**POSTER SESSION 1 – Sunday 8th September 10h45-11h15 AND 16h-16h30**

**Cell signaling, ionic channels and receptors (P1-P8)**

**P1: Estrogen receptors in the inner ear**

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Older women in the normal population tend to develop less severe hearing loss compared to males up to menopause. In Turner's Syndrome(loss of one X chromosome, 45,X) affecting 1:2000 new-born girls estrogen deficiency due to streak ovaries is the dominant problem. This leads to short stature, failure to enter puberty spontaneously and infertility. Ear and hearing problems are common among these patients and affects outer, middle and inner ear. The middle-aged women frequently complain of a rapid onset of social hearing problems, due to a premature aging of the ear (presbyacusis). Can estrogen have an impact on hearing?Aim: Thirty rats have been ovariectomized in order to study the effect of estrogen receptors and estrogen on the inner ear. Methods: The rats were supplemented with saline (controls), estrogen and estrogen receptor inhibitors or stimulators (tamoxifen, raloxifen, ICI) and were then stained immunohistochemically with antibodies against estrogen receptor alfa and beta.Results: Estrogen receptors are present in the inner ear of the rat, and will differ depending on substitution.Conclusion: Estrogen receptors are present in the inner ear proposing that estrogen may affect hearing, possibly in a protective way.

**P2: Expression pattern of H3-receptor subtypes in the vestibular nuclei of the rat**

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The neuronal histaminergic system acts on the vestibular system both peripherally and centrally. Vestibular nuclei (VN) possess at least three characterized histamine receptors (H1, H2 and H3).The recently cloned histamine H4 receptor is still poorly characterized, but the expression of this receptor is thought to be peripheral. Currently, three rat H3 receptor subtypes (H3A, H3B and H3C) have been characterized. The receptor isoforms are generated as a result of alternative splicing. They show a high homology to the cloned human H3 receptor and vary in the length of the third intracellular loop. The three subtypes have distinct CNS expression profiles and couple differentially to adenylate cyclase and MAP kinase signalling pathways. Subtype specific oligonucleotides were designed and the expression of the three subtypes in the VN of the rat was studied by in situ hybridization. By applying an oligonucleotide probe (H3X) detecting all different H3 subtypes, a clear expression pattern could be seen in the VN. It seems that all the three subtypes studied are expressed in the VN. Hybridization signals were detected in all parts of the vestibular nuclei. Apparently the H3 receptor isoforms act on the vestibulo-hypothalamo-vestibular loops in the VN. The specific mechanisms in restoration of vestibular function are not well understood. The presynaptic H3 receptors might contribute to central rebalancing. Our results show that the H3 receptor isoforms are expressed in the VN. Further studies are needed to evaluate the different activation mechanisms of H3 receptor subtypes in the VN.

**P3: Ionic currents of type I spiral ganglion neurones of the guinea pig**

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Type I spiral ganglion neurones provide the afferent innervation of the inner hair cells of the organ of Corti, and they produce a rapidly adapting action potential firing on stimulation. In this work patch-clamp measurements were conducted on enzymatically isolated spiral ganglion cells, to investigate the contribution of the various current components to the general membrane properties of these cells. The identity of the cells was confirmed by applying a neurone specific immunostaining (neurone specific enolase). The neurones possessed a hyperpolarization-activated non-specific cationic current ($I_h$), whose basic properties were not affected by the enzyme treatment. The

depolarization-activated $K^+$ current components were also examined in this study. When tetraethyl-ammonium ($TEA^+$) was applied in the extracellular solution, it inhibited a substantial portion of the peak current (10 mM $TEA^+$ reduced the peak current by 67± [at 0 mV; mean±SD; n=6]). The effect of 4-aminopyridine (4-AP) was also tested. 100 M 4-AP reduced the peak current by 56±13 % (n=7), but it did not alter the action potential firing pattern. The highly 4-AP-sensitive component suggested that type I spiral ganglion cells may express a dendrotoxin-I (DTX) sensitive current component. The extracellular application of DTX inhibited a low-threshold $K^+$ current, whose contribution to the total current was approximately 30 % at 0 mV (n=4). Unlike some other cells of the central auditory pathway (bushy cells and pricipal cells of the medial nucleus of the trapezoid body), the DTX-sensitive current was not crucial for the rapidly adapting firing of the type I spiral ganglion cells, as the cells produced only a single action potential at the beginning of a depolarizing stimulus even in the presence of DTX. This finding suggests that similar types of ionic currents may have different roles in determining the overall membrane characteristics of the various cell types of the central auditory pathway.

**P4: An improved procedure for the isolation of spiral ganglion cells of the guinea pig**

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Type I spiral ganglion cells are responsible for the sensory innervation of the inner hair cells of the organ of Corti. The functional investigation of these cells requires a stable and reliable isolation technique, which yields neurones in sufficient number and quality. The major purpose of this work was to refine and improve the techniques already available for the isolation of the spiral ganglion neurones. After the application of the general anesthesia, the guinea pigs were decapitated and the temporal bones removed. The guinea pigs were anesthesied, the modioli were removed, and they were incubated in enzyme (collagenase [30 mg/l] and pronase [120 mg/l]) containing artificial cerebrospinal fluid (aCSF) at 31 °C for 15-20 min. The solution was continuously bubbled with 95 %$O_2$/5 % $CO_2$. The enzyme exposure was terminated by transferring the tissue pieces to aCSF containing tripsin inhibitor (1 mg/ml). After the enzyme treatment the tissue pieces were carefully agitated and the cells were allowed to settle down onto poly-D-lysine coated cover slips. The enzyme treatment and the careful mechanical agitation facilitated the shreading of the myeline sheath enwrapping the cell bodies, which significantly enhanced our ability to apply the patch-clamp technique for the investigation of the membrane properties of the spiral ganglion cells. Immunocytochemistry was applied to demonstrate the presence and absence of the myelin covering the isolated neurones. The vitality of the cells was tested by functional measurements, in which the membrane potential, the action potential firing ability and the ionic currents of the cells were recorded. The average whole-cell capacitance of the neurones was 9±2 pF (n=51; mean±SD), while the resting membrane potential was –62±9 mV (n=19). We conclude that the technique presented in this study is suitable to yield isolated spiral ganglion cells in good condition, allowing their electrophysiological investigation.

**P5: Control of the lateral wall stiffness by efferent neurotransmitters in the OHCs**

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The lateral wall of the OHCs, as mechanical filter, plays probably an important role in the shape changes of these cells (slow an fast motility) (Holley et al., J. Cell Sci. 102:569, 1992; Arima et al., Cell. Tissue Res. 263:91, 1991). The efficacy of the OHC motility in the dynamic energy feedback to the basilar membrane is dependent upon the stiffness of the lateral wall of the OHCs. This may be controlled by efferent neurotransmitters, Ach and GABA. The lateral wall stiffness of the OHCs has been measured by the microdeformation technique under the control of a phase-contrast microscope (Oghalai et al., J Neurosci 18: 48, 1998) at constant application of negative pressure (6 cm $H_2O$). The neurotransmitters were dropped into the recording chamber in such a manner that the concentration of the neurotransmitters should be 50 mol/l. After the re-sedimentation of the cells the measurement was done within 5 minutes. Beside the passive deformation of the lateral wall of the OHCs an active response is found synchronously to a slow cell contraction which can be described by a Boltzmann function. A cochleobasally biased Ach-response and a

cochleoapically biased GABA-response was found in the lateral wall stiffness change. Stiffness was significantly decreased versus control experiments when Ach was given to the incubation medium of cochleobasal OHCs or GABA was given to cochleoapical OHCs. No stiffness change was found when cochleoapical cells were incubated with Ach or cochleobasal OHCs were incubated with GABA. The neurotransmitters increases the compliance and the contraction power of the target cells, nevertheless the effect of the Ach is higher on the cochleobasal cells than the effect of the GABA on the cochleoapical cells. Withdrawing of the $Ca^{2+}$ the effect of both Ach and GABA decreases significantly: the lateral wall becomes more stiff and the activity of the lateral wall drops off.

