Abstract book

44th Workshop on Inner Ear Biology

LONDON
16 – 19 September 2007

Sunday 16 September 2007

SYMPOSIUM:
Ear Nerve and Synapse: Emerging ideas in sensory-neural hearing impairment

Monday, Tuesday, Wednesday 17–19 September 2007

WORKSHOP:
Oral and Poster Presentations

PROGRAMME OVERVIEW

Conference Venue

The conference will take place at the Institute of Child Health, 30 Guilford Street, London WC1. The symposium and workshop will be held in the Kennedy Lecture Theatre in the Institute. The registration desks will be manned from Sunday 16th September.

The welcome reception will be held at the UCL Ear Institute, 332 Gray's Inn Road, London WC1 which is a 10 minute walk from the conference venue.

Sunday 16th September

9:00 onwards	Registraton
10:00 – 17:30	Symposium Scientific Session
18:00 – 19:30	Welcome Reception, UCL Ear Institute

Monday 17th September

9:00 onwards	Registration
9:30 – 17:30	Workshop Scientific Sessions
	Evening Free

Tuesday 18th September

9:00 onwards	Registration
9:30 – 17:30	Workshop Scientific Sessions
19:00 – 22:30	Dinner at Shakespeare Globe Theatre

Wednesday 19th September

9:30 – 12:00	Workshop Scientific Sessions
12:00 noon	Workshop ends

Coffee and light refreshments will be provided at the session breaks.

Posters should remain in place for all of Monday and Tuesday. Discussion time is scheduled during the meeting. In addition, a limited amount of space for the presentation of posters is possible on Wednesday. The size of poster boardse is 1.5m wide x 1m high.

We should like to acknowledge the generous support of the following sponsors of Inner Ear Biology 2007

Advanced Bionics	http://www.advancedbionics.com/
Cochlear	http://www.cochlear.co.uk
Deafness Research UK	http://deafnessresearch.org
EuroHear	http://www.eurohear.org
Jencons PLS	http://www.jencons.co.uk
Med-El	http://www.medel.com
Scientifica	http://www.scientifica.co.uk
UCL	http://www.ucl.ac.uk
Zeiss (UK)	http://www.zeiss.co.uk

Scientific Programme

University College London , 16 – 19 September 2007
All sessions will be held in the Kennedy Lecture Theatre,
UCL Institute of Child Health, 30 Guilford Street, London WC1

The poster display areas will be in the atrium and meeting rooms near the lecture theatre

Sunday September 16th

Symposium

Nerve, Ear, and Synapse: Emerging ideas in sensory-neural hearing impairment

(Supported by Deafness Research UK)

9.45-10.00		**Welcoming remarks**
10:00-10.30	S1	The Genetics of Human Auditory Neuropathies **Ignacio del Castillo**
10.30-11:00	S2	The molecular machinery for exocytosis at IHC ribbon synapses **Saaid Safieddine**
11:00 -11:30		BREAK
11:30-12:00	S3	The function of the IHC ribbon synapse **Tobias Moser**
12:00-12:30	S4	Dysfunction of synaptic sensory inner hair cell - afferent and –efferent complex: a putative source for the generation of tinnitus **Jean-Luc Puel**
12:30-14:00		Lunch

14:30 -15:30	S5	Hyperactivity in the dorsal cochlear nucleus: Evolution of a chronic tinnitus model **James A. Kaltenbach**
15:00-15:30	S6	Molecular approaches to tinnitus **Marlies Knipper**
15:30 -16:00		BREAK
16:00 -16:30	S7	Investigating differences in outcome for cochlear implantees **Colette McKay**
16:30 -17:00	S8	Perspectives on Auditory Neuropathy: Disorders of inner hair cell, auditory nerve, and their synapse **A Starr**
17:00 -17:15		General Discussion
		End of Symposium

18:00 – 19:30 **IEB 2007 Opening Reception**

UCL Ear Institute
332 Gray's Inn Road
London WC1

44th Inner Ear Biology Workshop

ORAL PRESENTATIONS

Monday September 17th

9.25-9.30.		**Welcome and Introduction to the workshop** **Jonathan Ashmore**
		Session 1: *Cochlear genetics and development*
9.30-9.45	O1	Identification of microRNAs in the Developing Cochlea and Vestibule **Lilach M. Friedman, Ginat Toren, Takunori Satoh, Ronna Hertzano, Martin Irmler, Johannes Beckers, Eran Hornstein, Donna M. Fekete, and Karen B. Avraham**
9.45-10.00:	O2	Mosaic complementation of the shaker1 allele suggests a novel role for myosin VIIa in hair cell stereocilia **Agnieszka K. Rzadzinska and Karen P. Steel**
10.00-10.15	O3	Hair cell differentiation becomes tissue specific by E9.5 in mouse inner ear **Tatsunori Sakamoto, Juichi Ito and Raj Ladher**
10.15-10.30	O4	Calbindin and S100 protein expression in the developing auditory system in mice. **Buckiova D. and Syka J**
10.30-10.45	O5	Characterization of a spontaneous recessive mutation arising in the mouse Tecta gene: A model for DFNB21 hearing loss **Miguel Ángel Moreno-Pelayo, Richard J. Goodyear, Angeles Mencía, Silvia Modamio-Høybjør, Kevin Legan, Leticia Olavarrieta, Felipe Moreno, and Guy P Richardson**
10.45-11.15		**BREAK**

Session 2: Hair cells I: Motility

11.15-11.30 O6 Outer hair cell somatic rather than hair bundle motility is the basis of the cochlear amplifier
Marcia Mellado Lagarde, Markus Drexl, Andrei N. Lukashkin and Ian J. Russell

11.30-11.45 O7 Salicylate and chlorpromazine induce nanoscale curvature changes in the outer hair cell plasma membrane
Jennifer N. Greeson and Robert M. Raphael

11.45-12.00 O8 Frequency Effect on Charge Transfer by Prestin: Insight from an Electro-Diffusion Model
Sean X. Sun, Matthew S. Chana, George Oster, William E. Brownell, and Alexander A. Spector

12.00-12.15 O9 Prestin's state dependence of anion binding
Santos-Sacchi J and Song L

12.15-14.30 **LUNCH**
and
POSTER SESSION 1 (odd numbers)

Session 3: Hair Cell Damage and Otoprotection 1

14.30-14.45 O10 Mechanism of Age-Related Hearing Loss
Donald Henderson, Eric Bielefeld, Guang-di Chen and Wei Ping Yang

14.45-15.00 O11 Otoprotection strategies against Cisplatin induced ototoxicity
Guiscardo Lorito, Pietro Giordano, Sathiyaseelan Theneshkumar, Alessandro Martini and Stavros Hatzopoulos

1.00-15.15 O12 Styrene ototoxicity and its protection
Guang-Di Chen, Chiemi Tanaka,Weiping Yang and Donald Henderson

15.15-15.30 O13 TNF-α exposure initiates apoptosis of auditory hair cells that can be prevented by dexamethasone: Mechanisms
Thomas R Van De Water, Scott Haake, Shibing Chen, Christine Dinh, Xiaoyun Nong, Adrien A Eshraghi, and Thomas J Balkany

15.30-16.00 **BREAK**

Session 4 Hair Cell Damage and Otoprotection 2

16.00-16.15 O14 BDNF protects against Pseudomonas aeruginosa Exotoxin A -induced hearing loss
Adnan Lidian, Leif Nordang, Monika Stenkvist-Asplund, Birgita Linder and Matti Anniko

16.15-16.30 O15 In the newborn rat cochlea, HSP70 is upregulated on a transcriptional and translational level during normoxic and hypoxic culture conditions
Agnieszka J. Szczepek, Johann Gross and Birgit Mazurek

16.30-16.45 O16 Adenosine signalling in the cochlea: an emerging role in neuroprotection
SM Vlajkovic, CX Guo, R Gupta, GD Housley and PR Thorne

16.45- 17.30 **The EuroHear Lecture:**

Regulation of cell division and patterning in the regenerating inner ear
Mark Warchol

Tuesday September 18th

Session 5 Cochlear mechanics

9.30-9.45	O17	Longitudinal change in the role of organ of Corti mass in cochlear tuning **Ombeline de La Rochefoucauld and Elizabeth S. Olson**
9.45-10.00	O18	Negative damping of the cochlear non-linear mechanical responses **Andrei N. Lukashkin, Mark N. Walling and Ian J. Russell**
10.00-10.15	O19	Combined electric and acoustic stimulation in the cochlea of the guinea pig **H. Christiaan Stronks, Huib Versnel, Vera F. Prijs and Sjaak F.L. Klis**
10.15-10.30	O20	Nonlinearity and origin of the round window recorded cochlear microphonic potential **Alfred L. Nuttall, Jiefu Zheng, Yuan Zou, Fangyi Chen and Tianying Ren**
10.30-10.45	O21	Aminoglycoside & Acoustic Ototoxicity in Meriones Unguiculatus **Nneka Eze and Elizabeth S Olson**
10.45-11.15		**BREAK**

Session 6 Cochlear homeostasis

11.15-11.30	O22	The dynamic characteristics of human organ of Corti homeostasis as inferred from post noise-burst OAE level fluctuations **David Kemp and Oliver Brill**
11.30-11.45	O23	Unitary permeability of gap junctions channels expressed in HeLa cells **Victor H. Hernandez, Mario Bortolozzi, Saida Ortolano and Fabio Mammano**

11.45-12.00	O24	Expression of Aquaporins and Vasopressin type 2 receptor in the lateral wall of the Cochlea and Inner Ear Fluid homeostasis **Takeda T, Nishioka R, Kakigi A, Nishimura M, Okada T, Takeda S and Taguchi D**
12.00-12.15	O25	Bridging the epithelial membrane barrier: Potassium spatial buffering by Kir4.1/AQP4 **B. Hirt, C. Gleiser, A. Eckhard1, M. Müller, H. Wolburg and H. Löwenheim**
12.15-12.30	O26	Coordinated control of Cx26 and Cx30 at the regulatory and functional level in the outer sulcus region **Saida Ortolano, Giovanni Di Pasquale, Giulia Crispino , Fabio Mammano, and John A. Chiorini**
12.30-14.45		**LUNCH** **and** **POSTERS 2 (even numbers)**

Session 7 Mitochondria

14:45-15:00	O27	Normal outer hair cell function contrasts with impaired function of auditory neurons in Friedreich ataxia due to faulty mitochondria **Fabrice Giraudet, Alain Martelli, Hélène Puccio, Odile Boespflug-Tanguy, Thierry Mom and Paul Avan**
15.00-15:15	O28	Use of fluorescent imaging techniques to assess mitochondrial function in cochlear explants and slices **Zoe F. Mann, Michael R. Duchen, Daniel J. Jagger and Jonathan E. Gale**
15.15-15.30	O29	Stress-induced changes in mitochondrial peroxiredoxin in mouse cochlear hair cells **Fuquan Chen, Su-Hua Sha and Jochen Schacht**
15.30-16.00		BREAK

Session 8 Stem Cell Therapies

16.00-16.15 O30 Synapse formation between embryonic stem cell-derived neurons and auditory hair cells in vitro
Masahiro Matsumoto, Takayuki Nakagawa, Ken Kojima, Tatsunori Sakamoto and Juichi Ito

16.15-16.30 O31 Bone Marrow Derived Cells in Inner Ear Repair
Brian T Tan and Runsheng Ruan

16.30 **IEB Business Meeting**

19:00 – 22.30 **Conference Dinner**

The UnderGlobe,
Shakespeare Globe Theatre,
Bankside
London SE1

Reception 19:00

Guided tours of the theatre 18:30 -19:00

Coaches will leave for St Paul's Cathedral at 17:30

departing from the Institute of Child Healt h, Guilford Street, there will be a short walk across the Millennium Bridge to the Theatre

Wednesday September 19th

Session 9: Hair cells II: Transducer and Synapse

9.30-9.45 O33 Transducer Gating in the Drosophila Ear
Joerg T. Albert, Bjoern Nadrowski and Martin C. Goepfert

9.45-10.00 O34 Defects In The Atp2b2 Gene Causing Hereditary Hearing And Balance Loss In Mice And Humans: A Functional Study Of Normal And Mutated PMCA2 Pumps
M. Bortolozzi, R. Ficarella, F. Di Leva, S. Ortolano, S.L. Spiden, A. Lelli, G.E. Shull, M. Brini, P. Gasparini, KP Steel, E. Carafoli and F. Mammano

10.00-10.15 O35 Calcium, Calmodulin-dependent Regulation of Inner Hair Cell Calcium Channels
Lisa Grant and Paul A. Fuchs

10.15-10.30 O36 Voltage-Gated Channels and Scaffolding Proteins Define Compartments in the Vestibular Calyx Ending
Anna Lysakowski, Sophie Gaboyard, Shilpa Chatlani, Steven D. Price, Ruth Anne Eatock and Jay M. Goldberg

10.30-11.00 **BREAK**

Session 10: Synapse and Nerve

11.00-11.15 O37 HCN Channels Shape the Time Course of Postsynaptic Potentials at the Inner Hair Cell Afferent Synapse
Eunyoung Yi and Elisabeth Glowatzki

11.15-11.30	O38	Instantaneous Rate vs Instantaneous Amplitude curves of Auditory Nerve Fibres are in close agreement with the transduction in the cilia of Inner Hair Cells. **J. Wiebe Horst, JoAnn McGee and Edward Walsh**
11.30-11.45	O39	Tonotopic frequency mapping on the basilar membrane of the Chinchilla (Chinchilla laniger) measured by labelling auditory nerve fibres with HRP and biocytine **Jean Smolders, Silvi Hoidis and Marcus Müller**
11.45-12.00	O40	Neural and receptor cochlear potentials in auditory neuropathy obtained by transtympanic electrocochleography (ECochG) **Rosamaria Santarelli, Arnold Starr, Henry J Michalewski and Edoardo Arslan**

12:00 **End of IEB 2007**

Posters

(Posters will be displayed on Monday and Tuesday from 9 am all day with timetabled discussion as indicated in the programme. The order below does not reflect the order in which the posters will be displayed. For poster assignments see listing of abstracts, and the index)

Effects of defensins on frog semicircular canal: evidence of interaction between the immune and nervous systems.
Andrianov GN, Nozdrachev AD and Ryzhova IV

Interaction between Cochlear Cells and Bone Marrow Cells
Susumu Baba, Hiroshi Iwai, Shinryu Lee, Mariko Omae, Susumu Ikehara, Toshio Yamashita

Evidence for K+ recycling in an organotypic model of mouse utricle.
S. Bartolami, M. Cavalier, Z. Ouaray and Ch. Chabbert

Age-related changes in the cochlear nucleus of C57BL/6J mouse.
Maria Visitación Bartolome.

Actin dynamics in avian sensory epithelia.
Jonathan Bird[1,2], Nicolas Daudet[2] & Jonathan Gale[1,2].

All-or-none delayed spike burst elicited by synaptic activation of unipolar brush cells in the vestibulo-cerebellum
Luisa Bottà, Sergio Masetto, Gianpiero Zucca and Egidio D'Angelo

Effects of hypothyroidism and lack of thyroid hormone receptors alpha and beta on the expression of $Ca_v1.3$ and BK channels
Brandt N,[1] Münkner S,[1] Braig C,[2] Winter H,[2] Knipper M,[2] Engel J[1]

A comparison of the cochlear and vestibular effects of gentamicin and kanamycin/furosemide co-treatment in guinea pigs
H.G. Bremer, H. Versnel, J.C.M.J. de Groot, F.W.J. Albers, S.F.L. Klis

Comparison of GAD levels in the inferior colliculus and auditory cortex of two rat strains during aging
Burianová Jana, Ouda Ladislav, Profant Oliver, Syka Josef

Expression of Histamine Receptors in the Mammal Vestibule
Sophie Gaboyard-Niay, Cécile Travo and Christian Chabbert

Illuminating the dynamics of the Notch signalling pathway during inner ear development
Elena Chrysostomou and Nicolas Daudet

Modulation of cell-cell and cell-extracellular matrix interactions during neuroblast migration
D. Davies and C. Nobes

Identifying Target Genes of the Hair Cell POU-Domain Transcription Factor Brn-3c
Emily Towers, Chrysostomos Tornari, Jonathan Gale and Sally Dawson

Expression of glycine receptors and gephyrin in the rat cochlea
Julia Dlugaiczyk[1], Wibke Singer[3], Bernhard Schick[1], Heinrich Iro[1], Kristina Becker[2], Cord-Michael Becker[2], Marlies Knipper[3]

Src inhibition prevents the initial cochlear damage induced by cisplatin chemotherapy
Fetoni[1] AR, Paludetti[1] G, Hu[2] BH, GD Chen2, Henderson[2] D.

Role of Coenzyme Q10 and his hydrosoluble multicomposite, Coenzyme Q10 terclatrate, in protection against noise induced hearing loss.
Fetoni AR, Fiorita A, La Greca C, Rizzo D, Troiani[1] D, Paludetti G.

Hair cell loss in connexin 30 deficient mice is accompanied by anomalous epithelial repair patterns.
Andrew Forge[1], Regina Nickel[1], Ruth Taylor[1], Daniel Jagger[1], Shoeb Ahmad[2], Wenxue Tang[2], Xi Lin[2]

Three-dimensional organisation of cytoskeletal components in the sensory epithelia of the inner ear.
Andrew Forge[1], Ruth Taylor[1],

A Subtractive Hybridization Strategy to Identify Barhl1 Target Genes in Sensory Hair Cells
Valentina Gburcik, Jonathan Gale and Sally Dawson

Scanning electron microscope imagining as a good supportive method in cisplatin induced ototoxicity.
Pietro Giordano, Sathiyaseelan Theneshkumar, Guiscardo Lorito, Alessandro Martini and Stavros Hatzopoulos.

Effect of noise exposure on middle latency response amplitudes in rats.
Grécová J, Popelář J, Syka J

Prevention of Accelerated Presbycusis by Maintenance of Systemic Immune Functions
Hiroshi Iwai, Susumu Baba, Shinryu Lee, Mariko Omae, Susumu Ikehara, Toshio Yamashita

Connexin 30 trafficking to the plasma membrane via a Golgi-independent pathway
John Kelly, Regina Nickel, Andrew Forge, Daniel Jagger

Effects of thyroid hormone deficiency on Ca^{2+} currents and exocytosis in cochlear inner hair cells
Stephanie Kuhn[1], Claudia Braig[2], Stefan Münkner[1], Harald Winter[2], Marlies Knipper[2] and Jutta Engel[1]

ERK1/2 activation in support cells during hair cell damage is a common signalling mechanism that may contribute to hair cell death
Manuela Lahne and Jonathan Gale

Association of HLA-DRB1*1101 allele with bilateral Meniere´s disease in mediterranean population
Jose A. Lopez-Escamez[1], Herminio Perez-Garrigues [2] Jose R Vilchez[2] , Andres Soto-Varela [3], Sofia Santos-Perez [3], , Ismael Aran [5], Miguel A. Lopez-Nevot[2]

Development of a bioreactor system aiming at the in vitro culture of the functionally mature Organ of Corti
H. Arnold, A. Müller, M. Müller, H. Löwenheim

AQP4 and Kir4.1 are colocalized in distinct supporting cell populations of the rat cochlea
A. Eckhard[1,2], B. Hirt[1,2], C. Gleiser[1,2], M. Müller[1], H. Löwenheim[1]

Functional analysis of water flow in cochlea supporting cells
C. Gleiser[1,2], B. Hirt[1,2],A. Eckhard[1], M. Müller[1], H. Löwenheim

Noise trauma in the guinea pig
M. Müller[1], M. Tisch[2], H. Maier[2], B. Hirt[1], H. Löwenheim[1]

Methodological study for isolation of stem cells from the spiral ganglion
S. Sandke, K. Aulwurm, J. Waldhaus, A. Gharabaghi, H. Löwenheim

Nestin expression in the organ of Corti during embryonic and postnatal development
J. Waldhaus[1], H. Winter[1], B. Hirt[1,2], M. Müller[1], H. Löwenheim[1]

Dynamics of Fgf3 expression in the developing mouse inner ear
Androulla Economou, Stephanie Cadot and Mark Maconochie

Effect of interference tones on DPOAE level/phase maps in rabbits
GK Martin,[1,2] BB Stagner,[1] T Fleck,[3] PF Fahey,[4] BL Lonsbury-Martin[1,2]

A computationally based large scale model of potassium flow in the cochlea
Pavel Mistrík[1], Peter Saffrey[1], Chris Mullaley[1], Renato Nobili[2], Fabio Mammano[2] and Jonathan Ashmore[1,3]

Upregulation of erythropoietin and erythropoietin receptor mRNA in organ of Corti, modiolus and stria vascularis of newborn rats
Mazurek B, Haupt H, Amarjargal N, Machulik A, Moller R, Ungethüm[1] U, Kuban[1] RJ, Szczepek, AJ, Gross J

The role of prestin and the tectorial membrane in the generation of electrically evoked otoacoustic emissions in mice.
Markus Drexl[1,2], Marcia Mellado Lagarde[1], Jian Zuo[3], and Ian J. Russell[1]

Number, Design and Function of Hair Cell Synapses along the Tonotopic Axis of the Cochlea
Alexander C. Meyer[1], Darina Khimich[1], Alexander Egner[2], Youri Yarine[3], Tobias Moser[1]

Ionic currents through $Ca_v1.3$ Ca^{2+} channels in mature mouse inner hair cells under mobile phone field exposure
S. Münkner[1], A. El Ouardi[2], J. Streckert[2], R. Vonthein[3], V. Hansen[2], J. Engel[1]

Differential modulation of $Ca_v1.3$ Ca^{2+} channels by Ca^{2+} binding proteins and significance for auditory hair cell function
Guiying Cui[1*], Alexander Meyer[2*], Irina Calin-Jageman[1], Jakob Neef[2], Françoise Haeseleer[3], Tobias Moser[2], and Amy Lee[1]

Molecular correlates of tinnitus in the auditory system: BDNF, Arg 3.1, and the role of GABA
Rama Panford-Walsh1, Wibke Singer1, Lukas Rüttiger1, Hyun-Soon Geisler1, Ulrike Zimmermann1, Iris Köpschall1, Karin Rohbock1, Elmar Oestreicher2, Helen Haas1 and Marlies Knipper1

Expression patterns of erythropoietin and erythropoietin receptor in the spiral ganglion of guinea pig after noise exposure
Jae Yong Park, MD, Dong-Hyun Kim, MD, Ah Young Kim, MD, and Yong Ho Park, MD, PhD

Expression patterns of KCNJ10 K+ channel in the cochlear lateral wall after acoustic trauma
Yong Ho Park, MD, PhD, Jae Yong Park, MD, and Ah Young Kim, MD

Presence and Characteristics of Spontaneous Otoacoustic Emissions in Children and adolescents
Pelánová J [1,2], Groh D [1,2], Popelář J [1], Kabelka Z [2], Syka J [1].

Mitotracker staining of mitochondria in outer hair cells of the guinea pig cochlea
Jim Pickles, Hema Indrasamy

Mouse pharmacological models of sensorineural hearing loss.
Poirrier AL, Kim TS, Vandenbosch R, Nguyen L, Lefebvre PP, Malgrange B.

Prestin self-association, lateral mobility and membrane cholesterol
Louise E. Organ, Jennifer N. Greeson, and Robert M. Raphael

Computational Model of Ion Transport in the Stria Vascularis
Imran Quraishi and Robert M. Raphael

Targeted Disruption of Otoa with EGFP demonstrates otoancorin is required for adhesion of the tectorial membrane to the spiral limbus.
P. Kevin Legan[1], Lindsey Welstead[1], Richard J. Goodyear[1], Christine Petit[2] and Guy P. Richardson[1]

Studying the distribution of inositol phospholipids in sensory hair cells with EGFP-tagged reporter constructs
Gowri D. Nayak, Richard J. Goodyear and Guy P. Richardson

NO-cGMP pathway and hearing loss
Lukas Rüttiger, Robert Feil*, Susanne Feil* and Marlies Knipper

Age-related changes in the rat auditory system
Natalia Rybalko, Jana Pelanová, Stanislava Hamsová, Josef Syka

Which interstereocilial links can be found in disorganized hair bundles of cadherin 23 mutants?
Agnieszka K. Rzadzinska, Karen P. Steel

Identification of IGF-I target genes implicated in inner ear development and functional maturation
Hortensia Sanchez-Calderon[1,2], Lourdes Rodriguez de la Rosa[1], Marta Milo[2] Jose R. Pichel[3], Matthew Holley[2] and Isabel Varela-Nieto[1]

Noise stress in a Otoprotective strategy against Cisplatin induced Ototoxicity
Sathiyaseelan Theneshkumar, Stavros Hatzopoulos, Guiscardo Lorito, Pietro Giordano, Sara Magosso , Lucia Bertolaso, Alessandro Martini.

Relationship between Accelerated Presbycusis and Immunosenescence
Shinryu Lee, Hiroshi Iwai, Susumu Baba, Mariko Omae, Susumu Ikehara, Toshio Yamashita

Identification of markers for pathological neuronal plasticity changes in the auditory system
Singer W, Rüttiger L, Panford-Walsh R, Reinbothe T, Rohbock K, Knipper M

Expression and immunolocalization of aquaporin-6 (Aqp6) in the rat inner ear
Daizo Taguchi[a], Taizo Takeda[b], Akinobu Kakigi[b], Teruhiko Okada[c], Rie Nishioka[b], Hiroya Kitano[d]

Effects of Aging on Inner Ear Morphology in Dogs Related to Brainstem Responses to Pure-Tone Auditory Stimuli
G. ter Haar[1], J.C.M.J. de Groot[2], A.J. Venker-van Haagen[1], W.E. van den Brom[1], F.J. van Sluijs[1] and G.F. Smoorenburg[2]

An atypical cell during development of the auditory organ : the inner pillar cell.
Nicolas Thelen, Brigitte Malgrange and Marc Thiry

Delivery of brain-derived neurotrophic factor to the guinea pig cochlea using Gelfoam
Versnel H, Sedee RJ, Agterberg MJH, De Groot JCMJ, Albers FWJ, Klis SFL

Speech-in-noise intelligibility does not correlate with efferent olivocochlear reflex in humans with normal hearing
Wolfgang Wagner, Kathrin Frey, Guido Heppelmann

Different appearance of apoptosis caused by platinum-derived anti-cancer drugs in the vestibule of guinea pig
Ken-ichi Watanabe, Shuta Inai, Ken-Jinnnoiuchi, Hess Alexander, Olaf Michel, Toshiaki Yagi

Expression of osmotically responsive cationic channel TRPV4 in the endolymphatic sac
H. Kumagami, M. Terakado, Y. Saino, A. Baba, D. Fujiyama, K. Takasaki, H. Takahashi

Human Cytomegalovirus and Factors Involved in Neurogenesis
Jane C. Murphy and John H. Sinclair

Region-specific transcriptional expression of neurotrophin-family genes in the cochlea of newborn rat
Gross J, Szczepek, AJ, Amarjargal N, Machulik A, Fuchs J, Ungethüm[1] U, Kuban[1] RJ, Haupt H, Mazurek B

Vestibular morphology and function in the German guinea pig
Sachie Kawaguchi[1, 2], Mats Ulfendahl[1], Mamoru Suzuki[2], Malou Hultcrantz[3]

Abstracts

Symposium Lectures

Oral Presentations

Poster Presentations

S1 The Genetics of Human Auditory Neuropathies

Ignacio del Castillo
Unidad de Genetica Molecular, Hospital Ramon y Cajal, Madrid, Spain

Human auditory neuropathy (AN) encompasses a variety of disorders characterized by normal otoacoustic emissions (OAE) and absent or severely distorted auditory brainstem responses (ABR) in the affected subjects. AN can result from environmental or genetic causes. It can be part of a systemic neurodegenerative disorder (Charcot-Marie-Tooth peripheral neuropathy, Friedreich ataxia, mitochondrial disorders…), or it can be an isolated clinical entity. The primary lesion in AN can be located in the inner hair cells, in the auditory nerve, or in the synapse in between. In the last four years, several genes have been shown to be involved in isolated AN.
Mutations in the *OTOF* gene, on 2p22-p23, are responsible for the DFNB9 subtype of autosomal recessive non-syndromic hearing impairment (NSHI). This gene encodes otoferlin, a protein containing six C2 calcium-binding domains, which is membrane-anchored through a C-terminal transmembrane domain. Most of the approximately 40 different mutations which are currently known in *OTOF* are private, each one being reported in only one family. A remarkable exception is Q829X, highly prevalent in Spain because of a genetic founder effect. Affected subjects show a very homogeneous phenotype, i.e. prelingual profound NSHI without associated inner ear malformations. ABR are absent, but younger subjects exhibit normal OAE, which can be lost later on in life. In adults, otoferlin expression in the inner ear is restricted to the inner hair cells. Based on this fact and on the good outcome of cochlear implantation in subjects carrying two mutant alleles of *OTOF*, we hypothesized that the primary lesion in this subtype of AN would be cochlear, specifically located in the inner hair cells. Consistently, recent data suggest that otoferlin is required for the exocytosis of the synaptic vesicles at the auditory inner hair cell ribbon synapse. Our epidemiological data indicate that mutations in *OTOF* constitute a major cause of isolated, inherited AN.
Specific missense mutations in the pejvakin gene, *DFNB59* on 2q31, have been shown to result in autosomal recessive isolated AN and severe NSHI both in humans and mice. In this case, the location of the primary lesion seems to be neural. Our data indicate that these mutations constitute a rare genetic cause of AN. Also, the AUNA1 locus was mapped to 13q14-q21 in a large American family segregating an autosomal dominant form of .,isolated AN, associated with postlingual progressive NSHI

S2 **The molecular machinery for exocytosis at IHC ribbon synapses**

Saaid Safieddine
CNRS/INSERM, Institut Pasteur, Paris, France

The hearing organ, the cochlea, allows sound detection, source localization and frequency discrimination. This unique performance depends on the properties of the inner hair cell (IHC) ribbon synapse that signals sound stimulus to the central nervous system (CNS) with high temporal precision and over a wide range of stimulus intensities. In addition to keeping the CNS constantly informed about surrounding sounds, the IHC synapse is specialized in maintaining a high level of tonic synaptic release over long periods of time, even in a quiet environment. When compared to the CNS synapses, the IHC ribbon synapse presents several striking differences, which are likely to be involved in these unique functional characteristics: i) The Ca^{2+} invasion of the ribbon synapse active zone upon xell depolarization occurs through L-type Ca^{2+} channels (instead of P/Q channels ay the CNS synapses); ii) otoferlin is proposed to be the major calcium sensor with a probable functional coupling with complexin III and IV (instead of synaptotagmin I, and complexin I, and II at the CNS synapses); iii) the unique molecular constituent of the ribbon synapse, namely RIBEYE, which is essential for synaptic ribbon assembly.

