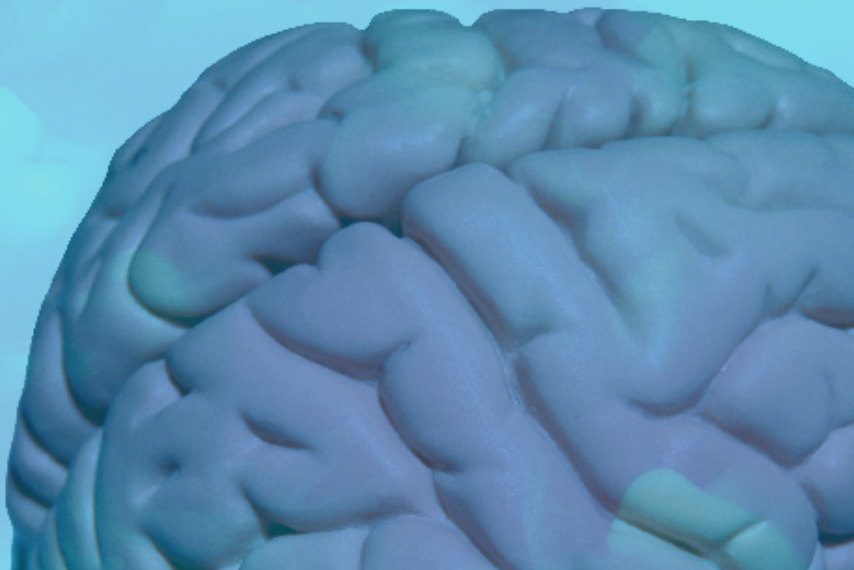
INNSBRUCK MEDICAL UNIVERSITY NEUROSCIENCE

Neuroscience Day 2010

Wednesday, 16th June 2010. Congresspark Igls.

Final Program and Abstract Book



08:00 - 09:00 Registration and Poster Set-Up

09:00 - 09:15 **Georg Dechant**
SNI Executive Committee Chair
Introduction and Opening

KEYNOTE LECTURE
Chair: Francesco Ferraguti

09:15 - 10:30 **Andreas Lüthi**
Friedrich Miescher Institute for Biomedical Research
Basel, Switzerland
„Defining the neuronal circuitry of fear."

PRESENTATIONS: SESSION I
Chair: Christoph Schwarzer

10:30 - 10:45 **Daniela Busti**
Institute of Pharmacology, IMU
„Structural and functional diversity of the intercalated cell masses of the amygdala."

10:45 - 11:00 **Bernhard Loy**
Institute for Neuroscience, IMU
„P38 Mitogen Activated Protein Kinase controls cholinergic differentiation of sympathetic neurons."

11:00 - 11:15 **Michael Fritz**
Department of Experimental Psychiatry, IMU
„Cocaine relapse prevention by social interaction and simga1 receptor activation: Development of an animal model."

Lectures

11:15 - 11:30 Break

PRESENTATIONS: SESSION II
Chair: Lars Klimaschewski

11:30 - 11:45 **Simone Mader**
Clinical Department of Neurology, IMU
„AQP4-IgG autoantibodies as biomarkers for Neuromyelitis optica and their relevance for disease pathogenesis."

11:45 - 12:00 **Iris Kastenberger**
Institute of Pharmacology, IMU
„Anxiogenic effects of oestrogen are mediated through the dynorphin-kappa opioid receptor system."

12:00 - 12:15 **Maria Auer**
Division of Neuroanatomy, IMU
„Effects of Rho inhibition on peripheral axon regeneration."

12:15 - 13:30 Lunch

POSTER SESSION

13:30 - 15:30 Poster Session

15:30 - 16:00 Coffee Break

16:00 - 16:15 Poster Prize Awards

Conference Venue

Congresspark Igls
Eugenpromenade 2
6080 Igls
T: 043 512 377364

Directions: Please use the highway exits A12 - Innsbruck Mitte, Ost or West or highway A13 exit Patsch and then drive to Igls and congresspark.

Organization

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ZEISS

Abstracts

T 1: Daniela Busti

Structural and Functional Diversity of Intercalated Cell Masses of the Amygdala

D. Busti[1], R. Geracitano[2], N. Whittle[3], Y. Dalezios[4,5], M. Manko[2], W.A. Kaufmann[1], K. Sätzler[6], N. Singewald[3], M. Capogna[2] and F. Ferraguti[1]

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Intercalated cell masses of the amygdala (ITC) are clusters of densely packed GABAergic cells or thin strands of neurones interspersed among the various amygdaloid nuclei. Despite increasing evidence indicating that ITC neurons play a pivotal role in fear extinction, many aspects regarding the anatomical and physiological properties of these neurons remain still unknown. Therefore, our work aimed at clarifying the spatial distribution of the ITC clusters within the amygdaloid complex. The main ITC nucleus and other ITC clusters located in the intermediate capsule (medial and anterior paracapsular clusters and intramedullary gray), in the external capsule (lateral paracapsular cluster) and dorsally to the lateral nucleus (supralateral cluster) have been identified in consecutive immunolabelled coronal sections of the entire mouse amygdala using neuronal markers enriched in ITC neurons, such as μ-opiod receptor and $GABA_A$ α3 receptor subunit, and three-dimensionally reconstructed. In addition, within the lateral nucleus of the amygdala, we detected a small cluster of densely packed cells with the same immunohistochemical properties of other ITC clusters that we named “intralateral clusters”. To define whether ITC clusters are constituted by distinct populations of neuron, we have analysed, by means of whole-cell patch-clamp recordings and biocytin labelling in acute slices of mouse amygdala, 51 ITC neurons of the medial and anterior paracapsular clusters. Unsupervised cluster analysis based on morphological and physiological features identified three main neuronal classes displaying an unexpected diversity in the pattern of their axonal projections. In particular, ITC neurons of the first group (n=17) showed a primary axonal branch running ventro-medially along the intermediate capsule to innervate the main ITC nucleus and establishing symmetrical type II synapses, as revealed by electron microscopy. Direct inhibitory connectivity between these neurons and the main ITC nucleus was also demonstrated by *in vitro* electrophysiological recordings indicating functional monosynaptic transmission (IPSCs) mediated by $GABA_A$ receptors. In addition, Zif268 expression mapping revealed that the medial paracapsular cluster and the main ITC nucleus show opposite activation upon fear conditioning and extinction. Specifically, the medial paracapsular cluster exhibited more Zif268 positive cells after fear conditioning compared to unconditioned mice whereas the main ITC nucleus was exclusively activated after extinction and extinction retrieval.
In conclusion, our findings suggest that ITC clusters are composed of distinct types of neurons with dissimilar connectivity and that distinct clusters differently contribute to regulate fear learning and extinction.

Acknowledgments
We thank Ms G. Schmid for the excellent technical assistance and L.M. Kastlunger for helping with the 3D reconstructions. This work was supported by the Innsbruck Medical University and FWF grant n° S10207 to F.F.

T 2: Bernhard Loy

P38 Mitogen Activated Protein Kinase controls cholinergic differentiation of sympathetic neurons

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Keywords: sympathetic, neurotrophic factor, cholinergic, signalling

We have investigated the signalling mechanisms that determine transdifferentiation of sympathetic neurons fom noradrenergic to cholinergic neurotransmission upon exposure to neuropoietic cytokines. Downstream of gp130/LIFRb ciliary neuronotrophic factor (CNTF) stimulated a rapid and sustained p38 MAPK activation in cultures of postnatal rat superior cervical ganglia (SCG) neurons. Inhibition of p38MAPK signalling with different classes of pharmacological compounds blocked the up-regulation of cholinergic marker genes but had no effect on noradrenergic marker expression. Overexpression of p38 in noradrenergic neurons was sufficient to cause a marked increase of Vacht and VIP expression in the absence of neuropoietic cytokines. Mouse SCG neurons isolated from p38beta deficient mice were severely impaired in their capacity to regulate cholinergic marker genes *in vitro*. *In vivo*, VAChT expression was reduced by 70% compared to wildtyp controls in the mouse stellate ganglion of p38beta deficient mice. These findings represent the first report of a phenotype for the p38beta mutant. Our findings reveal a non-canonical gp130 signaling mechanism in primary postmitotic neurons that involves p38 MAPK for specification of the neurotransmitter phenotype *in vitro* and *in vivo.*

T 3: Michael Fritz

Cocaine relapse prevention by social interaction and simga1 receptor activation: Development of an animal model

M. Fritz[1], R. El Rawas[1], A. Salti[2], S. Klement[1], M. Bardo[3], G. Dechant[2], A. Saria[1] and G. Zernig[1]
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Is social interaction as a non-drug ("alternative") reinforcer able to help the addict to reorient his/her behavior away from the drug of abuse? As the incentive salience of drug-associated stimuli is a key component of a drug's apparent reinforcing effect, we investigated, in the rat conditioned place preference (CPP) paradigm, if social interaction is able to decrease the incentive salience of cocaine-associated contextual stimuli. Therefore, singly housed, drug- and experiment-naive rats were trained in a 3 chamber CPP apparatus by receiving either a 15 mg/kg i.p. cocaine or saline injection. Rats that developed cocaine CPP were divided into 3 groups for different extinction trainings: Group 1 received normal saline extinction. Group 2 was given the possibility of social interaction in the formerly saline-paired compartment with a playfellow of the same weight and gender. To group 3, social interaction was made available while cocaine conditioning was continued. The first two groups were then challenged in a reacquisition test and brains were collected 2h later (i.e., 26h after the last cocaine injection) and processed for Zif268 immunocytochemistry. A single social interaction of singly housed rats was able to reverse cocaine CPP. Even more importantly, 4 episodes of social interaction were able to fully prevent cocaine-induced reacquisition of cocaine CPP. This behavioral effect was paralleled by a pronounced deactivation of Zif268 expression in AcbSh, VTA, Ce, and BLA. Even in continuing competition with cocaine, social interaction-associated contextual stimuli came to be preferred over cocaine-associated ones.

