



Zentrum für systemische Neurowissenschaften Hannover



MARIE CURIE ACTIONS

# **ZSN-Kolloquium 2009**

**11. September 2009  
to  
13. September 2009**

Maritim Berghotel Braunlage  
Am Pfaffenstieg  
38700 Braunlage

**Program**

**Friday, 11.09.09**

<b>Up to 14.00</b>	<b>Arrival</b>
<b>15.00- 15.10</b>	<b>Address of Welcome Prof. Baumgärtner</b>
<b>15.10.- 16.00</b>	<b>Prof. Alfred Effenberg</b> Institut für Sportwissenschaft, Leibniz Universität Hannover <b>Multisensory Motion Perception and Effects on Motor Control and Learning</b>
<b>16.00- 17.00</b>	<b>Chair: Prof. Tipold</b>
<b>16.00- 17.00</b>	<b>Maria Herrojo Ruiz</b> Department of Music-Physiology and Musician´s Medicine, HMTH <b>Electrophysiological Correlates of Motor Control and Error Monitoring in Healthy Pianists and Pianists with Musician´s dystonia</b> (Altenmüller, Dengler, Esser, Jäncke)
<b>17.00- 17.50</b>	<b>Chair: Prof. Tipold</b> Evaluation group: Prof. Tipold, PD .Dr. Claus, Prof. Löscher
<b>17.00- 17.15</b>	<b>Christopher Sinke</b> Clinic for Psychiatry, Social Psychiatry and Psychotherapy, MHH <b>Evaluating models of synaesthesia</b> (Emrich, Altenmüller, Zimmermann)
<b>17.15- 17.30</b>	<b>Janina Neufeld</b> Clinic for Psychiatry, Social Psychiatry and Psychotherapy, MHH <b>Sound induced synaesthesia – How do tonal aspects influence synaesthetic photisms?</b> (Emrich, Altenmüller, Zimmermann)
<b>17.30 - 17.40</b>	<b>Arne Liebau,</b> Department of Zoology, TiHo <b>Retinal spectral sensitivity and adaptation in nocturnal tree frogs</b> (Esser, Ngezahyo, Altenmüller)
<b>17.40 - 17.50</b>	<b>Daniel Schmidtke</b> Department of Zoology, TiHo <b>Neuroethological studies on the neuronal basis of three-dimensional allocentric spatial cognition in mammals</b> (Esser, Altenmüller, Gernert)
<b>19.00</b>	<b>Dinner</b>
<b>20.00</b>	<b>Meeting of the ZSN members</b> (Prof. Löscher, hall "Travemünde", ground floor) <b>Meeting of the groups of the three ZSN main topics</b> (Prof. Löscher, Prof. Altenmüller, Prof.Zimmermann)

**Saturday, 12.09.09**

<b>9.00 – 9.20</b>	<b>Introduction of the new PhD-students of 2009 (study books)</b> <b>Prof. Dr. Baumgärtner</b>
<b>9.20- 10.50</b>	<b>Chair: Prof. Ngezahayo</b> Evaluation group: Prof. Ngezahayo, Prof. Lanfermann,
<b>9.20-9.35</b>	<b>Annika Lehmbecker</b> Department of Pathology, TiHo <b>An inhalation study in rats to detect the differences in translocation behaviour of fine and ultrafine TiO<sub>2</sub> particles from nose to brain”</b> (Baumgärtner, Claus, Gernert)
<b>9.35-9.50</b>	<b>Nicole Schneider</b> Department of Neuropsychology, MHH <b>Glutamate transporters in retinal neurons</b> (Fahlke, Grothe, Hildebrandt)
<b>9.50- 10.05</b>	<b>Nadine Polascheck</b> Department of Pharmacology, Toxicology and Pharmacy, TiHo <b>Prophylactic treatment with the COX-2 inhibitor parecoxib after status epilepticus: Effect on epileptogenesis, behavioral alterations, and neuronal damage in rats</b> (Löscher, Petri, Claus)
<b>10.10- 10.20</b>	<b>Franziska Buttkus</b> Department of Music-Physiology and Musician´s Medicine, HMTH <b>Developing a new quantification method for musician’s dystonia in violinists</b> (Altenmüller, Dengler, Fahlke)
<b>10.20- 10.30</b>	<b>Melanie Langer</b> Department of Pharmacology, Toxicology and Pharmacy, TiHo <b>Comparison of rats from different breeders after induction of status epilepticus by prolonged electrical stimulation of the basolateral amygdala</b> (Löscher, Baumgärtner, Fahlke)
<b>10.30- 10.40</b>	<b>Christina Brauer</b> Animal neurology, Clinic of small animals, TiHo <b>Electroencephalographic recordings in dogs and cats: prevention of muscle artifacts and evaluation of two activation techniques</b> (Tipold, Baumgärtner, Petri)
<b>10.40- 10.50</b>	<b>Florian Hansmann</b> Department of Pathology, TiHo <b>In vivo demonstration of matrix metalloproteinase-3, -9 and -12 mediated demyelination</b> (Baumgärtner, Claus, Gerardy-Schahn)

**Saturday, 12.09.09**

<b>10.50 - 11.20</b>	<b>Coffee break</b> <b>Consultation of the evaluation group</b>
<b>11.20- 12.45</b>	<b>Chair: Prof. Baumgärtner</b> Evaluation group: Prof. Baumgärtner, Prof. Hildebrandt, Prof. Ponimaschkin
<b>11.20- 11.35</b>	<b>Sarah Knippenberg</b> Clinic of Neurology, MHH <b>Studies on the therapeutic potential of adult stem cells in the G93A animal model of amyotrophic lateral sclerosis (ALS)</b> (Petri, Brinker, Steinlechner)
<b>11.35- 11.50</b>	<b>Olga Baron</b> Department of Neuroanatomy, MHH <b>Role of FGF-2 during Development of the Substantia Nigra</b> (Grothe, Dengler, Lanfermann)
<b>11.50- 12.05</b>	<b>Roger Calixto</b> Clinic of Laryngology, Rhinology and Otology, MHH <b>Auditory implant project: Midbrain and Nerve Implants</b> (T. Lenarz, Dengler, Krauss)
<b>12.05- 12.15</b>	<b>Ieva Kalve</b> Department of Neuroanatomy, MHH <b>Application of genetically modified dopaminergic progenitor cells in a rat model of Parkinson's disease</b> (Grothe, Krauss, Bicker)
<b>12.15 - 12.25</b>	<b>Yunping Song</b> Clinic of Neurology, MHH <b>pH-dependency of recombinant glycine receptor channels</b> (Petri, Fahlke, Löscher)
<b>12.25 - 12.35</b>	<b>Stefanie Honndorf,</b> Department of Pharmacology, Toxicology and Pharmacy, TiHo <b>Investigation of the small-conductance calcium-activated potassium channel SK2 and the GABAA receptor subunit <math>\alpha 1</math> expression in the basal ganglia in the rat amygdala-kindling model of temporal lobe epilepsy</b> (Gernert, Grothe, Tipold)
<b>12.35- 12.45</b>	<b>Souvik Kar</b> Clinic of Laryngology, Rhinology and Otology, MHH <b>Determination of the apoptosis and cell survival in the rat cochlea following neomycin induced deafness</b> (Stöver, Grothe, Esser)

**Saturday, 12.09.09**

13.00-14.00	<b>Lunch</b> Consultation of the evaluation group
14.00-17.20	<b>Chair: Prof. Bicker</b>
14.00-15.00	<b>Dr. Schwartz, Malte</b> Animal neurology, Clinic of small animals, TiHo <b>Pathogenetical factors contributing to high IgA levels and marked neutrophilic pleocytosis in canine steroid-responsive meningitis-arteritis</b> (Tipold, Baumgärtner, Stangel, Anderson)
15.00-16.00	<b>Koutsoudaki, Paraskevi</b> Clinic of Neurology, MHH <b>Molecular basis of the axon-oligodendrocyte interaction during remyelination</b> (Stangel, Hildebrandt, Claus, Kuhlmann)
16.00-16.20	<b>Coffee break</b>
16.20-17.20	<b>Danai Dima</b> Clinic for Psychiatry, Social Psychiatry and Psychotherapy, MHH <b>Investigation of neural correlates of bottom-up and top-down processing with functional Magnetic Resonance Tomography and Event Related Potentials. Exemplified by the binocular depth inversion paradigm</b> (Emrich, Dietrich, Altenmüller, Vogeley)
17.20-18.30	<b>Feedback for the students</b>
18.30-19.00	<b>PhD- commission meeting</b> (hall "Travemünde", ground floor)
19.00 - 20.00	<b>Dinner</b>
20.00	<b>Meeting of the PhD-students</b> (Prof. Baumgärtner, Dr. Esser; (hall "Travemünde", ground floor)

