Abstract book

42nd Workshop on Inner Ear Biology

18. – 20. September 2005

and

Symposium on

Terminal Differentiation:
A Challenge for Regeneration

17. September 2005

IEB 2005 TÜBINGEN

Tübingen, Germany

Location

The 42nd Workshop on Inner Ear Biology, together with the Symposium on "Terminal Differentiation: A Challenge for Regeneration" will be held in the University town of Tübingen. Tübingen is situated on the River Neckar in Southern Germany, in the heart of Baden-Württemberg, between the Black Forest and the Swabian Alb. The University was founded in 1477 by Eberhard im Bart, Count and later first Duke of Württemberg. The University motto "Attempto" characterizes the scientific mission of this conference.

Organization

Local:
Nicole **Bayer**
Jutta **Engel**
Tony **Gummer**
Csaba **Harasztosi**
Mirko **Jaumann**
Marlies **Knipper**
Assen **Koitschev**
Renate **Lauf**
Hubert **Löwenheim**
Marcus **Maassen**
Paul-Stefan **Mauz**
Stefan **Münkner**
Markus **Pfister**
Stefan **Plontke**
Serena **Preyer**
Marc **Scherer**
Anne **Seeger**
Wolfgang **Wagner**
Hans-Peter **Zenner**
Ulrike **Zimmermann**

International:
Matti **Anniko**, Uppsala, Sweden
Karen **Avraham**, Tel Aviv, Israel
Stavros **Hatzopoulos**, Ferrara, Italy
Stefan **Heller**, Boston, U.S.A.
Matthew **Holley**, Bristol, U.K.
Philippe **Lefebvre**, Liege, Belgium
Jean-Luc **Puel**, Montpellier, France
Jochen **Schacht**, Ann Arbor, U.S.A.
Anneliese **Schrott-Fischer**, Innsbruck, Austria
István **Sziklai**, Debrecen, Hungary
Mats **Ulfendahl,** Stockholm, Sweden

Secretariat and Mailing Address

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We would like to thank the following institutions and companies for supporting the Inner Ear Biology 2005 Workshop and Symposium

Research Organizations:

Deutsche Forschungsgemeinschaft	http://www.dfg.de

Sponsors and Partners:

BIOPRO Baden-Württemberg GmbH	http://www.bio-pro.de
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Conference Venue

The Workshop and Symposium will be held in the main lecture theatre complex of the University, called the Kupferbau, Hölderlinstraße 5, located within 10 minutes walking distance from the city centre, and less than 10 minutes bus ride from most accommodation.

Registration: The registration desk is located in the Kupferbau and will be open on:
Saturday, 17. September: 08.00 – 18.00
Sunday, 18. September: 07.30 – 18.00
Monday, 19. September: 07.45 – 18.00
Please note that we can only accept payment in cash (**€**), no cheques, no credit cards.

Language: The official conference language is English.

Programme – Overview

Saturday, 17. Sept.

08.00 – 18.00	Registration
09.00 – 17.00	Symposium Scientific Sessions "Terminal Differentiation: a Challenge for Regeneration"
18.00 – 20.00	Welcome Reception: Kelter, Schmiedtorstr. 17

Sunday, 18. Sept.

07.30 – 18.00	Registration
08.15 – 08.30	Opening of the IEB Workshop
08.30 – 17.30	Workshop Scientific Sessions
18.00 – 19.45	Visit to the ENT-Clinic and Hearing Research Centre
20.00 – 23.00	After Work Party: Zentrum Zoo, Schleifmühleweg 86

Monday, 19. Sept.

07.45 – 18.00	Registration
08.15 – 17.30	Workshop Scientific Sessions
19.00 – 23.30	Gala Dinner: Sommerrefektorium Kloster Bebenhausen

Tuesday, 20. Sept.

08.30 – 11.45	Workshop Scientific Sessions
11.45 – 12.00	In Memoriam Sigurd Rauch (1916 – 2003)
12.00 – 12.30	Business Meeting
14.00 – 22.45	Congress Tour to the Swabian Alb

Lunch will be provided in the Conference building on Saturday to Tuesday. Lunch tickets are required – they were purchased with your registration or can be purchased at the registration desk.

Posters should remain posted for the entire length of the meeting.
Authors should be at their posters during poster presentation time.
Poster board dimensions: 1 m wide, 2 m high.

Accompanying Persons' Programme

Sunday, 18. September
"Wine museum & wine-tasting in Metzingen, Schloss Urach and Urach Waterfall"
Bus leaves from the Kupferbau at 09.30, return approx. 17.00. Fee: 40 €

Metzingen has a very long tradition of culturing and producing wine. We visit the wine culture museum which opened in 1979 and learn about preparation of the ground for growing wine, harvesting and producing wine. You will see a 12-m long wine press, a cooper's workshop and a distillery. Wine tasting is also included. After lunch, we visit Schloß Urach which is the only residence castle in Baden-Württemberg preserved from late medieval times. We then visit Urach Waterfall, where the water falls straight down for 37 meters and then on for 50 more meters over tufa stone polster.

Monday, 19. September
"Atomkeller Haigerloch and Burg Hohenzollern"
Bus leaves from the Kupferbau at 09.00, return approx. 17.00. Fee: 40 €

This tour leads us to Haigerloch - a small town at the border of the Swabian Alb, around 40 km from Tübingen. It was the site where the last German experiments on nuclear fission were conducted during World War II by the research group of Werner Heisenberg, Carl-Friedrich von Weizsäcker and Karl Wirtz. We will visit the town and the Atomkellermuseum. After lunch, we visit Burg Hohenzollern. Burg Hohenzollern is the ancestral seat of the prussian-brandenburg line, as well as of the electoral-catholic line of the House of Hohenzollern. It ranks among Europe's most beautiful and popular castles.

Congress Tour

Tuesday, 20. September
"Congress tour to the Swabian Alb, visiting the Nebelhöhle and Schloss Lichtenstein"
Bus leaves from the Kupferbau at 14.00, return approx. 22.45. Fee: 50 €

Nebelhöhle is one of the most important and popular show caves on the Swabian Alb. According to a legend, Herzog Ulrich von Württemberg used this cave as a hideout many years ago. In his 1826 published novel "Lichtenstein", the author Wilhelm Hauff mixes true historic facts and fiction producing a stirring adventure story. After visiting the cave, we will move on to Schloss Lichtenstein which is only 3 km away. You can decide if you would prefer to travel there by bus or enjoy a walk through the lovely countryside. If you'd like to walk, please bring suitable shoes. Schloss Lichtenstein is a small castle, perched on a rock high above the valley. It is a product of the romantic passion for castles in the nineteenth century and was built from 1840 to 1841 by Duke Wilhelm of Urach. After a tour through the castle, we will finish the tour with dinner at the "Altes Forsthaus Lichtenstein".

Scientific Programme

Symposium

"Terminal Differentiation: a Challenge for Regeneration"

In cooperation with BioRegio STERN and BIO PRO Baden-Württemberg GmbH

Saturday, 17. September

09.00 - 09.10		Introduction H. Löwenheim Tübingen Hearing Research Centre (D)
		First Session: **"Hair-Cell Function, Innervation and Cell-Cell Interaction: Implications for Hair Cell Regeneration"** **Chairpersons: C.J. Kros, Y. Raphael**
09.10 - 09.50	S1	Developmental maturation of inner hair cell function in mice C.J. Kros School of Life Sciences, University of Sussex, Brighton (UK)
09.50 - 10.30	S2	Physiology and pharmacology of the inner hair cell synaptic complex J.L. Puel Laboratoire de Neurobiologie de l'Audition-Plasticite synaptique, Universite Montpellier-1, Montpellier (F)
10.30 - 11.10	S3	Cellular interactions and maintenance of phenotype in the regenerating inner ear M.E. Warchol Dept. of Otolaryngology, Washington University School of Medicine, St. Louis, Missouri (USA)
11.10 – 11.40		Break

Second Session:
"Mechanisms of Regeneration in Model Organisms and Terminally Differentiated Tissues"
Chairpersons: M.C. Holley, M.E. Warchol

11.40 - 12.20	S4	Regeneration in the metazoans: Why does it happen? Lessons from simple model organisms A. Sánchez Alvarado Dept. of Neurobiology, University of Utah School of Medicine, Salt Lake City, Utah (USA)
12.20 - 13.00	S5	Mechanisms in the induction of lens regeneration P.A. Tsonis Department of Biology, University of Dayton, Dayton, OH, (USA)
13.00 – 14.30		Lunch

Third Session:
"Approaching Cellular Regeneration in Mammalian Tissues"
Chairpersons: J.-L. Puel, P.A. Tsonis

14.30 - 15.10	S6	Transdifferentiation of non-sensory cells as a strategy for hair cell regeneration Y. Raphael University of Michigan, Kresge Hearing Research Institute, Ann Arbor (USA)
15.10 - 15.50	S7	Developmental mechanisms in the context of regeneration M.C. Holley University of Sheffield, Department of Biomedical Sciences, (UK)
15.50 - 16.20		Break
16.20 - 17.00	S8	Applications of stem cells for the restoration of hearing loss A. Edge Mass. Eye and Ear Infirmary, Harvard University, Boston (USA)

IEB Workshop, Programme – Overview

Saturday, 17. September

18.00 – 20.00	IEB Welcome Reception in Kelter, Tübingen Suggestion for after the Welcome Reception: Walk through Tübingen and Dinner at the "Umbrisch-Provenzialischer Markt"

Sunday, 18. September

08.15 – 08.30	Opening
08.30 – 10.15	Oral presentations (Deafness genes / Development: O1 – O7)
10.15 – 10.45	Break
10.45 – 12.15	Oral presentations (Mechanics I: O8 – O13)
12.15 – 13.30	Lunch
12.45 – 14.30	Poster presentation I P1 – P11: Deafness genes / Development, P12 - P26: Ototoxicity, P27-P44: Mechanics
14.30 – 16.00	Oral presentations (Ototoxicity I: O14 – O19)
16.00 – 16.30	Break
16.30 – 17.30	Oral presentations (Stem cells / Pharmacology: O20 – O23)
18.00	Busses leave for a visit to the ENT-clinic and Hearing Research Centre
19.45	Busses leave the ENT-clinic for the After Work Party at Zentrum Zoo.

Monday, 19. September

08.15 – 08.45	***Spoendlin Junior Award Lecture***
08.45 – 10.00	Oral presentations (Protection: O24 – O28)
10.00 – 10.30	Break
10.30 – 12.00	Oral presentations (Ion channels and transporters: O29 – O34)
12.15 – 13.30	Lunch
12.45 – 14.30	Poster presentation II P45 – P59: Protection, P60 - P67: Ion channels and transporters, P68 - P77: Stem cells / Pharmacology, P78 - P90: Neurotransmission

14.30 – 16.00	Oral presentations (Stem cells: O35 – O40)
16.00 – 16.30	Break
16.30 – 17.30	Oral presentations (Neurotransmission: O41 – O44)
19.00	Busses leave at the Kupferbau for the Gala Dinner in Sommerrefektorium Kloster Bebenhausen. Return to Tubingen at approx. 23.30.

Tuesday, 20. September

08.30 – 10.00	Oral presentations (Ototoxicity II: O45 – O50)
10.00 – 10.30	Break
10.30 – 11.45	Oral presentations (Mechanics II: O51 – O55)
11.45 – 12.00	In Memoriam Sigurd Rauch (1916 – 2003), Founder of the IEB
12.00 – 12.30	Business meeting
12.30 – 13.45	Lunch
14.00	Congress tour to the Swabian Alb, visiting the Nebelhöhle and Schloss Lichtenstein. We finish the tour with dinner at 18:00 at the "Altes Forsthaus Lichtenstein". Busses return to Tübingen at about 22:45.

IEB Workshop

Detailled Programme

Sunday, 18. September

08.15 – 08.30		Opening
		Session A: Deafness genes / Development **Chairpersons: K. Avraham, B. Mazurek**
08.30 – 08.45	O1	Compensatory mutagenesis in the transmembrane domains of connexins involved in deafness A. Dgani, S. Fleishman, E. Ophir, V.H. Hernandez, F. Mammano, N. Ben-Tal, K.B. Avraham
08.45 – 09.00	O2	The fine structure of the stria vascularis in connexin30 and connexin26 deficient mice D.A. Koch, M. Kibschull, K. Willecke, K. Jahnke, E. Winterhager, J. Lautermann
09.00 – 09.15	O3	Usher syndrome type I and II are molecularly linked via the scaffold protein harmonin E. van Wijk, J. Reiners, H. te Brinke, T. Märcker, K. Jürgens, C. Cremers, R. Roepman, U. Wolfrum, H. Kremer
09.15 – 09.30	O4	Hearing loss in conditional Rb-knockout mice T. Weber, Y. Tian, J. Zuo
09.30 – 09.45	O5	Comparison of rehabilitation results in deaf patients with confirmed and non confirmed genetic background of hearing loss. M. Wróbel, M. Magierska, W. Szyfter, M. Rydzanicz, K. Szyfter
09.45 – 10.00	O6	BMP4 regulates survival of sensory progenitors during early development of ear sensory organs C. Pujades, B. Alsina, F. Giraldez
10.00 – 10.15	O7	Differential gene expression in the organ of Corti, the modiolus and the stria vascularis of newborn rats J. Gross, F. Fuchs, B. Mazurek
10.15 – 10.45		Break

Session B: Mechanics I
Chairpersons: J. Ashmore, J. Santos-Sacchi

10.45 – 11.00	O8	Advanced optical techniques for investigating the outer hair cell protein Prestin J.N. Greeson, L. Organ, R.M. Raphael
11.00 – 11.15	O9	Developmental expression of the outer hair cell motor in the mouse T. Abe, S. Kakehata, R. Kitani, J. Santos-Sacchi, H. Shinkawa
11.15 – 11.30	O10	Prestin is an oligomer J. Zheng, G.-G. Du, C. Anderson, A. Orem, P. Dallos
11.30 – 11.45	O11	OHC-induced motion of the organ of Corti M. Nowotny, A.W. Gummer
11.45 – 12.00	O12	A mathematical model of the regulation of OHC basolateral permeability and transducer operating point G.A. O'Beirne, R.B. Patuzzi
12.00 – 12.15	O13	NompC TRP channel is a true mechanotransducer channel in the *Drosophila* ear J.T. Albert, M.C. Göpfert
12.15 – 13.30		Lunch
12.45 – 14.30		**Poster presentation I** P1 – P11: Deafness genes / Development, P12 - P26: Ototoxicity, P27 - P44: Mechanics

Session C: Ototoxicity I
Chairpersons: A. Forge, J. Schacht

14.30 – 14.45	O14	2,3-dihydroxybenzoic acid attenuates kanamycin-induced volume reduction in mouse utricular type I hair cells S.Å. Severinsen, M. Kirkegaard, J.R. Nyengaard
14.45 – 15.00	O15	Noise Induced Hearing Loss (NIHL) and N-acetyl-cysteine (NAC): just a matter of time ? G. Lorito, P. Giordano, M. Martini, S. Hatzopoulos
15.00 – 15.15	O16	Minocycline and MDL28170 can be effective in reducing gentamicin ototoxicity E. Corbacella, I. Lanzoni, S. Magosso, M. Previati, D. Ding, R. Salvi

15.15 – 15.30	O17	Carbamathione prevents synaptic edema in acoustic overexposure in chinchilla X. Ge, R. Jackson, J. Coleman
15.30 – 15.45	O18	Hyposmotic activation-induced nitric oxide production in outer hair cells of the guinea pig cochlea N. Harada, H. Nakazawa, T. Yamashita
15.45 – 16.00	O19	Imaging and quantifying the human cochlear scalae A. Voie, E. Saxon
16.00 – 16.30		Break

Session D: Stem cells / Pharmacology
Chairpersons: M. Anniko, A. Salt

16.30 – 16.45	O20	Local drug delivery to the cochlea by the biodegradable polymer and gel J. Ito, T. Nakagawa, T. Kita, T. Endo, T. Tamura, K. Iwai, Y. Matsuoka, Y. Tabata, M. Higaki
16.45 – 17.00	O21	Experiences with delivery of nanoparticles to the mouse cochlea M. Praetorius, K. Baker, B. Schick, P.K. Plinkert, H. Staecker
17.00 – 17.15	O22	Making effective vector based drugs for hearing and balance disorders: Engineering adenovectors to optimize delivery to the inner ear H. Staecker, M. Praetorius, C. Hsu, D.E. Brough
17.15 – 17.30	O23	Scala tympani perilymph sampling from the cochlear apex A. Salt, R. Mynatt, S. Hale, S. Plontke
18.00		Busses leave for a visit of the ENT-clinic and Hearing Research Centre.
19.45		Busses leave the ENT-clinic for the After Work Party at Zentrum Zoo (Schleifmühleweg 86 – see city map).

Monday, 19. September

08.15 – 08.45		***Spoendlin Junior Award Lecture***
		In vitro dissection of PKCβ1 function in deafferented cochlear neurons F. Lallemend
		Session E: Protection **Chairpersons: J. Adams, L. Nordang**
08.45 – 09.00	O24	Non-sensory cells contribute towards protection of hair cells from acoustic trauma J.C. Adams
09.00 – 09.15	O25	Induction of heat shock proteins in the cochlea in normal and *Hsf1*-null mice T.-W.L. Gong, D. Fairfield, R.A. Altschuler, M.I. Lomax
09.15 – 09.30	O26	Neural stem cells suppress the shift in the hearing threshold caused by ischemia-reperfusion injury of the gerbil cochlea N. Hakuba, I. Morizane, K. Gyo
09.30 – 09.45	O27	Inhibition of caspases promotes recovery of hearing in a rat model of acute cochlear mitochondrial dysfunction K. Mizutari, K. Kamiya, Y. Fujinami, S. Nakagawa, M. Fujii, T. Matsunaga
09.45 – 10.00	O28	Brain-derived neurotrophic factor, a protector from toxic inner ear damage L. Nordang, A. Lidian, B. Linder, M. Anniko
10.00 – 10.30		Break
		Session F: Ion channels and transporters **Chairpersons: J.-L. Puel, I. Sziklai**
10.30 – 10.45	O29	The signal transduction pathway for the dopamine D1 receptor in the guinea pig cochlea X. Niu, B. Canlon
10.45 – 11.00	O30	Discoidin domain receptor 1 tyrosine kinase (DDR1) has an essential role in the inner ear A.-M. Meyer zum Gottesberge, B. Beirowski, T. Massing, M. Weber, O. Gross
11.00 – 11.15	O31	Aquaporin 2 in the saccus endolymphaticus: the subcellular localization B. Hirt, Z. Penkova, M. Müller, H. Löwenheim

11.15 – 11.30	O32	Vestibular function of experimental endolymphatic hydrops in guinea pig A. Kakigi, D. Taguchi, T. Takeda
11.30 – 11.45	O33	Effects of lithium on endolymph homeostasis and experimentally-induced endolymphatic hydrops T. Takeda, A. Kakigi, S. Takeda, S. Nishioka, S. Sawada, H. Azuma, K. Fukushima
11.45 – 12.00	O34	A novel gene for *Enlarged Vestibular Aqueduct Syndrome* (EVA-Syndrome) ? R. Birkenhäger, A.J. Zimmer, A. Aschendorff, T. Klenzner, J. Schipper, R. Laszig
12.15 – 13.30		Lunch
12.45 – 14.30		**Poster presentation II** P45 – P59: Protection, P60 - P67: Ion channels and transporters, P68 - P77: Stem cells / Pharmacology, P78 - P90: Neurotransmission
		Session G: Stem cells **Chairpersons: P. Lefebvre, G. van Camp**
14.30 – 14.45	O35	Neurite outgrowth of ES cell-derived neurons engrafted into the cochlea modiolus of guinea pigs T. Nakagawa, T. Okano, T. Endo, T. Kita, T.-S. Kim, M. Matsumoto, T. Ohono, T. Sakamoto, J. Ito
14.45 – 15.00	O36	Investigation of neural stem cell-derived donor contribution in the inner ear S. Volkenstein, C. Aletsee, R. Mlynski, D. Brors, A.M. Müller, S. Dazert
15.00 – 15.15	O37	Restoration of vestibular peripheral nervous systems by stem cell-derived neurons T.-S. Kim, T. Nakagawa, T. Kita, T. Higashi, M. Matsumoto, K. Kojima, T. Sakamoto, J. Ito
15.15 – 15.30	O38	Survival and integration of mouse embryonic stem cells in guinea pig cochlea R.A. Altschuler, N.L. Wys, D.M. Prieskorn, J.M. Velkey, K.S. O'Shea, J.M. Miller
15.30 – 15.45	O39	Differentiation of mouse spiral ganglion neurons in vitro A.J. Nicholl, D.I. Cacciabue-Rivolta, M.C. Holley

15.45 – 16.00 O40 Innervation of embryonic stem cell-derived neurons into auditory epithelia of mice
M. Matsumoto, T. Nakagawa, T. Higashi, T.-S. Kim, K. Kojima, T. Kita, T. Sakamoto, J. Ito

16.00 – 16.30 Break

Session H: Neurotransmission
Chairpersons: E. Glowatzki, T. Moser

16.30 – 16.45 O41 Persistence of neonatal Ca^{2+} action potentials and developmental arrest of ionic conductances in inner hair cells of hypothyroid rats
N. Brandt, C. Braig, H. Winter, S. Münkner, S. Kuhn, M. Knipper, J. Engel

16.45 – 17.00 O42 Non-synaptic communication of dopaminergic and glutamatergic cochlear neurotransmission
G. Halmos, B. Lendvai, T. Zelles, S.E. Vizi

17.00 – 17.15 O43 Input-output relations of auditory nerve fibers with low spontaneous spike rate
W. Horst, J. McGee, E. Walsh

17.15 – 17.30 O44 Analysis of the human inner ear
H. Rask-Andersen, M. Boström, M. Anderson, L. Pamulova, A. Schrott-Fischer, R. Gluckert, C. Pfaller

19.00 Busses leave at the Kupferbau for the Gala Dinner in Sommerrefektorium Kloster Bebenhausen. Return to Tübingen at approx. 23.30.

Tuesday, 20. September

Session I: Ototoxicity II
Chairpersons: J. Miller, J. Smolders

08.30 – 08.45	O45	Restoration of immune mediated hearing loss by adoptive cellular gene therapy or DNA vaccine T.J. Yoo, B. Zhuo, M. Kermany, C. Cai, Q. Cai, J. Zhang, I.H. Tarner, C.G. Fathman, P. Kim, J.W. Kim
08.45 – 09.00	O46	Time-related formation and distribution of cisplatin-DNA adducts in the guinea pig cochlea J.C.M.J. de Groot, M.W.M. van Ruijven, E.G.J. Hendriksen, G.F. Smoorenburg
09.00 – 09.15	O47	No-show D. Labbé, N. Koop, O. Michel
09.15 – 09.30	O48	Drugs, noise, age - a radical triad S.-H. Sha, H. Jiang, J. Schacht
09.30 – 09.45	O49	Platinum concentrations in various tissues and fluids in relation to ototoxic effects of cisplatin J.W. Sepmeijer, S.F.L. Klis, G.F. Smoorenburg
09.45 – 10.00	O50	Cochlear degeneration in old Fischer 344 rats D. Buckiova, J. Popelar, J. Syka
10.00 – 10.30		Break

Session J: Mechanics II
Chairpersons: B. Lonsbury-Martin, A.L. Nuttall

10.30 – 10.45	O51	Distortion product otoacoustic emissions in adult and old Fischer 344 rats J. Popelar, D. Groh, J. Pelánová, J. Syka
10.45 – 11.00	O52	Adaptation of distortion product otoacoustic emissions in L_1,L_2 space in humans D.K. Meinke, B.B. Stagner, G.K. Martin, B.L. Lonsbury-Martin
11.00 – 11.15	O53	The bounce phenomenon: investigation in humans Z. Kevanishvili, G. Hofmann, I. Burdzgla, M. Pietsch, Y. Yarin, T. Zahnert
11.15 – 11.30	O54	Biophysics of Drosophila hearing B. Nadrowski, J.T. Albert, M.C. Göpfert

11.30 – 11.45	O55	Individual characteristics of members of the SLC26 family in vertebrates and their homologues in insects M. Knipper, T. Weber, D. Oliver, H. Winter, M. Göpfert, J. Albert, C. Braig, J. Cimerman, U. Zimmermann
11.45 – 12.00		In Memoriam Sigurd Rauch, (1916 – 2003) Founder of the IEB A. Meyer zum Gottesberge
12.00 – 12.30		Business meeting
12.30 – 13.45		Lunch
14.00		Busses leave for Congress tour to the Swabian Alb, visiting the Nebelhöhle and Schloss Lichtenstein. We finish the tour with dinner at 18:00 at the "Altes Forsthaus Lichtenstein". Busses return to Tübingen at about 22:45.

Posters

Posters should remain posted for the entire length of the meeting.
Authors should be at their posters during poster presentation time.
Poster board dimensions: 1 m wide, 2 m high.

Deafness genes / Development

P1 Mutation of β_2-glycoprotein I and idiopathic sudden sensorineural hearing loss in a Korean population
J.Y. Byun, C.I. Cha, S.G. Yeo, B.Y. Cui, J.S. Cho

P2 Familial Meniere´s disease; clinical and genetic investigations
C. Frykholm, H.-C. Larsen, N. Dahl, J. Klar, U. Friberg

P3 Progressive degeneration of stria vascularis in the developing German waltzing guinea pig inner ear
Z. Jin, M. Ulfendahl, L. Järlebark

P4 Auditory function in cysteine string protein α knockout mice
N. Strenzke, D. Khimich, A. Bulankina, R. Fernandez Chacon, T. Südhof, T. Moser

P5 No-show
S. Noorbakhsh, M. Farhadi, A. Tabatabaei

P6 *GJB* mutations and computational modeling in patients with nonsyndromic hearing impairment from Hungary
T. Tóth, B. Haack, S. Kupka, I. Komaromi, L. Muszbek, N. Blin, M. Pfister, I. Sziklai

P7 Hearing loss in Igfl deficient mice
R. Riquelme, R. Cediel, J. Contreras, J.A. Morales, A. Diaz, I. Varela-Nieto

P8 Expression of β–I, β-III and α-V integrins in inner ear tissue, and OC-Two cells
I. Brunetta, S. Casalotti, A. Forge

P9 Characterization of cells cultured from hair cell epithelia of new-born mice
O. Gleich, J.-T. Fraenzer, F.P. Wachs, K. Licht, J. Strutz

P10 Distinct regulation of ion channels during final differentiation of outer hair cells
H. Winter, C. Braig, U. Zimmermann, M. Knirsch, H.-S. Geisler, I. Köpschall, K. Rohbock, J. Engel, M. Knipper

P11 Application of RT-PCR and in situ hybridization on human temporal bone
K. Sunami, D. Theil, T. Derfuss, S. Herberger, I. Paripovic, H. Yamane, M. Strupp, T. Brandt

Ototoxicity

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Symposium

"Terminal Differentiation: a Challenge for Regeneration"

Abstracts

S1 Developmental maturation of inner hair cell function in mice

C.J. Kros

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Mature inner hair cells (IHCs) respond to sound with fast, graded receptor potentials. However, before the onset of hearing at P12 IHCs fire spontaneous and evoked action potentials (Kros et al 1998). Developmental changes in ion channel expression underlie this switch in function.

Just after terminal mitosis at E14 small and slow delayed-rectifier K currents can be recorded which increase in size during development (Marcotti et al 2003a). From E16.5 IHCs express Ca and Na currents (Marcotti et al 2003b). This mix of currents enables the IHCs to fire spontaneous action potentials between E17.5 and P6. The Ca current is necessary for the action potentials whereas the Na current shortens the inter-spike interval. In the second postnatal week IHCs still fire action potentials in response to current injection, but no longer spontaneously, due to further changes in the K currents.

From just after birth until the onset of hearing, IHCs are transiently innervated by efferent nerve fibres and they hyperpolarize in response to ACh application (Glowatzki & Fuchs 2000), due to activation of an SK current by Ca flowing in through α9α10 nACh receptors (Marcotti et al 2004). Efferent activity would thus inhibit spontaneous action potentials. Surprisingly, the SK current is also important for sustaining repetitive action potentials, in the absence of ACh, as it is activated by Ca influx through the Ca channels too (Marcotti et al 2004).

The transition to a functionally mature sensory receptor comes about at the onset of hearing by the expression of a large and fast BK current, $I_{K,f}$ (Kros et al 1998). At the same time a KCNQ4 current, $I_{K,n}$, is expressed which sets the resting potential of mature IHCs (Marcotti et al 2003a; Oliver et al 2003). Developmental changes in the exocytotic machinery further contribute to the maturation of postsynaptic IHC signalling (Johnson et al 2005).

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Glowatzki & Fuchs (2000) Science 288:2366
Johnson et al (2005) J Physiol 563:177
Kros et al (1998) Nature 394:281
Marcotti et al (2003a) J Physiol 548:383
Marcotti et al (2003b) J Physiol 552:743
Marcotti et al (2004) J Physiol 560:691
Oliver et al (2003) J Neurosci 23:2141

S2 Physiology and pharmacology of the inner hair cell synaptic complex

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Within the cochlea, the sensory inner hair cells (IHCs), which transduce mechanical displacement of the basilar membrane into neural activity, release glutamate to act on postsynaptic receptor channels located on dendrites of primary auditory neurons. In turn the activity of the postsynaptic auditory dendrites is modulated by a variety of lateral efferent neurotransmitters. This presentation will review findings obtained at the IHC synaptic complex on cochlear slices and on an in vivo model of perilymphatic perfusion coupling gross cochlear and auditory nerve single unit recordings. These functional studies demonstrated that (1) in physiological conditions, the activity of auditory nerve fibers is mediated by AMPA, but not kainate or NMDA receptors, and (2) this activity is tonically modulated by dopamine, one of the lateral efferent neurotransmitters. Since we reported a structural and functional recovery after local application of AMPA [Puel et al., C R Acad Sci III 1995;318: 67–75], several studies have confirmed this regenerative capacity of auditory neurites using different excitotoxic protocols, such as a local application of kainate [Zheng et al., Hear Res 1997;105:65–76] or a noise trauma [Puel et al., Neuroreport 1998;9:2109–2114]. To summarize, after a massive destruction of all dendrites of the primary auditory neurons connected with the IHCs, there is a regrowth and a neoformation of synapses accounting for functional recovery. Here we will emphasize two points during this process of synaptic repair, namely the respective roles of NMDA receptors and of the lateral efferent neurotransmitters, which may account for pathological activities such as tinnitus.

S3 Cellular interactions and maintenance of phenotype in the regenerating inner ear

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The avian inner ear possesses a remarkable ability to regenerate sensory hair cells after acoustic trauma or ototoxic injury. Although several distinct cellular mechanisms may contribute to the production of replacement hair cells, the majority of such cells are produced via renewed proliferation within the injured sensory epithelium. Numerous studies have demonstrated that supporting cells can re-enter the cell cycle after epithelial injury and serve as progenitors for regenerated hair cells. It is not clear, however, whether all epithelial supporting cells can proliferate after injury or whether only a subpopulation of those cells participates in the regenerative process. As a first step towards the resolution of this issue, we have studied the expression of supporting cell markers in the avian cochlea and utricle. Supporting cells in the avian ear express a number of identified proteins, including several cell adhesion molecules (E- and N-cadherin, N-CAM), secreted extracellular matrix molecules (e.g., alpha and beta tectorin, laminin, tenascin), cell signaling molecules (e.g., the supporting cell antigen – SCA, a novel receptor tyrosine phosphate) and certain transcription factors (e.g., GATA3, PAX2). In this talk, I will describe the expression patterns of many of these markers during the early phases of epithelial repair and regeneration. So far, we have not found evidence for a distinct 'stem cell' population in the avian ear. Instead, our results are consistent with the notion that that all supporting cells constitute a single phenotypic class, which may undergo limited de-differentiation prior to cell cycle entry.

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S4 Regeneration in the metazoans: Why does it happen? Lessons from simple model organisms

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The problem of regeneration is fundamentally a problem of tissue homeostasis that involves either being able to replace cells lost to normal "wear and tear" (cell turnover), or injury (trauma). This is particularly significant for organisms possessing relatively long life spans, in which maintenance of all body parts and their functional integration is required for many years in order for the individual to thrive. Thus, the replacement of differentiated cells is a major challenge all multicellular organisms must face. Humans, for example, with life spans often reaching 80 years must replace an estimated 10 billion cells lost to cell turnover every day. Although such regenerative properties are widely distributed throughout the Metazoa, it is not entirely clear if regeneration in multicellular animals follows an evolutionarily conserved program or are the result of evolutionary convergence, i.e. a situation whereby individual species may have invented their own way to regenerate. While the latter possibility has not been formally ruled out, the limited number, extensive conservation and characteristic pleiotropy of signaling pathways involved in tissue formation and maintenance in all animals studied to date, suggests that regeneration (like development) may be the product of evolutionary conservation. The identification of a model organism in which this problem can be easily dissected and rapidly understood would allows to gain key molecular and biological insights on regenerative processes. To begin a systematic investigation of the mechanisms underpinning animal regeneration, we have chosen to introduce RNA-mediated genetic interference and other genomic and molecular biological approaches to the study of planarians, a classic model system of regeneration, tissue homeostasis, and stem cell biology. Our efforts thus far, have uncovered 240 genes functionally associated with regenerative processes. Here, I will discuss the implications of these findings in the context of regeneration in higher organisms.