T 4: Simone Mader

AQP4-IgG autoantibodies as biomarkers for Neuromyelitis optica and their relevance for disease pathogenesis

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Neuromyelitis optica (NMO), a severe demyelinating disease of the central nervous system (CNS), represents itself with optic neuritis and longitudinally extensive transverse myelitis. Serum NMO-IgG autoantibodies (Abs), a specific finding in NMO patients, target the water channel protein aquaporin-4 (AQP4), which is expressed as a long (M-1) or a short (M-23) isoform. The aim of our study was to analyze serum samples from NMO patients and controls for the presence and epitope specificity of anti-AQP4 Abs using an immunofluorescence assay with HEK293 cells expressing M-1 or M-23 human AQP4. We included 56 patients with definite NMO (n = 30) and high risk NMO (n = 26), 101 patients with multiple sclerosis (MS), 27 patients with clinically isolated syndromes (CIS), 30 patients with systemic lupus erythematosus (SLE) or Sjögren's syndrome, 29 patients with other neurological diseases and 47 healthy controls. Serum anti-AQP4 M-23 IgG Abs were specifically detected in 29 NMO patients, 17 patients with high risk NMO and two patients with myelitis due to demyelination (CIS) and SLE. In contrast, IgM anti-AQP4 Abs were not only found in some NMO and high risk patients, but also in controls. The sensitivity of the M-23 AQP4 IgG assay was 97% for NMO and 65% for high risk NMO, with a specificity of 100% compared to the controls. Sensitivity with M-1 AQP4 transfected cells was lower for NMO (70%) and high risk NMO (39%). The conformational epitopes of M-23 AQP4 are the primary targets of NMO-IgG, whereas M-1 AQP4 Abs are developed with increasing disease duration and number of relapses. Our results confirm M-23 AQP4-IgG as reliable biomarkers in patients with NMO and high risk syndromes. M-1 and M-23 AQP4-IgG are significantly associated with a higher number of relapses and longer disease duration. We are currently analyzing whether AQP4 Abs can fix and activate complement and are thus cytotoxic in vitro and in vivo. We further analyzed the pathogenicity of patient NMO IgG in vivo (Bradl et al, 2009). Therefore, human Ig from AQP4 Ab positive and negative patients and other controls were injected in Lewis rats with T cell mediated experimental autoimmune encephalomyelitis. Immunoglobulin from AQP4 Ab positive patients are pathogenic, they augment clinical disease and induce lesions in the CNS that are similar to those seen in NMO patients. AQP4-IgG are not only important diagnostic markers, they augment disease and induce NMO-like lesions in animals with T cell mediated brain inflammation.

Grant support: This study was supported by a research grant of the Interdisciplinary Center for Research and Treatment (IFTZ) of Innsbruck Medical University.

T 5: Iris Kastenberger

Anxiogenic effects of oestrogen are mediated through the dynorphin - kappa opioid receptor system

Mag. Iris Kastenberger[#], Dipl. Biol. Eduard Schunk[#], Christian Lutsch[#], Prof. Dr. Herbert Herzog* and Prof. Dr. Christoph Schwarzer[#]
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Background
The influence of ovarian hormones on behaviour is well accepted and oestrogen replacement therapy has proven beneficial in several cases of menopausal mood disorders. However, there are also adverse effects of such a therapy, especially in women who suffer from premenstrual syndrome. In fact, the neurochemical background of the actions of oestrogen on emotions is largely unclear.

Methods
Female wild-type and prodynorphin deficient mice were subjected to behavioural testing. Anxiogenic like effects observed in the pro-oestrous phase in wild-type, but not prodynorphin deficient mice was investigated in more detail in ovariectomized mice applying estrogen receptor specific agonists and kappa opioid receptor antagonists. Double-immunofluorescence was applied to identify c-Fos expression in dynorphinergic neurons.

Results
We provide evidence that activation of G-protein coulped oestrogen receptors (GPER) mediates anxiogenic effects in female mice. Moreover, we demonstrated that these effects are blocked by genetic deletion of prodynorphin or by pharmacological blockade of kappa opioid receptors. Our data suggest that anxiogenic effects caused by increasing oestrogen involve the release of dynorphin through stimulation of GPER. This is supported by increased c-Fos expression in dynorphinergic neurons of the hypothalamic paraventricular nucleus after oestrogen agonist treatment.

Conclusions
Our results support the hypothesis of individual sex steroid sensitivity as a cause of adverse effects of oestrogen replacement therapy, identifying GPER as a potential candidate to induce anxiogenic effects. Thus, the development of oestrogen receptor specific agonists may not only be important to reduce the risk for breast cancer, but may also reduce adverse effects on mood.

T 6: Maria Auer

Effects of RhoA inhibition on peripheral axon regeneration

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The small GTPase RhoA inhibits axonal regeneration. Activation of Rho and its effectors mediates growth cone collapse and retraction via phosphorylation of myosin light chain and interference with actin turnover. Increased RhoA activation was observed following peripheral axon lesion. Blocking RhoA activation or expression of a dominant negative construct results in process outgrowth by rat pheochromocytoma cells. In addition, the stimulation of axon outgrowth by neuronal growth factors possibly involves the inhibition of RhoA. In the present study, we assessed axonal outgrowth and regeneration in cultured primary sensory neurons in vitro and in the sciatic nerve lesion model in vivo in response to inhbition and down-regulation of RhoA, respectively. Dissociated dorsal root ganglia (DRG) neurons obtained from adult rats were transfected with RhoA siRNA plasmids also encoding EGFP. Pharmacologically, Rho activation was inhibited by Clostridium botulinum toxin C3. RhoA siRNA transfected or C3 treated DRG neurons exhibited a significant increase in total axonal length within a 24h period. RhoA GTP assays were carried out in adult DRG neurons treated with NGF or FGF-2. Compared to controls, both growth factors significantly decreased RhoA GTP loading. In vivo, we transected the right sciatic nerve of adult rats and implanted a 12 mm-silicon tubule bridging the nerve stumps. Conduits were filled with a collagen solution containing the Rho inhibitor C3. Controls received collagen or an enzymatically inactive mutant version of C3. Functional sciatic nerve regeneration was assessed monthly using the walking track, thermal algesimetry, and electrophysiological recording of compound digital sensory nerve action potentials and compound plantar muscle action potentials (supported by SPIN/FWF and COST B-30).

P 1

Altered Expression of GABA-A Receptor Subunits in Temporal Lobe Epilepsy

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Reduced GABAergic inhibition may be a cause of temporal lobe epilepsy (TLE) and has been demonstrated in animal models of TLE. The principal receptors involved are $GABA_A$ receptors ($GABA_A$-R) that are chloride channels constituted of 5 subunits. Subunits α_4, α_5, and δ are thought to mediate long-lasting extrasynaptic tonic inhibition, whereas other subunits (e.g. α_2, γ_2) are involved in postsynaptic phasic inhibition. In several animal models of TLE, a decreased expression of the δ subunit along with increased expression of γ_2 and α_4 subunits in the dentate gyrus (DG) has been reported [1,2]. In addition, immunostaining of extrasynaptic α_5 subunit-containing $GABA_A$-R was significantly reduced in the CA1 region of the hippocampus [3]. Possible changes in subunits α_4, α_5, and δ have not been investigated in human TLE so far.

We now investigated changes in the expression of $GABA_A$-R subunits α_4, α_5, and δ, compared with γ_2 in hippocampal specimens obtained at surgery from TLE patients with and without hippocampal sclerosis and in autopsy controls using immunohistochemistry and *in situ* hybridisation.

Increased mRNA levels for subunits α_4 (dentate granule cell layer: by 50 $\pm$ 8.1% of control, subiculum: 77 $\pm$ 14.4%; mean $\pm$ SEM), α_5 (81 $\pm$ 10.2%, 63 $\pm$ 10.0%), δ (110 $\pm$ 12.7%, 156 $\pm$ 30.8%), and γ_2 (117 $\pm$ 16.4%, 173 $\pm$ 20.6%) were observed in the dentate granule cell layer and the subiculum of sclerotic specimens. In non-sclerotic specimens, the levels of mRNAs were increased by: α_4, gcl: 92 $\pm$ 14.8%, sub: 104 $\pm$ 23.2%; α_5, 88 $\pm$ 9.5%, 136 $\pm$ 17.9%; δ, 112 $\pm$ 9.8%, 137 $\pm$ 17.6%; and γ_2, 160 $\pm$ 24.4%, 188 $\pm$ 23.9%. Similarly, increases in the immunoreactivity of subunits α_5 (by 72%), δ (55%), and γ_2 (149%) were detected in the dentate molecular layer of TLE patients. In the subiculum, increased immunolabeling was found for α_5 (by 54%), δ (130%), and γ_2 (83%).