**Sunday, 13.09.09**

<b>9.00-10.35</b>	<b>Chair: Prof. Dietrich</b> Evaluation group: Prof. Dietrich, Prof. Stangel, Prof. Berding
<b>9.00-9.15</b>	<b>Christoph Lindemann</b> Dept. of Neurosurgery, MHH <b>Effect of deep brain stimulation on cognitive and neuropsychiatric function in rat models of Parkinson's disease.</b> (Krauss, Gernert, Emrich)
<b>9.15-9.30</b>	<b>Regina de Campos Oliveira Ulrich</b> Center for Anaesthesiology and Critical Care Medicine, MHH <b>Effects of the co-application of the topical antiseptics amylmetacresol and dichloro-benzylalcohol on the voltage-gated neuronal sodium channel NaV1.2</b> (Haeseler, Kästner, Weissenborn)
<b>9.30-9.45</b>	<b>Barbara Schlingmann</b> Department of Biophysic, Leibniz University <b>Physiological characterisation of lens gap junction connexins</b> (Ngezahayo, Steinlechner, Claus)
<b>9.45-10.00</b>	<b>Arianna Maiolini</b> Animal neurology, Clinic of small animals, TiHo <b>Steroid responsive meningitis-arteritis in dogs: role of cytokines and toll-like receptors</b> (Tipold, Baumgärtner, Stangel)
<b>10.00-10.10</b>	<b>Janett Schaper-Rinkel</b> Department of Neuroanatomy, MHH <b>In vivo evaluation of exogenous polysialic acid effects on peripheral nerve regeneration after substance loss</b> (Grothe, Gernert, Gerardy-Schahn)
<b>10.10-10.20</b>	<b>Anna Fredericke Nölle</b> Department of Neuroanatomy, MHH <b>The molecular pathogenesis of Spinal Muscular Atrophy: Characterization of the interaction between SMN and the actin regulating protein Profilin</b> (Claus, Esser, Petri)
<b>10.20-10.30</b>	<b>Ingo Spitzbarth</b> Department of Pathology, TiHo <b>Microglial responses in organotypic spinal cord slice cultures - An <i>in vitro</i> model of canine spinal cord injury</b> (Baumgärtner, Bicker, Tipold)

<b>10.30-11.00</b>	<b>Coffee break</b> <b>Consultation of the evaluation group</b>
<b>11.00-12.00</b>	<b>Chair: Prof. Altenmüller</b> Evaluation group: Prof. Altenmüller, PD Dr. Ding, Dr. Haastert
<b>11.00-11.10</b>	<b>Tegenge, Million Adane</b> Department of Physiology and Cellbiology, TiHo <b>Nitric oxide and cyclic nucleotide signal transduction modulates synaptic vesicle recycling in human model neurons</b> (Bicker, Steinlechner, Hildebrandt)
<b>11.10-11.20</b>	<b>Alexander Karabatsiakis</b> Clinic for Psychiatry, Social Psychiatry and Psychotherapy, MHH <b>Error-related negativity (ERN) in remitted depression: an ERP study using the Eriksen Flanker task</b> (Diertrich, Steinlechner, Baumgärtner)
<b>11.20-11.30</b>	<b>Jelena Skuljec</b> Clinic of Neurology, MHH <b>Characterization of microglial phenotypes during de- and remyelination</b> (Stangel, Tipold, Baumgärtner)
<b>11.30-11.40</b>	<b>Vanessa Herder</b> Department of Pathology, TiHo <b>Cuprizone ameliorates spinal cord inflammation in a viral model of multiple sclerosis</b> (Baumgärtner, Stangel, Löscher)
<b>11.40-11.50</b>	<b>Gudi, Victoria</b> Department of Neurology, MHH <b>Identification of growth factors involved in the demyelination/remyelination in the murine cuprizone model</b> (Stangel, Tipold, Fahlke)
<b>11.50-12.00</b>	<b>Christine Müller</b> Department of Pharmacology, Toxicology and Pharmacy, TiHo <b>Differences in sensitivity to the convulsant pilocarpine in substrains and sublines of C57BL/6 mice</b> (Löscher, Hildebrandt, Dietrich)
<b>12.00-12.30</b>	<b>Consultation of the evaluation group</b> <b>Feedback for the students</b>
<b>12.30-12.40</b>	<b>Farewell note</b> <b>Prof. Löscher</b>

## Abstracts

### **The molecular pathogenesis of Spinal Muscular Atrophy:**

Characterization of the interaction between SMN and the actin regulating protein Profilin

Nölle A.<sup>1,3</sup>, Grothe C.<sup>1,3</sup>, Niedenthal R.<sup>2</sup>, Claus P.<sup>1,3</sup>

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<sup>3</sup>Center for Systems Neuroscience (ZSN) Hannover, Germany

Spinal muscular atrophy is a neurodegenerative disease characterized by a selective death of motor neurons. This defect is caused either by mutations or deletions in the survival of motoneuron (SMN). The pathogenesis of the disease is still unclear. SMN is known as an assembly protein for RNA-protein complexes and localize to the nuclear bodies. The number of SMN-positive nuclear bodies is decreased in SMA patients. Moreover, we have recently shown that the stability of these structures is regulated by nuclear fibroblast growth factor - 2 (FGF-2) (Bruns et al., 2009, PNAS, in press) and that the destabilization is not responsible for disease progression. However, SMN is also found in axons of motoneurons stressing the importance of a non-nuclear mechanism of disease development.

We previously analyzed the effects of SMN on neuronal differentiation and identified the Rho-Kinase (ROCK) pathway as an important modulator of SMN-dependent actin cytoskeletal regulation (van Bergeijk et al., 2007, FASEB J. 21: 1492-1502). How SMN does interact with the ROCK pathway? One putative molecular bridge between SMN and the ROCK pathway is the protein Profilin. Here, we analyzed the interaction of Profilin with SMN by a new in vivo protein-protein interaction assay (Niedenthal, 2009). This method employs the SUMO conjugating enzyme Ubc9 for fusion-dependent trans-sumoylation. Site-directed mutagenesis of SMN allowed the determination of the Profilin-binding site in SMN. Our data suggest that actin-regulating proteins downstream of ROCK are involved in SMN-dependent neurogenesis defects and that the SMN-Profilin interaction is the putative link between SMN and the ROCK-pathway. Importantly, analyses of this pathway could help to elucidate new molecular targets for treatment of spinal muscular atrophy.

This work is supported by the Georg-Christoph-Lichtenberg Fellowship.

### **Determination of the apoptosis and cell survival in the rat cochlea following neomycin induced deafness**

Souvik Kar, Kirsten Wissel, Verena Scheper, Thomas Lenarz, Timo Stöver

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Sensorineural hearing loss is associated with destruction of the hair cells in the cochlea. Since

inner hair (IHC) cells provide the afferent excitatory activation of the auditory neurons (AN), it is proposed that deafferentation of the AN leads to spiral ganglion neurons (SGN) degeneration following hair cell loss. In vitro and in vivo studies demonstrated that neurotrophic factors play key roles in protection of SGN following ototrauma. By the common hypothesis cell death provoked by deafferentation of neurons may reflect deprivation of the neurotrophic factors. However, gene expression profiling in the AN of deafened rats revealed that artemin, GDNF and BDNF were significantly upregulated in the rat cochlea 26 days following neomycin induced deafness. The goal of this project is the determination of the signalling of neurotrophic factors in the context of apoptotic mechanisms by gene expression analysis following 7, 14, and 28 days deafening. Differential gene expression of brain- (BDNF) and glial cell line-derived neurotrophic factor (GDNF), their corresponding receptors Trk B, p75<sup>NTR</sup> and GFR $\alpha$ 1, the pro-apoptotic signal molecules caspase 9, Bax and the anti-apoptotic signal molecules GLAST, Bcl-2 will be studied within this time interval. So far, total RNA was extracted from 20 modioli of normal hearing and deafened animal sacrificed after 7 days, respectively, and reverse transcribed. First, the appropriate housekeeping gene (HKG) as internal standard was established by using real-time PCR. The comparison of the amplification efficiency of 16 mammalian HKG in tissue samples from normal hearing and deafened animals disclosed Rplp2 (ribosomal protein) as the relevant HKG. Morphometric estimation of SGN degeneration following 7 days deafening revealed 1,8fold decrease of the SGN number indicating strong apoptosis process. Further results for the expression of HKG and Caspase, BDNF, GDNF, Bcl-2, Bax, GLAST on day 7 deafened will be presented in the poster.

Financial Support : Georg - Christoph - Lichtenberg –scholarship of the state of Lower Saxony to Souvik Kar

## **Retinal spectral sensitivity and adaptation in nocturnal tree frogs**

Arne Liebau, Karl-Heinz Esser

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Frogs have a very low absolute threshold of vision. They catch prey at light intensities where humans would neither see the frog nor the prey. This high visual sensitivity is mainly caused by the relatively low body temperature of these poikilothermic animals. First, the low body temperature leads to a low receptor-noise in the retina due to a reduced (re warm-blooded animals) number of spontaneous thermal isomerizations of the chromophor in the light-receptor cells. This improves the retinal signal-to-noise ratio and the detectability of photons at very low light intensities. Second, the low temperature of the retina extends the integration time of photoreceptors probably by slowing down the signal cascades that amplify the signal of photo isomerizations in the cell. This improves photon catch and leads to a further increase in sensitivity by the cost of loss of temporal resolution.