Transduction of the sound stimulus into an electrical signal occurs when the IHC depolarizes, resulting in an increase of Ca^{2+} influx raising the calcium concentration at the active zone. The Ca^{2+} ions bind to a calcium sensor, probably otoferlin with the help of complexin III and IV thereby triggering synaptic vesicle fusion with the plasma membrane. Despite significant progress in elucidating the molecular composition of the presynaptic active zone of the IHC ribbon synapse, we are still far from understanding the basic mechanisms underlying the unique functioning of this synapse. My presentation will focus on recent discoveries of molecular and cell biological processes that shed new light on how vesicle fusion at the ribbon synapse may occur in order to accomplish the high temporal precision and endless neurotransmitter release that characterize this synapse.

S3 **The inner hair cell ribbon synapse**

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Ribbon synapses are fascinating structures in the inner ear and the retina that encode sensory stimuli with high speed and reliability. The molecular structure and function of the synaptic ribbon, a conspicuous proteinaceous structure that is packed with synaptic vesicles and anchored to the presynaptic membrane at these synapses, has remained unclear. Biophysical analysis of inner hair cell ribbon synapses revealed kinetically distinct components of release. Each hair cell ribbon synapse holds a large readily releasable pool of vesicles (RRP) that supports high initial rates of transmitter release and is rapidly replenished after stimulation. Exocytosis of individual vesicles shows some degree of coordination at the hair cell ribbon synapse, which is different from many other synapses where vesicles are release independently of each other. Release at the hair cell ribbon synapse appears to be controlled by one or few Ca^{2+} channels nearby the vesicle(s). This Ca^{2+} nanodomain control of vesicle release likely contributes to achieving the temporal precision of sound coding for low stimulus intensities.
Genetic inactivation of the presynaptic scaffolding protein Bassoon impairs ribbon anchorage in mouse inner hair cells and attenuates auditory brainstem responses without changing otoacoustic emissions. Ribbon-deficient active zones displayed a strongly reduced RRP. *In vivo* recordings from auditory nerve fibers revealed reduced spontaneous and evoked spiking rates. Therefore, the synaptic ribbon seems important to stabilize a large RRP, which enables the high rates of synaptic transmission observed at the hair cell afferent synapse.

S4 Dysfunction of synaptic sensory inner hair cell -afferent and –efferent complex: a putative source for the generation of tinnitus

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Glutamate is the neurotransmitter of the sensory inner hair cells (IHCs). Receptor channels located on the nerve endings are preferentially activated by the glutamate agonist AMPA, indicating that AMPA-preferring receptors are functionally located at the sensory cell-afferent synapse whereas NMDA and kainate receptors are not. This questions about the role of NMDA and kainate receptors in these cells. While the NMDA receptors are involved in the salicylate-induced tinnitus, the role of kainate receptor still remains to be elucidated.

The lateral olivocochlear (LOC) efferents modulate auditory nerve activity. We examined the effects of acetylcholine (ACh), GABA, dopamine (DA), neuroactive substances present in the LOC efferents. Perilymphatic perfusion of ACh in presence of strychnine, which blocks the effect of the median efferent controlling the outer hair cell activity, increased the spontaneous and sound driven activities of single auditory nerve fibers. In contrast, perilymphatic perfusion of DA, or DA transporter inhibitors (increasing the endogenous content of DA) has an inhibitory effect on both spontaneous and sound-driven activity. In addition, perilymphatic perfusion of eticlopride, a selective D2 receptor antagonist, increased the spontaneous firing suggesting that DA exerted a tonic inhibition. Removal of the tonic inhibition may result to the development of tinnitus. In conclusion, LOC efferents can both excitate and inhibit the activity of auditory nerve fibers. This leads to the proposal that lateral efferents constitute a gain control acting at the initialization site of action potential to maintain the adequate firing of the auditory nerve fibers and may prevent the occurrence of tinnitus.

S5 Hyperactivity in the dorsal cochlear nucleus: Evolution of a chronic tinnitus model

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Efforts to understand mechanisms underlying chronic tinnitus depend on the establishment of animal models in which changes in neural activity caused by tinnitus inducing agents can be systematically studied. Numerous animal models have emerged in recent years, but few have modelled tinnitus in its chronic form. Over the past decade, a model has been introduced by our research group for the study of chronic, noise-induced tinnitus. At the core of this model is the finding that noise exposure causes chronic elevations of spontaneous neural activity in the dorsal cochlear nucleus (DCN). In this presentation, the evolution of the concept that this spontaneous hyperactivity is related to tinnitus will be reviewed. The hypothesis of a link between hyperactivity and tinnitus was initially based on the observation that hyperactivity displays a tonotopic profile similar to that of activity evoked in normal animals by a narrow band of noise or tonal stimulus. It was suggested that noise exposure induces the DCN to behave as though it is responding to a narrow band of noise or tone and therefore might carry a tone-signalling place code in the absence of sound. It was then demonstrated that the same noise exposure conditions that induce hyperactivity in the DCN also cause animals to experience tinnitus-like percepts; equally important, when measures of hyperactivity and tinnitus were performed in the same animals, those measures were found to be significantly correlated. Later studies revealed that noise-induced hyperactivity parallels several psychophysical attributes of tinnitus. For example, both hyperactivity and tinnitus, once induced, can be observed to persist following removal of eighth nerve input. In addition, both the degree of hyperactivity in the DCN and the severity of tinnitus are observed to increase with the degree of hearing loss. Also, hyperactivity reaches a peak of severity in a tonotopic region representing frequencies above that of the exposure tone, in agreement with psychophysical findings that the pitch of tinnitus induced by tone exposure is typically higher than the frequency of the tinnitus-inducing exposure tone. These findings point to the DCN as an important source of tinnitus producing signals. The hypothesis and supporting evidence will be presented that the emergence of tinnitus-related hyperactivity involves plastic alterations in the DCN which are triggered by changes of input from the inner and outer hair cell systems. These changes shift the balance of excitatory and inhibitory inputs to DCN neurons, which, in turn, results in disinhibition and hyperactivity of DCN principal neurons. (This work is supported by NIH-R01-03258).

S6 Molecular approaches to tinnitus

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Aberrant neuronal activity is known to lead to changes in neuronal plasticity. However, the molecular changes following sensory trauma and the subsequent response of the central nervous system are only poorly understood. We focused on finding a molecular tool for monitoring the features of excitability which occur following acoustic and ototoxic trauma to the auditory system. Of particular interest are genes that alter their expression pattern during activity-induced changes in synaptic efficacy and plasticity. The expression of brain-derived neurotrophic factor (BDNF) and the activity-dependent cytoskeletal protein (Arg3.1/arc) were monitored in the peripheral and central auditory system hours and days following tinnitus-inducing traumatic stimuli or salicylate treatment. Tinnitus induction was monitored in a rodent animal behavior model (Rüttiger et al., 2003, Hear Res). Excitatory input to the rat AI were investigated by local field potential (LFP) post pure-tone acoustic trauma using chronic implantion of multi- channel microelectrode arrays. BDNF and Arg3, were monitored at the mRNA and protein level in the cochlea and subcortical and cortical areas. We present here a summary of recent findings comparing and correlating the expression of activity dependent genes with tinnitus-behavior. The data are discussed in the context of using the monitoring of activity-dependent genes to screen for the pharmacological reversal of tinnitus.
Supported by a grant from the Deutsche Forschungsgemeinschaft Kni-316/3-3

S7 Investigating differences in outcome for cochlear implantees

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It is well known that the effectiveness of hearing devices in improving communication ability varies considerably between people who have similar hearing levels for simple tones. This is particularly true for cochlear implant (CI) recipients, who can be greatly affected by the physiological effects of auditory deprivation on peripheral and central auditory pathways. In this presentation I will review research aimed at assessing and understanding the perceptual effects of changes in the auditory pathway due to deafness.
At the peripheral level, many cochlear implantees have < 10% of surviving spiral ganglion cells. Histological studies have found no correlation between gross outcome with the CI and peripheral nerve survival. However, as CI design moves towards very place-focussed nerve activation and much higher rates of stimulation, this will no doubt be an important variable affecting both outcome or optimal fitting strategy. One way to assess peripheral neural survival in current (alive!) CI users is to measure the psychophysical or electrophysiological effects of changes in electrical pulse duration and interphase gap.
The ability of the 'deaf' auditory system to convey temporal information in speech signals can also be highly compromised in some individuals. Auditory Dis-synchrony is an extreme example of this. CIs can ameliorate this condition by restoring neural synchrony provided that the site of pathology is peripheral to the auditory nerve. An assessment of temporal resolution ability can predict whether a child will benefit from standard acoustic hearing devices, or alternatively would benefit from implantation.
Finally, the outcome of CIs tends to be better in people with more residual hearing and shorter durations of deafness. Is this an effect of having better peripheral nerve survival or of having less central auditory changes due to auditory deprivation? Also, what factors will determine whether such a person can gain significant benefit from combining acoustic and electric hearing? Some evidence is now emerging to begin to answer these questions

S8 Perspectives on Auditory Neuropathy: Disorders of inner hair cell, auditory nerve, and their synapse

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The term “auditory neuropathy" or "AN” was introduced in 1996 to describe a hearing disorder in children and adults reflecting altered function of the auditory nerve in the presence of preserved functions of cochlear outer hair cells. “AN” seemed appropriate since a peripheral neuropathy was identified in the majority of the subjects. Disorders of inner hair cells and their synapses with auditory nerve were also considered as alternative sites for these patients. Subsequently, temporal bone analyses in adult subjects confirmed that AN subjects with peripheral neuropathies showed loss of auditory ganglion cells and nerve fibers and preservation of numbers and morphology of hair cells. We do need temporal bone analyses of AN subjects without peripheral neuropathies to broaden our knowledge of the neuropathology of the disorder.

The hearing loss in AN reflects impaired auditory neural temporal processing. Auditory nerve and brainstem potentials (ABRs) are typically absent or abnormal consistent with deafferentation and/or impaired synchrony of discharge. Outer hair cell functions are normal. Clinical behavioural features reflecting abnormal temporal processing include 1) impaired speech comprehension beyond that expected for the degree of threshold elevation; 2) marked sensitivity of speech comprehension to noise masking; 3) absent binaural acoustic processes; and 4) minimal improvement with amplification.

AN can be identified at all ages, with varying degree of clinical expression and course, and is caused by a host of etiologies. Clinical tests do not distinguish between sites of the disorder (auditory nerve, synapses between nerve and inner hair cells, inner hair cell receptors). This can be unsettling and has led to a host of alternate descriptors: “Type I afferent neuron dysfunction”, “auditory dysynchrony”, “neural hearing loss”, "auditory synaptopathy", pre and post synaptic auditory neuropathy”.

I stress that “AN” is a clinical descriptor of a temporal processing disorder. The nerve can be affected pathologically or functionally leading to the same clinical picture or phenotype. The more we can appreciate about the variety of mechanisms that lead to this type of hearing disorder, the more likely we will be to develop proper nosologies, therapies, and counselling. At this conference. I hope to learn much about molecular mechanisms of function of inner hair cells, the synapses with auditory nerve terminals, and auditory nerve fibers .

Inner Ear Biology Workshop

17 - 19 September 2007

Session 1

Molecular biology and genetics

O1 Identification of microRNAs in the Developing Cochlea and Vestibule

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MicroRNAs (miRNAs) are 17-23 nt double-strand RNAs that can inhibit the translation of target mRNAs and affect, directly or indirectly, the expression of a large part of the protein-coding genes. MiRNAs are expressed differentially in different tissues. During the last years, miRNAs have been discovered as having important roles in development and disease of plants and animals. The vertebrate ear expresses specific miRNAs, as was suggested by recent studies in zebrafish and mice. Our goal is to identify miRNAs that contribute to the development and function of the mouse inner ear and may be involved in hearing and deafness in mammals, as well as their target mRNAs.
We have used bioinformatics and other prediction tools to identify potential miRNAs that may control inner ear development or function. Using expression microarrays and real time qRT-PCR to profile the miRNAs of the mouse inner ear, we deciphered the expression of miRNAs in cochleae and vestibules at various ages. Although most of the inner ear-specific miRNAs are expressed similarly in the cochlea and vestibule from the same age (e.g. miR-182), some miRNAs have a different expression pattern. The differential expression of miRNAs in the cochlea and vestibule may be responsible for some of the differences in the cochlear and vestibular transcriptomes and functions. MiRNAs with expression levels that changed over time or were different in cochleae and vestibules were selected for further study, including localization by in situ hybridization and a search for their targets. For a number of miRNAs, morpholino experiments in zebrafish demonstrated abnormalities in inner ear development and/or structure

O2 Mosaic complementation of the shaker1 allele suggests a novel role for myosin VIIa in hair cell stereocilia

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A female X-inactivation mosaic strategy was used to study the cellular function of myosin VIIa in auditory sensory hair cells. Mutations in *Myo7a* cause progressive disorganization of the bundle of mechanosensory stereocilia on the apical surfaces of hair cells and lead to deafness and vestibular dysfunction in mice and humans. BAC clones containing the wild type *Myo7a* gene were integrated by recombination-mediated cassette exchange (RMCE) into the *Hprt* locus on the X-chromosome and female mice were generated with one copy of the engineered X chromosome plus one wild type X-chromosome on a background of homozygosity for a mutant $Myo7a^{4626SB}$ allele on chromosome 7. Random X-inactivation of the X-chromosome containing the wild type *Myo7a* gene led to a fine mosaic of affected and unaffected hair cells, as the X-linked wild type *Myo7a* gene was able to complement the mutant allele on chromosome 7. The ability to compare two interspersed cell populations differing only in myosin VIIa expression revealed that stereocilia of mutant hair cells were longer, when actin polymerization was blocked they shortened faster than those of controls and the immunoreactivity of whirlin was prolonged at their tips. Our results strongly suggest that myosin VIIa plays a regulatory role in the elongation and turnover of stereocilial actin bundles and demonstrate the utility of X-linked mosaicism for high-resolution analysis of gene function.

O3 Hair cell differentiation becomes tissue specific by E9.5 in mouse inner ear

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Inner ear hair cells start their development from otic placodes, a pair of ectodermal thickening adjacent to hindbrain. Otic placode invaginates to form otic vesicle, then changes its morphology to form endolymphatic sac/duct, cochlea, vestibules, and semi-circular canals. A sensory patch in each structure other than endolymph contains common progenitor cells that give rise hair and supporting cells. The differentiation of these cell types is induced by the tissue interaction between otic tissue and surrounding structures. Identifying tissue interaction signals, which are necessary to develop those sensory cells, will not only help understanding the development, but also provide important information to differentiate and propagate sensory cells in vitro, a necessary technique to put inner ear regeneration therapy into practice.

Tissue culture is a standard method to study tissue interactions during embryogenesis. Serum, which usually is used in tissue culture, however, can confound results as it includes unidentified factors. In this study, we used a serum-free otocyst culture to investigate the tissue interactions that determine hair cell fate in mice otocysts. Otocysts cultured with surrounding tissues have the ability to produce mature hair cells in serum-free culture. Although isolated otocysts from E9.5 mice produced hair cells, those from E9.0 could not. This indicates that the mouse otocyst gains the ability to generate hair cells between E9.0 and E9.5 and that this ability depends on signals from the surrounding mesenchyme and/or the hindbrain

O4 Calbindin and S100 protein expression in the developing auditory system in mice.

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Calbindin D28K (CB) and S100 are calcium binding proteins known to be expressed in the inner ear of adult mice. Limited information exists about their occurrence during inner ear development. In this study, the expression patterns of CB and S100 were investigated immunohistochemically in C3H mice, from embryonic day 11 (E11) to postnatal day10 (P10). CB was expressed in the otocyst and vestibulocochlear ganglion (VCG) from E11. In the developing cochlea at E17, CB immunoreactivity clearly labeled precursors of the outer and inner hair cells, Kölliker´s organ and the stria vascularis. CB staining was also present in the VCG. Two days later, to this CB expression pattern was added the labeling of the inner hair cell afferent nerve fibers. Early postnatal CB expression encompassed VCG neurons, hair cells, their afferent nerve fibers and the cochlear lateral wall. The first signs of S100 immunostaining appeared at E14. At E17 S100 showed spatially restricted expression patterns in the cochlea. The Deiters, pillar, and inner hair cells, and partly the VCG, were S100-positive from E19. Postnatally, S100 staining also appeared in the inner hair cells and Deiters cells, in some VCG neurons and, in addition, in the spiral limbus, spiral prominence and the intermediate cells of the stria vascularis. This study demonstrates that the sites of CB and S100 expression in the mouse inner ear during embryonic and early postnatal development do not overlap, thus signaling independent developmental patterns.

The study was supported by grants AV0Z50390512, GACR 309/07/1336, IGA NR 8113-4 and LC 554.

O5 Characterization of a spontaneous recessive mutation arising in the mouse *Tecta* gene: A model for DFNB21 hearing loss

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The *TECTA* gene encodes alpha-tectorin (TECTA), a major non-collagenous component of the tectorial membrane (TM). Different mutations in *TECTA* lead to either dominant (DFNA8/A12) or recessive (DFNB21) forms of non-syndromic hearing loss in humans. All known missense mutations in *TECTA* reported thus far are associated with the dominant subtype, whereas those leading to the recessive form are all inactivating mutations. A transgenic mouse homozygous for a deletion in the entactin G1-like domain of Tecta (*Tecta*$^{\Delta ENT/\Delta ENT}$) has a TM that is deficient in non-collagenous components, is primarily composed of randomly organised collagen fibrils, and is completely detached from the surface of the organ of Corti. This mouse has been considered a potential model for DFNB21. In this study we have characterized a spontaneous missense mutation (A349D) in the mouse *Tecta* gene that is, unlike all previously reported missense mutations in *TECTA*, recessive and leads to a phenotype in which the TM resembles, but is not identical, to that of the *Tecta*$^{\Delta ENT/\Delta ENT}$ mouse. The *Tecta*$^{A349D/A349D}$ mouse also shows a completely detached TM that lacks the characteristic striated sheet matrix and is deficient in beta-tectorin (Tectb). A significant amount of Tecta is, however, detected and numerous dense granules are interspersed amongst the disorganised collagen fibrils, indicating that mutated *Tecta*^{A349D} is incorporated into TM but unable to interact with Tectb. Taken together, these findings suggest that recessive missense mutations in *TECTA* can result in a phenotype thought to cause DFNB21 hearing loss in humans, and for which the *Tecta*$^{A349D/A349D}$ mouse may represent a valuable model.

Session 2:

Hair cells I: Motility

O6 Outer hair cell somatic rather than hair bundle motility is the basis of the cochlear amplifier

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The outer hair cells of the organ of Corti, which are capable of both sensing and generating mechanical vibrations, are the source of cochlear amplification that ensures the exquisite sensitivity and enormous dynamic range of mammalian hearing. Two molecular mechanisms associated with the outer hair cells are purported to provide cochlear amplification. One of these is calcium-mediated hair bundle motility. This is associated with the gating of the outer hair cell mechanosensitive channels by sound-induced vibrations of the basilar membrane and involves interaction between the hair bundles and the overlying tectorial membrane to which they are attached. The other mechanism is somatic motility based on the motor protein prestin in the lateral membrane of the outer hair cell body. Here we investigate the involvement of these two mechanisms in amplifying electrically- and acoustically-elicited basilar membrane vibrations in the basal, high-frequency, region of the cochlea in wild type (*Tecta*$^{+/+}$) and mutant *Tecta*$^{\Delta ENT/\Delta ENT}$ mice. Electrical stimulation of the normal cochlea, as in *Tecta*$^{+/+}$ mice, drives both somatic-motility and hair bundle motility directly and acoustically- and electrically-elicited basilar membrane responses from *Tecta*$^{+/+}$ mice have very similar frequency-tuning, sensitivity and gain. The hair bundles of *Tecta*$^{\Delta ENT/\Delta ENT}$ mice are freestanding and cannot interact with the tectorial membrane. We show, however, that electrically elicited basilar membrane responses from *Tecta*$^{\Delta ENT/\Delta ENT}$ mice, where hair bundle motility cannot contribute to their amplification, are indistinguishable from those of *Tecta*$^{+/+}$ mice. Electrically evoked basilar membrane vibrations of both *Tecta*$^{+/+}$ and *Tecta*$^{\Delta ENT/\Delta ENT}$ mice are completely suppressed by salicylate, which has been shown to block outer hair cell somatic motility. We conclude, therefore, that outer hair cell somatic motility and not calcium-mediated hair bundle motility is the basis of cochlear amplification.

This work was supported by grants from the Deutsche Forschungsgemeinschaft to M. D., IBRO/ FENS to M. M-L, and from the MRC

O7 **Salicylate and chlorpromazine induce nanoscale curvature changes in the outer hair cell plasma membrane**

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The sensitivity and frequency selectivity of mammalian hearing requires the expression of the protein prestin which drives outer hair cell electromotility. Prestin is a multipass transmembrane protein and its function is sensitive to the amphiphilic compounds salicylate and chlorpromazine. The biophysical mechanism by which these drugs exert ototoxic effects is unknown. In the red cell, these compounds visibly alter membrane curvature resulting in the morphology changes of crenation (salicylate) and cup formation (chlorpromazine). As prestin is a polytopic protein, it likely interacts intimately with the surrounding membrane environment. There is growing recognition that the function of many membrane proteins are affected by the curvature of the membrane. To investigate whether this paradigm is applicable to prestin, we utilized fluorescence polarization microscopy (FPM), an optical imaging technique that measures the orientation of membrane-embedded fluorescent probes with respect to the plane of the membrane. Existing models for analyzing FPM data were extended to the cylindrical OHC. Our steady state model for the orientation of pyridinium, 4-[2-[6-(dioctylamino)-2-naphthalenyl]ethenyl-1-(3-sulfopropyl) (di-8-ANEPPS), a voltage-sensitive membrane probe, predicts the molecule is oriented at 27° with respect to the plane of the membrane. Following treatment with salicylate or chlorpromazine, although no gross morphological changes are detected, significant differences in the raw FPM data are observed. The predicted orientation changes for di-8-ANEPPS are consistent with nanoscale changes in plasma membrane curvature. These results demonstrate the sensitivity of FPM to nanoscale changes in membrane architecture, and suggest that salicylate and chlorpromazine may alter outer hair cell electromotility by modulation of the membrane curvature strain.

O8 **Frequency Effect on Charge Transfer by Prestin: Insight from an Electro-Diffusion Model**

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The membrane motor protein prestin is critically important to the active properties of the mammalian cochlea (Liberman et al., 2002), and prestin-transfected cells acquire such active properties as motility, force production, and nonlinear capacitance (Zheng et al., 2000). Transfer of an effective electric charge through the membrane, including the movement of intracellular chloride ions in and out of the membrane (Oliver et al., 2001; Fakler and Oliver, 2002) is essential to the protein function. The charge movement may be associated with conformational changes in prestin that are further accumulated into overall dimensional changes of the cell (Brownell, 2006). Active hearing is mainly observed in the high-frequency area of the cochlea, but how the corresponding electromechanical machinery functions at such frequencies is not fully understood. Here, we examine the frequency effect on prestin-associated charge movement. We propose an electro-diffusion model where ions of chloride bind the protein, and a combined charge (including some internal protein charges) moves through the membrane. While we imply conformational changes in prestin, we do not assume metastable states of the protein. Thus, our model can provide a physical basis for phenomenological kinetic rates used in models with two or three stable states of prestin. We found a twofold frequency decrease in charge transfer by prestin: first, it is because of the finite speed of the charge movement, and second, it is related to a phase shift between the transferred charge and applied field. We estimate the model parameters by using the available experimental data, including the diffusion coefficient that was chosen to match the experimental frequency decrease in outer hair cell nonlinear capacitance (Gale and Ashmore, 1997). As a result, we obtain the total transferred charge, its capacitive and resistive components, and the phase shift as functions of voltage and frequency. The proposed modelling can complement the biological and physiological approaches in further understanding of the structure and function of prestin.

O9 Prestin's state dependence of anion binding

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The motor protein, prestin, is responsible for our exquisite sense of hearing by providing the basis for cochlear amplification. Chloride and other anions are important modulators of prestin activity since they bind to this molecule and alter characteristics of the motor's displacement charge, or nonlinear capacitance (NLC; Oliver et al., 2001; Rybalchenko and Santos-Sacchi, 2003; Song et al 2005). Indeed, chloride has been recently shown to directly alter cochlear amplification in vivo (Santos-Sacchi et al, 2006). In addition to prestin's anion sensitivity, motor charge also responds to changes in membrane tension, and initial resting (holding) membrane potential. Here we report on studies to determine whether the state of prestin alters the protein's binding affinity for anions. Isolated OHCs were whole cell voltage clamped and NLC was measured under conditions of altered turgor pressure or holding potential while varying chloride or salicylate concentrations, an anion that competes for prestin's chloride binding site. Positive membrane tension induced by turgor pressure was found to shift the dose response curve of salicylate's reduction in NLC, indicating an increase in the IC50 for salicylate. Also the steady state holding potential altered the EC_{50} of chloride's effects on NLC magnitude. Both observations can be interpreted as a reduction in the affinity of anions for prestin, and may account for the effects of tension and prior voltage on OHC performance. We hypothesize that the conformational changes in prestin which occur during imposed membrane tension or hyperpolarization, namely an increase in the probability of residence in the expanded motor state, alters the binding site for anions. The induced reduction in binding affinity, and consequent unbinding of anions during the switch from compact to expanded state is contrary to the extrinsic voltage sensor model of Oliver et al (2001), where anion binding and ion movement through the membrane field is required to evoke motor expansion.