**P6: Arguments for steroidogenesis in the rat cochlea**

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Endolymph that bathes apical side of the organ of Corti (OC) exhibits features of an intracellular-like fluid, i.e. high potassium and low sodium concentrations. Steroid hormones and particularly mineralocorticoids are candidates to control the endolymph homeostasis since steroid receptors are widely expressed in the cochlea. At variance, experiments on adrenalectomized animals have shown that lack of steroids had little effects on endolymph ionic composition and on hearing abilities. We have thus hypothesized that a local production of steroids in the inner ear could regulate cohlear fluid exchanges. Using RT-PCR techniques, we have shown that transcripts encoding the P450 side-chain cleavage, the 3 hydroxysteroid dehydrogenase (3 HSD) and the 17 hydroxylase (17 OHase) were expressed in the lateral wall, the OC and the modiolus. mRNA encoding aldosterone synthase was expressed in the modiolus and in the lateral wall while the 11 1 hydroxylase was not detected at all. *In situ* hybridization (ISH) experiments confirmed that the 3 HSD transcripts were expressed in the spiral ligament, the modiolus and may be in hair cells of the OC. However, it was not possible to detect 17 OHase transcripts. Immunohistochemistry performed with an antibody raised against the various 3 HSD isoforms confirmed the localization found by ISH. These results suggest that enzymes of the steroid pathway leading to mineralocorticoids and sexual steroid hormones but not glucocorticoids might be expressed in the rat cochlea. Further confirmation is necessary. In particular, we intend doing experiments to confirm that enzymatic activities are present within cochlea tissues. This work is in line with the recent description of local production of steroid hormones in brain, heart and skin. In the cochlea, a local production of steroids could account for a paracrine role and could induce specific gene expression through steroid receptors or act through a non genomic mechanism.

**P7: Transcriptional control of the cochlear motor protein**

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Electrical stimulation during the hearing process induces rapid length changes of cochlear outer hair cells. The molecular motor (prestin) has recently been identified (Zheng, J. et al. 2000 Nature 405, 149-155). In our effort to find transcriptional regulators of the prestin gene we have identified a functional Thyroid Hormone Response Element (PresTRE) at position -416 in relation to the ATG codon of rat prestin (Weber, T. et al. 2002 PNAS 99, 2901-2906). We determined the start site of transcription in the rat using RACE-PCR. After alignment of the RACE sequence data with the homologous human genomic sequence we found that PresTRE is located downstream from the startpoint of transcription, probably in the second intron. We then started to analyse up to 1 kb upstream of the transcriptional start point of the human prestin gene using computer programmes. We were able to note a number of putative regulatory elements including another TRE. Fragments differing in their length from within these first 1 kb have been cloned. First studies analysing specifity and function of distinct binding sites in this region will be presented and discussed in the context of their function for normal prestin activation. This work was supported by a grant from the Federal Ministry of Education and Research (Fö. 01KS9602) and the Interdisciplinary Center of Clinical Research Tübingen (IZKF).

**P8: Potassium channel KCNQ4**

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Cochlear outer hair cells are responsible for the frequency-resolving capacity of the mammalian inner ear. Electrical stimulation during the hearing process induces rapid length changes of these cells, transduced by the novel motor protein prestin (Zheng et al., 2000, Nature 405). Recently Karkovets et al. 2000, PNAS 97 reported the expression pattern of a novel potassium channel, KCNQ4, in hair cells and neurons of the auditory and vestibular systems, the expression of which is linked to nonsyndromic dominant deafness, DFNA2. In outer hair cells and Type I vestibular hair cells this channel is presumed to be similar to the unusual potassium selective `leak` current, termed $I_{K,N}$, which is responsible for the repolarisation of the outer hair cells. We noted an alteration of the subcellular distribution of the outer hair cell motor protein prestin coincident to an alteration of the subcellular distribution of KCNQ4 prior to the onset of hearing. As the prestin expression itself as well as its subcellular distribution revealed as being under control of thyroid hormone (TH) (Weber et al Knipper, 2002, PNAS 99), we analysed the effect of TH on KCNQ4 expression and focused also on a particular molecular link between TH and the subcellular redistribution of prestin and KCNQ4. Data towards this aim, indicating a role of TH on KCNQ4 will be presented. Supported by a grant from the SFB 430 B3/Kni Tuebingen.

**Development and regeneration (P9-P16)**

**P9: Apoptotic hair cell death after transient cochlear ischemia in gerbils**

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Mechanisms of cochlear hair cell death following exposure to transient inner ear ischemia were histologically investigated in gerbils. Ischemic insult was applied to the animals by occluding the bilateral vertebral arteries for 15 min, since they congenitally lack the posterior cerebral communicating arteries and the cochlea is nourished by the vertebral arteries. Hoechst 33342 nuclear staining showed that the hair cells, especially the inner hair cells (IHCs), underwent sporadic degeneration through nuclear condensation with peak at 12 hours after the ischemia. Furthermore, nuclear DNA fragmentation was noted at the IHCs by TUNEL staining method. Transmission electron microscopy also revealed morphological changes of the hair cells characteristic to apoptosis, such as karyopyknosis, chromatin condensation and absence of tissue architecture disruption. These findings suggested that the IHCs were more vulnerable to ischemic insult than the outer hair cells (OHCs), and that apoptotic cell death was the major process in hair cell degeneration in this animal model.

**P10: Induction of neural cell from the bone marrow cells of Chinchilla**

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Neural cell have already been differentiated from human and rat mesenchymal stem cells(MSC). We have planned to apply this technique for the treatment of damaged inner ear neural cells. Chinchilla has been used as an experimental animal in otology, because the structure of their inner ear is similar to that of human. The object of this study is to confirm the neural differentiation for Chinchilla MSC using molecular biology technique.

18 Chinchilla weighing 400-550g was used. 20 mm^3 bone marrow (containing MSC) was aspirated from the femur. The harvested cells were cultured to a DMEM proliferation media containing 10% FBS. The media was then changed for an induction medium containing; butylated hydroxyanisole, dimethylsufoxide, valproic acid, forskolin, hydrocortison and insulin. Morphological change was recorded with a digital video-camera for 9 hours.

It was recognized that within 3 hours MSCs of Chinchilla had differentiated to exhibit neuron-like morphology.

The neural cells were successfully differentiated from Chinchilla mesenchymal stem cells by means of the induction medium for human MSC. We are now going to confirm the expression of NSE/SSC on these inducted cells by FACS.

**P11: Epidermal growth factor modulates the differentiation of both hair cell and support cell immunophenotypes in rat otic epithelial clonal line 1005 high density cultures.**

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Clonal cell line 1005 was established from embryonic day 12 rat otocyst epithelium by a combination of limited dilution and retroviral gene-marking methods. Descendants derived from clone 1005 passaged at low density in the serum free medium containing epidermal growth factor (EGF), expressed immature cell features (cell proliferation and expression of nestin) for up to six months of passage in cell culture. In contrast, cells from clone 1005 in culture for 1 week at high seeding density supplemented with EGF differentiated into either hair cell (i.e. expressed myosin VIIa) or support cell (i.e. expressed both cytokeratin and p27kip1) immunophenotypes. Clone 1005 cells seeded at high density without EGF supplementation expressed only low levels of either hair cell or support cell marker epitopes. These results support the assertion that EGF plays an important role in modulating inner ear sensory epithelial cell differentiation in vitro.

**P12: p27 expression in the adult mouse cochleae and its alteration after insults**

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Recent studies have revealed that p27, one of cyclin-dependent kinase inhibitor, plays an important role in the development of sensory epithelia of the inner ear. However, roles of p27 in the inner ear have not been fully understood. We, thus, examined p27 expression in the organ of Corti of normal adult mice and adult mice treated with cisplatin. In normal specimens, p27 expression was observed in cochlear supporting cells. Alteration of p27 expression was induced by the cisplatin treatment. For analysis of alteration of p27 expression in cochlear supporting cells, we divided cochlear supporting cells into four groups, inner sulcus cells, pillar cells, Deiters' cells and Hensen's and Claudius' cells. The quantitative analysis on each cell type of cochlear supporting cells has revealed different relationships between rates of p27-positive cells and those of residual cell numbers according to cell types. In short, supporting cells by inner hair cells demonstrated no significant changes in rates of p27-positive cells, while those by outer hair cells demonstrated significant decrease. In addition, the morphological analysis revealed significant loss of outer hair cells and remaining of inner hair cells. These findings suggest that the alteration of p27 in cochlear supporting cells may occur in response to the loss of hair cells.