S5 Mechanisms in the induction of lens regeneration

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Introduction: The process of lens regeneration in adult newts is one of the best examples of regeneration by transdifferentiation. After lentectomy, the pigment epithelial cells (PECs) of the dorsal iris only dedifferentiate and then differentiate to form a new lens. The restriction of this process from the ventral iris of the newt as well as in other animals introduces important questions on the regulation of regeneration. Based on the above, we have decided to search for molecules, which can be specific to the newt dorsal iris, and therefore involved in the regulation and induction of lens regeneration.

Methods: Ventral iris PECs were either transfected with known eye regulators or treated with soluble factors, which are involved in the BMP signaling.

Results: We have identified two pathways that are involved in the induction of lens regeneration. We will present data indicating that in one pathway six-3 and retinoic acid and in the other inhibition of the BMP pathway induce lens transdifferentiation from the ventral iris. Also we find quite unique regulatory events in gene expression indicating that gene levels must overcome a particular threshold for the induction of regeneration.

Conclusions: Induction of lens regeneration is possible from incompetent tissues.

S6 Transdifferentiation of non-sensory cells as a strategy for hair cell regeneration

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Introduction: Epithelial cells in the auditory epithelium are terminally differentiated. In birds and other non-mammalian species, supporting cells can change their phenotype and become new hair cells following lesions that eliminate the original hair cells. The ability to change the phenotype (transdifferentiate) is rare, and its molecular mechanism not well understood. We attempted to induce transdifferentiation of supporting cells to hair cells using genetic manipulation. Specifically, we tested whether induced over-expression of *Atoh1*, a gene that is necessary for hair cell differentiation, would induce a phenotypic transdifferentiation in non-sensory cells that remain in the deafened cochlea.

Materials and Methods: Adult guinea pigs were systemically deafened by kanamycin and ethacrynic acid, resulting in bilateral elimination of hair cell and profound deafness, verified by ABR audiometry. Four days after the insult, an adenovirus vector was inoculated into the scala media of the left cochlea. Gene inserts were *Atoh1, Atoh1-GFP* or controls (empty vector). At different time-points, ABRs were measured, animals sacrificed and ears assessed with SEM, epi-fluorescence or plastic sections.

Results: Remaining non-sensory cells showed robust expression of the *Atoh1* transgene. Two months after the inoculation, hair cells were found in the *Atoh1*-treated ear but not in control ears. Hearing thresholds improved in *Atoh1*-treated ears.

Conclusions: We have attempted to switch cell fate in non-sensory cochlear cells and induce transdifferentiation into new hair cells. The ability of *Atoh1* over-expression to induce such transdifferentiation provides proof for the principal that expressing developmental genes can be harnessed for hair cell regeneration therapy. Several important aspects of this technology, including safety, reliability and efficiently, need to be resolved or improved before clinical applications can be planned.

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S7 Developmental mechanisms in the context of regeneration

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The search for mechanisms of sensory regeneration in the mammalian inner ear has largely been informed by studies of development. This is unsurprising yet the molecular environment of adult sensory epithelia and neurons is quite different to that of the progenitor cells during embryonic development. In the chick, where regeneration occurs naturally, there is clear evidence for recapitulation of developmental signalling pathways. Regeneration fails in mammals, either because of a lack of inductive signals or the expression of inhibitory ones. However, the transcription factor Atoh1 can induce new hair cells in adult rodents and manipulation of the appropriate signalling pathways should be an important part of future regenerative therapies. We aim to elucidate the signalling pathways associated with gata3, another transcription factor that is essential for cochlear development, linked with human deafness and re-expressed during regeneration in the chick basilar papilla. We have used 3 conditionally immortal cell lines derived from the mouse otocyst to model early developmental events during the differentiation of hair cells and spiral ganglion neurons. We have identified genes associated with *gata3* by screening cells with Affymetrix microarrays during differentiation in vitro. Clustered gene expression profiles from all 3 cell lines revealed 30 genes consistently linked to *gata3* and 7 of these were associated with IGF-signalling. One of them was protein kinase B (PKB), which has a direct functional association with the cyclin-dependent kinase inhibitor p27kip1. Analysis of these molecules in vivo and in vitro revealed a relationship between gata3, the phosphorylation of PKB and the nuclear localisation of p27kip1. When *gata3* was knocked down, additional IGF-signalling molecules were down-regulated, including the IGF-1 receptor and the IGF binding proteins 4 and 7. We conclude that gata3 modulates cellular responses to IGF and that this may have important implications for the expansion of the spiral ganglion cell population during development and potentially during regeneration.

S8 Applications of stem cells for the restoration of hearing loss

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The lack of regenerative capacity of the adult mammalian cochlea is the main reason for the permanence of hearing loss. We have shown that stem cells from the vestibular system in adult mice undergo cell division in vitro and can be differentiated into multiple cell types and we have isolated stem cells from the newborn cochlea by their ability to form spheres. The ability for stem cells to form spheres in the mouse cochlea decreases about 100-fold during the second and third postnatal weeks, indicating that the lack of regenerative capacity of the adult mammalian cochlea is either a result of an early postnatal loss of stem cells or diminishment of stem cell features of maturing cochlear cells. Sphere-forming stem cells from the early postnatal organ of Corti give rise to cells that express multiple hair cell markers and acquire functional ion channels reminiscent of nascent hair cells. Spiral ganglion stem cells display features of neural stem cells and can give rise to neurons and glial cell types.

We have attempted to regenerate connections to hair cells using neurons derived from both inner ear stem cells and from neural progenitors derived from embryonic stem cells and we have attempted to replace auditory neurons in vivo using a gerbil model of primary neuronal degeneration and in organotypic culture of the organ of Corti. Inner ear stem cell-derived neurons formed synapses at sites of contact with hair cells in the in vitro model. In the gerbil model using ouabain treatment to eliminate spiral ganglion neurons and leave hair cells intact, injection of embryonic stem cell-derived neural progenitor cells into the base of the cochlea resulted in neuronal differentiation with extensive processes projecting from the central core of the cochlea toward the denervated organ of Corti 12 days after surgery. After 64 days, the grafts had sent out abundant processes that grew in fasciculating bundles that projected into the osseous spiral lamina and ultimately into the organ of Corti where they made contact with hair cells.

IEB Workshop, Abstracts

Session A:

Deafness genes / Development

Chairpersons: K. Avraham
B. Mazurek

O1 Compensatory mutagenesis in the transmembrane domains of connexins involved in deafness

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Connexins are a family of transmembrane (TM) proteins that form gap junctions between neighboring cells and allow intercellular communication. The wide range of involvement of connexins in pathological conditions in humans, and particularly the involvement of connexin 26 and 30 in hearing loss make it important to further investigate the molecular mechanisms and physical interactions leading to disease. Recently, Cryo-electron microscopy (cryo-EM) at 5.7Å in-plane resolution was used to compute a model structure of the gap junction transmembrane (TM) domain (Fleishman et al. 2004 *Mol Cell*). The final model, specifying the coordinates of the Cα atoms in the TM domain, provided a structural basis for understanding the different physiological effects of almost 30 mutations and polymorphisms in terms of structural deformations at the interfaces between the helices. The model, which needs to be validated, makes a prediction of the physical forces that stabilize the TM domains of connexins. To test these suggestions experimentally, we are taking the approach of compensatory mutagenesis, based on the assumption that the phenotypic effects of a disease-causing mutation in one helix of the protein can be rescued by a mutation simultaneously expressed on a neighboring helix. Sets of point mutations were introduced into connexin 26. Wild type, single and double mutant connexins were expressed in cell lines and tested for protein localization in the cell. For the mutant proteins that exhibit normal localization, we are using a second assay to test these mutant proteins' functionality. Coupled cells expressing the relevant mutations are tested by double patch-clamp for the electrical coupling of the cells and for the ability of the channel to transfer molecules between neighboring cells. Our results offer a first glimpse into the physical forces underlying disease in the TM domain of connexins.

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O2 The fine structure of the stria vascularis in connexin30 and connexin26 deficient mice.

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Introduction: Gap junctions consist of two hemi-channels (connexons), each build up from six subunits (connexins), and allow ions and small molecules to traverse between connected cells. Connexin26 (Cx26) and connexin30 (Cx30) are the predominantly expressed isoforms in the mouse inner ear and are suggested to be involved in the potassium recycling mechanism within the cochlea. Deficiency in Cx26 or Cx30 leads to histopathological damage of the organ of Corti and functional impairment with subsequent hearing loss. However, morphological changes of the stria vascularis and the spiral ligament have not yet been described.

Materials and Methods: We investigated the cochlear lateral wall of Cx30 (and Cx26) deficient and wild type mice by transmission electron microscopy at postnatal day 12(13), postnatal day 19(20) and at adult stage. Results: In Cx30 deficient mice we found a reorganization of the stria vascularis from a multilayered highly differentiated epithelium into a cuboid-like three-layered epithelium as well as oedematous changes in the spiral ligament. In Cx26 deficient mice we detected degenerative changes of all three different strial cell types.

Conclusions: Our results suggest that a dysfunction and subsequent degeneration of the stria vascularis may result in early hearing loss. However, we cannot exclude that the histological changes may be a consequence rather than the origin of cochlear dysfunction.

O3 Usher syndrome type I and II are molecularly linked via the scaffold protein harmonin

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Introduction: Usher syndrome (USH) is characterized by congenital deafness and retinitis pigmentosa and is the most common cause of combined deaf-blindness. Three types of the syndrome can be distinguished based on clinical diagnosis, USH1, USH2 and USH3. Until now 7 USH1, 3 USH2 and 1 USH3 loci have been identified. All USH1 proteins physically interact with the scaffold protein harmonin. The aim of our study is to verify whether the USH2 proteins and NBC3, a candidate for USH2B, are also part of the same supramolecular USH protein network through interaction with harmonin.

Material and Methods: Interaction studies were performed with yeast-two-hybrid analysis and verified with GST-pull down and co-immunoprecipitation assays. Immunohistochemistry was performed on cryosections of the mouse inner ear and retina using antibodies generated against the USH2 proteins, NBC3 and harmonin.

Results: In vitro and in vivo interaction studies revealed that the intracellular tails of USH2A, NBC3 and VLGR1b interact with the PDZ1 domain of harmonin via their C-terminal PDZ type 1 binding motif. Immunohistochemical subcellular localization of the USH1C and USH2 proteins showed colocalization at the synaptic regions and in the stereocilia of the inner and outer hair cells in the organ of Corti, and in the outer plexiform layer in the retina.

Conclusions: Our results provide the first evidence for the existence of a common supramolecular protein network of USH1 and USH2 proteins. All known USH1 and USH2 proteins are molecularly linked by the scaffold protein harmonin. In the inner ear, the USH protein network might play a role in the development and maintenance of the structure and organization of the stereocilia and in their function. In the sensory cells of both the retina and the inner ear, the USH proteins are thought to participate in the structural and functional organisation of the synaptic junction. Dysfunctioning or absence of one of the members of the USH protein network may lead to the degeneration of the stereocilia and dysfunctioning of the synapses in both hair cells and photoreceptors, thus causing deafness and retinitis pigmentosa.

O4 Hearing loss in conditional Rb-knockout mice

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Conditional knockout studies using a late embryonic Cre-expressing line showed that the retinoblastoma protein (Rb) is required for the normal development of inner ear sensory epithelia (Sage et al. 2005). Loss of Rb in the developing otocyst leads to the production of supernumerary hair cells in the vestibular and cochlear system, consistent with its role as cell cycle regulator. There is increasing evidence that in addition to its traditional role, Rb is also required for cell differentiation and maintenance of postmitotic cells. In this study we investigated whether Rb also plays a role during the postnatal maturation of inner ear hair cells by crossing conditional Rb knockout mice (Vooijs et al. 2002) with a hair cell specific postnatal Cre-expressing mouse-line (Tian et al. 2004).

The mice were viable and reached adulthood. They behaved normally in **rotor-rod tests**, indicating that ongoing expression of Rb in postmitotic hair cells is not critical for the maintenance of the vestibular system (N=7, controls N=26). **ABR-measurements** revealed severe deafness of homozygous Rb-knockout mice; hearing threshold elevations of 40-45 dB at all frequencies tested (click, 4, 16, 8 and 32 kHz) were observed (N=6). Heterozygous knockout mice and Cre-negative littermate controls had normal hearing (N=9). **Cross-sections** of the cochleae pointed to a normal morphology without obvious signs of degeneration or proliferation. Consistent with these results, the expression of prestin and myosin VII appeared to be normal.

This is the first study analyzing Rb's function in the postmitotic, adult mouse inner ear. Our results reveal a critical role of the tumor-suppressor Rb for hearing function which is apparently different from its role as cell cycle regulator. Interestingly, despite causing deafness, loss of Rb does not seem to induce rapid degeneration of the organ of Corti. Presently we concentrate our studies on the identification of the critical targets of Rb supposed to be causative for the observed phenotype.

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O5 Comparison of rehabilitation results in deaf patients with confirmed and non confirmed genetic background of hearing loss.

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Introduction: There are several parameters which have impact on progress in rehabilitation of deaf patients. Apart of individual factors such as IQ, duration of deafness, there are also environmental ones. Parents involvement, close environment - difficult to be objectively estimated - have influence on sound detection and speech understanding. It would be important to introduce prognostic tools for rehabilitation progress to indicate patients with worse or better results in order to adjust the rehabilitation program in addition to described variabilities. Authors took an attempt to find out if additional parameter – molecular defect interfere with achieved rehabilitation results.

Materials and Methods: Group of 51 subjects were selected from 165 CI patients with prelingual deafness operated at Otolaryngology Department, Poznan University of Medical Sciences in Poland. Molecular analysis was based on 35delG of *GJB2* identification with PCR-RFLP technique, a quick, simple and reliable tool. Logopedic assessment was performed with test of seven sounds, which assess detection, discrimination and identification of sounds at early stages – 1, 3 and 6 months after implantation.

Results: Conducted comparison analysis for patients with confirmed and non confirmed mutation in GJB2 gene revealed that for 35delG positive patients ability for sound detection of all seven sounds on first, third and sixth month was 11.1%, 81.5% and 100%, identification – 0, 29.6% and 77.7%; discrimination – 11.1%, 66.6% and 100% where as in patients without 35delG mutation – detection was on the level of 0, 62.5% and 95.8, identification 0, 12.5%%, 58.3% and discrimination 0, 62.5%, 95.8%.

Conclusions: Results indicate that patients with 35delG mutation achieve better results in rehabilitation.

O6 BMP4 regulates survival of sensory progenitors during early development of ear sensory organs

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Bone morphogenetic proteins (BMPs) are diffusible molecules involved in a variety of cellular interactions during development. *BMP4* expression accompanies the development of the ear sensory organs during patterning and specification of neuronal of sensory cell fates yet, there is no understanding of the role of BMP4 in these developmental processes. The present work was aimed at exploring the effects of BMP-signaling on the development of sensory hair-cells. For this purpose, we studied gene expression, cell proliferation and cell death in isolated chick otic vesicles that were grown in vitro in the presence of recombinant BMP4 or the BMP-inhibitor Noggin. *Cath1* was used as a marker for hair-cell specification. BMP4 reduced the number of *Cath1* positive cells and, conversely, Noggin increased the size of the sensory patches and number of *Cath1*-positive cells. The effect of BMP4 was irreversible and only occurred before cell fate specification. *Lfng* and *FGF10*, were both expressed in the prosensory domain before *Cath1*, and the expression persisted after BMP4 or Noggin, suggesting that the specification of the prosensory field was not affected by BMP signaling. The expression of *BMP4* was suppressed by BMP4 and induced by Noggin. Analysis of Brdu incorporation and P-Histone-3 labeling indicated that the effects of BMP4 were due to the reduction in the number of actively proliferating progenitors. Indeed, BMP4 induced death of cell progenitors within the prosensory domain of the otic vesicle. The effects of BMP4 were reduced by inhibition of DNA-synthesis with Hydroxyurea, but not blocked by the mitotic inhibition with Nocodazolee, suggesting that BMP4 exerts a pro-aptotic effect of during the G2-phase of the cell-cycle. We like to propose that BMP4 is crucial for regulating progenitors survival and for the establishing the final number of sensory hair-cells. Supported by MEC and FIS/ISCIII.

O7 Differential gene expression in the organ of Corti, the modiolus and the stria vascularis of newborn rats

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Introduction: The cochlea consists of three main complex cellular structures, each serving a specific function: Organ of Corti (OC), modiolus (MO) and stria vascularis (SV). Hair cells of the OC and neurons of the spiral ganglion located in the MO are highly vulnerable. Understanding of gene expression profiles of the main structures of the cochlea is important, if new strategies for hair cell and neuron protection are to be developed.

Methods: Microarray technology was used to analyze the regional gene expression in the cochlea of 3-5 day old Wistar rats. The membranous rat cochleae were prepared, and the cochlea was dissected into the OC, SV, and MO. Total RNA was prepared using RNAeasy kit (Qiagen, Hilden, Germany). Hybridization to the Affymetrix Rat Neurobiology U34 Array (1200 gene transcripts) and detection of signals were performed by the Laboratory for Functional Genome Research, Charité - University Medicine Berlin (U. Ungethüm, R.-J. Kuban).

Results and Conclusions: The specific gene expression of OC is characterized by genes involved in the regulation of calcium (Ca^{2+}) and potassium (K^+) homeostasis (S100 Ca^{2+} binding protein A1, plasma membrane Ca^{2+} transporting ATPase, K^+ channel Kcnj 16) and neurotransmission (high affinity glutamate transporterSlc1a3, γ aminobutyric acid receptor Gabbr1). The specific gene expression of MO is characterized by genes involved in growth and maturation (e.g. insulin-like growth factor (Igf)2, prion protein). The specific gene expression of SV is characterized by genes involved in ion homeostasis (e.g. K^+, sodium transporting ATPases Atp1a1, Atp1b2), blood flow (inducible nitric oxide synthase 2) and the oxygen radical defense system (Heme oxygenase Hmox1). The analysis of insulin-like growth factor (Igf) associated genes points to the latter's specific role in inner ear development. The insulin-like growth factor binding protein (Igfbp)3 is highly expressed in OC, the Igfbp6 and Igf2 in MO and Igfbp2 and Igf1 in SV.

Session B:

Mechanics I

Chairpersons: J. Ashmore
J. Santos-Sacchi

O8 Advanced optical techniques for investigating the outer hair cell protein Prestin

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Prestin, a transmembrane protein, confers electromotility to cochlear outer hair cells (OHCs) by converting changes in membrane potential into mechanical forces manifested as axial length changes. Although the complete mechanism of electromotility is not presently known, this somatic deformation is responsible for the remarkable sensitivity and frequency selectivity of the mammalian auditory system. In order to characterize the electromotile process and its role in OHC function, a complete understanding of the constituents of the plasma membrane, both lipids and proteins, as well as their interactions, is crucial. Unfortunately, ex vivo, OHCs have a short lifetime making it necessary to study prestin in cellular model systems such as human embryonic kidney cells (HEKs). We have implemented fluorescence recovery after photobleaching (FRAP) to evaluate the lateral diffusion of prestin and voltage-sensitive dyes in HEK cells. Mobility data provides information on the extent of interaction between membrane components and the effect of the membrane environment on prestin. We are also utilizing fluorescence resonance energy transfer (FRET) to assay the degree of prestin self-association. Preliminary FRET experiments reveal significant prestin-prestin interactions, consistent with the gradual recovery observed in FRAP experiments. The use of these two advanced techniques in tandem allows for more sophisticated and quantitative studies of the activity of prestin and the mechanism of electromotility than through use of each technique alone.

O9 Developmental expression of the outer hair cell motor in the mouse

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The development of motor protein activity in the lateral membrane of the mouse outer hair cell (OHC) from P5 to P18 was investigated under whole cell voltage clamp. Voltage-dependent, non-linear capacitance (Cv), which represents the conformational fluctuations of the motor molecule, progressively increased during development. At P12, the onset of hearing in the mouse, Cv was about 70 % of the mature level. Cv saturated at P18 when hearing shows full maturation. On the other hand, C_{lin}, which represents the membrane area of the OHC, showed a relatively small increase with development, reaching steady-state at P10. This early maturation of linear capacitance is further supported by morphologic estimates of surface area during development. These results, in light of recent prestin knockout experiments, show that rather than the incorporation of new motors into the lateral membrane after P10, molecular motors mature to augment nonlinear capacitance. Thus, current estimates of motor protein density based on charge movement may be exaggerated. A corresponding indicator of motor maturation, the motor's operating voltage midpoint, V_{pkcm}, tended to shift to depolarized potentials during postnatal development, although it was unstable prior to P10. However, after P14, V_{pkcm} reached a steady state level near –67 mV, suggesting that intrinsic membrane tension or intracellular chloride, each of which can modulate V_{pkcm}, may mature at P14. These developmental data significantly alter our understanding of the cellular mechanisms that control cochlear amplification.

O10 Prestin is an oligomer

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Introduction: Prestin has 744 amino acids and provides the molecular basis for outer hair cell (OHC) electromotility, which is crucial for sensitivity and frequency selectivity in the mammalian cochlea. It is thought that these abundantly expressed motor proteins (prestin) constitute the 11-nm particles present in the OHC's lateral membrane. Because the estimated size of a prestin monomer is too small to form an 11-nm particle, the possibility of prestin's oligomerization is studied.

Materials and Methods: Prestin cDNA was transfected into different expression systems including yeast and mammalian cell lines. Prestin protein was studied in LDS-PAGE/Western blot or PFO-PAGE/Western blot. Various reducing reagents including β-mercaptoethanol, dithiothreitol and ethanedithiol, different detergents, 6 M urea and chemical cross-linking reagents were used to treat prestin-expressing cells.

Results and Conclusion: Two bands were consistently found in different host cells including prestin-expressing OHCs and prestin-expressing yeast in LDS-PAGE/Western blot. The size of the higher molecular-weight band is about twice that of the lower prestin band. These data imply that prestin may exist as a dimer or multimer. Prestin dimer is resistant to various reagents, detergents and 6 M urea, indicating that prestin dimer is very stable structure. We also used PFO (perfluo octanoiate) to study prestin's oligomerization. PFO was reported as a "biological detergent," less disruptive of interactions within protein oligomers, and thus permitting molecular mass determination of multimeric proteins. In prestin-transfected yeast and mammalian cell lines, four prestin bands were consistently observed in PFO-PAGE/Western blots. These bands probably correspond to prestin's monomer, dimer, trimer and tetramer. Finally, by using chemical cross-linking reagents, we demonstrated that prestin in OHCs is also present as an oligomer. [Supported by Grant DC00089, DC006412 and The Hugh Knowles Center].

O11 OHC-induced motion of the organ of Corti

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The somatic electromotility of the outer hair cells (OHCs) plays an essential role in amplification of the motion of the organ of Corti (OoC) (LIBERMAN *et al.*, 2002). Electromechanical force produced by the OHCs amplifies the motion of the OoC, which is somehow coupled to the hair bundle of the inner hair cells (IHCs). To investigate the role of OHC somatic electromotility, we developed an *in vitro* preparation in which the electrically induced vibration pattern of the reticular lamina (RL) and tectorial membrane (TM), upper and lower surface, could be measured.

In the guinea-pig cochlea (n = 81), a transepithelial electrical stimulus was applied with electrodes placed in *scala vestibuli* and *scala tympani*. The stimulus was multi-tone and contained 81 frequencies (480 Hz - 70 kHz) with equal amplitude but random phase. Transversal motion was measured with a laser Doppler vibrometer, at three different places along the cochlea (characteristic frequency 0.8, 3 and 24 kHz).

A complex vibration pattern of the RL was found. The RL motion exhibited two pivot points - one at the pillar cells and the other at the Hensen´s cells. In contrast, TM motion was in-phase along its entire lower and upper surfaces. This leads to counterphasic motion of the RL and TM above the IHCs. This motion causes radial fluid motion inside the subtectorial space, which is theoretically capable of deflecting the IHC stereovilli.

The experiments showed an additional possibility of OHC-induced amplification - fluid motion in the subtectorial space which will add vectorially to the travelling wave-induced shearing motion between RL and TM.

Lieberman,M.C., Gao,J., He,D.Z., Wu,X., Jial,S., Zuo,J. Nature 419, 300-304 (2002).

O12 A mathematical model of the regulation of OHC basolateral permeability and transducer operating point

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The cochlea presumably possesses a number of regulatory mechanisms to maintain cochlear sensitivity in the face of disturbances to its function. Evidence for such mechanisms can be found in the time-course of the recovery of CAP thresholds during experimental manipulations, and in observations of slow oscillations in cochlear micromechanics following exposure to LF tones (the 'bounce phenomenon') and other perturbations. To increase our understanding of the regulatory processes within the cochlea, and OHCs in particular, we have developed a mathematical model of the OHC that takes into account its known electrical properties, and includes the effect of fast and slow-motility of the cell body on transducer operating point and apical conductance. Central to the operation of the model is a putative intracellular 2nd-messenger system based on cytosolic Ca^{2+} concentration. Cytosolic Ca^{2+} is involved in regulation of i) the operating point of OHC MET channels via slow motility and axial stiffness; ii) the permeability of the basolateral wall to potassium via Ca^{2+}-sensitive potassium channels; and iii) the cytosolic concentration of Ca^{2+} itself, via extrusion from the OHC (via the Ca^{2+}-ATPases in the plasma membrane) and Ca^{2+}-induced Ca^{2+}-release (CICR) from intracellular Ca^{2+} storage organelles. The permeability of the OHC basolateral wall determines the standing current through the OHCs (and therefore a component of EP regulation), and in the presence of sound, affects the magnitude of the AC receptor potential that drives the prestin-mediated somatic electromotility and active gain. The mathematical model we have developed provides a physiologically-plausible and internally-consistent explanation for the time-courses of the cochlear changes observed during a number of different perturbations. We show how much of the oscillatory behaviour can be attributed to oscillations in cytosolic calcium concentration, and present results from the model for a number of simulations, including DC current injection into scala media, perilymphatic perfusions, and exposure to LF tones, and compare the results of these simulations to experimental data recorded from the guinea pig.

O13 NompC TRP channel is a true mechanotransducer channel in the *Drosophila* ear

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Introduction: Mechano-electrical transducer channels (METs) are gated in the straightest way possible: directly by the forces of the mechanical stimuli themselves. As a consequence, the unequivocal identification of METs requires that their direct mechanical gating is demonstrated in a native environment. Only then, true METs can be distinguished from downstream channels involved in electrical signal amplification.

Materials and Methods: Using a computer controlled scanning laser Doppler vibrometer we recorded the displacement response of the fly's antennal sound receiver to electrostatically applied external forces.

Results: The response of wild type receivers to force steps was complex. When actuated by a force, the receiver displayed an initial, pronounced movement in the direction of the force that was followed by a fast movement in the opposite direction and a subsequent slow excursion to a steady-state position. The initial peak in the receiver's displacement response scaled nonlinearly with the applied force and coincided with the phasic electrical nerve activity. Loss-of-function mutations in *nompC* virtually abolished both the mechanical nonlinearity and the nerve response in the range of forces analyzed.

Conclusion: Here, we show that *Drosophila* NOMPC, a transient-receptor-potential (TRP) channel reportedly required for the sensation of touch and sound (1,2), constitutes a true MET in the fly's antennal ear. We found that the mechanical gating of METs impacts on the mechanics of the ear. Including a gating related, nonlinear compliance and mechanical correlates of transducer adaptation this impact accords with a tethered mechanism of channel activation via gating-springs (3). These findings establish *Drosophila* NOMPC as the first true, mechanically gated transducer channel for hearing and show that the gating of this channel governs the mechanical performance of the entire ear.

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Session C:

Ototoxicity I

Chairpersons: A. Forge
J. Schacht

O14 2,3-Dihydroxybenzoic acid attenuates Kanamycin-induced volume reduction in mouse utricular type I hair cells

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Sensory hair cells are delicate structures which can be damaged by exposure to aminoglycosides. The aim of this study was to estimate cellular quantitative changes in the utricular balance organ of mice to assess a possible damage to cells after treatment with the aminoglycoside kanamycin. Antioxidant therapy with 2,3-dihydroxybenzoic acid (DHB) was also introduced to attenuate the cell damaging effect of kanamycin.

Mice were injected twice daily with either saline, kanamycin or kanamycin + DHB for 15 days and perfusion fixed three weeks after last injection. Total volume of the utricle as well as number of hair and supporting cells was estimated using stereological methods. Volume of hair cells (HCI & HCII), supporting cells (SC) and neural elements were estimated on electron micrographs.

Mean volume of the utricle was $3.65 \cdot 10^{-3}$ mm^3 (0.07) in control animals and $3.50 \cdot 10^{-3}$ mm^3 (0.06) in kanamycin + DHB-treated animals. In animals treated with kanamycin the volume was reduced to $3.14 \cdot 10^{-3}$ mm^3 (0.04) which was significantly different from the two other groups.

Although hair and supporting cell numbers remained unchanged regardless of treatment, cell volume was significantly affected. Mean volume of HCI was 270 μm^3 in control animals. Kanamycin-treated animals had a HCI mean volume of 209 μm^3 and were significantly different from kanamycin + DHB-treated animals ($P=0.04$), which had a HCI mean volume of 286 $microm^3$, but only tended to be different from controls (0.09). Mean volume of HCII and SC did not differ significantly between the groups ($P>0.05$). We did not find any apoptotic cells (no caspase-3 reactivity).

This experiment demonstrates that two kanamycin-injections of 900 mg/kg/day do not alter the number of sensory hair cells in the utricular macula of mice. However, a significant decrease in volume of the utricular sensory epithelia indicates that the tissue was affected by the kanamycin treatment. Mean volume of HCI and total volume of the utricular macula did not decrease in animals co-treated with the antioxidant DHB, indicating that antioxidant therapy could be a successful way of otoprotection against aminoglycoside-induced inner ear damage.

O15 Noise Induced Hearing Loss (NIHL) and N-acetyl-cysteine (NAC): just a matter of time ?

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Introduction: The cellular mechanisms which lead to NIHL involve the reactive oxygen species (ROS). Studies on animals have suggested that the cochlear level of the ROS increases significantly after exposure to continuous noise. Recent studies on glutathione (GSH) and on N-acetyl-cysteine (NAC) have shown that these molecules can protect the cochlea from the ROS derived damage, increasing the levels of the endogenous cellular defences. The purpose of this study was to evaluate not only the oto-protective efficacy of NAC, but also the timing of its administration, and if the timing itself influences the oto-protection.

Materials and Methods: Twenty-eight male Sprague Dawley albino rats were divided in 4 groups treated with diverse NAC administration modalities: Group A received 4 injections during 2 days, group B received 1 injection prior to noise exposure, group C received 1 injection 24h after noise exposure and group D served as the control (no NAC. The single injection dosage was 375 mg/kg while the control group received equal volume saline. The animals were exposed to 8 kHz, 105 dB SPL continuous noise for 4 hours. Cochlear functionality was evaluated by the recordings the distortion product otoacoustic emissions (DPOAE) and auditory brainstem responses (ABR). DPOAE recordings were obtained with a 50-40 dB SPL asymmetric protocol for the frequencies 4 to 16 kHz. ABR responses were elicited by tone-burst at 8, 12 and 16 kHz. Pre-noise and post-noise (after 168 hours) recordings were collected.

Results: ABR thresholds shifts were found significantly different ($P > 0.05$) between groups A, B, C and D. The best results (minimum threshold elevation) were obtained from group B (total of 375 mg/kg). Analysis of the DPOAE responses has shown that the exposed animals of groups A, B and C were characterized by partial to almost complete (normal) cochlear functionality.

Conclusions: The role played by the timing of NAC injection is very important: the data suggest that the best protection scheme is accomplished by receiving NAC before noise (group B). Administering NAC after noise exposure limits considerably the protection efficacy at higher frequencies.

O16 Minocycline and MDL28170 can be effective in reducing gentamicin ototoxicity

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While gentamicin (GM) side effects on cochlear structures and functions are well known, the intracellular molecular mechanisms which lead to aminoglycoside ototoxicity are not completely understood. Ototoxicity occurs through hair cell damage and death, with an higher sensitivity to GM in outer in respect to inner hair cells, and in the basal more than in the apical part of the cochlea. Free radicals have been detected and indicated as responsible of GM toxicity. Hair cell death frequently occurs by apoptosis, with the activation of protease cascades such as caspase or calpain, and with the release of cytochrome c from mitochondria to cytosol. In this study we used as potential otoprotector Minocycline, a second-generation tetracycline antibiotic which was reported to inhibit caspases and prevent the release of cytochrome c, and MDL28170, a dipeptide able to act as a selective calpain inhibitor. The organs of Corti of postnatal day 3 (P3) rat were dissected and kept in culture. After in vitro treatment tissues were fixed and labeled with fluorescent falloidin, and hair cells were counted under a fluorescent microscope. Cochlear organotypic cultures were treated with GM alone or in combination with different doses of minocycline, and MDL28170. Treatment with GM induced a dose-dependent loss of outer hair cells (OHC) and inner hair cells (IHC). Addition of Minocycline to the GM-treated cultures greatly reduced the amount of GM induced hair cell damage. The greatest protection was achieved with 100 μM of Minocycline. Application of Minocycline alone had no adverse effects on hair cell survival. MDL 28170 safety range was investigated, and concentrations up to 200 μM resulted not significantly oxic. In cultures treated with highest concentration of GM, MDL28170 showed a dose-dependent protective effect These data can support a role of calpains and cytochrome c in GM induced hair cell apoptosis.