Supported by NIDCD grant DC000273 to JSS.

Session 3

Age and Ototoxicity

010 Mechanism of Age-Related Hearing Loss

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The Fischer 344 rat has been used as a model to study age-related hearing loss (ARHL). Various reports have shown that the Fischer 344 develops outer hair cell (OHC) losses, VIII nerve fiber degeneration, atrophy of stria vascularis, and possibly changes in middle ear mechanics. This project is focused on the changes in the OHC population with the goal of better understanding the triggers and mechanisms of age-related Fischer 344 OHC death. Our results show that from ages 23 months to 26 months, the percentage of OHC loss increases from 20% to 40%, mostly in the base of the cochlea. Most of the OHC die by apoptosis as indicated by propidium iodide (PI) labeling of the OHC nuclei, and caspase-3 staining. The apoptotic process can be triggered by either caspase-8 or -9. The extent of hair cell loss is less than would be expected by the hearing loss. However, in the older animals, there are large regions where the OHC are present (as indicated by prestin or PI staining) but their mitochondria are dysfunctional (as indicated by very weak succinate dehydrogenase staining). Finally, the progressive loss of OHC may be mediated by persistent oxidative stress as reflected in both Bcl and Bax labeling.
[Research supported by NIH grant #1R01DC00686201A1 to Dr. Henderson].

O11 Otoprotection strategies against Cisplatin induced ototoxicity

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This study has evaluated the pharmacological protection of three different agents against cisplatin (CDDP) induced ototoxicity using the Sprague Dawley rat model. D-methionine (D-Met), L-N-acetylcysteine (L-NAC) and Ebselen have been tested alone at three different dosages in order to find which was the most effective otoprotecting agent and which concentration gave the best results. D-Met has been tested at the dosages of 300, 350 and 400 mg/kg; L-NAC at 275, 375 and 475 mg/kg; Ebselen at 4, 8 and 12 mg/kg body weight. The hearing function was evaluated by using Auditory Brainstem Responses (ABR), Distortion Product OtoAcoustic Emissions (DPOAE) and Transient Evoked OtoAcoustic Emissions (TEOAE). These tests were performed before (PRE) and after 96 hours (PS96) from the CDDP treatment (14 mg/kg i.v. by slow infusion). After the last recording session cochleae were extracted and sent for Scansion Electronic Microscopy (SEM) imaging. The three agents were provided i.p. to the animals 1 hour after the CDDP treatment; the effectiveness of CDDP was calculated by the individual weight loss (side effect) of the animals.
The electrophysiology results at time PS96 have shown that the groups which had taken 350 and 400 mg/kg D-Met and 475 mg/kg L-NAC presented a significantly better hearing preservation vs. the other groups.
SEM images revealed that animals of the 350mg/kg D-Met group had the best hair cell preservation in the organ of Corti. Other D-Met dosages (300 and 400 mg/kg) showed less hair cell preservation. In the case of L-NAC, the 475 mg/kg dosage showed the best hair cell preservation when compared to the other L-NAC dosages (275 and 375 mg/kg). The hair cell preservation was found less than the D-Met, in particular in comparison with the 350 mg/kg D-Met group. Ebselen showed the least hair cell preservation, which was proportional to Ebselen dosage (12 mg/kg Ebselen showed the maximum preservation among the Ebselen groups). The SEM data from the 12 mg/kg Ebselen group have shown some hair cell protection, but the electrophysiology data did not confirm the SEM findings.

O12 Styrene ototoxicity and its protection

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Styrene is one of the most ototoxic industrial solvents and many workers are exposed daily to the solvent. It is known that styrene exposure causes outer hair cell loss leading to hearing loss in the middle frequency region. This report will discuss: (1) the location-selectivity of styrene toxicity in the cochlea; (2) the mechanism underlying styrene-induced cochlear cell death; (3) the relationship between cochlear cell loss and functional loss; (4) accumulation of styrene-induced injury in the cochlea after a long-term exposure; and (5) protection against styrene ototoxic activities in the cochlea. Rats were exposed to styrene by oral gavage at different doses up to 800 mg/kg once per day for 5 days per week for 3 to 24 weeks. Hearing loss was assessed by cochlear compound action potential (CAP) recording after styrene exposure. Hair cell losses were counted after the functional measurement. Apoptosis was defined when a broken or condensed nucleus was recognized. Active caspase-8 and 9 were detected to determine cell death pathway. Styrene level in the cochlea varied and its distribution was consistent with the damage, with the lowest styrene level in the basal turn and no hair cell loss in the area. Styrene first targeted Deiters cells (DCs) and then outer hair cells (OHCs) in the third row, then to the second and first rows. Caspase-dependent apoptotic cell death appeared to be the main cell death pathway in the cochlea after styrene exposure. However, cell death in the third row did not always result in a hearing loss. A complete loss of OHCs and DCs in the third row was observed in a few cases without threshold shift. It seems that cochlear cells have different susceptibility to styrene exposure. Hair cell loss was depended on styrene exposure level instead of exposure time. The styrene-induced hearing loss and hair cell loss could be reduced by application of antioxidants, indicating an involvement of free radical production in the styrene ototoxicity. (Research supported by NIOSH grant 1R01OH008113 to Dr. Henderson

013 TNF-α exposure initiates apoptosis of auditory hair cells that can be prevented by dexamethasone: Mechanisms

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Background: TNF-α expression is linked to trauma-induced inflammation of the cochlea. Expression of TNF-α and its receptors are up regulated in the cochlea following a physical trauma. Systemic and local dexamethasone administration can treat sudden idiopathic sensorineural hearing loss.
Material and Methods: Organ of Corti explants (P-3 rat) were exposed to escalating doses of TNF- to determine if exposure to TNF- α can directly initiate the loss of auditory hair cells. Using a TNF-α dose level (i.e. 1ug/ml) that eliminates >90% of an explant's hair cells we tested the protective effect of a pancaspase inhibitor (Boc-D-fmk) and determined a dose response curve for dexamethasone in preventing the loss of hair cells. Inhibitors of: 1) endogenous corticosteroid production: 2) NFκ-B activity; 3) TNF- α receptors; 4) PI3K; and 5) PKB (AKT) helped define mechanisms involved in dexamethasone protection against TNF- α induced hair cell loss.
Results: TNF- α exposure initiated a dose dependent, base to apex pattern of auditory hair cell loss within exposed explants with the explant's outer hair cells more susceptible to TNF- α than its inner hair cells. Treatment with a pancaspase inhibitor prevents loss of TNF- α exposed hair cells. Dexamethasone protection of auditory hair cells in the explants was dose dependent. The protective effect of dexamethasone against TNF- α ototoxicity is partially prevented by blocking: 1) corticosteroid receptors; 2) endogenous synthesis of corticosteroids; 3) NFκ-B activity; and 4) the activity of PI3K and PKB activity.
Conclusions: 1) TNF- α induces apoptosis of auditory hair cells; 2) dexamethasone protects hair cells against TNF- α induced apoptosis; 3) dexamethasone acts through its receptors by activating anti-apoptotic signaling (e.g. PKB and NFκ-B) in organ of Corti explants. Dexamethasone has been shown to limit the amount of hearing loss that occurs in a guinea pig model of electrode trauma induced hearing loss. Therefore dexamethasone is a good candidate drug for local delivery (e.g. biorelease) in conjunction with cochlear implantation for conservation of residual hearing.
(Supported by Advanced Bionics Corporation, Valencia, CA)

Session 4

Otoprotection

014 BDNF protects against Pseudomonas aeruginosa Exotoxin A - induced hearing loss

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Introduction: The Brainderived neurotrophic factor (BDNF) is a peptide within the neurotrophin family with protective effects in noise-induced hair cell loss and toxic inner ear damage following exposure to cisplatin.The Exotoxin A (PaExoA) from Pseudomonas aeruginosa, the most common microorganism in chronic suppurative otitis media, induces sensorineural hearing loss in rats. Our previous study showed that BDNF given simultaneously with the toxin protected the inner ear from damage.
The aim of this study was to investigate if BDNF has a protective effect when given 12 - 72 h after PaExoA .

Material and Methods: Three groups of Sprague-Dawley rats were used (n=16). Group A (control group, n=8) received 15µg/20µl PaExoA injected into the round window niche. Group B (n=4) received the same dose PaExoA and BDNF was applied 12 h later. Group C (n=4) received 15µg/20µl PaExoA and BDNF after 72 h.
Brainstem response audiometry (ABR) was performed on day 0 (control), and repeated on days 7,14,21,28 and 35 analysing the thresholds shifts.

Results: Exposure to 15µg /20µl PaExoA caused persistent and significant ABR impairment in the control group after 35 days. A single dose of BDNF given 12 h after PaExoA significantly reduced hearing loss. When BDNF was given 72 h after PaExoA no protective effect could be measured.

Conclusion: BDNF protects the inner ear from PaExoA-induced sensoryneural hearing loss when given 12 h after exotoxin but not when given after 72 h.

O15 **In the newborn rat cochlea, the heat shock protein 70 is upregulated on a transcriptional and translational level during normoxic and hypoxic culture conditions**

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Heat shock proteins (HSPs) are induced in a variety of cells under stress conditions. To better understand stress response in the inner ear, we analyzed expression of selected HSPs in tissues freshly isolated from a newborn rat and subjected to 24 h *in vitro* organotypic normoxic and hypoxic cultures of the organ of Corti (OC), modiolus (Mod) and stria vascularis (SV). The genes analyzed included mitochondrial stress gene HSP60, cytoplasmic stress gene HSP70 and endoplasmic reticulum (ER) stress gene HSP90. Using the expression micorarrays, we demonstrated that the transcripts of three analyzed heat shock proteins were constitutively present in fresh tissues, HSP60 and 90 being highly expressed and inducible HSP70 (iHSP70) being expressed on a low level. After 24h of hypoxic or normoxic culture, the expression of HSP70 was significantly upregulated, whereas the expression of HSP60 was somewhat downregulated and that of HSP90 remained unchanged. These results were confirmed by a real-time quantitative RT-PCR. Increased HSP70 was mirrored by respective protein translation, as shown by immunoblotting of respective tissue lysates with the monoclonal antibody against iHSP70. In the freshly isolated, cochlear neonatal rat tissues, the iHSP70 protein was below the immunoblot sensitivity threshold, whereas expression of HSP60 protein was strong in these samples. In the normoxic organotypic culture we found iHSP70 translation in OC and Mod, but not in SV. Short-term mild hypoxia upregulated iHSP70 protein expression about 3 fold in OC and about 2 fold in Mod as compared to normoxia and induced *de novo* iHSP70 expression in SV. Taken together, our results demonstrate upregulation and differential distribution of inducible HSP70 within the inner ear after preparatory mechanical insult represented by normoxic culture conditions and after mechanical insult combined with mild hypoxia. Injury alone induced iHSP70 translation in Mod and OC, whereas injury and hypoxia induced iHSP70 translation in all three cochlear regions tested. Induction of iHSP70 indicates cytoplasmic stress. In addition, presence of iHSP70 could likely have anti-apoptotic potential well known to be displayed by HSP70.

016 **Adenosine signalling in the cochlea: an emerging role in neuroprotection**

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Adenosine is a constitutive cell metabolite that can be released from cells via specific bi-directional transporters. In the extracellular space adenosine is a signaling molecule for P1 (adenosine) receptors involved in a variety of physiological responses in mammalian tissues. Extracellular adenosine acts as a local modulator with a generally cytoprotective function. The role of adenosine in hearing is, however, unclear. In this study we investigated the expression and distribution of adenosine receptors in rat cochlea and their role in cochlear function. Using RT-PCR, four subtypes of P1 receptors were identified: A_1, A_{2A}, A_{2B} and A_3. Adenosine receptor distribution was demonstrated by immunohistochemistry using receptor-specific antibodies. Adenosine receptors were differentially expressed in the organ of Corti, spiral ganglion neurones, lateral wall tissues and cochlear blood vessels. Adenosine (non-specific P1 receptor agonist, 1 mM), CCPA (A_1 receptor agonist; 100 □M) and Cl-IB-MECA (A_3 receptor agonist; 200 μM) applied to the round window membrane did not alter sound-evoked cochlear potentials (compound action potential, summating potential), whilst A_{2A} receptor agonist CGS21680 (200 μM) slightly improved auditory thresholds. Expression levels of adenosine receptors were altered during sustained noise exposure (4.5 kHz octave band, 24 hours at 100 dB SPL or 110 dB SPL). A_1 and A_3 receptors were up-regulated, whilst expression of the A_{2A} receptor remained unchanged. Our study thus established the expression pattern of adenosine receptors in rat cochlea and demonstrated that A_{2A} receptor activation can increase hearing sensitivity. The distribution of A_1 and A_3 receptors in sensory and neural tissues of the cochlea and their up-regulation during noise exposure suggest that these receptors are well positioned to provide cochlear protection, for example from noise exposure.
All of the studies described were approved by the University of Auckland Animal Ethics Committee. Supported by the Auckland Medical Research Foundation, Royal National Institute for the Deaf (UK), Deafness Research Foundation (NZ), and Health Research Council (NZ).

THE EUROHEAR LECTURE

L1 **Regulation of cell division and patterning in the regenerating inner ear**

Mark Warchol

Washington University, St Louis, MI, USA

Session 5:

Cochlear Mechanics

O17 Longitudinal change in the role of organ of Corti mass in cochlear tuning

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The plunging motion of the stapes launches a traveling wave down the cochlea that peaks at frequency dependent locations. The physical basis for the wave is the interaction between the cochlear fluid mass, the stiffness of the organ of Corti complex (OCC: the cellular tissue of the organ of Corti, the basilar and tectorial membranes), fluid and/or tissue viscosity and possibly the OCC mass.
In 3D computer models, tuning can arise through the interaction of the cochlear fluid mass and the OCC stiffness without local mass: due to the decreasing OCC stiffness from base to apex, the wave slows and its wavelength □ decreases as its best frequency place (peak position) is approached and then passed. However, other models explicitly include the OCC mass: the local resonance enhances the slowing and growing of the wave as the stiffness decreases. To decide whether the OCC mass plays a role in tuning the frequency variation of the wavenumber *k* of the cochlear traveling wave (proportional to 1/λ) was measured (in vivo, passive gerbil cochleae) and compared to theoretical predictions.
The experimental wavenumber is found by taking the phase difference of basilar membrane motion between two adjacent longitudinal positions and dividing by the distance between the two points ($k=\Delta\varphi/\Delta x$). As expected, *k* increased (λ decreased) as the frequency approached its best frequency. *k* depends on the phase response which is not very sensitive to cochlear condition so our results also apply to active response. The theoretical wavenumber is a solution of the dispersion relation of a 3D cochlear model developed by de Boer with OCC mass and stiffness the free parameters. When OCC mass is included in the theoretical *k*, the curve grows more rapidly than it does when only the fluid has significant mass. The experimental data are better fit by the model that includes OCC mass. However, extending the study to slightly more apical locations (from BF~ 40 kHz to 12 kHz) the role of mass is diminished. The notion of local resonance seems to only apply in the very base.

Supported by the NIDCD and the Emil Capita Foundation

O18 **Negative damping of the cochlear non-linear mechanical responses**

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The mammalian cochlea is extremely sensitive to sound and possesses exceptionally sharp frequency selectivity. This fine tuning is due largely to the tuning of the basilar membrane. In 1948 Gold pointed out that the remarkable sensitivity and frequency selectivity of the cochlea required a power amplification mechanism to counter the otherwise damped passive, responses of this sensory organ to sound. Gold suggested that the activity of the amplifier could formally be described by introducing a negative damping constant, which would compensate for the positive damping of basilar membrane vibrations by the intracochlear fluids. For almost sixty years, the existence of a non-linear active process, commonly called the cochlear amplifier, which pumps energy into the vibrations of the cochlear structures, has been inferred through measurement of neural, electrical, mechanical and acoustical responses from the cochlea. Evidence for the existence of the cochlear amplifier has involved cochlear manipulations, including death, that were designed to target the amplifier, although none of the manipulations so far reported have targeted the amplifier exclusively. Other evidence, including the recording of spontaneous acoustic emissions and evaluation of the power fluxes within the cochlea, requires assumptions that are not universally held. We have taken a new approach to demonstrate the existence of the cochlear amplifier that requires neither uncertain assumptions nor cochlear manipulations to exclude or reduce the impact of the amplifier. Here we prove that energy is indeed produced in the cochlea on a cycle-by-cycle basis. Using laser interferometry we show that the non-linear component of basilar membrane responses to sound stimulation leads the forces acting on the membrane. This is possible only in active systems with negative damping, i.e. in systems with energy production. Supported by the Medical Research Council.

O19 **Combined electric and acoustic stimulation in the cochlea of the guinea pig**

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Patients with severe to profound high-and mid-frequency hearing loss, but residual low-frequency hearing are nowadays considered as potential candidates for cochlear implantation (Kiefer et al., *Audiol Neurotol* 2005). Preservation of residual hearing can improve speech intelligibility and esthetic value of sounds after implantation. However, the improvement by the addition of a hearing aid to a cochlear implant is variable (Gantz et al., *Laryngoscope* 2005; 115:796-802). This raises the issue of how electric and acoustic stimulation interact in the cochlea.

In this study the effects of ipsilateral electric stimuli on acoustically evoked cochlear potentials were examined using a forward masking paradigm in guinea pigs. After exposure of the cochlea in anaesthetised animals, extracochlear stimulation electrodes were placed on the round window and on, or near, the basal turn. Recording electrodes were placed on the apex of the cochlea and on the bulla wall. The acoustically evoked compound action potential (CAP) was measured in response to pure tone bursts from 0.5-16 kHz at variable acoustic levels. Electric maskers consisted of 10 biphasic, alternating pulses presented at 1 kHz. Current levels and masker-to-probe intervals were varied.

Electric masking reversibly reduced the amplitude of acoustically evoked CAPs. Masking increased with higher electric current levels and shorter masker-to-probe intervals, and was more pronounced at high tone frequencies (8 and 16 kHz) of low acoustic levels. At high acoustic frequency and low acoustic level, the observed amount of masking could be as high as 90%, while CAP latency increased by no more than 0.2 ms. Masking of CAPs elicited by lower acoustic frequencies was less pronounced, probably due to the fact that the electric masker was presented basally on the cochlea. These results support the idea that high frequency cochlear regions can be stimulated electrically without affecting low frequency hearing, either by limiting the use of apical electrodes in conventional implants, or by using short electrode arrays (Gantz and Turner, *Acta Otolaryngol* 2004).

This work was funded by the Heinsius-Houbolt Foundation, The Netherlands

020 Nonlinearity and origin of the round window recorded cochlear microphonic potential

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The cochlear microphonic (CM) is a field potential that primarily originates from the sound-stimulated outer hair cells (OHC) in the cochlea, which is readily recorded from the round window niche. The location of the OHCs generating the CM is complex, but experimental work has provided evidence that OHCs nearby the round window dominate. The CM waveform has been shown to saturate with high sound levels. This saturation generally has asymmetry such that a spectral analysis shows a dc term, as well as even and odd order harmonics. In this study, we show that distortion in the CM from the guinea pig, takes another form when the cochlea has normal sensitivity. At sound levels of 50 to 90 dB SPL, low-frequency pure tones below 2 kHz produced an unusual waveshape characterized by a notch, which occasionally is so prominent to appear as a frequency doubling of the waveform. This CM distortion was sound level and frequency dependent. Total harmonic distortion of the CM at 500 Hz showed two separate peaks, one at about 60-70 dB SPL and the other at high sound levels (>90 dB SPL). In addition, the distortion increased when the scala media was perfused with artificial endolymph containing NMGD-Cl to replace KCl. Application of TTX on the round window or scala tympani perfusion with low concentration quinine significantly reduced CAP response but did not remove the CM distortion. The time waveform of the CM is related to the ensemble shape of the OHC mechanical transduction function and\other nonlinearities in the equivalent electrical circuit of the transduction current path. The saturating shape of the Boltzman activation function for stereociliary channels accounts for saturation and asymmetry of the CM distortion. To account for other types of distortion, there are several possibilities: A non-monotonic activation function, addition of a distorted but out of phase CM signal or neural contamination. These possible mechanisms on the origin of the round window recorded CM will be discussed.

Supported by NIH NIDCD DC 00141

021 Aminoglycoside and Acoustic Ototoxicity in *Meriones Unguiculatus*

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The stiffness of the cochlear partition is determined by the point stiffness of the organ of Corti and basilar membrane. The pillar cells are an important anatomical factor in the overall stiffness of the partition. Longitudinal coupling of the cochlear partition is a mechanical property that might influence the travelling wave. Low longitudinal coupling is commonly used in cochlear models unlike the models of the cochlear amplifier, which assign significant coupling. The transversely arranged collagen fibres of the basilar membrane support low coupling. In contrast, the paired pillar cells, which are tightly connected at their heads, may be implicated in significant longitudinal coupling of the cochlear partition.

Intracochlear neomycin causes complete destruction of the organ of Corti after 4 days in the guinea pig. Intratympanic neomycin, in the guinea pig, also causes loss of the organ of Corti but the time period is longer at 3 months. Combination of the two modalities has a synergistic effect on the damage to the organ of Corti.

The initial aim is to severely damage the pillar cells using neomycin and acoustic overstimulation in order to eliminate the longitudinal coupling provided by them. The final aim is to measure intracochlear pressure and motion responses to sound stimuli in the damaged cochleae and compare these responses to those in normal cochleae.

Ototoxicity to neomycin was observed most dramatically after intratympanic injection. Cochlear damage progressively increased in a time dependent manner. At 6 months, there was complete loss of all hair cells and supporting cells at the base of the cochlea. After intracochlear neomycin, minimal damage to the supporting cells was evident at the base, after 4 weeks. Disruption was limited to the inner and outer hair cells. Combination treatment appeared to extend the cochlear damage from the basal to the middle turn in the 2-week period.

Pillar cells appear extremely robust and seem to be the cells most resistant to ototoxic and acoustic damage in the gerbil. In this study, complete disruption of the pillar cell system has been noted several months after intratympanic neomycin injection.

Sesson 6

Cochlear Homeostasis

022 The dynamic characteristics of human organ of Corti homeostasis as inferred from post noise-burst OAE level fluctuations

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Hearing sensitivity is normally very stable except for example after exposure to intense sound. It is known that brief acoustic over-stimulation induces oscillations in the intensity of otoacoustic emission on a time scale of 2 mins. New data from 22 human ears is presented for a variety of exposures which produce minimal TTS . Results were highly consistent across individuals. We consider the oscillations in OAE intensity to be a direct correlate of changes in the organ of Corti as OHCs responds to the changing stimulation and modulating the gain of the 'cochlear amplifier'. We have developed a model to fit our data with which we can accurately predict the form of the oscillation for different exposures durations. The linearity demonstrated with respect to stimulus duration indicates that these oscillations are not the result of fatigue but rather the transient response of a simple under-damped resonant system. Such under-damped behaviour is a natural property of a negative feedback system with delay and high gain. We suggest that the homeostatic mechanism of the organ of Corti which stabilises hearing with respect to stimulus intensity comprises such a system negative feedback system. Our experiments and other research lead us to conclude that this mechanism operates locally to outer hair cells and involves an oscillation of their micro-mechanical and electro-physiological status.

We propose that this OAE technique provides an sensitive, safe and objective means of studying an individual's homeostatic mechanism parameters. For small perturbations caused by exposures which do not substantially elevate hearing threshold the feedback mechanism responds as a linear control system. With increasing exposures we do see signs of non-linearity (ie system fatigue) which we propose are associated with the onset of noise induced temporary threshold shift (TTS) . OAE tracking of the cochlea's response to sub-TTS noise exposures might be a practical way to quantify an individual ear's robustness.

023 Unitary permeability of Gap junction channels expressed in hela cells

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In a gap junction channel, each cell contributes a hexamer of connexins forming a connexon, which interacts with another connexon from the adjacent cell. Mutations in connexin genes are linked to a variety of human diseases, including cardiovascular anomalies, peripheral neuropathies, skin disorders, cataracts and deafness. Endogenous ions and low molecular weight species have been shown to cross gap junction channels, including glycolytic intermediates, vitamins, amino acids, nucleotides and second messengers as InsP3 and cAMP. Defective permeation of cAMP through gap junctions between adjacent cytoplasmic loops of myelinating Schwann cells has been hypothesized to underlie certain forms of CMTX disease. InsP3 permeability defects detected by a Ca^{2+} reporter system in supporting cells of the auditory sensory epithelium have been recently implicated in genetic deafness. Direct measurement of endogenous messengers' transit has been so far problematic due to lack of selective reporters. Procedures that have gained acceptance in assaying the permeability of connexins are dependent on the introduction into living cells of exogenous markers which are then traced in their individual intercellular movements. With the aim of quantitatively monitoring the change of second messenger concentrations in single living cells in real time, we have measured directly the flux of cAMP and InsP3 through recombinant connexin channels using novel FRET ratiometric biosensors. Simultaneous measurement of junctional conductance, by the dual whole cell patch clamp technique, combined with knowledge of unitary conductance of homotypic channels allowed estimation of the number of active gap junction channels thus determining the unitary permeability to InsP3 and cAMP. The flux of LY was quantified similarly, for comparison. Results were analyzed in terms of an all-atom model of the HCx26wt connexon derived from the recently model of mouse Cx32. This approach may have a general impact as it provides fast and reliable estimates of connexin permeability to second messengers and permits to investigate their role in the physiology and pathology of cell-cell communication.

O24 Expression of Aquaporins and Vasopressin type 2 receptor in the lateral wall of the Cochlea and Inner Ear Fluid homeostasis

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Purpose: Expression and localization of AQPs and V2-R in the lateral wall of the cochlea were investigated and water homeostasis of the stria vascularis was discussed.
Materials and Methods: In Experiment 1: In molecular biological study, the expression of AQP2, 3, 4, 7, 8, 9 and V2-R mRNAs was investigated. In histochemistry study, the localization of AQPs and V2-R was observed using immunofluorescence and immunoelectron microscopes.
In Experiment 2: The effects of argine vasopressin (AVP), bumetanide and V2-antagonist (OPC-31260) on the stria vascularis were morphologically investigated by TEM.
Results: Experiment 1: AQP2, 3, and V2-R were expressed in the strial basal cells, and AQP-7, -9 were expressed in the apical site of the marginal cell. Immunoelectron microscopic studies revealed that V2-R was localized on the medial site of the basal cells.
Experiment 2: Intraperitoneal application of AVP resulted in an enlargement of the intrastrial space. Bumetanide also produced an enlargement of the intrastrial space. However, combined application of bumetanide and OPC-31260 did not cause the marked enlargement of the intrastrial space.
Conclusion: AQP2, 3 and V2-R is localized in the basal cells, located at the boundary between the perilymphatic system and the endolymphatic system. Since osmolality is generally thought to be higher in the endolymphatic compartment, water might enter into the intrastrial space via these water channels. Water in the intrastrial space is speculated to enter the marginal cells through NKCC1 and to flow out through AQP-7 and -9 located in the apical membrane. AVP application accelerates the water entry into the intrastrial space via AQP2 water channels located in the basal cells. Meanwhile, bumetanide inhibits NKCC1 to block water influx into the marginal cells, resulting in the water retention in the intrastrial apace. OPC application is thought to decrease bumetanide-induced intrastrial enlargement by reduction of water entry via AQP2 water channels. These results imply that AQPs water channels, especially AQP2 water channel, and Na-K-Cl cotransporter play a great role in water homeostasis of the stria vascularis.