**P13: Transplantation of neural stem cells into the mouse inner ear**

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Neural stem cells have been paid particular attention as a material for transplantation approaches to degenerative diseases in nervous and sensory systems. However, there have been few reports on transplantation approaches for inner ear sensory systems. In this study, we examined potential of neural stem cells as donor cells for cell therapy for inner ear disorders caused by the loss of sensory cells. Neural stem cells obtained from GFP mice were used as donor cells. We transplanted neural stem cells into the inner ear of normal mice or mice affected by aminoglycoside. The medium containing neural stem cells were injected from the second turn of cochleae or lateral semicircular canal. Histological analysis 1-4 weeks later showed that transplant-derived cells survived in the inner ear and some of them were integrated into inner ear tissues including vestibular sensory epithelia. A major part of transplant-derived cells was localized in the perilymphatic space, and over 90% of them exhibited glial fibrillary acidic protein (GFAP). Few of transplant-derived cells in the perilymphatic space were positive for microtubule-associated protein-2 (MAP-2). In addition, transplant-derived cells that exhibited a hair cell marker was observed in vestibular epithelia damaged by aminoglycoside, although the numbers were very limited. These findings indicate that neural stem cells have potential for a material for transplantation approaches to

inner ear dysfunction.

**P14: Ephs and ephrins in the neonatal mouse inner ear**

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The distributions of the Eph-class receptors EphA4 and EphB1, and their ligands ephrin-A2, ephrin-B1 and ephrin-B2, were analysed by immunostaining in the neonatal mouse inner ear. Complementary patterns of EphA4 and its potential ligand ephrin-A2 were found, with ephrin-A2 in many of the structures lining the cochlear duct and within the cochlear nerve cells, and EphA4 in the deeper structures underlying the cochlear duct and in the cells lining the nerve pathway. EphB1 and its potential ligands ephrin-B1 and ephrin-B2 showed a segregated layered expression in the lateral wall of the cochlear duct, (the external sulcus), which together with EphA4 expressed in the area, form a four-layered structure with an alternating pattern of receptors and ligands in the different layers. This arrangement gives the potential for different bidirectional Eph-mediated interactions between each of the layers. The results suggest that the Eph system in the cochlea may have a role in maintaining cell segregation during phases of cochlear development. Supported by the Garnett Passe and Rodney Williams Memorial Foundation.

**P15: Morphological analysis of overproduction of hair cells and Deiters'cells in the E19 rat organ of Corti.**

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Although the phenomenon of overproduction of hair cells (HCs) and Deiters'cells (DCs) has been reported in vitro and in vivo, their progenitor cells within the sensory epithelium are not yet identified. In the present work, we used the capacity of E19 rat organ of Corti to produce spontaneously supernumerary HCs and DCs in culture after 5 days in vitro as a model for the identification of HCs and DHs progenitors. By immunolabeling with bromodeoxyuridine, we showed that production of supernumerary cells occurred in the absence of cell division. Examination of semithin and ultrathin sections in the organ of Corti revealed the following points : 1.the cuticule plate of outer HCs, located in the most distal row, was incomplete, 2. when two inner HCs were present, their ultrastructure was identical, 3. All HCs, including rows of supernumerary HCs, were supported by a supporting cell, 4.the last outer HCs was separated from Hensen's cells by one or two tectal cells, 5. tectal cells and outer HCs shared morphological characteristics, 6. beneath the tectal cells lain cells that we have named "undertectal cells", they presented an ultrastructural morphology similar to that of both DCs and Hensen's cells. Quantitative analysis of cell types present in the organ of Corti demonstrated further that, when the number of HCs increased: 1. the total number of cells remained constant, 2. the number of supporting cells increased, 3. the number of tectal cells decreased and of Hensen's cells decreased. This study also indicated that a supernumerary inner HC could only be observed when the number of HCs was equal and/or higher than 6. Together, all these data suggest that the tectal cells differentiate into outer HCs and are subsequently replaced by the differentiation of Hensen's cells. Similarly, the supernumerary DCs which derived from the differentiation of undertectal cells which themselves are replaced by Hensen's cells. Hensen's cells constitute in this model the population of progenitors of both cell lineage. It also suggest that the differentiation of HCs into outer or inner HCs is related to the positioning of the Corti tunnel.

**P16: Development of electrical excitability in the neurons of the rat vestibular nucleus in microexplant culture**.

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The intrinsic excitability of mature neurons in the medial vestibular nucleus (MVN) is one of the highest observed in the brain. Everyone of them is able to respond to 1-sec steps of intracellularly-injected current by a sustained firing (tonic neurons) whose maximum can reach up to 150-200 spikes/sec. The present study is a first step in an attempt to identify the factors which determine and maintain this electrophysiological phenotype. We cultured with success for 28 days microexplants of MVN obtained by punching brainstem slices from newborn rats with a hollow needle. On a laminin-coated substrate, neurons migrated outwards from the microexplants.Their maturation were monitored by

submitting them to 1-sec steps of intracellularly-injected current, using the patch clamp whole-cell technique. During the first week in vitro, half of the recorded neurons could respond only by a few action potentials (phasic neurons) whereas the other half behaved as tonic neurons. The immature phasic phenotype disappeared over time and, during the fourth week, all the neurons were tonic neurons. The maximum firing rate of the tonic neurons increased from a mean of 16.1 spikes/sec during the first week in vitro to 40.6 spikes/sec during the fourth week. However, this maturation remained incomplete: none of the cells responded with a sustained firing higher than 60 spikes/sec. It could be that a complete maturation were dependent on the innervation of the MVN by the vestibular nerve, an hypothesis which will be tested in a future work.

**Aging effect in the inner ear (P17-P18)**

**P17: Suppression tuning of DPOAEs in the CBA/CaJ mouse strain during postnatal development.**

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Many species exhibit enhanced susceptibility to noise-induced hearing loss (NIHL) during postnatal development. Mice reach adulthood at ~8 wk of age, but NIHL resistance does not mature until ~3 mo of age. Several methods of predicting NIHL susceptibility using DPOAEs have been suggested (e.g., monitoring efferent-related DPOAE changes). Neonatal humans exhibit both increased susceptibility to noise-induced hearing loss and abnormalities in DPOAE response suppression tuning curve (STC) parameters, attributed to cochlear immaturity. Thus, STCs were studied in mice as a potential predictor of NIHL susceptibility and cochlear immaturity during postnatal development. In this study, STCs were recorded in CBA/CaJ inbred mice at ages from 6 wk to 3 mo. Iso-suppression response areas were recorded with primary tones held at constant frequencies ($f_2$=11- and 22-kHz; $f_2/f_1$=1.25) and levels ($L_1$=$L_2$=45, 55, 65, and 75 dB SPL) and a suppressor tone presented in a frequency-level combination matrix. STCs were described by plotting the suppressor level necessary to induce a criterion change in DPOAE level (e.g., -6 dB) across suppressor frequency. STC parameters, including center frequency (CF), suppression threshold, and $Q_{10dB}$ were assessed and compared across age groups. Unlike neonatal humans, in which $Q_{10dB}$ was significantly higher than in adults, no systematic changes in STC parameters were discernible across ages. Thus, despite immature NIHL susceptibility, young mice exhibit normal suppression of the cochlear nonlinearity. Supported by: DC00613, DC03114, Lois Pope LIFE Foundation.