O17 Carbamathione prevents synaptic edema in acoustic overexposure in chinchilla

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Introduction: Glutamate has been proposed to be the excitatory neurotransmitter between inner hair cells (IHC) and afferent nerve endings. Both classes of ionotropic glutamate receptors, NMDA (N-methyl-D-aspartate) and AMPA (α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid), have been shown to be present on afferent dendrites below IHC. In the event of an acoustic overexposure, excessive glutamate produced and glutamate excitotoxicity occurred. The first sign of glutamate excitotoxicity is afferent dendrite edema. This edema is dependent on the entry of excessive amounts of sodium and chloride ions, together with water, into the cell. It was demonstrated that such edema could be blocked by glutamate NMDA receptors antagonist, MK 801, and AMPA receptors antagonist, GYKI 53784. However, these compounds have unacceptable side effects. To find an alternative, this study investigated the protective effect of Carbamathione (CARBA), a glutamate NMDA and non-NMDA receptors antagonist, and a compound that is known to have minimum side effects.

Materials and Methods: Sixteen female chinchillas were equally divided into two groups. Each group received noise exposure (NE= octave band, 105 dB SPL, centered at 4 kHz for six hours) and treated with either saline or CARBA (5.2 mg/kg). Treatments were given one hour before and after noise and twice daily for two days prior to and after NE. Animals were humanely euthanized at either two hours or three weeks post-NE. Cochleae were processed for microscopy.

Results: In the saline group, the acute effects of noise trauma were severe afferent dendrite edema and disruption of the synapse in all four chinchillas studied at two hours post-NE. Mild edema remained in three of four animals at three weeks post-NE. In the group pre-treated with CARBA,dendrites remained intact without edema in three of four animals, and a lightly swollen dendrite could be observed in one animal at two hours post-NE. No edema was noted in all four animals at three weeks post-NE.

Conclusions: The findings of this study indicated that afferent dendrite edema resulted from acoustic overexposure could be prevented by CARBA in a chinchilla model.

O18 Hyposmotic activation-induced nitric oxide production in outer hair cells of the guinea pig cochlea

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Previous studies showed that hyposmotic activation induced an increase of intracellular Ca^{2+} concentrations ($(Ca^{2+})_i$) in outer hair cells (OHCs) of the guinea pig cochlea (Harada, Ernst and Zenner, 1993, 1994). Recent study also showed that nitric oxide (NO) inhibited the ATP-induced Ca^{2+} response in cochlear inner hair cells by feedback mechanism (Shen, Harada and Yamashita, 2003). To investigate whether interaction between NO and Ca^{2+} mobilization during hyposmotic activation may occur, we investigated the role of NO on hyposmotic activation-induced Ca^{2+} response in OHCs using NO sensitive dye DAF-2 and Ca^{2+} sensitive dye fura-2. Simultaneous measurements of cell movement and NO production revealed that hyposmotic cell swelling was accompanied by NO production in OHCs. Hyposmotic activation failed to induce NO production in the Ca^{2+}-free solution, suggesting that NO production induced by hyposmotic activation may be dependent on the Ca^{2+} influx from the extracellular space. A non specific NOS inhibitor, L-NG-nitroarginine methyl ester enhanced the hyposmotic-induced $(Ca^{2+})_i$ increase while a NO donor, S-nitroso-N-acetylpenicillamine inhibited it. These results suggest that NO inhibits the hyposmotic activation-induced Ca^{2+} response by feedback mechanism. A selective neuronal NOS inhibitor, 7-nitro-indazole (7 NI) did not inhibit the hyposmotic activation-induced NO production and Ca^{2+} response while 7 NI inhibited the both ATP-induced NO production and Ca^{2+} response in OHCs. Double immunofluorescent staining of nNOS and P2X receptors using single OHC showed the co-localization of nNOS and P2X receptors in the apical region of OHCs. Double immunoflurescent staining of nNOS and eNOS using single OHC showed that expression of eNOS was apparent in the cell membrane whereas the expression of nNOS was absent in the cell membrane. Our results suggest that the Ca^{2+} influx during hyposmotic activation may be the principal source for eNOS activity in the basolateral cell membrane of the OHCs whereas the ATP-induced Ca^{2+} influx may be the principal source for nNOS activity in the apical region of the OHCs.

O19 Imaging and quantifying the human cochlear scalae

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Introduction: While the scala volumes of a number of animal species have been documented, there are few reports of the human cochlear scalae. Most prior studies have been limited to the scala tympani volume alone. One study (Thorne et al. Laryngoscope, 1999) used magnetic resonance imaging to define the 3D structure of the cochlea but due to limited resolution of the method, Reissner's membrane could not be adequately resolved so that scala media could not be differentiated from scala vestibuli. In the present study we have imaged a human inner ear using Orthogonal Plane Fluorescence Optical Sectioning (OPFOS), reconstructed and measured the scalae.

Materials and Methods: A human cochlea was harvested from a body donor, trimmed of excess bone, decalcified, dehydrated, cleared, and treated with a fluorescent dye. Using the OPFOS apparatus, and a 6 micron sectioning increment, an image stack of optical sections through the specimen was acquired. The resulting data was imported, segmented, rendered and displayed using Amira.

Results: The scanned cochlea produced 2667 images with pixel dimensions of 6.12 microns. In Amira, the data set approximated cubic voxels and could be manipulated and displayed as 3D structures. Reissner's membrane was clearly resolved throughout the cochlea, permitting all 3 cochlear scalae to be segmented with confidence. Scala dimensions (length and cross-sectional area as a function of distance) were measured. Additionally, we mapped and measured the round window membrane and its orientation with respect to the scalae, and the 3D configuration of the helocotrema.

Conclusions: This is the first study in which the human cochlea has been imaged in 3D with sufficient resolution to resolve the three scalae and intricate cochlear features such as the helicotrema and round window membrane. This study demonstrates the value of OPFOS as a high-resolution, 3D imaging tool. Reconstructed images provide anatomically-correct details for teaching. They can also be used quantitatively to establish the dimensions for the cochlear scalae which are of value in modeling drug diffusion or the mechanical properties in the human cochlea.

Session D:

Stem cells / Pharmacology

Chairpersons: M. Anniko
A. Salt

O20 Local drug delivery to the cochlea by the biodegradable polymer and gel

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Introduction: An obstacle of inner ear medicine is non-traumatic and nontoxic delivery of therapeutic molecules to the cochlea. The use of biodegradable polymers or gels, which enable controlled and sustained release of drugs, could contribute to develop safe and convenient methods for local drug delivery to cochleae. This paper describes the efficacy of biodegradable polymers and gels for local drug delivery to cochleae.

Materials and Methods: In this study, we used two biodegradable materials, gelatin hydro gels and poly lactic/glycolic acid (PLGA) nanoparticles. Hydro gels immersed otoprotective agents, brain-derived neurotrophic factor (BDNF) for spiral ganglion (SG) protection against degeneration due to hair cell loss or insulin-like growth factor 1 (IGF1) for hair cell protection against noise trauma, were applied on the round window membrane (RWM) of guinea pigs or rats. Protective effects were assessed historogically and functionally. The potential of PLGA nanoparticles for drug delivery to cochleae was investigated by local application of PLGA nanoparticles encapsulating rhodamine using guinea pigs. The distribution of rhodamine fluorescence in the cochlea was analyzed histologically.

Results: Local application of BDNF using biodegradable gels successfully protected SG degeneration in both histology and functionality. In addition, sustained delivery of BDNF in the perilymph was demonstrated by ELISA. Local application of IGF1 attenuated hearing loss and hair cell degeneration. These findings indicate the efficacy of biodegradable gels for cochlea drug delivery. Histological analysis of cochleae treated with PLGA nanoparticles demonstrated delivery of PLGA nanoparticles into the basal portion of the scala tympani, indicating that PLGA nanoparticles can pass through the RWM.

Conclusion: The biodegradable polymers or gels can be utilized for local drug delivery to the cochlea.

O21 Experiences with delivery of nanoparticles to the mouse cochlea

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Gene transfer in the inner ear has been approached mainly using viral vectors. Recent studies have suggested that gene therapy has the potential to treat sensorineural hearing loss through the transfer of math1. The safety profile of non viral gene therapy makes this form of gene delivery ideal for use in the cochlea. Cochlear non viral gene delivery has been examined by several studies and found to be inefficient compared to viral vector gene transfer. We have examined two separate approaches to non viral gene delivery in the inner ear. We compared the delivery of silica nanoparticles to delivery of a range of delivery of polymer based gene delivery. Two separate approaches to the inner ear were used: Vectors were delivered via application to the round window or delivery into the cochlea via a fenestration of the utricle.

Distribution of transgene was assessed in the cochlea as well as in the contra lateral ear and in the brain stem. Transfection of the sensory hair cells and the spiral ganglion cells was seen in the cochlear and vestibular organ on both sides, suggesting that the vector spread through the cochlea aqueduct as previously demonstrated in viral vectors. The distal portions of the central auditory pathway (dorsal cochlear nucleus, superior olivary complex) were found to be transfected with the nanoparticles indicating an anterograde axonal transport. Non viral gene transfer using advanced non viral vectors may provide a delivery system to the sensory hair cells and spiral ganglion cells and may even be suited to transfection of neurons of the central auditory pathway.

O22 Making effective vector based drugs for hearing and balance disorders: Engineering adenovectors to optimize delivery to the inner ear

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Introduction: Several studies have demonstrated that exogenous delivery of the atonal homolog math1 is capable of generating both exogenous supernumerary hair cells and is capable of converting residual supporting cells to hair cells after aminoglycoside ototoxicity. There are many challenges facing effective delivery of math1 to the inner ear including correct targeting of the vector to the right cell type, achieving even distribution of the vector through the inner ear and achieving adequate expression of the transgene in the target cell. Three main aspects of vector delivery can modified: The vector coat, the vector backbone and the method of delivery.

Methods: Ad5 based adenovectors underwent modification of their capsid to ablate binding to CAR, integrins or heparin. The effect of capsid on delivery was examined in vitro and in vivo in a mouse model of aminoglycoside ototoxicity. Conversely the effect of capsid modifications that increased binding to integrins, heparin or targeted cellular receptors were examined.

Results: We have demonstrated that in the mouse the normal entry receptors for adenovirus are at a very low level in the inner ear which is inconsistent with the amount of gene delivery seen. Using capsid modified adenovirus vectors we have determined that efficiency of gene delivery in the inner ear is dependent on target volume and local vector concentration. Adenovirus capsids ablated of normal receptor interactions (CAR and integrin) are still capable of gene delivery suggesting that vector entry may occur through alternative pathways. While the ablated vector maintains efficient delivery within the inner ear, it has been shown to have a restricted tropism for gene delivery to tissue outside of the inner ear.

Conclusions: Delivery to the inner ear can be improved with tropism modified vectors.

O23 Scala tympani perilymph sampling from the cochlear apex

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Introduction: Local drug delivery to the inner ear is becoming widely used in the clinical treatment of diseases such as Meniere's disease. There is therefore an increasing need to document the pharmacokinetics of drugs applied to the inner ear. In many published studies, perilymph for analysis was obtained from the basal turn of scala tympani (ST) through the round window membrane (RWM) or through a perforation of the bony capsule. As the cochlear aqueduct enters ST at the base, such samples are highly contaminated with CSF. A recent study estimated that 10 µL samples taken through the RWM contain less than 20 % perilymph. In the present study, an alternative method for obtaining ST perilymph samples with less CSF contamination has been evaluated.

Methods: Perilymph samples were obtained from the cochlea apex of guinea pigs. Prior to perforating the otic capsule, the bone was dried, coated with cyanoacrylate and a "cup" was formed from two-part silicone glue. This permitted fluid to be collected into capillary tubes when the apex was perforated without any loss or contamination. Samples were obtained following local application of the marker TMPA by injection or by irrigation of the RWM, or following application of gentamicin to the RWM.

Results; In experiments where a 10 µL volume was collected from the cochlear apex, the concentrations were consistent with the samples containing the entire ST contents (volume 4.7 µL), "rinsed through" with a similar volume of CSF. Simulations based on measurements made with TMPA-selective electrodes in ST correlated well with the measured samples. In experiments where repeated, 1 µL samples were taken, longitudinal gradients of TMPA and gentamicin along ST were demonstrated.

Conclusions: Sampling perilymph from the cochlear apex is technically straightforward. The perilymph content of large samples from the apex is higher than that of an equivalent volume taken through the RWM. If sequential, small samples are taken, the longitudinal gradient of drug along the perilymphatic space can be demonstrated. This method offers substantial advantages over sampling from the basal turn of ST. Supported by NIH/NIDCD grant DC01368.

Session E:

Protection

Chairpersons: J. Adams
L. Nordang

O24 Non-sensory cells contribute towards protection of hair cells from acoustic trauma

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Studies of hair cell death following acoustic trauma have naturally focused primarily upon hair cells but there are now reasons to believe that many other cochlear cells are involved in the responses of the organ to acoustic trauma. Using NF κB-GFP reporter mice, quantitative RT-PCR, and immunostaining, cellular responses to loud noise exposures were assessed.

Results with the NF κB-GFP reporter mouse indicate that cochlear cells that most reliably activate NF κB following exposure to loud sounds are not in the organ of Corti, but rather are connective tissue cells within the spiral ligament and spiral limbus. The predominant cell type that shows NF κB activation is the type I fibrocyte. Type I fibrocytes also express glial derived neurotrophic factor (GDNF) and its receptor GFR-α1. These gene products are up-regulated following noise exposures, perhaps through the actions of NF κB. In contrast, sensory cells do not activate NF κB following noise exposure nor do they express GDNF or its receptor. However, several groups have reported that GDNF administration protects hair cells from acoustic trauma and other insults. It therefore appears that protection of hair cells from acoustic trauma can be mediated by the activity of non-sensory cells. These observations indicate that hair cell death following noise exposures that do not physically disrupt the organ of Corti but nevertheless ultimately result in hair cell death involves activities of non-sensory cells, perhaps through changes in the ionic or metabolic environment of the cochlea. Strategies for preventing hair cell loss due to acoustic trauma should therefore include consideration of the concerted functions of both sensory and non-sensory cells.

O25 Induction of heat shock proteins in the cochlea in normal and *Hsf1*-null mice

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Hearing loss is a major health problem for both the young and old. Excessive noise can cause either transient or permanent hearing loss. Approaches for protecting the cochlea from noise damage throughout life would be highly desirable. Our research has focused on the classic protective mechanism regulated by the heat shock transcription factor (HSF1). Many different stresses, including heat and noise, activate HSF1, leading to induction of genes for heat shock proteins (HSP), the downstream target genes regulated by HSF1. To examine the role of HSF1 in the cochlea, we used mouse models that lack HSF1 and showed that wild-type, but not *Hsf1*-null mice, recover hearing following exposure to mild noise (Fairfield et al., J. Neurosci. Res., in press). In this study, we used both whole body hyperthermic stress and noise stress of wild-type and *Hsf1*-null mice to identify the HSP genes activated by HSF1 in the cochlea. Heat shock was accomplished by raising the body temperature to 3.3 °C above basal temperature for 15 min, followed by a 30 min recovery period at room temperature. For noise stress we exposed mice for 2 hr to broad band noise, 2-20 kHz, at different intensities ranging from 98 dB SPL to 120 dB SPL. We isolated cochlear RNA 4 hr following the noise exposure and measured the relative levels of mRNA for eight HSP genes by quantitative RT-PCR. Five HSP genes showed significant induction following heat stress in wild-type, but not in *Hsf1* null mice. These results indicate that heat induction of HSPs requires HSF1. We observed lower levels of HSP mRNAs following noise stress. The 98 dB SPL noise produced only a temporary threshold shift in wild-type mice 2 weeks following the noise, whereas the 120 dB noise exposure caused considerable permanent threshold shift (PTS). Induction of HSP genes increased with the intensity of the noise exposure. However, we observed some induction of *Hsp70.1* and *Hsp70.3* in *Hsf1*-null mice. These studies suggest that induction of genes for HSP70 following noise stress is mediated by additional signaling pathways.

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O26 Neural stem cells suppress the shift in the hearing threshold caused by ischemia-reperfusion injury of the gerbil cochlea

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Neural stem cells (NSCs) are multipotent progenitor cells that have self-renewal activity. The use of NSCs has recently provided a novel strategy for production of cells of the central nerve system (CNS), including neurons, astrocytes, and oligodendrocytes. In this study, we assessed the utility of NSCs for the amelioration of ischemia-reperfusion injury of the gerbil cochlea. NSCs were injected into one inner ear through the round window, one day after bilateral ischemic insult; the other ear served as the untreated control. Immunostaining for nestin showed that the distribution of NSCs was concentrated in the organ of Corti at the basal cochlear turn. To evaluate the engraftment of NSCs, neurosphere cells were transduced with adenoviral vectors expressing β-galactosidase before transplantation, and their presence in the cochleae was demonstrated using X-gal staining 3 days after transfer. The hearing ability on the transplanted side was compared with that on the control side by sequential recordings of the auditory brainstem response (ABR). On the fourth day of ischemia, the injury-induced shift in the ABR threshold was remarkably suppressed on the NSC-transplanted side as compared to the control side. These results suggest that transplantation of NSCs is therapeutically useful for prevention of the damage to hair cells that otherwise occurs after transient ischemia of the cochlea.

O27 Inhibition of caspases promotes recovery of hearing in a rat model of acute cochlear mitochondrial dysfunction

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Introduction: Mitochondrial dysfunction within the cochlea is thought to be an important cause of sensorineural hearing loss. Recently, we established a novel rat model presenting with acute hearing impairment by local administration of the mitochondrial toxin 3-nitropropionic acid (3-NP). Depending on the amount of 3-NP, the degree of hearing impairment varied. By administration of 3-NP at a concentration of 300 mM, permanent threshold shift was observed at 20 kHz and 40 kHz. In this study, we examined whether inhibition of caspases induce protective effects on hearing impairment in this model.

Materials and Methods: Animals and surgery: Male Sprague-Dawley rats weighing between 160 and 200 g (approximately 6 weeks old) were used. The animals received intraperitoneal injection of pan-caspase inhibitor z-Val-Ala-Asp(Ome)-fluoromethylketon(zVAD) for 3 days. As a control study, DMSO was used. On the second day of 3 days of zVAD treatment, local administration of 3-NP into the round window niche was conducted under general anestesia. Auditory brainstem response (ABR) was recorded before surgery and at several time points after surgery.

Results: The ABR threshold at 8 kHz completely recovered to the preoperative level at 7 d after the 3-NP administration in zVAD group, while it took 21 d in the control group. The ABR threshold at 20 kHz started to improve at 3 d after the 3-NP administration and recovered to the preoperative level at 14 d after the 3-NP administration in zVAD group, whereas it remained significantly elevated even at 28 d after the 3-NP administration in control group. Both zVAD and control groups the ABR threshold at 40 kHz showed significant threshold shifts immediately after 3-NP administration and they persisted for at least 28 d.

Conclusions: Inhibition of caspases significantly promoted recovery of hearing in a rat model of acute mitochondrial dysfunction. These results indicate that inhibition of apoptosis by administration of pan-caspase inhibitor may be a useful strategy to improve hearing impairment caused by acute cochlear energy failure such as cochlear ischemia.

O28 Brain-derived neurotrophic factor, a protector from toxic inner ear damage

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Introduction: Peptides of the neurotrophin family is known to interact with specific membrane receptors and prevent neuronal death during embryonic development. Neurotrophins have shown a protective effect in both noise-induced hair cell loss and in ototoxicity of Cisplatin. The aim of this study was to investigate if Brain-derived Neurotrophic Factor (BDNF) prevents Inner Ear Damage caused by Pseudomonas Exotoxin (PaExoA).

Material and Methods: PaExoA was injected as single dozes simultaneously with BDNF into the round window niche of the rat. Brainstem Response Audiometry was used to measure the effect of the treatment by deciding the thresholds shifts previous to and after injections.

Results: A single doze of BDNF was able to protect the inner ear from hearing loss when increasing concentrations of (PaExoA) were injected.

Conclusion: BDNF seems to be an effective protectant from toxic inner ear damage when toxin and BDNF was given simultaneously.

Session F:

Ion transporters and transporters

Chairpersons: J.-L. Puel
I. Sziklai

O29 The signal transduction pathway for the dopamine D1 receptor in the guinea pig cochlea

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Dopamine released from lateral efferent fibres modulates the activity of the auditory nerve, but the signalling mechanism by which this is mediated is not known. The present study investigated the signal transduction pathway for the dopamine D1 receptor in the cochlea. D1 receptor immunolabeling was localized to the spiral ganglia neurons and at the base of the inner hair cells. Western immunoblotting on whole cochlear preparations revealed positive bands for the D1 receptor and for DARPP-32. A D1 receptor agonist, SKF 38393, enhanced the amplitude of cochlear compound action potential (CAP) from the auditory nerve, an effect that was abolished by H89, a protein kinase A (PKA) inhibitor. Conversely, SKF 83566, a D1 receptor antagonist decreased the amplitude of CAP, while forskolin, a PKA activator prevented this effect. Furthermore, it was found that the level of glutamate receptor 1 (GluR1) phosphorylation at the PKA site (Ser^{845}) was increased by the D1 agonist, but decreased by D1 antagonist. These results provide direct evidence that the D1 receptor is located under the inner hair cells and plays a modulatory role in auditory nerve activity, which is mediated by a PKA phosphorylation of GluR1.

O30 Discoidin domain receptor 1 tyrosine kinase (DDR1) has an essential role in the inner ear

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Basement membranes (BM) are responsible for tissue compartmentalization and maintenance of organ architecture. Originally believed to serve as a selective barrier and scaffold to which cells adhere, it has become evident that the individual components of the BM are regulators of biological activities such as cell growth, differentiation, and migration. The type IV collagen is the most abundant basement membrane protein, and in association with laminins provides the major suprastructural frameworks of the BM.

Recently discovered Discoidin Domain Receptors 1 and 2 (DDR1 and DDR2) are tyrosine kinase receptors activated by triple-helical collagens. Aberrant expression and signaling of these receptors have been implicated in several human diseases linked to accelerated matrix degradation and remodeling including tumor invasion, atherosclerosis, liver fibrosis, and malformation of the ductal morphogenesis of the glands. A deficit of DDR1 leads to alteration of the glomerular basement membrane in the kidney (Gross et al. 2004).

The objective of this study is to characterize (i) the distribution of the DDR1 receptors in the inner ear and (ii) ultrastrucural alterations caused by DDR1 deficiency in DDR1+/- mice.

DDR1 immunostaining was detected mainly in the stria vascularis, in the supporting cells of the organ of Corti and less in the spiral ganglion and nerve. Otopathological changes of DDR-/+ mouse inner ear were closely related to the location of the DDR1. The most progressive alterations were related to the stria vascularis, where alteration of the capillary BM and accumulation of the electron dense compounds in the epithelial cells, presumably collagen, occurs. Our preliminary results stress that DDR1 play an important role in the stria vascularis and is essential for the function of the inner ear.

O31 Aquaporin 2 in the saccus endolymphaticus: the subcellular localization

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Idiopathic endolymphatic hydrops is the histopathological hallmark of Meniere's diseases and other volume regulation related inner ear disorders. From the correlation of elevated levels of antidiuretic hormone (AVP) and the occurrence of endolymphatic hydrops as seen in the clinical observations and the animal experiments (Takeda 1995, Kumagami et al 1998), a kidney-like V_2-receptor (V_2-R) mediated, aquaporine 2 (AQP-2) dependent water regulation system in the saccus endolymphaticus has been postulated. To identify the direct effect of AVP on the endolymphatic sac the molecular basis of such an AVP/ V_2-R/ AQP-2 mediated volume regulation system was investigated by immunohistochemical techniques in rat. First, the V_2-R and AQP-2 have been detected in wholemount preparations of the saccus endolymphaticus on cellular level. Second AQP-2 could be localized on subcellular level at the basolateral membrane in tissue tec embedded and resin embedded specimen. Third, ADH dependent AQP-2 translocation could be induced by V_2-R stimulation and analysed in wholemount preparations and sections. The presence of the V_2-R and AQP-2 in the saccus endolymphaticus strongly suggest its functional role in endolymph volume regulation. The experiments might have direct implications for the pharmacological treatment of inner ear diseases associated with idiopathic endolymphatic hydrops.

O32 Vestibular function of experimental endolymphatic hydrops in guinea pig

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There are many reports of auditory function of experimental endolymphatic hydrops. However, its vestibular function has rarely reported. In this study, we investigated the vestibular function of the hydropic guinea pig by measuring the gain of horizontal vestibulo bar ocular reflex (HVOR) with an infrared system. Four pigmented guinea pigs underwent unilateral obliteration of endolymphatic sac. Before the surgery, HVOR gain under sinusoidal rotation with a maximal head velocity of 45 deg/sec and frequency range between 0.05 and 0.8 Hz was analyzed. After the obliteration surgery, HVOR gain decreased. This result indicates that the endolymphatic hydrops causes vestibular dysfunction and that this experimental animal seems to be suitable for vertigo model of Meniere's disease.

O33 Effects of lithium on endolymph homeostasis and experimentally-induced endolymphatic hydrops

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Introduction: Water homeostasis in the inner ear is regulated in part via the vasopressin (VP)-aquaporin 2 (AQP2) system in the same fashion as in the kidney. The VP-AQP2 system in the kidney is well known to be inhibited by lithium, resulting in polyuria due to a decrease in reabsorption of water in the collecting duct of the kidney. Therefore, lithium is also likely to inhibit the VP-AQP2 system in the inner ear, and consequently to exert some influence on water homeostasis of the inner ear.

Materials and Methods: In this study, we investigated the effects of lithium on AQP2 expression in the rat inner ear (Experiment 1), and on the cochlear fluid volume in hydropic ears of guinea pigs (Experiment 2). In Experiment 1, lithiume-treated rats were fed the lithium chow with a concentration of 60 mmol/kg dry food for 4 weeks. The immune reactivity of AQP2 in the cochlea and endolymphatic sac was observed with a confocal laser scanning microscope system. The expression of AQP2 mRNA was examined in the cochlea and endolymphatic sac using a quantitative PCR method. In Experiment 2, guinea pigs with electro-cauterization of the endolymphatic sac were fed a standard diet, or lithium chow with a lithium concentration of 6 and 60 mmol/kg dry foods. After 4-week feeding, all animals were sacrificed to quantitatively assess the volumetric changes of the scala media.

Results: Lithium application decreased the immune reactivity of AQP2 in the stria vascularis and the epithelial layer of the endolymphatic sac. Quantitative PCR study revealed that lithium intake reduced AQP2 mRNA expression significantly. In morphological study, lithium intake significantly reduced endolymphatic hydrops dose-dependently.

Conclusions: These results indicated that lithium acts the VP-AQP2 system of the inner ear functions in the same fashion as in the kidney, consequently producing the dehydratic effect to the endolymphatic compartment. The previous favorable clinical response in an open trial of lithium treatment of Meniere's disease may reflect the decompression of endolymphatic hydrops resulting from lithium-induced inhibition of VP-AQP2 system in the inner ear.

O34 A novel gene for *Enlarged Vestibular Aqueduct Syndrome* (EVA-Syndrome) ?

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Background: Pendred syndrome (MIM 274600), an autosomal-recessive disorder is characterized by sensorineural deafness and goiter. This syndrome is one of the most common forms of syndromic deafness. Hearing loss is prelingual in the majority of the cases, only a subset of patients have a progressive hearing loss later in life. The deafness is associated with temporal bone abnormalities ranging from isolated enlargement of the vestibular aqueduct (EVA, LAV) to Mondini dysplasia, a more complex malformation that also includes cochlear hypoplasia. Both, EVA and Mondini dysplasia are easily recognized by computer tomography. PDS is caused by mutations in the *SLC26A4* gene, it is located on Chromosome 7q31 and is composed of 21 exons that encode a 780 amino acid protein. This gene product, a transmembrane protein, is called pendrin. Functional studies in Xenopus laevis oocytes and Sf9 cells have shown that pendrin is a transporter of iodide and chloride. In this study we analyzed several multiplex families with EVA and hearing loss to distinguish between the Pendred- and EVA-Syndrome (MIM 603545).

Methods: Individual exon and intron transitions of the *SLC26A4/PDS* gene of patients were PCR amplified. Direct automatic sequencing of variant fragments was performed with the same primers, on an automatic genetic analyzer. A genomewide linkage analysis was undertaken using the Affymetrix 10K GeneChip mapping 10K Xba SNP array.

Results: We identified 15 different mutations in the *SLC26A4* gene so far, 6 of these mutations are novel. In 30 % of our patients we could not identify any mutation. These patients carry potential mutations in regulatory domains such as promotor regions, or alternatively the possibility of a distinct locus for a gene of autosomal recessive deafness with enlarged vestibular aqueduct. With a genomewide linkage analysis it was possible to identify genome regions where a gene is located which is, in addition to the *SLC26A4/PDS* gene, responsible for the development of the *Enlarged Vestibular Aqueduct Syndrome*.

Conclusions: Our results indicate evidences of a second gene which is involved in the development of the *Enlarged Vestibular Aqueduct Syndrome.*

[1] Everett LA et al. Nat Genet. 1997 Dec;17(4): 411-422
[2] Scott DA, et al. Nat Genet. 1999 Apr;21(4): 440-443

Session G:

Stem cells

Chairpersons: P. Lefebvre
G. v. Camp

O35 Neurite outgrowth of ES cell-derived neurons engrafted into the cochlea modiolus of guinea pigs

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Introduction: The loss of spiral ganglion neurons (SGNs) compromises auditory function, and reduces the effectiveness of cochlear implants, which can improve impaired hearing by stimulating SGNs. Embryonic stem cells (ESCs) are candidates for use as transplants to restore nervous systems, as neural-inducing methods have been established for this cell type. The aim of this study was set to estimate the activity of ESC-derived neurons for neurite outgrowth after engraftment in the cochlea modiolus.

Materials and Methods: EGFP-labeled ESCs were used as a source for transplants. Mouse ESCs were engrafted into the basal portion of the cochlea modiolus in normal guinea pigs following neural induction by the stromal cell-derived inducing activity. The left temporal bone and brain stem were dissected out en block 1-3 weeks later. Cryostat sections were produced and the mid-modiolus sections were provided for histological analysis. Immunostaining for EGFP and β III-tubulin was performed to distinguish ESC-derived neurons and their neurites from host tissues.

Results: The majority of ESC-derived neurons were located within cochlear nerves in the basal end of the modiolus as a cluster. Grafted cells exhibited the expression of growth-associated protein 43, which reflects neurite elongation and synaptic sprouting. ESC-derived neurites reached to spiral ganglions in the middle portion of cochleae for the peripheral direction, and to the brain stem for the central direction. A few ESC-derived neurons migrated into the middle portion of the cochlear modiolus or into the inner auditory canal.

Conclusion: These findings demonstrate that ESC-derived neurons have the potential for neurite outgrowth to both peripheral and central auditory system, indicating a high potential of ESC-derived neurons as transplants for the treatment of SGN degeneration.

O36 Investigation of neural stem cell-derived donor contribution in the inner ear

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Background: Utilization of the enormous proliferation and multi-potential differentiation potentials of somatic stem cells represents a possible therapeutical strategy for diseases of the inner ear. In the current study, the potentials of murine neural stem cells to contribute to the developing inner ear was investigated.

Methods: Fetal brain-derived neural stem cells from the E 14.5 cortex of BCL2- and Lac-Z double transgenic mice were isolated and expanded for four weeks in neurobasal media supplemented with bFGF and EGF. Neural stem cells of male animals were harvested, injected into blastocysts and the blastocysts were transferred into pseudo-pregnant foster mothers. The resulting mice were investigated six months post partum for the presence of donor cells. Auditory brainstem response audiometry (ABR) was performed in six animals. To visualize donor cells Lac-Z staining was performed on sliced cochleas of two animals. In addition, the cochleas of four female animals were isolated and genomic DNA of the entire cochlea was analyzed for donor contribution by Y-chromosome-specific YMT2B-PCR.

Results: All animals had normal ABR-thresholds. The 342 bp male-specific PCR product indicating the presence of male donor cells were detected in the cochleas of three of the four female animals investigated. In two animals, male donor cells were detecetd unilateral, in one animal bilateral.

Conclusion: The results suggest that neural stem cells possess the ability to integrate into the developing inner ear.

O37 Restoration of vestibular peripheral nervous systems by stem cell-derived neurons

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Introduction: Stromal cell-derived inducing activity (SDIA) induces embryonic stem (ES) cells to differentiate efficiently into neural precursors and neurons when cultured for 1 week with stromal cell lines (PA6 cells). Whether these SDIA-treated ES cells are capable of replacing the neural tissues in the inner ear remains unknown. This study examined the potential of ES cell-derived neurons in restoring the vestibular peripheral nervous systems.

Materials and Methods: Mouse vestibular sensory epithelia on post-partum day 3 were isolated by thermolysin treatment, and cultured in serum-free medium for 24 h. Vestibular epithelia were then co-cultured with SDIA-treated ES cells for 7 days. Organotypic cultures of vestibular epithelia without ES cells were used as controls.

