis, superior olivary complex, lemniscus lateralis), midbrain structures (colliculus inferior) and diencephalon structures (corpus geniculatum mediale) can be isolated, the electrocochleography mainly reflects the activity of the auditory nerve (compound action potential, CAP) and the receptor potential of the inner hair cells (summating potential, SP) and outer hair cells (cochlear microphonics, CM). Since the ABR, CAP and IHC/OHC signals derive from different sources differential inspection of the evoked potentials offers the opportunity to judge the function of particular sensory and brain structures. However, estimating the hearing threshold on the basis of different measures may lead to varying results dependent on the criteria and the method used.
The hearing thresholds of rats and mice derived from ABR and CAP recordings were analysed and compared in respect to absolute threshold, single peak threshold, growth function (input/output function) and delay times. Based on automated threshold judgements the correlations of IHC threshold, OHC threshold, CAP threshold and the response of selected brainstem nuclei will be discussed.

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P 54

Steroid treatment of acute noise-induced hearing loss

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Noise exposure, either by impulse noise or long-term exposure, causes a temporary threshold shift (TTS) and eventually a permanent threshold shift (PTS). Otoprotective drugs attempt to prevent PTS in noise-induced hearing loss. For the evaluation of such otoprotective properties reproducible in vivo noise trauma models was established.
Guinea pigs were exposed to impulse noise (142 dB SPL rms). Audiograms were monitored by measuring responses of the auditory nerve before, immediately after, 7 and 14 days after the noise trauma. Then whole mounts of the basilar membrane were prepared to count hair cells and to plot cytocochleograms. To study otoprotection either dexamethasone, prednisolone or methylprednisolone was applied to the round window of the cochlea with osmotic minipumps for 2 weeks at various concentrations.
Impulse noise exposure conditions resulted in a severe TTS in the complete hearing range. Within one week threshold recovery was observed in all exposure conditions, in the second week after exposure a further but minor recovery was seen. Hair cell loss was observed across the entire length of the cochlea, except for the very basal and apical ends. Dexamethasone (1 and 4 mg/ml), prednisolon (25 mg/ml) and methylprednisolon (12.5 mg/ml) resulted in an significant improved threshold recovery as compared to controls treated with artificial perilymph. Dexamethasone and prednisolone also resulted in reduced hair cell loss.
Our results suggest that locally applied high-dose anti-inflammatory steroids are potential candidates for the treatment of acute noise-induced hearing loss.

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P 55

Otolithic function and brain activation in humans

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The ascending projections from the otolithic organs to the central nervous system are not yet fully understood in humans. A patient with unilateral vestibular deafferentation such as schwannoma sometimes shows abnormal tilts of subjective visual vertical (SVV). This is assumed to be caused by a dysfunction in the otolithic organs and graviceptive pathways. However, it is also unknown which cerebral cortex area participates in the graviceptive pathways from the otolithic organs. In the present study, using multi-channel functional near-infrared spectroscopy (fNIRS), the brain activation in the SVV tests was investigated in both 6 healthy subjects and 5 patients with vestibular schwannoma (VS). In the healthy subjects, activation was observed in the ipsilateral superior temporal gyri, and the insular gyri of the both cerebral hemispheres. Although the active areas in patients with VS were similar to those in healthy subjects, these were observed in the contralateral hemisphere predominantly. The cortical processing mechanism in the cerebral hemispheres likely plays an important role in SVV.

P 56

Glucocortidoid receptors and 11β-hydroxysteroid dehydrogenase isoforms in the rat and human inner ear