Our data show marked changes in $GABA_A$-R subunit expression and indicate a reorganisation of $GABA_A$-R in TLE patients. Upregulation of subunits α_5 and δ is in contrast with findings in animal models and may indicate unchanged or even increased tonic inhibition by GABA in the hippocampus of TLE patients.

Funded by EU Grant FP6 Epicure (LSH-CT-2006-037315) and FWF (P 19464).

[1] Schwarzer et al. (1997), Neuroscience 80:1001-1017.
[2] Nishimura et al. (2005), Neuroscience 134:691-704.
[3] Houser & Esclapez (2003), Hippocampus 13:633-645.

P 2

ENDOGENOUS DYNORPHIN IN EMOTIONAL CONTROL AND STRESS RESPONSE

CHRISTIAN LUTSCH[1]; IRIS KASTENBERGER[1]; HERZOG HERBERT[2] & SCHWARZER, CHRISTOPH[1]
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Cerebral control of stress and anxiety involves several neurotransmitter systems. Beside serotonin, noradrenaline or catecholamines, also several neuropeptide systems are considered to be involved in generating symptoms of anxiety and stress. These systems act in a circuit connecting amygdalar and hypothalamic nuclei, the pituitary and adrenal glands, regulating the physiological response via ACTH and corticosterone release.
In this study, we investigated anxiety and stress related behaviour of male germ-line prodynorphin knockout (dynKO) mice on the genetical background of Balb/c. Male dynKO mice exhibited about 2-fold ambulation in the open field intermediate and center areas. DynKO mice travelled a longer distance (2-fold) and spent more time on open arms of the elevated plus maze test. In the light-dark test only one out of ten Wild-type (WT) mice entered the light area, while four out of seven dynKO explored this aversive environment. In stress related tests, dynKO mice displayed a tendency to increased immobility in the tail suspension test and unchanged immobility at the late stage of the forced swim test. Of note is the fact that dynKO mice displayed less immobility in the beginning of the forced swim test as compared to WT mice. In line with this, only little differences in the expression of □FosB were observed after these tests in brain areas related to stress coping.
Our data support the anxiogenic effects of endogenous dynorphin observed in C57bl/6N mice recently. Although trait anxiety is markedly higher in Balb/c than in C57bl/6N mice, the key features of increased exploratory drive and mostly unchanged stress coping abilities were also observed in this strain.

P 3

An inducible transgenic MSA model: generation and characterization.

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Multiple System Atrophy (MSA) is a fatal, rapidly progressing, adult onset neurodegenerative disease. Patients present with parkinsonism, ataxia and autonomic failure in various combinations. The median onset of MSA is 53 years of age and survival beyond ten years after disease onset is unusual. The major hallmark of MSA is the appearance α-synuclein (αSYN) positive glial cytoplasmic inclusions (GCIs). The exact mechanism of how oligodendroglial αSYN inclusions cause neuronal loss in the striatum and substantia nigra pars compacta (striatonigral degeneration, SND), cerebellum, pons, inferior olives (olivopontocerebellar atrophy, OPCA), and the intermediolateral column of the spinal cord, is not fully understood. Currently there are no efficient treatment options. MSA patients, in contrast to parkinsonian patients, respond only poorly to L-dopa treatment. Therefore there is great need for development of alternative treatment options.
There are several animal models used as test beds for the development new therapies for MSA. One approach includes specific neurotoxins to cause the MSA specific neuronal loss. Another strategy, which has become of more interest since the discovery of αSYN in MSA is the creation of transgenic models to constitutively express alpha-syn and thereby lead to formation of GCIs. A combination of neurotoxins in transgenic animals is currently the most reliable way to reproduce both, neuronal loss and GCIs that are characteristic for human pathology.
As MSA is a disease which affects the elderly, a more appropriate animal model would be to activate the pathological MSA-mechanisms later in life. For this reason, the aim of the current project is to generate an inducible model of MSA, to 'switch on' the MSA-like pathology in adult mice. To do this, we will use the tamoxifen-inducible PLP-Cre system to overexpress αSYN specifically in oligodendrocytes. The transgene has been created as the work of a previous PhD student in our lab and oocyte injection performed by Dr. Yannoutsos.
The aim of my thesis is the characterization of the αSYN-tg animals as well as the cross-breeding with the PLP-Cre mice, comprehensive phenotypic characterization including characterization of motor behavioral, autonomic function as well as neuropathology.
Acknowledgements
This study is generously supported by the FWF.

P 4

Fine-tuning of Cav1.3 calcium channel currents by C-terminal alternative splicing

Gabriella Juhasz-Vedres, Mathias Gebhart, Martina J. Sinnegger-Brauns, Jörg Striessnig and Alexandra Koschak

L-type Ca^{2+} channels (LTCCs) such as Cav1.3 in the CNS are mainly located on the dendrites and cell soma of cortical, hippocampal and dopaminerg neurons (Hell et al., 1993). Ca^{2+} influx through Cav1.3 channels mediates depolarization-induced activation of gene transcription which process is particularly effective in activating CREB which factor is required for neuronal plasticity. However in spontaneously active adult dopaminergic neurons (DNs) such as subsantia nigra pars compacta (SNc) Cav1.3 LTTCs are believed to contribute to the maintenance of rhythmic firing. The threshold of nifedipine-sensitive calcium currents evoking action potentials (APs) underlying pacemaking and the slow membrane oscillations were seen to activate at potentials between -60 and -40 mV (Nedergaard et al., 1993). The activation threshold of full length Cav1.3 splice variant is ~40mV when coexpressed with their auxilliary in tsA-201 cells (Koschak et al., 2001) These properties are suitable for pacemaking in DNs. However different modifications such as alternative splicing (AS) can modify channel gating. Previous analysis of long (CaV1.342) and short (Cav1.342S) human C-terminal splice variants revealed that gating is modulated by an intramolecular C-terminal mechanism (CTM) affecting the negative activation range and slows calcium-dependent inactivation (CDI). In our investigations we identified another C-terminal lacking Cav1.3 splice variant (Cav1.3 43S) in mouse SNc and hippocampal tissues. Its gating properties were similar to Cav1.342S: the voltage dependence of Cav1.343S I_{Ca} activation was negatively shifted (mV:Cav1.342:-2.7; Cav1.343S:-12.4, $p<0.0001$) and channel inactivation was faster (% inactivation, 250ms Vmax:Cav1.342L:63.6%; Cav1.343S: 87%, $p<0.0001$) which is due to pronounced CDI. Interestingly, coexpression of its C-terminal with Cav1.343S but not Cav1.342S restored long Cav1.342 channel like gating. Simulating HCN or DN AP-like brief stimulations, short channels showed more pronounced accumulation which is due to slower recovery and faster inactivation leading to less Ca^{2+} influx in comparison to Cav1.342. Moreover we observed an increased coupling efficiency of Cav1.342S which could be due to a higher single channel open probability. In contrast to long form, short splices were shown modestly important in CREB signalling. Its function may be to enable the activation of the long form due its higher operating voltages in tissues when they localized together. We conclude that Cav1.3 splices may give rise to different functions in different cells where physiological function critically dependent on their activity.

P 5

Alexandra Koschak[1], Shahid M. Baig[2], Mathias Gebhart[1], Claudia Dafinger[3], Gudrun Nürnberg[4], Niels Brandt[5,6], Jutta Engel[5,6], Amjad Ali[2], Ilyas Ahmad[2], Martina J. Sinnegger-Brauns[1], Matteo E. Mangoni[10], Muhammad Farooq[2], Habib U. Khan[7], Peter Nürnberg[4,8], Hanno J. Bolz[3,9] and Jörg Striessnig[1]

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Gating properties of a human disease-causing mutation in $Ca_V1.3$ L-type calcium channels