Results: Immunohistochemistry for class III β-tubulin revealed massive extensions of neurites from SDIA-treated ES cells into vestibular epithelia. Some of the neurites from SDIA-treated ES cells attached to the basal or basolateral portion of sensory hair cells. Immunohistochemistry for synaptophysin indicated the formation of synaptic connections between neurites from SDIA-treated ES and sensory hair cells. ES cell-derived neurons exhibited expressions of afferent and efferent neurotransmitters and N-methyl-D-aspartate receptor subunit 1, an afferent post-synaptic marker.

Conclusions: Under co-cultured conditions with vestibular epithelia, the connection between SDIA-treated ES cells and vestibular epithelia may consist of synaptic formation and SDIA-treated ES cells may differentiate into glutamate-sensitive neurons. SDIA-treated ES cells appeared to have the potential in replacing the neural tissues in vestibular epithelia.

O38 Survival and integration of mouse embryonic stem cells in guinea pig cochlea

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Introduction: Degeneration of the auditory nerve, secondary to loss of inner hair cells leads to hearing impairment and compromises cochlear prosthesis function, which depend on auditory nerve excitation. Stem cell implants to replace lost auditory nerves could improve the efficacy of cochlear prostheses. As a first step to develop this technology, we studied survival and differentiation of mouse embryonic stem (ES) cells placed into guinea pig scala tympani.

Methods: Five days following systemic kanamyin- ethacrynic acid deafening of guinea pigs, a 5 µl bolus containing 0.1 or 2.5 million B5 mouse ES cells (Hadjantonakis et al, 1998) that robustly express enhanced green fluorescent protein (eGFP) was injected into the scala tympani via the round window. Ten µg/ml GDNF was administered into the scala tympani over 14 d, followed by assessment of survival and differentiation of the ES cells.

Results*:* eGFP labeled cells were observed in masses adhering to the borders of scala tympani, most numerous under the osseous spiral lamina and the basilar membrane of the basal turn. Half of the animals had large masses of ES cells in the first turn, while other animals had only a few layers of ES cells in the first turn. Over 50 % of the ES cells in the aggregated mass were TUJ1 immunoreactive (IR), indicating a neuronal phenotype. In more apical turns, there were fewer ES cells and a smaller percentage (10-20 %) was TUJ1 IR. The majority of the ES cells that were not TUJ1 IR were labeled for glial fibrillary acidic protein. Vesicular glutamate transporter (VGT)2 IR was found with a comparable distribution to TUJ1, in adjacent sections, although fewer cells were labeled. VGT1 did not immunolabel ES cells. Calretinin IR was observed in numerous ES cells in the same regions as TUJ1 and VGT2 immunostaining, while parvalbumin IR ES cells was relatively rare with no more than one - two cells per section.

Conclusion: These studies show stem cells survival in scala tympani and can differentiate into a neuronal phenotype with auditory nerve characteristics.

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O39 Differentiation of mouse spiral ganglion neurons in vitro

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We studied the differentiation of spiral ganglion neurons (SGNs) in a conditionally immortal cell line derived from neuroblasts in the ventral otocyst at embryonic day E10.5. The cell line, US/VOT-N33 (N33), may provide an in vitro model of SGN differentiation and we have explored its potential for transplantation to the auditory nerve. It was selected for expression of the transcription factor GATA3 and the neuronal marker β-3-tubulin. We screened it under differentiating culture conditions for a range of marker proteins and for its response to several growth factors, including insulin-like growth factor 1 (IGF1), fibroblast growth factors (FGF1, FGF2), brain derived growth factor (BDNF) and neurotrophin 3 (NT3). Growth factors were added to N33 cultured in serum-free media containing Neurobasal and 2 % B27 supplement (Invitrogen-Gibco). We also co-cultured N33 with primary cochlear epithelia removed from mouse pups at post-natal day P3. For this purpose the cells were stably transfected with Enhanced Green Fluorescent Protein (EGFP). Under differentiating conditions N33 expressed the transcription factors Islet1 and Brn3a but down-regulated Pax2 and then NeuroD, as occurs in native neuroblasts. In serum free media, cell morphology remained unchanged through differentiation. In the presence of FGF1 and FGF2 the cells developed a bipolar phenotype similar to SGNs and groups of cells adhered to each other to form small elongated bundles. All cells of this type expressed β-3-tubulin. No other growth factor caused this response. IGF1 was more potent in stimulating cell proliferation measured with a pulse of Bromodeoxyuridine (BrdU). BDNF and NT3 did not induce the formation of bipolar cells. N33 displayed active migratory behaviour in co-culture with P3 tissue and was repelled from epithelial surfaces but not from native SGNs, alongside which it extended parallel neuronal processes. N33 thus behaves in a very similar manner to native SG neuroblasts. Its ability to migrate, to differentiate and to localise with native SGNs in vitro suggests that it can provide an effective model for SGN differentiation and for cell transplantation into the ear.

O40 Innervation of embryonic stem cell-derived neurons into auditory epithelia of mice

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Introduction: The loss of spiral ganglion neurons (SGNs) is often encountered in the pathogenesis for sensorineural hearing loss, and spoils clinical benefits of cochlear implants. Embryonic stem (ES) cells have been expected as transplants for replacement of neural systems. In this study, we investigated the potential of ES cell-derived neurons for innervation into the organ of Corti using explant culture systems.

Materials and Methods: Mouse ES cells genetically labeled with EGFP gene were used. We used the co-culture of ES cells with PA6 cells, mouse skull bone marrow cells, as a method for neural induction of ES cells. Colonies that formed on the PA6 layer during the 6 days of culture were collected, and prepared as cell suspensions. Auditory epithelia obtained from P3 mice were co-cultured with ES cell suspensions for 7 days. Auditory epithelia cultured without supplement of ES cells were used as controls. At the end of the culture period, specimens were fixed with 4 % PFA and provided for histological analysis in whole mounts or cross sections. Immunohistochemistry for myosin VIIa was performed to identify the location of the hair cells in the auditory epithelia. The locations of ES cell-derived neurons and their neurites were determined by the expression of β-III tubulin (TuJ1) and EGFP. The formation of synaptic connections was estimated using immunohistochemistry for synaptophysin, a marker for synaptic vesicles.

Results: After co-culture with auditory epithelia for 7 days, immunostaining for TuJ1 demonstrated substantial elongation of neurites from ES cell-derived neurons around and within cultured auditory epithelia. Some neurites of ES cell-derived neurons were identified adjacent to or surrounding hair cells. Expression of synaptophysin was observed in the neurites of ES cell-derived neurons adjacent to the basal or basolateral portion of hair cells.

Conclusion: These findings indicate a high potential of ES cell derived neurons as transplants for replacement of spiral ganglion neurons.

Session H:

Neurotransmission

Chairpersons: E. Glowatzki
T. Moser

O41 Persistence of neonatal Ca^{2+} action potentials and developmental arrest of ionic conductances in inner hair cells of hypothyroid rats

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Thyroid hormone (TH) is essential for normal hearing function. Lack of TH in the critical developmental period between E17 and P12 leads to morphological and functional deficits in the organ of Corti and the auditory pathway. To investigate the effects of TH on inner hair cells (IHC) we used hypothyroid rats treated with the thyreostatic drug methimazol (MMI) during pre- and postnatal life.

Using the whole-cell patch clamp method we measured spontaneous and evoked Ca^{2+} action potentials (AP) and K^+ and Ca^{2+} currents in hypothyroid animals (P2-P34) and euthyroid controls. Ca^{2+} APs are assumed to play a crucial role for differentiation of IHCs and the auditory pathway. APs were present in control IHCs from P3 - P12 and abruptly vanished in parallel with the expression of a rapidly activating big K^+ conductance (BK conductance). No APs could be elicited with current injection beyond P12 in IHCs of control animals while in IHCs of hypothyroid mice, APs persisted until at least P25. The frequency of spontaneous APs in IHCs of hypothyroid rats was considerably higher than in control animals (10-25 Hz versus 0.5-3.5 Hz).

IHCs of hypothyroid rats did not express the BK conductance until P25, a finding that was confirmed by immunocytochemistry. The Ca^{2+} current, which in IHCs of control rats peaked around P9 and declined to about 50 pA at P15, stayed 3.5 fold elevated in hypothyroid IHCs until P20.

In conclusion, (i) IHCs of hypothyroid rats generate Ca^{2+} APs in the first three weeks; (ii) they are unable to generate graded receptor potentials after the onset of hearing in control rats which explains deafness on the level of the IHCs; (ii) the expression of the BK channel and the reduction of the $Ca_v1.3$ Ca^{2+} channel expression are both regulated by TH.

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O42 Non-synaptic communication of dopaminergic and glutamatergic cochlear neurotransmission

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The synaptic network of the sensory epithelia of the organ of Corti is widely investigated and our knowledge is expanding quickly. Although non-synaptic neurotransmission is present in the central nervous system, its possible role in the cochlear synaptic transmission has not been implicated yet. In non-synaptic (volume) transmission transmitters use the extracellular space as communication channel and they reach distant targets by diffusion. As the concentration of transmitters is very low in this type of interneuronal interaction, the target receptors have very high affinity. These receptors are of high importance as they might be the primary targets of neuromodulator drugs. In our previous studies we investigated the possible interactions between the dopaminergic and glutamatergic cochlear neurotransmission using *in vitro* microvolume superfusion method We found that cochlear dopamine release is influenced by group II mGluR and $GABA_AR$. This data functionally indicates a non-synaptic effect of glutamate, released by the inner hair cells on the dopaminergic lateral olivocochlear efferent fibers.

O43 Input-output relations of auditory nerve fibers with low spontaneous spike rate

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Introduction: Auditory nerve fibers can be classified according to their spontaneous spike rate. Fibers with low spontaneous spike rate differ in terms of their level dependence from fibers with high spike rate. This can be seen in rate-level curves. In addition to that, at low levels there is a different behavior in the processing of complex stimuli. Low spont. nerve fibers underestimate small amplitudes in complex waveforms. This suggests nonlinear behavior in terms of an expansive static nonlinearity. This was studied in detail using pure tones.

Materials and Methods: Healthy anesthetized cats were stimulated with pure tones. Responses of single auditory nerve fibers were collected and analyzed in terms of period histograms. From the spectrum of the period histogram as determined by means of FFT, we determined the phase shift of the response with respect to the stimulus. After phase shifting the stimulus waveform by the same value, the pure tone stimulus and period histogram were compared bin by bin. This yields an input-output curve in terms of instantaneous spike rate vs. pressure amplitude

Results: Input-output curves were determined for fibers with high and low spike rates. Whereas rate level curves deal only with intensity on a dB scale, the present input-output curves deal with instantaneous pressure on a linear scale and give information on the processing of the rarefaction as well as the condensation phase of the stimulus.

Conclusions: The present method yields instantaneous input-output relations. This type of information is by definition not present in rate-level curves. The input-output relation exhibits strong expansive behavior for low spont. fibers. The data contain new information for modeling the IHC-nerve fiber synapse.

O44 Analysis of the human inner ear

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Introduction: Our knowledge about the inner ear is mostly based on animal studies. We performed a systematic analysis of the human inner ear with special interest on the spiral ganglion using surgically obtained material. This may provide tissue with better preservation suitable for both ultrastructural (TEM and SEM) and immunohistochemical analyses. In addition, this obtained tissue was also excellent to use for cultivation of human inner ear tissue especially the spiral ganglion cells.

Material and Methods: Human spiral ganglion cells (hSG) were obtained during petroclival meningeoma surgery (ethical and patient consents were obtained). Adult hSGs were developed in vitro as single cell cultures using NT-3, GDNF and BDNF as neurotrophic factors. Cochleas were EDTA-decalcified after gluteraldehyde fixation and processed for TEM and high resolution SEM.

Results: The use of surgical material gave excellent preservation of tissue suitable for fine structure analysis. The hSG perikarya were non-myelinated and surrounded by thin satellite cells often encircling groups of type I cell bodies. These cells were often incomplete and cell membranes often abutted each other at these sites. Here specialised membrane contacts were revealed possibly representing cadherin complexes. This interaction occurred more often in the apical region of the cochlea, which may affect pattern of retrograde degeneration following hair cell loss. hSG were cultured even though, more difficult, compared to animals. Signs of regeneration and new formation of hSG cells could be observed after treatment with nerve growth factor stimulation using time lapse video microscopy. A model was developed for studying effects of electrical stimulation on cultured human cells.

Conclusions: The use and analysis of surgically obtained human inner ear tissue may give new valuable information that is hard to obtain from cadaver material. Culture techniques of hSG cells may allow, for the first time, investigations of factors influencing auditory nerve growth including influence of growth cone steering such as electrical/magnetic stimuli, “guidance” molecules and other chemical factors.

Session I:

Ototoxicity II

Chairpersons: J. Miller
J. Smolders

O45 Restoration of immune mediated hearing loss by adoptive cellular gene therapy or DNA vaccine

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Autoimmune disease such as autoimmune inner ear disease including Meniere's disease are common and often devastating disease. The main feature of this disease is the development and persistence of inflammatory processes in the apparent absence of pathogens, leading to destruction of the target tissues. It may be possible to establish disease remission and to re-acquire immune homeostasis by transiently introducing immune regulatory elements by adoptive cellular gene therapy.

Mice and guina pigs were immunized with tubulin and hearing loss was induced. Light microscopic images of 200 µg and 300 µg demonstrate low density of the Spiral ganglion as well as cochlear hair cells damage.

Increased ABR thresholds were shown in BALB/c mice and guinea pig. Serum antibody reactivity against β-tubulin were elevated. CD4 T cells as well as Th1 cytokines mediated autoimmune response were involved. CD4+CD25+ lymphocytes were linked to autoimmune inner ear disease for the first time. Adoptive Immunotherapy via T cell delivery of the IL-12p40 subunit were performed by i.v. injection of $2x10^6$ T-cell hybridomas transfected with IL-12 P40 gene. ABR as well as DPOAE threshold for tubulin immunized mice at 2 weeks and 6 weeks were measured. Distortion product traces of 8, 16, 32 KHz frequency at 80 dB SPL intensity in 300 µg tubulin immunized were measured. DPOAE show low response to immunized group. Therapeutic group show restoration of hearing level 6 weeks after the therapy. In guinea pig experiment, after the second hearing test, 250 µg of DNA vaccine (Immugen vaccine 007) was injected into the guinea pig right thigh intramuscularly. After 1 weeks, then we performed hearing test again. They have restored the hearing loss.

In summary, T-cells, cytokines as well as antibodies play a significant role. In this autoimmune hearing loss. CD4+CD25+ regulatory T-cells decrease as the lesion got worse. T-cell hybridoma transfected with IL-12p40 were able to restore 90-95 % of hearing loss in mice. In guinea pigs DNA vaccine also could restore the hearing loss.

O46 Time-related formation and distribution of cisplatin-DNA adducts in the guinea pig cochlea

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Introduction: Chronic administration of the antineoplastic agent cisplatin results in functional and morphological changes in the organ of Corti, stria vascularis and spiral ganglion. However, it remains unclear whether these changes are linked together or develop in parallel. In order to identify the first site(s) of cisplatin uptake and accumulation in the cochlea we have performed a time-sequence study and used immunohistochemical detection of cisplatin-DNA adducts as an indirect means to trace cisplatin.

Materials and Methods: Albino guinea pigs were treated with daily i.p. injections of cisplatin (2 mg/kg) for 2, 4, 6, 8, 10, 12, 14 or 16 consecutive days. Following intravascular perfusion with an aldehyde fixative, cochleas were microdissected and cochlear segments containing the organ of Corti, lateral wall and spiral ganglion were processed for cryosectioning (Van Ruijven et al. [2005] Hearing Research 203: 112-121). Cryosections (0.5 µm) were incubated with a polyclonal antiserum (NKI-A59) containing antibodies against cisplatin-DNA adducts. Immunoreactivity was visualized using an indirect peroxidase-labelled extravidin-biotin method.

Results: Immunoreactivity for cisplatin-DNA adducts was first obvious in the nuclei of the OHCs and Deiters' cells in the basal turn after 6 days. Nuclear immunoreactivity in the lateral wall was observed in the marginal cells and spiral ligament fibrocytes after 8 days. The IHCs followed after 10 days and the type-I spiral ganglion cells after 14 days. Immunoreactivity for cisplatin-DNA adducts demonstrated a distinct longitudinal gradient. From day 12 onward, immunoreactivity could also be detected in the middle and apical turns. After 16 days, all cells and cochlear turns demonstrated nuclear immunoreactivity.

Conclusions: The first occurrence of cisplatin-DNA adducts in OHCs and the Deiters' cells between 4 and 6 days together with our finding that the first signs of OHC loss also occur within this time window (Van Ruijven et al. [2004] Hearing Research 197: 44-54) suggest that OHC loss and subsequent degeneration of the organ of Corti are a direct consequence of cisplatin uptake and accumulation in these cells.

O47 No-show

D. Labbé, N. Koop, O. Michel

O48 Drugs, noise, age - a radical triad

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Auditory dysfunctions frequently show very similar patterns of pathology. Loss of outer hair cells is a hallmark of drug-induced, noise-induced, and age-related hearing loss. In addition, at least drugs (cisplatin and aminoglycoside antibiotics) and noise share a progression from the base of the cochlea to the apex. Such similarities suggest that the underlying mechanisms may also be similar. Considerable evidence indicates that this is indeed the case. While this evidence in the past has been gathered from a variety of animal species, we have now established the CBA mouse as a model for aminoglycoside-induced, noise-induced, and age-related hearing loss. Such a common model not only allows for better comparisons of mechanisms of pathology but also enables molecular biological or genetic studies of the commonalities and differences of these pathologies.

We report here on these animal models. CBA mice chronically treated with aminoglycosides, exposed to noise, or of advanced age (18 to 24 months) all showed the established pattern of trauma-induced hair cell loss which was accompanied by functional deficites as evidenced by ABR recordings. Investigations of appropriate markers showed evidence for the involvement of reactive oxygen species and the activation of apoptotic and necrotic pathways in all three pathologies. While similarities were obvious, differences in cellular responses were also evident. These studies may provide further insight into cochlear survival and cell death mechanisms and guide the dsign of protective interventions.

Dr. Schacht's research on these topics is supported by grants DC03685 and DC06457 from the National Institutes on Deafness and Other Communication Disorders, National Institute of Health.

O49 Platinum concentrations in various tissues and fluids in relation to ototoxic effects of cisplatin

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Introduction: Previously we have shown the effects of an ototoxic insult caused by cisplatin on the electrocochleographic thresholds and the endocochlear potential (EP) in guinea pigs in time. In the present work we are trying to relate the platinum concentration in several tissues and fluids to the ototoxic effects.

Materials and Methods: Guinea pigs, equipped with permanent round-window electrodes, were treated daily with an i.p. injection of 1.5 mg/kg cisplatin until the compound action potential threshold shifted by 40 dB. Cisplatin treatment was then stopped but we continued electrocochleography for 0, 2/3, 5 or 7 days, after which the EP was measured. Additionally, the platinum concentration was determined in blood, perilymph, liver and kidney with atomic absorption spectrometry (AAS) and in the cochlea with induction coupled plasma mass spectrometry (ICP-MS).

Results: Platinum concentrations in perilymph were measurable at day 0 (100 µg/L) and dropped below the detection limit afterwards. In blood, the platinum concentration dropped gradually from around 1300 µg/L to 400 µg/L after 7 days. Preliminary results show a high concentration of platinum in liver, kidney and cochlea. These concentrations appear to be higher than the concentration in blood if we compare them on a weight to weight basis. An attempt will be made to determine platinum concentrations in various subsections of the cochlea (organ of Corti, lateral wall and spiral ganglion). Electrophysiological results were in line with previous work. Briefly, CAP thresholds partially recover within a week, especially for the lower frequencies. In parallel, the EP, which decreases with cisplatin treatment, recovers.

Our results will be discussed in relation to the electrophysiological effects.

O50 Cochlear degeneration in old Fischer 344 rats.

D. Buckiova, J. Popelar, J. Syka

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The reason why hearing function deteriorates faster in the Fischer 344 (F 344) albinotic strain of rats than in other strains is not completely known. According to a recent publication (Popelar et al., Neurobiol. Aging, 2005, in press) the hair cells in old F 344 rats are morphologically almost intact, but the animals' hearing loss is profound. In this study we used histological methods to characterize other aspects of the inner ear morphology of F 344 rats. Paraffin sections were either stained with conventional hematoxylin & eosin or with Masson´s trichrome staining for collagen. The animals were 18-24 months old; rats of the Long-Evans strain of a similar age were used as controls.

In control animals the cochlea was found to be without any pathological changes. In contrast, a distorted tectorial membrane with no contact with the organ of Corti was observed in almost all F 344 rats examined. The pillars of Corti were degenerated, and the tunnel was not present in some samples. The stria vascularis in Long Evans animals consisted of three layers of cells, was strongly vascularized, and contained darkly stained pigmented cells. A damaged layer of marginal cells, no pigmented cells and a reduced vascularization were typical findings in the stria vascularis of F 344 animals. In addition, degenerated spiral ganglion cells, mainly in the basal region, were observed in this strain.

Masson's trichrome staining demonstrated the presence of collagen fibrils in the tectorial membrane, the spiral ligament and the basilar membrane in the inner ear samples from Long-Evans rats. The staining for collagen in the tectorial membrane was similar in both F 344 and Long-Evans rats. However, in F 344 rats a decreased number of collagen fibrils was found in the spiral ligament and also in the basilar membrane. Similarly, there was a decrease in the number of fibrocytes producing basilar membrane collagen (Type IV).

In conclusion, degenerative changes were found in the cochlea of F 344 rats but not in Long Evans animals. The alterations were confined to the organ of Corti, stria vascularis and spiral ganglion. These findings provide additional evidence for the explanation of the hearing loss in old Fischer 344 rats.

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Session J:

Mechanics II

Chairpersons: B. Lonsbury-Martin
A.L. Nuttall

O51 Distortion product otoacoustic emissions in adult and old Fischer 344 rats

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The inbred rat strain Fischer 344 (F344) is often used as an animal model for studying the mechanisms underlying age-related hearing loss. Our previous results (Popelar et al., Neurobiol. Aging, 2005, in press) demonstrated a significant increase in hearing thresholds (based on auditory brainstem response evaluation) and a continuous decrease in the amplitudes of 2F1-F2 distortion product otoacoustic emissions (DPOAEs) measured with ILO 96 equipment in the F2 frequency range 1-6.3 kHz during ageing in this rat strain. The aim of the present study was to record DPOAEs across a wide frequency range from 1 to 40 kHz using an Etymotic probe and a TDT system in adult (12-month) and old (20-month) F344 rats and to compare the results with the previously obtained low-frequency DPOAE recordings. Low-frequency hearing loss, observed in young (1-month-old) F344 rats, changed to a rapid deterioration of hearing thresholds across the whole frequency range in old animals. DPOAEs were absent in young rats at frequencies below 2 kHz and this low-frequency limit increased with age. Simultaneously there was a decrease in DPOAE amplitude at higher frequencies, resulting in a complete disappearance of low-frequency (1-6.3 kHz) DPOAEs in half of adult rats. However, moderate DPOAE amplitudes were still measurable in these rats at higher frequencies using Etymotic/TDT equipment. The deterioration in the DPOAEs further progressed with age, and in most of the old rats no DPOAEs were detected at any frequency, except in a few cases in which small DPOAE amplitudes were still measurable in the frequency range 10-20 kHz. The results demonstrate a deficit in low-frequency DPOAEs in young F344 rats, which progresses to high-frequencies in old animals. Since pronounced hair cell loss has been observed only at the most basal and apical parts of the organ of Corti in old rats, the results suggest the involvement of other cochlear and/or extracochlear (middle ear) pathologies during the ageing process in F344 rats.

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O52 Adaptation of Distortion Product Otoacoustic Emissions in L_1,L_2 Space in Humans

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Introduction: The present study provided systematic information on L_1,L_2 levels that optimize the measurement of ipsilateral DPOAE adaptation in humans. Performance of the experiment was based on the expectation that a comprehensive exploration of the 'space' of the DPOAE stimulus-level parameter would facilitate the identification of primary-tone levels that may prove most effective for quantifying adaptation clinically.

Materials and Methods: The adaptive properties of $2f_1$-f_2 DPOAEs at a single f_2 of 1.55 kHz were investigated in 12 normal-hearing young adults. DPOAE adaptation was defined as the difference in emission levels between the initial 92-ms baseline measure using a standard protocol and another measure obtained during the final 92 ms of prolonged 1-s primary-tones. Adaptation investigated under both monaural and binaural stimulus-presentation conditions was evaluated as a function of the levels of the primary tones in a matrix of L_1,L_2 settings, which varied from 45-80 dB SPL, in 5-dB steps. These differences were averaged across subjects to create contour plots of mean adaptation in L_1,L_2 space. Adaptation activity was also evaluated for the f_2-f_1, $2f_2$-f_1, and $3f_1$-$2f_2$ DPOAEs.

Results: The $2f_1$-f_2 DPOAE revealed consistent regions of 1 to 2 dB of suppression, typically at level combinations in which L_1>L_2, along with other areas of 3 to 4 dB of enhancement, but only for the binaural condition, at primary-tone level combinations where L_1<L_2. Evaluating adaptation activity for the f_2-f_1, $2f_2$-f_1, and $3f_1$-$2f_2$ DPOAEs was not successful, because these emissions were either immeasurable (i.e., f_2-f_1), or only present in a subset of subjects over a very narrow range of primary-tone frequencies and levels that did not support systematic analyses.

Conclusions: The adaptive properties of $2f_1$-f_2 DPOAEs suggest that a potentially important area for obtaining adaptation measures exists in the L_1,L_2 space at which L_1 is lower than L_2. This overall finding suggests that such adaptation effects may be related to a notch in the DPOAE response/growth function.

O53 The bounce phenomenon: investigation in humans

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The bounce phenomenon implies an alteration of hearing after presentation of low-frequency loud tones. Only rare attempts have been offered previously to study the bounce in humans via objective methods, the most of the respective investigations being performed on animals. To compensate the deficiency, in present experiments the event has been searched in normally hearing adults while click-evoked otoacoustic emission (OAE) has been utilized for qualitative estimation of hearing changes. After 250 Hz frequency, 95 dB SPL intensity tone exposure during 3 minutes OAE augmentation has been found in all subjects tested. The increase peaked at 1 minute of the postexposure time. It was followed by the decrease, that attaining a maximal value after 3 minutes. The recovery took some additional minutes afterwards. The bounce indices have been compared under linear and non-linear OAE registration modes. The greater OAE increments as well as decrements were found under non-linear mode. The differences, however, never reached statistically significant levels. The dependence of the bounce upon the exposure tone intensity has been estimated. At relatively low intensities, 65, 70, 75 dB SPL, the process has been manifested in a selective OAE increase. At higher intensities, 80, 85, 90, 95 dB SPL, a bipolar behavior was evident: an increase in OAE magnitudes was followed by a decrease. At highest intensity, 100 dB SPL, a pronounced OAE decrement has been observed that hardly been preceded by any increment. The subjects with high bounce indices in one ear demonstrated similar high indices in other ear too. Correspondingly, lower bounce levels were typical for particular subjects irrespective of the ears tested. It has been concluded thus that the bounce carries more individual than ear-specific characteristics. In control experiments, the dynamics of OAE have been traced under continuous presentation of test-stimuli only. No reliable OAE alteration was found over the whole inspection time of 10-15 minutes. It has respectively been stated that OAE changes in bounce experiments were the result just of particular inner-ear processes but not of regular stimulus applications.

O54 Biophysics of Drosophila hearing

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Introduction: In Drosophila melanogaster, antennal organs (arista) mediate the detection of sound. The motility of mechanosensory neurons is thought to play a major role in this process.

Materials and Methods: Based on experimental observations of the mechanical response of the arista to force steps, we present a simple physical model of the active mechanics underlying Drosophila audition. This model, which is inspired by the work on vertebrate hair-cells, is based on an interplay of active motor motility, calcium feedback on the motor activity, and the mechanical gating of the mechanotransducer channels.

Results: We show that this model allows to understand qualitatively and quantitatively the response of the fly's antenna to force steps. Furthermore, we show that this system is able to display spontaneous oscillations which mimick experimentally observed large-amplitude oscillations occasionally observed in the fly.

Conclusion: We propose that Drosophila audition exploits critical oscillators for amplification. Our hair-cell inspired model well explains the mechanical performance of the fly's ear. This suggests that the fundamental physical principles exploited by the ears of vertebrates and insects may be the same.

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O55 Individual characteristics of members of the SLC26 family in vertebrates and their homologues in insects

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The ten-member SLC26 gene family encodes anion exchangers of which SLC26A5 appears to be restricted to the outer hair cells of the inner ear. Here, the so-called prestin protein acts as a molecular motor, thought to be responsible for active mechanical amplification in the mammalian cochlea. We introduce special characteristics of the SLC26A5 which may have relevance for other members of the family as well. As such, data indicate to a characteristic transcriptional control mechanism of which thyroid hormone surprisingly takes a role not only as an enhancer of expression, but also as a regulator of the subcellular redistribution of the prestin protein. Of significance for other members of the SLC26 family may be the observation that the failure of the subcellular redistribution of prestin protein prior to the onset of hearing leads to severe deficit of mature prestin function. Data will furthermore be argued in the context that prestin-related SLC26 proteins in the auditory organs of non-mammalian vertebrates and insects are widespread, possibly ancestral constituents of auditory organs and are likely to serve salient roles in mammals and across taxa.

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Poster Presentations

Posters should remain posted for the entire length of the meeting.
Authors should be at their posters during poster presentation time.
Poster board dimensions: 1 m wide, 2 m high.

P1 Mutation of β_2-glycoprotein I and idiopathic sudden sensorineural hearing loss in a Korean population

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Background and Objective: β_2-glycoprotein I (β_2GPI) also referred to as Apolipoprotein H(apoH), has been implicated in several physiologic pathways including lipid metabolism, coagualtion, production of hypertension and clearance of apoptotic bodies in circulation. The plasma β_2GPI levels have a wide range of interindiviual and interracial variation, so the plasma apoH level is thought be under genetic control. The codon316 polymorphism in the fifth domain of β_2GPI is known to be a major determinant of the plasma β_2GPI level. Since β_2GPI-dependent antiphospolipid antibody are thought to play a causal role in hypercoagulable states, this genetically determined variation can affect the pathogenesis of hypercoagulable state. Recently vascular thrombosis has become the one of the risk factors of the sudden sensorineural hearing loss. Thus we ananlyzed the frequencies of these mutations of β_2GPI in patients with idiopathic sudden sensorineural hearing loss and any correlation with the audiologic finding and clinical data.

Methods: Exon7 and 8 of β_2GPI, which encode for its fifth domain, were amplified by polymerase chain reaction, and the presence of mutations were determined by restriction digestion and single strand conformation polymorphism analysis. And then we performed DNA sequencing in sudden sensorineural hearing loss patients and in healthy control.

Results: From a total 46 patients studied, we found 10.8 % were heterozygous for the mutation at exon 8 (codon316), but we did not find any mutations in healthy control. We did not find a significant association of this mutation with either audiologic and clinical data, but there was a trend favoring the frequent detect polymorphism in flat type hearing loss and in patients with vertigo.

Conclusion: The polymorphism of codon316 in β_2GPI might be associated with the pathogenesis of sudden sensorineural hearing loss in Korean population.

P2 Familial Meniere´s disease; clinical and genetic investigations

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Introduction: Five to 13 % of Meniere´s disease (MD) patients have a family history of the disease. The cause of both the sporadic and inherited forms of MD remains undefined despite a number of candidate genes involved in hearing loss. A Swedish family that presented with MD during five generations was clinically and genetically investigated to assess possible modes of genetic transmission and attempt to identify a common causative gene.

Materials and Methods: Twelve patients with MD presented in a family tree comprising 50 members in five generations. Six had MD strictly according to AAO-HNS-95 criteria while three had partial expression of the disease. Anecdotal information from relatives regarding MD symptoms was obtained for three members in the first two generations. A genome wide linkage scan was performed on family members.

Results: The pattern of inheritance was autosomal dominant with incomplete expression in some affected members. The mean age at onset of disease was 64.5 years in the 3rd, 43 in the 4th and 25 in the 5th generation. Five candidate regions with a lod score of >1 were identified. Two additional families with autosomal dominant MD were analyzed for these regions and a cumulative Zmax of 3.46 was obtained for a single region on chromosome 12p. In two of the families, a shared haplotype was found extending over 7 Mb between markers AFM338wh5 and D12S1591, which suggests a common ancestral origin. The minimal overlapping region segregating those with MD in all three families was established by refined mapping to only 463 kb.

Conclusions: In a large 5-generation family the MD trait was transmitted in an autosomal dominant pattern. The decreasing age at onset of disease with succeeding generations could indicate anticipation. A molecular genetic study of this family and two additional families with autosomal dominant MD has defined a novel candidate region for the disease on chromosome 12p12.

P3 Progressive degeneration of stria vascularis in the developing German waltzing guinea pig inner ear

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Introduction: The German waltzing guinea pig is a new strain of animals with recessively inherited cochleo-vestibular impairment. Cochlear morphological analysis of homozygous animals (gw/gw) indicates that the primary defect is located in the stria vascularis, although the underlying genetic substrate is yet unknown. Voltage-dependent K^+ channel subunit KCNQ1, inwardly rectifying K^+ channel KCNJ10 (Kir4.1), and tight junction protein Claudin-11 are expressed in strial marginal, intermediate and basal cells, respectively. TYRP-2 is a melanocyte-specific marker. The above functional cell markers were used to characterize the degeneration of stria vascularis in the developing German waltzing guinea pig inner ear.