How does such a high visual sensitivity influence the spectral range of vision? Because of the Gaussian shape of the absorption spectra of visual pigments, a lower threshold should lead to a broader bandwidth of vision. In the present study, we used the red-eyed tree frog (*Agalychnis callidryas*) as experimental animal. Considering the lifestyle of the species (nocturnal insect hunter) it could be assumed that *A. callidryas* has a very low absolute threshold of vision.

In a first behavioral experiment we measured prey-capture rate under different spectral light conditions and found the visible spectrum extending from the UV into the NIR (near-infrared) range. At present, we are recording full-field electroretinograms (ERGs) to determine quantitatively the spectral sensitivity of the eye of *A. callidryas*.

### **Electroencephalographic recordings in dogs and cats: prevention of muscle artifacts and evaluation of two activation techniques**

C. Brauer, S.B.R. Kästner, H.C. Schenk, J. Tünsmeier, A. Tipold

Department of Small Animal Medicine and Surgery, University of Veterinary Medicine, Hannover, Germany

Electroencephalography (EEG) in veterinary neurology is limited because anaesthesia is necessary for artefact free recordings. Stimulation methods are frequently used in human medicine to improve the diagnosis of epilepsy. This study evaluated the effect of photic stimulation and hyperventilation on the EEG in healthy dogs and dogs and cats with seizures. Animals were anaesthetized with propofol. Rocuronium bromide, a peripheral muscle relaxant, was administered to prevent muscle artefacts. Visual and quantitative analysis were used to determine background activity of the EEG and events superimposing this background activity in healthy dogs. EEGs of animals suffering from seizures were analyzed visually.

Ten healthy beagle dogs, 65 dogs suffering from idiopathic epilepsy, 46 dogs suffering from symptomatic epilepsy and 12 epileptic cats were anaesthetized with propofol constant rate infusion. After electrode placing recording was started using a five channel monopolar montage. Administration of rocuronium (0.4 mg/kg IV) followed. Activity without stimulation was measured for three minutes followed by photic stimulation. Flash frequency was gradually increased until 50 Hz and then decreased. Subsequently to a recording interval of three minutes without stimulation animals were hyperventilated for at least 180 seconds and until an endtidal CO<sub>2</sub> (EtCO<sub>2</sub>) concentration of 30 mmHg was reached. Post hyperventilation recording lasted for another three minutes.

Visual and quantitative analysis of the EEG recordings did not reveal paroxysmal discharges or differences in background activity between periods with and without stimulation in healthy dogs. Photic stimulation and hyperventilation were not able to elicit pathological EEG activity in any of the epileptic dogs. Photic stimulation induced photic driving in the majority of the epileptic cats. Further studies will show if photic driving will also be elicited in healthy cats and if this finding is helpful in diagnosis of pathological changes of the brain in cats.

This study was supported by a Georg-Christoph-Lichtenberg Scholarship donated by the Department of Science and Culture of the federal state of Lower Saxony, Germany

## **An inhalation study in rats to detect the differences in translocation behaviour of fine and ultrafine TiO<sub>2</sub> particles from nose to brain**

Annika Lehmbecker<sup>1,2</sup>, Susanne Rittinghausen<sup>2</sup>, Wolfgang Baumgärtner<sup>1</sup>

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Particulate matter (PM) is of great concern because of its effects on public health. Fine and ultrafine particles are widely distributed throughout the environment. They are used in a variety of industries and they are crucial for workplace safety as well. Ultrafine particles are supposed to cause more severe alterations than their fine counterparts because of their huge surface area in comparison to size. Those effects may play a roll in the pathogenesis of neurodegenerative diseases like Alzheimer or Parkinson disease.

Titanium dioxide (TiO<sub>2</sub>) is an insoluble and nearly inert material which causes as fine particle only minimal changes whereas its ultrafine particles cause inflammatory reaction in the lung.

The aim of the present study was to investigate the differences in translocation and morphological alterations in nasal and brain tissue after inhalation of fine and ultrafine TiO<sub>2</sub> particles by light and electron microscopy and immunohistochemistry.

Female Hanover Wistar rats were exposed by inhalation for 6 hours per day for 21 days with 25 mg/m<sup>3</sup> or 5 mg/m<sup>3</sup> ultrafine TiO<sub>2</sub> or 45 mg/m<sup>3</sup> or 9 mg/m<sup>3</sup> fine TiO<sub>2</sub> or clean air respectively. Animals were euthanized 3, 28 and 90 days after recovery. Subsequently noses and brains were removed, formalin fixed, paraffin embedded and the sections were stained with hematoxylin and eosin for histological examination. No obvious morphologic alterations were detected in the paraffin sections of nose and brain. Future studies will focus on ultrastructural alterations with special emphasis on intermediate filaments, cell proliferation and death.

## **Evaluating models of synaesthesia**

C. Sinke, J. Neufeld, W. Dillo, M. Zedler, H.M. Emrich

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Synaesthesia is a condition in which stimulation in one sensory modality leads to an involuntary perception in another unrelated modality. For example can music elicit taste, color or shapes. But not only couplings between modalities but also within a modality are observed. In the most investigated form of synaesthesia, grapheme-color synaesthesia, achromatic letters and numbers have a certain color. Psychophysiological and neuroimaging experiments have shown that this condition is real and not an illusion of the affected people (Ramachandran 2001). For example show synaesthetes reaction time differences in a modified stroop task whereas non-synaesthetes do not (Bergfeld-Mills 1999). In addition have fMRI experiments shown an additional activation of V4, an area thought to process color information, compared to non-synaesthetes (Sperling 2006).

Currently the neuronal mechanisms are still unclear but there are different models available. The first one, called local- crossactivation states that direct connections between the involved brain areas are responsible for the additional perception. Another model, disinhibited feedback, explains synaesthetic perception with a weaker inhibition of the involved areas from higher multimodal areas. The third model, called hyperbinding, states that in general synaesthetes

have a hypersensitive binding mechanism which elicits the synaesthetic perception (Hubbard 2007). We now want to test these hypotheses with dynamic causal modeling (DCM) on fMRI data. DCM is a tool that is developed for the analysis of effective connectivity using experimentally designed inputs and fMRI responses (Friston 2003). The goal is to use the different explanatory models (i.e. cross-activation, disinhibited feedback, hyperbinding) and look which explains best our fMRI activation. Therefore 20 grapheme-colour synaesthetes are presented with letters which trigger synaesthesia and signs which do not trigger synaesthesia and the resulting activation pattern is modeled with DCM.

This work is supported by a scholarship of the MHH, center for mental health.

Ramachandran and E. M. Hubbard (2001), "Psychophysiological investigations into the neural basis of synaesthesia," *Proc. R. Soc. B.* 268, 979–983

C. Bergfeld-Mills, E. Howell Boteler, and G. K. Oliver (1999), "Digit Synaesthesia: A Case Study Using a Stroop-type Test," *Cognitive Neuropsychology* 16, no. 2, 181–191

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K.J. Friston, L. Harrison, and W. Penny (2003), "Dynamic causal modeling." *NeuroImage* 19, no. 4, 1273-1302

### **Nitric oxide and cyclic nucleotide signal transduction modulates synaptic vesicle recycling in human model neurons**

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2. Center for Systems Neuroscience (ZSN) Hannover, Germany

Numerous studies in both vertebrates and invertebrates implicate an involvement of nitric oxide (NO) signaling in neuronal proliferation, migration, neurite outgrowth and synaptic maturation processes. Nevertheless, it is unknown whether NO plays a role in the development of the human nervous system. We established a model of developing human model neurons from a well characterized teratocarcinoma cell line (NT2) as spherical aggregate culture. Recently, we have showed that NO and cyclic GMP signal transduction positively regulates the migration of cells out of the NT2 aggregates. In this study, we followed the maturation of NT2 cell aggregates to form functional neurons by immunocytochemical methods and imaging synaptic vesicle recycling. Cells migrate out of NT2 aggregates to form fully mature postmitotic neurons that express typical presynaptic proteins (synapsin and synaptotagmin I) along the neurites. Imaging synaptic vesicle recycling by employing the fluorescent dye (FM1-43) showed that upon depolarization mature NT2 neurons display presynaptic vesicle exocytosis in a calcium dependent manner. The level of presynaptic proteins increases with the length of in vitro

culture, indicative of a presynaptic maturation processes. Human NT2 neurons express the neuronal isoform of nitric oxide synthase (nNOS) and soluble guanylyl cyclase (sGC), the major receptor for nitric oxide (NO). Pharmacological manipulation revealed that NO and cyclic nucleotide signal transduction modulates presynaptic vesicle exocytosis in human model neurons. Future study will focus on the cellular mechanism by which NO regulates vesicle releases from human NT2 neurons.