$Ca_v1.3$ voltage-gated L-type calcium channels (LTCCs) mediate window currents at negative membrane voltages allowing them to function as pacemaker channels in the sinoatrial node (SAN) and neurons and to trigger neurotransmitter release in cochlear inner hair cells. Mice lacking $Ca_v1.3$ □1-subunits are congenitally deaf and show SAN dysfunction. We have identified families with deafness and SAN dysfunction resulting from a homozygous mutation in the Cav1.3 *CACNA1D* gene encoding $Ca_V1.3$ □1-subunits. The mutation leads to an amino acid insertion in a pore-forming transmembrane S6 helix.
To determine the functional consequences of the mutation we expressed wildtype (WT) and mutant (MUT) $Ca_v1.3$ channel complexes in tsA201 cells and analyzed their biophysical properties by whole-cell patch-clamp. Although expressed at protein levels comparable to WT, MUT channels did not conduct significant Ca^{2+} currents (I_{Ca}) (peak I_{Ca} 1.2 ± 0.14 pA/pF, n = 12) in contrast to WT (15.9 ± 1.7, n = 17). However, ON-gating currents were readily recorded in both WT and MUT channels during depolarizations to the reversal potential where contaminating ionic current is absent during test pulses but can be quantified from the tail currents (I_{tail}) upon repolarization. In WT, integrated ON gating charge (Q_{ON}) increased with increasing ionic current whereas no I_{tail} was detectable in MUT channels even at Q_{ON} that resulted in robust I_{tail} in WT. MUT Q_{ON} exhibited a typical non-linear voltage-dependence of activation, and neither the size of Q_{ON} ($p = 0.2$, Mann Whitney) nor its voltage-dependence was affected by the Ca^{2+} channel activator BayK8644 (5μM). Amplitude and Q_{ON} were significantly smaller than in WT. MUT channel ON-gating current also showed pronounced kinetic differences from WT evident as a significantly shorter width, faster time-to-peak, and shorter time constant of decay ($p<0.05$, Mann-Whitney test).
The presence of intra-membrane charge movement in the absence of ionic currents implies that voltage-sensors in MUT channels move but either fail to trigger pore opening or opened MUT channels cannot conduct Ca^{2+}. Since the mutation is not located within voltage-sensing S4 helices the pronounced kinetic changes of gating currents suggest that the mutation in the pore indirectly alters gating by affecting the mechanistic coupling that exists between S4 voltage-sensor movements and the gate mediating pore opening. Our data also for the first time provide direct evidence that loss of $Ca_v1.3$ function causes deafness and SAN dysfunction in humans.

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P 6

Serotonergic neurodegeneration after inflammation and search for peripheral blood biomarkers

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Neurodegeneration and associated inflammatory processes in the central nervous system are common characteristics of several brain disorders accompanied by dysfunction of different neurotransmitter systems. A reduction of the serotonergic neurotransmitter system has been linked to mood disorders and especially depression. A lack of the neurotransmitter acetylcholine directly correlates with cognitive impairment and is a hallmark in Alzheimer's disease (AD). The aim of this project was (1) to study survival of dorsal raphe (doR) serotonergic neurons in rat brain slices after inflammation and (2) to search for blood biomarkers in AD patients.
We characterized organotypic brain slices of the doR and incubated slices with or without interferon-γ (IFNγ), β-amyloid (Aβ), tumor necrosis factor-α (TNF-α), lipopolysaccharid (LPS), brain derived neurotrophic factor (BDNF) or glial cell line-derived neurotrophic factor (GDNF). We performed immunohistochemistry and counted the number of tryptophanhydroxylase (TPH+) positive and indolamine-2,3-dioxygenase (IDO+) positive cells. Furthermore we measured proinflammatory cytokines of doR brain slice extracts by Multiplex Elisa. For biomarker search we determined telomere length, different cell adhesion molecules (CAMs) and chemokines in monocytes of AD and healthy subjects.
In doR brain slices, LPS, IFNγ, Aβ and TNFα treated slices displayed reduced TPH+ neurons compared to controls. The growth factors BDNF or GDNF counteracted the decrease in TPH+ neurons. The number of IDO+ neurons was significantly elevated in slices incubated with LPS, IFNγ and TNFα, but when treated with Aβ the number of IDO+ neurons did not change compared to control slices. Proinflammatory cytokines were increased, when slices were incubated with LPS. In AD monocytes, CAMs (ICAM-3, P-selectin) were significantly reduced, telomere length was significantly shortened and specific chemokines (MIP-1δ, MIG) were significantly elevated.
In summary our data show that inflammation markedly affects the number of TPH+ and IDO+ cells and also shows that inflammation leads to increased levels of proinflammatory cytokines in doR brain slices as well as increased levels of chemokines in monocytic cells of AD patients, which can possibly be used as diagnostic markers for neuroinflammatory and/or neurodegenerative processes. It will be another aim to search for peripheral biomarkers in depressive patients.
This study is supported by Moodinflame (EC) and Austrian Science Funds (L429).

P 7

Infusion of an AAV-NPY vector into the basolateral amygdala reverses accelerated fear acquisition in NPY KO mice

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Neuropeptide Y (NPY) has important functions in emotional processes such as anxiety and depression. To investigate a possible role of NPY in the processing of fear, we subjected NPY and Y1, Y2 and Y4 receptor knockout mice to *Pavlovian* fear conditioning, a simple form of associative learning. Fear conditioning was performed by 5 times presentation of a tone (30s white noise) co-terminating with a mild foot shock (2s, 0.7 mA). Extinction of fear was performed 24 h later by repetitive presentation (30s, 40 times) of the tone in the absence of a foot shock.
Compared to wild type controls, NPY KO mice acquire higher freezing levels during fear conditioning and show impaired extinction. After Y1 receptor deletion faster conditioning and delayed extinction was observed, whereas Y2 KO mice respond equally as controls. Unexpectedly, Y4 KO mice showed normal acquisition but impaired extinction of conditioned fear.
Local, viral vector mediated re-expression of NPY in the basolateral amygdala of NPY KO mice resulted in a significant delay in the acquisition of conditioned fear. In addition, there was a trend towards improved extinction.
Our data using NPY KO mice indicate that NPY has a protective role in the acquisition of fear and facilitates extinction of conditioned fear. The basolateral amygdala may be a key site for NPY mediated modulation of acquisition and extinction of conditioned fear. Results from Y receptor KO mice suggest that the Y1 receptor mediates NPY induced modulation of fear acquisition whereas both, Y1 and Y4 receptors are involved in fear extinction. (Supported by the Austrian Science Fund S10204)

P 8

Status epilepticus induced epileptogenesis in the rat traced by video-supported EEG-telemetry

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Background: Temporal lobe epilepsy is the most common focal epilepsy in humans and is often associated with severe neuronal damage in major areas of the hippocampal formation. The subiculum, however, the major output area of the hippocampus, remains largely preserved and has therefore been proposed to critically contribute to seizure generation. We now established a video-supported EEG-telemetry system in freely moving rats to correlate spontaneous recurrent seizures with neurochemical and morphological changes in parahippocampal areas of kainic acid treated rats. Therefore we investigated cell losses of different neuronal subclasses in correlation to frequencies of seizures.
Methods: Male Sprague-Dawley rats (ca. 250 g) were implanted with reusable EEG-transmitters with electrodes epidurally above the hippocampus. After one week, rats were injected with kainic acid (10 mg/kg, i.p.) leading to initial status epilepticus. At the same time video-supported EEG monitoring was started and continued 24 h/day for 3 months. Estimation of neuronal loss was assessed by Neuron specific nuclear protein- and Parvalbumin-immunoreactive immunohistochemistry.
Results: Initial status epilepticus was interrupted by i.p. injection of diazepam 2 h after the first generalized seizure, but recurred 4 ± 1.1 h later and lasted again for several hours (12 ± 2.4 h). All rats exhibiting initial status epilepticus developed spontaneous recurrent seizures. Seizure frequency increased 6 weeks after seizure onset from 4 to 13 seizures/week. Rats with pronounced neuronal loss in the subiculum (Neuron specific nuclear protein-immunoreactive surviving cells: up to 64% in proximal subiculum; Parvalbumin-immunoreactive surviving cells: up to 53% in superficial subiculum) exhibited increased numbers of spontaneous seizures.
Conclusion: Kainic acid-induced status epilepticus leads to a fast development of epilepsy characterized by an early onset and high frequencies of spontaneous seizures. The degree of neurodegeneration in the subiculum correlated with the number of spontaneous seizures. Parvalbumin-immunoreactive cells, a subclass of GABAergic interneurons responsible for perisomatic inhibition, are particularly vulnerable in this model of Temporal lobe epilepsy.

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P 9

SIGNIFICANCE OF GP130 SIGNALING IN NEURONAL REGENERATION

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Background: The cytokine interleukin-6 (IL-6) activates target genes involved in inflammation and pain. It acts by binding to plasma membrane receptor complexes containing the common signal transducing receptor subunit gp130. Subsequently and by activation of JAK, STAT and MAPK signaling pathways, IL-6 regulates various target genes leading to its broad effects. Still, it is poorly understood how IL6/gp130 interaction participate in injured peripheral neurons. In the present work, we have investigated the role of gp130 in the regeneration of peripheral sensory neurons using conditional knock-out mice (SNS-gp130$^{-/-}$) (Andratsch et al., J. Neurosci., 2009 Oct; 29 (43): 13473-13483).
Methods: Biochemical approaches were used. A nerve injury model, obtained by a one minute crush of the sciatic nerve, and sensory and motor behavioral tests were used to monitor the recovery process *in vivo*.
Results: Dorsal root ganglion neurons (DRGs) from SNS-gp130$^{-/-}$ mice showed a significantly reduced neurite extension and number of neurite bearing neurons in culture as compared to gp130$^{fl/fl}$ and wild-type animals. Furthermore, the substitution of growth factors as NGF or BDNF did not rescue neurite outgrowth in cultured neurons from SNS-gp130$^{-/-}$ in contrast to gp130$^{fl/fl}$ mice.
In vivo, after nerve crush injury regeneration of the sciatic nerve was monitored for 25 days by determining mechanical and heat thresholds and motor capabilities in control and knock-out mice. Recovery of sensitivity was similar in wt and gp130$^{fl/fl}$ mice but significantly delayed in SNS-gp130$^{-/-}$ mice. No significant difference was observed in recovery of motor capabilities between the groups.
In order to elucidate the effects of IL-6/gp130 on peripheral regeneration and to find potential signaling partners we used Affymetrix® gene expression analysis of DRG explants from SNS-gp130$^{-/-}$ mice and gp130$^{fl/fl}$ controls. We found down-regulation of two regeneration-associated genes (RAG), Atf3 and Sprr1a. Using L4-L6 DRG explants the mRNA expression in SNS-gp130$^{-/-}$ mice was determined by quantitative PCR. mRNA of both Atf3 and Sprr1A was lower in non-injured SNS-gp130$^{-/-}$ DRG explants compared to gp130$^{fl/fl}$ mice. qRT-PCR showed an increase in mRNA expression of both genes after sciatic nerve injury at 3 and 7 days in both animals strains.
Conclusion: Our data suggest that regeneration of neurons is significantly inhibited in SNS-gp130$^{-/-}$ *in vitro* and *in vivo*. We found that genes that are associated with nerve injury or regulation of neurite outgrowth were significantly down-regulated in SNS-gp130$^{-/-}$ mice. Experiments addressing the functional importance of gp130/IL6 signaling in the outgrowth process of peripheral neurons are ongoing.
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P 10

Functional integrity of malformations of cortical development in a language network: an fMRI study.