Materials and Methods: Cochleae from guinea pigs (gw/gw and +/+; embryonic day E30, 35, 40, 45, 50 and 60, postnatal day 0 and 60; $n \geq 2$) were harvested. Total RNA from micro-dissected cochlear lateral wall served as templates for semi-quantitative RT-PCR. Antibodies against KCNQ1, KCNJ10 and Claudin-11 were used in immunohistochemical assay.

Results: We observed: 1) the reduced expression of Kcnq1, Kcnj10 and Tyrp2 mRNA in the cochlear lateral wall of gw/gw inner ear 2) loss of KCNJ10 and Claudin-11 protein expression in the gw/gw strial intermediate cells and basal cells, respectively 3) reduced expression of KCNQ1 protein in the gw/gw strial marginal cells from E35 until birth, and loss of expression at the adult stage.

Conclusions: The stria vascularis in the developing German waltzing guinea pig inner ear is progressively degenerating from E40. Dysfunctional or deficient melanocytes/intermediate cells are involved in the mechanism underlying the stria vascularis degeneration.

P4 Auditory function in cysteine string protein α knockout mice

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Cysteine string protein α (CSPα) is an abundant synaptic vesicle protein that contains a DNA-J domain characteristic of Hsp40-type co-chaperones. An essential role for CSPα in preserving the integrity of photoreceptor ribbon synapses has recently been demonstrated in addition to the known age-dependent neurodegeneration of the neuromuscular junction and the calyx of Held synapse in CSPα-deficient mice. We therefore set out to examine the function of CSPα in the ribbon synapses of inner hair cells. ABR recordings demonstrated a progressive hearing loss in CSPα-knockout mice between the third and fourth postnatal week. Similarly, otoacoustic emission amplitudes decreased significantly during this time period. In contrast, inner hair cell calcium currents and exocytoxis in patch clamp experiments were normal in CSPα knockout-mice. There was no change in synaptic ribbon number or architecture. Immunohistochemistry showed CSP to be expressed in inner hair cells and in efferent nerve terminals of the auditory nerve. Surprisingly, CSP immunoreactivity was not abolished in inner hair cells of CSPα-knockout mice, suggesting that normal inner hair cell function is due to expression of CSPβ or –γ, previously only detected in testis. Hearing impairment could either be due to dysfunction of central auditory pathway synapses, defects of cochlear efferent control or due to middle ear ventilation problems caused by neuromuscular dysfunction. Middle ear effusion was never observed after paracentesis, but hearing thresholds and DPOAE were improved significantly one week after paracentesis in some cases.

P5 No-show

S. Noorbakhsh, M. Farhadi, A. Tabatabaei

P6 *GJB* mutations and computational modeling in patients with nonsyndromic hearing impairment from Hungary

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Introduction: Although hereditary hearing impairment (HI) is a very heterogeneous disorder, variants in one gene, *GJB2*, account for up to 60 % of autosomal recessive non-syndromic (NS) sensorineural HI cases in most populations. To date, three different connexin encoding genes have been associated with HI and a digenic inheritance of mutations in two of these connexin genes was proven to lead to HI.

Methods: We performed DNA sequence analysis of *GJB2* (Cx26), *GJB3* (Cx31), *GJB6* (Cx30) and of the 5' non-coding region of *GJB2* in 335 patients with moderate-profound NSHI from Hungary (143 familial and 192 sporadic cases). Results: Mutations were detected in 196 patients (58 %). 106 patients were homozygous for the c.35delG (32 %), 57 were c.35delG heterozygous (17 %). In all, 21 different mutations in *GJB2* were detected. 38 patients showed only one single *GJB2* mutation (c.35delG, p.R127H or p.G59V). We screened these cases for other mutations. The –3170G>A splice site mutation in the 5' non-coding region of *GJB2* was detected in nine individuals. The 309kb deletion in *GJB6* [Δ(*GJB6*-D13S1830)] was observed only in two patients with c.35delG. Three common *GJB3* polymorphisms (c.357C>T, c.798C>T, c.813+53G>A) were detected not resulting in a amino acid change. One patient with a c.35delG heterozygous genotype carried the c.94C>T (p.R32W) missense mutation in *GJB3*. Altoghether, two new *GJB2* mutations resulting in amino acid replacements were detected (c.176C>G (p.G59V); c.51C>A (p.S17Y). In one case with p.G59V mutation no second *GJB2* defect was found, while the other patient had a [c.35delG] + [c.177G>T (p.G59V)] genotype. Mutation p.S17Y occurred in compound heterozygosity with the c.35delG mutation. These missense mutations affect conserved amino acids in the 1. intracellular domain (p.S17Y) and in the 1. extracellular domain (p.G59V) of Cx26.

Conclusions: Our results confirm the importance of *GJB* mutations in the Hungarian population. The pathogenic role of the given mutations was examined by computational modeling, where we predicted the possible conformational changes caused by each mutation thus accounting for the modified functional properties of the Cx26 protein.

P7 Hearing loss in Igfl deficient mice

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Insulin like growth factor-I (IGF-I) plays a central role in inner ear development. IGF-I is a member of the insulin gene family and modulates otic cell proliferation and survival during chicken inner ear development. Humans lacking the IGF-1 gene present sensorineural deafness. The analysis of the Igf-1-/- mouse inner ear evidenced profound morphological alterations on the cochlea and cochlear ganglion cells. Here we show that these morphological alterations correlate with an altered auditory function. Auditory brainstem responses (ABR) were obtained in wild type and Igf-1-/- mouse. Click and pure tone stimuli (4, 8, 16 and 32 kHz) were delivered by an insert earphone and threshold responses were obtained for each mouse. Measurements were obtained on the postnatal day 30. Igf-1-/- mouse present hearing loss as indicated by threshold elevation in ABR responses, both by click and pure tone stimuli. The click data showed a mean threshold elevation across all knock out mice of 23 dB compared with their wild type siblings. The pure tone stimuli also showed a threshold elevation, although the elevation does not affect equally to each of the four tested frequencies. The higher threshold differences were found at the higher tone frequency used (32 kHz). Peak I latency was significantly increased, although inter-peak latencies (IPL) seem to be similar between the animal groups. On the other hand, RMN analysis of the Igf-1-/- mice brain showed central morphological alterations.These data confirm that Igf-1-/- mice present hearing loss, as predicted from the morphological alterations of their inner ear. We propose that the Igf-1-/- mice is a novel animal model of sensorineural hearing loss.

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P8 Expression of β–I, β-III and α-V integrins in inner ear tissue, and OC-Two cells

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Introduction: Integrins are a large family of trans-membrane glycoproteins which act as receptors for extracellular matrix (ECM) proteins, and contribute to cell-cell interactions. Integrins play an important role in the development and maintenance of epithelial tissues and potentially of the inner ear. Functional integrins receptors consist of α/β heterodimer. To date 18 α and 8 β subunits and 24 different heterodimer have been described.

Material and Methods: β1, β3, and αV expression was detected either by Western blots and/or RT-PCR using tissue or mRNA derived from cochlea and OC-2 cells, the latter grown at 32 °C with γ interferon (undifferentiated). β1, β3, and αV subunits were also immuno-precipitated from adult mice cochleas and from OC-2 cells. Characterisation of β1, β2, and αV cellular distribution was performed by immunostaining of inner ear sections and analysed by confocal microscopy.

Results and Conclusions: The expression of all the three subunits were detected by either Western Blot or RT-PCR in adult mouse cochlea as well as in the OC-2 cell line. Immuno-precipitations suggest that β1, β3, and αV subunits do not form heterodimer among them. Immunostaining results indicate that αV is mainly expressed on the lateral walls of supporting cells while β1 is localised at the interface with the basilar membrane.

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P9 Characterization of cells cultured from hair cell epithelia of new-born mice

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As previously reported (Licht K., Wachs F.P., Strutz J., 2003, Abstr. 26th ARO Midwinter Meeting, p259-260) cells harvested from inner ear tissue of new born mice and cultured following protocols established for the isolation of neural stem cells from brain tissue differentiate into morphologically distinct types and spheres form within 1-2 weeks. Here we further characterize these cultures by immunohistochemistry using antibodies for the proliferation marker BrdU, neural markers doublecortin and β3-tubulin and the glial marker glial-fibrillary-acidic-protein (GFAP). In addition, we analyzed the effect of externally applied vascular-endothelial-growth-factor (VEFG) and examined the expression of the VEGF-receptor Flk-1.

In spheres from primary cultures that were immunostained as whole mounts we observed expression of doublecortin and a typical neuronal morphology in a small subset of cells. In addition, adherent cells in primary cultures on chamber slides were analyzed. In experiments where BrdU had been added to the culture medium we observed GFAP/BrdU double labelled cells within 2-3 days of culture. Cells expressing neural markers that also had incorporated BrdU were less frequent and occurred only after more than a week in culture. These data show that cells from inner ear tissue of new born mice proliferate in culture. Subsets of cells differentiate and some express either neural or glia markers.

In a second series of in vitro experiments we were able to show a dose dependent effect of VEGF on the growth of dissociated spheres from primary cultures. Consistent with this VEGF effect we found expression of Flk-1 in a subset of cells by immunohistochemistry and expression of Flk-1 mRNA was observed in RNA isolated from spheres formed in primary cultures.

These data show that the inner ear of new born mice contains cells showing stem-cell characteristics. These cells respond to VEGF and can generate various other cell types.

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P10 Distinct regulation of ion channels during final differentiation of outer hair cells

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Cochlear outer hair cells (OHCs) terminally differentiate prior to the onset of hearing function. It was recently shown that thyroid hormone (TH) enhances the expression of the motor protein prestin (Weber et al. 2002) via liganded TH receptor β (TRβ) while it activates the expression of KCNQ4 by derepression of unliganded TRα1 during this developmental period (Winter et al. 2005, submitted). We raised the question if the expression of additional ion channels expressed in OHCs might be up-regulated in a thyroid hormone-dependent manner during this time. By using immunohistochemistry the expression of the potassium channels BK and SK2 in outer hair cells was analysed under hypothyroid conditions. TR mutant mice were also analysed to determine the TR-specificity of BK and SK2. Data suggest the same mechanism of regulation which was previously described for KCNQ4 expression (Winter et al. 2005, submitted) pointing to a permissive role of TH on BK and SK2 expression by mediating the derepression of unliganded TRα1. Accordingly, BK and SK2 expression were analysed in OHCs of mice bearing a dominant negative TRα1 mutant receptor. Considering a steep rise of TH plasma levels at the time when final differentiation of OHCs occur through up-regulation of KCNQ4, BK and SK2 (Knipper et al. 2000) it is interesting to note that a transient peak of 5'-deiodinase type 2 activity is observed at the same time in the cochlea and was suggested to be responsible for a local amplification of TH levels (Campos-Barros et al. 2000). Therefore the expression pattern of 5'-deiodinase type 2 was analysed. Data are discussed in the context of a mechanism in which TH is needed to unlock repressive apo-TRα1 leading to an onset of BK and SK2 expression during final differentiation of OHCs.

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P11 Application of RT-PCR and in situ hybridization on human temporal bone

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Investigations of the auditory and vestibular systems are not possible without the application of molecular techniques. However, the basic techniques for studies of human temporal bone have not yet been established or standardized. The purpose of this study was to establish techniques for extracting RNA from human temporal bones and to make sections for in situ hybridization using human temporal bones.

Materials and methods: Autopsy samples were used to collect RNA. RNAlater® was perfused through the round and the oval windows within 12 hours after the subject's death. Subsequently micro-dissections were performed to collect the parts of the internal ear (ampulla, utricles, stria vascularis, organ of Corti, and spiral ganglia), which were placed in TRIzol® reagent. RNA was then extracted from each part and measured with a spectrophometer. An oligo(dT) and a random primer were used to synthesize cDNA, which was then amplified with a set of β-actin primers to verify that RNA had been successfully transcribed. For in situ hybridization, samples were perfused with 4 % paraformaldehyde. The remaining bony part of the cochlea was removed and then using a microwave method decalcified in EDTA solution. A digoxigenine-labeled Poly-A probe was used as a positive control for in situ hybridization.

Results: RNA was extracted from each sample, and β-actin RT-PCR was positive in each sample of the internal ear. A positive staining pattern was seen in the histological sections.

Conclusion: These procedures can be used as standards for future studies of the auditory and vestibular systems at molecular levels.

P12 Effects of SERCA inhibitors and PMCA blockers on the survival of rat cochlear hair cells during ischemia in vitro

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Introduction: An important mechanism underlying cochlear hair cell (HC) susceptibility to hypoxia/ischemia is the influx of calcium (Ca^{2+}) via voltage- or N-methyl-D-aspartate-activated Ca^{2+} channels. Two mechanisms contribute to maintaining low Ca^{2+} levels: uptake of Ca^{2+} into intracellular stores via smooth endoplasmic reticulum calcium ATPase (SERCA) and extrusion of Ca^{2+} via plasma membrane calcium ATPase (PMCA).

Methods: The effects of the SERCA inhibitors thapsigargin (10 nM-10 µM) and cyclopiazonic acid (10-100 µM) and of the PMCA blockers eosin (1.5 - 10 µM) and o-vanadate (1-5 mM) on the inner and outer hair cells (IHCs/OHCs) were examined in normoxia and ischemia using an in vitro model of the newborn rat cochlea.

Results: Exposure of the cultures to ischemia resulted in a significant mean loss of 43 % of the number of IHCs and 20 % of that of OHCs. Thapsigargin and cyclopiazonic acid changed the numbers of HCs neither in normoxia nor in ischemia. Eosin and o-vanadate led to a decrease in the numbers of IHCs and OHCs. Analysis of mRNA expression of various PMCAs showed clear differences (PMCA1A 0.26 $\pm$ 0.10, PMCA2A4 0.14 $\pm$ 0.06, PMCA2A1 0.10 $\pm$ 0.05 arbitrary units; mean $\pm$ SD; n = 6; p = 0.00), but without regional or conditional changes.

Conclusions: The effects of eosin and o-vanadate indicate that PMCAs have an important role to play in protecting the HCs from ischemic cell death, but the regulation occurs at the posttranscriptional levels.

P13 Tuning in the cochlea after ischemic loss of tuning? A DPOAE-based investigation

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Introduction: Distortion-product otoacoustic emissions (DPOAEs) are widely thought of as by-products of nonlinear sound processing by cochlear outer hair cells (OHC). Being easily collected by non-invasive means, DPOAEs are thus expected to serve as indicators of OHC function. OHCs are responsible for cochlear gain and tuning, so that when they are impaired, both characteristics vanish as shown by innumerable basilar membrane and nerve fiber measurements. In the meantime, DPOAEs may survive, if only at high stimulus levels (70-75 dB SPL), and it has been suggested that this happens only if enough OHCs remain structurally intact. Acute or chronic strial dysfunction is particularly prone to giving rise to cochlear deafness with residual high-level DPOAEs.

Material and Methods: This work addresses the issue of whether residual tuning can be detected at the DPOAE level when OHCs remain present albeit disabled. A gerbil model of acute cochlear ischemia was used, whereby DPOAE suppression tuning curves (STCs) were derived in the presence of a 3rd, suppressor tone of fixed frequency.

Results: Before ischemia, normal isosuppressor STCs exhibited a high degree of tuning, not surprisingly, remindful of other types of cochlear tuning curves. Yet more than 70 min after onset of complete ischemia, DPOAE STCs remained hardly unchanged, actually as long as surviving OHCs could generate residual DPOAEs. Yet, the latter were extremely vulnerable to mild overstimulation.

Conclusion: These results suggest that some kind of tuning exists in an inactive cochlea, perhaps because some OHC structure exhibits a passive natural resonance. The implication of this putative structure in DPOAE generation as well as in vulnerability to overstimulation suggests that it may have to do with stereocilia bundles. The present results raise the question of the possible interest of such a residual resonance for perception and intelligibility.

P14 Susceptibility to impulse noise trauma in three mice strains: CAB/Ca, C57/6J, CDH 23

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Noise-induced hearing loss (NIHL) is a very common inner ear disorder. There are many factors influencing an NIHL such as species differences, genetic factors, sex, pigmentation and ageing. Genetic factor might play critical role in NIHL.

Susceptibility to impulse noise trauma in different mice strains was rarely reported. The aim of the present study is to test our hypothesis that susceptibility to impulse noise trauma might differ among CBA/CA, C57/6J and CDH23.

We exposed three strains of mice to 160 dB pSPL at 50 and 100 impulses, and compare susceptibility to impulse noise trauma in the mice. We found that there was significant difference among these mice indicating that genetic background is important in impulse noise-induced hearing loss.

P15 Withdrawn

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P16 Prestin mRNA expression in the adult rat organ of Corti in relation to noise-induced hearing loss

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Introduction: The sensitivity of hearing strongly depends on the amplification of the sound-induced vibrations by outer hair cells (OHCs) on the basis of the expression of the motor protein prestin. Recently, we have found the prestin mRNA expression to increase after moderate noise-induced hearing loss (NIHL) in the guinea pig. The present study aimed at confirming this expression pattern of prestin mRNA in the adult rat in relation to NIHL.

Methods: Anesthetized rats (Wistar, 110-200 g) were unilaterally exposed to a selected impulse noise of Lpeak 167 dB SPL (1/s) for 2.5 - 4 min in order to induce different degrees of NIHL. Unexposed animals served as controls. The distortion product otoacoustic emissions (DPOAE, f2 1.5-16 kHz) were measured before exposure and one week post-exposure. Prestin mRNA was determined by competitive RT-PCR with an internal cRNA standard. Three apical, middle and basal parts of the organ of Corti were grouped according to the degree of NIHL measured in the exposed ears. In some animals, the OHC loss was examined using phalloidin staining.

Results: In the unexposed animals, the prestin mRNA expression significantly increased from the basal to the apical cochlear parts (12-157 fg/µg total RNA). In the exposed ears, a mean NIHL of 10-30 dB was measured. The prestin mRNA expression increased in the apical fragment at a mean NIHL of about 20 dB. In the corresponding frequency range, an OHC loss of about 25 % was observed. In the contralateral ears, an increase in the prestin mRNA expression was measured corresponding to a DPOAE-improvement of about 5-10 dB.

Conclusions: In both guinea pigs and rats, an apical-basal gradient in the prestin mRNA expression was shown to exist. In both species, NIHL of about 20 dB was associated with an increase in prestin mRNA expression. Additionally, the increases in the prestin mRNA of the contralateral ears were confirmed suggesting an upregulation in intact OHCs.

P17 Differential gene expression in the cochlea following exposure to intense impulse noise

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Introduction: Intense noise exposure damages the sensory hair cells of the inner ear leading to cell death and consequently hearing loss. Biological processes are regulated by expression of different genes or subsets of genes and further knowledge about which genes that are activated following noise trauma will reveal more about the processes involved. In the present project we use the Affymetrix GeneChip for gene expression analysis to detect genes that are differentially expressed after intense impulse noise exposure.

Methods: Rats were exposed to a single blast wave (194 kPa/199 dB) and the cochleae were removed at 3, 24, 72 hrs following exposure. Total RNA was isolated from whole cochleae, labeled and hybridized to Affymetrix Rat 230 GeneChip. RNA from a single cochlea was hybridized to each chip in order to minimize inter-animal variation. The signals were normalized using the Robust Multiarray Analysis (RMA) approach, which is optimal for analyzing differential expression of genes with low expression level. Changes in gene expression between control animals (anaesthetized but not exposed) and exposed rats were compared as well as changes in gene expression with time. Regulated genes were confirmed by real time PCR.

Results: At 3 hours after exposure to the blast wave, a number of genes showed a significant up-regulation. These include immediate early genes known to be involved in the immediate stress response of the tissue and other transcription-related genes. Also, genes possibly involved in growth/differentiation responses and oxidative stress were up-regulated. Ongoing experiments include additional time points.

Conclusions: We have successfully implemented the GeneChip technology to detect differentially expressed genes in a rat model for noise-induced hearing loss. Several regulated genes have been identified. It is hoped that with understanding of the specific pathways it could be possible to prevent noise-induced hearing loss in the future; either by blocking apoptotic pathways or stimulating protective or regenerative pathways.

P18 Influence of ischemia on the activation of hypoxia-inducible factor and mRNA expression of its target genes in the rat cochlea

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Introduction: Hypoxia-inducible factor (HIF-1) is a key transcription factor for the adaptation of cells and tissues to hypoxia.

Methods: To study the influence of hypoxia on HIF-1 activation and gene expression of the cochlea, we used an in vitro hypoxia model of the cochlea of newborn rats (incubation in Billups-Rothenburg chamber, 5-13 hours, pO2 level of the atmosphere 5-10 mmHg). For the determination of HIF-1 activity, we used the pGL3-Epo-HRE3-SV40-Luciferase reporter gene construct. Gene-expression of HIF-1 α and HIF-1 target genes was determined by microarray analysis. Organ of Corti, modiolus and stria vascularis were pooled separately (n=6), total RNA was prepared with RNAeasy Kit (Qiagen), and the RNU 34 Chip (Affymetrix) was used for microarray.

Results: The HIF-1 activity increased about 10-fold in the organ of Corti and modiolus and 3-4-fold in the stria vascularis after the 13-h ischemia. This activation pattern is very similar to the patterns induced by pure hypoxia. On the microarray chip, there are 19 HIF-1 sensitive genes. Under normoxic conditions, ten of them were significantly expressed in all three regions, five genes showed only a significant expression in the stria vacularis. Under hypoxic conditions, in all regions of the cochlea, the expression of hemoxygenase, nitric oxide synthetase and transferrin receptors increased. Transforming growth factor β 3 was downregulated. Moreover, in the modiolus, transferrin and vascular cell adhesion molecule 1 were downregulated. In the stria vascularis, the expression of insulin-like growth factor binding protein 2 decreased.

Conclusions: The activation of the HIF-1 transcription factor occurs by oxygen deficiency, independently on of glucose deficiency. The regulation of HIF-1 target genes is regional specific and influenced, in addition to HIF-1, by other transcription factors.

P19 The normal physiological place-frequency map in the mouse cochlea is shifted by noise exposure

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Genetically manipulated mice play a prominent role in in vivo research of the auditory system. A prerequisite for the correlation of structural and functional properties of the inner ear is the exact knowledge of the cochlear place-frequency map.

We labelled physiologically characterized auditory nerve fibres at their innervation site in the cochlea of normal hearing CBA/J mice. From the characteristic frequency (CF) and the innervation site in the cochlea a place-frequency map (physiological map) was established for CFs between 7.2 and 61.8 kHz, corresponding to locations between 90 and 10 % basilar membrane length (base = 0 %, apex = 100 %, mean length: 5.13 mm). The relation between normalized distance from the base (d) and frequency (kHz) can be described by a simple logarithmic function: d(%) = 156.5 – 82.5*log(f), with a slope of 1.25 mm/octave of frequency.

Relative to the physiological map the place-frequency relation derived from noise exposed C57BL/CBA F1 hybrid mice (anatomical map, Ou et al., 2000, Hear. Res. 145) is shifted by about an octave towards lower frequencies. We suggest that noise trauma alters basilar membrane vibration, resulting in a shift of the most sensitive frequency of auditory nerve fibers towards lower frequencies. To quantify the frequency shift in the mouse, we measured changes in CF from the same cochlear nucleus unit after acute noise exposure (2 octaves bandwidth, centered at CF, 110 dB SPL, 4 min.). Noise exposure resulted in a frequency dependent CF-shift, increasing from 0.8 octaves at a (pre-exposure) CF of 10 kHz to 1.2 octaves at 50 kHz. The CF-shift in the mouse is about twice that reported in guinea pig and cat. Considering that the slope of the place-frequency map in mouse (1.25 mm/octave) is about half that in cat and guinea pig, the absolute shift in mm along the basilar membrane is similar in all three species.

The results indicate that desensitisation of the inner ear after noise exposure leads to a shift of the cochlear place-frequency relation and can explain the shift of the anatomical map after noise damage relative to the normal physiological map.

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P20 Safety range evaluation of antioxidants on organotypic cultures and cell lines

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In the last years several evidencies linked the ototoxicity due to use of cisplatin (CDDP), an antineoplastic drug commonly used in cancer therapies, and gentamicin (GM), a world-wide used aminoglycoside, to intracellular ROS production. Actually the use of antioxidants or protective agents, such as melatonin, ascorbic acid, a-tocopherol acid succinate, glutathione, N-acetylcysteine (Lopez-Gonzales et al., 2000) was shown to protect hair cells against CDDP damage and prevent hearing loss. On the other hand, some of these antioxidant, in particular ascorbic acid and tocopherol, showed a double face effect, being able on one side to protect cell proteins and membranes acting as free radical scavengers, on the other side to surprisingly potentiate the redical attack. Our work was addressed to examine the safety range of ascorbic acid and Trolox (a soluble form of tocopherol) using a p53-negative continuosly growing tumor cell line (HL60); dopaminergic neurons obtained after NGF-induced differentiation of PC12; and ex vivo organotypic cultures of organs of Corti. In these models, we determined cell viability, GSH concentration and protein carbonylation. Results shown the existence of a cytotoxic activity of high concentration of ascorbic acid or Trolox, and of an additive effect when these antioxidant are added together. The possible protective effect of several compounds, as p53 and MAPK pathway inhibitors and metal chelators, is examined.

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P21 Effects of Carboplatin on local field potentials in the inferior colliculus of awake chinchillas

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Carboplatin preferentially damages inner hair cells in the inner ear of the chinchilla. We investigated the consequences of selective inner hair cell (IHC) loss, on local field potentials (LFP) in response to clicks, measured at different locations in the central nucleus of inferior colliculus (IC) in chronically operated unanaesthetized chinchillas treated with carboplatin.

Animals were implanted with a recording chamber and holder on the skull which allowed stereotactic microelectrode recordings from the IC. Click stimuli were presented in free field at different sound pressure levels from a sound-source located lateral to the contralateral ear. After control measurements the animals were given a total dose of 80 mg/kg carboplatin in two doses of 40 mg/kg/24h each, resulting in a partial loss of IHCs. LFPs were recorded repeatedly with electrodes (1 MOhm) from the same stereotaxic recording coordinates at regular intervals post carboplatin over a period of at least 30 days.

The general waveform of the LFPs in the IC consisted of two positive peaks and two negative peaks in between. Peak latencies ranged from 4 to 15 ms. Waveforms were generally similar in any dorsoventral penetration track except for a shift in latencies corresponding to the tonotopic frequency gradient. From caudal to rostral and from lateral to medial locations in the IC, LFP waveforms differed considerably in prominence of positive or negative peaks. Peaks of rostrolateral LFPs were mostly negative whereas LFP waveforms gradually changed to positive in the caudomedial direction. After carboplatin-induced IHC loss, LFPs in general lost their complexity. Early components (positive 4-6 ms, negative up to 10 ms) were reduced, later components (>10 ms) in caudal and medial locations were often enhanced.

The results show that selective IHC loss causes locally different, topically organized changes in the IC. The increase of late LFP components after carboplatin treatment may reflect central compensation of peripheral losses.

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P22 Expression of tissue factor and tissue factor pathway inhibitor in the stria vascularis

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Disturbance of cochlear blood flow is considered one of the causes of acute sensorineural hearing impairment, such as sudden deafness, acoustic trauma, Meniere's disease and other conditions. Although there has been much research concerning regulation of cochlear blood flow, the mechanism of such regulation is not yet clear. It has been reported that blood flow in the stria vascularis is slower than that in the spiral ligament. It is therefore thought that blood flow in the stria vascularis is vulnerable. Inhibition of thrombus formation may therefore be important in maintaining blood flow in the stria vascularis. In this study, we demonstrated the expression of tissue factor (TF) and tissue factor pathway inhibitor (TFPI) and alterations of such expression following endotoxin treatment.

Experimental Design: We examined the expression of TF and TFPI in sections of rat stria vascularis using immnofluorescence methods. Normal saline and 0.2 mg/ml lipopolysaccharide (LPS) were delivered via the round window membrane. Tissue was exposed to normal saline for 2 hours, and to LPS for 2 hours, 5 hours, 12 hours, 1 day, 3 days, and 7 days. After treatment, animals were sacrificed and examined for TF and TFPI expression using immunohistochemistry.

Results: TF was detected in a part of the stria vascularis. TFPI was detected in the capillary surface of the stria vascularis. TF expression was weak in normal condition, but increased after treatment with LPS. On the other hand, expression of TFPI was abundant in normal condition but decreased after the treatment.

Conclusion: TF and TFPI existed in the stria vascularis, and might be related to thrombus formation. Our findings suggest that TF and TFPI play important roles in maintaining blood flow in the stria vasularis.

P23 Changes in purinoceptor distribution and intracellular calcium levels following moderate noise exposure in the outer hair cells of the guinea pig

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Introduction: Among the cells of the inner ear, the outer hair cells (OHCs) are the most important targets of noise-induced effects, being the most sensitive cell types. The aim of this study was to examine the effect of moderate-loud noise (80 dB SPL, 14 days) on the intracellular calcium levels and on the expression pattern of purinoceptors in the membrane of the OHCs of the guinea pig.

Material and Methods: After dissection, guinea pig OHCs were isolated by enzymatic digestion (1 mg/ml type IV. collagenase, 10 min.). The incubation medium contains (in mM) NaCl 142, KCl 4, $MgCl_2$ 1, $CaCl_2$ 2. The solution was adjusted to 308 mOsm and to pH 7.4. We monitorised the change of the intracellular Ca^{2+} level with fura-2 AM. Intracellular [Ca^{2+}] levels were calculated from the ratio of measured fluorescence intensities at excitation wavelengths of 340 nm and 380 nm. We used polyclonal antibodies to detect the purinergic receptors of the OHC.

Results: In noise-treated animals, the resting intracellular calcium concentration increased, as compared to the non-treated animals and was slightly higher in the cells of the basal (218.5±29.2 nM, n=8) than in the apical turns (181.1±24.4 nM, n=11). After the application of 180 μM ATP, the intracellular calcium level rose by 60±22 nM in cells from the apical, and by 44±10 nM in cells from the basal turns, significantly less than in non-treated animals. The expression of the P2X1, P2X2, P2X4, P2X7 and P2Y1, P2Y4 receptor subtypes was suppressed, while the expression of the P2Y2 subtype did not decrease.

Conclusion: Noise-induced changes in the intracellular calcium homeostasis and subsequently in the calcium dependent regulatory mechanisms, may be in the background of the noise-induced hearing loss.

P24 Latent Herpes virus infection in human vestibular and spiral ganglia

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Although the etiologies of vestibular neuritis and sudden deafness remain unclear, the reactivation of Herpes Simplex Virus type-1 (HSV-1) has been associated with these syndromes. It is known that HSV-1 can persist in the nuclei of sensory neurons of the trigeminal and facial ganglia, where the latency associated transcript (LAT) can be detected in abundance. The aim of this study was to test if LAT can be detected in situ in human vestibular and spiral ganglia.

Materials and methods: Autopsy samples were used to prepare temporal bones for histological sections. A digoxigenine- and biotine-labeled antisense probe to the stable intron of LAT was used for in situ hybridization. The sections were then sequentially immunostained using a pan T cell marker (CD3).

Results: The vestibular and spiral ganglia did not stain positive for LAT, even when the staining was amplified with the Tyramide Amplification System®. While infiltrations of T cells positive for the CD3 marker were mostly found in the trigeminal ganglia, they were not detected in the vestibular and spiral ganglia. We therefore could not prove the presence of HSV-1 by detecting LAT.

Conclusion: The absence of LAT in vestibular and spiral ganglia does not support the hypothesis that vestibular nerve palsy and sudden deafness are caused by viral reactivation.

P25 The protein therapy using super anti-apoptotic FNK decreases cisplatin-induced hearing loss

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Introduction: Cisplatin (CDDP), a platinum-derived anti-cancer drug, induces apoptosis by DNA damage and is widely applied for the patients with head and neck cancer. On the other hand, CDDP has some side effects, such as ototoxicity, nephrotoxicity, and myelosuppression. The ototoxocity is one of the reasons to stop the chemotherapy. The super anti-apoptotic protein FNK, constructed from Bcl-x_L, exhibits the stronger activity to inhibit cell death. The fusion protein PTD-FNK of the HIV/Tat protein transduction domain (PTD) and FNK can enter into the cell body. PTD-FNK can be delivered to the brain to reduce ischemic injury, when *i.p.* injected into gerbils. Here, we investigated whether PTD-FNK protects inner ear from CDDP-induced hearing loss.

Materials and Methods: PTD-FNK was *s.c.* injected 2 hours before the injection of CDDP. CDDP (17 mg/kg) was *i.p.* injected into the mice (C57/BL6). 7 days after the injection, ABR was recorded and animals were sacrificed. CDDP (1.5 mg/kg) also injected the tumor carrying mice and measured the tumor growth.

Results: When CDDP (17 mg/kg) were *i.p.* injected into mice (C57/BL6), the survival rate was 57 %. On Day 7, all of the survived mice exhibited the elevation of ABR threshold. CDDP (1.5 mg/kg) inhibited an increase in the volume of tumor, when injected into tumor-carrying mice.

Conclusions: PTD-FNK didn't affect the activity of CDDP to inhibit the tumor growth. PTD-FNK seems to penetrate the blood-inner ear barrier to protect inner ear from CDDP-induced ototoxicity. These results suggest that PTD-FNK has great potential for clinical applications to prevent death of normal cells caused by side effects of anti-cancer drugs.