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Glucocorticoid receptors (GRs) are present in the inner ear and corticosteroids are thought to play a role in fluid homeostasis and sensory transduction. The concentration of corticosteroids in the target cells are regulated by 11β-hydroxysteroid dehydrogenase (HSD). HSD is an enzyme complex responsible for the conversion of hormonally active cortisol to inactive cortisone and two isoforms of the enzyme (HSD-1 and HSD-2) have been cloned and characterized. However, the precise distribution of GRs, the isoforms of HSD, and physiological roles of corticosteroids in the inner ear have not been fully understood to date. Therefore, we investigated the distribution of GRs and the isoforms of HSD in the rat inner ear tissues using PCR and fluorescence immunohistochemistry. In addition, the immunohistochemical localization of GRs, HSD-1 and HSD-2 in the semicircular canals obtained from patients with vestibular schwannoma were investigated. GRs were present in all the rats' inner ear tissues including the cochlea and the semicircular canals. For instance, in the inner sulcus cells, GRs were predominantly present in the basolateral membrane. HSD-1 was also detected in all the inner ear tissue of the rat. However, the localization of HSD-1 varied in each inner ear tissue. Although mRNA of HSD-2 was detected in each inner ear tissue, no positive reaction was observed in the immunostaining of HSD-2. In the human semicircular canals, GRs are present in the basolateral membrane of the epithelium. A physiological study was also conducted to determine physiological action of cortisol through the use of the culture of the rat cochlea and the semicircular canals. Cortisol seems to have protective properties against hypoxia in the cochlea and the semicircular canals. Our results suggest that different local steroid regulation by GRs and the isoforms of HSD is present in each part of the inner ear.

P 57

Uptake and toxicity tests of hyperbranched poly-L-lysine on PC12 and OCk-3 cells

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Several viral vectors are currently tested for drug delivery in the inner ear. In the present study we used for the same purpose hyperbranched poly-L-lysine particles (Mn 15 kDA, PDI 4). We evaluated uptake and potential toxicity of poly-L-lysine in two cell lines, Ock-3, from the inner ear of Immorthomouse™, and PC12, from rat pheochromocytoma, at concentrations between 10^{-9} and 10^{-5} M, for time intervals between 24 and 72 hours.
Polymer uptake and cell viability were measured by fluorescent microscopy and flow cytometry. Hyperbranched poly-L-lysine was taken up by cells in a time- and dose-dependent way. At the lowest concentration (10^{-9} M), no toxicity was observed in both cell lines. As expected, cell viability decreased with increasing sample concentrations.
Apoptosis mechanisms underlying cytotoxicity were investigated by Western blot (PARP and p53) and by immunocytochemistry (annexin V and DAPI).
The results supported the hypothesis that in both lines cell death occurred by apoptosis, although nuclear fragmentation was not detected. At low concentrations the polycation could be useful as a non-viral system for drug delivery in the inner ear.

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P 58

Clinical outcomes of the MP3000™ sound-coding strategy optimization study in Freedom™ recipients

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MP3000 is a sound-coding strategy based on psycho-acoustic masking, which has been implemented on the Nucleus® Freedom cochlear implant system. The objectives of the study were to optimize the MP3000 parameter settings, to investigate the preference and speech intelligibility for MP3000 in comparison to ACE™ and to assess battery life.
A prospective, multicentre study was conducted in nine different European countries, including a total of 37 cochlear implant centres, recruiting 247 Freedom recipients. At study entry, threshold (T) and comfort (C) levels were optimized. Different numbers of maxima and masking function slopes were tested to optimize the MP3000 parameters. Speech intelligibility was assessed in Dutch, English, French, German, Polish, Italian and Spanish for MP3000 and ACE according to an ABABA design to compensate for learning effects. Battery life was logged in diaries and recipients were asked to indicate their preferred coding strategy and parameter settings.

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No significant difference in either speech intelligibility (p=0.4) or preference (p=0.2) for MP3^{000} and ACE were observed. T- and C-level profiles increased by 6 and 7 CLs when converting ACE to MP3^{000}. Most recipients preferred MP3^{000} with higher numbers of maxima and narrow masking functions. The average battery life increased significantly by 24% (p<0.001).
MP3^{000} provides a sound coding strategy with the benefit of significantly increased battery life, without affecting speech intelligibility. MP3^{000} is equally preferred as ACE, the coding strategy that the Freedom recipients were accustomed to before entering the study.

Sponsored by Cochlear Europe.