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Background: Functional reorganization of cerebral cortex occurs in subjects with malformations of cortical development (MCD). In some MCD, functional MRI (fMRI) shows blood oxygen level-dependent (BOLD) activation in malformed cortices, suggesting their functional integrity. This raises concerns about post-surgical deficits in patients with MCD and medically refractory epilepsy. We aimed to assess functional integrity of MCD in language neural network in patients with epilepsy and MCD by use of fMRI.
Methods: Forty-six patients (26w/20m) aged 15-73 years (mean 33.5 years) with MCD and epilepsy underwent MRI (1.5-T) and fMRI. Single-subject image analysis was performed with statistical parametric mapping (SPM5).
MCD diagnosis was based on MRI and classified according to Barkovich et al., 2005. MCD were located either in vicinity of language cortex – Broca's and Wernicke's areas or subependymally along lateral ventricles.
We used word generation task: Patients had to generate silently words staring with letters “K” and “S” and words in categories “Animals” and “Tools”.
Subsequently, seven patients underwent epilepsy surgery. Histological classification of focal cortical dysplasia (FCD) was based on nomenclature proposed by Palmini et al. 2004.
Results: Fifteen patients had periventricular nodular heterotopias (PNH); nine – polymicrogyria (PMG); five - FCD type II; seven – FCD type I; six– tuberous sclerosis (TS); one – dysembryoplastic neuroepithelial tumour; one - hemimegalencephaly; one – subcortical laminar heterotopia (SLH); and one – TS with PMG.
Majority of patients (44/46) had focal epilepsy; 32/46 (70%) were pharmacoresistant. Mean age at seizure onset was 15 years (range 1-61); mean epilepsy duration for time of fMRI– 12.5 years (range 1-58).
Shift of BOLD activation from MCD affecting language cortex was observed only in patients with FCD type II (3/5) and TS (2/6). Malformed cortices involving language cortex harboured BOLD activation in patients with FCD type I (6/7), PMG (4/10) and SLH (1/2). In PNH situated along lateral ventricles BOLD activation was not observed. In four patients no BOLD activation was registered. Neither shift of BOLD activation from affected language cortices nor their BOLD activation, were influenced by age at seizure onset, seizure frequency during the first year of epilepsy, epilepsy duration, seizure outcome or handedness.
Conclusions: In patients with MCD affecting language cortices, word generation task fMRI suggests functional integration of MCD due to abnormal neuronal migration (SLH) or organization (FCD type I, PMG) and shift of function from MCD due to abnormal neuronal proliferation/apoptosis (FCD type II, TS).

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P 11

Cell death and neuroprotection of rat cholinergic neurons in organotypic brain slices after oxygen glucose deprivation or ethanol exposure

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Alzheimer's disease is a severe chronic neurodegenerative disorder characterized by beta-amyloid plaques, tau pathology, cerebrovascular damage, inflammation, reactive gliosis, and cell death of cholinergic neurons. Ischemia or increased ethanol consume are risk factors which cause cell death of cholinergic neurons. Nerve growth factor (NGF) is so far the most potent substance to protect cholinergic neurons against neurodegeneration. However, also other molecules are of particular interest like the astroglial derived S100b. The aim of the present study was (1) to explore whether S100b is a potent neuroprotective factor for cholinergic neurons after ischemic events and (2) to investigate ethanol-induced neurodegeneration as well as neuroprotection by NGF.

Organotypic rat brain slices of the nucleus basalis of Meynert (nbM) were used to study survival of cholinergic neurons. Oxygen glucose deprivation (OGD) was induced in a hypoxia chamber by flushing the chamber with N_2 (25 l/min) for 5 min in low glucose medium for 3 days. For the ethanol experiment slices were incubated with 1 mM, 10 mM, 50 mM, 100 mM and 500 mM ethanol for 7 days. Cholinergic neurons were detected by using the immunohistochemical marker for the enzyme choline acetyltransferase (ChAT).

Our data showed that OGD markedly induced neurodegeneration of cholinergic nbM neurons. This cell death was counteracted by 50 µg/ml S100b. Application of nerve growth factor (10 ng/ml) or fibroblast growth factor-2 (2 ng/ml) was less protective. Ethanol caused neurodegeneration of the cholinergic neurons after 7 days, most potently at 50 mM ethanol, while higher concentrations of ethanol did not further induce a decline. NGF partly counteracted the ethanol-induced decrease.

In conclusion, organotypic rat brain slices provide a potent tool to study neurodegeneration of cholinergic neurons after oxygen glucose deprivation or ethanol exposure. Neurodegeneration of cholinergic neurons was counteracted by astroglial S100b or NGF.

In further studies we will explore the intracellular pathways involved in OGD or ethanol-induced neurodegeneration of cholinergic neurons.

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P 12

Neuroprotective effects of kappa opioid receptors in epilepsy

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We recently demonstrated that endogenous prodynorphin-derived peptides mediate anticonvulsant, antiepileptogenic and neuroprotective effects via kappa opioid receptors (KOP). Here we show acute and delayed neurodegeneration and its pharmacology after local kainic acid injection in prodynorphin knockout and wild-type mice and neuroprotective effect(s) of KOP activation in wild-type mice. Prodynorphin knockout and wild-type mice were injected with kainic acid (3 nmoles in 50 nl saline) into the stratum radiatum of CA1 of the right dorsal hippocampus. Knockout mice displayed significantly more neurodegeneration of pyramidal cells and interneurons than wild-type mice two days after treatment. This phenotype could be mimicked in wild-type animals by treatment with the KOP antagonist GNTI and rescued in knockout animals by the KOP agonist U-50488. Minor differences in neurodegeneration remained three weeks after treatment, mostly because of higher progressive neurodegeneration in wild-type mice compared with prodynorphin-deficient animals. Progressive neurodegeneration, but not acute neuronal loss, could be mostly blocked by U-50488 treatment. Our data suggest that endogenous prodynorphin-derived peptides sufficiently activate KOP receptors during acute seizures, and importantly in situations of reduced dynorphinergic signalling – like in epilepsy – the exogenous activation of KOP receptors might also have strong neuroprotective effects during excitotoxic events.

P 13

FGFR1 endocytosis and trafficking in human glioma cells

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Fibroblast growth factor receptors (FGFRs) are members of the receptor tyrosine kinase (RTK) family. Extracellular binding of the ligand, i.e. one of the FGF isoforms, leads to receptor dimerization, autophosphorylation and activation, thereby initiating a cascade of intracellular signaling events. FGFR activation is usually followed by rapid endocytosis and degradation of the receptor/ligand complex via the endosomal/lysosomal pathway which leads to signal termination.

Four different FGFRs (FGFR1-4) are produced by alternative mRNA-splicing. FGFR1 is the most abundant in the nervous system exhibiting several diverse functions in development and during regeneration. Depending on the cell type, the activation of the receptor recruites a number of signaling molecules relevant for cell proliferation and brain development. FGFR1 is commonly over-expressed in many types of cancer and often genetically altered leading to ligand-independent self-sustained signaling and consequently to tumor growth. A better understanding of FGFR1 trafficking could help to develop different strategies for tumor growth inhibition.

The goal of our studies is to explore the internalization, trafficking and degradation of the receptor tyrosine kinase FGFR1 in the human glioma cell line U373 which may result in novel therapies to regulate tyrosine kinase signaling. For this purpose, cultured cells are transfected with FGFR1 constructs fused to fluorescent marker proteins. The distribution of the receptor on the cell membrane as well as in intracellular compartments labeled with specific fluorescent markers for endosomes or lysosomes is visualized via confocal fluorescence microscopy and structured illumination using the ApoTome (Zeiss) method. The effects of FGF-2 applied to transfected glioma cells and of the lysosomal inhibitor leupeptin are studied in detail.