**P18: Oxidative stress in the aged cochlea of C57BL/6 mice**

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Introduction: C57BL/6 mice have been widely used as an animal model for presbycusis, age-related auditory deficits. In C57BL/6 mice, hearing loss starts at high frequencies at 3-4 months of age and proceeds to lower frequencies at 12-15 months. Recently evidence has been accumulated to demonstrate that aging in various organs such as heart, blood vessels and brain are closely related to oxidative stress state. Oxidative stress is typically characterized by lipid peroxidation. Thus, the present study was undertaken to investigate a correlation of changes of lipid peroxidation in cochleae with the aging process of cochleae of C57Bl/6 mice. Materials/methods: Three different age groups of 7 week- (n=6), 7-8 month- (n=6), 12 month- (n=6) old mice were used. Auditory brain stem responses (ABR) were measured to evaluate auditory function. The cochleae were fixed in 4% paraformaldehyde overnight after cardiac perfusion, decalcified in 10% EDTA and embedded in paraffin. Mid-modiolar sections were incubated with a primary antibody to malondialdehyde (MDA), which is a marker of lipid peroxidation. A biotinylated secondary antibody was used for accentuation. Processing was ultimately performed by an HRP-streptavidin complex and nickel-enhanced DAB, with subsequent observation under a light microscope. Results/conclusions: Electrophysiological recordings showed that in the mice of the 7-8 month- and 12 month-old groups, ABR thresholds were significantly elevated at clicks, 4 kHz, 6 kHz, 8 kHz and 12 kHz, compared with those in the 7 week-old group. Immunohistochemical investigations revealed that MDA was strongly detected in the stria vascularis and spiral ganglion cells of cochleae of 7-8 month-old mice, compared with 7 week-old mice. The stria vascularis of 12 month-old mice showed an atrophy and

weaker immunostaining to MDA than that of 7-8 month-old mice. These results suggest that oxidative stress state is associated with the aging process of cochleae of C57BL/6 mice.

**Central and peripheral auditory morphology (P19-P20)**

**P19: Vasodilator-stimulated phosphoprotein in the mouse cochlea**

B. Schick, M. Praetorius, M. Eigenthaler, M. Müller, U. Walter, M. Knipper

Vasodilator-stimulated phosphoprotein (VASP) is an actin-binding protein associated with actin filament formation, actin-based motility, establishment of highly dynamic membrane regions and processes of focal adhesion.
Polymerase chain reaction, western blot-analysis, in-situ-hybridisation and immunohistology were used to investigate VASP-expression in the neonatal and adult mouse cochlea. Hearing measurements were performed on VASP-/- knock-out-mice including DPOAE, Click-BERA and frequency-dependent BERA.
In the neonatal mouse cochlea VASP-expression was proved by mRNA-RT-PCR-examination and Western blot analysis. In-situ-hybridisation and immunohistology proved VASP-expression in vascular endothelial cells and spiral ganglion cells including their axons. In the adult mouse cochlea VASP-expression in spiral ganglion cells is not present, but intensive staining has been found in the pillar cells and vascular endothelial cells. DPOAE`s, Click-BERA and frequency-dependent BERA indicated normal hearing in adult VASP-/- knock-out-mice. Noise exposure (86 dB) at 11,1 kHz over a time period of 24 hours lead to a statistically significant (p=0,006) temporary threshold shift at 7,9 kHz in VASP-/- knock-out mice.
VASP is temporarily expressed in the neonatal spiral ganglions indicating presumptive functional role in axonogenesis. In pillar cells of adult organs of Corti VASP has been identified as a further actin-binding protein. Observation of a temporary threshold shift half an octave below the specific noise exposure frequency in VASP-/- knock-out-mice indicated possible role of pillar cell stiffness in noise protection in the organ of Corti.

**P20: Prestin-like immunoreactivity in outer hair cells of the mustached bat (Pteronotus parnellii)**
Marianne Vater [1], Thomas Weber [2], Marlies Knipper [2]
1) Dept. Biochemistry and Biology, University of Potsdam, Germany 2) Mol. Neurobiology, HNO-Hearing Research, University of Tübingen, Germany
Mustached bats posses the most sharply tuned cochlea known to nature and their hearing range extends from frequencies of about 5 kHz up to 120 kHz. Strongly enhanced cochlear tuning to 61 kHz, the dominant echolocation frequency is created by specialized micromechanical resonance and is physiologically vulnerable which suggests the contribution of an active mechanism. In order to study, whether the basis of the mammalian cochlear amplifier that resides in specialized properties of the outer hair cell (OHC) lateral wall is also established in a cochlea that is highly specialized for hearing at ultrasonic frequencies, we investigated the immunolabelling pattern to antibodies directed against prestin. Prestin is the most likely candidate for the OHC motorprotein (Zheng et al. 2000, Nature 405,149-155). Strong prestin-like immunoreactivity was exclusively found in the lateral wall of OHCs throughout the mustached bat cochlea. The labelling extended from below the cuticular plate down to the attachment site with the Deiters cup and there were no obvious differences among rows of OHCs or OHCs in different cochlear locations. This suggests a conservative nature of OHC-organization and function even at the highest frequencies of hearing.

**POSTER SESSION 2 – Monday 9th September 10h45-11h15 AND 16h-16h30**

**Genetic: human hereditary deafness (P21-P23)**

**P21: Hearing impairment related *GJB2* mutations in the Hungarian population**
Tímea Tóth[1], Susan Kupka[2,3], Nikolaus Blin[2], Hans-Peter Zenner[3], Markus Pfister[3]and István Sziklai[1]

1) Medical and Health Science Center, Department of Otolaryngology, University of Debrecen, Hungary 2) Department of Anthropology and Human Genetics, University of Tübingen, Germany 3) Department of Otolaryngology, University of Tübingen, Germany

Mutations in the *GJB2* gene, encoding the gap-junction protein connexin 26, have been shown to be responsible for a major part of non-syndromic hereditary prelingual hearing impairment (HI). The 35delG mutation is the most frequent type of mutation in several populations. Mutation analysis of the *GJB2* gene were performed in 18 families (68 patients) and 69 sporadic cases demonstrating congenital hearing loss from the Northeastern population of Hungary. Mutations in the *GJB2* gene were detected in 14 families (55 patients) (77.7%), and 40 persons (57.97%) out of a pool of sporadic cases of HI. Mutation 35delG was the most common one, accounting for 61.76% of all *GJB2* HI related alleles. All patients showing homozygosity for 35delG were sequenced. In total, seventeen different *GJB2* mutations were detected. By segregation analysis in families we determined the mode of inheritance and the relevance of these mutations concerning the development of HI in our patients. These studies demonstrate, that most of these genetic defects are responsible for the HI phenotype.

**P22: Two new otosclerosis families not linked to known loci or collagen 1 genes**

Carole Faghel, Kris Van Den Bogaert, Guy Van Camp
Department of Medical Genetics, University of Antwerp (UIA), Belgium

Otosclerosis is a localized disorder of the otic capsule characterized by a disordered bone metabolism. Clinical otosclerosis results in hearing impairment due to the fixation of the stapes footplate and has a prevalence of 0.3-0.4% in the Caucasian population. Otosclerosis has been reported to be autosomal dominantly inherited, although many cases may be of complex etiology. Until now three loci for autosomal dominant otosclerosis have been localized. The first, *OTSC1*, on chromosome 15q25-26 was reported in 1998. In 2001 we found a second locus, *OTSC2*, on chromosome 7q34-36 and recently a third locus, *OTSC3,* was reported localized on chromosome 6p21.2-22.3. Strikingly similar histopathology and clinical manifestations with mild forms (type1) of osteogenesis imperfecta and association between a COL1A1 genetic marker and otosclerosis lead to the speculation of a common genetic etiology of otosclerosis and osteogenesis imperfecta. In this study we performed linkage analysis for the three otosclerosis loci and for the collagen type I loci, COL1A1 and COL1A2, in two large otosclerosis families, one Dutch and one Belgian. All loci could be excluded for the two families. In these families at least one other gene is responsible for the otosclerosis phenotype and the results also do not support the hypothesis of a common genetic etiology between otosclerosis and osteogenesis imperfecta.