P 59

Applicability of the phosphodiesterase inhibitor rolipram as neurotrophic factor for spiral ganglion cells

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Nanoparticles may be suitable carriers of pharmacological substances. They can be used as non-viral, biodegradebable and cell-specific vectors to increase the efficacy of heretofore existing application methods. Brain-derived neurotrophic factor is an important survival factor for spiral ganglion cells (SGC), as has been demonstrated in numerous in vitro and in vivo studies. Composed of 119 amino-acids with a molecular mass of 14 kDa BDNF may be too massive to be incorporated in nanoparticles. Therefore, it may be reasonable to identify smaller molecules with neurotrophic properties for the incoperation in nanoparticles. Rolipram ($C_{16}H_{21}NO_3$), a phospodiesterase inhibitor, appears due to his small molecular size as suitable. However, the effect on the survival of SGC remains to be elucidated yet. We therefore hypothesize that rolipram acts neuroprotective on SGC cultures.
Cultivation of SGC after extraction from neonatal Sprague-Dawley rats and cultivation for 48 h in a serum-free medium and the addition of rolipram. The neuroprotective properties of rolipram were compared with those of BDNF (50 ng/ml) and negative controls (medium without the addition of any growth factors).
Similar survival rates were achieved under treatment with BDNF or rolipram. Both factors significantly increase survival of SGC when compared to negative controls. Rolipram and BDNF act neuroprotective on SGC derived from neonatal rats in vitro. Provided that rolipram is as effective in in vivo experiments as demonstrated in vitro, it may be an ideal candidate for the application to the inner ear via nanoparticles.

P 60

The role of the auxiliary Ca^{2+}-channel α2δ3 subunit for signal transmission in the auditory brainstem and acoustic startle reflex pathway

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Voltage-activated Ca^{2+} channels consist of a pore-forming α1 subunit (SU), a β and an α2δ SU. Expression of α1 SUs is cell specific, and the co-assembly with auxiliary subunits β and α2δ may also be rather specific. The α2δ SUs are required for trafficking and stabilizing α1 SUs in the plasma membrane, thereby defining the amplitude and biophysical properties of Ca^{2+} currents. Mice deficient for the gene CACNA2D3 coding for the α2δ3 SU showed an impared hearing phenotype with slightly increased ABR hearing thresholds over the entire frequency range, reduced amplitudes of ABR waveforms but normal DPOAE thresholds and amplitudes. Additionally, the acoustic startle response was also reduced in the $\alpha2\delta3^{-/-}$ mice (knockout). To test if hearing and acoustic startle deficits were caused by reduced presynaptic Ca^{2+} currents in the IHCs, Ba^{2+} currents were recorded in inner hair cells (IHC) pre- and post-hearing onset. Ba^{2+} currents were slightly different between wild-type and knockout IHCs, but this difference most likely does not account for the impaired knockout phenotype. Analysis of α2δ3 expression in the cochlea using lacZ reporter staining and in situ hybridization revealed its expression in spiral ganglion neurons and cochlear root neurons. To test if the presynaptic channels along the hearing and startle response pathways were affected, we performed immunohistochemistry for the fast P/Q and N-type Ca^{2+} channels. In brainstem nuclei and pontine giant neurons, we found differences in the P/Q as well as N-type expression patterns between wild-type and knockout animals.
In conclusion, the hearing and startle deficits of $\alpha2\delta3^{-/-}$ mice are not due to malfunction of IHCs or OHCs, but may rather be caused by altered presynaptic Ca^{2+} channels and currents of spiral ganglion neurons, root neurons and giant neurons further up the hearing and startle pathway.

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P 61

Pharmacokinetics of gentamicin entry into the cochlea following systemic applications