Our present data suggest that U373 cells respond to FGF stimulation with increased proliferation (cell rounding, reduction of cellular processes and increase of BrdU incorporation) after receptor activation and internalization. Treatment of cells with FGF enhances colocalization of FGFR1 with endosomes as well as with lysosomes. Leupeptin leads to an increased receptor accumulation in lysosomes. FGFR1 is partially recycled to the cell membrane as indicated by colocalization of the receptor with transferrin. The distribution of endosomes, lysosomes and receptor containing vesicles throughout the cell is demonstrated after threedimensional reconstruction of a confocal image stack.

Our results contribute to a better understanding of FGFR trafficking in glioma cells and can therefore help to establish new treatment strategies to reduce RTK-mediated cell proliferation.

P 14

The role of Sprouty2 in peripheral nerve regeneration

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Sprouty proteins are negative feedback inhibitors of fibroblast growth factor (FGF) signaling. They limit the intensity and duration of FGF receptor activation, thereby, controlling growth and differentiation processes. Mammals exhibit four Sprouty isoforms which are functionally conserved in vertebrates. We investigated the expression of Sprouty isoforms in adult sensory neurons from dorsal root ganglia (DRG) and analyzed their role in axon growth by down-regulation of Sprouty applying short hairpin RNAs (shRNAs). Sprouty2 revealed the highest expression level in adult DRG neurons, whereas Sprouty1 and -4 were expressed at significantly lower levels. Accordingly, down-regulation of Sprouty2, but not of Sprouty1 or -4 promoted elongative axon growth by DRG neurons, whereas overexpression of Sprouty2 inhibited axon growth. Furthermore, down-regulation of Sprouty2 enhanced FGF-2-induced activation of ERK and Ras, which are key signaling molecules involved in axonal elongation. Our data demonstrate the significance of Sprouty2 for in vitro regeneration by adult sensory neurons. To identify the functional significance of Sprouty2 in axon regeneration in vivo we will investigate peripheral nerve regeneration in Sprouty2-/- mice applying the sciatic nerve lesion model. Crush and transection lesions will be performed and lesioned Sprouty2-/- mice will be treated with FGF-2. Axonal regeneration will be studied morphologically and electrophysiologically as well as by behavioral testing of motor and sensory functions (supported by Daniel-Swarowski-Funds).

P 15

Effects of RhoA inhibition on peripheral axon regeneration

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The small GTPase RhoA inhibits axonal regeneration. Activation of Rho and its effectors mediates growth cone collapse and retraction via phosphorylation of myosin light chain and interference with actin turnover. Increased RhoA activation was observed following peripheral axon lesion. Blocking RhoA activation or expression of a dominant negative construct results in process outgrowth by rat pheochromocytoma cells. In addition, the stimulation of axon outgrowth by neuronal growth factors possibly involves the inhibition of RhoA. In the present study, we assessed axonal outgrowth and regeneration in cultured primary sensory neurons in vitro and in the sciatic nerve lesion model in vivo in response to inhbition and down-regulation of RhoA, respectively. Dissociated dorsal root ganglia (DRG) neurons obtained from adult rats were transfected with RhoA siRNA plasmids also encoding EGFP. Pharmacologically, Rho activation was inhibited by Clostridium botulinum toxin C3. RhoA siRNA transfected or C3 treated DRG neurons exhibited a significant increase in total axonal length within a 24h period. RhoA GTP assays were carried out in adult DRG neurons treated with NGF or FGF-2. Compared to controls, both growth factors significantly decreased RhoA GTP loading. In vivo, we transected the right sciatic nerve of adult rats and implanted a 12 mm-silicon tubule bridging the nerve stumps. Conduits were filled with a collagen solution containing the Rho inhibitor C3. Controls received collagen or an enzymatically inactive mutant version of C3. Functional sciatic nerve regeneration was assessed monthly using the walking track, thermal algesimetry, and electrophysiological recording of compound digital sensory nerve action potentials and compound plantar muscle action potentials (supported by SPIN/FWF and COST B-30).

P 16

Highly Flexible System based on Mammalian Cell Culture for Identification of new Protein-Protein Interactions

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Protein production in mammalian cells offers great advantages for proteins that can not be expressed in bacteria or that have certain eukaryotic posttranslational modifications (PTMs), such as glycosylation. The establishing of stable cell lines and the screen for high producing clones is labour intense and time consuming. For getting good expression often gene amplification is needed, which requires additional selection rounds. Therefore at least several months are needed to get a high producing clone. But if the protein of interest is of any harm to the cell, the stability of the clones is an additional concern, which might lead to the complete loss of expression after some passages. A pull-down system that offers the advantages of mammalian protein production without the need for stable clones would be of great help in finding new protein-protein interaction.
We developed a system that uses Chinese Hamster Ovary (CHO) cells, grown in suspension and large scale transient transfection for protein production. In combination with the ONE-STrEP tag (IBA, Göttingen) and state of the art mass spectrometry we have a highly flexible system for pull down experiments in our hands. This technology will help us to identify new interaction partners and will lead to a better understanding of biological processes in the future.

P 17

The low-voltage-activated, high amplitude Ca^{2+} channel splice variant CaV1.1-ΔE29 reveals the molecular mechanism underlying the specific gating behaviour of CaV1.1

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The Ca^{2+} channel α_{1S} subunit (CaV1.1) is the voltage sensor of skeletal muscle excitation-contraction (EC) coupling. Upon membrane depolarization it rapidly triggers Ca^{2+} release from internal stores. Recently, we identified and characterized a new splice variant of skeletal muscle α_{1S} with very distinct expression and gating properties (Tuluc et al. 2009, Biophys. J. 96, 35-44). Quantitative RT-PCR analysis shows that the new splice variant contributes little to the total CaV1.1 mRNA in adult mouse muscle but, in day 16.5 embryonic muscle it is the dominant splice variant accounting for ~80% of CaV1.1 mRNA. In new-born muscle it still represents ~50% of CaV1.1 mRNA, thereafter its expression decreases rapidly dropping to adult levels 3 weeks after birth. The newly described splice variant lacks exon 29, resulting in a 19 amino acid deletion in the extracellular loop linking transmembrane helices IVS3 and IVS4. Expression of rabbit α_{1S} with the deleted exon 29 (*i.e.* α_{1S}-ΔE29) in dysgenic myotubes demonstrates that this new splice variant is capable of skeletal muscle-type EC coupling. In contrast to the full-length α_{1S}, α_{1S}-ΔE29 has an 8-fold increased Ca^{2+} current amplitude and faster activation kinetics. Furthermore, the voltage-dependence of activation is shifted 30mV to lower potentials. Therefore, the low amplitude, slow activating and poor voltage sensitivity Ca^{2+} current of the classical CaV1.1 is determined by the specific sequence of exon 29. Whereas secondary structure prediction shows that in the other high voltage activated Ca^{2+} channels the extracellular loop linking transmembrane helices IVS3 and IVS4 is forms a coil, the corresponding region in α_{1S} contains a small beta-sheet which is deleted in α_{1S}-ΔE29. Convertion of the beta-sheet into a coil in α_{1S} by mutating two amino acids fully recapitulates the changes in the kinetic properties induced by deletion of exon 29. Furthermore, the amplitude of the Ca^{2+} current is increased 3-fold and the voltage dependence is left shifted by 15 mV. Currently we are analyzing additional α_{1S} and α_{1S}-ΔE29 chimeras to better understand the molecular mechanisms underlying the specific gating behavior of CaV1.1.

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P 18

Characterizing Cultured Cerebellar Granule Cells to Analyze Nuclear Targeting of Calcium Channel β4b Subunit

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Voltage-gated calcium channels (VGCC) mediate calcium influx in response to membrane depolarization and regulate numerous cellular functions. Auxiliary β subunits are critical determinants of membrane expression and gating properties of VGCCs. Four distinct β subunit isoforms have been identified, all of which are expressed in brain. In our previous work we discovered the localization of the neuronal β_4 subunit in nuclei of cerebellar granule cells and Purkinje cells (*Subramanyam et al., 2009, Channels)*. Moreover, this unexpected finding was corroborated by heterologous expression of β_{4b}-V5 in dysgenic skeletal myotubes and primary cultured hippocampal neurons.

Here we established and characterized cultured cerebellar granule cells (CGCs) to further analyze the mechanism and function of β_4 nuclear targeting in its native neuronal environment. CGCs were isolated from whole cerebella of six days old BALB/C mice and were allowed to differentiate in culture for 7-9 days. Immunofluorescence labeling of CGCs with anti-tau and anti-MAP2 indicated elaborate axonal networks and few short dendrites, respectively. Loading CGCs with the fluorescent calcium indicator Fluo4-AM demonstrated the spontaneous activity of the neurons. Immunolabeling with synapsin-1 and vGlut revealed a high density of presynaptic specializations which were demonstrated to be functional nerve terminals by depolarization-induced uptake and release of FM 1-43 dye**.** Double immunofluorescence labeling further demonstrated the localization of calcium channel $Ca_V1.2$ and $Ca_V2.1$ subunits as well as β_1 and β_4 subunits in clusters on the soma, the dendrites, and along the axons. However no nuclear targeting of endogenous β_4 subunit was observed at any point of development. In contrast, heterologously expressed β_{4b}-V5, but not β_{2b}-V5 or β_{4a}-V5 was targeted into the nuclei. Yet, neither blocking electrical activity with TTX or depolarization with KCl, nor blocking nuclear export with Leptomysin-B caused an accumulation of endogenous β_4 in nuclei, or the export of heterologous β_{4b}-V5 from the nuclei. In conclusion, cultured CGC recapitulate isoform-specific nuclear targeting of heterologous β_{4b}-V5 but not of endogenous β_{4b}. The difference to cerebellar granular cells in situ may arise from lower expression levels of β_4 observed in cultured CGCs by quantitative RT-PCR.