**P23: Patients affected with Fabry disease have an increased incidence of progressive hearing loss and sudden deafness: an investigation of 22 hemizygotes.**

Pierre Bonfils[2], Dominique Germain[2], Paul Avan[2]
1) European Hospital Pompidou, Paris, France 2) School of Medicine, Clermont-Ferrand, France

Fabry disease (FD, OMIM 301500) is an X-linked inborn error in the metabolism of glycosphingolipids due to the deficient activity of α-galactosidase A, a lysosomal enzyme. While the progressive systemic deposition of uncleaved glycosphingolipids throughout the body is known to have protean clinical manifestations, few data are available with respect to the cochlear involvement. Cochlear function was investigated by means of non invasive tests in 22 consecutive hemizygous males (age 19-64 years, mean 39) affected with classic FD. Conventional audiometry, tympanometry, ABR, otoacoustic emissions were measured in all patients, and compared with the outcome of a comprehensive assessment including heart, brain and kidney functions prior to enzyme replacement therapy. Eleven patients with classic FD were found to have abnormal hearing. Nine of them had progressive hearing loss and six patients had experienced sudden deafness. In addition, some degree of high-frequency hearing loss was noticeable in the 7 patients with normal or near-normal hearing, despite their young age at the time of examination. The incidence of hearing loss was significantly increased in FD patients with kidney failure or cerebrovascular lesions, whereas there was no correlation with left ventricular hypertrophy. Finally, tinnitus was also found in six patients. This is the first evidence of a high occurrence of

both progressive hearing loss and sudden deafness in a sample of male patients affected with classic Fabry disease. That this disease affects the cochlea through damage to blood vessels, sensory cells and / or neurons remains to be ascertained.

**P23bis: Investigating and treating progressive hearing loss associated with lysosomal storage diseases using mouse models**

Anne K. Hennig1, Mark S. Sands1, and Kevin K. Ohlemiller2
1)Division of Stem Cell Biology, Washington University School of Medicine, and 2) Central Institute for the Deaf; St. Louis, MO USA

Lysosomal storage diseases are usually caused by inherited deficiencies in the activity of a lysosomal enzyme. As a result, substrate accumulates in lysosomes, leading to lysosomal distension and progressive impairment of many organ systems. Progressive hearing loss occurs in a subset of the lysosomal storage diseases, notably those resulting from impaired degradation of proteoglycan carbohydrates (the mucopolysaccharidoses [MPS]) or glycosphingolipids. In human patients, the hearing loss is often overlooked or poorly characterized because of the severity of other clinical features. The MPS VII mouse, which has a single nucleotide deletion in -galactosidase, provides an accurate animal model of the human form of MPS type VII. Studies to characterize the hearing loss in these mice show a conductive component present at the earliest age examined (4 weeks), with a sensorineural component appearing at 10-12 weeks of age. These mice can be successfully treated with recombinant enzyme, stem cell transplantation, or gene therapy. Each of these treatments decreases the hearing impairment to some degree, but each has limitations. By investigating the impact of each of these treatments on the hearing loss, we can build a knowledge base that will be valuable in optimizing treatment for human patients.

**Otoprotection (P24-P28)**

**P24: Reduction of acoustic trauma by systemic and local magnesium application**

Heidemarie Haupt, Fred Scheibe, Ovidiu König
Dept. of ORL, Charité Hospital, Humboldt University, Berlin, Germany

We have recently demonstrated in the guinea pig that post-exposure magnesium (Mg) application (s.c. injection) may reduce hearing loss caused by acute impulse noise and that a high Mg level in the perilymph (PL) may an important role to play in bringing about that effect. However, the Mg values fluctuated as a result of the injections. Therefore, we tried to improve the therapeutic efficacy of Mg in acoustic trauma by modified applications. The animals were exposed to an impulse noise series ($L_{peak}$ = 167 dB; $L_{eq,1s}$ = 127 dB) for 4 min and subsequently treated with $MgSO_4$ or saline as a placebo either by systemic implantation of osmotic infusion pumps or by local applications on the round window membrane. The threshold shifts of the auditory brainstem response, compound action potentials and distortion product otoacoustic emissions were measured one week post-exposure. The Mg status of the animals and the damage to the stereocilias were determined by atomic absorption spectrometry and scanning electron microscopy, respectively. The systemic application of Mg via osmotic pumps resulted in the same therapeutic efficacy in acoustic trauma as that found after systemic injections, whereas the local application was more effective with a concomitant high Mg increase in PL. The functional results were confirmed by the morphological findings. In addition to the Mg effect, the hearing loss in the placebo groups was also partially lower when saline was given locally instead of being applied systemically. This effect might be due to a mild cochlear hypothermia that occurred after opening the bulla tympanica. Therefore, additional tests are needed to confirm the improved efficacy of local Mg.

**P25: The effect of intracochlear drug administration against the acoustic trauma**

Hiroshi Yamashita, Kazuma Sugahara, Hiroaki Shimogori. Department of Otolaryngology, Yamaguchi University School of Medicine, Ube, Yamaguchi, Japan

Study about the plasticity of the inner ear has been advanced, and many kinds of drugs may be useful for the recovery of the inner ear function. Because these drugs will be used in practice, we made the animal model of the drug delivery

system and have examined the effect of these drugs for the functional recovery of the inner ear. Guinea pigs have been used for this animal model. Fine catheter was inserted into the tiny hole that was made adjacent to the round window. The catheter connected to an osmotic pump filled with saline (control) or drugs. Hearing threshold was evaluated with ABR before pump implantation and 3, 5, 7, and 14 days after operation. ABR hearing thresholds in these animals didn't change. To investigate the effect of some drugs on threshold shift following noise exposure, some drugs were administered to the guinea pig inner ear by osmotic pump and ABR examinations were performed after noise exposure. We demonstrated the results as follows. Acidic fibroblast growth factor and ATP are effective for the protection against noise exposure. And also a few drugs are useful for the treatment of the inner ear diseases.

**P26: Long-term effect of intracochlear administration of betamethasone on peripheral vestibular disorder in the guinea pig**

Hiroaki Shimogori, Osamu Horiike, Takuo Ikeda, Hiroshi Yamashita. Department of Otolaryngology, Yamaguchi University School of Medicine, Ube, Yamaguchi, Japan

We evaluated the long-term effect of steroid hormone on vestibular function in guinea pigs with peripheral vestibular disorder. The right lateral semicircular canal was srugically transected in 12 guinea pigs, and after surgery, animals were treated with 1 mg/ml of betamethasone in saline (n = 6), or saline only (n = 6), which was administered directly into the scala tympani by osmotic pump for a week. Pre-treatment, and at 1 and 4 m post treatment, pendular rotation tests were performed, and vestibulo-ocular refrex (VOR) gain value and VOR gain ratio (right VOR gain value/left VOR gain value) were calculated. At 4 months after operation, the VOR gain ratio in the steroid group was near the mean value and was statistically greater than that in the saline group. Results indicate that in the vestibular periphery steroid hormones may play an important role to maintain well-balanced vestibular function.

**P27: Cochlear sensitivity to temporary threshold shifts is affected by sedation/anaesthesia.**

Fabrice Giraudet, Kathleen Horner, Yves Cazals
INSERM EPI 9902 - Marseille - France

Recent data suggest that sympathetic activity might influence sensitivity to acoustic trauma. We describe here the temporal recovery pattern of temporary threshold shift (TTS) following exposure to 8 kHz pure tone at 96 dB SPL for 1 or 10 min first in awake, before and after sympathectomy, and then in sedated and anaesthetised normal guinea pigs. Unilateral ablation of the superior cervical ganglion reduced TTS in both ears. Maximum TTS induced by 1 or 10 min exposure was centred over one half-octave shifted frequencies throughout recovery. In contrast for test ears, 30 min after the trauma, TTS was reduced for first half-octave frequencies - with significantly less TTS at 12.5 kHz. After an intramuscular injection of xylazine, the TTS was also reduced for half-octave frequencies. The one-octave frequencies was either not affected or slowed. Xylazine is a pre-synaptic  2 adrenoreceptor agonist which blocks noradrenaline release from the sympathetic system. The data suggest that under normal physiological conditions, noradrenaline can modulate olivocochlear efferent acetylcholine release. Ketamine together with xylazine also reduced TTS. Ketamine is a NMDA receptor antagonist. Since glutamate is the principal neurotransmitter at the inner hair cell/afferent synapse, the data suggest ketamine-induced reduction of glutamate excitotoxicity during acoustic overstimulation and/or reduced sympathetic output. The data provide further supporting evidence for sympathetic modulation of cochlear protection and emphasises the potential role of stress in determining susceptibility to acoustic trauma.