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Gentamicin is an aminoglycoside antibiotic in clinical use for the treatment of gram-negative bacterial infections. Nephrotoxicity and ototoxicity due to long-term systemic applications are well-documented side effects of aminoglycosides. The vestibulotoxic properties are used for the treatment of Menière’s disease patients to reduce the severity of vertigo attacks. Deafening has been reported after local round window membrane (RWM) applications in Menière’s patients. Recent experimental pharmacokinetic studies showed basal-apical gradients following RWM applications and uptake of the drug in the vestibule without passing through the helicotrema. Thus, high concentrations in the base of the cochlea could be responsible for damage after local applications in humans. We were interested whether a basal-apical gradient of gentamicin was present in the perilymph after continuous systemic applications.
Ten equal 1 µl samples of perilymph were taken sequentially from the apex of each cochlea. The gentamicin concentration of each perilymph sample was measured using a fluorescence polarization immunoassay.
With a dosage of 300 mg/kg body weight, the highest concentration was measured in the first perilymph sample aspirated from the cochlea, representing the apical part of the cochlea. In each experiment, concentrations declined in subsequent samples, that originated from more basal cochlear locations. This contrasts with sample concentrations measured following local applications to the RW niche for 3 hours, in which the concentration in the first perilymph sample was low and the peak concentration was found in the fourth sample, originating from the basal part of the cochlea.
With systemic application of gentamicin, perilymph concentration at apical locations increases more rapidly than at basal locations, demonstrating that drug entry is non-linear along the length of the cochlea.

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Future goals include comparison of experimental data with predictions from computer simulations calculated with a modified three-dimensional model of the guinea pig cochlea.

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P 62

Notch signaling regulates cochlear stem cells maintenance and sensory cell-fate determination

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Mammalian cochlear hair cell loss is irreversible and leads to permanent hearing loss. To restore hearing, it is necessary to generate new functional hair cells from endogenous cells or from exogenously transplanted hair cell progenitors. Although recent studies have suggested the presence of hair cell progenitors in the postnatal cochlea, the specific signaling pathways responsible for their maintenance and cell fate determination have not been reported.
We set up an in vitro model that examines the signaling events involved in cochlear stem/progenitor cells maintenance and differentiation along the cochlear sensory pathway. In this culture system, cochlear stem/progenitor cells derived from the postnatal mouse inner ear generate cellular spheres that can be expanded for extensive passages (up to the 12th generation). These spheres contain Abcg2, Jagged1, Sox2 and Notch1 positive stem/progenitor cells that can divide and generate hair cell-like cells, i.e. immunopositive for specific hair-cell markers, including myosin VIIa and Math1.
We demonstrate that reducing Notch signaling with a gamma-secretase inhibitor decreases the number of spheres generated following treatment of the stem/progenitor cell cultures. Additionally, activation of Notch by an exogenous soluble form of a Notch ligand, i.e. Jagged1 protein, promotes sphere formation and the sensory potential of cochlear stem/progenitor cells (i.e., hair cell and support cell immunophenotypes).
Our findings suggest that Notch1/Jagged1 signaling plays a role in maintaining a population of Abcg2 sensory stem/progenitor cells in the postnatal mammalian cochlea. Successful isolation and propagation of hair cell progenitors will facilitate studies on mechanisms of hair cell differentiation and regeneration which are crucial for repairing hearing loss.

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46th Workshop on Inner Ear Biology

P 63

Estrogen and hearing

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The objective of this study was: Are female sex steroids the key to preserved hearing in the aging human, both males and females?
Morphology, immunohistochemistry, auditory brainstem recordings (ABR) and PCR will be used to measure hearing and to localize and quantify estrogen, progesterone and androgen receptors in the inner ear.
Hearing loss appears to be more profound in elderly males than females. There are also well-known sex differences in the auditory brainstem recordings (ABR), where women have shorter latencies than men. Moreover, women at menopause with hormonal replacement therapy have a slightly better hearing than non-substituted women and women with Turner syndrome (45, X), who are biologically estrogen deficient, show an early presbycusis. The menopause seems to be a crucial period for increasing hearing problems in the normal female population.These findings are supported by animal experiments. Presently, if estrogen receptor beta is knocked out a severe progressive hearing loss is present that leads to early deafness in mouse. The inter-estrogen receptor action and effects from other sex hormones like progesterone and androgens, must also be considered and here no androgen or progesterone receptors could be visualized.
Conclusion: Knowing how sex steroids can alter the hearing abilities might give us important clues as to how estrogen might preserve hearing in humans and also that substitution of selective receptor stimulators can be beneficial.

AUTHOR INDEX

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46th Workshop on Inner Ear Biology

46th Workshop on Inner Ear Biology

NOTES

NOTES

NOTES

NOTES

NOTES

NOTES

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