## **Sound induced synaesthesia – How do tonal aspects influence synaesthetic photisms?**

J Neufeld, H M Emrich

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Synaesthesia (Greek: syn = together; aesthesis = perception) is a neurological condition in which stimulation of one sensory modality or processing stream leads to one or more other unrelated perceptual experiences. In sound-induced synaesthesia, different sounds like music, pure tones or environmental noises trigger the experience of so called photisms – visual perceptions which have a certain shape and size and often a colour and/or structure. Previous investigations found a correlation between pitch and lightness and pitch and size in synaesthetes and as intermodal analogies in non-synaesthetes: For most non-synaesthetes, high pitched tones are associated with lighter colours and smaller forms than low pitched ones. In most synaesthetes there is a similar interrelation but the interindividual differences are greater than in non-synaesthetes and the intra-individual consistency is higher. The aim of the current study is to investigate the brain activity which is associated with sound-induced synaesthesia via functional Magnetic Resonance Imaging (fMRI) and to find out which tonal parameters influence the synaesthetic experience and the related brain activity most: besides the relation between pitch and lightness, which is similar for synaesthetes and non-synaesthetes, we will focus on timbre and aspects of harmony and mode. Therefore, 20 tone-colour synaesthetes and 20 matched controls will be investigated. A first testing revealed activation of early visual areas during the presentation of all auditory stimuli (pure tones and chords) in a sound-photism synaesthete while there is no such activation in a control. Furthermore, synaesthetes and controls will be tested in their memory for melodies and other musical aspects and in their overall mnemonic abilities to find out if sound-induced synaesthesia correlates with a facilitated auditory memory.

This study is supported by a grant of the medical school of Hanover, centre of mental health.

## **Physiological characterisation of lens gap junction connexins**

Barbara Schlingmann, Anaclet Ngezahayo

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Gap junction channels connect the cytoplasm of adjacent cells and allow an exchange of ions and metabolites (up to 1kDa) between cells. In vertebrates, gap junction channels are formed by pairs of hemichannels inserted into the membrane of interacting cells. The hemichannels are hexamerized connexins (Cx) with a high conserved structure. The conductance of gap junctions is regulated by transjunctional voltage, intracellular  $Ca^{2+}$  and pH as well as phosphorylation. The sensitivity to these modulators varies between different connexins. Especially in the non-vascularised ocular lens, gap junctions are part of the pump-leak transport system and play a crucial role in transport of metabolites between the periphery and the core. Two different connexin classes are expressed in lens fibres: the Cx46 class with human and rat Cx46 (hCx46 and rCx46), chicken Cx56 (cCx56) and bovine Cx44 (bCx44) and the class of Cx50 with the human and rat Cx50 (hCx50 and rCx50), the chicken Cx45.6 (cCx45.6) and the bovine Cx49 (bCx49). The two-electrode-voltage-clamp technique has revealed that Cx46 hemichannels formed in *Xenopus* oocytes are voltage dependent and could be blocked by extracellular  $Ca^{2+}$  and acidification of the extracellular space. For rCx46, it was found that protein kinase C dependent phosphorylation reduced the conductance and induced a voltage dependent inactivation of the hemichannels. Furthermore, it was postulated that casein kinase dependent phosphorylation regulates the formation of functional rCx46 hemichannels. The aim of the present project is to ablate specific parts of the connexins and to introduce specific point mutations in order to characterize the connexin structures involved in regulation of rCx46 hemichannels in comparison to other connexins of the Cx46 class such as hCx46 and cCx56. A particular focus will be the PKC and casein kinase dependent phosphorylation motives. Understanding the regulation of Cx46 hemichannels will give an insight into the mechanism of cataract pathogenesis.

Financed by Tansregio TR37 NANOTOME

### **Prophylactic treatment with the COX-2 inhibitor parecoxib after status epilepticus: Effect on epileptogenesis, behavioral alterations, and neuronal damage in rats**

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Inflammatory pathways in epileptic brain are suggested to play a central role in the pathophysiology of epilepsies. Cyclooxygenase 2 (COX-2), an inflammatory mediator, enhances neuronal excitability, decreases seizure threshold and may exacerbate brain injury. Recently, Jung et al. (Neurobiol. Dis. 23, 237-246, 2006) reported that the COX-2 inhibitor celecoxib, administered after a pilocarpine-induced status epilepticus (SE) in rats, prevented neuronal damage and microglia activation in the hippocampus and decreased the likelihood of developing spontaneous recurrent seizures (SRS).

We performed experiments in which we administered the highly selective COX-2 inhibitor parecoxib after a pilocarpine-induced SE in rats. Starting 90 minutes after SE parecoxib was administered twice daily at a dose of 10 mg/kg i.p. over 18 days. Eight weeks after SE induction, the occurrence of SRS was detected by EEG- and video-monitoring over one week for 24 hours per day. As epilepsy is often associated with memory impairment and behavioral

problems, we implemented a behavioral test battery to investigate whether parecoxib treatment is able to weaken epilepsy-associated behavioral alterations.

Our data show that parecoxib does neither prevent epileptogenesis nor reduce seizure frequency, duration, or severity. Parecoxib slightly diminishes impaired visuospatial learning in epileptic rats but does not prevent other epilepsy-associated behavioral alterations. Nevertheless, neurodegeneration was less in parecoxib-treated animals even six months after SE induction in the CA1 region of the hippocampus as well as in the piriform cortex, indicating long-lasting neuroprotective effects of this selective COX-2 inhibitor.

This work is supported by the Georg-Christoph-Lichtenberg Fellowship by the State of Lower Saxony.

### ***In vivo* demonstration of matrix metalloproteinase-3, -9 and -12 mediated demyelination**

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Matrix metalloproteinases (MMPs) are important enzymes mediating extracellular matrix remodeling and contributing to central nervous system diseases such as multiple sclerosis. *In vitro* most MMPs cleave myelin basic protein (MBP), a major component of myelin sheaths. However, little is known about their activity *in vivo*. The aim of this study was to compare the demyelinating potential of MMP-3, -9 and -12 *in vivo*.

Groups of 2-4 SJL-mice were stereotactically injected with 440 ng activated recombinant murine MMP-3, -9, -12 or diluent only into the inferior cerebellar peduncle (ICP). At 12, 24, 72 and 168 hours post injection (hpi) transcardial perfusion and necropsy were performed. One hour before perfusion Evans blue was administered intra-peritoneally. Brains were post-fixed in 10% formaldehyde and embedded in paraffin wax. Immunohistochemistry was performed employing an antibody directed against MBP. Blood brain barrier (BBB) permeability was evaluated by Evans blue and IgG immunofluorescence.

Immunohistochemistry revealed a severe and progressively increasing loss of MBP-immunoreactivity within the ICP of MMP-3-injected mice. In contrast, MMP-9 and -12 induced a moderate degree of demyelination most prominent at 12 and 24 hpi respectively. A significantly increased Evans blue fluorescence was detected in MMP-9 and -12-injected mice, whereas increased IgG-fluorescence was present in MMP-3, -9 and -12-injected mice.

In summary, demyelination was induced *in vivo* by stereotactically injected MMP-3, -9 and -12. The observed differences in the spatio-temporal degree of demyelination and BBB integrity indicate a specific pathomechanism for each MMP.

Supported by a scholarship of the German National Academic Foundation.

## Application of genetically modified dopaminergic progenitor cells in a rat model of Parkinson's disease

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Parkinson's disease is a progressive neurodegenerative disease with a world wide increasing prevalence. Primary symptoms result from the loss of dopaminergic (DA) neurons in the *substantia nigra pars compacta*. Neither pharmacological therapies nor surgical approaches are able to stop the neurodegenerative process. Exogenous cell replacement therapy is considered to be a promising therapeutic tool for the treatment of patients with late stage Parkinson's disease, although just 5-10% of the grafted dopaminergic neurons survive the transplantation procedure.

Our work is concentrated on augmentation of DA neuron survival rate during the post-transplantation interval. As a cell source for transplantation we use ventral mesencephalic neuronal progenitor cells (NPCs) from E12 rat embryos and the unilateral 6-OHDA rat model of Parkinson's disease is used. We are developing a concept of genetically modifying NPCs to overexpress neurotrophic factors. Our recently developed *in vitro* strategy is allowing us to obtain high numbers of transfected neurons in the cell suspension which is transplanted into the neurotoxin lesioned striatum. Using EGFP (enhanced green fluorescence protein) expression plasmid containing the most efficient promoter construct, we have been able to detect strong EGFP signal after 2 weeks *in vivo*.

To optimize the *in vitro* and transfection protocols, different pre-transplantation conditioning of NPCs has been tested. Currently, tyrosine hydroxylase (TH; marker for DA neurons) immunoreactive neurons in the transplants are being quantified by means of stereology and the fiber density of the grafted DA neurons is being evaluated, to define the most efficient procedure.

Expression plasmids of neurotrophic factors, e.g. CDNF (conserved dopamine neurotrophic factor), GDNF (glial cell derived neurotrophic factor), BDNF (brain derived neurotrophic factor), and MANF (mesencephalic, astrocyte-derived neurotrophic factor) will be used in the next experiments.

The effect of intrastriatal transplants will be analyzed functionally by apomorphine and amphetamine induced rotation tests.

This work is supported by the Georg-Christoph-Lichtenberg Fellowship by the State of Lower Saxony, Germany (IK).