**P28: IB1/JIP-1 peptides protect hearing and auditory hair cells from noise and aminoglycoside-induced apoptotic death.**

Azel Zine[1], Jin Wang[3], Christophe Bonny[2], François de Ribaupierre[1] and Jean-Luc Puel[3]
1) Institut de Physiologie, 2) Division de Génétique Médicale, CHUV, Université de Lausanne, Suisse; 3) INSERM U.254 Université de Montpellier I, France

Hearing loss can be caused by a variety of factors including acoustic trauma or ototoxic insults that principally affect the

sensory hair cells which die via an apoptotic pathway associated with the c-Jun amino terminal kinase (JNK), a member of the stress-activated family of MAP kinase. We have studied the effects of IB1/JIP-1, a scaffold protein that prevents the interaction between JNK and its numerous targets such as c-Jun , on the traumatized cochlea. As model systems, we used guinea pigs cochleas exposed to an acoustic trauma and organotypic cultures of neonatal mouse cochleae. In organotypic cochlear cultures, IB1/JIP1 prevented totally neomycin-induced hair cell loss. This finding and the observed increase of phosphorylation of one JNKs target, the transcription factor c-Jun, in stressed hair cells are demonstrations of the ability of IB1/JIP-1 peptides to prevent hair cell loss. In vivo, IB1/JIP1 was delivered to one ear via a mini-osmotic pump in guinea pigs exposed to a sound trauma (6 kHz, 120 dB SPL, 30 min). Protection was assessed physiologically by the change in $8^{th}$ nerve compound action potential threshold and histologically by hair cells survival counts. Animals ears that were perfused with IB1/JIP1 peptides showed less threshold shift and less hair cell loss than non-treated contralateral ears. These results indicate that JNK pathway is involved in both neomycin and sound induced hair cell loss and that its blocking by a small permeable peptide acting on the intracellular signaling cascade, such as IBI/JIP-1 might be of therapeutic value to confer morphological and functional protection.

**Ototoxicity: apoptosis and cell death (P29-P35)**

**P29: Hypoxia- and ischemia vulnerability of inner and outer hair cells**

Birgit Mazurek, Elisabeth Winter, Heidemarie Haupt, Volker Jahnke, Johann Gross
Dept. of ORL, Charité Hospital, Humboldt University, Berlin, Germany

Hearing loss is among the most frequent disorders of the sensorial system. Hypoxia and ischemia are thought to be important factors in bringing about inner ear damage. In order to study the effect of these determinants on the organ of Corti, we used an *in vitro* hypoxia and ischemia model of the newborn rat cochlea. The specimens were exposed to a $pO_2$ level of 5-10 mm Hg in artificial perilymph with (hypoxia) and without glucose (ischemia) for 6-8 hours using a Billups-Rothenburg chamber. The number of inner and outer hair cells (IHC/OHC) was counted. Glucose deprivation did not cause any significant hair cell loss (3-4%). Oxygen deprivation caused a mean significant OHC loss of 8% after 8h and an IHC loss of 8-14% after 6 and 8h, respectively. The combined oxygen-glucose deprivation (ischemia) increased the hair cell loss significantly (OHC to 5-19%, IHC to 25-39%). The vulnerability of IHC to ischemia was significantly higher than that of OHC. The minimal effect of glucose deprivation on the cochlea may be explained by stored glycogen. The fact that oxygen deficiency alone does not affect the hair cells more seriously may be due to the high glycolytic rate. Nevertheless, we found that hair cells are more sensitive to oxygen deficiency than to glucose deficiency. The damage will increase dramatically if the cochlea is deficient in both oxygen and glucose. In this case, the maintenance of energy level depends on the availability of endogenous substrates. Because their amount is limited and low, a rapid and strong decrease of ATP may occur. This might be the key factor for the higher vulnerability of hair cells to ischemia in relation to hypoxia.

**P30: Acoustic stimulation causes the expression of inducible nitric oxide synthase (iNOS/NOS II) in the vestibule of guinea pigs**

Ken-ichi Watanabe[1)], Shunta Inai[1)], Alexander Hess[2)], Olaf Michel[2)] and Toshiaki Yagi[1)]
1)Department of Oto-Rhino-Laryngology, Nippon Medical School, Japan, 2)Department of Oto-Rhino-Laryngology, University of Cologne, Germany

A loud acoustic stimulation is known to cause the inner ear disturbance. We immunohistochemically examined the vestibule of guinea pigs after the acoustic stimulation. Animals were divided into two groups; control (n=6) and the acoustic stimulation group (n=6). The temporal bones were fixed via cardiac infusion of fixative and immunohistochemically stained for inducible nitric oxide synthase. The temporal bones in the control group did not show any inducible nitric oxide synthase. Immunoreactivity for inducible nitric oxide synthase was detected in the supporting cells of the sensory epithelium and the dark cell areas. These findings suggest that free radicals are involved in the pathogenesis of noise induced inner ear damage. This phenomenon could lead to vestibular damage, as seen in noise

induced inner ear damage.

**P31: Free radical scavenger prevents ototoxicity after transient ischemia of the cochlea in gerbils**

Toshiki Maetani, Nobuhiro Hakuba, Masafumi Taniguchi, Jun Hyodo, Yoshitaka Shimizu, Kiyofumi Gyo.
Department of Otolaryngology, Ehime University School of Medicine, Japan

Ischemia-reperfusion injury is one of the major causes of sensory hearing loss (SHL). Due to the lack of a suitable experimental model of cochlear ischemia, the mechanisms of ischemia-reperfusion injury remain unclear and treatments for it are still controversial. Using a technique called experimental hindbrain ischemia, we successfully developed a chronic animal model of transient cochlear ischemia in Mongolian gerbils. We demonstrated that cochlear ischemia causes a progressive inner hair call (IHC) loss until the seventh day after ischemia. IHCs are thought to play an important role in information transfer to the central nervous system. Therefore, protection from IHC degeneration should provide new insight into the treatment of ischemia-induced hearing disturbance. In this study, the protection effects of free radical scavenger on progressive IHC damage in gerbils were examined. The edaravone(3mg/kg), a potent free radical scavenger, was administered intravenously one hour after the ischemic insult. Hearing was assessed by sequentially recording auditory brain stem response (ABR) threshold before and after the ischemia. The degree of hair cell loss in the organ of Corti was evaluated in specimens stained with rhodamine-phalloidin. On the seventh day after ischemia, the ABR threshold shift and progressive IHC loss were significantly reduced in cochleae treated with edaravone. These results suggest that the free radical scavenger is useful for protection against hair cell damage, which otherwise eventually occurs after transient ischemia of the cochlea.