P26 Age-Related Hearing Impairment (ARHI) and the ISO 7029 standards for presbyacusis

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In collaboration with the ARHI-consortium.

Introduction: Age-related hearing impairment (ARHI) is a complex trait caused by an interaction of genetic and environmental factors but the exact etiology of inner ear damage in ARHI remains unrevealed. A consortium with representatives from 10 European centers has been assembled to investigate the genetic and environmental compounds of ARHI.
Here, audiometric results of the coordinating partner are compared to normative data for presbyacusis from the International Organisation for Standardisation (ISO) standard 7029.

Methods: Using population registers from a suburb of Antwerp, inhabitants aged between 55 and 65 were invited to voluntary audiological, otological and clinical examination. Exclusion criteria were based on medical conditions, clinical examination and audiological evaluation to exclude other types of hearing impairment than ARHI. Our audiological data were weighed against to the current ISO 7029 standards. The comparison has been made by using a sex and age independent mathematical conversion of the hearing thresholds to a Z-score that is based on ISO 7029 standards.*

Results:
The study included 852 volunteer with a mean age of 61.4 years and a mean Z-score of 0.29. The population consisted of 451 females and 401 males. The average Z-score for females was 0.41 and is significantly different from the average male Z-score of 0.16. (t-Test, $p<0.001$). The mean audiometric thresholds are symmetric and down-sloping as expected.

Conclusions: Our analyses on audiological data show notable differences from the current presbyacusis standard. There is no apparent reason why females in our highly screened population would have worse hearing than males. Possibly there is an underestimation of ARHI in females by the currents ISO 7029 standards. This can have important consequences for genetic studies that use these standards to correct for presbyacusis. The Z-score conversion, however, remains a valid instrument to quantify and study ARHI.

P27 The role of plasma membrane Ca^{2+} ATPase 2 (PMCA2) in the regulation of calcium concentration in the outer hair cells

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Calcium (Ca^{2+}) is a ubiquitous intracellular signal responsible for the control of many cellular processes like fertilization, learning, proliferation, development, exocytosis and mechanoelectrical transduction. However, exceeding its normal concentration, Ca^{2+} can be highly toxic and lead to cell death. Thus, it is very important cells to tightly regulate the cytosolic Ca^{2+} concentration [Ca^{2+}].

Mechanoelectrical transduction channels of hair cells allow for the entry of appreciable amounts of Ca^{2+}, which regulates adaptation and triggers the active mechanical amplification in the hair bundles. Most of the Ca^{2+} that enters through transduction channels and basolateral voltage gated Ca^{2+} channels is extruded by plasma membrane Ca^{2+} ATPases (PMCAs).

Deafwaddler (dfw2j) mice harbor a mutation in the Atp2b2 gene that encodes the plasma membrane calcium ATPase type2. These mice are deaf, ataxic, and epileptic. In this study we used Fura-2 Ca^{2+} imaging to investigate the regulation of [Ca^{2+}] during mechanical stimulation of hair cells. Compared to the wild type, the outer hair cells (OHC) of both the mutant (dfw2j) and heterozygotes showed an elevated initial [Ca^{2+}] followed by a prolonged decay. In the inner hair cell (IHC) of deafwaddler mice we observed no changes of [Ca^{2+}] homeostasis. We attribute the OHC phenotype of dfw2j mice to the absence of PMCA2, based on its more abundant presence in OHCs compared to IHCs and its localization in the stereocilia and cuticular plate .

In the second part we pharmalogically blocked all present isoforms of PMCA with Carboxyeosin (25 µM) or with basic ringer solution (pH 8.5) and observed an increase of the initial ratio and a slower decay of calcium transients in OHCs and in IHCs as well. This result shows the importance of PMCA and principally of PMCA2 in the [Ca^{2+}] regulation of OHCs.

P28 Electrically evoked otoacoustic emission and cochlear amplification changes from alterations of organ of Corti extracellular and intracellular chloride ion concentrations

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Introduction: Cochlear sensitivity and the electrically evoked otoacoustic emissions (EEOAE) depend on the proper function of prestin, the outer hair cell (OHC) motor protein. Intracellular chloride ions modulate prestin function. Further, there is evidence that chloride acts in ways other than simply carrying capacitive charge. The intracellular chloride ion concentration can induce a shift of prestin's operating voltage range and can affect the 'gain' of the OHC voltage to length relationship as the cell's operating point shifts. The purpose of this study is to investigate how chloride ions influence organ of Corti sensitivity and the amount of EEOAE (as an indirect measure of changes in prestin activity).

Materials and Methods: Guinea pigs were surgically prepared for laser Doppler velocimeter measurements of basal membrane (BM) motion in the basal turn. Electrodes to stimulate the organ were attached to the cochlea. A perilymphatic perfusion system allowed applications of artificial perilymph (AP) with different chloride concentrations (by substitution with gluconate) to be delivered into the basal turn (2 µL/min). The AP could also contain tributyltin (TBT) (50 µM), a chloride iontophore.

Results: Lowering extracellular chloride to 5 µM greatly reduced cochlear amplification and the EEOAE. Washout with AP completely restored the control levels. TBT combined with 0 µM extracellular chloride nearly abolished the EEOAE. We also observed that TBT improved the magnitude of the BM velocity response by 3-6dB near the best frequency (approximately 17 kHz) for low sound level stimuli in certain conditions.

Conclusion: These results indicate that the electrochemical drive for chloride is important for normal activity of prestin. TBT can alter intracellular chloride concentration and is able to modulate the gain of OHC electromotility.

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P29 Revisiting selected auditory data

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A generally accepted assumption is that the cochlear receptors are only sensitive to mechanical stimulation. There is no doubt that some sensitivity is a result of mechanically stimulating the hair cells (HC), but is it possible that mechanical stimuli are not the sole source of HC stimulation? For the 20 dB above threshold, BM motion is not required for cochlear activity (Offutt, 1986, Hear Res). Compound action potentials were little changed at those levels when the BM motion was nulled as indicated by the nulling of the cochlear microphonics (CM).

The inner hair cells (IHC) are about 12 dB more sensitive than the outer hair cells (OHC) as measured by intracellular recordings at ~800 Hz (Dallos, 1985, J Neurosci). However, the OHC have a 20 to 30 dB greater sensitivity than IHC as determined by CM that were measured with differential electrodes in the scala media of uninjured cochlea (Pierson and Møller, 1980, Hear Res). These data argue against the generally accepted processes of cochlear function.

In the1930's there were at least two opposing concepts of how the ear functioned. There were many experiments showing sensitivity to electrical stimulation and many Europeans, especially in Stockholm and Leningrad, thought the ear was primarily an electroreceptor. In opposition were those who thought the ear was only mechanically sensitive. This assumption of total mechanical sensitivity is flawed because it is based in-part on the hypothesis that used the negative argument there were no known HC that were electroreceptors (Stevens, 1937, JASA). Now there are many known HC that are electroreceptors with sensitivity to both anodal and cathodal (e.g. to 0.01 μV/cm) stimuli.

Responses to electrical stimuli are usually considered to be due to electrophonics and mechanical stimulation is the generally accepted mode of cochlear action. Nevertheless, since 1970 I have been interpreting and evaluating published data using the assumption of electrical sensitivity that has been expanded to include cathodal sensitivity by the IHC. It was unanticipated how comfortably the published data fit the assumption of electroreception by the IHC.

P30 Effect of salicylate on the elasticity, bending stiffness, and strength of lipid membranes

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Salicylate is a small amphiphilic molecule which has diverse effects on membranes and membrane-mediated processes. We have utilized micropipette aspiration of giant unilamellar vesicles to determine salicylate's effects on lecithin membrane elasticity, bending rigidity and strength. Salicylate effectively reduces the apparent area compressibility modulus and bending modulus of membranes in a dose-dependent manner at concentrations above 1 mM, but does not greatly alter the actual elastic compressibility modulus at the maximal tested concentration of 10 mM. The effect of salicylate on membrane strength was investigated using dynamic tension spectroscopy (DTS), which revealed that salicylate increases the frequency of spontaneous defect formation and lowers the energy barrier for unstable hole formation. The mechanical and dynamic tension experiments are consistent and support a picture in which salicylate disrupts membrane stability by decreasing membrane stiffness and membrane thickness. The tension-dependent partitioning of salicylate was utilized to calculate the molecular volume of salicylate in the membrane. The free energy of transfer for salicylate insertion into the membrane and the corresponding partition coefficient were also estimated, and indicated favorable salicylate-membrane interactions. The mechanical changes induced by salicylate may affect several biological processes, especially those associated with membrane curvature and permeability. In particular, the mechanical results shed light on the mechanism by which salicyalte affects outer hair cell electromotility.

P31 Voltage dependence of the force required for membrane tether formation: a theoretical analysis

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The mechanical properties of cellular membranes can be studied by forming a long, thin, bilayer tube (a tether) from the membrane surface. Recent experiments on human embryonic kidney and outer hair cells (OHCs) demonstrate the force needed to maintain a tether at a given length depends upon the transmembrane potential. Since the OHC tether force is highly sensitive to the holding potential, these results suggest that the unique electromechanical properties of the OHC membrane contribute to the voltage response of the tether. Here we develop a theoretical framework to analyze how two proposed mechanisms of OHC electromotility, piezoelectricity and flexoelectricity, affect tether conformation. To determine the contribution of each form of coupling to tether equilibrium, both forms of electromechanical energy are introduced into a thermodynamic expression for tether formation. By minimizing this energy expression with respect to the tether length and radius, the equilibrium tether conformation is obtained. While both forms of coupling are predicted to lead to experimentally observable changes in tether force, piezoelectric coupling is predicted to cause an increase in tether force with depolarization while flexoelectric coupling is predicted to lead to a decrease in force. The results of this analysis indicate tether experiments can provide insight into electromechanical behavior of the OHC membrane.

P32 The tuning of the cochlea

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In a first approximation the inner ear uses a wavelet transform when analyzing sound. The present study gives a detailed account on the specific wavelet which naturally appears in this context. Based on the resulting functional model of the cochlea a prediction was made about hearing phases. Experiments have since confirmed this prediction.

P33 Evaluation of cochlear function in patients with tinnitus using spontaneous and transitory evoked atoacoustic emissions

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Introduction: Otoacoustic Emissions (OAEs) are defined as sound energy emitted by the cochlea and propagated towards the base of the cochlea into the external canal, where it can be recorded objectively and non-invasively The well established finding of frequency stability support the notion that some structural feature within the organ of Corti is most likely involved in the generation of these emissions: OAEs have been suggested to originate from an overshoot of cochlear amplification and control processes located in Out Hair Cells (OHC) contraction. The aim of this paper has been to investigate the cochlear function and the basic properties of OAEs in patients with tinnitus using Spontaneous Otoacoustic Emissions (SOAEs) and Transitory Evoked Otoacoustic Emissions (TOAEs).

Materials and Methods. We have analyzed the incidence, amplitude and spectral content of hearing thresholds, SOAEs and TOAEs in a 44 ears sample in patients with tinnitus. We have measured incidence, frequency, number of peaks and amplitude of emission in spectral bands from 500 to 5000 Hz. A correlation was determined between the OAEs results and the results obtained using hearing thresholds.

Results. We have not found statistical differences at any spectral band of 500, 1000, 2000, 4000 and 8000 Hz neither at hearing thresholds means between the sample of ears with tinnitus and without tinnitus. SOAEs were only present in 1 of the 44 ears tested (2.27 %) and it was a 17 dB SPL amplitude peak at 2770 Hz frequency. TOAEs, however, were displayed in some spectral band in all the ears. We have compared TOAEs parameters between the sample of ears with tinnitus and without tinnitus in spectral bands between of 500, 1000, 2000, 4000 and 5000 Hz, and we have only found statistical differences at 4000 Hz spectral band, $p=0.02$. Comparation of TOAEs parameters between ears with tinnitus and ears without tinnitus in the same patient have only found statistical differences at 4000 Hz spectral band, $p=0.011$. In both cases there were not statistical differences at 500, 1000, 2000 and 5000 Hz frequencial bands neither at mean TOAEs amplitudes for every group.

Conclusions. We have not found significative relations between tinnitus and OAEs registration. The biological and clinical significance or importance of OAEs in relation to pathological changes in the cochlea in tinnitus cases is not entirely clear at the moment and OAEs may be not useful in the study of tinnitus.

P34 Withdrawn because of Hurricane Katrina

J. Waguespack, H. Farris, A. Ricci

P35 Frequency dependent morphometry of stereocilia of inner and outer hair cells (SEM investigation on guinea pig cochlea)

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The tonotopic frequency mapping along the cochlea goes along with a change of the shape of cochlear membranes but also with a change of stereocilia dimensions. The differences in stereocilia height along the cochlea were first found by Retzius (1884) and were later confirmed by many other authors. However, the length of the shorter rows of the guinea pig stereocilia bundle as well as the diameter of the stereocilia were not examined.The morphometry of the stereocilia of all rows of hair cells was examined by means of scanning electron microscopy (SEM Philips XL30 ESENS). The specimen was dehydrated using a conventional preparation-method and sputtered with an extremely thin layer of gold. Length and diameter of the stereocilia were measured with up to 50,000 times magnification and were cartographically analysed. The height and diameter-differences as well as shape and position of the stereocilia bundles within the basilar membrane were precisely measured. The study showed that not only the height and diameter of the tall stereocilia changes by a logarithmic law but also the height and diameter of the middle stereocilia. The height of the short stereocilia in the bundle changes along the basilar membrane, however the diameter remains constant. The stereocilia bundles of the outer hair cells become smaller from row 3 towards row 1. This size-gradation is more dominant in the low frequency area of the cochlea. Furthermore the bundle geometry (angle formed by the bundle) and their arrangement (distance between adjacent bundles and rows) also vary along the basilar membrane.

Conclusions: Changes of stereocilia and bundle dimensions can be found along the basilar membrane as well as between the rows of a section of the basilar membrane. These data can be used for further refinement of simulation models.

P36 Functional and morphological characterization of efferent synapses in cochlear hair cells of nAChR α10 'knockout' mice

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The postsynaptic cholinergic receptor at the olivocochlear (OC)-hair cell synapse is encoded by the α9 and α10 nAChR subunit genes. While α9 functions as a homomeric receptor in heterologous expression assays, expression of the native hair cell-like physiology requires α10 co-expression. Changes in the level of α10 mRNA during normal postnatal development correlate with increased sensitivity of IHCs to ACh; however, the precise function played by the α10 gene in auditory processing is unknown. We have therefore generated α10-'knockout' mice to assess the role of the α10 gene in the development and function of the OC system. Homozygous α10 mutant mice breed normally and show no overt behavioral phenotype. Antibody staining of nerve terminals revealed hypertrophied OC synaptic terminals on OHCs in α10 null mice reminiscent of those described for the α9 knockouts. Whole cell electrophysiological recordings of IHCs obtained from acutely excised P8-9 organs of Corti revealed no ACh-inducible responses or K-induced synaptic activity in IHCs of α10 knockouts. In preliminary experiments, however, ACh-evoked responses in 1 out of 4 OHCs (P11-12). Wildtype and heterozygous littermates showed robust ACh-evoked responses and K-induced synaptic activity in all IHCs and OHCs tested at the same ages. In IHCs from α10 knockouts, no changes with respect to wild type and heterozygous littermates, were observed in the amplitude, voltage sensitivity, reversal potential and activation kinetics of total K-currents before or after the onset of hearing. Relative to wild type littermates, no changes in threshold were observed in the α10 null mice as assessed by ABR across a frequency spectrum spanning 8-32 kHz. DPOAE amplitudes during OC stimulation, however, were enhanced compared to the suppression observed in wildtype controls. These results suggest that the lack of the α10 subunit gene has a different impact on auditory physiology than that observed in the α9 null mice.

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P37 Prestin is an electrogenic anion transporter

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The outer hair cell motor protein, prestin, is a member of the epithelial anion transporter superfamily SLC26A. Members of this family are found in a wide range of tissues and can act as electrogenic counter-transporters and as sulphate transporters. Although prestin (SLC26A5) is described as an 'incomplete' transporter (Oliver et al., Science 292:2340 (2001)), its low rate of transport measurable by exploiting the high protein density naturally expressed in outer hair cell basolateral membranes. We have developed a preparation of organ of Corti of the guinea pig where currents can be recorded in whole cell tight seal recording conditions under visual control with stability for periods in excess of 90 mins and with little outward sign of deterioration. In enzyme-free conditions solutions can be bath applied several times, allowing study of the anticipated small currents.

Recording cells with low intracellular chloride levels (Cl_i<10 mM) produced lower slope conductance at hyperpolarised potentials than with Cl_i high. The data are compatible with a membrane permeability to chloride, as suggested in the literature, but where the permeability itself depends on Cl_i. The charge movement, associated with electro-motility, depended on both Cl_i and the replacement anion; when both gluconate and sulphate were used in the same preparation sulphate supported charge movement (and electromotility) but shifted by ca. 90 mV more positively. Voltage clamp data suggested that sulphate enters the cell at sufficiently depolarised potentials. We have also studied the effects of bicarbonate as the buffer and find evidence from voltage clamp a) that bicarbonate is coupled to chloride movement and b) that the countertransport is electrogenic. The apparent stochiometry is consistent with 2:1 HCO_3 / Cl counter-transport.

Conclusion: Although molecular crowding of prestin in the membrane is likely to be the origin of electromotility, we provide evidence that the protein shares functional properties implied by membership of the SLC26A superfamily. There are ensuing consequences for the mechanism of stability of hair cell feedback in the cochlea.

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P38 ATP a candidate co-regulator of cochlear micromechanical properties

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Introduction: A force feedback within the cochlear partition is provided by the OHC electromotility. Its dynamic adjustment to actual requirements is regulated by several mechanisms, including the efferent innervation. (Zenner 1986, Hear. Res. 22:83-901; Dulon and Schacht 1992, Am. J. Otol. 13:108-112). Noise exposure may activate a regulatory response of OHCs which can modify the cochlear micromechanical properties and the effectiveness of the cochlear amplifier (Batta et al., 2004, Eur. J. Neurosci. 20:3364-3370; Friedberger et al., 1998, Neurobiol. 95:7127-7132). The previously described differential expression of purinerg receptors in OHCs (Szűcs et al., 2004, Hear. Res. 196:2-7) and interaction between ATP, ATP agonists-antagonists and cochlear potentials (Munoz et al., 1999, Hear. Res. 138:56-64; LeBlanc and Bobbin 1999 Hear. Res. 138:192-200) arise the possibility of the ATP mediated co-regulatory mechanism.

Methods: The effect of noise exposure and ATP on the micropipette aspiration evoked stiffness changes, regulatory stiffness response of the OHC lateral wall and stretch evoked slow cell motility (Batta et al., 2004, Eur. J. Neurosci. 20:3364-3370) were examined using a pseudocolour image analysis method (Batta et al., 2003, Pflüg. Arch. 447:328-336).

Results: ATP decreased the OHC lateral wall stiffness, increased the regulatory stiffness response and stretch induced cell shortening. The ATP was less effective than ACh or GABA, the efferent neurotransmitters of OHCs. However ATP can compensate the noise induced increasing of OHC lateral wall stiffness and the decreasing of stretch induced OHC shortening whilst the regulatory stiffness response unaltered.

Discussion: Our data suggest that ATP can work as a co-regulator of cochlear gain control and adaptation procedure by adjustement of OHC mechanical properties. Additional results may give answers about the komplex effectivity of ATP in cochlea.

P39 Yeast-based screening for putative interaction partners of prestin

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Membrane-based electromotility, the phenomenon of rapid length change produced by electrical stimulation of an outer hair cell, is mediated by the voltage-dependent motor protein prestin. Prestin, identified as a member of the SLC26 superfamily of anion-bicarbonate transporters (Zheng et al. 2000) is expressed in the cochlear outer hair cells. It is now established that prestin is redistributed towards the lateral membrane prior to the onset of hearing (Weber et al. 2002). Recent studies in our laboratory show that the loss of the redistribution during hypothyroidism leads to outer hair cells which are able to generate motility but no force (Zimmermann, Knipper et al., in preparation). It is unknown whether the prestin redistribution and generation of force can be mediated by prestin alone or by other cytoskeletal anchor proteins. To address this issue, the identification and isolation of putative novel prestin anchor proteins based on protein-protein interaction remains the imperative method. Using the motor protein as a bait in a yeast two-hybrid interaction screen, based on a rat cochlear cDNA expression library, the first putative results will be presented.

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P40 Developmental changes of morphology and transducer current during early maturation of rat outer hair cells

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The inner ear in developing rats is known to be particularly susceptible to ototoxic influences with a maximum sensitivity occurring during periods of anatomical and functional maturation of the cochlea. The mechanosensitive molecular complex which is formed by a link connected to the gate of a channel pore is a very likely to be the point of toxic action. Therefore we studied the early development in rat outer hair cells during this “critical” period (P4-P10) in order to correlate the morphology of developing mechanosensitive hair bundles with transduction current responses during maturation.

Morphology was examined by high resolution scanning electron microscopy (SEM) while transducer currents were measured using patch clamp in response to displacement of individual stereocilia by atomic force microscopy (AFM).

Despite considerable morphological changes of the stereocilia and their linking proteins (links) the receptor currents elicited by deflecting stereocilia of the tallest row remained uniform.

Our data imply that even in this early stage of development an adult-like mechanism of mechanoelectrical transduction is already present and its molecular function adapt to the dramatic morphological changes of the surrounding microstructural components of the hair bundle without measurable effects. Therefore membrane specializations like links or other intracellular molecular targets rather than the transduction channel itself seem more likely to be the target of toxic drugs during the “critical” period of cochlear maturation. Different hypotheses explaining these observations are presented.

P41 Local calcium signals in the hair bundle of outer hair cells

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Entry of calcium ions into the stereocilia, and their intracellular regulation, is crucial for mechanoelectrical transduction. The goals of the present study were to investigate the origin of calcium signals and localise transduction channels in the hair bundle of freshly isolated outer hair cells (OHCs) from an adult mammalian hearing organ, the guinea-pig cochlea.

OHCs were mechanically isolated from the adult guinea-pig cochlea. Calcium transients were evoked by deflection of the stereocilia using a fluid-jet stimulator. To facilitate calcium entry into the hair bundle, calcium concentration in the fluid-jet solution was 4 mM (extracellular 100 μM). Intracellular calcium changes were monitored using the acetoxymethyl ester form of the fluo-3 dye and the fluorescence signals were detected by a confocal laser scanning microscope.

Averaging the fluorescence signals in the whole hair bundle, we recorded stimulus-evoked calcium transients with average onset time constant (τ) of 0.26±0.19 s. The decay of the intracellular calcium signal after removal of the fluid-jet stimulus was also exponential: τ = 3.15±1.31 s. To investigate whether mechanoelectrical transduction channels are also involved in this calcium signal, the open channel blocker dihydrostreptomycin (DHSM, 100 μM) was applied. DHSM significantly increased the onset time constant to τ = 2.14±1.36 s. The effect was reversible (τ = 0.75±0.24 s) after washout. A time delay (< 1 s) was found in the apical part of the hair bundle relative to its basal area. This delay was absent in the presence of DHSM.

In conclusion, the results suggest that calcium entry into the stereocilia of these cells is not restricted to the transduction channels and supports the hypothesis that transduction channels are located at the tip region of the middle row, far from the tip of the tallest stereocilia.

P42 Speed of intracellular transport in the guinea-pig outer hair cell

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Introduction: Hair cells are known to exhibit rapid endocytic activity around the cuticular plate. FM1-43 is an excellent reagent for investigating mechanisms of vesicle cycling. Using FM1-43, we have previously provided evidence that trapped vesicles at the apical pole are transported to the basal pole in guinea-pig cochlear outer hair cells (OHCs), (ARO 2004). We demonstrate here measurements of the speed of intracellular vesicle movement from the apical part of the cell.

Materials and Methods: OHCs were mechanically isolated from the adult guinea-pig cochlea. FM1-43 (10 µM) was applied locally to OHCs by pressure injection through a grass capillary. Fluorescence changes of FM1-43 were monitored using confocal laser scanning microscopy.

Results: To observe intracellular transport from the apex to the base of the cell more clearly, fluorescence responses at various positions along the cell axis were analyzed. Fluorescence signals appeared with a delay from the puff onset in cytosolic areas between Hensen's body and the basal pole. The presence of intracellular traffic from apex to base along the cell axis could be distinguished due to different onset times of the fluorescent signals from the various areas. The speed of intracellular vesicle movement was approximately 0.36 ± 0.03 µm/s (n=15).

Conclusions: It is common in polarized cells that molecules are transported from one side of the cell to the other within membrane-bounded carriers. The speed of 0.36 ± 0.03 µm/s, calculated here, is consistent with values obtained from other studies for vesicle movement along microtubules, which range from 0.02 to 2 µm/s (Nakata et al., 1998; Toomre et al., 1999; Kipp et al., 2002; Mundy et al., 2002).

Although in this study there is no direct proof that the intracellular transport system relates to the afferent synapse or the fast electromotility of OHCs, intracellular traffic has been demonstrated here from the apical surface to specific regions in OHCs. Identifying transported molecules remains one of the most important goals for future research.

P43 The accuracy of the hair cell transducer channel

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Introduction: Mechanically activated transducer channels in cochlear outer hair cells (OHC's) transduce sound encoded mechanical signals into electrical signals. Entry of extracellular Ca^{2+} through these channels modulates transduction by reducing their open probability. The channel's open probability can be adequately described by a differentially activating two-state model [1]. Also a direct relationship was demonstrated between the gating spring stiffness (K_s) and the accuracy ($\sigma_{min} = 2kT/[K_s \cdot D] = 5.4$ nm, where D is the distance between the engaging positions of the closed and open conformational state) with which hair bundle position can be detected as a result of intrinsic channel stochastics.

In rat OHC's, altering the extracellular Ca^{2+} concentration from 1.5 mM to 20 μM shifts the operational range about 20 % in the negative direction (at $p_{open} = 0.5$) and causes a doubling of the hair bundle's passive stiffness, suggested to be due to an increased K_s [2].

Various parameters that may affect the transducer channel's open probability were simulated.

Material & Methods: Experimental data on mouse OHC's recorded in 1.3 mM extracellular Ca^{2+} were taken as reference and a differentially activating two-state model was used to generate fits [1].

Results: Decreasing the energy gap between the engaging positions with 1.5 kT, which is associated with lowering the extracellular Ca^{2+} concentration, shifts the current-displacement curve in the negative direction. It does not affect the operational range, nor the accuracy. An energy gap of 5kT shows opposite effects.

Modelling an almost doubled K_s (12.9 μN/m) we observe a negative shift, a decrease of the operational range but an improved accuracy (σ_{min} ~ 3 nm) at the hair bundle's resting position. Decreasing K_s shifts the current-displacement curve in the positive direction, broadens the operational range and degrades the accuracy.

Conclusion: An optimal accuracy at the hair bundle's resting position might be reached under normal endolymph conditions assuming a Ca^{2+}-dependent K_s.

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P44 Identification of hair bundle proteins using mass spectrometry

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The goal of this study is to identify hair-bundle proteins using a proteomics approach. We isolated hair bundles from more than 200 chicken and 400 mouse vestibular organs using the twist-off method (Gillespie and Hudspeth, 1991). Proteins in these samples will be separated by one-dimensional gel electrophoresis, digested in gel with trypsin, extracted, and sequenced using nano-liquid-chromatography tandem mass spectrometry (GeLC-MS/MS). Localisation of identified proteins to hair bundles will be confirmed using immunohistochemistry. We expect to identify proteins that are involved in a variety of cellular functions characteristic for the bundle, including ATP metabolism, ion transport, and actin turnover. Unfortunately, the most interesting set of proteins, those involved in hair-cell transduction, may be too scarce for successful identification using this broad approach. To specifically identify these proteins, an alternative differential approach will be applied. Several transduction components like the transduction channel and tip link redistribute out of the bundle when tip links are cleaved by calcium chelators. The protein composition of control and EGTA-treated hair bundles will be directly compared using the isotope-coded affinity tag (ICAT) method. The ICAT method, when coupled with the high sensitivity of protein sequencing by mass spectrometry, permits identification of proteins that are differentially expressed in two distinct samples. This is the first attempt of a direct and comprehensive identification of hair-bundle proteins on the protein level and promises to identify many proteins of interest for the hearing research field.

P45 Delayed BDNF treatment and brief electrical stimulation to enhance spiral ganglion cell survival in deafened guinea pigs

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Several studies indicate that electrical stimulation and application of neurotrophic factors can enhance spiral ganglion cell (SGC) survival in deafened animals. We are interested in SGC survival in animals which have been deaf for a clinically relevant period. We used a period of two weeks after which in deafened guinea pigs a significant SGC loss is observed. Structure and function of SGCs were investigated in deafened guinea pigs treated with brain-derived neurotrophic factor (BDNF) and/or receiving daily brief electrical stimulation (BES).

In the control group, guinea pigs were implanted with a round-window electrode and deafened by co-administration of kanamycin and furosemide. In the treated groups, 2 weeks after the deafening procedure, the right cochleas were implanted with an electrode and cannula. In the BDNF-treated group the cannula was attached to a mini-osmotic pump (flow rate: 0.25 μl/h) filled with BDNF (100 μg/ml). BDNF was administered to the cochlea for 4 weeks. In the BES group, the animals received ES approximately 30 minutes per day, 6 days a week, for 4 weeks. An additional group of animals received both BDNF and BES. In all animals, compound action potentials (CAPs) and auditory brainstem responses (ABRs) were recorded regularly in the experiment. In most animals, electrically evoked ABRs were recorded as well. Acoustical stimuli were broadband clicks and tone pips of various frequencies (2-16 kHz). The electrical stimulus currents were 100-1000 μA, pulse width was 20 μs. At the end of the experiment animals were sacrificed for histology.

In the control group, SGC densities started to decrease significantly between 2 and 4 weeks and reached a 70 % loss, 8 weeks after deafening. We found a significant effect of BDNF treatment: SGC densities in BDNF-treated (right) cochleas were 2 to 3 times higher than in untreated (left) cochleas. We will relate the SGC densities found in the BDNF- and/or BES-treated animals to functional data as obtained with CAPs, ABRs and eABRs.

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P46 Expression of pigment epithelium-derived factor (PEDF) in the rat cochlea

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PEDF is a protein showing neurotrophic/neuroprotective and anti-angiogenic activity (Barnstable and Tombran-Tink, 2004, Retinal and Eye Research, 23:561-577). PEDF has been best characterized within the eye, but it is also expressed in the brain and non-neuronal tissues. Here we characterize the expression of PEDF in the rat cochlea.

The cochleae, brains, eyes and kidneys of young adult rats were fixed in 2.5 % paraformaldehyde. Because the cochlea is encapsulated in bone all tissue samples were decalcified in buffered 0.1 M EDTA for 2 weeks and subsequently embedded in paraffin. Immunohistochemistry demonstrating PEDF expression was performed on 10 µm sections.

Virtually no staining was seen in controls, while highly differentiated PEDF-expression conforming to previously published patterns were present in the kidney, eye and brain. Consequently, decalcification by EDTA is compatible with the immuno-histochemical localization of PEDF.

In the cochlea, PEDF expression was most prominent in the basilar membrane below the organ of Corti. PEDF expression was also prominent in the stria vascularis and was found in cochlear ganglion neurons, in the spiral ligament and the limbal region. Also blood vessel endothelia showed a strong expression. The same expression pattern was observed demonstrating antibody binding either by a ABC-HRP-DAB procedure or by rhodamine fluorescence.

The strong PEDF expression in the basilar membrane might prevent blood vessel formation that would disturb cochlear micromechanics and interfere with the mechano-electrical transduction in the organ of Corti. PEDF expression in cochlear ganglion neurons might serve a neuroprotective function possibly protecting these neurons from excessive glutamate released by the inner hair cells. Further studies especially in senescent animals are necessary to evaluate the potential role of PEDF in age dependent strial degeneration and the associated atrophy of capillaries (Gratton et al., 1996, Hearing Research, 102:181-190).

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P47 Expression of NF-kB in spiral ganglion neurons during rat cochlear development

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NF-kB denotes a family of dimeric transcription factors, the prototype of which is the p50:p65 dimer, involved in transcriptional regulation in many cells, including neurons. In the present study, we investigated the presence of the inducible transcription factor NF-kB during the development and maturation of the cochlear nervous system using in situ hybridization, western blotting, and immunohistochemical analysis. Both transcripts and proteins for p50 and p65 were detected in spiral ganglion neurons during embryonic and postnatal periods, with a more pronounced expression in the former stage. Strikingly, during the postnatal period, p65 immunostainings show a restricted expression in afferent type II neurons until P10, and no staining thereafter, a time-lapse correlating with the period during which the first cochlear action potentials could be recorded. P65 immunostaining reappeared in type II neurons in the mature cochlea. These various transitory NF-kB expression patterns in cochlear nervous system suggests that NF-kB may play a fundamental role in the maturation of the architecture and the physiology of the peripheric auditory nervous system.