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P 19

Short- and long-term changes in the expression of GABAA receptor subunits in the rat parahippocampal region after kainic acid induced status epilepticus

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Temporal lobe epilepsy (TLE) is the most common form of focal epilepsies in adults and there is broad evidence for changes in the balance of excitation and inhibition. Fast inhibitory neurotransmission in the brain is mainly mediated by gamma-amino-butyric acid (GABA) acting on ionotropic GABAA receptors. These receptors form heteropentameric ligand gated Cl^- channels assembled of five protein subunits that belong to different subunit classes. Subunit composition largely defines the pharmacological properties of the receptors and their subcellular localization. In previous studies, our group has characterized the dynamic regulation of GABAA receptor subunit mRNA-expression in the rat hippocampus in the kainic acid (KA) model of TLE. As recent electrophysiological studies from human patients and from animal models of TLE suggest active roles for the subiculum and the entorhinal cortex (EC) in the initiation of spontaneous epileptic seizures, this study was designed to investigate changes in GABAA receptor subunit mRNA expression in these regions during epileptogenesis.
Status epilepticus (SE) was induced in male Sprague-Dawley rats by i.p. injection of 10 mg/kg KA. Rats were sacrificed 24 hrs, eight days, one month or three months after chemically induced SE. Expression of mRNAs for subunits α1-5, β2-3, γ2 and δ in the subiculum and in the EC were assessed by *in situ* hybridization (ISH).
ISH for α1 and α3 mRNA revealed an initial downregulation at early time intervals after SE, followed by an upregulation at later stages eventually reaching control levels. α4 mRNA was significantly increased after 1 month in the proximal subiculum, but returned to control values after 3 months. α2 mRNA expression was decreased in the subiculum at early and very late intervals after KA-induced seizures. α5, β2, and β3 mRNAs were significantly downregulated in all areas 24 hrs and at late intervals after KA-induced SE. γ2-mRNA was downregulated in the deep layers of the EC after 24 hrs, but returned to control-values at later time intervals. In the superficial layers of the EC, γ2-mRNA was upregulated eight days, one and three months after KA-injection. In contrast, mRNA for the δ subunit was downregulated after 24 hrs, but did not return to control values at later intervals after SE.
These results suggest a distinct regulation of mRNA expression for different subunits. The selective upregulation of the γ2-subunit - mainly expressed by synaptically located GABAA receptors in controls – together with a downregulation of mRNA for preferentially perisynaptically or extrasynaptically located subunits (α5 and δ) may reflect changes in the balance of phasic versus tonic inhibition in parahippocampal brain regions during epileptogenesis. Alternatively, these changes may result in GABAA receptors with changed subunit composition and thus changed pharmacological properties.
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P 20

Structural changes of GABAergic synapses upon fear conditioning in basolateral neurons of the mouse amygdala.

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The amygdaloid complex is engaged in a number of behavioural and regulatory functions, which include autonomic and motor responses to emotionally relevant sensory stimuli. The basal nucleus (BA), which is mostly composed of projection neurons and a few interneurons, plays important roles in fear learning and memory. In Pavlovian fear conditioning, in which initially a conditioned stimulus (CS) is repeatedly paired with an aversive stimulus (US), animals learn to associate CS with US, hence attaching emotional significance to sensory stimuli. Conditioned fear can be suppressed when the CS is repeatedly presented alone, a phenomenon known as fear extinction. Previous studies suggest that the mechanisms underlying fear conditioning involve inhibitory neurotransmission through $GABA_A$ receptors. $GABA_A$ receptors are generally composed of two α, two β and one γ subunits. The 16 $GABA_A$ receptor subunits present in the brain from multiple receptor subtypes with different, biophysical and pharmacological properties. Recent work showed that fear conditioning induces a dramatic downregulation of benzodiazepine binding sites and transcripts for gephyrin and some $GABA_A$ receptor subunits in the BA, which were restored to control levels after fear extinction.
In this work, we analysed by means of the novel freeze-fracture replica immunolabelling technique (SDS-FRL) the synaptic and extrasynaptic content of the $GABA_A$-γ2 subunit in the BA of mice that underwent fear conditioning as well as extinction. Using this technique, we could also measure the synaptic area of GABAergic synapses. Immunogold particles for $GABA_A$ subunits tend to concentrate in clusters of intramembrane particles (IMP) on the protoplasmic face of the plasma membrane, indicating that labelled IMP clusters represent GABAergic synapses. The average size of GABAergic synapses in control mice was 0.030±0.019 μm^2. Fear conditioned animals showed a significantly ($P < 0.05$, one-way ANOVA) larger (0.041±0.026 μm^2) average synaptic size, whereas in mice that underwent extinction it was similar to controls (0.033±0.021 μm^2). No differences could be detected in both synaptic and extrasynaptic labelling density for $GABA_A$-γ2, although a clear tendency for a lower density in fear conditioned animals was observed, which however did not reach statistical significance.
We also analyzed by *in situ* hybridization the mRNA levels of $GABA_A$-γ2 among the 3 groups, which were found highly similar in the BA as well as in other amygdala nuclei. These results suggest that fear conditioning produces an enlargement of GABAergic synapses maintaining the number of $GABA_A$ receptors substantially unaltered.

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P 21

Cell Fate Analysis of Embryonic Stem Cells during *in vitro* Differentiation to Mesencephalic Dopaminergic Neurons

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Methods for generating mesencephalic dopaminergic (DA) neurons from embryonic stem (ES) cells for cell replacement therapy are frequently based on a five step protocol published first by Lee SH *et al.,* (Nat Biotechnol. 2000).
In order to optimize this protocol, we have defined PCR probe sets for more than 100 molecular markers. Molecular profiles based on these markers allow a follow up of cell fate decisions *in vitro* and *in vivo*. These profiles enabled us to modify the "classical" protocol, which is based on treatment of cells for a fixed number of days in vitro (DIV), by a more flexible regimen that takes into account the actual progress of cells through series of cell lineage decisions. With this strategy, we generated a cell population which displays the typical molecular profile of E13 ventral mesencephalon.

The established molecular profiles are useful, first to guide ES cell derivatives through cell fate decisions occurring *in vitro* and second to characterize the cellular composition of the transplant before and after grafting *in vivo*. In the future, we aim to define molecular profiles that help to predict the survival rate and efficacy of dopaminergic cells in transplants.

P 22

Identification and characterization of Nogo protein complexes

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RTN-4/Nogo proteins are myelin-associated proteins intimately implicated to impair axonal regeneration and structural plasticity in the adult mammalian nervous system. Apart from this well characterized function, recent *in vitro* studies have suggested that related to their subcellular localization to the endomembrane system, Nogo proteins may subserve additional roles, such as regulation of membrane curvature and protein trafficking [1-3].

To further investigate this hypothesis and to unravel possible molecular mechanisms of Nogo function in neurons, we aimed to identify interaction partners of Nogo in adult mouse brains.

For this purpose, we performed pull-down experiments using NiR, the N-terminal domain of Nogo-A and Nogo-B. Recombinant Nogo domain protein NiR (-GST) was used as bait to interact with proteins from mouse brain lysate. The pull-down products were analyzed by Mass Spectrometry (MS). This approach identified a number of proteins, majority of which had been classified as signal transduction, cytoskeleton dynamics related, and synaptic-vesicle and vesicle trafficking (endocytosis/exocytosis) associated.

We are currently in the progress of validation of these interactions for selected candidates, by performing co-immunoprecipitation, and colocalization studies by advanced microscopy techniques. Furthermore, in-detail biochemical characterization e.g. site/domain specificity of these potential interactions are being conducted.

As an outlook, we plan to address the functional importance and physiological significance of Nogo interactions. In brief, we envision to investigate the effect of these interactions in neurons, to close the gap of knowledge of how does Nogo function at a cellular level, for instance via the study of subcellular structures and related cellular mechanisms, such as endocytosis and exocytosis [4].

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References

1. Rafelski and Marshall, 2008, Nature Reviews Molecular Cell Biology, v.9: 593-602.
2. Voeltz *et al*., Cell, 2006, 124: 573-586.
3. Hu *et al*., Cell, 2009, 138: 549-561.
4. Shupliakov *et al*., Science, 1997, 276: 259-263.