## **Error-related negativity (ERN) in remitted depression: an ERP study using the Eriksen Flanker task**

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Depression is characterized by different changes in cognition and behaviour. Abnormalities in error processing have been implicated in the etiology and maintenance of major depressive disorders (MDD). It is proposed that the anterior cingulate cortex (ACC), a part of the limbic system, contributes to cognition by detecting the presence of conflict and error commitment and to alert systems involved in top-down controls to resolve this conflict. Disturbances in cognition and behaviour like in depression and other mental disorders may result from alterations in performance monitoring functions associated with this region of the brain.

One electrophysiological correlate of behavioural changes in depressed patients is the error-related negativity (ERN), a large negative deflection generated in the ACC with a mediofrontal distribution in the EEG. The ERN amplitude changes in acute depression showing a significantly smaller ERN compound that might represent attenuated effects of error evaluation and action monitoring. To our knowledge the characteristics of the ERN in remitted depressive patients have been insufficiently described.

Aim of the study is to extend the knowledge of the ERN compound in remitted depression using the Eriksen Flanker task, a speeded decision making task that elicits the ERN.

Nineteen fully or partially remitted depressed women (mean age = 51.6, SD = 8.02; status of remission > 6 months, BDI score <19) and nineteen non-depressed female comparison subjects (mean age = 50.1, SD= 8.29) participated in the study. Across all conditions remitted depressed did not show significant differences ( $p = <0.05$ ) in ERN amplitude after erroneous button response relative to the comparison group that might reflect restored error evaluation processing. In contrast to the control subjects remitted patients did show significantly longer reaction times for stimuli processing compared to controls that might reflect mild cognitive impairment residuals due to the history of depression.

This work is supported by a Georg-Christoph-Lichtenberg scholarship of the state of Lower Saxony to Alexander Karabatsiakis

## **Studies on the therapeutic potential of adult stem cells in the G93A animal model of amyotrophic lateral sclerosis (ALS)**

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Adult stem cells have recently come into the focus of neurological research. It was shown that hematopoietic stem cells derived from human umbilical cord blood (hUCBC) could differentiate into cells with neuronal and glial like phenotypes in vitro. While a direct replacement of degenerating neurons by stem cells does currently not appear feasible in motor neuron disorders, they could possibly protect motor neurons by release of neurotrophic factors. hUCBC delayed ALS symptoms in a mouse model after intravenous administration. Because of the

blood-brain-barrier, the establishment of local administration of the cells appears to be beneficial.

Injection of hUCBC into the spinal cord of G93A ALS transgenic mice has not been done yet. In the present study we therefore established a reproducible method of intraspinal transplantation of hUCBC. The effects of the transplantation will now be assessed by survival analysis, evaluation of motor performance and immunohistological and molecular biological approaches. hUCBC were isolated via Ficoll density gradient centrifugation and CD34 or CD133 associated MACS beads. The stem cell enriched population then was expanded in presence of stem cell growth factors for 8 days. 100.000cells/ $\mu$ l in a volume of 1 $\mu$ l per side were administrated bilaterally into the ventral horn region of lumbar spinal cord. For better localization of the transplant in the spinal cord, in some experiments hUCBC were lentivirally transduced to expressed enhanced green fluorescent protein (eGFP). Besides the examination of locomotor activity and survival time, we will perform immunohistochemical analysis to detect surviving stem cells as well as positive effects of the transplants on motor neuron survival.

The surgery was well tolerated and did not cause any persisting motor impairment of the animals. A survival study of G93A mice transplanted either at the presymptomatic stage (d40) or after symptom-onset (d90) is currently ongoing. First results of the effects of transplantation on survival time and motor performance will be shown.

## **Role of FGF-2 during Development of the Substantia Nigra**

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Massive loss of dopaminergic neurons in the substantia nigra pars compacta (SNpc) leads to characteristic symptomatology of Parkinson's disease. Understanding of the processes involved in the development of SNpc may contribute to an improvement of several therapeutic approaches, like the intrastriatal cell replacement strategies.

Previous *in vitro* and *in vivo* studies indicate that basic fibroblast growth factor (FGF-2) plays an important role in the development and maintenance of the ventral mesencephalic dopaminergic system (Grothe and Timmer, Brain Res. Rev. 54, 2007). Our group has studied the physiological function of the endogenous FGF-2 system by evaluation of the adult nigrostriatal system of FGF-2 deficient and overexpressing mice, as well as mice lacking FGF-receptor 3 (FGFR3). The loss of endogenous FGF-2 revealed an increased volume of SNpc and higher number of tyrosine hydroxylase immunoresponsive dopaminergic neurons, whereas the overexpression of FGF-2 showed an opposite effect. In addition, FGFR3 seems to be crucially involved in regulation of the number of dopaminergic cells in adult SNpc (Timmer et al., J. Neurosci. 27, 2007). The consequent challenge is to define the developmental stage where the FGF-2 signalling is critical for proper development of SNpc. FGF-2 may affect cell differentiation, migration or cell death.

At the moment we are evaluating the mice deficient for FGF-2 morphometrically at different developmental stages between E14 and P28, which include two important phases of development of SNpc in mice: differentiation and maturation. Our preliminary data suggests that the pivotal role of FGF-2 signalling is taking place before the maturation stage, as morphological

differences already occur at P0 stage suggesting an altered differentiation or migration of nigral dopaminergic neurons. Further experiments should delineate the certain time window where FGF-2 is acting. Molecular biological characterization of several marker genes for dopaminergic neurons may depict altered physiological events during differentiation phase of the SNpc development.

This work is supported by the DFG grand for Prof. C. Grothe

### **Effect of deep brain stimulation on cognitive and neuropsychiatric function in rat models of Parkinson's disease**

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Deep brain stimulation (DBS) is an established treatment for movement disorders like Parkinson's disease (PD). The typical disturbance of motor functions in this disease due to the progressive loss of dopamine (DA) neurons in the substantia nigra can be improved by stimulation of the subthalamic nucleus (STN). The STN is a critical node of the basal ganglia motor loop but it also has a central position within associative and limbic circuits.

Recently more attention has also been paid to the cognitive and neuropsychiatric disturbances caused by PD itself and by therapeutic approaches like dopaminergic medication and DBS. Therefore, we investigate the impact of high frequency DBS of the STN on the behavioral outcome in two different PD rat models.

In the 6-hydroxydopamine (6-OHDA) rat model the retrograde degeneration of dopaminergic neurons in the substantia nigra is induced by bilateral injection of 6-OHDA into the striatum. Rats with 6-OHDA lesions make more errors during continuous alternation, indicating disturbed learning and memory. Additionally, the apomorphine and MK801 induced PPI-deficit is stronger in 6-OHDA lesioned rats compared to controls, indicating enhanced sensitivity for psychoactive drugs.

The haloperidol rat model is a common transient Parkinson model. Haloperidol is a dopamine-2-receptor antagonist that leads in higher doses to a cataleptic Parkinson-like state often used to analyze antidepressive substances.

In the next step we will investigate the behavioral changes in freely moving, high frequency STN stimulated rats, in the food-choice-test and the food-preference-test for motivation, in object recognition for cognitive functions, in PPI for altered psychiatric state, and in locomotor activity for motor impairment.

## ***In vivo* evaluation of exogenous polysialic acid effects on peripheral nerve regeneration after substance loss**

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Endogenous polysialic acid (polySia) is a homopolymer of sialic acid residues. When bound to the neural cell adhesion molecule polySia plays a crucial role in plasticity during processes related to learning and memory as well as regeneration in the nervous system. We are developing polySia-based scaffolds in order to increase peripheral nerve regeneration (PNR) across long gaps. We did demonstrate before that polySia substrate has no negative effects on primary neurons and glia cells *in vitro*, here we present first *in vivo* results. In a first study a 10 mm rat sciatic nerve gap was bridged by silicone tubes filled with growth factor reduced matrigel<sup>TM</sup> (matrigel) (1) alone, (2) plus soluble polySia, (3) plus Schwann cells (SC), (4) plus polySia and SC. Eight weeks after surgery, polySia treated animals showed significantly enhanced numbers of regenerated myelinated axons. Also the outcome of motor recovery (electrodiagnostic measurements) was positively influenced. Pre-labeling of transplanted SC and retrograde tracing experiments revealed no negative effects of exogenous polySia on SC survival and regenerating motor and sensory neurons. In a second study a 13 mm nerve gap was either bridged by tubes filled with Matrigel + polySia + SC like the experimental group 4 (see above) or by autologous nerve grafts (clinical standard). Over 10 weeks the functional recovery was monitored in Rotarod-test and sciatic function index (for motor recovery), as well as pinch- and withdrawal-test (for sensory recovery). While the pinch-test revealed a higher speed of sensory recovery after transplantation of exogenous polySia-containing nerve bridges, the other tests showed no differences between the two transplantation conditions. Finally, electrodiagnostic measurements showed reinnervation of the gastrocnemius muscle in 30% of the polySia-grafted animals but 100% in the group implanted with autologous grafts. We demonstrate here biocompatibility of exogenous polySia and show its potential to improve PNR.

Financially supported by DFG-FOR-548/2

## **Neuroethological studies on the neuronal basis of three-dimensional allocentric spatial cognition in mammals**

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By recording from single cells and small cell clusters in the hippocampus of microbats my project sets out to investigate how three-dimensional allocentric space is represented in the mammalian brain. The recording will be done with implanted micro-electrodes and a miniaturised amplifier/telemetry system while the experimental animals are performing spatial

orientational tasks. The neuronal activity will be examined for dependencies on parameters such as (i) position in 3D space, (ii) flight direction (iii) production of echolocation calls.