O25 **Bridging the epithelial membrane barrier: Potassium spatial buffering by Kir4.1/AQP4**

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In cerebral astrocytes and retinal Müller cells the aggregation of Kir4.1 and AQP4 and its non-uniform distribution across the cell membrane facilitates osmotically compensated potassium uptake as well as potassium release in separated cell and tissue domains (potassium spatial buffering). The potassium recycling mechanism of the cochlea follows similar principles as potassium is routed towards the lateral wall and limbus. However, in contrast to glial cells of the CNS it still remains unclear how potassium conquers the intra/extracellular barrier of the supporting cell syncytium of the cochlea.
This study analyses the Kir4.1/AQP4 complex in the cochlea and displays the analogy to glial cells. Colocalization of Kir4.1 and AQP4 can be visualized in supporting cells by immunhistochemistry and confocal laser scanning microscopy. Cell types close to the transduction process show strongest staining pattern along both the medial and lateral route of the transcellular potassium recycling pathway. Complemetary RT-PCR of cochlear tissue reveals all AQP4 isoforms known to be expressed in astrocytes and retinal Mueller cells. In electron microscopy orthogonal arrays of particals, the ultrastructural correlate of AQP4 channel clusters can be identified by freeze fracture analysis and TEM in analogy to astrocytes and Mueller cells. Confocal live cell imaging of cochlear wholemount preparations after hyposomotic shock confirm the immunhistochemical and ultrastructural findings and show highest ability of transmembrane water permeation in supporting cells close to the transduction process.
Taken together the molecular and functional analysis the Kir4.1/AQP4 channel system in the cochlea demonstrates considerable similarities between inner ear supporting cells and glial cells of the CNS. Our results suggest that the Kir4.1/AQP4 channel system plays a crucial role in potassium spatial buffering mechanisms of the cochlea and might be involved in both the transcellular and extracellular potassium recycling pathway.

This work is supported by fortune program (F 1541021)

026 **Coordinated control of Cx26 and Cx30 at the regulatory and functional level in the outer sulcus region**

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In the cochlea, two connexin isoforms, connexin 26 (Cx26) and connexin 30 (Cx30), account for the majority of gap junction channels, most likely forming a heteromeric assemblies, which interconnect adjacent non-sensory cells(1-3). The genes encoding both connexins (GJB2 and GJB6) are found in the DFNB1 complex locus. Mutations in this locus are responsible for 30%-50% of all cases of congenital deafness in Mediterranean countries.(4) In this work we transduced postnatal day 5 (P5) cochlear organotypic cultures from two mouse models, Cx30 knock out (KO) and $Cx26^{loxP/loxP}$, with Bovine Adeno Associated Virus vectors (pCx30GFP, pCRE-IRES-GFP) to study the mechanisms underlying the expression control of Cx26 and Cx30.In selected sub population of supporting and epithelial cells of the outer sulcus region, immunohistochemistry and qPCR experiments revealed that Cx26 is down-regulated in Cx30 KOs, both at the protein and at the mRNA level. Reciprocally, we found Cx30 to be down-regulated in parallel with Cx26 in outer sulcus cells of $Cx26^{loxP/loxP}$ cochlear cultures, transduced with pCRE-IRES-GFP. Gap-FRAP measurement of dye permeability showed that delivery of the recombinant Cx30 protein to Cx30 KO cultures resulted in restoration of the normal pattern of protein expression and formation of functional channels. Altogether our data highlight for the first time the mutual control in the expression of these two connexins at the regulatory and functional level in the outer sulcus region of the immature cochlea in vitro, as well as the potential of a gene therapy approach to ameliorate a defect due to connexin dysfunction.

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Session 7

Mitochondria and stress

O27 **Normal outer hair cell function contrasts with impaired function of auditory neurons in Friedreich ataxia due to faulty mitochondria**

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Friedreich's ataxia (FA) is due to faulty function of a ubiquitously expressed mitochondrial protein: frataxine, involved in mitochondrial iron homeostasis. The progressive iron mitochondrial accumulation is associated with a defect in respiratory chain complexes resulting in an excessive production of free radicals.

Besides the typical neurological disorders of FA, some patients, notably among those with severe disorders, complain about auditory discomfort or decreased intelligibility in noise. As cochlear sensory cells are known to present a high density of mitochondria and to be highly sensitive to oxidative stress, their possible involvement in Friedreich's hearing dysfunction would be particularly important to assess.

The goal of this study is to evaluate, in patients (15 males, 25 females, mean age 37.3 y) and in a knock-out mouse model of FA (29 mutant mice, 32 wild type mice), the micromechanics of the inner ear and the responses of the auditory pathway. Hearing assessment of patients showed normal or near normal pure-tone audiometry, distortion-product otoacoustic emissions (DPOAEs) were in the normal range throughout the usual frequency spectrum. The observed decrease of speech intelligibility in noise came together with either absence of auditory-evoked brainstem responses (ABR), or progressive decay of ABRs with prolonged acoustic stimulation. In mice, DPOAEs were normal and although present, ABR waves exhibited increased latencies.

This profile of deafness matches the definition of an auditory neuropathy with normal cochlear hair cell function and absent or abnormal ABR. FA's neuropathy is most evident with prolonged acoustic stimulation. This is logical as FA involves mitochondrial dysfunction and energetic deficit, and it is well acknowledged that auditory neurons exhibit a large density of mitochondria. Meanwhile, although also richly supplied in mitochondria, cochlear outer hair cells keep generating a normal cochlear amplification and normal OAEs. Thus, cochlear gain does not seem to heavily rely on mitochondrial function and hearing aid fitting is not an appropriate answer to FA complaints, even though a number of patients had been proposed such a "solution" in the past.

O28 Use of fluorescent imaging techniques to assess mitochondrial function in cochlear explants and slices

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A major function of mitochondria is the production of ATP but these organelles also have a pivotal role in Ca^{2+} homeostasis, regulation of intracellular redox potential and cell death. Mitochondrial mutations have been linked with aminoglycoside hypersensitivity, accelerated age-related hearing loss and sudden onset hearing loss. Here, we employ fluorescence imaging techniques to study mitochondrial physiology and metabolism in hair cells and supporting cells (SCs) from basal and apical cochlear turns.

The mitochondrial transmembrane potential (ψ_{mt}) is central to oxidative phosphorylation.. We assess ψ_{mt} using the fluorescent lipophilic cation tetrametylrhodamine ethyl ester (TMRM). Analysis of TMRM fluorescence reveals a base-to-apex difference in ψ_{mt} of inner hair cells (IHCs) in P3 cochleae. To further investigate base-to-apex differences in mitochondrial function, we utilise auto-fluorescence from reduced pyridine nucleotides (NAD(P)H) and oxidised flavoproteins (FAD^{2+}). Data obtained using the metabolic inhibitors cyanide and FCCP to manipulate intermediary metabolism suggest a base-to-apex variation in redox state of IHCs . No differences in redox state were seen in either outer hair cells (OHCs) or supporting cells.

Fluorescence lifetime imaging (FLIM) of NAD(P)H auto-fluorescence provides additional insight into cell metabolism. NAD(P)H lifetimes will change depending on whether it is immobilised or free (Lakowicz *et al.*, 1992). Typically lifetimes can be fit with double exponentials, giving two decay constants ($\tau1$: ~ 0.3 ns for free and $\tau2$: ~ 1-2 ns for the bound form). In the cochlea, we observe significant differences in $\tau2$ of OHCs (2.5 ns) and SCs (3.1 ns).. This suggests a greater proportion of bound NAD(P)H in SCs. We also observe differences in the relative weighting of the two lifetimes. Support cells show a weighting of 55% $\tau1$ vs 45% $\tau2$ and OHCs 68% $\tau1$ vs 32% $\tau2$. Treatment with pyruvate (10 mM) causes a change in the weighting in SCs ($\tau1$ increasing from 55% to 62%) whereas there is no effect in OHCs. Pyruvate promotes oxidation of cytosolic NADH but reduction in the mitochondrial NADH pool. Based on the data presented here and that from other cell types we suggests that $\tau2$ is derived mostly from NADH bound to cytosolic enzymes (involved in glycolysis) while the signal detected as $\tau1$ is dominated by free intramitochondrial NADH. These measurements are consistent with a more glycolytic role for support cells compared to the more oxidative role of OHCs.

029 Stress-induced changes in mitochondrial peroxiredoxin in mouse cochlear hair cells

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Age-, noise, and drug-dependent hearing loss are accompanied by oxidant stress in the cochlea. Antioxidants such as glutathione decrease, followed by an upregulation of enzymes that maintain redox homeostasis in an attempt to counter the oxidative insult. Peroxiredoxins are recently characterized components of an antioxidant defense that catalyze the inactivation of pro-radicals such as hydrogen peroxide and the reduction of protein sulfhydryl groups. The family of peroxiredoxins is currently comprised of six members of which peroxiredoxin 3 (Prx-3) is a mitochondrion-specific H_2O_2-scavenging enzyme that acts as a critical regulator of the intracellular redox balance. Its depletion results in increased intracellular levels of H_2O_2 and sensitizes cells to apoptotic signaling, thus making Prx-3 a crucial mediator of stress-induced apoptosis.
During chronic kanamycin treatment in the mouse, Prx-3 was first upregulated at times when hair cells were still intact and then precipitously decreased preceding outer hair cell death. The rise and fall of Prx-3 was reflected in organ culture. After gentamicin treatment for 8 hr, Prx-3 was increased in hair cells and their morphology remained undamaged. By 16 hr – shortly before hair cell death - Prx-3 decreased significantly. In a mouse model of age-related hearing loss, Prx-3 initially increased with advancing age in the cochlea but decreased around the time of impending hair cell death. A strong correlation between Prx-3 levels and cell fate was underscored by studies of protection. Antioxidant co-treatment (e.g., dihydroxybenzoic acid) with aminoglycosides maintained a high level of Prx-3 in outer hair cells and promoted their survival. These studies suggest a pivotal role for Prx-3 in hair cell death and survival.

Acknowledgement: The authors' research on acquired hearing loss is supported by research grants DC 03685 (drugs) and AG 025164 (age) from the National Institutes of Health.

Session 8

Stem cells

030 Synapse formation between embryonic stem cell-derived neurons and auditory hair cells in vitro

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Introduction: Recent investigations have indicated possible regeneration of spiral ganglion neurons (SGNs) by cell transplantation. Embryonic stem cells (ESCs) can be a sourse for replacement of SGNs, because they have a high ability to differentiate into neurons and several neural inducing methods have been established. Previous studies have demonstrated projection of neurites from ESC-derived neurons to auditory sensory epithelia. However, the potential of ESC-derived neurons for synapse formation with auditory hair cells (HCs) have not been elucidated. We set the aim of the present study to examine the ability of ESC-derived neurons to form synaptic connections with HCs in vitro.
Materials and Methods: Mouse ESCs labeled with EGFP were used. We used the co-culture of ESCs with PA6 cells, mouse skull bone marrow cells, as a method for neural induction of ESCs. Auditory epithelia obtained from P3 mice were co-cultured with ESC-derived neurons for 7 days. We performed histological analysis. HCs were identified using the expression of myosin VIIa. ESC-derived neurons and their neurites were determined by the expression of β-III tubulin and EGFP. We estimated expression of synapsin I and synaptophysin, markers for synaptic vesicles in co-cultured specimens. Transmission electron microscopy was employed for morphological evaluation of synaptic contacts between HCs and ESC-derived neurons.
Results: EGFP-expressing ESCs co-cultured with auditory epithelia for 7 days exhibited the expression of β-IIItubulin as reported elsewhere, indicating differentiation into neurons. We observed that colonies of ESC-derived neurons projected many neurites toward inner hair cells (IHCs). The expression of synapsin-1 and synaptophysin was found in the nerve endings of ESC-derived neurons adjacent to IHCs. Transmission electron microscopy demonstrated synaptic contacts between nerve endings of ESC-derived neurons and IHCs.
Conclusion: The present findings elucidate the capacity of ESC-derived neurons for making synaptic connections with IHCs, suggesting a clinical feasibility of ESC-derived neurons as transplants in cell therapy for SGN regeneration.

031 Bone Marrow Derived Cells in Inner Ear Repair

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Hair cell loss in the inner ear leads to hearing impairment and balance dysfunction. Remaining hair cells are unable to reconstitute the degenerated tissue, resulting in permanent functional deterioration. Recent studies suggested that bone marrow derived cells (BMDC) can contribute to the regeneration processes by engrafting into degenerated tissues and differentiating to various somatic cell types, thus promoting structural and functional repair. This high degree of plasticity prompted us to determine whether BMDC could be recruited and take part in the regenerative process when an injury is inflicted in the inner ear. We transplanted whole bone marrow cells isolated from C57BL/6 transgenic mice that ubiquitously express enhanced green fluorescent protein (eGFP) into lethally irradiated wild C57BL/6 mice. After successful haematopoietic reconstitution, we induced inner ear injury by acoustic deafening. Histological analysis showed the recruitment of BMDC in the deafened cochlea where robust infiltration of $eGFP^+$ cells was observed across most regions in the cochlea. Majority of the infiltrated $eGFP^+$ cells expressed the common leukocyte antigen (CD45), hence most BMDC preserve their haematopoietic identity. To examine the potential contribution of these BMDC in inner ear regeneration, BMDC was mobilised by stem cell factor (SCF) and granulocyte colony stimulating factor (G-CSF) into the peripheral blood. Despite higher circulating BMDC in the peripheral blood by cytokine treatment, immunotyping of the BMDC in the deafened cochlea by various stem, sensory and supporting cell markers were negative. The cell lineage of the infiltrated cells was identified and there is little evidence in this model to show that BMDC contribute to inner ear repair in acoustic deafened mice

Session 9

Hair cells II: Transduction and Synapse

033 **Transducer Gating in the Drosophila Ear**

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The sense of hearing has hidden its primary transducer channels till this day. This striking gap in our understanding of the molecular bases of audition is largely due to the peculiar way auditory mechanotransducers (aMETs) are gated: As opposed to the indirect activation of transducer channels for chemical or optical stimuli, aMETs are gated directly by the stimuli they are meant to transduce, namely the sound-induced vibration themselves. Under in situ conditions, the gating mechanisms of aMETs cannot be probed directly, yet in some cases it is possible to infer this mechanism from the mechanics of stimulus receiving structures: if the receiver and the transducers are mechanically linked so that receiver movements directly gate the transducers, the gating of the channels will inevitably modulate the mechanics of the receiver over that range of forces (and displacements) at which gating takes place. Such nonlinear modulation of receiver mechanics by transducer gating has been documented for isolated vertebrate hair cells[1] and, most recently, for the Drosophila ear[2]. In the fly, the third antennal segment and its lateral arista constitute a sound receiver, vibrations of which are directly picked up by the primary mechanosensory neurons of Johnston's organ in the second segment of the antenna. Deflecting this receiver with force steps betrayed a nonlinear compliance in the receiver's mechanics that qualitatively accords with a direct transducer activation. Quantitatively, this gating compliance was found to conform to the gating spring model of mechanotransduction in vertebrate hair cells, suggesting that fly and vertebrate mechanotransducers for hearing operate in equivalent ways. Here, we describe how the gating spring model can guide the molecular dissection of auditory mechanotransduction in *Drosophila* by making clear quantitative predictions about the phenotypes that associate with distinct transducer defects.

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This work was supported by the VolkswagenFoundation.

034 Defects In The Atp2b2 Gene Causing Hereditary Hearing And Balance Loss In Mice And Humans: A Functional Study Of Normal And Mutated PMCA2 Pumps

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Ca^{2+} enters the stereocilia of hair cells through mechanoelectrical transduction channels opened by the deflection of the hair bundle and is exported back to endolymph by an unusual splicing isoform (*w*/*a*) of plasma-membrane calcium-pump isoform 2 (PMCA2). Ablation or missense mutations of the pump cause deafness and loss of balance, as described for the G283S mutation in the deafwaddler (*dfw*) mice. A single a.a. change, G293S, in the pump in combination with a substitution, T1999S, in cadherin 23 was also found causing hearing loss in a human family. We performed functional analyses in CHO cells overexpressing the G283S or G293S mutant pump, determining that Ca^{2+} extrusion following transient Ca^{2+} elevations, induced by $InsP_3$ receptors, was significantly reduced compared to controls transfected with the wild type pump. We also investigated Ca^{2+} extrusion in hair cells in organotypic cultures of inner ear sensory epithelia from neonatal mice. Confocal Ca^{2+} imaging showed that the dissipation of stereociliary Ca^{2+} transients, induced by Ca^{2+} photoliberation, was compromised by various degrees in *dfw*, PMCA2 knockout mice as well as in a novel strain, Oblivion, carrying the single point mutation S877F .

035 Calcium, Calmodulin-dependent Regulation of Inner Hair Cell Calcium Channels

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Steady-state calcium influx through voltage gated calcium channels ($Ca_v1.3$) controls tonic transmitter release at the inner hair cell (IHC) ribbon synapse. Graded receptor potentials evoked by sound are encoded by modulation of steady-state gating. Calcium dependent inactivation (CDI) may serve as a self regulating, negative feedback process to optimize steady-state open probability of IHC $Ca_v1.3$ channels, establishing set-point and dynamic range for the ribbon synapse. Here we demonstrate that CDI of calcium channels in rat IHCs is calcium-, and calmodulin-dependent. Further, CDI is strongly temperature-dependent, and varies with IHC maturation. A voltage-ramp protocol was used to show that small, 'steady-state', deflections around the resting membrane potential produced inactivation within the IHC's functional range. This inactivation was calcium-dependent, as indicated by substitution of calcium with barium, and by a strong reliance on the calcium driving force. There was a striking decline in CDI after the onset of hearing (P12). However, this age-dependent reduction in CDI could be overwhelmed by inhibition of endoplasmic calcium ATPase (SERCA). Finally, in pre-hearing IHCs with a greater inactivating capability, introduction of either of two calmodulin (CaM) inhibitors, CaM inhibitory peptide or E_6 berbamine, markedly reduced CDI. We therefore identify CaM as one of the molecular components essential for CDI of IHC calcium channels and as a candidate site for modulation of inactivation. The confirmation of the involvement of CaM in IHC CDI expands on previous work showing that co-expression of $Ca_v1.3$ with CaBPs in HEK cells markedly diminishes CDI (Yang *et al*., 2006 J. Neurosci. 26:10677). Thus, CaM-like calcium binding proteins (CaBPs), which are expressed in IHCs, could modulate CDI by interacting with CaM binding sites on $Ca_v1.3$.

Supported by grants DC000276 and DC005211 from the National Institute for Deafness and Communication Disorders, NIH.

036 Voltage-Gated Channels and Scaffolding Proteins Define Compartments in the Vestibular Calyx Ending

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Two questions are important in understanding the operation of calyx endings. 1) How do ionic currents spread from the inner face of the calyx, where synapses are located, to the outer face, where the spike-trigger site is presumably located? 2) Is the trigger site located on the outer face? One possible solution is that synaptic currents are enhanced by voltage-gated currents. Recent studies are consistent with this suggestion: synaptic potentials are enhanced by persistent Na^+ currents and depressed by persistent K^+ currents (Holt et al., J. Neurophysiol., 2007). To better understand the role of voltage-activated currents, we studied their distribution, as well as that of scaffolding proteins, in the calyx ending. Tissues were stained with antibodies and viewed by confocal or EM. Antibodies were obtained from Matthew Rasband ($Na_V1.6$, $Na_V1.2$, Caspr1, □IV spectrin), Vann Bennett (AnkG and AnkB), Elior Peles (Caspr3) and Thomas Jentsch (KCNQ4 and KCNQ5); other antibodies were obtained from Chemicon. Molecules differ in their distribution on inner and outer faces. $Na_V1.5$, a TTX-insensitive isoform, is found on the inner face and could enhance synaptic transmission. The hemi-node stains positively for a pan-Na antibody, and for AnkG, □IV spectrin, AnkB, markers of initial segments, where spikes arise. As such, they could define the spike initiation site. ERG1 and ERG2 staining are concentrated in the upper half of the inner and outer faces, while KCNQ4 and KCNQ5 staining are more intense on the lower part of the inner face. When active, these K^+ channels could depress synaptic transmission. MiRP1, which can function as an accessory protein for ERG channels, is found at the base of the calyx and may alter the properties of inner-face channels. Scaffolding proteins link ion channels to the membrane. Caspr1 and Caspr2 are found on the inner- and outer-faces of the calyx membrane, respectively, while Caspr3 is found at the base. Tenascin-C, an ECM protein, is found in the synaptic cleft. Questions remain: How do these elements fit together to determine calyx function? Do differences observed between calyces in calyx and dimorphic afferents reflect function?

(Supported by DC-02058, DC-02290, DC-02521.)

Session 10

Postynaptic mechanisms and Nerve

037 HCN Channels Shape the Time Course of Postsynaptic Potentials at the Inner Hair Cell Afferent Synapse

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Neurons in the auditory pathway are specialized in rapid and reliable signaling. Here we study the features of synaptic transmission and identified the role of postsynaptic voltage-gated ion channels at the inner hair cell (IHC) afferent synapse, the first synapse in the auditory pathway.
Whole cell patch clamp recordings were done on afferent dendrites of auditory nerve fibers in excised apical turns of the 7-14 day-old rat cochleae. We investigated a slowly activating inward current that activated during prolonged hyperpolarizing voltage steps to -90 or more negative. CsCl (2 mM), $BaCl_2$ (2 mM), and ZD7288 (50 µM) blocked this current by 87±14 (n=9), 14±8 % (n=4) and 93±11 % (n=5), respectively. Such pharmacological profiles indicate that this current is mediated by hyperpolarization-activated cyclic nucleotide-gated cation (HCN) channels. cAMP analogs (200 µM cAMP internal and 200 µM 8-Br-cAMP external) increased the amplitude and activation kinetics of the current, and shifted the activation voltage to more positive values ($V_{0.5}$ from -103.6 ± 2.8, n=3, to -91.1± 2.2 mV, n=4). CsCl hyperpolarized the resting potential by 2±1 mV and slowed down the EPSP decay time constant by 46±31 % (n=5). This indicates that HCN channels play an important role in maintaining resting membrane potential and shaping EPSP waveforms. These results also raised a possibility that various neuromodulators found in the cochlea could modify the shape of EPSPs and afferent nerve excitability by cAMP-HCN channel pathway.
Supported by NIDCD DC006476 to EG

038 Instantaneous Rate vs Instantaneous Amplitude curves of Auditory Nerve Fibres are in close agreement with the transduction in the cilia of Inner Hair Cells.

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Action potentials ("spikes") were collected from single auditory nerve fibres in cats. By ordering the moments of occurrence of spikes on a time scale of one period of the stimulus we determined period histograms (PHs). The traditional view is that PHs resemble filtered and half-wave rectified versions of the stimulus waveform. In PHs of complex stimuli, however, there is a small but definite difference in shape between nerve fibres of high spontaneous (HS) rates and low spontaneous (LS) rates (Horst et al., JASA 88(6) 1990). Near threshold, the PHs of HS fibres show fairly linear responses, those of LS fibres show a more expansive character. To investigate this input-output (IO) behavior, we compared the waveforms of pure tone stimuli and their corresponding PHs. Instantaneous IO-curves were determined by relating the instantaneous discharge rate (IR) bin by bin to the instantaneous stimulus amplitude (IA). Such IO-curves turned out to be robust, time-invariant curves for levels ranging at least from synchronization threshold to rate threshold. IR-vs-IA curves are fundamentally different from rate-level curves, in which no phase or other instantaneous information is present. IO-curves for nerve fibres with various spontaneous spike rates did show expansive behaviour for pure tone stimuli. This was especially clear for average rates up to 10 spks/s as found in responses of LS and MS (medium spont.) fibres. When plotted on a logarithmic vertical scale and linear horizontal scale, all IO-curves became fairly linear irrespective of spontaneous rate. This indicates an exponential relation between IR and IA.
The exponential relation can be explained by the "sandwich model" of the transduction in the cochlea: a sigmoid IO-relation is sandwiched between a bandpass and a low pass filter. This IO-relation represents the transduction in the cilia of the IHCs and can be described by the Boltzmann relation. For small inputs, this relation can be approximated by an exponential relation. Thus, the exponential relation of the PHs can be attributed to the transduction in the cilia.
The data suggest a parsimonious description for transduction for all fibres: Transduction can be expressed by a single exponential IO-curve, variably attenuated for fibres with different spontaneous rates; i.e., the lower the spontaneous rate, the larger the attenuation.

039 Tonotopic frequency mapping on the basilar membrane of the Chinchilla (Chinchilla laniger) measured by labelling auditory nerve fibres with HRP and biocytine

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We recently reported that the frequency-place map of the basilar membrane of the mouse, based on tracing of auditory nerve fibres (physiological map), was shifted by about one octave towards lower frequencies compared to that determined from hair cell damage induced by sound (anatomical map). We also showed that sound-trauma-induced shifts of neural frequency tuning can explain the observed shift of the frequency map.
From our results we hypothesized that the sound-trauma-based frequency map in the chinchilla might also be shifted towards lower frequencies. We therefore determined the physiological map of the chinchilla by recording frequency responses of neurons in the cochlear nucleus, followed by iontophoretic application of HRP or biocytine and tracing of labelled auditory nerve fibres towards the cochlea. The distance of labelled innervation sites from the basal end of the basilar membrane was determined from midmodiolar sections of the cochleae. HRP labelling was mainly confined to the somata of the spiral ganglion cells, whereas biocytine predominantly labelled afferent boutons below the inner hair cells. The physiological frequency-place map was established for CFs from 0.135 - 21.2 kHz, corresponding to 84 and 1% distance from the base respectively. The map was best described by a mono-exponential function. The slope of the map was 2.4 mm/octave for a mean basilar membrane length of 20.11 mm.
The present data set indicated that the chinchilla anatomical map is shifted towards lower frequencies compared to the present physiological map but that the shift is smaller than in the mouse. Apart from methodological differences in determination of the anatomical maps (behavioural versus ABR tone thresholds in chinchilla and mouse respectively), this difference may relate to the larger slope (2.4 mm/oct) and length (20.11 mm) of the chinchilla map compared to that of the mouse (1.25 mm/oct and 5.13 mm). In addition a smaller neural frequency shift after sound damage in chinchilla might occur compared to the mouse, because the sound-trauma-induced CF shift decreases with lower CF and the chinchilla frequency range is about two octaves below that of the mouse.

040 Neural and receptor cochlear potentials in auditory neuropathy obtained by transtympanic electrocochleography (ECochG)

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Auditory neuropathy (AN) is a recently identified disorder of auditory nerve characterized by prominent auditory temporal processing deficits. Both auditory nerve and brainstem activities recorded as far-field potentials are typically undetectable whereas cochlear outer hair activities (otoacoustic emissions and/or cochlear microphonics) are normal. We recorded cochlear receptor (summating potential [SP]; cochlear microphonics [CMs]) and auditory nerve (compound action potential [CAP]) activities by transtympanic electrocochleography (ECochG) in 15 adults and children with the clinical picture of AN. Data from eight children and adults with delayed onset AN will be presented. Test stimuli were 0.1 ms clicks which were presented in free-field from 60 to 120 dB SPL. Measures were compared with the ECochG results from 16 children who had normal thresholds of CAPs.
Cochlear potentials from AN were significantly prolonged in duration and reduced in peak amplitude compared to controls. CAPs were identified in 4/16 AN cochlea only to high intensity stimuli. In the remaining ears it was difficult to separately identify the CAP and the SP within the broad cochlear potential. In contrast, CMs were identified in all AN subjects and did not differ significantly in amplitude from controls. Receptor summating potentials (SPs) when identified were of normal amplitude. Rapid stimulus rates were used in six of the AN subjects to help distinguish the generator sources of the prolonged cochlear potentials by taking advantage of different amount of adaptation involving CAP and SP. In controls, rapid stimulus rates were accompanied by amplitude reduction of CAP approximately twice that for SP while the duration of cochlear potentials was unaffected. In AN rapid stimulus rates were accompanied by a reduction of duration to control values in all and a reduction in amplitude in the majority consistent with a neural origins for the abnormal cochlear potentials. We suggest that the prolonged and attenuated cochlear potentials in AN reflect pre- and postsynaptic disorders of inner hair cell synapses with auditory nerve terminals.