**P32: A Mouse Model for Rapid Degeneration of Spiral Ganglion Neuron**

Tae-Soo Kim[1], Takayuki Nakagawa[1], Ji-Eun Lee[1,2], Fukuichiro Iguchi[1], Tsuyoshi Endo[1], Yasushi Naito[1], Juichi Ito[1]
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The rescue and repair of injured spiral ganglion neurons (SGNs) are important to prevent the hearing loss. Previous reports showed slow SGN degeneration model, and mouse experimental models have wide application as transgenic animals, so research on topics related to the survival of SGNs needs a mouse experimental model in which SGNs degenerate in a well-defined manner. The aim of this study, hence, is to establish a mouse model for rapid SGN loss. C57/BL6 mice were used as experimental animals. We injected 10 l of cisplatin (2.5mg/ml in normal saline) into the posterior semicircular canal and examined SGN loss in the middle turns of cochleae after 3, 7 and 14 days. We used TUNEL assay for cell death analysis, and performed immunohistochemistry for Neurofilament 200kD and peripherin to determine the neuron type in degenerative SGN. TUNEL positive cells in SGN were observed from 3 to 7 days after cisplatin treatment. The numbers of TUNEL-positive cells were most prominent at day 7. The mean of the SGN density and the numbers of Neurofilament 200kD positive cells decreased over a time course, while the numbers of peripherin-positive type 2 neurons did not decrease. This indicates that cisplatin toxicity induced apoptotic cell death in type 1 neurons. The rapid time course of SGN loss in this model should be useful for studying mechanisms of cell death in SGNs and its prevention. In addition, this model can be used as a recipient model for regeneration of SGNs by transplant approaches.

**P33: time-course of oto-vestibular toxicity from cisplatin.**

B. Sergi[1], A. Ferraresi[2], D. Troiani[2], G:B: Azzena[2], G. Paludetti[1], M: Maurizi[1] and A.R. Fetoni[1]
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Cisplatin (CDDP), an antitumor agent widely used in treatment of head and neck cancers, has dose-limiting effects such as ototoxicity and nephrotoxicity. As concerns the labirynthine organs investigation has been limited, both in humans and in experimental animals, to the mechanisms leading mainly to hearing loss. Toxicity of the vestibular system from CDDP has not been detailed with the exception of a few case reports indicating a different time-course of toxicity onset and

recovery. The present study was designed to monitor vestibular function during CDDP treatment and to compare the vestibular alterations with the auditory loss. Albino guinea pigs were divided into CDDP experimental group (n=8; 1.5 mg/kg/day i.p for 6-9 days) and control-saline group (n=2). Horizontal and vertical VORs and CAPs were recorded daily prior to CDDP therapy. Changes in cochlear function were characterised as CAP threshold shifts. Alterations of vestibular function were evaluated by computing VOR gain and phase. Morphological changes were analysed by SEM. CDDP induced progressive high-frequency threshold shifts of 70 dBSPL and consistent reduction of VOR responses. The onset of vestibular function impairment was observed at the 3rd day as evidenced by decreased VOR gain. The progression of impairment was not linear and after CDDP final treatment VOR gains decreased at 50-70% of initial values. The morphological observations confirmed the functional data. The results on vestibulotoxicity did not parallel the ototoxic effects in that vestibulotoxic onset is an early event but the magnitude of final gain loss is inferior.

**P34: Substance P as an antiapoptotic factor for cultured postnatal auditory neurons**

François Lallemend[1], Philippe P. Lefebvre[1], Jean-Michel Rigo[1], Ingrid Breuskin[1], Laurent Nguyen[1], Thomas R. Van De Water[2], Gustave Moonen[1] & Brigitte Malgrange[1]

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Although neuropeptides have been extensively studied in the cochlea for their presence, their physiological roles therein are currently poorly understood. In that respect, many neuropeptides including enkephalins, CGRP, dynorphins, somatostatin, substance P and TRH have been demonstrated in the lateral efferent nerve endings that contact the peripheral axonal projections of the type I spiral ganglion neurons. Several studies suggest that neuropeptides have trophic actions influencing neuronal survival, phenotype and neurite sprouting. In the adult nervous system, these neurotrophic properties may have important implications in repair and synaptic reorganization following peripheral nerve injury. In the present study, we first looked for the expression of neuropeptide receptors by RT-PCR of mRNA extracts prepared from P3 spiral ganglia. Transcripts that encode for somatostatin, substance P, CGRP, TRH and opioids receptors were detected in the ganglion mRNA preparations. The presence of NK1 receptors, the substance P (SP) high affinity receptor, on cultured auditory neurons (ANs) was then demonstrated by immunostaining. Using a fluo-3 base calcium imaging system, we showed that SP induces an increase of the intracellular calcium concentration in ANs in culture, arguing for functional NK1 receptors on these neurons. The activation of the NK1 receptor by SP or a specific agonist showed a survival-promoting effect on ANs suggested by its ability to protect them from cell death induced by serum deprivation. Finally, analyses on caspase activation and DNA fragmentation defined this effect as an antiapoptotic one.
*This work was supported by the Fonds National de la Recherche Scientifique of Belgium.*

**P35 : Local therapeutic strategy against cisplatin-induced ototoxicity in guinea pig.**

Jing Wang, Ruth V. Lloyd Faulconbridge , Ana Fetoni, Matthieu Guitton, Remy Pujol, Jean-Luc Puel

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Cisplatin (*cis*-diamine-dichloroplatinum II) is an effective cytotoxic drug, used in the treatment of a range of neoplasms. Unfortunately, sensorineural hearing loss is a serious side effect of cisplatin chemotherapy which greatly impairs patients' quality of life. One way to protect the cochlea without affecting the antitumoural activity of cisplatin is to apply protective agents directly into the cochlea. This study was design to develop therapeutic strategy using local application of putative protective agents. In the guinea pig, cisplatin treatment (2 mg/kg, I.P., for 5 days) results in high-frequency hearing loss (up to 60 dB), and the loss of outer hair cells from the basal turn. Morphological analyses and specific DNA labelling reveal fragmented hair cell nuclei and cytochrome c redistribution into the cytoplasm of the hair cells of the basal turn, suggesting an apoptosis mechanism of hair cell death. Two strategies were used to rescue the cochlea. The first used sodium thiosulfate, a drug known to bind to the platinum molecule of cisplatin to form an inactive platinum-thiosulfate complex which is not taken up into cells. The second strategy compared the efficiency of anti-apoptotic agents. Here, we show that an intracochlear perfusion of sodium thiosulfate as well an intracochlear perfusion of z-DEVD-fmk, a specific caspase-3 inhibitor, rescued hair cells from apoptotic death. In contrast, D-JNK, a cell-permeable peptide inhibitor of the

c-Jun-N-terminal kinase, acting upstream in the apoptotic cascade does not. This is probably because the activation of the $NH_2$-terminal Jun kinase is required for DNA repair. In human, local application of drugs is achieved through the tympanic membrane using a catheter placed into the round window niche. The present results, especially those obtained with sodium thiosulfate, constitute a great hope for prevention of deafness in those patients having to undergo cisplatin chemotherapy.

**Cochlear fluids (P36-P38)**

**P36: Local inner ear therapy: A technical approach**

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2) Cochlear Ltd. Sydney, Australia

Today, cochlear implantation is the standard procedure to treat the deaf. However, there is still a need to improve the performance of the CI-systems. Animal experiments suggest that local therapy of the inner ear could be a promising new approach to possibly enhance cochlear implant performance by pharmacological intervention. One of the critical issues is to deploy a way for drug delivery to the human inner ear. The objective of the study was to investigate the possibility of a modification of a cochlear implant electrode array for the purpose of drug delivery to the cochlea. The tip of the Nucleus Contour electrode array was cut to open up the lumen of the array and a connecting piece was developed in order to connect the electrode to a pump. The feasibility of the array for drug delivery was investigated using an Alzet mini-osmotic pump as well as a mechanical pump. The stability of the connection was tested for leakage and resistance against tractive forces. Furthermore, the system was applied to temporal bones to evaluate its applicability to a human cochlea. The modified Contour electrode is easy to handle in temporal bones and can be used to simulate drug delivery to the inner ear. The connection to the pump was sealed for all tested pump rates (up to 20 ml per hour for 20 min) and resisted tractive forces of up to 50 N. The described modified electrode array could provide a safe and easy-to-handle possibility to combine electrical stimulation with the beneficial effects of a local drug therapy to the inner ear.