Intense studies during the last decades revealed that the hippocampus plays an important role in mammalian spatial cognition, with so-called place cells being the most likely candidate for the neuronal substrate of a cognitive spatial map. Whenever place cells have been investigated in freely moving animals hitherto, the experiments have almost exclusively taken place in a two-dimensional experimental environment. This can partially be referred to the limited movement capabilities of the preferentially used model organisms (rodents and primates), but even where place cells have been described in bats they could only be characterised two-dimensionally. Recognising the enormous potential bats have as a model organism for spatial cognition my project sets out to investigate the three-dimensional response characteristics of place cells by recording from flying microbats and to provide new insights into the neuronal mechanisms that underlie allocentric mammalian spatial cognition.

Funding by the Studienstiftung des deutschen Volkes (doctoral scholarship to DS).

### **Characterization of microglial phenotypes during de- and remyelination**

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Present immunomodulating therapies for multiple sclerosis (MS) are only partially effective and do not promote the regeneration and remyelination. Microglia play an important role in central nervous system (CNS) inflammation and recent studies suggest their involvement in neuroprotective events. The effective phagocytosis of myelin debris by microglia/macrophages as well as expression of certain immunoregulatory surface markers, including chemokine receptors, and secretion of cytokines seem to play an important role in remyelination. There are indications that different microglial phenotypic characteristics, which additionally could be region-specific, are determining their roles in the CNS. Feeding mice with cuprizone and its subsequent retrieval from the food lead to complete demyelination and remyelination respectively in both white (corpus callosum) and gray (cortex) matter and is accompanied with the accumulation of microglia in these areas. The absence of the peripheral inflammatory component and an intact blood-brain barrier make the cuprizone model particularly convenient for the investigation of the intrinsic microglial function during de- and remyelination.

We examined microglial phenotypes in the cuprizone model in order to identify factors which could promote regeneration in the CNS. 8 week old C57BL/6 mice were fed for 5 weeks with food containing 0,2% cuprizone, following one week of normal food and were sacrificed at week 3, 3.5, 4, 4.5, 5 and 6, parallel with the non-treated mice which were sacrificed at week 0, 3 and 6 (controls). Microglia were separately isolated from dissected corpus callosum and cortex, stained for various surface and intracellular markers (CD11b, CD45, F4/80, MHC-II, CD40, CD80, CD86, CCR5, CD32/16, IL-12, IL-10, IFN- $\gamma$ , TNF- $\alpha$ , CD200R, TREM-2b) and measured with flow cytometry (FACS). Since the experiment is still in progress, the expression pattern of microglial pro- and anti-inflammatory factors will be presented on the poster.

This work has been supported by Georg-Christoph-Lichtenberg Stipendium of Lower Saxony, Germany

## **Differences in sensitivity to the convulsant pilocarpine in substrains and sublines of C57BL/6 mice**

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In rodents, the cholinomimetic convulsant pilocarpine is widely used to induce status epilepticus (SE), followed by hippocampal damage and spontaneous recurrent seizures, resembling temporal lobe epilepsy. In the present study, we compared the effects of pilocarpine in three (C57BL/6) B6 substrains (B6JOla; B6NHsd; B6NCrl) that were previously reported to differ in several behavioral and genetic aspects. In B6JOla and B6NHsd, only a small percentage of mice developed SE independently of whether pilocarpine was administered at high bolus doses or with a ramping up dosing protocol, but mortality was high. The reverse was true in B6NCrl, in which a high percentage of mice developed SE, but mortality was much lower compared to the other substrains.

However, in subsequent experiments with B6NCrl mice, striking differences in SE induction and mortality were found in sublines of this substrain coming from different barrier rooms of Charles River. In B6NCrl from barrier 8, administration of pilocarpine resulted in a high percentage of mice developing SE, but mortality was low, whereas the opposite was found in B6NCrl mice from four other barriers of the same vendor. We supposed a spontaneous mutation risen inside of pilocarpine-sensitive mice of barrier 8. Pilocarpine-sensitive female mice of barrier 8 and pilocarpine-resistant male mice of barrier 9 were used to generate a hybrid F<sub>1</sub>- generation. Only male F<sub>1</sub>-mice exhibited a significantly higher pilocarpine sensitivity than male parental generation of barrier 9. These results strongly indicate that a spontaneous mutation with recessive inheritance could have risen on the X-chromosome. Supporting our hypothesis F<sub>3</sub>-offsprings of B6-mice exhibited a SE-induction rate which did not differ from pilocarpine-sensitive animals of barrier 8.

These differences in B6 substrains and sublines offer a unique opportunity to identify the genes and pathways involved and contribute to a better understanding of the underlying molecular mechanisms of seizure susceptibility.

## **STEROID RESPONSIVE MENINGITIS-ARTERITIS IN DOGS: ROLE OF CYTOKINES AND TOLL-LIKE RECEPTORS**

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"Steroid Responsive Meningitis-Arteritis" (SRMA) is a highly painful disease characterized by inflammatory-stenosing lesions of the arteries especially in the cerebrospinal meninges and coronary vessels as well as by massive suppurative inflammation of the meninges. It is a recognized animal model for human immune mediated vasculitis.

Previous neuroimmunological studies suggested that concomitant elevation of immunoglobulins A (IgA) levels in both serum and cerebrospinal fluid (CSF) are specific for SRMA throughout the different stages of the disease and also during long-term treatment with glucocorticosteroids. Specificity and sensitivity of elevated IgA levels in 525 paired CSF and serum samples were evaluated to clarify how specific these findings are for the clinical diagnosis of SRMA in dogs. Serum and CSF IgA levels were significantly higher in dogs in the acute form of SRMA in comparison to dogs with other diseases. The sensitivity for simultaneous elevation of IgA levels in serum and CSF was 91 % with a specificity of 78 %.

Consequentially, examination of specific cytokine patterns in CSF and serum is performed to clarify underlying causes for increased IgA production (interleukin-6, IL-6 and Transforming growth factor  $\beta$ , TGF- $\beta$ ) and increased vascular permeability (Vascular endothelial growth factor, VEGF). IL-6 and VEGF were analysed using an ELISA technique and will be examined functionally with cell culture methods. Up to now values from 30 dogs with SRMA were compared with 30 dogs affected with other diseases. Preliminary findings showed that CSF IL-6 levels were increased in dogs during the clinical manifestation of SRMA.

It is hypothesized that SRMA is caused by environmental factors. Since isolation attempts to find an infectious agent failed, we planed an indirect method and examined Toll-like receptors (TLRs, a recent discovered family of pattern recognition receptors). Expression of TLRs is measured using immunophenotyping and flow cytometry.

### **pH-dependency of recombinant glycine receptor channels**

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Glycine receptor channels are expressed throughout the CNS (Central Nervous System), working as a primary inhibitory neurotransmitter. Glycinergic neurotransmission play an important role in voluntary motor control, reflex response, reciprocal and recurrent inhibition. Since fluctuations of the blood pH value occur under some physiological and pathological conditions, effects of altered pH on glycine receptor function become an interesting topic for inhibitory neurotransmission. We studied the altered kinetics of glycinergic currents under both alkaline and acidic conditions, using the patch clamp technique in combination with fast agonist application system.

Our results showed a negative functional effect on  $\alpha 1$  homomeric glycine receptor channels under alkaline conditions (pH =8.5). The relative area-under-current curve (rAUC), relative current amplitude of steady state (rCdes) significantly reduced. When the extracellular pH regulated to 6.2, reduced rAUC was observed. This negative effect became more pronounced when a more acidic pH was reached. Under pH 5.2 and pH 4.5, the glycinergic current desensitized faster. Both fast and slow component of the time constants are significantly smaller than those under pH 7.2. Reduced rAUC and rCdes were observed as well. On  $\alpha 1\beta$  heteromeric

glycine receptors, both alkaline and acidic extracellular pH (pH =8.5 and 4.5) negatively modulated the glycinergic currents, but in a less pronounced way.

Our study provided a potential cause for an impaired glycinergic inhibitory neurotransmission as pH fluctuations occur in the CNS especially under pathological conditions like epileptic seizure or ischemia.

This work was supported by a Georg-Christoph-Lichtenberg scholarship of the state of lower saxony to Y. Song and a grant of the Deutsche Forschungsgemeinschaft to J. Bufler and K. Krampfl (DFG, Bu938/8-2). The authors thank Ms. J. de la Roche for expert help with cDNA preparation and maintenance of cell cultures, and A. Niesel and J. Kilian for technical support.

### **Auditory implant project: Midbrain and Nerve Implants**

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The overall goal of this project is to develop and test novel auditory implants beyond the cochlear implant. For my project I have worked on implants for the auditory nerve and midbrain. The first phase of this project tested the next generation of the Auditory Midbrain Implant (AMI), currently in clinical trials. The AMI, which is implanted into the inferior colliculus (IC), targets the restoration of auditory sensation in patients who no longer have an intact auditory nerve by stimulating the brainstem directly. For these experiments we used 2 human implants in a guinea pig model to evaluate the cortical responses to stimulation of multiple sites of the IC simultaneously. To do so we recorded from the primary auditory cortex with a penetrating multi-shank Michigan probe during AMI stimulation. Offline we analyzed the cortical evoked potentials, which correspond to the synaptic activity entering the cortex.