POSTERS

P1 **Which interstereocilial links can be found in disorganized hair bundles of cadherin 23 mutants?**

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Cadherin 23 is a member of a transmembrane family of cadherins. Cadherin 23 localizes to hair cell stereocilia and is thought to be involved in the formation of interstereocilial links, including tip links. Loss-of-function mutations in the *Cdh23* gene cause deafness and vestibular dysfunction in zebrafish, mice and humans. Progressive disorganization of the stereocilia on the apical surface of hair cells lacking cadherin 23 can be observed as early as E18.5 and leads to hair cell degeneration in older mice.
Here we analyzed in great detail the stereocilia bundles of outer and inner hair cells of waltzer mutant mice and their littermate controls. We used OTOTO sample preparation and field emission scanning electron microscopy in order to avoid coating artifacts. The overall shape of the bundle, it's organization, the shape of individual stereocilia and presence of interstereocilial links were evaluated. In the disorganised hair bundles of adult $Cdh23^{2J}/Cdh23^{2J}$ mice there was little difference in height amongst remaining stereocilia, all stereocilia showed rounded tips and stereocilia fusion was observed in the basal turn of the cochlea. The stereocilia of waltzer mutants were tightly connected to each other by horizontal links, and tectorial membrane attachment crowns were easily recognizable. At P15 however there was no fusion between stereocilia, differences in length between different rows were more pronounced, and a few stereocilia with pointed tips were found, confirming the progressive character of degeneration of waltzer hair bundles.

P2 **Identifying Target Genes of the Hair Cell POU-Domain Transcription Factor Brn-3c**

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Hair cells play a critical role in hearing and balance, and loss of these cells is a common factor in many cases of hearing loss. Hair cell survival depends on the temporal and spatial regulation of gene expression, controlled by transcription factors. The POU domain transcription factor Brn-3c (Brn3.1, POU4f3) is required for hair cell differentiation and survival, and transgenic mice lacking Brn-3c are profoundly deaf. In addition, a mutation in the human homologue has been identified as a locus responsible for adult onset sensorineural hearing loss. Despite the apparent importance of Brn-3c in maintaining hair cells, little is known about how this promotes survival. We aim to identify target genes whose expression is regulated by Brn-3c and investigate their role in hair cell survival. We identified potential targets by performing a subtractive hybridisation screen on OC2 cells, a cell line derived from the embryonic organ of Corti, comparing gene expression in cells with increased or decreased levels of Brn-3c. Selected targets were assessed on the basis of: i) *expression*, expression of candidate genes/gene products was examined either by immunofluorescence at different stages of development, or using qPCR to measure mRNA levels in rodent hair cells; ii) *evidence for regulation by Brn-3c*, Brn-3c binding sites were identified in the promoters of potential target genes and reporter constructs containing these sites tested in cell lines to determine their effect on promoter activity in the presence and absence of Brn-3c. The role of these candidate Brn-3c targets is currently being evaluated and further elucidation of the genes and molecular pathways involved will aid the development of strategies to promote hair cell survival.

P3 **A Subtractive Hybridization Strategy to Identify Barhl1 Target Genes in Sensory Hair Cells**

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The predominant cause of hearing loss is the damage and subsequent death of the sensory hair cells in the inner ear. We know little about pro-survival hair cell genes; our primary aim is the elucidation of molecular candidates of survival pathways in hair cells. Targeted deletion of the Barhl1 homeobox gene in mice produces a phenotype of progressive post-natal hearing loss due to loss of hair cells (Li et al., 2002). Given this phenotype for a transcription factor Barhl1 is likely to act as a key regulator of gene expression at the head of hair cell survival pathways. Levels of Barhl1 were manipulated in an organ of Corti cell line derived from the immortomouse (OC-2): (1) transient transfection was optimized to efficiently overexpress Barhl1, (2) an siRNA strategy was developed to knock down Barhl1 expression. We used the PCR-select cDNA Subtraction System (Clontech) to perform subtractive hybridization on cDNA from cells with manipulated Barhl1 expression level and we identified and cloned cDNAs which were differentially expressed under these conditions. RT-PCR analysis confirmed the Barhl1 regulated expression of four identified genes. We have shown by bandshift assays that at least two of these genes have Barhl1 binding sites in their promoters. The candidates obtained by subtractive hybridization will be analyzed further in cell lines and in *ex vivo* organ explants to assess changes in Barhl1 and candidate gene product expression during hair cell damage. The functional role of those genes will be assessed by manipulating gene expression or protein levels in *ex vivo* explants. This approach may lead to identification of genes that regulate how hair cells respond to damage and thus reveal them as targets for therapeutic or protective intervention.

P4 **Interaction between Cochlear Cells and Bone Marrow Cells**

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A substrain of the senescence-accelerated mouse (SAM), the SAMP1 mouse, is an animal model for accelerated senescence including the age-related acceleration of both immunological dysfunction and hearing loss caused by the impairment of spiral ganglion cells. In the present study, we examine 1) whether the accelerated presbycusis in the SAMP1 mice can be prevented by bone marrow transplantation (BMT) using allogeneic bone marrow cells (BMCs) of BALB/c mice, a non-presbycusis-prone mouse strain and 2) whether the presbycusis in the SAMP1 mice can be transferred to BALB/c mice by BMT using BMCs of SAMP1 mice.
Young recipient mice were irradiated with 9 Gy and then reconstituted with BMCs from allogeneic mice.
SAMP1 mice that had been transplanted with BMCs from BALB/c mice (abbreviated as [BALB/c to SAMP1]) showed prevention of the development of immunological and cochlear dysfunctions and apoptosis of spinal ganglion cells.
BALB/c mice that had been transplanted with BMCs from SAMP1 mice (abbreviated as [SAMP1 to BALB/c]) indicated the development of immunological impairment, hearing loss, and cochlear pathology.
These findings indicate that some types of accelerated presbycusis do not result from defects in the cochlea but do from defects in the hematopoietic stem cells (HSCs) and immunocompetent cells derived from the HSCs. If this is the case, either allogeneic BMT, which replaces abnormal HSCs with normal HSCs and reconstructs a normal immune system in the recipients, or autologous BMT using genetically modified bone marrow cells, could become a new strategy for the treatment of presbycusis.

P5 **Upregulation of erythropoietin and erythropoietin receptor mRNA in organ of Corti, modiolus and stria vascularis of newborn rats**

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Protection against apoptosis by erythropoietin (EPO) has been observed in several types of CNS neurons and glial cells. In a previous study, we found that rhEPO attenuates the ischemia-induced loss of hair cells. In the present study, we wanted to know if endogenous EPO plays a role in the inner ear. Because organ of Corti, modiolus and stria vascularis tissues are functionally connected with each other, we determined the mRNA levels in these three regions using real time RT-PCR. We used freshly prepared tissue and tissue cultured for 24 h under normoxia or hypoxia. The basal expression of EPO- and EPO-receptor (EPOR) mRNA in freshly prepared tissue of all regions was very low. However, after 24 h in culture, a dramatic increase of these two transcripts was measured under normoxic and hypoxic conditions. To evaluate the functional network of endogenous EPO- and EPOR in the cochlea, we analyzed the expression of three groups of genes using microarray: (1) transcription factors, which regulate the expression of EPO. Hypoxia inducible factor 1/HIF-1alpha mRNA level was found increased in culture by a factor of 2-6. (2) Genes, which are associated with the EPO function (e.g. transferrin, transferrin receptor and cell cycle progression factor early growth response1/Egr1), they were differentially upregulated by a factor of 2-5. (3) Genes induced in EPO-signaling pathway (e.g. signal transducer and activator of transcription 3/Stat 3, Janus kinase 2/Jak2 and mitogen activated protein kinase1,-3/Mapk1/Mapk3). Expression of Stat3 and Jak2 was upregulated whereas Mapk3 was downregulated. Obtained results let us conclude that under normal physiological conditions, EPO and EPOR are present in the cochlea but on a minimal level. Preparatory stress, culture and exposure to hypoxia increase transcript expression of both EPO and EPOR.

P6 **Expression and immunolocalization of aquaporin-6 (Aqp6) in the rat inner ear**

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Recently, multiple subtypes of aquaporins (Aqps) have been reported to be expressed in the inner ear. As to Aqp6, which is unlike other members of the Aqp family in its intra-cellular distribution pattern and its function, the mRNA was confirmed to be expressed in the rat cochlea and endolymphatic sac by using reverse transcription-polymerase chain reaction (RT-PCR). However, its localization in the inner ear has not yet been evaluated. In the present study, Aqp6 mRNA expression in the rat inner ear was investigated in the vestibulum as well as in the cochlea and endolymphatic sac using the RT-PCR method, and detailed immunolocalization of Aqp6 in the rat inner ear was investigated using immunohistochemical methods including immunofluorescence microscopy and immunoelectron microscopy. We obtained novel data showing that not just Aqp6 mRNA but also Aqp6 protein is expressed in the cochlea, endolymphatic sac and vestibule. The immunoelectron microscopic studies revealed that the immunolabeled gold was diffusely seen in the intracellular area of the stria vascularis, endolymphatic sac and vestibule, but never in the plasma membranes. Since Aqp6 expression is localized in the main site of absorption and/or resorption of the endolymph, Aqp6 might play some role in the homeostasis of endolymph in the inner ear. However, its lack of expression on the plasma membrane indicates that Aqp6 does not take direct part in water flux via the plasma membrane.

P7 **Connexin 30 trafficking to the plasma membrane via a Golgi-independent pathway**

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Gap junctions consisting of connexins 26 (Cx26) and 30 (Cx30) are essential for hearing in mammals, and are the two predominant connexin subtypes found in the cochlea. Whereas the majority of connexins, such as Cx43, have been shown to traffic to the plasma membrane (PM) via the Golgi apparatus (Laird, Biochem. J. 394,527-543, 2006), it has been suggested that Cx26 trafficking may occur via a novel Golgi-independent pathway (Martin, J. Cell Sci. 114, 3845-3855, 2001). Gap junctions in the cochlea are composed primarily of Cx26/Cx30 heteromeric connexons, which are likely to oligomerize prior to insertion in the PM. Thus it seems likely that Cx26 and Cx30 trafficking pathways show common features. We have investigated the trafficking of mouse connexin 30 (mCx30) *in vitro*, with particular attention to potential Golgi interactions.

In Hela cells stably expressing mCx43, Cx43 immunofluorescence was localised to gap junction plaques at the PM, and co-localised with the cis-Golgi marker GM130. In comparison, cells stably expressing mCx30 displayed distinct gap junction plaques, but displayed little discernible intracellular labelling, particularly at the Golgi apparatus. Brefeldin A (BFA), which disrupts the Golgi apparatus, reduced the number of mCx43 gap junction plaques and completely dispersed intracellular expression. However, mCx30 localisation was not affected by BFA. In separate patch clamp experiments, BFA caused a 59% reduction ($p<0.0001$) in the intercellular transfer of neurobiotin between mCx43 expressing cells, consistent with the observed decrease in the number of gap junction plaques. BFA did not significantly affect neurobiotin transfer between mCx30 expressing cells ($p=0.6562$).

These data suggest that mCx30 may traffic to the PM via a pathway that is shared by Cx26, and that is unaffected by the disruption of the Golgi apparatus. It will be important to determine the sub-cellular site of Cx26/Cx30 oligomerization, and how the heteromeric channels are trafficked subsequently to the membranes between cochlear supporting cells.

Supported by Deafness Research UK, the BBSRC and the Royal Society.

P8 **Mitotracker staining of mitochondria in outer hair cells of the guinea pig cochlea**

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Outer hair cells of the guinea pig cochlea were stained with the membrane-permeable and fixable dye Mitotracker Red, which is preferentially sequestered by mitochondria with a normal mitochondrial potential. Outer hair cells showed a number of pools of mitochondria: one pool was situated just below the cuticular plate, and in some hair cells continued in a column down the centre of the hair cell. A second major pool was visible just underlying the basolateral membrane of the hair cell. In side view, mitochondria of this pool were distributed with a meshwork or reticular appearance. Following kanamycin intoxication, staining was lost as hair cells were lost. However, occasionally, staining could be still visible in the apical-most pool, while all specific staining was lost deeper in the cell, and while cellular structures were still present. The results give information on the distribution of mitochondria in hair cells, and suggest that different pools of mitochondria in the hair cells might show different vulnerabilities to the effects of kanamycin.

Key words: Hair cell; guinea pig; mitochondria; Mitotracker Red; kanamycin; aminoglycoside.

P9 **Actin dynamics in avian sensory epithelia.**

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Sensory epithelia help partition the ionic compartments that are necessary for mechano-transduction in the inner ear. How these epithelia respond to hair cell damage is critical for the preservation of homeostasis and sensory function. In other systems, the actin cytoskeleton is a key determinant of repair processes that remove damaged cells from an epithelium. Here, we describe a live-cell imaging approach to investigate the cellular and cytoskeletal events that underlie repair in sensory epithelia of the chick inner ear. We used *in ovo* electroporation of retroviral RCAS plasmid DNA at embryonic day 2.5 to induce sustained expression of β-actin-EGFP in the developing chick otocyst. Explant preparations of E19 transgenic utricles were imaged using a Nipkow confocal system allowing us to monitor the 3D distribution of β-actin-EGFP over extended periods (>48 hours). In response to aminoglycoside antibiotics, we observed an extensive and highly dynamic remodelling of the supporting cells' actin cytoskeleton. Supporting cells constricted apically around a hair cell to excise and eject its stereocilial bundle. The same supporting cells extended lamellipodia basolaterally to engulf the hair cell soma. Dual-channel imaging of β-actin-EGFP and fluorescent DNA dyes confirmed the phagocytic uptake of hair cell material into the supporting cells. These experiments reveal the dynamic nature of supporting cells and demonstrate the coordinated cytoskeletal and cellular activity critical for hair cell removal and epithelial repair.

Supported by Deafness Research UK and The Royal Society

P10 Targeted Disruption of *Otoa* with EGFP demonstrates otoancorin is required for adhesion of the tectorial membrane to the spiral limbus.

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The tectorial membrane is an extracellular matrix unique to the inner ear. It is attached to the surface of the cochlea via the microvilli of interdental cells in the spiral limbus and at the tips of outer-hair-cell hair bundles. Otoancorin is a 124.6 kDa GPI-anchored protein that is mutated in the non-syndromic deafness DFNB22. It shows similarity with mesothelin, a mucin binding protein, and is expressed on the surface of the spiral limbus. Together these observations suggest it mediates adhesion of the tectorial membrane to the spiral limbus. To investigate this hypothesis, we have created an otoancorin null mutant mouse by gene targeting in ES cells. The first coding exon of *Otoa* was replaced with sequence encoding EGFP, preventing expression of *Otoa* whilst allowing sites of *Otoa* expression to be visualised. In $Otoa^{EGFP/+}$ mice, EGFP expression is observed as early as E10.5 in the presumptive sensory epithelia of the otocyst. The sites of expression at later stages correlate largely with those described for otoancorin using antibodies. Strong expression is seen in the interdental cells of the spiral limbus, the border cells and inner phalangeal cells of the developing greater epithelial ridge, and the supporting cells of the vestibular end organs. In $Otoa^{EGFP/EGFP}$ mice, the tectorial membrane is detached from the spiral limbus but remains associated with the organ of Corti. It develops almost normally, except for the marginal band which is malformed and Hensen's stripe which is absent. In the vestibular organs, the cupulae and otoconial membranes remain attached. These results provide further evidence that otoancorin is a novel cell surface receptor for the tectorial membrane and suggest that additional molecules may be required to anchor fully the extracellular matrices of the vestibular end organs to the surface of the sensory epithelium.

Supported by The Wellcome Trust

P11 AQP4 and Kir4.1 are colocalized in distinct supporting cell populations of the rat cochlea

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In cerebral astrocytes and retinal Mueller cells the aggregation of the water channel aquaporin 4 (AQP4) and the potassium channel Kir4.1 in distinct membrane domains facilitates osmotically driven waterfluxes accompanying glial potassium uptake and release. Potassium spatial buffering controls extracellular ion and water homeostasis in neuronal tissues. In analogy epithelial cells are suggested to mediate "potassium recycling" in the cochlea along distinguished intracellular routes. Transcellular potassium fluxes in the endocochlear supporting cell-epithelium are proposed to be mediated by gap-junctional coupling. Molecular substrates facilitating uptake of extracellular potassium near hair cells and its release to the fibrocytes are still unknown. Immunohistochemical staining of cryo-embedded rat cochleae was analysed by confocal laser scanning microscopy to determine i) the cellular expression of AQP4 in the cochlear duct ii) the subcellular localization of AQP4 and the potassium channel Kir4.1 iii) sites of subcellular colocalization of AQP4 and Kir4.1. As shown previously (Takumi et al., Eur. J. Neurosci. 10, 1998) water channel protein AQP4 was detected in supporting cells along proposed medial and lateral potassium recycling routes. In addition we found labelling for AQP4 in epithelial cells flanking fibrocytes of the lateral wall and the spiral limbus. Epithelia neighbouring inner and outer hair cells as well as cells close to fibrocytes show a colocalization of AQP4 and Kir4.1. The identified colocalization of Kir4.1 and AQP4 resembles a system which enables transmembranous potassium fluxes accompanied by osmotically driven intracellular volume changes. This analysis indicates an endocochlear Kir4.1/AQP4-system similar to that of brain and retinal glial cells.

Supported by: fortüne program (F 1541021)

P12 Methodological study for isolation of stem cells from the spiral ganglion

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The production of specialized differentiated neurons derived from stem cells has been proposed as a revolutionary technology for regenerative medicine. Stem cell implantation into the inner ear has also been proposed and attempted. The inner ear spiral ganglion is populated by bipolar neurons connecting the peripheral sensory receptors, the hair cells, with central neurons in auditory brain stem nuclei. The isolation of stem cells from the spiral ganglion has recently been demonstrated on the basis of the formation of spheres, self-renewal and multipotent linage (Oshima et al, 2007).
In order to optimize the isolation process we systematically compared conditions for the isolation of stem cells from the cochlear spiral ganglion. The investigated parameters include developmental age, variations in the isolation media, number of cells in primary culture and filter size for generation of the primary cell isolates. Culture were supplemented with FGF (fibroblast growth factor), EGF (epithelial growth factor) and IGF(insulin-like growth factor) were maintained over up to 14 days. The various conditions were evaluated regarding their sphere forming capacity qualtitatively and quantitatively using cell counting, immunocytochemistry and FACS analysis. The results demonstrate the most efficient isolation of stem cells from spiral ganglia at postnatal day 4, PBS-glucose isolation media and a 40µm isolation filter size. Stem cell characteristics of the sphere population were characterized via the stem cell markers Nestin, Prominin and Jagged 2. These experiments demonstrate that exposure to different environmental conditions can result in different sphere forming capacity in the isolated population of spiral ganglion neurons in culture.
Supported by: BMBF 0313837B

P13 Studying the distribution of inositol phospholipids in sensory hair cells with EGFP-tagged reporter constructs

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The inositol phospholipids (PI) are key components of many signalling pathways, including those that regulate the actin cytoskeleton. The broad spectrum inositol lipid phosphatase, Ptprq, is a component of the shaft connectors linking adjacent stereocilia within a hair bundle, and has been shown to be essential for the correct maturation and maintenance of hair-bundle structure (Oganesian et al., 2003; Goodyear et al., 2003). In frog saccular hair cells, immunofluorescence studies have shown that Ptprq and phosphatidyl inositol (4, 5) biphosphate (PtdIns 4,5 P2) have a reciprocal distribution, with PtdIns 4,5 P2 being excluded from those regions where Ptprq is expressed (Hirono et al., 2004). In this study we have used EGFP-tagged domains from various PI-binding proteins to study PI distribution in sensory hair cells.
The pleckstrin-homology (PH) domain of PLCδ1, a PtdIns 4,5 P2 reporter, labels the hair bundles and basolateral membranes of hair cells. The PtdIns 3,4 P2 reporter, the TAPP1 PH domain, localises along the entire length of the stereocilia, and is found in the cytoplasm and nucleus of hair cells. The PH domain of GRP1, a PtdIns 3,4,5 P3 reporter, intensely labels hair bundles and shows a uniform cytosolic distribution within the hair cell. A PtdIns 4P reporter, the PH domain of FAPP1, labels the hair bundle, the kinocilium, and a region surrounding the basal body. This reporter construct also concentrates in the Golgi apparatus. The FYVE domain protein TAFF1, a PtdIns 3P reporter, does not label the hair bundle but localises in dense cytoplasmic granules that are presumably part of the endosomal system.
Thus far, the reporters for PtdIns 4,5 P2, PtdIns 3,4,5 P3 and PtdIns 3P indicate that the distributions of these PIs in wild type and Ptprq null mutant hair cells are broadly similar, suggesting other inositol phospholipid species, e.g., PtdIns 5 P or PtdIns 3,5 P2, may be the major target of Ptprq.
Supported by The RNID and The Wellcome Trust.

P14 Three-dimensional organisation of cytoskeletal components in the sensory epithelia of the inner ear.

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The arrangement of the structural components of the hair cell and the hair bundle is crucial to understanding how a hair cell works, how the hair bundle operates, and how mutations in genes for these components cause deafness. With this as a goal, we are investigating means to visualise at high resolution the cytoskeleton in cells of the sensory epithelia of the inner ear. The cytoskeleton of hair cells and supporting cells is exposed in tissue that has been rapidly frozen then freeze-fractured and deeply etched. The three-dimensional organisation of cytoskeletal elements can be visualised, but the procedure is difficult to apply and immunolocalisation of molecular components is difficult. It does however, provide a basis for examining the preservation and three-dimensional organisation of the cytoskeleton by scanning electron microscopy of samples that have been de-membranated by incubation with detergent prior to fixation. We have applied this methodology to the inner ear of mammals (mice and guinea pigs) and of newts. Filamentous structures with the dimensions of microfilaments and of microtubules are revealed in the apical cytoplasm of supporting cells. Crosslinks between microfilament networks and the plasma membrane at the level of the tight junction between supporting cells and hair cells are exposed both in supporting cells and hair cells. In the newt inner ear, in particular, the microfilaments forming stereocilia and cross-links between them are revealed. Following solubilisation of the membrane immunogold labelling can be applied to localise particular molecular elements of the cytoskeleton using back-scatter detection in the SEM. In the preliminary work presented here, immunogold labelling of microtubules in pillar cells is shown. These results indicate the feasibility of the methodology to localise and define the arrangement of the cytoskeletal components in hair cells and supporting cells, and their structural relationships, in three-dimensions

P15 ERK1/2 activation in support cells during hair cell damage is a common signalling mechanism that may contribute to hair cell death

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Cellular stress results in the activation of members of the Mitogen Activated Protein Kinase (MAPK) family including c-Jun N-terminal kinases (JNK), p38 kinases and Extracellularly Regulated Kinases 1 and 2 (ERK1/2). JNK signalling is known to play a role during sensory hair cell (HC) death in the cochlea. Recently, we have shown that following mechanical damage of the sensory epithelium ERK1/2 are transiently and specifically activated in cochlear supporting cells (SCs) surrounding the damage site. We now investigated whether ERK1/2 are also activated when HCs are specifically targeted with aminoglycosides such as neomycin.
Cochlear explants were prepared from postnatal day 1 rat pups. After 1 day in vitro they were exposed to neomycin (1mM) for 8 or 24 hrs. Explants were labelled with (i) an antibody to the dually phosphorylated form of ERK1/2; (ii) a DNA stain and (iii) fluorescent phalloidin to label actin rich stereocilia at the apical surface of HCs. HC death was quantified by counting pyknotic nuclei. After 8 hrs of neomycin treatment there was a significant increase in pyknotic inner HC nuclei compared to untreated controls and ERK1/2 activation was observed in clusters of SCs surrounding dying HCs. By 24 hrs, pyknotic outer HC nuclei were observed and again ERK1/2 were activated in SCs. U0126, an inhibitor of MEK1/2, the MAPK kinase was used to investigate the role of activated ERK1/2 during neomycin-induced HC death. At 8 hrs, neomycin-induced inner HC death was significantly reduced in the presence of 10 μM U0126. However after 24 hrs, this protective effect was overcome and there was no significant difference between U0126-treated and untreated explants. In outer HCs a reduction in cell death was observed at both 8 and 24 hrs in the presence of U0126; however the effect was not statistically significant. In summary, these data suggest that ERK1/2 activation in SCs is a common signalling event during HC damage and that activation contributes to the subsequent HC death.

P16 Different appearance of apoptosis caused by platinum-derived anti-cancer drugs in the vestibule of guinea pig?

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Platinum-derived anti-cancer drugs, such as Cisplatin (CDDP) and Carboplatin (CDBCA), are known to be effective in the patients with head and neck cancer. It is also reported that CDDP causes inner ear damage while CBDCA induces less ototoxicity than CDDP. The focus of ototoxicity mainly is focused on the cochlear disturbance. Little papers reported on vestibulo-toxicity. We examined apoptotic changes in the vestibule of guinea pigs after injection of CDDP or CBDCA using immunohistochemical and electrophysiological techniques.
Three days after the injection of CDDP or CBDCA solution, the temporal bones were immunohistochemically examined for the presence of fragments of single-stranded DNA. The auditory brain stem response was recorded before and three days after the injection.
We detected fragments of single-stranded DNA in the dark cell area and the sensory epithelium of the CDDP-treated vestibule. In this group, the threshold of the auditory brainstem response was significantly elevated, however, in the CBDCA group, no apparent change of the threshold was detected. In the CBDCA group, fragments of single-stranded DNA were detected in the dark cell area and the sensory epithelium. The number of cells that stained positive for single-stranded DNA, was less than that in the CDDP group.
Our findings indicate that CDDP potentially has a possibility of the vestibulo-toxicity. CBDCA induces less apoptosis than CDDP and that this phenomenon contributes to the vestibulo-toxicity of CDDP.

P17 Scanning electron microscope imagining as a good supportive method in cisplatin induced ototoxicity.

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Cisplatin is widely used in cancer treatment. Ototoxicity is an important and dose-limiting side-effect of cisplatin therapy. Cisplatin has a specific pattern of damaging the outer hair cells (OHC) and the inner hair cells (IHC) of the organ of Corti. The OHCs in the basal segment of the cochlear are more susceptible to cisplatin than the OHCs in basal cochlear segment. This sensitivity to cisplatin decreases towards the apex of the organ of Corti. Presently many staining methods are being used to asses the morphological changes of the hair cells.

Auditory brain stem response (ABR) and Distortion Product (DPOAE) and Transiently evoked otoacoustic emissions (TEOAE) are used to assess hearing function in animal models treated with cisplatin. As a supportive method we used scanning electron microscope (SEM) to evaluate the surface morphological changes of the organ of Corti. SEM stands ahead of other morphological methods as it gives a clear image of the organ of Corti and the hair cells, providing information on the apical surface of the hair cells and the arrangement of their hair bundles. Sprague-Dawley rats were exposed to cisplatin alone and cisplatin with different protection molecules in order to study the morphological changes in the organ of Corti. After 96 hours from the treatment the animals were sacrificed, the cochleae were explanted and fixed using 2.5% glutaraldehyde and 1% paraformaldehyde solution and prepared for electron microscope imaging.

Cochleae were viewed under SEM and photos were made to compare the correlation of electrophysiological data which were obtained from ABR, DPOAE and TEOAE. We have also learnt that OHCs are more affected than IHCs by cisplatin and this pattern varies with different protectors in the base to apex gradient.

P18 **A comparison of the cochlear and vestibular effects of gentamicin and kanamycin/furosemide co-treatment in guinea pigs**

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Aminoglycoside antibiotics and loop diuretics are known to damage the auditory and vestibular sensory epithelia. Especially the combined administration of kanamycin and furosemide is known for its devastating effect on the cochlear hair cells, but the effect on the vestibular system is less well known. The aim of our study was to investigate the immediate effect of a co-administration of kanamycin and furosemide on both the cochlear and vestibular system and to compare it to the effects of chronic gentamicin treatment.
Methods: Fifteen albino guinea pigs were used in this study. Five animals were injected with a single dose of both kanamycin (400 mg/kg, im) and furosemide (100 mg/kg, iv), 5 animals received gentamicin (100 mg/kg, ip) for 10 days and 5 untreated animals served as a control group. Cochlear function was assessed by measuring auditory brainstem responses (ABRs) and vestibular function by measuring vestibular short-latency evoked potentials (VsEPs). The latter were evoked by stimulating the head with mechanical impulses.
Results: There was no effect of gentamicin on cochlear function: the ABR thresholds were completely normal. Combined kanamycin and furosemide administration resulted in a significant elevation of the mean ABR thresholds (≈60 dB). There was a significant effect of gentamicin on vestibular function: VsEP thresholds were elevated and VsEP amplitudes showed a decrease. In contrast, co-administration of kanamycin and furosemide had no signficant effect on the VsEP.
Conclusion: Kanamycin/furosemide co-treatment as applied here has mainly cochleotoxic effects, whereas gentamicin has mainly vestibulotoxic effects.