P48 Erythropoietin in the acoustic trauma

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Erythropoietin (EPO) is a 165 aminoacid glycoprotein with a molecular mass of 30 kD. It is produced mainly in the kidney of the adult and in the liver of the foetus. In the hematopoietic system EPO regulates red cell production by preventing apoptosis of erythroid progenitor cells in the bone marrow. Beside this role it has been shown in the recent years that EPO plays an important role in the CNS. EPO mRNA and protein are found in the brain in a variety of mammals including humans (Marti et al., 1996). EPO and its receptor is expressed in most cerebral cell types, including neurons, endothelial cells, microglial cells and astrocytes (Marti et al., 1996; Siren et al., 2001; Brines et al., 2000). EPO has been suggested to play a role in brain development as this hormone and its receptor are abundantly expressed in the embryonic brain (Dame et al., 2001). Although the expression in the brain of the normal adult is low it is thought to be activated by oxygen deficiency and metabolic stress. Under hypoxic conditions EPO gene expression is induced by the hypoxia-inducible transcription factor (HIF). The protective function of EPO has been shown in preclinical models of ischemic, traumatic, toxic and inflammatory injuries (Brines et al., 2004). Brain derived EPO therefore may be an endogenous protective agent for nervous tissues against different forms of tissue hypoxia and ischaemia. We analyzed the EPO/EPO-receptor system under the conditions of the acoustic trauma. Data are discussed in the context of EPO as a putative protective substance of the inner ear.

P49 Can Erythropoietin protect the inner ear from toxic effects?

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The most sensitive part in the inner ear are the hair cells, they can not regenerate and their damage leads to an irreversible hearing loss. Therefore we try to find substances that can protect the hair cells from damage. Erythropoietin (Epo) may be a possible candidate. Epo is known as the primary regulator of erythropoiesis, preventing apoptosis and promoting differentiation of the red blood cell precursors. Several new studies have shown that Epo has also a neuroprotective effect. Many in vitro studies documented an inhibition of cellular apoptosis by Epo. Different in vivo studies documented a reduction of structural damage when Epo was applied, for example in animal models for brain-, spinal cord- and retina pathology. Epo exerts its effects by binding to the Epo-Receptor. This raised the question, if the inner ear expresses the Epo-Receptor. In newborn rats we found by RT-PCR Epo and Epo-Receptor mRNA in the organ of corti, the spiral ganglion and the stria vascularis. Immunohistochemistry was performed to localize the Epo-Receptor. Outer- and inner hair cells and supporting cells of the organ of corti, the spiral ganglion cells and the stria vascularis stained for the Epo-Receptor. The presence of the Epo-Receptor in the organ of corti supports the theory that Epo may be able to prevent the inner ear hair cells from apoptosis. We will study, if Epo effects on damaged hair cells in vitro.

P50 Functional intervention on tinnitus in a rat *in vivo* animal model

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Behavioural conditioning studies on rats have been proven to be a valid animal model for the evaluation of acute and chronic phantom auditory experience (tinnitus). We developed an animal model for induced phantom auditory sensations in rats (Rüttiger et al., 2003). Tinnitus was induced by the administration of sodium salicylate (350 mg/kg body weight) given three hours before testing. As an alternative method we use acoustic trauma as a paradigm to induce tinnitus in the behavioural approach. Several Tinnitus inducing paradigms - as salicylate treatment and acoustic trauma - were recently shown in our laboratory to lead to a differential change in the expression of activity dependent genes in cochlear spiral ganglion neurons, midbrain auditory nuclei like the inferior colliculus, and auditory cortical tissues (Tan et al., submitted). Our aim was to characterize the relationship between behaviourally manifested tinnitus sensation, general hearing function, responsiveness of single neurons in auditory midbrain and the expression pattern of activity dependent genes. Additionally, the effect of a variety of drugs was tested under situations leading to tinnitus.

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P51 Proteolytic cleavage of BDNF

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Brain-derived neurotrophic factor (BDNF) is a member of the neurotrophin family of signalling molecules, which are essential for the growth, survival and differentiation of neuronal cells. BDNF is synthesized in the cell as a precursor protein (proBDNF) and is cleaved by the intracellular protease furin to release mature BDNF (mBDNF) which was previously believed to be the only active form of BDNF. The intracellular processing of BDNF is not however a complete reaction; proBDNF is also released from the cell and is cleaved by a variety of extracellular proteases including plasmin and matrix metalloproteinase-3 (mmp-3). The signalling activity of mBDNF was thought to be mediated mainly via interaction with its high affinity receptor TrkB and to a lesser extent via the less specific p75 neurotrophin receptor ($p75^{NTR}$). proBDNF, however, may act independently of mBDNF by preferentially binding to $p75^{NTR}$ instead of TrkB. The result of this may be a completely different cell fate, as the $p75^{NTR}$ uses multiple signalling pathways in order to promote cell death, or to inhibit cell growth. If the fate of BDNF-responsive cells depends upon the activation of distinct pathways determined by the form BDNF (pro or mature), it immediately becomes important to know what the balance of the respective signalling molecules is, and which conditions lead to the activation of a particular signalling pathway. In this context we are using immunohistochemistry, *in situ* hybridization and molecular techniques to investigate the role that proteolytic cleavage of BDNF may play in the plasticity changes that occur in the central auditory pathway following various forms of trauma.

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P52 The outcome of *Atoh1* over-expression in deaf guinea pig cochlea

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Introduction: The bHLH transcription factor *Atoh1* (previously *Math1*) is an essential gene for hair cell (HC) differentiation during cochlear development. We examined the outcome of *Atoh1* gene over-expression in the mature deafened cochlea.

Materials and Methods: Mature guinea pigs were systemically deafened in one of two ways. A. kanamycin (sc) followed by ethacrynic acid (iv), resulting in nearly complete bilateral elimination of cochlear HCs and leaving supporting cells (SCs) intact. B. Unilateral (left) intra-cochlear neomycin, resulting in complete elimination of both SCs and HCs. Four or seven days after the insult, recombinant adenovirus vectors (Ad.*Atoh1*, Ad.*Atoh1-GFP*, Ad.empty or Ad.*GFP*) were inoculated into the scala media of the left cochlea. ABRs were measured to confirm loss of function. SEM was used to observe the surface of the auditory epithelium and epi-fluorescence to view whole-mounts stained for GFP, myosin VIIa and rhodamine-phalloidin.

Results: At two months after Ad.*Atoh1* inoculation of kanamycin-deafened animals, the auditory epithelium contained numerous cells with mature stereocilia bundles. Cross sections revealed normal appearing inner HCs, whereas new outer HCs were poorly differentiated. Epi-fluorescence in *Atoh1-GFP* treated ears revealed HCs positive for GFP. ABR thresholds in *Atoh1* treated ears were significantly better than contralateral ears and control groups.
In neomycin-deafened cochleae, surface morphology revealed a small number of immature looking HCs, and a lack of the typical organization of the organ of Corti.

Conclusions: Our data suggest that in ears deafened with kanamycin and ethacrynic acid, overexpression of transgenic *Atoh1* can induce surviving SCs to transdifferentiate into new HCs. The new HCs can improve auditory thresholds of the deafened animals. In contrast, in ears deafened by neomycin, regeneration of hair cells by *Atoh1* treatment was very limited. The data suggest that survival of differentiated SCs is necessary for *Atoh1*-induced transdifferentiation into new hair cells.

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P53 Effects of neurotrophic factors in chemically deafened guinea pigs

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Introduction: A primary cause of deafness is the damage of receptor cells in the inner ear. Sensory neurons become dependent on target-derived neurotrophic factors for survival after their axons have grown to their peripheral and central targets. Hair cells seem to provide SGCs permanently with nutritive growth factors, so that hair cell loss results in degeneration of SGCs. The effects of neurotrophic factors, either BDNF / aFGF or GDNF were tested in chemically deafened guinea pigs mimicking the clinical situation in humans. Neurotrophic factors may be important for the survival of neurons and their peripheral processes.

Methods and Discussion: Studies were performed in guinea pigs deafened with kanamycin (450 mg/kg) SQ 2 hours prior to ethacrynic acid (60 mg/kg). Baseline aABR confirmed deafness. NTFs were applied locally via mirocannula-osmotic pump to the scala tympani. Control groups included normal hearing animals and animals that survived 3 days to 7 weeks post deafening. In this study we examined morphological and quantitative changes of primary neurons in the Rosenthal´s canal and of their peripheral processes following deafness. The mean density and diameter of primary neurons was assessed for each turn. Influence of neurotrophin treatment post deafening and changes of peripheral process composition in comparison with untreated deafened controls are shown using immunohistochemistry to label efferent and afferent fibers in the cochlea on electron and confocal microscopic level. In the present study we quantified for the first time afferent and efferent nerve endings post habenula perforata in the different treated animal groups using the image-pro 3D Constructor version 5.0 plug-in to process confocal data of double-stained whole mount preparations.

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P54 Protective effects of Idebenone against a noise induced hearing loss

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Background: Intervention strategies to prevent noise-induced hearing loss (NIHL) have been developed and they target various points in the cell death process. The generation of reactive oxygen species (ROS) is involved in the cascade of events that induce to cochlear damage. In this study we used Idebenone, an ubiquinone derivative with protective effects against oxydative stress-induced lipid peroxidation, as protective treatment for NIHL.

Methods: Hartley albino guinea pigs (250-300 g) were used. Acoustic trauma was induced by a continuous pure tone of 6 kHz, at 120 dB SPL for 40 minutes. In the experimental group (n=6), Idebenone was injected intraperitoneally at a dose of 5 mg/kg b.w., one-hour prior noise exposure and once daily for the following three days. The control group (n=6) received the same doses of vehicle alone. Compound action potentials (CAPs) were measured at 2-20 kHz at different time points. Morphological changes were analysed by scanning electron microscopy. Necrotic or apoptotic OHCs in the cochleae were identified with immuno-histochemical stains. Statistical analysis was performed.

Results: The acoustic threshold measured 1 hour after acoustic trauma was elevated to 70-90 dB in the higher frequencies of the CAP audiogram, with a maximum threshold elevation ranging between 12 and 20 kHz. During the first 24 h following the acoustic trauma, there was a partial recovery of CAP thresholds of about 20 dB to reach a final threshold elevation of about 50-70 dB at 7 days after noise exposure, but there was no further improvement over the remaining experimental weeks. The animals treated with idebenone showed a similar temporary threshold shift but a clear improvement in the recovery of CAP thresholds were observed 7 days after noise exposure with significantly reduced permanent threshold shift and hair cell loss.

Conclusion: These results suggest that the anti-oxidant action of Idebenone is associated to the prevention of hair cell loss and plays a significant role in reducing noise-induced hearing loss. Further studies will be needed to determine the clinical applications of these results on noise induced hearing loss.

P55 Ca^{2+}-responsive transcriptional regulators in the auditory system differentially alter BDNF expression upon tinnitus – inducing paradigms

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Aberrant neuronal activity and plasticity changes are thought to be the basis for a variety of neurological disorders inducing epilepsy, phantom pain and phantom noise (tinnitus). We recently showed that brain-derived neurotrophic factor (BDNF), c-fos and the activity-regulated cytoskeletal gene (Arg 3.1) is changed in cochlear neurons (BDNF, c-fos) and auditory cortex (BDNF, Arg 3.1) upon a variety of tinnitus-inducing paradigms such as salicylate treatment or acoustic trauma (Tan et al., submitted). We therefore were interested in the signalling pathway by which salicylate and/or acoustic trauma change the expression of BDNF in cochlear or central auditory neurons. Transcription of the brain-derived neurotrophic factor (BDNF) gene is regulated in a calcium- and neuron-selective manner. Calcium entry into the cell leads to phosphorylation of the transcription factor cAMP-response element (CRE) binding portein (CREB) which binds to the calcium response element (CaRE) 3. Calcium response factor 1 (CaRF) acts on CaRE1 which is required for activity-dependent transcription of BDNF. CaRE2 contains an E-box element and binds the upstream stimulatory factors USF1 and USF2. While it is well known that calcium entry alters the recruitment of transcriptional regulators to the BDNF promoter, it is completely elusive how these transcription factors are regulated. The current view is that the activity dependent recruitment requires overall protein availability. We were able to amplify and clone CaRF, CREB, USF1 and USF2 from postnatal and adult cochlear tissue. Considering the current view of relatively stable transcriptional regulators we were surprised to note a differential change in the expression of these distinct regulators, subsequent to tinnitus-inducing stimuli. The data reveal a so far unknown activity-, injury- or stress-induced response of the transcriptional regulators of BDNF.

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P56 Purinergic activation of ERK1/2 in the neonatal rat cochlea mimics activation by hair cell damage

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Extracellularly regulated kinases 1 and 2 (ERK1/2) are members of the MAPK family of signalling molecules that play critical roles in cell survival and proliferation. Recently, using a phospho-specific antibody, we showed ERK1/2 activation in response to laser or mechanically induced damage in the neonatal rat cochlea. ERK1/2 activation spread along the length of the cochlea coil and occurred predominantly in Deiters' and inner phalangeal cells. Neighbouring hair cells did not show ERK1/2 activation. In response to hair cell damage ATP is released (Gale et al., 2004). Degradation of extracellular ATP by treatment with the ectonucleotidase apyrase reduced the longitudinal spread of the ERK1/2 signal, suggesting involvement of purinergic receptors in damage-induced ERK1/2 activation. We tested whether exogenous ATP is able to mimic the damage response. Brief (20 s) local application of 100 µM ATP activated ERK1/2 in Deiters' and in some cases, inner phalangeal cells but not hair cells, a pattern almost identical to damage.

Purinergic receptors are subdivided into two main clasess, the ionotrophic P2X and the metabotrophic P2Y receptors. P2X receptor subtypes only respond to ATP whereas P2Y receptor subtypes differentially respond to ATP, UTP, UDP and ADP. Therefore we investigated whether UTP, UDP and ADP are able to induce ERK1/2 activation. Unlike ATP, application of 100 µM UTP (20 s) activated ERK1/2 in Deiters' cells, in most cases only the second and third rows, but failed to activate inner phalangeal cells. UDP (100 µM) and ADP (100 µM) failed to elicit an ERK1/2 response. Thus, ability of ATP and UTP and inability of UDP and ADP to activate ERK1/2 suggests a role for $P2Y_2$, $P2Y_4$ or $P2Y_{11}$ receptors. Preliminary data obtained in the absence of extracellular calcium suggests that we cannot exclude the involvement of P2X receptors. Using commercial antibodies we observed $P2Y_2$, $P2Y_4$ and $P2X_2$ expression in neonatal rat cochlea supporting cells, with $P2X_2$ expression being particularly intense in the Deiters' cells and pillar cells. Further investigation of the purinergic receptor subtypes involved in the damage-induced ERK response is ongoing.

P57 Sound conditioning activates glucocorticoid receptors in the cochlea

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Sound conditioning is a well studied paradigm where low level of acoustic stimuli prevents subsequent damage in the cochlea. Our working hypothesis is that sound conditioning, through its locally targeted action, activates stress activating pathways and thereby alters the level of the glucocorticoid receptor (GR) in the cochlea to modulate cochlear sensitivity. Male CBA mice were exposed to acoustic trauma with or without sound conditioning (8-16 kHz, 89 dB, 15 min). Another group of mice were treated with the glucocorticoid synthesis blocker (metyrapone) and receptor antagonist (RU486) prior to sound conditioning. Immediate post trauma, the trauma only group showed 40-50 dB hearing loss between 8-20 kHz. Sound conditioning prior to trauma significantly lowered the ABR threshold shift at all frequencies compare to the trauma group. Pre-treatment with drugs (Metyrapone+RU486) abolished the protective effect. The combined treatment of sound conditioning + trauma showed significantly higher corticosterone levels when compared with the trauma alone group. Immunohistochemical analysis showed increase nuclear translocation of GRs in the spiral ganglion neurons in sound conditioning + trauma group compared to the trauma alone group. Pre-treatment with drugs before sound conditioning prevented the nuclear translocation of GRs. We conclude that sound conditioning activates the HPA axis, stimulates systemic stress levels and activates GRs in spiral ganglion. These combined effects contribute to the overall protection of the cochlea against acoustic trauma.

P58 A rat model for vibration induced hearing loss

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Introduction: Sensorineural hearing loss after middle ear surgery has been explained by noise generated by drilling neglecting the vibration generated by the burr. The aim of the present study was to evaluate the role of temporal bone vibration in the etiology of the hearing loss in a rat model.

Methods: An electromagnetic shaker was used for vibration on the middle of the skull of rats at different durations and intensities. The hearing threshold was measured by auditory evoked brainstem response. In total 14 rats were tested (10 vibrated rats, 4 control rats).

Results: Different parameters (frequency and duration) were tested to get a good hearing loss after vibration. Vibration with 500 Hz for 45 Min resulted in a mean immediate threshold shift of 32 dB measured directly after vibration. This hearing loss could be different for both ears. Moreover these parameters are specific for the rat since in guinea pig vibration with 250 Hz for 15 Min was shown to be sufficient for the induction of a mean immediate threshold shift of 42 dB. By monitoring the hearing loss within the following days post vibration (1, 2, 3, 7, 14 days) a stepwise recovery with almost full recovery after 7 days was found in most cases. Rarely the hearing loss developed only 1 day after vibration. Control experiments with rats exposed to the noise caused by the vibration (95 dB) were carried out to show that this hearing loss was not induced by the noise.

Conclusion: An animal model for vibration induced hearing loss was established. This animal model may be used in assessing the severity of temporal bone vibration.

P59 Identification of environmental risk factors for age related hearing impairment (ARHI)

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Introduction: Age related hearing impairment has a prevalence of 50 % at the age of 80 years and therefore is one of the major sensory impairments in elderly people. It might be influenced by different diseases, noise and hereditary factors. Factors like hypertension, high cholesterol or smoking may also increase the probability for the development of hearing loss. Aim of this study was to identify environmental factors correlated with age related hearing loss.

Methods: A total of 1536 patients (mean age 74 years) participated in this study at Tampere University Hospital in the years 2003 - 2004. After removing patients with possible hearing loss due to diseases of central neural system, ear infection or ear diseases 744 was left in the study. A pure-tone audiometry with air conduction was performed for all participants. The participants filled out a detailed questionnaire to survey medical history and environmental risk factors that may affect the hearing as well as quality of life. Environmental risk factors will be identified by statistical analysis of audiological and environmental data. Data were analysed with SPSS for Windows 11.5. The hearing loss at speech region (500 Hz - 4000 Hz) was modelled with age, quality of life, smoking and cholesterol treatment as independent variables using general linear models (GLM).

Results: Age (0.052), quality of life (0.056) alone were found significant. Cholesterol was not significant (0.107), but its interaction with quality of life (0.019) as well as its interaction with age were found significant factors as well its interaction with age and quality of life (0.061). Furthermore a correlation was found with age and quality of life (0.004) as well.

Conclusion: Age is the main factor behind hearing loss. Cholesterol seems to be an important factor because of its interaction. Although quality of life is significant in the model, it does not mean that it is a factor behind hearing loss. Merely this suggest that individual aging which reflects also the quality of life is a significant factor.

P60 Block of mouse inner hair cell Ca^{2+} currents by phenylalkylamines and benzothiazepines

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Whole-cell calcium currents in mouse neonatal inner hair cells (IHC) are carried predominantly by voltage gated L-type calcium channels of the $Ca_v1.3$ subtype (Platzer et al., 2000). They have an unusual resistance towards dihydropyridine blockers such as nimodipine or nifedipine compared to the $Ca_v1.2$ calcium channels (Platzer et al., Cell, 2000; Michna et al., J. Physiol., 2003). We aimed at determining the block of IHC Ca^{2+} currents by the two other classes of L-type calcium channel blockers, phenylalkylamines (e.g. verapamil) and benzothiazepines (e.g. diltiazem).

Calcium currents were measured in IHCs aged P3-P7 with 5 mM Ca^{2+} as charge carrier (bath solution (mM): NaCl 100, $CaCl_2$ 5, $MgCl_2$ 1, glucose 5.6, HEPES 10, TEACl 35, 4-aminopyridine (4-AP) 15; pipette solution (mM): cesium methansulfonate 105, $CaCl_2$ 0.1, $MgCl_2$ 4, Hepes 5, EGTA 5, CsCl 20, sodium phosphocreatine 10, Na_2ATP 4, GTP 0.3).

Both verapamil and diltiazem inhibited the current by about 90 % at concentrations of 3 mM, respectively. The block was largely reversible. For verapamil, IC_{50} was 199.3 $\pm$ 18.6 µM and the Hill coefficient was 0.64 $\pm$ 0.02. IC_{50} for diltiazem was 296.6 $\pm$ 83.8 µM and Hill coefficient was 0.93 $\pm$ 0.16.

In conclusion, phenylalkylamines and benzothiazepines block the L-type Ca^{2+} currents in mouse IHCs with concentrations that are in the same range as those needed to block $Ca_v1.2$ channels (Lacinova et al., J. Pharmacol. Exp. Ther. 1995), which is in contrast to the action of dihydropyridines. Blocking concentrations of both verapamil and diltiazem are in the same range as those required for block of turtle hair cell Ca^{2+} currents, respectively, while Hill coefficients are smaller (Schnee and Ricci, J. Physiol., 2003).

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P61 Voltage-activated calcium channel β subunits in cochlear hair cells

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Voltage gated calcium channels consist of four subunits (SU): the pore forming α_1 and the auxiliary $\alpha_2\delta$, γ and β SU. The α_1 SU $Ca_v1.3$ plays a key role in mammalian hearing. It is expressed in inner (IHC) and outer (OHC) hair cells and carries >90 % of the depolarization-evoked calcium current that is required for exocytosis. β SU modulate biophysical properties such as opening time, activation and inactivation (Meir et al., 2000) and membrane trafficking of the α_1 SU (Bichet et al., 2000), and are encoded by four distinct genes (Cacnb1 – Cacnb4) giving rise to the proteins β1- β4.

We aimed at investigating the occurrence of β SU in the organ of Corti, particularly in IHC and OHC, by using RT-PCR and immunohistochemistry. For hair cell-specific RT-PCR, 10-50 IHCs (resp. OHC) of freshly dissected explants of the apical organ of Corti (age: P18) were harvested with micropipettes under the microscope, followed by cDNA first strand synthesis of pooled IHC (resp. OHC) mRNA and nested PCR with β SU-specific primers. Using total cDNA of the organ of Corti, all four β SU isoforms could be detected. IHC cDNA probes were positive for β1, β2, β4, and partially for β3. OHC cDNA probes were positive for β2, β3 and β4, and partially for β1.

To localize β SU in the organ of Corti, immunohistochemical stainings of cochlear cryosections were performed with β SU-specific antibodies (kind gift of Veit Flockerzi, Homburg). Basolateral membranes of IHCs were stained with anti- β1, β2, β3 and β4, and the basal poles of OHC were positive for β1, β2, β3. It was however hard to distinguish between staining of HC membranes and their efferent/afferent fibres. Outer and inner spiral bundles were clearly stained with antibodies against β1 and β3. Spiral ganglia neurons showed membrane stainings for β2 and β4.

In conclusion, all four β SU isoforms are expressed in the organ of Corti, each with a specific expression pattern. Further studies are needed to identify the β SU composition of $Ca_v1.3$ channels in IHC and OHC.

P62 Regulation of endocytosis in Reissner's membrane

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It is speculated that Reissner's membrane plays an important role in water transport of the cochlea. Recently, vasopressin and cAMP-mediated pathways have been suggested to contribute to water transport in the cochlea. However, regulation of water transport and effects of c-AMP-mediated pathways containing vasoressin in epithelial cells of Reissner's membrane have been obscure. Thus, in the present study, endosomal transport of water in epithelial cells of Reissner's membrane of rat was investigated by fluorescein isothiocyanate (FITC)-dextran. The cellular location and the number of FITC-labeled endosomes in cultured epithelial cells of rat Reissner's membrane were examined by an epifluorescence microscope under changes of osmolality, administration of the antidiuretic hormone, vasopressin; a stimulator of β-adrenergic receptor, isoproterenol; and an inhibitor of Na^+/K^+-ATPase, ouabain. In isotonic solution, FITC-labeled endosomes were visualized after 3 minutes of treatment and the maximum increase of FITC-dextran endocytosis was observed within 15 minutes of treatment. Under hypotonic conditions, a small but significant increase of FITC-dextran endocytosis was observed, however, an increase of FITC-labeled endosomes was not observed under stimuli of vasopressin, isoproterenol, and ouabain. We concluded that endocytosis of water transport in the epithelial cells of Reissner's membrane is mainly dependent on changes of osmotic pressure in endolymph.

P63 SK2 and BK in the inner hair cell

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Inner hair cells express multiple potassium channels, like the SK2 or BK channel. Before the onset of hearing a transient efferent innervation is found on inner hair cells, mediated by nicotinic cholinergic receptor α9- and α10 subunits (AchRα9/10) and SK2 channels, which are calcium activated and responsible for the potassium efflux. The SK2 channel is localised at the basal pole of inner hair cells. This inhibitory synapse disappear around the onset of hearing (Katz et al., 2004), at the same time when the BK channel is upregulated (Pyott et al., 2004; Hafidi et al., 2005). The presumed physiological role of BK channels is a decrease of the membrane time constant even at the resting potential and fast repolarization of the receptor potential. Both contribute to phase-locked receptor potentials up to high sound frequencies (Kros et al., 1998).

In conclusion a striking temporal overlap becomes visible between SK2 and AchRα9/10 downregulation and BK upregulation, questioning a common molecular mechanism of regulation. Using various knockout mutants, immunohistochemistry, molecular and biochemical techniques, we try to unravel the mechanism which guides the final differentiation of IHC on the level of BK and SK2.

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P64 Aquaporin 5 in the cochlea: the subcellular distribution reveals water shunt at the endolymph-perilymph barrier

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Aquaporins act as water-specific membrane channel proteins and have been identified in the cochlea. In order to maintain auditory sensory function water homeostasis and ionic composition of the cochlear fluids must be tightly regulated. In particular the composition of the extracellular endolymph is essential for sensory transduction. The lateral wall of the cochlear duct has been proposed to play a major role in endolymph secretion. Contradicting results regarding the expression of the Aquaporin-5 (AQP5) in the lateral wall of the cochlea have previously been described. This study identifies species differences in tissue and cellular distribution in the lateral wall of the mouse, rat, gerbil and guinea pig cochlea. Developmental gradients along the cochlear duct show pancochlear expression during postnatal development and a reduction to the apical quadrant in adult rats. This loss of AQP5 expression coincides with the loss of direct contact between outer sulcus cells and the endolymphatic fluid space. At the subcellular level AQP5 demonstrates a polarized expression in the apical plasma membrane of outer sulcus cells in direct contact with the endolymph. This is the first demonstration of an aquaporin mediated water shunt between the endolymphatic and perilymphatic fluid domains of the inner ear.

P65 Expression of TRPV4 receptor in outer hair cells of the guinea pig cochlea

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Previous study showed that cell swelling induced by hyposmotic activation led to an increase of intracellular Ca^{2+} concentrations ($(Ca^{2+})_i$) in outer hair cells (OHCs) of the guinea pig cochlea (Harada, Ernst and Zenner; 1993,1994). Recently, transient receptor potential vanilloid 4 (TRPV4) has been proposed as an osmosensor channel (Wissenbach et al., 2000). To investigate whether TRPV4 channel mediates the hypotonic swelling-induced Ca^{2+} response in OHCs, we investigated the possible role of TRPV4 receptors on the hypotonic activation-induced Ca^{2+} signaling in the OHCs of the wild-type and TRPV4-/- mice cochlea. Results showed that TRPV4 mRNA and protein were expressed in the cochlear inner hair cells (IHCs), OHCs, and spiral ganglion cells (SGCs) by means of RT-PCR, single-cell RT-PCR, and immunohistochemistry whereas they were negative in the TRPV4-/- mice cochlea. Hypotonic activation and 4α-phorbol 12,13-didecanoate (4α-PDD), a TRPV4 synthetic activator, increased the $(Ca^{2+})_i$ of OHCs isolated from the wild-type mice cochleae, which was inhibited by red ruthenium, a TRPV4 inhibitor. In contrast, OHCs from TRPV4-/- mice failed to exhibit the Ca^{2+} response both to hypotonic and 4α-PDD stimulation. We conclude that the functional expression of TRPV4 is involved the hypotonic stimulation-induced Ca^{2+} response in cochlear OHCs.

P66 Modulation of voltage-gated Ca^{2+} channels of vestibular hair cells

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Ca^{2+} inflow through voltage-gated Ca^{2+} channels of vestibular hair cells sustains transmitter release at the cytoneural junction. It is therefore important to establish how these channels are regulated by voltage and/or by intracellular factors. Hair cells isolated from frog semicircular canals were recorded in the whole-cell configuration, using intra- and extra-cellular solutions designed to block all but the voltage-gated Ca currents. In the presence of 1 mM ATP in the pipette, about 60 % of the cells displayed a Ca^{2+} current formed by a mix of L- and drug-resistant (R2) components, while the remaining 40 % exhibited an additional drug-resistant fraction (R1), which inactivated in a Ca^{2+}-dependent manner. If the pipette ATP was raised up to 10 mM, R1 progressively enhanced as intracellular ATP equilibrated with the pipette solution, and became apparent in all recordings (instead of in 40 % of them). In cells exhibiting the R1 fraction, ATP dialysis produced a mean increase of the R1 component of about 380 %, whereas L and R2 increase was of about 170 %. Cells initially lacking the R1 current had a similar raise in R2 and L fractions, while R1 raised from 0 to about 25 pA. During the ATP effect, cell perfusion with 10 µM nifedipine produced a 70 % decrease of the plateau current without affecting R1, as in control experiments. These results indicate that ATP modulation was mainly targeted to the R1 channel. Despite the presence of internal ATP, long depolarizations (>5 s) produced a decay of the current to a steady level: larger the depolarization, faster the decay. The steady level was outward at +20 mV; the decay was fully reversible on returning to the holding potential. Ca channel blockade, probed during current decay with the fast application of 200 µM Cd, reduced the total current of the same amount. This shows that the decay was not produced by the increase of outward Cs^+ flow through the Ca^{2+} channel, reducing Ca^{2+} inflow. Rather, total current decay was generated by the progressive activation of an outward current flowing through a different channel type. Long depolarizations might unblock the K^+ channels, which become able to carry outward Cs^+ current.

P67 Numerical simulation of ion transport in the stria vascularis of the cochlea

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Cochlear mechanoelectrical transduction is driven by gradients in electrical potential and potassium concentration across the organ of Corti. Both of these gradients are believed to be produced by active potassium transport from the stria vascularis into the endolymph, a fluid compartment apical to the organ of Corti. The stria vascularis consists of two layers, an apical layer of metabolically active marginal cells and a basal layer made of of several cell types. Cells of the basal layer are connected by gap junctions to each other and to substrial fibrocytes thought to supply the stria with potassium. Between the layers is an electrically distinct fluid space. We present a computational analysis of the steady state function of the tissue based on experimentally identified ion transporters and channels. The transepithelial current and voltage as well as the intracellular ion concentrations are calculated in open and short circuit configurations for each layer. The two layers are also modeled in series. The model confirms that marginal cells are a better current source than voltage source. The electrical activity of the basal layer depends on the current provided by the marginal cell layer. Contributions of individual transporters and channels to the system´s behavior are presented and synergism between the Na/K-ATPase and Na/K/Cl cotransporter in the marginal cell is demonstrated. These results have important implications for a variety of genetic and drug-induced causes of hearing loss involving cochlear ion transport. [Supported by the Whitaker Foundation]

P68 Low power LASER promotes hair-cell regeneration following gentamicin exposure in postnatal organotypic culture of rat utricles

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In normal postnatal mammalian inner-ear sensory epithelium, regeneration of hair cells is a very rare event, but following ototoxic damage there is a hair-cell renewal response with partial restoration of the vestibular sensory epithelium. One of the methods studied is the use of low power LASER to enhance this renewal response. We have established an long-time organotypic culture of 2-7-day-old rat utricular maculae to study aminoglycoside-induced vestibular hair-cell renewal. In an our series of experiments utricles were exposed to 1 mM of gentamicin for 48 h and then allowed to recover in culture medium or in medium supplemented with the irradiation of low power LASER daily. In a parallel control series, explants were not exposed to gentamicin. Whole-mount utricles were stained with FM1-43 that is efficient marker for identifying live hair cells in cultured tissues and their live hair cell visualized directly through tissue culture dish with cover glass bottom by Confocal microscope at specified time repeatedly. Loss of hair-cell was nearly complete 2 days after exposure to gentamicin, a peak of regeneration reached after 21 days in culture medium but within 15 days in medium added irradiation of low power LASER. These results suggest that low power LASER promote spontaneous hair-cell regeneration following gentamicin damage in utricular explants.

P69 Survival and integration of Sox-1-GFP mouse embryonic stem cells in auditory brain stem slice cultures

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For a clinical therapeutic approach the use of stem cells in a cell replacement therapy is promissing for the regeneration of sensory function. Previously we have used embryonic neuronal tissue and stem cells to initiate a neuronal repair paradigm into the inner ear.