What we discovered was that multi-site stimulation of the IC yields non-linear responses dependant on interstimulus delay. The first region of interest was +-10ms, which showed a marked potentiation dependant on rostral caudal placement of stimulation. This potentiation was then followed by a marked depression with almost a full recovery by 100ms or more. Our results suggest this potentiation may be temporal integration taking place at the collicular or thalamic level.

In the second phase of this project, the auditory nerve implant, acute experiments in cats are ongoing and have yet to be analyzed. In this project we are developing an implant that stimulates directly the auditory nerve, overcoming the main drawback of cochlear implants: stimulation through the bony wall of the modiolus. Once concluded and analyzed with a positive outcome, we will begin chronic implant experiments. These final experiments will evaluate the long term stability, safety and induced neural plasticity of the implant.

Funding provided by SFB 599, Cochlear Ltd. and Medizinische Hochschule Hannover

## **Keeping the gate – experience-dependent neuronal plasticity and stability in an output station of the amygdala**

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The mammalian amygdala plays a key role in the processing of emotions and eliciting of emotional reactions. Sensory information is filtered, evaluated and may lead to behavioural and physiological responses. The central nucleus of the amygdala, which is the major output nucleus, seems to be important for analysing the emotional relevance of sensory information. Via amygdaloid projections to the brainstem and the hypothalamus, the output nucleus mediates stress reactions and impairs the autonomic nervous system.

The present project sets out to study the effects of aversive experiences (i.e. immobilization stress) on the neuronal structural level of the central nucleus of the amygdala. In addition, behavioural as well as physiological alterations after immobilization are investigated to determine the stress level of the experimental animals (a well-established bat model, *Carollia perspicillata*).

For the behavioural experiments, stressed animals and controls were tested in a custom-made 3D-plus maze for bats, an experimental design that consists of four arms (two open and two enclosed ones) and serves as behavioural test for anxiety, which is supposed to correlate with the stress level of the animals. To verify the behavioural data stress hormone levels of the animals were determined. Glucocorticoids, like cortisol, are released in response to stressful situations. Fecal sampling was chosen to exclude the necessity of handling of the animals. In contrast, sampling of blood or saliva may be stressful itself. Another advantage of fecal sampling is that each individual can be used as its own control. On the one hand, this leads to a reduction of the total number of animals used for research. On the other hand this minimizes variation of the results due to individual variability of the animals.

To study the effects of aversive experiences on the neuronal structural level of the central nucleus of the amygdala the corresponding brains were prepared for morphometric analysis of cell density, spine density and morphometry.

This work is supported by a Georg-Christoph-Lichtenberg Fellowship of the Ministry for Science and Culture of Lower Saxony.

## **Microglial responses in organotypic spinal cord slice cultures - An in vitro model of canine spinal cord injury**

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Spinal cord injury (SCI) represents a common traumatic disease in veterinary and human medicine. The pathogenesis of inflammatory events during SCI remains largely undetermined and is discussed controversially. Since dogs are frequently affected by SCI, the aim of the study was to establish an in vitro model to investigate glial responses in traumatized spinal cord

tissue. Therefore, organotypic slice cultures of spinal cord cross sections (350  $\mu\text{m}$  thickness) were incubated in medium at 37°C for 3, 6, 9 and 12 days, respectively. Subsequently, slices were processed for light and electron microscopy. Further, glial responses were characterized by immunohistochemistry using major histocompatibility complex class II (MHC-II)- and myeloid/histiocyte antigen (Mac-387)-specific markers as well as lectin histochemistry using the lectin of *Bandeireaea simplicifolia* (BS-1) as a marker for microglial cells. Real time quantitative polymerase chain reaction (Real time qPCR) was performed on frozen tissue slices for the quantification of the cytokines tumor necrosis factor (TNF)- $\alpha$ , interleukin-(IL)-2, IL-6 and IL-10. Histology revealed a swelling of axons and myelin sheaths as well as the occurrence of gitter cells starting at day 3 in culture. Electron microscopy confirmed myelinophagia by ameboid gitter cells. The number of BS-1- and MHC-II-positive gitter cells significantly increased in a time-dependant manner. Real time qPCR demonstrated a transient increase of the expression of TNF- $\alpha$  and IL-10 at day 3 and an upregulation of IL-6 during the whole culture period. The results demonstrate that organotypic spinal cord slice cultures represent a useful model to study basic glial reactions associated with SCI on a morphological and molecular level. Furthermore, the results suggest that phagocytic microglial cells might have immunomodulatory and -activating functions and play a role in the initiation and regulation of inflammatory events during canine SCI by the expression of pro- and anti-inflammatory inflammatory cytokines.

Supported by Georg-Christoph-Lichtenberg Scholarship to Ingo Spitzbarth, ZSN-Hannover

### **Investigation of the small-conductance calcium-activated potassium channel SK2 and the GABA<sub>A</sub> receptor subunit $\alpha$ 1 expression in the basal ganglia in the rat amygdala-kindling model of temporal lobe epilepsy**

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Experimental and clinical data show an involvement of the basal ganglia in the propagation and modulation of seizure activity. Considerable plastic network changes were described in the rat amygdala-kindling model of temporal lobe epilepsy.

In the present study kindling-induced basal ganglia network changes were determined on subcellular level. SK2 channels are widely expressed in neurons and play an important role in dendritic excitability and synaptic plasticity including spontaneous activity of SNr neurons. In addition, experimental data revealed alterations of GABAergic inhibition within basal ganglia brain regions of kindled rats. We hypothesize that alterations of subunit composition of GABA<sub>A</sub> receptors and/or changes in the expression pattern of SK2 channels could be involved in kindling-induced basal ganglia network changes.

The expression pattern of the SK2 channels and the GABA<sub>A</sub> receptor subunit  $\alpha$ 1 were investigated in kindled rats and two groups of nonepileptic controls. Adult female rats were kindled once daily by stimulation of the right basolateral amygdala until ten secondarily

generalized seizures were elicited. The number of SK2 positive neurons was counted stereologically in the SNr and the subthalamic nucleus (STN) of kindled rats and controls. Analysis of kindling-induced changes in the distribution of GABA<sub>A</sub> receptor subunit  $\alpha 1$  in the SNr was performed by semi-quantitative image analysis of the optical density in stained sections by a computer-assisted system. The resulting data showed no significant differences in the expression pattern of the GABA<sub>A</sub> receptor subunit  $\alpha 1$  in the anterior and posterior SNr and of the SK2 positive neurons in the anterior and posterior SNr and the STN of kindled rats compared to control rats. While statistical significance was not reached for the GABA<sub>A</sub> receptor subunit  $\alpha 1$ , a trend reflecting an upregulation was seen bilaterally in the anterior SNr. Ongoing studies investigate further GABA<sub>A</sub> receptor subunits in the basal ganglia of kindled rats.

This study is supported by a 'Georg-Christoph-Lichtenberg-Stipendium des Niedersächsischen Ministeriums für Wissenschaft und Kultur' and 'Deutsche Forschungsgemeinschaft: GE1103'.

### **Developing a new quantification method for musician's dystonia in violinists**

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Musician's dystonia (MD) is a task-specific movement disorder with a loss of voluntary motor control in highly trained movements. Involuntary flexion or extension of individual fingers fundamentally impairs technical skills on the instrument and provokes irregularities in playing. Current methods to evaluate severity of MD include rating scales and a few objective methods. Most of these methods do not provide fine enough resolution to quantify severity of MD and have not yet been examined for reliability and validity. The only method found to be objective, reliable, valid and responsive was the Musical Instrument Digital Interface (MIDI)-based Scale Analysis, a method which however is only available for pianists<sup>1</sup>.

We are currently developing a method for objective quantification of the temporal and spatial properties of playing of the right hand in violin players. Clinical experience in our outpatient clinic shows that symptoms of MD lead to an impaired temporal regularity in finger pressing on the string and probably also to less accurate pitch depending on the severity of MD.

A violin with an added electrical circuitry is used for measuring. Voltage in the electrical circuitry changes over the length of the string and is measured while playing. The onset of the output signal can be analysed for temporal irregularities, and its amplitude for misplacement of the fingers on the board affecting pitch accuracy.

The aim of this work is to study motor output in professional violinists and to develop an objective method to quantify symptoms of MD in violinists reliably and validly.

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This work is supported by the Center for Systemic Neurosciences Hanover and the “Georg-Christoph-Lichtenberg”-Stipendium of lower saxony, Germany.