P19 **Role of Coenzyme Q10 and his hydrosoluble multicomposite, Coenzyme Q10 terclatrate, in protection against noise induced hearing loss.**

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The use of antioxidants in NIHL prevention and eventual therapy is allowed. It has been demonstrated that Coenzyme Q10 prevents oxidative injuries resulting from mitochondrial dysfunction and inhibits lipid peroxidation in several oxidative conditions. The aim of this study is to compare functionally and histopathologically the effectiveness of CoQ10 and his hydrosoluble multicomposite, CoQ10 terclatrate. NIHL was induced in guinea pigs by a continuous pure tone (6 kHz, 120 dB SPL, 40 minutes) in control group (I, n=6) and in treated animals. Guinea pigs were injected one hour before noise exposure and once daily for the following 3 days with Coenzyme Q10 (10 mg/Kg; n=6) that corresponded to dose of 100 mg/Kg of hydrosoluble Coenzyme Q10 terclatrate (n=6). Changes in cochlear function were characterized by means of ABR threshold shifts, registred before and 24 hours, 3 days, 7 and 21days after noise exposure. Missing and apoptotic cells were identified with scanning electron microscopy (SEM) 21 days after noise exposure and immunohistological staining (TUNEL Assay, 4-HNE). In control animals, the maximum threshold shift of 60 dB was observed 24h after noise exposure and a spontaneous recovery was observed after 3 days; in the following 3 weeks a constant decrease of threshold shift was measured. In animal treated with CoQ10 e con CoQ10ter a significant threshold improvement was observed after acoustic trauma. In CoQ10ter treated animals, TTS and PTS were significantly better than in the control group and CoQ10 treated guinea pigs. According to functional data, the most severe OHC loss was observed in a small area of the cochlea of about 3-4 mm with a decreasing pattern in the transitional area and a typical gradient from the first to the third row. Cochleae of the CoQ10ter protected group showed a better preservation from NIHL as compared to CoQ10. Immunohistochemical studies confirm that CoQ10ter significantly reduced the initial stage of apoptosis. In conclusion, the efficacy of both antioxidants in our experimental study shows effectiveness in protection from NIHL by reducing the effects of oxidative stress, increasing the mitochondrial respiratory chain and inhibiting lipid peroxidation in the cochlea.

P20 Src inhibition prevents the initial cochlear damage induced by cisplatin chemotherapy

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Cisplatin remains the standard platinum compound for many human malignancies. Because both the therapeutic effects and the ototoxicity of cisplatin are dose-dependent, there is great interest in developing effective strategies to protect or rescue the auditory organ from cisplatin ototoxicity without affecting the antitumoral activity of cisplatn citotoxicity occurs by way its capacity to induce DNA damage leading to the apoptosis. When the repair mechanism fail, p53 is phospholylated and activated in the nuclei and then traslocated into the mitochondria. In previous study has been demonstrated that when p53 was inhibited in vitro, by using a specific p53 Inhibitor, cochlear and vestibular hair cells apoptosis were prevented. Recently, in our lab has been shown that the src protein tyrosine kinase (PTK) signaling cascade my be involved in apoptotic cell death of sensory cells in the cochlea. In the current study we investigated weather the KX1-004 a src inhibitor, protects against the cisplatin induced ototoxicity. Fisher 344 rats were used as subjects. Animal were treated with cisplatin at dose of 16 mg/kg injected intraperitoneally (ip) in 30 minutes. In the experimental group, KX1-004 was administered at the dose of 5 mg/kg i.p, one hour before cisplatin and once per day for 2 days after chemotherapy. Interestingly, KX1-004 prevents the onset of cisplatin–induced damage, it significantly decreased the ABR thresholds shift measured at 1, 3 and 5 days preventing the higher frequencies hearing loss. Cisplatin caused an upregulation of p53 that was inhibited by src inhibitor as showed by immunohistochemical staining and Western blot analysis of the total cochlea. Furthermore, it prevented cisplatin DNA fragmentation, suppressed the apoptotic and necrotic hair cell degeneration. These results suggest that the src inhibition could have a dual action of preventing cisplatin-induced hearing loss and enhancing the tumor killing efficacy of cisplatin in patients undergoing chemotherapy.

P21 Noise trauma in the guinea pig

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Noise exposure, either by impulse noise or long term exposure, causes a temporary threshold shift (TTS) and eventually a permanent threshold shift (PTS). Otoprotective drugs attempt to prevent PTS in noise induced hearing loss. For the evaluation of such otoprotective properties reproducible *in vivo* noise-trauma-models are required. With these models we wish to predict a threshold loss, as a result of defined noise exposures using narrow band noise and impulse noise. Guinea pigs were exposed with narrow band noise (8 kHz±1/2 octave) at sound pressure levels of 110 and 120 dB SPL rms with duration of 60, 90 and 120 min. The impulse noise (142 dB SPL rms), filtered (0.25 - 4 kHz) consisted of noise bursts of 500 ms duration with 15, 30, 45, 60 and 120 repeats. Audiograms were monitored by measuring responses of the auditory nerve before, immediately after, 7 and 14 days after the noise trauma. Then whole mounts of the basilar membrane prepared to count hair cells and to plot a cytocochleogram. All noise exposure conditions resulted in a severe TTS in the complete hearing range. Within one week threshold-recovery was observed in all exposure conditions, in the second week after exposure a further but minor recovery was seen. Increasing exposure time and/or sound pressure level of the narrow band noise lead to increasing PTS and a widening of the affected frequency range. With the 120 dB SPL 120 min narrow band exposure hair cell loss expanded beyond the respective tonotopic place. Interestingly shorter exposure time did not result in hair cell loss despite the development of a PTS. In the impulse noise exposed guinea pigs PTS was seen in the complete hearing range; also hair cell loss was observed across the entire length of the cochlea, except for the very basal and apical ends. These noise paradigms form a basis for the development of the guinea pig animal model in otoprotection. In such a model the otoprotective potential of locally applied drugs can be investigated under different conditions. These results can be used as a basis for the planning of clinical studies for the treatment of acute noise trauma.

Supported by: BMVg M/SAB 1/5 /A014

P22 Expression patterns of KCNJ10 K+ channel in the cochlear lateral wall after acoustic trauma

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Background and Objectives: It is well known that noise induced hearing loss is due to sensory hair cell loss and other neuronal damage. But recently noise exposure also could damage lateral wall of cochlea such as stria vascularis and spiral ligament. K+ is the major cation in endolymph and important to maintain homeostasis within the cochlea. We have investigated the expression patterns of K+(KCNJ10) channel in noise –induced cochlear damage. Materials and Method: Twenty adult male guinea pigs(300~350 g) were included in this study. In experimental group (n=16), acoustic trauma was induced by continous broad band noise for 2 hr to 115 dB SPL and broad band noise for 6 hr to 120 dB SPL with 3 consecutive days. After noise exposure, auditory brainstem response threshold shift and hair cell loss were evaluated. A study for KCNJ10 K+ channel expression was examined by immunohitochemical staining. Results: After noise exposure, auditory brainstem response showed transient threshold shift and permanent threshold shift in accordance with noise exposure. And the expression patterns of CKNJ10 K+ channel were changeable in TTS group. But there were no change of expression patterns in PTS group. conclusion: In the cochlear lateral wall, KCNJ10 K+ channel expressions were affected with noise exposure and these change might be associated with maintain the homeostasis in the cochlea.

P23 Identification of markers for pathological neuronal plasticity changes in the auditory system

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It is well known that tinnitus leads to changes in neuronal activity in the central nervous system. These changes in activity are caused by disinhibition of cortical and subcortical neurons. The molecular changes following sensory trauma and the subsequent response of the central nervous system are, however, only poorly understood. Of particular interest are genes that alter their expression pattern during activity-induced changes in synaptic efficacy and plasticity. As described in Tan et al. (2007) gene expression changes in brain-derived neurotrophic factor (BDNF) and the activity-dependent cytoskeletal protein (Arg3.1/arc) are seen after acoustic trauma. This trauma induces hearing loss and tinnitus as shown in rodent animal behavior models. To date, the effect of sound intensity on gene expression changes has not been studied. We therefore exposed rats to various sound intensities for a certain time and frequency. We then analyzed the effect of the different intensities on tinnitus induction and gene expression in the auditory system. Our results suggest the presence of an activity threshold, beyond which a pathological situation occurs. It is known that Arg3.1/arc expression is up-regulated following sensory stimulation (e.g. sound, environmental enrichment) and that it plays an important role in activity-induced plasticity changes, balancing the efficacy of synapses (for review see: Neuron, November 9, 2006: 52 (3)). In contrast to an enhanced Arg3.1/arc expression after physiological stimuli, we observed a decline following pathological stimulation leading to tinnitus. Altered Arg3.1/arc expression in neurons may therefore be a useful tool to monitor pathological plasticity changes.

This work was supported by the Deutsche Forschungsgemeinschaft Kni 316/3-2, 3-3, Fortüne 816-0-0 and the European Commission, Marie Curie Training Site, HEARING (QLG3-CT-2001-60009)

P24 An atypical cell during development of the auditory organ : the inner pillar cell.

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Although the structure of the auditory organ in mature mammals, the organ of Corti, is clearly established, its development is far to be elucidated. Using cytochemical and immunocytochemical methods at the light and electron microscope levels, we examine its spatio-temporal development in rat from embryonic day 16 (E16) to E19. At E16, whatever the region of the cochlear duct studied (base, medium, apex), the organ of Corti is not present. We demonstrate that the organ of Corti develops from a non-proliferating cell zone that is located in the junctional region between two edges of the dorsal epithelium of the cochlear duct and characterized by the presence of numerous microvilli. Using the periodic acid-thiocarbohydrazide-silver proteinate method, we reveal that the first cells to develop in this zone are the inner pillar cells, a particular nonsensory supporting cell type; they arise in the base of the cochlear duct at the boundary between the two ridges at E16. The cell differentiation in this prosensory region continues according to a base-to-apex gradient, the inner hair cells appear in the greater epithelial ridge at E17 and the outer hair cells in the lesser epithelial ridge at E18. At E19, the different cell types of the Corti's organ are in place. We also show that the development of inner pillar cells within the prosensory domain does not involve Notch1 signalling. These results highlight the central role that could play the inner pillar cells in the organ of Corti development.

P25 Identification of IGF-I target genes implicated in inner ear development and functional maturation

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Insulin-like growth factor-I (IGF-I) is a key factor during inner ear development for the control of cell survival and differentiation. Three different homozygous mutations of the human *IGF-1* gene are associated with decreased intrauterine and postnatal growth rates, mental retardation and sensorineural deafness. IGF-I deficit in the mouse also causes hearing loss and severely affects postnatal survival, differentiation and maturation of the cochlear ganglion cells. By using Affymetrix GeneChip arrays to compare the expression profiles obtained from the cochlea of the *Igf-1*$^{-/-}$ null and *Igf-1*$^{+/+}$ wild-type mice, we have identified new IGF-I target genes implicated in inner ear development and maturation.
Microarray data presented very high variability, which could be associated to biological variability and to non specific hybridization. At this developmental stage, tissue specific genes are low expressed. Therefore, to improve the sensitivity of the gene expression estimates and detect tissue specific gene expression, the data were analyzed using gMOS family models (Bioconductor, package *puma*). The fold changes were computed using Probability of Positive Log Ratio that proved to reduce the number of false positives. Differentially expressed genes with a fold change greater than 1.5 were selected. 73 genes were found to be down-regulated and 32 up-regulated. Expression level changes were confirmed by quantitative RT-PCR using TaqMan probes and *in situ* hybridization. Among the genes validated, we found that *Fgf15*, *Ush1c*, *Cntn2*, *Slc19a2* and *Tub* are new targets of IGF-I in the developing cochlea.

This work was partially supported by the Royal Society and the “Fundación Mutua Madrileña”. HSC, LRdR and MM are fellows of CSIC-FSE I3 programme, MEC and Wellcome Trust Advanced Training Fellowships, respectively.

P26 Presence and Characteristics of Spontaneous Otoacoustic Emissions in Children and adolescents

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Spontaneous otoacoustic emissions (SOAEs) are sounds recordable in the external acoustic meatus and generated by mechanical activity of the cochlear outer hair cells. Previous studies have shown that typical SOAEs are characterized by one or more peaks in the frequency spectrum with great stability in individual ears over time. However, the amplitude of individual peaks may change with aging as well as with changes in the auditory threshold. In the present study, the occurrence and characteristic features of SOAEs were studied and their relation to other audiometric parameters compared in a group of 126 normal hearing children and adolescents aged from 6 to 25 years. SOAEs were present in 70.8% of the individuals in either one ear (55.4%) - with a higher occurrence in the right ear (70.1%) - or in both ears (44.6%). They were present more frequently in girls (81.6%) than in boys (58.1%). The frequency of the individual peaks of the SOAEs ranged between 0.5 and 6 kHz in all measured spectra, with a maximum in the speech-related range, especially at around 2kHz. The presence of SOAEs was significantly correlated with larger amplitudes of transiently evoked otoacoustic emissions (TEOAEs) and distortion-product otoacoustic emissions (DPOAEs). The audiometric parameters were compared in four age groups, each spanning five years of age. In the group of 21-25-year-olds, individual hearing thresholds at 16 kHz were significantly lower in individuals with SOAEs than in those without SOAEs. Taking into account the fact that high frequency hearing threshold, TEOAEs and DPOAEs start to decrease at ages over 20 years, the disappearance of SOAEs could be a sign of incipient hearing impairment in young subjects.

P27 Effects of thyroid hormone deficiency on Ca^{2+} currents and exocytosis in cochlear inner hair cells

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Thyroid hormone is essential for the development of hearing. In rats and mice, it is necessary in the phase of final differentiation of the inner ear between birth and onset of hearing at postnatal day (P) 12. In this work, we analyzed the effects of thyroid hormone deficiency on Ca^{2+} current development and exocytosis of the sound-transducing cochlear inner hair cells (IHCs). To this end, we performed current and membrane capacitance measurements using three models of thyroid hormone deficiency, hypothyroid rats and mice (treated with a thyreostatic drug) and athyroid $Pax8^{-/-}$ mice (lacking the follicular cells in thyroid glands). Additionally, IHCs of deaf mice lacking the thyroid hormon receptor β ($TR\beta^{-/-}$ mice) were analyzed.

IHCs of neonatal (P9) control and hypothyroid rats showed comparable exocytosis and voltage-activated Ca^{2+} currents. After the onset of hearing, the amplitude of both parameters was reduced in control rats, but the efficiency of exocytosis (i.e. capacitance increase normalized to the Ca^{2+} influx) had increased at P19. In P19 IHCs of hypothyroid rats, the size of Ca^{2+} currents and exocytosis resembled those of neonatal cells. Consequentially, the efficiency of exocytosis was reduced compared to mature control IHCs. Analysis of IHCs of hypothyroid and $Pax8^{-/-}$ versus wildtype mice yielded similar results. In conclusion, thyroid hormone deficiency prevents developmental downregulation of Ca^{2+} currents and the increase in exocytosis efficiency by mechanisms yet to be defined.

In IHCs of $TR\beta^{-/-}$ mice, both Ca^{2+} currents and exocytosis were present, indicating that deafness of these animals is not caused by lack of transmitter release from the IHC.

Otoferlin, a protein thought to be the Ca^{2+} sensor for vesicle fusion in IHCs (Roux et al, Cell 2006), was not present in IHCs of hypothyroid animals. Robust exocytosis in these cells therefore questions the presumed role of otoferlin for exocytosis.

Supported by the Forschungsschwerpunkt Baden-Württemberg and the graduate programme of the University of Tübingen 'Function and pathophysiology of sensory hair cells'.

P28 **Nestin expression in the organ of Corti during embryonic and postnatal development**

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Cellular development of the mammalian organ of Corti follows a precise spacio-temporal pattern. At first neuroblasts delaminate from the otic cup to build the cochleovestibular ganglion. Later in the ventral part of the otocyst a primordium develops. It will give rise to support and hair cells of the organ of Corti. Nestin is an intermediate filament cytoskeleton-associated protein. It is expressed in CNS stem- and neural crest cells and accepted as a neural progenitor marker. Here we present a mouse model expressing GFP under a Nestin promoter region (Mignone et al., 2004, J Comp Neurol 469). The model shows that Nestin is expressed in the primordium at stage E13.5, confirmed by double labelling with the progenitor markers Sox2 and Jag1. At E15.5 the cochlear duct is still extending and the primordium is labelled by the Nestin-GFP-signal, migrating to its apical end. In the basal turn the cells in the organ of Corti are already postmitotic and committed to Myosin VIIA-positive hair cells and p27-positive supporting cells. Despite having initiated differentiation these cells continue to show Nestin-GFP-positive labelling at different intensities. At P7 expression of Nestin-GFP becomes restricted to Deiters cells, inner phalangeal cells and inner border cells. At P28 only a slight expression remains in the first two rows of Deiters cells in the basal part of organ of Corti. These data implicate that the onset of Nestin expression coincides with the prolifertive progenitor cells stage in the primordial region. The fact that the progenitor cell marker Nestin continues to be expressed in a subpopulation of postmitotic supporting cells may indicate that these cells retain progenitor cell characteristics at postmitotic developmental time points.

Supported by: BMBF 0313612A

P29 **Dynamics of Fgf3 expression in the developing mouse inner ear**

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The fibroblast growth factors (FGFs) are required for early inner ear development in a number of different vertebrate species, such as mouse, chick and zebrafish. Recent studies have focussed on the inductive roles in the early inner ear, revealing that in the mouse, Fgf3 expression from the hindbrain is required in a redundant manner with additional FGFs to induce the otic vesicle from the neighbouring neuroectoderm. However, Fgf3 is expressed in additional domains in the developing inner ear. We have identified the regulatory regions from the mouse Fgf3 gene that are responsible for endogenous inner ear expression, and are using these regulatory regions to drive a lacZ reporter in transgenic mice. This is enabling us to investigate the precise temporal and spatial localisation of Fgf3 expression throughout mouse inner ear development.

P30 Modulation of cell-cell and cell-extracellular matrix interactions during neuroblast migration

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Cells destined to become the neurones of the cochlear-vestibular ganglion (CVG) originate within the otic epithelium. Early in development they detach from their neighbours and migrate out of the epithelium to form the CVG. This process involves modulation of cell-cell and cell-substratum interactions that are mediated by adhesion molecules.

The aim of this study was to investigate the role of adhesion molecules in neuroblast migration out of the otic epithelium. Murine otocyst explants, derived from E10.5 embryos, were cultured on fibronectin (FN). After 24hrs the cultures were fixed and immunostained or the epithelial cells and neuroblasts were separately harvested for RNA preparation. Immunohistochemistry revealed that expression of members of the cadherin family of cell-cell adhesion molecules was up-regulated during neuroblast migration. Moreover, qRT-PCR showed that E-cadherin was down-regulated and N-cadherin was up-regulated in migrating neuroblasts, as observed in epithelial-mesenchymal transitions. The integrin family of adhesion molecules is the major mediator of cell-ECM interactions. The level of β1 integrin expression was similar in epithelial cells and migrating neuroblasts. However, the expression of α6 integrin was decreased in neuroblasts that had migrated out of the epithelium. Immunohistochemistry revealed that down-regulation α6 coincided with exit from the epithelium rather than neuronal determination or initiation of migration. Inhibition of β1 integrins resulted in decreased neuroblast migration across the substrate and mounding of the neuroblasts. This suggests that β1 integrins control interaction with the FN substrate and that inhibition of β1 integrins acts to reinforce cell-cell interactions. Eph/ephrin interactions often mediate cell-cell repulsion. Therefore, we are currently investigating their role in neuroblast delamination. Preliminary results have revealed that expression of ephrins and Eph receptors are modulated during the early development of the CVG.

P31 Illuminating the dynamics of the Notch signalling pathway during inner ear development

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The Notch signalling pathway has ubiquitous roles during development of the inner ear. An early phase of Notch activity, dependent on the Notch ligand Serrate1 (Jagged1 in mammals) promotes the formation and/or expansion of inner ear sensory patches. Subsequently, lateral inhibition mediated by Delta1/Notch signalling is responsible for producing the regular mosaic of hair cells surrounded by supporting cells within the sensory patches. Notch activity regulates the expression of Serrate1 and Delta1 in opposite ways, and this could in theory produce complex patterns of Notch activity within individual cells or a group of interacting cells. To investigate this topic, we are exploring the potential use of reporters that consist of DNA regulatory sequences sensitive to Notch activity that drive the expression of fluorescent proteins. We are using *in ovo* electroporation of day-2 chicken embryo to determine whether these reporters 1) can faithfully reveal the endogenous pattern of Notch activity in the otic cup and 2) are sensitive to experimentally-induced changes of Notch activity. Results from these preliminary experiments will be critical to decide whether these reporters could be used to monitor the dynamics of Notch activity throughout inner ear development.

P33 Region-specific transcriptional expression of neurotrophin-family genes in the cochlea of newborn rat.

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The neurotrophins (NTs) play a pivotal role in cochlear development and maintenance. Here, we performed expressional analyses of the NTs gene family members in three distinct cochlear regions subjected to different conditions. We used cochlear regions that were freshly prepared or subjected to 24h normoxic or hypoxic organotypic culture. Organ of Corti (OC), modiolus (MOD) and stria vascularis (SV) were prepared from neonatal Wistar rats (3-5 day old). Presence and level of expression of NTs transcripts in above tissues was estimated using the Affymetrix microarray and confirmed using real time RT-PCR. To characterize the organotypic cultures, a number of tests were performed, including scoring the hair cells, measuring the LDH activity, calcein AM, PI and Hoechst 33342 staining, and estimation of neurofilament 200 mRNA levels. Overall, our findings demonstrated that organotypic cultures of OC, MOD and SV maintain the viability under normoxic and less under hypoxic conditions. We found that the expression of NTs is region-specific and changes under normoxic and hypoxic culture conditions. The brain derived nerve factor (Bdnf) transcript was expressed on a low level in all cochlear regions studied. The transcriptional expression of neurotrophin-3 factor (Ntf3) was found mainly in OC. Neurotrophic tyrosine kinase receptor 2 (Ntrk2), nerve growth factor receptor (Ngfr; =p75NTR) and GAP-43 (a marker of axonal outgrowth, synaptogenesis and synaptic remodelling) were expressed similarly in OC and MOD. The Ntrk2 transcript expression was decreased in OC and MOD under hypoxic and normoxic culture conditions. In contrast, Ngfr was decreased in OC and increased in MOD. The strong increase in the expression of Gap43 observed in MOD culture may be an indicative of regenerative potential. Similarly, the differential transcriptional regulation of p75NTR and TrkB could reflect the cochlear adaptation to culture stress and an attempt to prevent cell death.

P34 Human Cytomegalovirus and Factors Involved in Neurogenesis

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Human cytomegalovirus (HCMV) accounts for 10-15% of congenital sensorineural deafness in the UK and is the most common cause of neuronal impairment in neonates. This is partly because of HCMV's high global seroprevalence of 60-90% and the high transmission rate of the virus across the placenta from pregnant women undergoing primary infection. The clinical outcome for infants infected *in utero* appears to be determined, at least in part, by the viral load in the amniotic fluid and the gestational age at time of infection. However, our understanding of HCMV-induced neuropathology and the stages of fetal neuronal development most at risk from HCMV infection has been limited by difficulties in studying congenital infections and the lack of an animal model, due to the species specificity of HCMV. Deafness and neuronal disabilities caused by congenital HCMV are likely to result from damage to foetal neurons. Therefore we have been studying the disruptive effects of the virus in retinoic acid-differentiated human N-teratocarcinoma (T2) cells, which exhibit properties characteristic of early developmental stage neuronal precursors. HCMV appears to alter the growth pattern of normally non-replicating differentiated T2 cells by forcing them to start the division process. We have shown that HCMV infection induces changes in cellular proteins such as neurofilament, and are investigating the effect of the virus on proteins involved in sensorineural development.

Whilst HCMV affects cellular development, viral replication is determined by the differentiation status of cells. Only upon differentiation into HCMV-permissive cells can viral genes be expressed from the HCMV major immediate early promoter (MIEP). Within this region, we have identified several consensus binding sequences for the transcription factors GATA-2 and GATA-3, shown to be involved in mouse neurogenesis and morphogenesis of the ear. Therefore, we are investigating the role that these proteins play in differentiation dependent HCMV regulation. As the MIEP is the strongest known eukaryotic promoter, understanding it's tissue specific restrictions could benefit gene therapy treatments for human diseases, including deafness.

P35 Differential modulation of $Ca_v1.3$ Ca^{2+} channels by Ca^{2+} binding proteins and significance for auditory hair cell function

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Ca^{2+}-dependent inactivation (CDI) of voltage-gated Ca^{2+} channels is a negative feedback regulation by incoming Ca^{2+} ions, due to calmodulin binding to the $Ca_v\beta_1$-subunit. In contrast to their properties in transfected cells, $Ca_v1.3$ L-type Ca^{2+} channels in auditory hair cells display little CDI. Since Ca^{2+}-binding proteins related to calmodulin (CaBP1 and CaBP4) modulate CDI of Ca_v1 channels, we tested their role in limiting CDI of $Ca_v1.3$ channels in auditory hair cells. In biochemical analyses, both CaBP4 and CaBP1 interacted with the cytoplasmic N- and C-terminal domains of the $Ca_v1.3$ β_1-subunit ($\beta_1$1.3). In electrophysiological recordings of transfected HEK293T cells, CaBP4 had relatively minor effects compared to the strong suppression of $Ca_v1.3$ Ca^{2+} current inactivation by CaBP1. Consistent with these findings, inactivation of $Ca_v1.3$ Ca^{2+} currents was only marginally increased in inner hair cells from CaBP4$^{-/-}$ mice. Moreover, Ca^{2+}-dependent exocytosis and auditory function were not impaired in CaBP4$^{-/-}$ mice. RT-PCR and immunocytochemical analyses supported the expression of CaBP1, CaBP2, and CaBP4 in inner hair cells. However, CaBP2 did not affect inactivation of $Ca_v1.3$ Ca^{2+} currents in transfected cells. Therefore, the lack of an auditory defect in CaBP4$^{-/-}$ mice may be due to sufficient modulation of $Ca_v1.3$ by CaBP1. Our results reveal unexpected diversity in the role of CaBPs as Ca_v1 modulators, and argue against an essential role for CaBP4 in conferring slow inactivation of L-type Ca^{2+} currents in auditory hair cells.

P36 Ionic currents through $Ca_v1.3$ Ca^{2+} channels in mature mouse inner hair cells under mobile phone field exposure

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The increasing use of mobile phones over the last years led to an increased public concern with respect to possible health risks involved. In response to those public concerns the WHO orchestrates a world-wide research program to which this study contributes via the "German Mobile Telecommunication Research Programme" organised by the "Federal Office for Radiation Protection".

During use, mobile phones are in close proximity to the ear and most of their emitted energy is directly absorbed by the area around the ear. We therefore investigated whether mobile phone field exposure had any influence on the functioning of the voltage-activated L-type Ca^{2+} channel subtype $Ca_v1.3$ in mature mouse inner hair cells. This specific Ca^{2+} channel is driven by the receptor potential of the hair cells, triggers exocytosis via Ca^{2+} influx and is known to be sensitive to the cells' metabolic state.