**P37: Extracellular potassium concentration-related lateral wall stiffness in outer hair cells**

Zsolt Farkas, Tamas J Batta, Istvan Sziklai

Debrecen University, Healt Science Center, ORL Clinic, Debrecen, Hungary

$K^+$ induced outer hair cell (OHC) shortening has been described many years ago [Zenner et al., Hear.Res.18 :127-133 (1985)] The underlying cellular mechanism, however, is not yet clarified. Osmotic action, metabolic changes like intracellular free $Ca^{2+}$ concentracion elevation and subsequent phosphorilation of cytoskeletal proteins have been considered [Dulon et al., Hear.Res.32:123-130 (1988)].Isolated apical turn OHCs (L=65-85 m) of guinea pigs were examined under inverted microscope in the presence of different $K^+$ concentrations: 12.5, 25, 37.5 mM. Test solutions were applied by a puffer pipette in a 100 m distance from the OHCs. The flow rate was 1.5 l/min. Osmotic concentrations were adjusted to 300 mosm.kg$H_2O^{-1}$.The stiffness of the lateral wall of OHCs were measured by a microdeformation technique under the control of a phasecontrast microscope during constant application of negative pressure (6 cm $H_2O$). Cell length changes and deformation of the lateral membrane of OHCs were measured off-line on digitized images of the cells. The resolution of cell length and lateral wall deformation measurements were 6 pixels/ m and 12 pixels/ m, respectively. OHCs were challenged first by the incubation medium (Hank's), then by different $K^+$ concentrations. Each $K^+$ concentration steps were applied for 3 minutes and the stiffness measurements were carried out during the last 2 minutes.Even by this slow perfusion rate, the stiffness of the lateral wall of OHCs increased. When different $K^+$ concentrations were applied the active behavior of lateral wall stiffness could not be measured. Control experiments did not show significant differece comparing to 12.5 mM $K^+$ induced stiffness change. Higher $K^+$ concentracions induced a concentration dependent increase in stiffness of the lateral wall of OHCs.Conclusions are, that OHCs are highly mechanosensitive against low fluid perfusion rates, and that $K^+$ causes increase of the lateral wall

stiffness of OHCs.

**P38 : Detection of aquaporin 2 in the epithelium of human endolymphatic sac by immunohistochemistry**

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The endolymphatic sac is thought to be involved in the homeostasis of the volume and/or composition of endolymph by a double mechanism of fluid reabsorption and luminal secretion of osmotically active proteins and glycoconjugates. In most epithelial structures, transepithelial water fluxes occur through membranous water channels named aquaporins. Among them, aquaporin 2 (AQP-2) has been specifically localized in the renal collecting duct and its membranous insertion is regulated by antidiuretic hormone. The presence of AQP-2 in the endolymphatic sac remains controversial. The aim of the present study was to detect the presence of AQP-2 in a human endolymphatic sac by immunohistochemistry. The sac was sampled during a translabyrinthine approach for removal of a vestibular schwannoma and immediately fixed in 10% formaline. Rabbit polyclonal anti-aquaporin 2 antibody was used (Cluzeaud *et al.*, Am J Physiol 1998; 275: C1602-9). Positive immunostaining was observed in a subset of endolymphatic sac epithelial cells. AQP-2 was mostly localized in the cytosol and also more rarely in the basolateral membrane. This result suggests that water flux through the endolymphatic sac occur at least partly through AQP-2 water channels. Considering the predominant cytosolic localization of AQP-2, water flux through this channel is probably low or null in this endolymphatic sac. In other patients, membranous insertion may be upregulated by antidiuretic hormone, as receptors to this hormone have been localized in the rat endolymphatic sac. In conclusion, in human endolymphatic sac, the presence of AQP-2 water channel suggests that this aquaporin is involved in the regulation of the volume and/or composition of endolymph.

**Clinical otorhinolaryngology (P39-P41)**

**P39: A behavioral animal model for salicylate induced Tinnitus in rats**

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A behavioral model was developed on rats for the examination of salicylate induced Tinnitus, limiting periods of punishment according to the new recommendations of animal rights. Rats were trained on actively moving in a specially adapted behavioral cage (Skinner box) while a surrounding tone was played (65 - 70 dB SPL, white noise). Active accesses of the rat to one of two liquid feeders were followed by a liquid food reward. Randomly, the surrounding tone was replaced by silent periods (40 - 120 sec), during which no reward was given. Animals learned to become active during sound experience and to suppress activity during silence. Animals treated with salicylate (350 mg / kg body weight 3 h before testing) showed a significiantly increased activity during periods of silence compared to animals treated with saline. The behavioral change is presumed to be the result of a specific salicylate effect on the auditory system: control animals conditioned on a light-dark paradigm did not show behavioral changes after salicylate, validating that the changes observed in auditory conditioned rats were not due to any unspecific impairments of learning or locomotion. The overall activity spectrum was unaffected. From the behavioral effect onto different sound pressure levels, the assumed internal sound experience of rats treated with 350 mg / kg b.w. salicylate was estimated to be at least 45 dB SPL, and had a frequency characteristic different from a sinusoidal pure tone of 5, 8 or 11 kHz. Our animals model is the basis for ongoing experiments on the examination of otoactive drugs in the context of clinical Tinnitus research. Supported by DFG Grant Kni 316/3-2 and Fortuene 972-0-0Tuebingen

**P40: A behavior animal model to study the frequency characteristics of salicylate induced Tinnitus in rats**

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In an operant conditional paradigm, rats had to learn to become active during periods of sound and to suppress their activity during periods of silence. Three hours after the administration of Sodium-salicylate (salicylate, 350 mg / kg body weight) rats were not able to differentiate between periods of sound and periods of silence presumptively because of the tinnitus inducing effect of salicylate. We showed (see poster Rüttiger et al.) that animals could be conditioned on broad band white noise tones as well as pure tone stimuli (5, 8, 11 kHz). Learning did not transfer when switching from the familiar tone stimulus to a new unfamiliar tone (e.g. from white noise tones to sinusoidal pure tones) indicating that the animals were able to recognize the tones differentially. When however 3 different broadband frequency bands were presented (1 - 5.6 kHz; 4 - 16 kHz; 11.3 - 50 kHz, variing between 10 - 70 dB) no significant difference between the experimental groups trained on different frequency bands was found, indicating that the learning transfer with the switch to another frequency band was almost complete. This suggests that the precise frequency range was not of relevance for learning and that rats experienced the broadband stimuli as equivalent sound indicators. Whether rats experience their salicylate induced Tinnitus as a broadband white noise sound is subject of ongoing experiments examining the discrimination ability of rats onto different broadband noise stimuli. Supported by DFG Grant Kni 316/3-2 and Fortuene 972-0-0Tuebingen

**P41: Temporal bone density measurement by CT scan in otosclerosis**

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The aim of this study was to assess the bone density in Hounsfield units (HU) around the bony labyrinth in otosclerosis and its comparison with a control population. 11 patients with otosclerosis, and 34 control patients (acoustic neurinomas) were included in this study. In these 2 groups, the mean age was 42 years (24-55), and 46 years (20-71), and the sex ratio was 0.27, and 0.70 respectively. All the patients had a clinical examination, an audiometry, a CT scan with axial and coronal views on both ears. In the otosclerosis group, audiometry showed a unilateral involvement in 6 patients, and a bilateral hearing loss in 5 patients. The bone density was measured in HU at fissula ante fenestram (FAF), and 5 other anatomical points in the bony labyrinth. In the control group, the density at FAF was not significantly different from other anatomical points in the labyrinth of the same ear.The density at FAF was lower in otosclerosis than in control patients ($1737 \pm 86.1$ versus $2054 \pm 13.0$ UH, $P < 0.01$, unpaired t test). This density was not significantly different between the involved side and the unaffected side on audiometry in unilateral forms, and between the more involved and the less affected sides on audiometry in bilateral forms. In the otosclerosis group, the FAF density was lower in ears with a preserved stapedial reflex in comparison with the control group ($1753 \pm 191.2$ versus $2065 \pm 12.8$ UH, $P < 0.001$ unpaired t test). Our observations suggest the presence of bilateral otosclerotic foci with variable audiometric expression in all patients. The bone density measurement by CT scan is a simple method to enhance the sensitivity of this diagnostic tool.