Here we have studied the differentiation and incorporation of Sox1-GFP mouse embryonic stem-(ES) cell into the rat postnatal brainstem slice culture. *Sox-1* is the earliest known specific marker of neuroectoderm in the mouse embryo. GFP is not detectable in undifferentiated ES cells but becomes evident after neural differentiation that allows direct analysis of the conversion of pluripotent ES cells into neuroectoderm *in vitro*. Previously it was shown that in adherent monoculture Sox1-GFP mouse ES cells can differentiate into neurons and glial cells. We have implanted Sox1-GFP mouse ES cells into auditory brainstem slice from SD postnatal rats (P 11-15). Using tissue chopper 300-µm-thick slices encompassing vestibulocochlear nerve and cochlear nucleus were prepared and propagated using membrane interface methods described by Stoppini. After placing slices on membranes the cochlear nucleus was labeled by DiI. *In vitro* transplantation was conducted at day 5±2 in culture. A total volume of 0.5 µl of Sox1 cell suspension in concentration of $1x10^4$/µl was deposited next to the vestibulocochlear nerve and cochlear nucleus. Two weeks after Sox-1 cell transplantation co-cultures were fixed and immunostained with antibodies raised against neuronal and glial markers. We have demonstrated that following deposition next to the slices Sox1 cells actively migrate toward the cochlear nucleus pre-labeled with DiI. The morphological and immunohistochemical data indicated that grafted Sox1 cells were able to differentiate into neurons and glial cells by two weeks after implantation. These results demonstrate that organotypic slice co-cultures of the auditory brainstem with Sox1 cells present a useful model to study the regeneration of the respective neurons and that co-cultured Sox1 cells generate neurons and glial cells capable of integrating into the brain circuitry.

P70 Mesenchymal stem cell transplantation targeting cochlear fibrocytes accelerates the hearing recovery in a rat model of acute mitochondrial dysfunction

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Local energy shortage in cochlea is thought to cause severe hearing loss. Recently, we have developed a novel rat model of acute cochlear mitochondrial dysfunction using a mitochondrial toxin, 3-nitropropionic acid (3NP) which results in hearing loss as a consequence of local energy shortage (Neuroreport 2004; 15:1597-1600, Audiol Neurootol 2005; 10:220-233). The rats treated with 300mM 3NP showed a temporary threshold shift (TTS) which reached the peak threshold at 1 d after administration and gradually recovered until 4-5w, although there remained a residual hearing loss at higher frequencies. In histological analysis and TUNEL assay, focal and drastic apoptosis of cochlear fibrocytes in the lateral wall and spiral limbus were found, although the organ of Corti exhibited no histological changes and TUNEL positive cells. The area of apoptosis in the lateral wall and spiral limbus were clearly demarcated and localized along the potassium recycling route within the cochlea. In Bromodeoxyuridin (BrdU) incorporation assay, active regeneration of fibrocytes was observed around the area of apoptosis in the lateral wall. These results suggest that reconstruction of the potassium recycling route by regeneration of cochlear fibrocytes led to the hearing recovery. To rescue the residual hearing loss of 3NP treated rat, we tried rat mesenchymal stem cell (MSC) transplantation into the cochlear perilymph from semicircular duct at 3 days after 3NP administration. Before the transplantation, MSCs were prelabeled by BrdU. At 11 days after the transplantation, a number BrdU-positive cells were detected around scala tympani and scala vestibule, and some of them were detected also in the damaged spiral ligament. The hearing recovery ratio after 3NP administration was significantly increased by the MSC transplantation in rats which exhibited BrdU-positive cells in the spiral ligament. These results suggest that reorganization of fibrocytes in the cochlear lateral wall is essential process in hearing recovery after acute sensorineural hearing loss due to cochlear energy shortage, and MSC transplantation would be a promising therapy against such hearing loss.

P71 Withdrawn

Characterization of progenitor cells in the human embryonic inner ear

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Sensorineural hearing loss is characterized by loss of sensory hair cells and/or damage to the associated spiral ganglion neurons. These cells are incapable of regeneration in mammals, including humans. Present treatment options for individuals with severe to profound sensorineural hearing loss include hearing aids or cochlear implantation. One of the new approaches that is being investigated to improve the survival or replacement of the damaged hair and neuronal cells is the implantation of embryonic stem or progenitor cells into the inner ear. Another possible source of suitable cells for transplantation would be human cell lines derived from the embryonic/fetal cochlea. With this aim we isolate the auditory progenitors from human embryonic/fetal cochleas (5-11 gestational weeks). The terminal differentiation of the auditory cells is not yet established at this stage, which gives us the opportunity to study multipotent capability and self-renewal properties of these cells. In order to define suitable tissues for isolation from the embryonic/fetal inner ear we first isolated human cochlea anlage (9 gestational weeks). The tissue was decalcified, fixed in paraformaldehyde and cryosectioned. Immunohistochemistry with primary antibodies against β-tubulin and NeuN (neuron specific markers) as well as Nestin (neural precursor marker) was applied to characterize the developing auditory precursors. A large number of neuronal precursors and neurons were immunoreactive for β-tubulin and NeuN within the spiral ganglia region as well as their neurites infiltrating the developing sensory epithelia. Many cells were also positively stained for Nestin antibody indicating the presence of immature neural precursor cell population, which suggests that these cells still maintain a proliferative and self-renewal capacity. Thus, such cells are suitable for isolation, expansion as well as induced segregated differentiation in in vitro systems. There is a significant need for a self-renewalable human in vitro system that could be a feasible experimental tool to study the differentiation potential of human sensorineural auditory cells and for therapeutic purposes in the future.

P72 Can the human auditory nerve undergo regeneration?

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Introduction: It has been shown that neural stem cells (NSC) located at various sites of the central nervous system (CNS) are able to proliferate in vitro and form neurospheres (Reynolds and Weiss, 1992). A technique was developed to culture human spiral ganglion (hSG) cells obtained during petroclival meningeoma surgery (Tylstedt and Rask-Andersen, 2001). In this study adult hSG and guinea pig SG (gpSG) cells, directly cultured or developed from expanded inner ear progenitor cells, were analysed using time lapse video microscopy (TLVM) (Rask-Andersen et al., 2005) and immunocytochemical staining.

Material and Methods: SG cells were enzymatically treated and seeded on uncoated dishes. Spheres were expanded with EGF and bFGF and differentiated on fibronectin/collagen or poly-L-ornithine coated dishes with NT-3, GDNF and BDNF to cells expressing both GFAP/S-100 and β-tubulin, indicating multipotency. Cultured neurons were maintained for several weeks.

Results: Neurospheres showed expression of nestin and incorporation of 5'-Bromo-2-deoxyuridine (BrdU). Differentiating neurons were immunopositive for both tyrosine kinase receptor B (TrkB) and C (TrkC) suggesting that they were inner ear specific. Human neuroblasts underwent cell division, verified with TLVM, and formed mature β-tubulin positive neurites. TLVM showed that neurites regenerated and perikarya formed clusters through translocation (speed approximately 30 µm/h). During this "ganglionization" process, the growth cones were essential for navigation, branching and elongation.

Conclusions: This study indicates that the adult human inner ear contains neural progenitor cells, suggesting that the auditory nerve may undergo self-renewal and repair during life. It is possible that SG cells may be substituted and used as graft material in cell therapy to regenerate tissue in patients with neurological defects including sensorineural hearing disorders.

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P73 *In vitro* and *in vivo* non-viral gene delivery to the cochlea

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Degeneration of auditory hair cells is considered a major cause of sensorineural hearing loss in humans. Earlier studies showed that the Atoh1 gene is vital for hair cell development, necessary and sufficient for hair cell differentiation. Many employ viral vectors to deliver various gene of interest into the cochlea and results are encouraging. One group managed to deliver the Atoh1 gene and transfect the non-sensory cells of the cochlea using an adenoviral vector which brought about hair cells regeneration and partially restore hearing in deaf guinea pigs. However the use of viral vectors as gene delivery vehicles has potential risks and raises certain safety concerns. Hence we are exploring the use of non-viral vectors for inner ear gene therapy applications. Our preliminary studies demonstrated polyethylenimine's ability to transport the Atoh1 gene and successfully transfect the supporting cells of mature guinea pigs. Transfection efficiency is boosted by the use of an osmotic pump after 1 week of sustained release into the cochlea. We hypothesize that if the expression is sustained long enough, we will be able to bring about transdifferentiation of supporting cells and regeneration of sensory hair cells like in the case where the adenoviral vector was used. Advantages of non-viral vectors include non-immunogenicity, unlimited size of transfer expression cassettes and ease of large scale production.

Until now, there isn't a representative in vitro model or cell line for cochlea transfection studies of mature guinea pigs. We transfected supporting cells isolated from the sensory epithelia of mature guinea pigs and compared the transfection efficiency results with that in the in vivo case. So far, the transfection efficiency results correlates well. Hence gene delivery vector efficiency could be first assessed using these primary cell cultures as a guide to the vectors' efficiency in vivo.

In conclusion, non-viral vectors potentially provide clinicians with an easy and safer approach for cochlear gene therapy and primary culture of supporting cells could be used as a preliminary assessment model for the screening and optimizing of these non-viral vectors.

P74 The effects of partial inner hair cell loss on neural response properties in the central nucleus of the Inferior colliculus in chinchillas

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A better understanding of central changes occurring after peripheral damage of hearing may lead to improvement of strategies in the development of inner ear implants and conventional hearing aids. For this purpose we searched for indications of reorganization in the auditory system after carboplatin-induced peripheral hearing loss. Carboplatin causes partial inner inner hair cell (IHC) loss, in contrast to most other ototoxic drugs which preferentially damage the outer hair cells. Thus carboplatin treatment leads to reduced afferent input into the central auditory system, but the afferent responses remain sensitive and sharply tuned, because outer hair cell function is not impaired.

We examined the effects of carboplatin-induced partial IHC-loss on excitatory and inhibitory reponse areas, CF thresholds, rate intensity functions and local field potentials (LFPs) of extracellular multi-unit-activity in the central nucleus of the inferior colliculus (ICc) in the same awake chinchillas before and after at least 4 weeks of recovery from carboplatin treatment. One-tone and two-tone stimuli were used to determine excitatory and inhibitory responses. Chinchillas were implanted with a recording chamber and holder on the skull for stereotaxic microelectrode recordings from the ICc in awake restrained animals. With an adequate dose of carboplatin (80 mg/kg) a reduction of the IHC density (40-60 %) occurred in all except for the most basal areas of the cochlea.

Response properties from over 200 multi-units before and after carboplatin treatment were recorded from the same stereotaxic locations in the ICc. Response properties before carboplatin treatment were similar to those reported for anaesthetized animals, except that there was a larger variety in inhibitory response regions. After carboplatin-induced partial inner hair cell loss we observed an increase of the negative LFP amplitudes, enhancement of multi-unit transient (onset) responses and a reduction of two-tone-inhibition.

These results indicate that reduced afferent input due to partial loss of IHC may induce compensatory mechanism at the level of the auditory midbrain.

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P75 Effect of a temporary inactivation of the auditory cortex on time resolution in the rat

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Previous studies have shown that the auditory cortex (AC) plays an important role in an animal´s ability to resolve the time parameters of an acoustical signal. Most of the data were obtained in experiments that used a permanent ablation of the AC. In our study, a temporary inactivation of the AC by the GABA agonist muscimol was used. The gap detection threshold and the ability to discriminate the duration of a gap were examined by a behavioral method before and after muscimol application to the surface of the AC (left, right and bilateral inactivation of the AC were performed sequentially in the same animal). An avoidance conditioning procedure was used for training and testing. One group of thirsty rats was trained to stop drinking when a gap occurred in a continuous noise. Another group was trained to discriminate between two different durations of gaps. For discrimination, gaps with durations of 60 ms or 10 ms were used as warning and safe stimuli, respectively. Unilateral and bilateral inactivation of the primary AC resulted in a worsening of the gap detection threshold, which increased from 1.6-1.8 ms to 5-8 ms. The maximal effect was observed thirty minutes after muscimol application. A deficit in the ability to discriminate between gaps of different durations was revealed only after left-side and bilateral inactivation of the AC. In this case the greatest decrease in discrimination performance was observed 1-2 hours after muscimol application. The following day, the rats' ability to detect a gap or to discriminate between two gap durations was fully recovered. The results point to a role for the AC in the time resolution of acoustical signals and suggest that some aspects of this function might be lateralized to the left hemisphere.

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P76 Longterm experiments with an auditory midbrain implant

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Auditory brainstem implants with electrodes positioned in the cochlear nucleus are used for the auditory rehabilitation of patients with neural deafness. Alternative concepts for targeted tonotopic stimulation of the higher auditory areas, such as Colliculus inferior (IC), are currently under investigation. In acute experiments electrically in IC evoked potentials can be registered at the auditory cortex using surface and/or deep insertion electrodes and compared with acoustically induced cortical signals. The running step is testing safety and functionality in chronic experiments. In collaboration with Cochlear Ltd. (Sydney), a 4 mm 20-channel rod electrode was developed, electrode contacts being arranged in a circle on a rod at 200 m-6 intervals. This electrode inserted in IC allowed measuring acoustically evoked potentials in acute experiments and also electrical stimulation of IC. Parallel to a non-stimulated control group daily stimulation with commonly used speech processor and impedance measurements are performed. After 3 months stimulation the brain was fixed for histological analyses. The behavior of the implanted cats did not change in any way with or without electric stimulation during longterm studies. Experiments with electrical stimulation in IC during speech processor a few up hearing threshold evoked no avoidance reaction. There is no visible pain reaction. In behaviour tests animals with deafness (neonatal or adult) react on short loud sounds with attention and visual searching. The impedance was stable during implatation time of 3 month. Histological analyses of IC showed a damage zone limited around the insertion channel. There was no chronic inflammation. There was a thin endothelia cell layer and a few new capillaries around the electrode track. Tissue behind the damage-zone appears to be undamaged and healthy. There a lot of questions concerning the best implantation angle of the electrode and the speech processing strategies, nevertheless the implantation of this multi channel electrode in IC has to be considered safe. Supported by Cochlear and SFB 955

P77 Hearing relative phases for two harmonic components

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Current models of basilar membrane motion such as the gammatone auditory filterbank predict that the first eight harmonics in a complex are resolved. A new functional model of the cochlea (Reimann, submitted J. Acoust. Soc. Am. (2005)) proposes much broader filters in which harmonics beyond the first are unresolved. This results in modulation of the membrane at the rate of F1 which is sometimes audible. The strength of the F1 perception is dependent on the relative phases and levels of the components. We provide experimental validation of the Reimann model by measuring threshold for a change in F1 loudness as a function of relative phase. In Experiment I, threshold was determined by presenting the listener with two low-numbered harmonics for 3 s. During the central section of the stimulus the phase of the higher harmonic was changed gradually. Listeners responded to whether the perception varied or remained constant. Overall stimulus level was 75 dB and the higher harmonic was 6 dB quieter. Four pairs of harmonics were used: F2 and F3, F3 and F4, F4 and F5, F3 and F5. Experiment II confirmed that it was the relative phase between the two components that produced the audible change in perception and not an absolute phase change. This procedure was similar to the first experiment but the phase of the lower harmonic was also changed. The amount of phase change applied to the higher harmonic however was a proportion of the phase change applied to the lower one; five scalars were used: 0.5, 1, 1.41, 1.5, 2. The results show that all listeners can detect a small change in the relative phase of the components in five of the conditions with the lowest-numbered harmonics. The average value for these five conditions was 0.43 radians in 600 ms. A baseline condition showed the rate of phase change necessary to produce an audible pitch change was 5.91 radians in 600 ms. The Experiments show that changes in the relative phase of two low-numbered harmonics are audible, indicating that auditory filters are broad on the high side at normal listening levels.

P78 Dendritic potassium channels shape postsynaptic potentials at the inner hair cell afferent synapse

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Inner hair cell (IHC) ribbon synapses in the mammalian cochlea are the first site of chemical transmission in the auditory pathway. Postsynaptically, fast transmission is mediated by fast-gating AMPA receptors at the afferent dendrites of auditory nerve fibers. To achieve rapid and reliable signaling, however, the receptor currents must be reliably converted into suprathreshold postsynaptic potentials with short latency. Here, we compare excitatory postsynaptic currents (EPSCs) and potentials (EPSPs) in afferent dendrites to identify dendritic mechanisms enabling rapid and reliable transmission. In 7-12 day-old apical turns of the rat cochlea we recorded spontaneous or (by 15 mM K^+ extracellularly) evoked postsynaptic events from IHC afferent dendrites using whole-cell patch clamp. As shown before, EPSC amplitude distributions were highly skewed with a peak at about 30-40 pA and values up to 800 pA. EPSP waveforms were less skewed with a peak at about 15-20 mV and EPSP amplitudes of 1 to 40 mV (in 1 µM TTX). EPSP waveforms were 2-4 times slower than EPSC waveforms. These results suggest that passive properties and also postsynaptic conductances might play a role in shaping EPSPs. Voltage step protocols revealed outward K^+ currents activating as low as -70 mV. This low-threshold K^+ conductance was completely blocked by 4 mM 4-aminopyridine (4-AP) and partially reduced by 60 nM α-dendrotoxin, a relatively selective blocker of Kv1 potassium channels. 4-AP reversibly and significantly slowed down the EPSP decay time constant by about 20 % while EPSP rise time and amplitude were not significantly changed. 4-AP had no effect on the EPSC waveform suggesting that 4-AP did not affect the release machinery nor AMPA receptor kinetics *per se*. These results suggest that low-threshold K^+ channels play a significant role in shaping the EPSP waveform in afferent dendrites.

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P79 Role of synaptic ribbons in hair cell transmitter release and hearing

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The function of the synaptic ribbon at the inner hair cell (IHC) afferent synapse is still largely unknown. Here we show that the large scaffolding protein Bassoon is expressed in the IHCs as early as at day of birth. IHCs of the mouse mutant for Bassoon showed impaired ribbon anchoring and reduced Ca^{2+} current. The lack of active zone-anchored synaptic ribbons reduced the presynaptic readily releasable vesicle pool (RRP) and impaired synchronous auditory signaling as revealed by recordings of exocytic IHC capacitance changes and sound evoked spiral ganglion neuron (SGN) activation. Both exocytosis of the hair cell RRP and the amount of synchronously activated SGNs co-varied with the number of anchored ribbons during development. However, the ribbon-deficient IHCs displayed sustained exocytosis with normal Ca^{2+} dependence. Despite normal kinetics of endocytosis, accumulation of tubular and cisternal membrane profiles in Bassoon mutant IHCs suggests role of Bassoon and/or ribbon in membrane recycling. Data on RRP recovery in ribbon-deficient hair cells will be presented. We conclude that the ribbon maintains the readily releasable pool of vesicles at the plasma membrane of IHC active zone, which is essential for normal hearing.

P80 Electrical stimulation of spiral ganglion cell cultures

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Introduction: Deafness leads to hair cell loss followed by the loss of spiral ganglion cells (SGC). In animal studies it has been shown that electrical stimulation of spiral ganglion cells is effective for SGC protection following deafness. However, it is still unknown what the most effective stimulation conditions are to protect spiral ganglion cells. Therefore, we have built a device to rapidly test cultured SGC in the presence of electrical fields (AC).

Materials and Methods: The device was first tested for pH and temperature behaviour of the culture media under stimulation conditions. Initial experiments with fibroblasts (NHDF) were carried out using stimulus trains of 50 biphasic rectangular pulses (10 ms, 50 Hz) at constant voltage (10-60 V) followed by an interburst interval of 19 s. After cultivation, fibroblasts were harvested, stained with propidium iodide and analyzed by flow cytometry (FACS). Additionally the electrical field strength in the cultivation chambers was determined under the different stimulation conditions.

Results: During electrical stimulation, the pH of the culture media remained constant at pH 7.4 whereas the temperature of the culture media remained in acceptable conditions for cell cultivation purposes (37 °C) at voltages of up to 40 V (480 V/m in the cultivation chamber). Cultivated and electrically stimulated fibroblasts showed no increased rate of damage when compared to unstimulated fibroblasts at voltages of up to 40 V. Initial experiments with cultured SGC from rats (P5) showed a remarkable outgrowth of dendrites when BDNF was added to the cultured cells, which was even enhanced by additional electrical stimulation. Under our starting conditions (biphasic pulses, 30 V) electrical stimulation alone caused only very few cells to grow out again.

Conclusion: These results demonstrate that the developed cell culture device is suitable for experiments analysing the effects of an electrical stimulation on cultivated cells, especially spiral ganglion cells.

P81 Innervation of the apical part of human cochlea

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Introduction: Our knowledge about the innervation pattern of the human cochlea is still incomplete and rests to a large extent on animal research. We performed a light (LM) and transmission electron microscopy (TEM) study of the apical turn of a freshly fixed human cochlea obtained during petroclival meningeoma surgery.

Materials and Methods: The cochlear tissue was histologically processed after removal during petroclival meningeoma surgery. Cochlea was serially sectioned perpendiculary to the long axis of the modiolus. At regular distances semi-thin sections were re-embedded and prepared for TEM. The nerve fibres/fascicles were traced from the spiral ganglion to the level of the inner hair cells (IHC). It allowed a comprehension of the tonotopic organisation of the apical portion of the cochlear nerve.

Results: The apical turn was innervated by 3694 myelinated nerve fibres (MNF) representing about 10 % of total number of fibres within the cochear nerve. The total number of unmyelinated nerve fibres (uMNF) was 513. A majority belonged to the efferent olivo-cochlear system of the intraganglionic spiral bundle, but also single fibres representing type II afferent neurons innervating outer hair cells (OHC).

Conclusion: Morphological characteristics and neural degeneration pattern of the apical turn of the human cochlea suggest that eletrical stimulation of this area might be beneficial for functional outcome improving and speech recognition in cochlear implant patients.

P82 Reliability of the chick cochlear hair cell afferent fiber synapse

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The cochlear hair cell-afferent fiber synapse converts continuous graded hair cell receptor potentials into cochlear nerve discharges that encode information about acoustic stimuli utilizing all-or-none action potentials or spikes. However, the response of a cochlear nerve unit to repeated identical stimuli will vary from trial to trial. We characterized the variability of spike patterns *in vivo* of cochlear nerve unit responses to repeated 40 ms tone bursts at each unit's characteristic frequency (CF) by measuring noise entropy (NE) and found that NE increased with higher CF's. Variability in spike patterns manifests as either fluctuations in spike count or spike timing and constrains the ability of the cochlear nerve to encode acoustic stimuli reliably. Much of the variability may be due to stochastic neurotransmitter release and thus, understanding this variability promises insight into synaptic function. To determine whether increases in variability of spike count or timing contribute to increased variability of spike patterns, we measured the Fano factor (FF) and coefficient of variation of interspike intervals (CV_{ISI}), respectively. Both FF and CV_{ISI} increased with higher CF's. The results suggest that spike count and timing are more variable with higher CF's and that both contribute to an increase in variability of spiking patterns. Therefore, encoding of high frequency stimuli in the cochlear nerve faces a greater challenge than stimulus encoding at low frequencies. The tonotopic gradient of hair cell ribbon number, size, voltage-gated calcium channels, calcium buffering capacity, and BK channels may help overcome this challenge.

P83 Localization of pre- and post-synaptic proteins at the chick cochlear hair cells afferent fiber synapse

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We employed immunocytochemistry and fluorescent laser scanning confocal microscopy to localize putative epitopes of the pre-synaptic ribbon protein, RIBEYE, C-terminal binding protein (CtBP), the post-synaptic glutamate receptor, (GluR2), and afferent fibers (NF-200) in the avian cochlea. Anti-GluR2, CtBP, RIBEYE staining exhibited a punctate, patch-like pattern concentrated at the base of the hair cell. Three-dimensional reconstruction of serial image stacks confirmed that the apparent receptor patches lie along the hair cell's baso-lateral surface. A marked gradient in GluR2 patch staining was observed along the radial axis from the neural to abneural edge of the cochlear duct, consistent with the labeling of synaptic contacts between the hair cell and its afferent fibers. A noted co-localization of the synaptic ribbon to the glutamate receptor patches was observed when the cells were double-labeled cells with both anti-GluR2 and anti-RIBEYE antibodies. Approximately 80 % of the receptor patches examined in each tissue section overlapped with aggregates of RIBEYE staining. At 2 days of age post-hatch, 83 ± 5 % (7 sections from 4 birds), and at 6 days, 78 ± 9 % (9 sections from 4 birds) of receptor patches were co-localized with a synaptic ribbon. Ongoing studies are focused on the development sequence of the synaptic ribbon-glutamate receptor morphological complex formation and distribution.

P84 Calcium buffering at the auditory hair cell afferent synapse

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Calcium modulates neurotransmitter release and calcium-activated potassium channels such as BK channels necessary for resonance. Intracellular calcium buffers localize calcium signaling within cellular regions but also affect functionality of calcium-dependent processes. We explored calcium buffering in isolated cochlear tall hair cells of White Leghorn chickens (8-21-days-old). We approximated endogenous calcium buffering properties by comparing recordings in the presence of endogenous buffer (perforated patch: Nystatin, 200 µg/ml) to recordings in the presence of various calcium buffers with known properties and concentrations (whole cell). We monitored both calcium-triggered vesicle fusion (capacitance changes) and calcium-activated potassium currents as dependent measures. For capacitance measures, cells were depolarized with voltage clamp from holding potentials of -81 mV to -21 mV for durations ranging from 50 ms to 500 ms. Endogenous calcium buffering capacity was better mimicked by millimolar concentrations of a fast buffer (1.6 mM BAPTA) than by submillimolar concentrations of a slow buffer (0.2 mM EGTA). To estimate calcium buffering using smaller time scales, we measure BK potassium current under various calcium-buffering conditions. Cells were voltage clamped to a holding potential of -81 mV and then clamped to testing potentials (-111 to 79 mV) for 3 ms. I_k remaining in the presence of Iberiotoxin is subtracted from I_k measured under control conditions, revealing the portion of the current attributable to BK channels. BK tail currents were analyzed for shifts in current/voltage relationship under buffering conditions. Weak buffering was seen to shift resonance activation to lower voltages while strong buffering shifted activation to higher voltages.

P85 Reliability and variability at the cochlear hair cell afferent fiber synapse

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The cochlear hair cell afferent fiber synapse converts the continuous graded hair cell receptor potential into trains of all-or-none action potentials or spikes that encode information about acoustic stimuli. However, the response of a single cochlear nerve unit to repeated identical stimuli varies from trial to trial. Variability in spike patterns manifests as either fluctuations in spike count or spike timing and constrains the ability of the cochlear nerve to encode acoustic stimuli reliably. We are concerned with characterizing the variability of these nerve discharges and understanding how pre-synaptic mechanisms contribute to such variability. In pursuit of this objective, our lab combines a range of techniques including single-unit recording from cochlear nerve fibers, patch-clamp recording of isolated cochlear hair cells, and computational modeling of hair cell synaptic exocytosis. Noise entropy calculations were employed to quantify the reliability of nerve firings and revealed that reliability of a unit's firing pattern is inversely proportional to its characteristic frequency. Whole-cell and perforated patch-clamp recordings have been utilized to measure endogenous mobile calcium buffering of both electrical resonance and synaptic vesicle exocytosis. It appears that the buffer equivalent of both cellular functions is best approximated by a high concentration of a fast calcium buffer. The effect on synaptic vesicle exocytosis of endogenous buffer properties and several other factors are being probed in a fully stochastic model of the hair cell ribbon synapse. Preliminary findings suggest that variability in the amplitude of exocytosis is strongly influenced by the spatial relationship between calcium channels and synaptic vesicles; however, the time course of exocytosis is remarkably insensitive to model perturbations.

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P86 Sources of variability at the hair cell ribbon synapse

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Phase-locking of the auditory nerve to a periodic acoustic stimulus requires the hair cell afferent synapse to release vesicles with marked temporal precision. We have developed a computational model of the hair cell pre-synaptic active zone to study the timing of exocytosis down to the resolution of single vesicle fusions. Our biophysically detailed model is fully stochastic and composed of several stages: gating of calcium channels, buffered diffusion of calcium, and fusion of synaptic vesicles. In order to better understand what limits the temporal precision of this synapse, we are selectively manipulating different stochastic elements of the model in order to identify the sources of "jitter" in synaptic vesicle exocytosis. Preliminary findings suggest that variability in the amplitude of exocytosis is strongly influenced by the spatial relationship between calcium channels and synaptic vesicles; however, the time course of exocytosis is remarkably insensitive to model perturbations.

P87 Dopamine transporter is essential to maintain fast synaptic transmission in the guinea-pig cochlea

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The efferent lateral olivocochlear (LOC) system, originating from the lateral superior olive, modulates the activity of afferent dendrites of primary auditory neurons below the sensory inner hair cells (IHC). The LOC innervation may use several neuroactive substances such as Ach, DA, GABA, CGRP, enkephalins and dynorphins (Eybalin, 1993). In a previous study (Ruel *et al.*, 2001), we showed that DA, which is present only in the LOC system, exerts a tonic inhibition on both spontaneous and sound-evoked activity of single auditory nerve fibers. In the cochlea, extracellular DA concentration must be highly regulated to maintain the physiological properties of auditory neurons to respond to sound stimulation. To address this hypothesis, we first investigated the cellular localization of dopamine transporter (DAT) in the guinea-pig cochlea using immunocytochemistry. Confocal microscopy analysis of vibratome sections showed that DAT was mainly localized around the basolateral part of IHC. The absence of colocalization with synaptophysin, a specific marker of efferent nerve fibers, suggests that the supporting cells selectively express DAT. Then, we analyzed the functional consequences of inhibiting dopamine uptake by recording cochlear potentials *in vivo* during intracochlear perfusions of DAT inhibitors, i.e. nomifensine and BTCP. Cumulative intracochlear perfusion of increasing concentrations of nomifensine or BTCP (0.1-300 µM) caused a dose-dependent reduction of the amplitude of compound action potential of the auditory nerve and an increase in N1 latency. In contrast, no significant change was seen in potentials that reflect the activity of hair cells (i.e. cochlear microphonic and summating potential) demonstrating a clear postsynaptic effect. IC_{50} calculated for nomifensine and BTCP were 44 µM and 72 µM, respectively. Finally, a significant increase of extracellular DA concentration was measured by HPLC in cochleas treated with DAT inhibitors. These results demonstrated that DA released from LOC efferent nerve endings is a major inhibitory neurotransmitter in the regulation of auditory nerve activity and their responsiveness to natural sound stimulation.

P88 Withdrawn because of Hurricane Katrina

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P89 Defective synaptic maturation in the inner hair cells of hypothyroid Pax8$^{-/-}$ mice

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Inner hair cells are the genuine sensory cells of the cochlea encoding sound information into neuronal signals at their afferent synapses. The afferent synapses undergo a major structural remodeling during ontogenesis which results in very different functional properties. Before the onset of hearing inner hair cells fire Ca^{2+} action potentials driving exocytosis at multi-ribbon active zones. This pre-sensory stimulation is most likely important for development and maintenance of the auditory pathway. This pre-sensory activity is made possible by a specific ion-channel ion channel make up of the immature IHC, which express a high density of $Ca_V1.3$ Ca^{2+} channels toward the end of the first postnatal week and mainly K_V-type K^+ currents. The Ca^{2+} current density subsequently declines to lower levels in IHCs of hearing mice, which display single ribbon active zones and express BK K^+ channels that ensure graded potentials. The regulation of the described developmental synaptic remodeling is unknown, but thyroid hormone (TH) is an attractive candidate.

In this study we investigated whether these changes in the Ca^{2+} current could be also regulated by TH. To this aim we have made perforated patch-clamp recordings of the Ca^{2+} currents in IHCs of hypothyroid Pax8$^{-/-}$ mice, which are profoundly deaf. We observed unusually large Ca^{2+} currents, even at advanced ages (P19), together with robust capacitance changes of up to a few hundreds of femtofarads, both hallmarks of immature IHCs. Immunohistochemistry confirmed an immature synaptic organization in IHCs of the hypothyroid mice. These results, although preliminary, seem to pinpoint the importance of TH in the functional maturation of IHCs as one prerequisite for normal hearing.

P90 Single L-type Ca^{2+} currents in chick embryo type I and type II hair cells

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Present experiments were undertaken in order to characterize the elementary Ca^{2+} channel properties in semicircular canal type I and type II hair cells. Cell-attached recordings were performed in chick embryo crista slice preparations, at late developmental stages. The patch pipette solution contained Ba^{2+} as the permeant cation (70 or 5 mM), K^+- and Cl^-- channels blockers, and Bay K 8644 (5 μM) to better resolve L-channel openings. The bath solution had a high K^+ content (135 mM) in order to set the hair cells' resting membrane potential close to 0 mV. With 70 mM Ba^{2+} in the pipette solution, brief openings were first detectable at –60 mV. The unitary current amplitude linearly decreased with depolarisation, with an average slope conductance was 20 pS. Mean open time (to) and mean open probability (Po) increased significantly with depolarisation: to was 2.4 ms at –50 mV (±1 SD) and 5.2 ms at –20 mV (±3 SD); Po was 0.08 at –40 mV (±0.04 SD) and 0.28 at –20 mV (±0.15 SD), with a maximum Po of 0.42 at 0 mV (±0.26 SD). When Ba^{2+} concentration in the patch pipette solution was lowered to 5 mM, single channel openings were already apparent at -80 mV. No time-dependent inactivation of Ca channels nor run-down was found, since single channel activity did not change significantly during 500 ms depolarising pulses or during the experiment. When P/Q- and N-type Ca^{2+} channels blockers (omega-agatoxin IVA 2 μM and omega-conotoxin GVIA 3.2 μM) were added to the pipette solution, the single channel activity was still present and unaffected. In a few cells, following cell-attached recording, we could achieve the whole-cell configuration. $I_{K,L}$, the signature current of type I hair cells, was still recognizable, which allowed us to confirm the type I hair cells' identity.

Present results demonstrate that chick embryo type I and type II semicircular canal hair cells express similar L-type Ca^{2+} channels, which in vivo might activate at voltages more negative than – 60 mV.

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