Ca^{2+} channel currents (charge carrier: Ba^{2+}) were measured in whole-cell patch clamp experiments in an acute preparation of the apical organ of Corti of NMRI mice aged 18±2 days. Outward K^+ currents were blocked by extracellular application of TEA and 4-AP and by intracellular use of Cs^+. Ba^{2+} currents were recorded for 5 min prior to exposure, during a 20 min exposure phase and for 15 min after exposure (40 min recording time). All recordings were carried out for SAR values corresponding to 0.02, 0.2, 2, 20 W/kg (averaged over the liquid volume in the bath chamber) and a sham. The exposure conditions were randomised and blinded, i.e. the specific measurement condition was unknown until the data was completely sampled. Three different exposure signal types simulating GSM 900, GSM 1800 and UMTS communication signals were used, and at least 15 cells were measured for each exposure type and intensity.

The following parameters were extracted from IV-relationships and current traces: Maximum current, voltage of half-activation, steepness of activation, series resistance and leak resistance. Maximum current, voltage of half activation and steepness of activation were subsequently used in statistical tests to evaluate a possible influence of the exposure on the properties of the $Ca_v1.3$ channels.

P37 Effects of hypothyroidism and lack of thyroid hormone receptors alpha and beta on the expression of $Ca_v1.3$ and BK channels

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Lack of thyroid hormone (TH) in the critical period of final differentiation leads to deafness due to morphological and functional deficits in the organ of Corti and perhaps also the auditory pathway. We investigated the effects of TH deficiency on the expression of Cav1.3 Ca2+ channels and of the fast activating K+ (BK) channels in rat and mouse IHCs using patch-clamp recordings.
In control rat IHCs, the Ca2+ current showed a developmental peak (P9-P11) and subsequent down-regulation whereas the peak in IHCs of hypothyroid rats was delayed (P19) and almost doubled in amplitude. The BK current was expressed in IHCs of control rats from P12 onwards yet was not expressed in hypothyroid IHCs until P27. Thereafter, part of the hypothyroid organs of Corti showed a mosaic expression of the BK current, with activation time constants twice as large as those of control IHCs. In the latter, iberiotoxin (100 nM) blocked only part of the BK current, leaving a fast conductance with activation time constants similar to those of BK-expressing hypothyroid IHCs. This confirms the heterogeneity of BK current properties in control IHCs (Marcotti et al., 2004) and suggests incomplete acquisition of mature BK currents in hypothyroid IHCs.
To define the role of TH receptors (TR) alpha and beta on Cav1.3 and BK channel expression, IHCs of TR knockout mice (TRalpha-/- and TRbeta-/-) and control mice (TRalpha+/-beta+/-) were analysed. $Ca_v1.3$ currents in IHCs of TRalpha-/- and TRbeta-/-mice were not different from those of control mice, still suggesting the possibility that TRalpha exerts a TH-dependent repression of Cav1.3 expression in the period of developmental downregulation. BK channel expression was normal in TRalpha-/- but severely delayed in TRbeta-/- mice, confirming data of Rusch et al. (PNAS 1998). As recent data demonstrate that BK-/- mice can hear, the reason for deafness of TRbeta-/- mice must be caused by other reasons than delayed BK channel expression.

Supported by DFG En 294/2-4, DFG Kni-316/3-1; 4-1,Fortüne 1446-0-0

P38 Role of Ca^{2+} binding proteins in hair cell presynaptic function

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Inner hair cells (IHC) release glutamate in a Ca^{2+}-dependent manner to faithfully transfer sound information to spiral ganglion neurons. They express several Ca^{2+} binding proteins, of which some probably act as mobile Ca^{2+} buffers to spatiotemporally restrict the presynaptic Ca^{2+} domains. To investigate their role in hair cell presynaptic function and hearing, we examined the auditory phenotype of a triple knockout mouse deficient of the Ca^{2+} buffers calbindin, parvalbumin alpha and calretinin. Hearing was tested by recordings of otoacoustic emissions, auditory brainstem responses and single auditory nerve fiber activity. The presynaptic function of single IHCs was studied by perforated patch-clamp recordings of Ca^{2+} currents and exocytic membrane capacitance changes. Both exocytosis of the readily releasable vesicles and sustained secretion was compared to those of age-matched wild-type IHCs. The results show that despite buffer deficiency hearing thresholds of the mutants were not significantly altered. There was a tendency of auditory nerve fibers to show increased sound-driven spike rates. The kinetic properties of the synaptic release at the ribbon synapse differ significantly in the buffer deficient IHCs, which showed a higher rate of sustained exocytosis. We conclude that mobile Ca^{2+} buffers play a role in the regulation of synaptic transmission. However, calbindin, parvalbumin alpha and calretinin are not essential for hearing, at least during transient sound stimulation. Future work is required to clarify a potential compensation for the Ca^{2+} buffer deficiency, for example, by mitochondrial Ca^{2+} uptake.

P39 Number, Design and Function of Hair Cell Synapses along the Tonotopic Axis of the Cochlea

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The ribbon synapses of cochlear inner hair cells (IHCs) transmit acoustic information to spiral ganglion neurons (SGNs). SGNs vary in their spontaneous firing rate, sound threshold and frequency response. It remained unknown in how far this relates to different properties of the individual ribbon synapses.

Here, we first show by quantitative immunohistochemistry that the number of ribbon synapses varies along the tonotopic axis and is maximal in the region of highest sound sensitivity in the mouse cochlea. Confocal microscopy of the organ of Corti following immunostaining for RIBEYE, a major component of the synaptic ribbon and for AMPA-receptor subunits GluR2 and 3 was performed to estimate the number of afferent synaptic contacts as colocalized spots of pre- and postsynaptic immunofluroescence.

Using patch-clamp recordings we demonstrated that fast and sustained exocytosis of IHCs scaled with the synapse number at different positions in the apical cochlea, while their total number of $Ca_v1.3$ Ca^{2+} channels did not vary. Size, charge and kinetics of the calcium current did not vary with the tonotopic position of the hair cells. To assay the morphological size of active zones from IHCs at different tonotopic positions, we used STED and 4Pi high resolution microscopy that both deliver images at an optical resolution better than 100 nm. We found no difference in size of neither synaptic ribbons nor presynaptic Ca^{2+} channel clusters at different tonotopic positions.

Our data argue for a uniform design of the ribbon-type active zones of IHCs. Therefore, fiber properties should be largely determined by postsynaptic mechanisms. In conclusion, the cochlea may use a maximum of neural information channels per hair cells in the range of best hearing. Moreover, a significant number of extrasynaptic Ca^{2+} channels may contribute to the total Ca^{2+}-Influx upon depolarization.

Acknowledgments: This work was supported grants of the DFG (CMPB Goettingen), HFSP and EC (Eurohear) to T.M.

P40 Evidence for K^+ recycling in an organotypic model of mouse utricle.

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The high K+ concentration in the endolymph is required for hair cells to transduce balance stimuli. The maintenance of this high K+ concentration partly depends on K+ recycling: Once hair cells are depolarized following K+ influx through stereocilia transducer channels, basolateral outward currents are activated to extrude K+ in the perilymph. Then, a connective tissue network of gap junction presumably conveys K+ towards dark cells which are in charge to secrete K+ back into the endolymph. Recently, we developed a murine organotypic model of utricle able to regenerate its endolymphatic compartment (called cyst) in order to investigate the regulation of endolymph homeostasis. Utricles dissected from neonate mice were grown in 3D matrix. After 2 days *in vitro* (div), utricles regenerate the cysts, easily recognizable under a dissecting microscope. Electrophysiological recordings show that an intracystic potential is maintained between – 1.5 and – 3.5 mV during the first 9 div. Afterwards, potential values become less steady. Using K+ selective electrodes, K+ accumulation was then estimated within cysts. K+ accumulates from div 2 to 5 where its concentration reaches 82.8 +/- 4.2 mM and remains fairly high for the following 6 div (level range: 60.1 +/- 8.8 to 95.0 +/- 7.7 mM). Bath application of 1 mM ouabaine (a selective Na+ K+ ATPase blocker) induces a decrease in K+ endocystic concentration, in a time-dependent fashion: a significant 34.4 % inhibition is obtained after a 15 min treatment, which further reaches 70.8 % at 40 min. Conversely, bath application of gentamicin (1 mM) increases the K+ endocystic concentration: a 90 min treatment induces a significant 27.7 +/- 2.4 mM rise of K+ within cysts, with respect to a 71.7 +/- 3.8 mM control level. Since ouabaine is known to inhibit the ability of dark cell to secrete K+ in the endolymph and since gentamicin is a blocker of the transduction channel hence obliterating transduction currents mediated by the influx of K+ from endolymph to hair cells, the present data suggest that functional K+ recycling pathway does exist in this vestibular organotypic model

P41 Functional analysis of water flow in cochlea supporting cells

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AQP4 mediated osmotic equilibration plays a major role in potassium (K^+) spatial buffering of glial cells. Glial AQP4 plays a role the clearance of K^+ from areas of high neuronal activity which is paralleled by water flux. In the inner ear K^+ is the charge carrier for sensory transduction, however little is known about AQP4 dependent K^+spatial buffering and recycling mechanisms which play a crucial role in hearing function. Cochlear supporting cells show some analogy to glial cells. This study makes a functional comparison between cochlear supporting cells and glial cells in osmotically induced changes in cell volume. Cell volume changes in response to a osmotic challenge were measured in whole mount preparations of the Organ of Corti and primary cultured astrocytes by a confocal laser scanning time series technique. The change in cell volume can be monitored over time by detection of the change in fluorescence intensity (F/F0) emitted by calcein loaded cells. All cells investigated revealed an increase in volume after exposure to hypoosmotic solution. Differences in the onset, amplitude and slope of the F/F0 change were detected. The maximum of F/F0 was observed in Hensen cells. Comparable, but lower maxima of F/F0 were measured in Deiter`s cells and Inner Pillar Cells. Astrocytes showed the lowest change in F/F0. Deiter`s cells and Inner Pillar cells show a delayed onset of swelling compared to astrocytes and Hensen cells. The differential allocation of AQP-isoforms might account for the observed differences. Delayed onset may be caused by cytoplasmatic AQPs that have to to shuttle to the membrane.. Alternatively the coupling of Deiter`s and Inner Pillar Cells via water permeable gap junctions may delay the onset of the swelling in only reflect the volume regulation of neighbouring cells. The functional analysis of water flow in the cochlea show marked similarities and differences between inner ear supporting cells and glial cells of the CNS. The measuring of cell volume kinetics by confocal laser scanning technique provides an adequate method for the functional description of AQPs in the supporting cells of the cochlea.

Supported by: fortüne (F1541021)

P42 Computational Model of Ion Transport in the Stria Vascularis

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The proper operation of the cochlea depends on generation of a large endocochlear potential and maintenance of a high potassium concentration in the endolymph. Both these tasks are performed by the stria vascularis, which contains a complicated network of transport proteins that operate synergistically to establish the endocochlear potential and transport potassium into the endolymph. We have constructed a computational model of ion transport in the stria vascularis based on available experimental data. We first consider an isolated layer of marginal cells and find that they do not make a significant direct contribution to the endocochlear potential but are capable of sustaining considerable potassium flux into the endolymph. Next, we expand the model and show that the inclusion of the channels and transporters expressed in the intermediate and basal cells is sufficient to generate the endocochlear potential. A particularly interesting prediction is the sensitivity of strial function to the potassium concentration in the intrastrial space. This is captured by a mathematical relationship between the marginal cell pump currents, intermediate cell conductances and intrastrial potential. The removal of marginal cell and basal cell tight junctions compromises cochlear function in a manner consistent with experimental results in claudin-11 null mice. The model is useful for determining the dependence of the system on the expression levels of different ion transporters and channels, which can be used to predict the effects of genetic mutations and drug interactions. For example, the model is consistent with proposed mechanisms of loop diuretic ototoxicity and can explain why deafness results from potassium and chloride transport deficiencies, such as Jervell and Lange-Nielsen syndrome and Bartter's syndrome, type IV. These results demonstrate the utility of compartmental modeling to investigate the role of ion homeostasis in inner ear physiology and pathology

P43 Expression of osmotically responsive cationic channel TRPV4 in the endolymphatic sac

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In the present study, the expression pattern of transient receptor potential vanilloid (TRPV) 4, one of the subfamilies of the osmoreceptor TRPV, was investigated in the endolymphatic sacs of rats and patients with vestibular schwannoma and Menière's disease by immunostaining. In the rat endolymphatic sac, TRPV4 was expressed predominantly in the apical membrane of the mitochondria-rich cells. Furthermore, to confirm the role of TRPV4 in the endolymphatic sac, cell volume regulation by TRPV4 was also examined in an organotypic culture of the rat endolymphatic sac. Cell swelling followed by regulatory volume decrease was observed in a hypotonic condition, however, regulatory volume decrease was blocked by Gd^{3+}, which is an inhibitor of TRPV 4. Thus, it was confirmed that TRPV4 plays a role in cell volume regulation in the endolymphatic sac. Furthermore, expression of TRPV 4 was confirmed also in the endolymphatic sac of patients with vestibular schwannoma, while in Menière's disease, degeneration caused a decrease of the endolymphatic sac epithelium, and TRPV4 was present only in the remaining epithelium. It was suggested that TRPV4 plays a physiological role in cell and fluid volume regulation in the human endolymphatic sac as an osmoreceptor, and quantitative decrease of TRPV4 may cause abnormal osomolarity regulation, and thus may be related to the pathogenesis of endolymphatic hydrops in Menière's disease.

P44 A computationally based large scale model of potassium flow in the cochlea

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Cochlear potassium (K^+) transport is facilitated by concerted action of ion channels, co-transporters and gap junctions, mutations in any of which lead to impaired K^+ homeostasis and hearing loss. While a K^+ circulating path from the stria vascularis to hair cells and a return via the fibrocytes of the spiral ligament is a generally accepted model, the quantitative details are unclear. We approached this problem by computing a large scale model of K^+ circulation in the cochlea using as input macroscopic cochlear mechanics. Time and frequency domain solutions were solved using a MATLAB implementation of the Modified Nodal Analysis method which can be applied to circuits of arbitrary complexity. Simulations indicate important differences between responses of hair cells in a cochlear network over isolated cells. The major findings are: 1) a reduced receptor potential attenuation in the 200-30,000 Hz range, from -20 to -2 (-9) dB/decade for OHCs (resp. IHCs), 2) 7 dB (14 dB) augmentation of receptor potentials in the low frequency region for OHC (resp. IHCs), and 3) a sensitivity of all these parameters on the longitudinal coupling. These findings are compatible with the suggestion that OHC amplification can be driven by potentials generated within the cochlea over a much wider hearing range than is generally thought. Supported by EuroHear Integrated Project.

P45 **Prestin self-association, lateral mobility and membrane cholesterol**

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The outer hair cell (OHC) transmembrane protein prestin converts changes in membrane potential to axial cellular deformations that increase the sensitivity of mammalian hearing. A full understanding of how prestin endows the OHC lateral wall membrane with piezoelectric-like behavior necessitates characterizing the interactions of prestin with its surrounding plasma membrane environment. Cholesterol is a major component of mammalian cell membranes, and the organization and mobility of many membrane proteins are sensitive to membrane cholesterol concentration. We hypothesize that prestin self-association, prestin diffusion and the diffusion of lipids in different regions of the OHC are affected by membrane cholesterol levels. To investigate this hypothesis, the quantitative techniques of fluorescence resonance energy transfer (FRET) and fluorescence recovery after photobleaching (FRAP) are used to monitor specific prestin-prestin interactions and to study prestin and lipid lateral mobility. FRET results show that prestin-prestin complexes are reversibly dissociated when cholesterol is extracted from the membrane. FRAP results in prestin-transfected HEK cells demonstrate the diffusion coefficient of prestin is not sensitive to cholesterol depletion, but is increased by cholesterol loading. FRAP experiments in living OHCs reveal that lipid mobility is increased in the basal region but decreased in the lateral wall region when the plasma membrane is loaded with cholesterol. Together these results demonstrate that manipulations of the membrane microenvironment affect the intermolecular interactions between prestin and lipids. Electrophysiological recordings have previously demonstrated that prestin function, as assayed by the nonlinear capacitance, is sensitive to membrane cholesterol. Our results thus suggest that the temporal and spatial organization of prestin and the mechanical properties of the OHC plasma membrane are important for proper auditory function

P46 **The role of *prestin* and the tectorial membrane in the generation of electrically evoked otoacoustic emissions in mice.**

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Electrically evoked otoacoustic emissions are sounds emitted from the inner ear when alternating current is injected into the cochlea. They are usually attributed to the motile responses of outer hair cells, one type of cochlear receptor cell, and show a typical temporal structure, which consists of a short- and a long-delay component. The short-delay component can still be measured after almost complete elimination of outer hair cells. The function, therefore, of outer hair cells as sole generators of electrically evoked otoacoustic emissions is questionable. We used *prestin* knockout mice, which do not have *prestin, a cochlear motorprotein,* in the outer hair cell lateral walls, and *Tecta*$^{\Delta ENT/\Delta ENT}$ mice, in which the tectorial membrane is vestigial and does not interact with the outer hair cell stereocilia. Electrically evoked otoacoustic emissions of *Tecta*$^{\Delta ENT/\Delta ENT}$ mice are slightly reduced at frequencies above 40 kHz, but enhanced at frequencies below this. Emission amplitudes of *prestin*$^{-/-}$ mice are dramatically reduced, although still present. Long-delay components are absent in *prestin*$^{-/-}$ mice. *Tecta*$^{\Delta ENT/\Delta ENT}$ mice have delay spectra similar to *Tecta*$^{+/+}$ mice, only the short-delay component is slightly reduced. *Prestin*-based somatic electromotility is therefore the main generator of electrically evoked otoacoustic emissions, but we suggest that a tectorial membrane-dependent process is the cause for the residual emission seen in *prestin*$^{-/-}$ mice.

This work was supported by grants from the Deutsche Forschungsgemeinschaft (DR 625/2-1) to M. Drexl, from the NIH (DC06471, CA21765) and ALSAC to J. Zuo, from IBRO/ FENS to M. Mellado-Lagarde and from the MRC to I.J. Russell

P47 Effect of interference tones on DPOAE level/phase maps in rabbits

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DPOAE level/phase maps similar to those first collected by Knight and Kemp were acquired from 4 normal-hearing rabbits and from 2 rabbits with noise-induced hearing loss. To construct DPOAE ratio vs level and phase plots, DPOAEs were measured in DPOAE-frequency steps of ~44 Hz from 0.5-6 kHz in response to primary-tone sweeps at 3 levels (L_1,L_2=65,65; 60,55; 55,45 dB SPL) using constant f_2/f_1 ratio increments of 0.025 from 1.025-1.5. DPOAE level was directly plotted, while phase was corrected for primary-tone phase variation and unwrapped before plotting. Maps were collected with and without an interference tone (IT) placed at various locations within the DPOAE level/phase space. ITs were presented on alternate trials throughout the protocol to minimize any effects due to time-dependent changes in DPOAEs. IT effects were further evaluated by taking vector differences of the maps collected with and without ITs. The $2f_1$-f_2 DPOAEs showed horizontal 'wave-fixed' phase bands at standard f_2/f_1 ratios of 1.21 and evidence of vertical 'place-fixed' phase banding for closely spaced f_2/f_1 ratios. When present, $2f_2$-f_1 DPOAEs showed vertical phase bands. ITs placed 44 Hz below $2f_1$-f_2 or $2f_2$-f_1 DPOAEs produced minimal or no effects as revealed by the vector-subtraction residual, and had negligible effects on notches in related DP-grams. Increasing IT levels yielded a residual with horizontal phase bands consistent with effects at the f_2 place. ITs placed 44 Hz below $2f_1$ or $2f_2$ or at higher frequencies had considerable consequences in that they tended to eliminate phase ambiguities in the DPOAE level/phase maps, vertical phase banding, and DP-gram notches. Vector differences were large and had predominately horizontal phase banding. The results clearly showed that ITs near the DPOAE place in rabbits affect place-fixed emissions much less than do very high-frequency ITs. This outcome was unexpected based upon a 2-source model in which ITs are capable of revealing a DPOAE-generation source near the DPOAE place in humans.

(Supported by NIDCD DC000613, DC003114, VA/RR&D C4494R).

P48 Delivery of brain-derived neurotrophic factor to the guinea pig cochlea using Gelfoam

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Several studies have shown that treatment with neurotrophins protects spiral ganglion cells (SGCs) in hair-cell deprived cochleas. In most of these studies the neurotrophins are applied by means of a cannula which is attached to an osmotic minipump and is inserted into the cochlea. Other application methods that might be more suited for clinical use have been developed. One method would be round window application of Gelfoam infiltrated with neurotrophins. We have examined whether this method indeed results in survival of SGCs in deafened guinea pigs.
Guinea pigs were deafened by means of co-administration of kanamycin and furosemide. The functional effect of the deafening procedure was confirmed by recording auditory brainstem responses (ABRs) to acoustic click stimuli. Two weeks after deafening, Gelfoam cubes (1x1x1 mm) were soaked in either 3 µl or 6 µl of a 1 mg/ml solution of brain-derived neurotrophic factor (BDNF) and deposited on the round window of the right cochlea. Subsequently, a gold-ball electrode was placed on the round window, to be used as stimulus electrode. Alternating monophasic pulses were delivered through this electrode to electrically evoke ABRs (eABRs). Two or four weeks after deposition of the Gelfoam, both left (untreated) and right (BDNF-treated) cochleas were fixed and processed for histological examination.
We found that treatment with 6 µg BDNF on the round window was effective, whereas treatment with 3 µg BDNF was not. Two weeks after deposition of the Gelfoam, the SGC packing densities were significantly larger in cochleas treated with 6 µg BDNF than those in the untreated cochleas (paired comparison). This effect was observed in the basal turn. Preliminary data also indicate an effect of BDNF four weeks after deposition of the Gelfoam. eABR amplitudes were relatively stable during four weeks.
We conclude that Gelfoam-based delivery of BDNF can be applied to preserve SGCs, provided the proper dose of BDNF is used.

P49 Development of a bioreactor system aiming at the in vitro culture of the functionally mature Organ of Corti

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Due to the difficult anatomical approach and complex cytoarchitecture basic research of the mammalian inner ear is still a challenge. *In vitro* culture models allow for the investigation of physiology, development, degeneration and regeneration of hearing as well as pharmacological drug screening and ototoxicity studies. *In vitro* cultures provide defined experimental parameters leading to enhanced reproducibility. Substantial progress has been made in culturing the inner ear in the past, however these techniques still require fragmentation and isolation of the sensory epithelia from its natural environment. We developed a culture technique, using a horizontally rotating culture vessel (Synthecon, USA) that facilitates the organ culture of the entire and intact inner ear within the bony labyrinth. As has been shown for several tissues, creation of simulated microgravity conditions results in low shear stress, enhanced mass transfer and cell-cell-association as well as better paracrine and autocrine cellular communication. The bony part of the basal turn and the apex are removed to facilitate perfusion of the perilymphatic space. Previous studies have shown that inner ears of postnatal day 7 mice can be maintained in culture for up to seven days. Experiments cultivating later stages of the functionally mature inner ear failed so far. It is known, that the increased vulnerability of the maturing cochlea correlates with the development of the endocochlear potential. Its maintenance is energy consuming and depends on aerobic metabolism. We speculated that hypoxic conditions as in the standard *in vitro* situation (about 140 mmHg pO_2) result in hair cell loss in mature ears. We extended our system by introducing an oxygenator to achieve oxygen partial pressures up to 500 mmHg in a continuous flow of media within the rotating culture vessel. First results show, that high level oxygenation results in enhanced survival of outer hair cells in 21 day old mice cultured for 24 hours. Areas of complete hair cell loss were reduced compared to non-oxygenated culture conditions.
Supported by: Ministerium für Ernährung und ländlichen Raum Baden-Württemberg

P50 Expression of Histamine Receptors in the Mammal Vestibule

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Histamine and related agonists are commonly used in antivertigo treatments. Until recently, these drugs were believed to act only on the central nervous system. Recently pharmalogical studies proposed that histamine may also act directly on vestibular endorgans. However up to now, evidence for the presence of histamine receptors in vestibular endorgans were missing. Using immunocytochemistry, we demonstrated that the three types of histaminergic receptors, HR1, HR2 and HR3 are expressed in the mammal vestibular endorgans. Immunoreactivities against all three subunits of histamine receptors were found in the soma of Scarpa's ganglion neurons, with restriction of HR2 to the subpopulation of neurons that lack calretinin. HR1 and HR3 were detected in the sensory epithelia, in both hair cells and supporting cells. The distinct expression of the histamine receptors reported here may account, at least in part, for the discrepancies in the actions of histamine related drugs on the vestibular sensory information. These observations open a new field of functional investigations to check how these histamine receptors may modulate the vestibular sensory information.

P51 **Expression of glycine receptors and gephyrin in the rat cochlea**

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The efferent cochlear feedback system modulates cochlear nerve activity and balances interaural sensitivity. Despite the wealth of information about glycinergic neurotransmission in the central auditory system, the inhibitory glycine receptor (GlyR) has not yet been regarded as a target molecule of efferent transmission in the mammalian cochlea. Glycine receptors are ligand-gated pentameric postsynaptic chloride channels composed of temorospatially regulated α subunits and a structurally homologous β polypeptide. To date, four genes encoding ligand-binding α subunits (α1-4) and one gene encoding the β subunit are known in vertebrates. The cytoplasmic protein gephyrin anchors the GlyR complex to the cytoskeleton. Here, we describe the detection of glycine receptors and gephyrin in the rat cochlea by RT-PCR, whole mount *in situ* hybridization and immunohistochemistry. Using RT-PCR, GlyR α3, β and gephyrin transcripts were amplified from rat cochlea (P14 onwards). GlyR α3, GlyR β and gephyrin were detected in the developing and adult rat cochlea. The expression pattern of GlyRs before and after the onset of hearing implies a possible modulatory role of these inhibitory receptors in the efferent cochlear feedback system. Further immunohistochemical and electrophysiological studies are required to elucidate the impact of GlyRs on cochlear signal transduction.

P52 **Mouse pharmacological models of sensorineural hearing loss**

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95% of deafness in our countries is due to the loss of sensory hair cells and neurons. Animal models are vital to understand the degeneration of hair cells and spiral ganglion neurons and to test otoprotective strategies. Mice are known to be resistant to ototoxic drugs. The development of a murine model of deafness is nevertheless of the utmost interest for two reasons. First, such a model allows more flexibility and offers transgenic studies potential. Second, we might understand the mice resistance to ototoxic molecules, and to deduce from it new therapeutic approaches. The aim of this study is to develop a murine model of deafness. We evaluate the ototoxicity of kanamycin, gentamicin, furosemide and cisplatin administered locally or systemically in mice.

In the systemically treated groups, adult mice received either kanamycin, cisplatin, or the combination of kanamycin and furosemide. We compare subcutaneous and intraperitoneal injections in the kanamycin and cisplatin treated mice. We compare the combination of intraperitoneal kanamycin and intravenous furosemide with the combination of intraperitoneal administration of kanamycin and furosemide. In the locally treated groups, we administered kanamycin, gentamicin or cisplatin in the vestibule or trough the round window.

We assess the hearing function by auditory evoked potential. We sacrificed the mice after different time of survival and we evaluate the progressive hair cell and spiral ganglion neurons loss. We correlate the hearing function, the hair cells evaluation and the neuronal count. We assessed several ototoxic molecules in mice, several ways of administration, several doses and several time of survival. The development of a murine model of deafness is an indispensable prerequisite to the understanding of hair cells and auditory neurons degeneration and the development of valuable therapeutic strategies.