**Effects of the co-application of the topical antiseptics amylmetacresol and dichloro-benzylalcohol on the voltage-gated neuronal sodium channel NaV1.2**

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**Introduction:** The drug combination of amylmetacresol (AMC) and dichlorobenzylalcohol (DCBA) is commonly used for topical treatment of upper respiratory tract infections. In addition to their bactericidal and anti-inflammatory properties, these substances have pain-relieving effects that have not been characterized systematically before. Since earlier studies demonstrated a local anesthetic-like activity of phenol derivatives, it is the objective of this in vitro-study to analyze effects of a co-application of the phenol-based antiseptics amylmetacresol and dichlorobenzylalcohol on the voltage-gated sodium channel, an important target site for analgetic drugs.

**Material and methods:** The  $\alpha$ -subunit of the voltage-gated neuronal sodium channel NaV1.2 was stably transfected in human embryonic kidney cells (HEK 293). Sodium inward currents were elicited using whole-cell patch clamp experiments. The effect of the co-application of DCBA, in concentrations of 100, 300 and 500  $\mu$ M, respectively, with different micro molar concentrations of AMC on the resting state and on the fast inactivated state of the sodium channel was analyzed.

**Results:** The co-application of DCBA and AMC reversibly blocked depolarization-induced inward sodium currents. It showed a voltage-dependent inhibition of the sodium channel NaV1.2. The inactivated channel state was blocked preferentially, indicated by an apparent concentration-dependent shift of the channel availability curve towards more hyperpolarized potentials. This is a general characteristic of local anesthetic-like activity.

**Conclusion:** The combination of AMC and DCBA reversibly blocks voltage-gated sodium channels. The local anesthetic-like effect of this combination could explain the analgetic property of these topical antiseptics.

This work has been supported by a grant from Reckitt Benckiser.

## **Identification of growth factors involved in the demyelination/remyelination in the murine cuprizone model**

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In recent years in vitro and in vivo studies indicate that neurotrophins, and neurotrophic cytokines support migration, proliferation, differentiation of glial cells and regulate myelin synthesis. Using these powerful agents to protect glial cells and neurons from damage or enhance remyelination may open new possibilities for Multiple Sclerosis (MS) therapy.

Cuprizone feeding is a commonly used animal model to study experimental de- and remyelination. Cuprizone intoxication leads to oligodendrocyte death and a subsequent reversible demyelination in the corpus callosum (cc) and in the cortex (ctx). The time course and dynamics of demyelination/remyelination differ in the corpus callosum and in the cortex. To address this question and investigate the implication of growth factors in demyelination/remyelination in the white and grey matter the profile of growth factors expression in the cc and ctx was analysed using laser microdissection and real-time PCR techniques.

Demyelination was induced in 8-week-old male C57BL/6 mice by feeding 0.2% cuprizone for 4,5 weeks. Gene expression of BDNF, CNTF, IGF-1, LIF, Neuregulin and TGF- $\beta$  were analysed in the cc and ctx at 9 time points (Demyelination phase: week 1, 2, 3, 4, 4.5; Remyelination phase: week 5, 5.5, and week 6). The mRNA expression pattern of growth factors in the white and grey matter will be presented on the poster.

This work is supported by a Georg-Christoph-Lichtenberg scholarship of the state of Lower Saxony to Viktoria Gudi

## **Glutamate transporters in retinal neurons**

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Processing of visual information already starts in the retina where photoreceptors, bipolar cells and ganglion cells are connected in a vertical signal transmission pathway. Glutamate is the major excitatory neurotransmitter in the retina and tonically released by photoreceptors in the dark. Fast removal of glutamate from the synaptic cleft is required to terminate synaptic transmission and to keep the neurotransmitter concentration below neurotoxic levels. This function is performed by a family of five excitatory amino acid transporters (EAATs) which couple the uptake of glutamate stoichiometrically to the cotransport of three sodium ions and one proton, and the countertransport of one potassium ion. In addition to this transport current, a passive flux of chloride ions was observed which is uncoupled from substrate translocation. The aim of this study is the functional characterization of glutamate transporters in the mammalian retina. Main focus will be set to EAAT5 which is known to be mainly expressed in the retina. By Immunohistochemical staining of mouse retina we detected EAAT5 exclusively

presynaptically in the axon terminals of the photoreceptors. Confocal microscopy of heterologously expressed, GFP-tagged mouse EAAT5 in the mammalian cell line tsA201 revealed an almost complete expression of the transporter in the membrane. Patch-Clamp measurements of the heterologously expressed EAAT5 showed that these transporters are functional. With  $\text{SCN}^-$  ( $\text{NO}_3^-$ ) as charge carrier we measured a voltage- and glutamate-dependent anion current with a  $\text{IC}_{50}$  for glutamate of 27  $\mu\text{M}$ . To our surprise we were not able to detect any glutamate uptake activity after application of radiolabeled glutamate. These results indicate that EAAT5 in photoreceptors is not responsible for glutamate uptake. Instead, the anions entering the axons through EAAT5 after glutamate activation may lead to a reduction of the glutamate release in the dark.

Cuprizone ameliorates spinal cord inflammation in a viral model of multiple sclerosis

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Theiler's murine encephalomyelitis virus (TMEV)-infection induces a demyelinating leukomyelitis in susceptible mice and serves as an important animal model for chronic progressive multiple sclerosis. In the present study, viral infection was combined with application of cuprizone to investigate the impact of toxic demyelination on the progression of Theiler's murine encephalomyelitis in SJL-mice.

TMEV-infected SJL-mice were fed with cuprizone for five weeks starting 35 days post infection (dpi). A RotaRod<sup>®</sup>-test was performed weekly to study motor coordination. Formalin-fixed, paraffin-embedded spinal cord samples were taken at 42, 49, 70, 98, 147, 196 and 245 dpi. A semiquantitative scoring system was used to evaluate the degree of inflammation by histology. In addition, the distribution of TMEV as well as B-cells (CD45R) and T-cells (CD3) were evaluated by immunohistochemistry.

Animals treated with cuprizone and TMEV (group 1) showed a temporary (84 until 140 dpi) improved motor coordination compared to TMEV-infected animals without cuprizone treatment (group 2). Histopathologically, the severity of leukomyelitis was reduced in group 1 compared to group 2 on 98, 147 and 196 dpi. Additionally, on 147 dpi the numbers of CD3- and CD45R-positive cells in the spinal cord were decreased in group 1 compared to group 2. Group 1 showed less TMEV-positive cells in the spinal cord on 196 dpi compared to TMEV-infected animals without cuprizone treatment.

In conclusion, cuprizone administration has a beneficial effect upon the development of virus-induced spinal cord inflammation in SJL-mice indicating that cuprizone might act as an immunomodulator and/or inhibitor of virus spread.

This work is supported by the Georg-Christoph-Lichtenberg Fellowship by the State of Lower Saxony.

## Comparison of rats from different breeders after induction of status epilepticus by prolonged electrical stimulation of the basolateral amygdala

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Post-status-epilepticus models, in which rats develop spontaneous recurrent seizures (SRS) 4 to 6 weeks after the induction of a status epilepticus (SE) are suitable models for temporal lobe epilepsy (TLE) – the most common type of epilepsy in adult humans. Rats in which the SE is induced by prolonged electrical stimulation of the basolateral amygdala (BLA) via a depth electrode represent a well established post-SE-model. However, over the last years a drift in the outcome of this model was observed: A reduced fraction of animals developed a self sustained SE (SSSE) after electrical stimulation and also the number of animals which showed the typical neurodegeneration of the hippocampus after the experience of a SE was lower than in earlier experiments. Accordingly, former trials showed that fewer animals than expected developed SRS 9 weeks after induction of SE [1]. This indicates a genetic drift in the Sprague Dawley rats from Harlan, which we used over recent years for these experiments, resulting in a lower sensitivity of the BLA to electrical stimulation.

Therefore, we want to investigate whether rats of other breeders or other rat strains than Sprague Dawley (SD) rats from Harlan Winkelmann (GER) or Harlan (NL) usually used by our group are more susceptible to BLA stimulation in this model of TLE. Interestingly, recent experiments of our lab showed that Wistar rats from different breeders exhibit high sensitivity to amygdala kindling [2], another model of TLE, which is also induced by electrical stimulation of the BLA, so that Wistar rats could constitute an interesting alternative strain for SE induction by BLA stimulation.

In a first series of experiments, we stimulated SDs from Harlan (NL), Charles River (GER) and Janvier (F) and also Wistar-Han rats from Janvier via the BLA and compared them with results of SDs from Harlan Winkelmann (GER). We found, that the incidence of rats developing a generalized SE (type 3) or a partial SE with generalized seizures (type 2), which is necessary for the development of SRS in SDs [3], was lower in SDs from all compared breeders than in SDs of Harlan Winkelmann. Even a lower fraction of rats with SE was reached in Wistar-Han rats.

Furthermore, preliminary data show that the SD rats of Harlan, which experienced a type 2 or 3 SE, show significant less severe neurodegeneration of the hippocampus than did SD rats of Harlan Winkelmann.

In ongoing studies using continuous video- and EEG-monitoring, we want to investigate if and how many of the rats of Charles River and Janvier develop SRS. Neurodegeneration in the hippocampus will be investigated by evaluating thionine stained brain sections and also by immunohistochemistry.

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## ZSN-Colloquium 2009

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This project is supported by a Georg-Christoph-Lichtenberg Fellowship by the State of Lower Saxony.