P53 **All-or-none delayed spike burst elicited by synaptic activation of unipolar brush cells in the vestibulo-cerebellum**

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The vestibulo-cerebellum regulates ocular movements and controls head and trunk posture, thus contributing to the correct positioning of the body in space. The cellular network involved is analogous to that of other cerebellar regions, except for the dense presence in the granular layer of unipolar brush cells (UBCs). By using the patch-clamp technique in combination with the tissue slice preparation, we investigated the electrophysiological properties of UBCs located in the vestibulo-cerebellum *lobi* I (*lingula*) and X (*nodulus*) of P18-33 wistar rats. In loose patch-clamp recordings, UBCs did not usually show any action potential activity (n = 7). In whole-cell recordings, UBCs showed a mean membrane capacitance (Cm) of 11.06 pF (± 3.17; n = 60), a mean input resistance (Rm) of 0.89 GΩ (± 0.53), and a mean resting potential (Vr) of –67.46 mV (± 5.80). Most UBCs were silent at rest; depending on Vr value, depolarizing current step injection evoked a burst of action potentials (Vr more negative than –76 mV; n = 8) or an adapting discharge (n = 52). However, in all UBCs depolarizing current step injection generated a repetitive discharge or a burst when Vr was artificially set at -65 mV or –80 mV. These properties correspond to those recently reported by Diana et al. (2007) (but see also Russo et al. 2007). Single-pulse stimulation of mossy fibers elicited an all-or-none excitatory post-synaptic potential (EPSP), consistent with a single synapse activation. When Vr was more negative than -58 mV, or artificially hyperpolarized to -80 mV, activation of the EPSP was coupled with a low-threshold calcium spike (LTS: threshold at -67.93 ± 8.11 mV; n = 7), and in most cells generated a burst of fast sodium spikes (threshold at -60,31 ± 7.64; n = 5) with a remarkable delay (19.05 ± 24.34 ms). The probability of EPSP-LTS generating a burst of action potentials increased upon repetitive stimulation. Yet, interestingly, the time-course of the response remained controlled by the LTS. In conclusion, the all-or-none delayed bursts generated by UBCs is likely to add a specific contribution to cerebellar elaborate of sensory vestibular inputs.

P54 **NO-cGMP pathway and hearing loss**

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NO is a short-lived, endogenously produced gas that acts as a signalling molecule in the body. It easily penetrates membranes and regulates the function of neighboring cells via synthesis of cGMP by activation of guanylyl cyclase (GC). Cyclic GMP acts on cGMP dependent protein kinase type I (cGKI), resulting in smooth muscle relaxation and growth regulation.
From a functional view, the NO-cGMP-cGKI signalling pathway may be crucial for the adequate response to general cell plasticity following a traumatic situation rather than maintaining basic metabolic functions. Therefore NO and cGMP may also play a major role in degenerative processes during acoustic trauma, hearing loss and hair cell loss. To obtain a first hint on function of cGMP in the inner ear we performed hearing tests in wildtype and cGKI-deficient mice (cGKI-/-) by measuring brainstem responses (ABRs) and the distortion products of the otoacoustic emissions (DPOAEs) to test the integrity of inner hair and outer hair cell function. Hearing was determined before and following traumatic acoustic overstimulation (noise, 4-16 kHz, 1 h, 120 dB SPLrms) for wildtype and cGKI-/- mice aged 2-3 months. Data will be presented and discussed in the context of histological expression of nitric oxide synthase and cGKI, hearing function and hair cell loss after noise trauma in wildtype and cGKI-deficient mice.
Supported by Deutsche Forschungsgemeinschaft supported by Deutsche Forschungsgemeinschaft Grants DFG Ru419, DFG Kni316/3-2, DFG Ru571/4-1, SFB 430-B3, and by Fortune 816-0-0.

P55 **Expression patterns of erythropoietin and erythropoietin receptor in the spiral ganglion of guinea pig after noise exposure**

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Background and objectives : Erythropoietin(EPO) is produced in the kidney and locally in the CNS and acts through binding to Erythropoietin receptor(EPO-R). Apart from playing an essential role in erythropoiesis, recent research has shown that EPO has neurotrophic and neuroprotective functions in the CNS and finds the EPO, EPO-R in the inner ear. The aim of this study investigates distribution and expression of EPO, EPO-R in the inner ear after noise exposure. Material and Methods : Normal guinea pigs were exposed to noise. 10 of them were sacrificed at 1 hour after noise-exposure(group B) and 10 animals were sacrificed on 7 days after noise-exposure (group C). 4 were normal control without noise exposure (Group A). Auditory function was evaluated by ABR for 7 days. Noise-induced morphological changes of cochlea were studied by phalloidin stain. The expression of EPO and EPO-R was examined by immunofluorescence. Results : The hearing threshold shift reached a level of 40 dB SPL at 8 kHz on day 1 after noise exposure and underwent a partial recovery on day 7. Increased expression of EPO and EPO-R were observed in noise-exposed animals at the level of spiral ganglion cells. Conclusion : It is suggested that the noise exposure affects the distribution and expression of EPO and EPO-R in the inner ear.

P56 **Noise stress in a Otoprotective strategy against Cisplatin induced Ototoxicity**

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Ototoxicity is an important and dose-limiting side-effect of cisplatin therapy, apart from neurotoxicity and nephrotoxicity. Many agents with chemo-protective ability have being tested against cisplatin-induced ototoxicity. From those, D-Methionine (D-MET) and L-N-Acetylcysteine (L-NAC) have shown promising oto-protection. Our lab has conducted a pilot study to moderate the employed otoprotector dosages of D-MET and L- NAC, by using noise stress. Sprague-Dawley rats were exposed to white noise (90 dB SPL, 5 min) after the intraperitoneal injection of an otoprotector. We have used two otoprotective scenarios one with D-MET and one with L-NAC. The dosages injected (300mg/kg of D-MET or 275 mg/kg of L-NAC) are less effective when given alone. After the injection of the otoprotector the animals underwent an intravenous acute cisplatin treatment (14mg/kg). Post treatment data were collected after 96 hours after which the animals were sacrificed and their cochleas were explanted.
The (electrophysiology and morphology) results have revealed that, when D-MET 300mg/kg and L-NAC 275mg/kg are given after an acute noise stress, they show similar protection to non noise and protected animals, which received 350mg/kg of D-MET and 475 mg/kg of L-NAC as protection molecules.
We have found that noise stress plays an important role in increasing the efficacy of the oto- protectors which were less effective when given alone. We postulate that the combined protection effect could be due to the increase of blood circulation in the organ of Corti organ, which results in an increase in the distribution of the protection molecule. It is also possible that, hair cells undergo adaptation due to the acute noise stress, which prepares them better for higher levels of cochlear stress (induced by cisplatin). In this context, we assume that in a systemic otoprotection administration model, the reduction of the protector molecule dose will reduce the cisplatin-complex formation hence increase the cisplatin anti-neoplastic efficacy.

P57 Speech-in-noise intelligibility does not correlate with efferent olivocochlear reflex in humans with normal hearing

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STUDY OBJECTIVES: Data from animal experiments indicate a role of the olivocochlear efferents in the discrimination of sound intensity and frequency in noise, abilities which strongly influence speech intelligibility. The objective of this study was to investigate this relationship in humans.
METHODS: Speech-in-noise intelligibility (SI_{noise}) was evaluated in three independent sessions by determining the ratio speech level/noise level, at which 50% of the words are understood (i.e. speech reception threshold, SRT). Efferent activity was inferred measuring contralateral suppression of distortion product otoacoustic emissions (CS of DPOAE). For optimum measurement conditions, the study was restricted to subjects (n=49) with valid DPOAE down to primary tone levels L1/L2=47/20 dB SPL. Two CS paradigms were used: A) f2= 1–6 kHz, L2=60-20 dB SPL, L1 = L2*0.4 + 39 dB SPL, B) f2= individually selected frequency with dip in DPOAE fine structure, L1=50-60/L2=35-45 dB SPL, both varied in 1 dB-steps. Contralateral acoustic stimulation consisted of 60 dB SPL BBN. CS measurement was performed in duplicate.
RESULTS: SRT was on average - 6.66 dB (-4.50 to -7.65, SD 0.63). CS increased with decreasing primary tone levels, with mean absolute CS values in the range of 0.6 to 6 dB SPL. Test-retest repeatability of CS was good. For statistical evaluation, CS, absolute CS, and the range between min. and max. CS were considered. In neither approach any statistical correlation between CS and SI_{noise} became apparent.
Conclusion: Our data showed no relationship between SI_{noise} and olivocochlear efferent activity – as measured by CS of DPOAE - in normally hearing humans. Future studies might include subjects with minor to moderate hearing loss, however accepting less optimal measurement conditions. The goal remains to clarify the potential impact of the efferent system on sound discrimination whose impairment is a key problem in perceptual hearing loss.

Supported by Deutsche Forschungsgemeinschaft (WA 1677/2-1) and grant fortüne 1469-0-0 (University of Tübingen)

P58 Association of HLA-DRB1*1101 allele with bilateral Meniere´s disease in mediterranean population

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Autoimmunity appears to be associated with the pathogenesis of some inner ear diseases, including rapidly progressive bilateral sensorineural hearing loss (SNHL), sudden SNHL and Meniere disease (MD). Bilateral MD is an aggresive form of the disease affecting both ears which usually determines severe SNHL and chronic dysequilibrium. The aim of the study was to analyze HLA-DRB1* and DQB1* class II alleles in patients with bilateral Meniere`s disease (MD).

Eighty patients from two ethnically defined groups with definite bilateral MD (diagnostic scale of the American Academy Otolaryngology), were compared with normal controls from the same origin in a multicenter study. DNA was obtained from whole peripheral blood by QiagenTM protocol (Qiagen, Valencia, CA, USA) and HLA typing was performed for low resolution with SSO technique from Dynal (Dynal, Oslo, Norway), using loci specific primers. High resolution typing was performed by a sequence based typing (SBT) kit from Applied BiosystemsTM and sequences were analyzed by MathToolsTM software in a ABI PRISM 377 DNA Sequencer. The strength of association between bilateral MD and DRB1 and DQB1 alleles was estimated using odds ratios (ORs) and 95% confidence intervals. Levels of significance were determined using Fisher´s exact test. Probability values (p) were corrected by multiplying the p-value by the number of alleles compared.

The alelle HLA-DRB1*1101 was associated with bilateral MD in the mediterranean population (OR= 3,65 (95% confidence intervals,1,5-9,1), corrected p=0,029); the alellic group HLA-DRB1*11 was more frequently found in this group (OR= 3,30 (1,5-7,8, corrected p= 0,012). However, this allele was not associated in the group from Galicia (northwest of Spain). No differences were found in the distribution of alleles for the gene HLA-DQB1* between patients and controls.

The alelle HLA-DRB1*1101 and the allelic group HLA-DRB1*11 may determine increased susceptibility for bilateral MD in mediterranean populations.

P59 Molecular correlates of tinnitus in the auditory system: BDNF, Arg 3.1, and the role of Gaba

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Phantom auditory sensation (tinnitus) is known to be associated with altered neuronal excitability. Brain derived neurotrophic factor (BDNF), the activity-dependent cytoskeletal protein (Arg3.1/Arc), and the immediate early gene c-Fos are known to be affected by changes in excitability and plasticity. Using RT-PCR, in situ hybridisation and immunohistochemistry, the expression of these genes was monitored following local (cochlear) or systemic application of salicylate, which is known to induce tinnitus in humans and rodents. Induction of tinnitus was verified in an animal behavioural model. Regardless of the traumatic paradigm used, a common pattern became evident: (1) BDNFmRNA expression was increased in the spiral ganglion neurons of the cochlea and (2) Arg3.1 expression was significantly reduced in the auditory cortex (AC). These findings introduce Arg3.1 and BDNF expression as a molecular “fingerprint” for trauma induced plasticity changes in the peripheral and central auditory system. Furthermore, we were able to manipulate the observed gene expression changes via pharmacological intervention. Our data demonstrate the potential role of molecular analysis in the diagnosis of auditory pathology and suggest a novel treatment for the alleviation or elimination of tinnitus.

P60 Effect of noise exposure on middle latency response amplitudes in rats.

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Noise exposure affects not only the peripheral, but also the central part of the auditory system. Previously, we found enhanced cortical middle latency response (MLR) amplitudes after noise exposure, but with large inter-individual variability. The aim of this work was to study the relationship between hearing threshold shifts, enhanced MLR amplitudes and changes in the slope of MLR amplitude-intensity functions (AIFs) after different noise exposures. MLRs were recorded with electrodes implanted on the surface of the rat auditory cortex; hearing thresholds were assessed on the basis of auditory brainstem responses recorded from subcutaneous electrodes. Rats were exposed twice for one hour to broad-band noise of 118 dB SPL (first exposure) or 122 dB SPL (second exposure); the interval between the exposures was three weeks. One day after the first exposure, hearing threshold shifts ranged between 5-45 dB at frequencies of 8-32 kHz, maximal MLR amplitude enhancement reached 0-250% and the slope of MLR AIFs increased to 8 from pre-exposure values of 4.04. The almost full recovery of thresholds and MLR amplitudes to pre-exposure values was observed during two weeks. The second noise exposure resulted in a more pronounced hearing threshold shift of about 80 dB, but the maximal MLR amplitude enhancement was similar to that measured after the first exposure. However, the slope of the MLR AIFs increased to 11.03. The results demonstrate a strong correlation between hearing threshold shifts and the slope of MLR AIFs over a wide range of noise exposure intensities, whereas maximal MLR amplitude enhancement, reached at moderate noise intensity, can not be increased by higher intensity exposure. Thus, changes in the slope of MLR AIFs characterize more the effect of noise exposure on the central part of the auditory system, whereas MLR amplitude enhancement is limited by the neuronal capacity of the auditory cortex of individual animals.
Supported by grants AV0Z50390512, GACR 309/07/1336, IGA NR 8113-4 and LC 554 and GACR 309/03/4095.

P61 Prevention of Accelerated Presbycusis by Maintenance of Systemic Immune Functions

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In recent years, considerable research efforts have focused on protection from and treatment for acute deafening with cochlear cell degeneration using local inoculation of nerve growth factors, stem cells, or genes. There have been, however, no successful reports of prevention of progressive hearing loss including presbycusis, the most frequent disability of aged people. In the present study, we show that the age-related dysfunctions of the systemic immune system in an animal model of accelerated presbycusis (SAMP1) are corrected by allogeneic bone marrow transplantation (BMT), and that this presbycusis is prevented; BMT protects the recipients from age-related hearing impairment and the degeneration of spiral ganglion cells as well as the dysfunctions of T lymphocytes which have close relation to immune senescence. No donor cells are infiltrated to the spiral ganglia, confirming that this experimental system is connected with the systemic immune system and does not contribute to transdifferentiation or fusion by donor cells but to the direct maintenance of ganglion cells by locally infiltrated donor immunocompetent cells. Therefore, another procedure which attempts to prevent the age-related dysfunctions of the recipient immune system is the inoculation of syngeneic splenocytes from young donors. These mice show no accelerated hearing loss, compared with the recipient mice with inoculation of saline or splenocytes from old donors. Further studies on the relationship between age-related systemic immune dysfunctions and neurodegeneration mechanisms may provide additional information pertinent to the treatment of presbycusis, for which there has been no effective therapy.

P62 Age-related changes in the cochlear nucleus of C57BL/6J mouse

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The cochlear nuclei (CN) receive projections from ascending fibers (all the primary afferent cochlear fibers), descending auditory pathways and internuclear connections.

Synaptophysin is a calcium binding glycoprotein located at the presynaptic membrane and the synaptic vesicle. The age–related changes in the CN were revealed by synaptophysin immnoreactivity using an anti-SY-38 monoclonal antibody (SY-38). The distribution of SY-38 immunoreactivity in different mammals CN showed that the terminal synaptic boutons divided this CN complex in three main subnuclei: the anterior ventral (AVCN), the posterior ventral (PVCN) and the dorsal (DCN) cochlear nuclei (Bartolome, et al., 1993; Gil-Loyzaga et al., 1998).

Age-related changes in CN complex were analyzed in the following age groups: 1, 3, 6, 9, 15, 18 and 24 months old C57BL/6J mice.

From 1 and 3 months old C57BL/6J mice, the distribution of SY-38 terminal synaptic boutons in CN complex corresponded with the control animals. The axonal boutons were clearly defined in contact with different types of neurons (cell bodies and dendrites) in AVCN and PVCN subnuclei. The DCN showed a dense immunostained network of SY-labelled puncta. The SY-38 immunostained terminal boutons were found scattered in the neuropil.

Changes in SY-38 immunolabelling distribution in CN complex begin at 6 months of age. From 6 to 15 months old C57BL/6J mice were observed some qualitatively differences with respect to control mice (1 and 3 months old). In PVCN subnucleus a decrease of terminal synaptic boutons was observed in the soma and the dendritic tree of the octopus cells, and the neuropil. In DCN subnucleus the deep area was the most affected by age-related changes.

At 18 and 24 months old C57BL/6J mice, the SY-38 immnunolabelling distribution in AVCN subnuclei was surrounding the few spherical cells. In contrast to the octopus cells (PVCN) that they were not distinguished. A diffuse network was observed in the central and deep area of DCN subnuclei, the two areas more affected by age-related changes. The neuropil SY-38 immunolabelling in both the VCN and the DCN was very diffuse.

The findings suggest the changes in the presynaptic membrane and the synaptic vesicle are consequence of the age-related hearing loos in the auditory brainstem subnuclei. The loss of terminal synaptic boutons, which they must be corresponded to the descending auditory pathway and/or the internuclear connections, since the majority of primary afferent fibers have been disappeared at 15 months old C57BL/6J mice.

Supported by: Ministerio de Educación y Ciencia SAF2004-0360-E

P63 Relationship between Accelerated Presbycusis and Immunosenescence

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The current study aimed to analyze the relationship between accelerated age-related hearing loss (presbycusis) and age-related impairment of immune functions (Immunosenescence), which is affected by pathogenic environments, and to devise a strategy for the prevention of presbycusis using the SAMP1 mouse, an animal model for accelerated senescence that shows hearing loss caused by the impairment of spiral ganglion cells in the cochlea. When these mice were bred in different pathogenic environments, we found that the development of age-related diseases such as immunosenescence, presbycusis, and degeneration of spiral ganglion cells was delayed in the mice bred under clean conditions. It is conceivable that pathogen-induced infections impose a severe stress on the host, impairing the host immune functions. A reduction in the number of pathogens may therefore prevent the acceleration of the aging process. These findings indicate that not only the gene backgrounds but also systemic immune functions affect the development of presbycusis in SAMP1 mice. Further studies into the relationship between systemic immune functions and the neuro-generation system may provide additional information about the treatment for age-related diseases.

P64 Age-related changes in the rat auditory system

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Rat strains differ substantially in the aging of their hearing function: some strains, such as the Fischer 344, lose their hearing abilities in early adulthood, whereas in others, for example the Long Evans strain, the presbycusis is relatively mild and occurs later in life. The aim of our study was to investigate auditory sensitivity (hearing threshold and gap detection threshold) and auditory discrimination ability (frequency discrimination and discrimination of gap duration) in pigmented rats (Long Evans strain), aged 2 to 34 months, using behavioral and electrophysiological methods. In the oldest rats, elevated hearing thresholds were observed at the highest frequencies, but these changes were not significant. The thresholds of gap detection and the ability to discriminate gap durations, estimated by behavioral methods, became significantly worse with age. With increasing age a tendency appeared towards an increase in the frequency difference limen (FDL), but significantly higher values of FDL were present only at 32 kHz. Middle latency responses (MLRs) recorded in very old rats exhibited prolonged latencies, decreased amplitudes and degraded MLR waveform morphology with increasing stimulation rate in comparison with middle-aged adult rats. The results indicate that aging in Long Evans rats is accompanied by deteriorated hearing function and suggest that the central auditory system may be involved in some of the age-related changes.

Supported by grants AV0Z50390512, GACR 309/07/1336, IGA NR 8113-4 and LC 554.

P65 Comparison of GAD levels in the inferior colliculus and auditory cortex of two rat strains during aging

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Age-related changes in neurochemistry are known to occur in the central nervous system. Gamma-aminobutyric acid (GABA) is the major inhibitory neurotransmitter in the mammalian neocortex and is critically involved in shaping neuronal responses in the central auditory system. In the current study we evaluate changes in the levels of glutamate decarboxylase (GAD), the key enzyme in the formation of GABA, in the inferior colliculus (IC) and auditory cortex (AC) of young and old animals of two rat strains - Long Evans (LE), a strain with normal aging and preserved hearing function up to late senescence, and Fischer 344 (F344), a fast aging strain with an early onset of hearing impairment. GAD is present in two isoforms, GAD65 and 67; immunohistochemistry and western blot techniques were used to investigate changes in GAD67 content. GAD67-immunoreactive(-ir) neurons were present in all subdivisions of the IC and in all layers of the auditory cortex in the examined animals. Age-related changes in GAD67 immunoreactivity comprised a significant decrease in the number of GAD67-ir neurons in the AC of old LE rats, especially in the superficial layers (I-IV). A decrease in the numerical density was accompanied by a tendency towards a decrease in the optical density of GAD67-ir neurons in the AC. In the IC of old LE animals, a tendency towards a decrease was observed in both the numerical and optical densities of GAD67-ir neurons, especially in the central nucleus of the IC. Western blots revealed a striking difference in the amount of GAD67 protein between the IC and the cortical regions. The density of GAD67 protein was almost three times higher in the IC compared to the auditory cortex and visual cortex in young animals of both strains.

Supported by grants AV0Z50390512, GACR 309/07/1336, IGA NR 8113-4 and LC 554.

P66 Effects of Aging on Inner Ear Morphology in Dogs Related to Brainstem Responses to Pure-Tone Auditory Stimuli

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Background: Age-related hearing loss (ARHL) is the most common form of hearing loss in humans and is increasingly recognized in dogs as well. The aim of this study is to describe the morphological changes in the cochleas of dogs with ARHL and to relate these findings to auditory thresholds obtained with brainstem-evoked response audiometry.
Animals: This study was carried out in a group of 10 healthy old dogs (mean age: 12.7 years). Three healthy 9-month-old dogs served as a control group.
Methods: Auditory testing was performed during a light plane of anaesthesia and auditory thresholds were determined by recording brainstem responses to pure-tone auditory stimuli (1, 2, 4, 8, 12, 16, 24, and 32 kHz). Next, the jugular venes and common carotid arteries were cannulated under deep surgical anaesthesia, immediately followed by euthanasia and perfusion fixation with a tri-aldehyde mixture. The temporal bones were dissected out of the skull and immersed in the same fixative. After decalcification, the cochleas were divided along a midmodiolar plane and embedded in epoxy resin. Semithin (1 µm) sections were cut and the number of outer hair cells (OHCs) and inner hair cells (IHCs) was counted at 6 different locations along the basilar membrane. Spiral ganglion cell (SGC) packing densities and strial cross-sectional area were determined as well.
Results: Mean auditory thresholds were dramatically higher in old dogs, for all frequencies tested, with the highest thresholds occurring at the middle and higher frequencies. Loss of OHCs and, to a lesser degree, of the IHCs was predominantly present in the basal turn. A decrease in SGC packing densities, indicating loss of SGCs, was observed in all cochlear turns, but the highest loss of SGCs was seen in the basal turn. Strial cross-sectional area was reduced primarily in the basal turn.
Conclusions: ARHL occurs in dogs and is comparable to presbyacusis in humans. The largest threshold shifts are observed in the high-frequency region. Histologically, ARHL in this specific population of old

dogs is of the mixed type, demonstrating loss of OHCs, IHCs and SGCs as well as strial atrophy, predominantly in the basal cochlear turn.

P67 Hair cell loss in connexin 30 deficient mice is accompanied by anomalous epithelial repair patterns.

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Connexin (cx)30, together with cx26, comprises gap junctions that connect supporting cells in the organ of Corti. Cx30 and cx26 are also both present between cells in the spiral ligament and in the stria vascularis. In humans, mutations in the *Cx30* gene cause deafness, and in mice with targeted disruption of *cx30*, endocochlear potential fails to develop and there is a loss of hair cells (Teubner et al., 2003: Hum Mol Gen 12: 13-21) . Using scanning electron microscopy and thin sections for transmission electron microscopy we have examined the organ of Corti of cx30-/- mice at various ages from 17 – 174 days old in animals that had been assessed for auditory sensitivity using ABR. We have also analysed the stria vascularis of the same animals by thin-sectioning and freeze-fracture. In all animals, from 17 days onwards, there was a threshold shift of over 90db at 32 kHz. At lower frequencies hearing loss was severe even at 17d and progressively worsened; by 174d threshold shifts were greater than 90db at all tested frequencies (4-32kHz). At 17d of age there was scattered loss of outer hair cells (OHC) in the basal coil but all hair cells were present more apically. OHC loss increased with age and progressed apically, but loss of inner hair cells was evident only in samples taken at 174d when OHC loss was almost complete. Thus, as reported previously, the profound deafness is unlikely due directly to hair cell loss. Loss of OHC was accompanied by repair of the sensory epithelium by supporting cells, but features of this repair differed from that seen with acquired hearing loss. In many cases individual hair cells were replaced by the heads of only one supporting cell, and, unusually, inner pillar cells often replaced first row OHC at the reticular lamina. The phalangeal processes of Deiters' cells also failed to expand when OHC were lost. Lack of cx30 may therefore restrict co-ordination of repair responses by Deiters' cells. Thin sections revealed localised separations of adjacent plasma membranes in the cell body regions of Deiters' cells at the sites where large gap junctions are seen in the organ of Corti of normal animals, suggesting loss of gap junction plaques. In the lateral wall, freeze-fracture revealed exceptionally large gap junction plaques associated with the membranes of strial basal cells, and numerous gap junctions between ligament fibrocytes. The stria was thinner than normal at 17d and became thinner still with time. However, we were unable to detect the anomalies of the tight junctions between capillary endothelial cells that others have reported (Cohen-Salmon et al., 2007: PNAS 104, 6229).

P68 Effects of defensins on frog semicircular canal: evidence of interaction between the immune and nervous systems.

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Defensins are one of the major groups of antibiotic peptides that are recognized as mediators of innate and adaptive immunity. We hypothesized that the role of defensins in the living organisms is more wide ranging, and that these natural antibiotics influence physiological processes such as neurotransmission. The present study was undertaken to determine the role of human defensins NP-1 (HNP-1) in the excitability of the peripheral vestibular system in the frog.

Using multiunit recording of action potentials from the semicircular afferents, vestibular receptors of the frog, *Rana temporaria*, were examined for the effect of bath-applied HNP-1 in concentrations between 0.00001 and 1 nM. HNP-1 decreased afferent activity at doses of about 0.0001 nM. The nature of the membrane receptors mediating effects of defensins has not been resolved. The firing evoked by L-glutamate and its agonists AMPA, kainate, NMDA and ACPD can be modulated by HNP-1 in an inhibitory way suggesting that one possible site for defensin action is on the postsynaptic membrane of the synapse. No significant modification of the net amplitude of the acethylcholine response was observed in the presence of HNP-1. The inhibitory action of HNP-1 did not depend on atropine application. This suggests that no apparent interaction exists between defensin and muscarinic-like facilitation of vestibular afferent firing. The inhibitory effect of HNP-1 on afferent discharge was antagonized by naloxone indicating that defensins may be involved in the interaction with opioid receptors on vestibular hair cells. Thus, it is possible to suggest that one site of action of these peptides is the defensin receptor, the activation of which induces an increase in the production of endogenous opioid peptides and stimulation of the opioid receptors. An alternative possibility exists that defensins can act directly through the mechanism of activation of the opioid receptors.

P69 Vestibular morphology and function in the German guinea pig

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Objective: The German Waltzing guinea pig is a special strain of animals with a recessively inherited inner defect, resulting in deafness and a severe vestibular dysfunction. In the cochlear part the hearing loss is a result of a collapse of Reissner's membrane and absence of scala media.

Methods: German Waltzing guinea pigs (homozygous and wild type) of different ages ranging from embryologic (E) age 50days to adult animals were investigated. The living animals were tested with 6 different vestibular tests and the E animals were controlled according to breeding. Morphology of the vestibular parts (ampulla, saccule and utricle) was shown in the light and transmission electron microscope.

Results: A collapse of the membranous labyrinth was found already at E50 with a progress over time. Vestibular dysfunction was noted already from birth.

Conclusions: Vestibular atelectasis, described in 1998 by Merchant and Schuknecht, is a vestibular disease in humans that has been shown to have the same morphology as the reported vestibular dysfunction in the German Waltzing guinea pig. Due to this similarity, this animal can be a good model for vestibular research.

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Notes