P 23

Two distinct pools of large-conductance calcium-activated potassium channels in the somatic plasma membrane of central principal neurons

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Although nerve cell membranes are often assumed to be uniform with respect to electrical properties, there is increasing evidence for compartmentalization into subdomains with heterogeneous impacts on the overall cell function. Such microdomains are characterized by specific sets of proteins determining their functional properties. Recently, clustering of large-conductance calcium-activated potassium (BK_{Ca}) channels was shown at sites of subsurface membrane cisterns in cerebellar Purkinje cells, where they likely participate in building a subcellular signaling unit, the 'PLasmERosome' (Kaufmann et al., 2009, J. Comp. Neurol. 515: 215-230). By applying SDS-digested freeze-fracture replica labeling (SDS-FRL) and postembedding immunogold electron microscopy, we have now studied the spatial organization of somatic BK_{Ca} channels in neocortical layer 5 pyramidal neurons, principal neurons of the central and basolateral amygdaloid nuclei, hippocampal pyramidal neurons and dentate gyrus granule cells to establish whether there is a common organizational principle in the distribution of BK_{Ca} channels in central principal neurons.
In all cell types analyzed, somatic BK_{Ca} channels were found to be non-homogenously distributed in the plasma membrane, forming two pools of channels with one pool consisting of clustered channels and the other of scattered channels in the extrasynaptic membrane. Quantitative analysis by means of SDS-FRL revealed that about 2/3 of BK_{Ca} channels belong to the scattered pool and about 1/3 to the clustered pool in principal cell somata. Overall densities of channels in both pools differed in the different cell types analyzed, although being considerably lower compared to cerebellar Purkinje cells. Postembedding immunogold labeling revealed association of clustered channels with subsurface membrane cisterns and confirmed extrasynaptic localization of scattered channels. This study indicates a common organizational principle for somatic BK_{Ca} channels in central principal neurons with the formation of a clustered and a scattered pool of channels, and a cell-type specific density of this channel type.

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P 24

Synaptic release and synapse formation in neuronal calcium channel □₂□ subunit knockdown/knockout models

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Auxiliary $\alpha_2\delta$ subunits are involved in the trafficking and modulation of voltage-gated calcium channels (VGCCs). Furthermore, recent findings suggested a function of $\alpha_2\delta$ subunits in synapse formation. However, whether and how $\alpha_2\delta$ subunits regulate synapse formation and presynaptic transmitter release in differentiated neurons of the central nervous system is largely elusive. Because neurotransmitter release depends on calcium influx to the third-fourth power, modifications in the composition and hence properties of presynaptic VGCCs are expected to result in altered synaptic release rates. Here we analyzed synapse density and synaptic release by shRNA knockdown and overexpression of $\alpha_2\delta$-1 in highly differentiated cultured mouse hippocampal neurons (DIV 17-24) of wildtype or $\alpha_2\delta$-null background. Consistent with a potential role of $\alpha_2\delta$-1 in presynaptic function overexpressed pHlourin-tagged $\alpha_2\delta$-1 was highly enriched in the axonal plasma membrane. Activity-induced uptake and release of fluorescent FM dyes provides a direct measure of synaptic release properties. Accordingly 57% of the decrease in FM dye fluorescence induced by 40 mM KCl could be blocked by agatoxin and conotoxin, revealing its dependence on presynaptic P/Q- and N-type channels. Lentiviral transfection of $\alpha_2\delta$-1 shRNA resulted in a consistent and robust mRNA knockdown to 21±10 % of controls after 24 DIVs. Surprisingly however, $\alpha_2\delta$-1 knockdown did not affect synaptic release rates. Furthermore the density of functional FM-positive synapses was similar in both conditions. To exclude functional compensation by other $\alpha_2\delta$ isoforms, we are analyzing cultures from $\alpha_2\delta$-2 mutant and $\alpha_2\delta$-3 knockout mice. Preliminary experiments employing $\alpha_2\delta$-1 shRNA knockdown in $\alpha_2\delta$-3 knockout neurons indicate no significant difference in kinetics and amplitude of synaptic release in comparison with $\alpha_2\delta$-3 deficient neurons. To address the putative role of $\alpha_2\delta$-1 in synapse formation we co-immunolabeled the presynaptic marker synapsin with the postsynaptic marker PSD95. Pre- and postsynaptic specializations were similarly observed in differentiated 21-31 DIV old $\alpha_2\delta$-1 knockdown/ $\alpha_2\delta$-3 knockout neurons; both in efferent and in afferent synapses of the transfected neuron. Together these results suggest that synapse formation and P/Q- and N-type channel-dependent transmitter release in differentiated cultured hippocampal neurons does not rely on $\alpha_2\delta$-1, and that potential $\alpha_2\delta$-1 mediated effects are not compensated by $\alpha_2\delta$-3.

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P 25

Characterization of a BK-channel subunit α conditional knock out mouse

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Big conductance potassium channels (BK-channels) are expressed in most mammalian tissues, in the nervous system BK-channels are found in axon terminals, somata and dendrites. BK-channels play a variety of roles and they are involved in neurotransmission, blood flow, uresis and immunity.
BK-channel subunit α germ-line KO mice lack functional BK-channels and exhibit intention tremor and abnormal gait, severe cerebellar ataxia and Purkinje cell dysfunction. Therefore, they are not suitable for most paradigms of behavioral testing.
BK-channel α subunit conditional KO mice allow region-specific gene inactivation. To overcome the problems of germ-line KO mouse regarding cerebellar ataxia, the conditional KO mice are crossed onto mice expressing Cre under the control of Cam-Kinase II α promoter. In these mice we expect BK function lacking in principal neurons outside the cerebellum, quite likely in the hippocampus and the amygdala, both brain regions play a crucial role in learning, memory and anxiety. As the phenotype of this animal model is not expected to exhibit cerebellar ataxia, further experiments on a functional level like behavioral testing regarding anxiety or learning and memory should be feasible.
The main aim of this project is to characterize conditional BK KOs on neurochemical and behavioral level. So far, we have established a reliable *in situ* hybridization protocol for detection of BK α subunit mRNA and identified suitable antibodies for Western Blots and immunohistochemistry. The outcoming of these investigations will form the basis of further investigations.
The distribution pattern of the BK-channel demonstrated in Immunohistochemistry coincides to the reported distribution and fits to the distribution pattern of the BK-channel mRNA demonstrated in *in situ* hybridization.

P 26

Investigating gene deregulation in the hippocampus of the NgR2 knockout mouse

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Abstract:
NgR2 is a member of the Nogo-receptor family, members of which are known to bind inhibitors of axonal regeneration in the CNS. Thus far the only known ligand of NgR2 is myelin associated glycoprotein (MAG). High expression of NgR2 mRNA has been reported in neurons of the rodent CNS, including hippocampal formation and amygdala. We have generated a NgR2 knockout mouse line to study NgR2 function. Behavioral studies have shown that under mild stress conditions, Ngr2-/- mice have a behavioral phenotype of reduced anxiety and depression. An anxiolytic effect was seen in the increase in exploratory time in the open field test, and decreased depressive-like behavior was observed in decreased immobility time in the tail suspension test. To investigate the underlying molecular mechanism linking this receptor to behavioral changes, potential deregulated genes are being investigated in adult NgR2-/- CNS at the mRNA and protein level. The ATF/CREB family of transcription factors, in particular CREB, have been implied in the molecular changes associated with depression. In this study, increased levels of activating transcription factor 3 (ATF3), an adaptive response gene, were found in areas implicated in regulation of depression and anxiety including hippocampus and amygdale, in NgR2-/- mice compared to wildtype controls. Interestingly, preliminary results suggest there is no phosphorylation of c-Jun in parallel, suggesting ATF3 does not heterodimerize with c-Jun to mediate its downstream effects. Increased levels of phosphorylated CREB were also seen in hippocampus, amygdala and hypothalamus. ATF3 is known to be to be regulated by MAPK signaling pathways, in particular the p38 pathway, of which CREB is known to play a role upstream of ATF3. Further investigation is needed to confirm the role of this pathway in this model and to investigate downstream effects on targets which could be mediating the behavioral phenotype seen.

P 27

Identification of non-coding RNAs involved in neuronal differentiation of embryonic stem cells through RNP selection.

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Non-coding RNAs are important for development, represent a significant portion of the transcriptome and have been shown to be involved in differentiation processes (Dinger, M.E. et al 2003). We aim to identify differentially regulated non-coding RNA species involved in neural cell differentiation, by combining an *in vitro* and an *in vivo* approach. An important *in vitro* model system not only to study development but also organ repair and stem cell therapy are embryonic stem cells, which have the ability to self renew and to differentiate into functional cell types. A significant portion of the stem cell transcriptome appears to correspond to non-coding transcripts, as both microRNAs and longer non-coding RNAs have been identified in those cells. An attractive *in vivo* system are dorsal root ganglia (DRGs), since they can be isolated in different developmental stages and are an important model system for axonal regeneration and pain.

Most, if not all biologically functional identified non-coding RNAs in Eukarya are known to be associated with proteins forming ribonucleo-protein (RNP) particles. In order to isolate non-coding RNAs that are likely to be functional, we have generated RNP libraries (Rederstorff, M. et al 2010) from various stages of mouse embryonic stem cells differentiating upon addition of FGF2 and different developmental stages of DRGs, derived from embryonic, postnatal and adult mice.

The RNP libraries raised were high-throughput sequence and revealed a significant amount of house keeping non-coding RNAs and candidates mapping to intergenic or intronic regions, which could potentially be novel functional non-coding RNAs, present in varying percentages between different libraries.

Candidates are verified by Northern blots and will be validated in a high-throughput manner by microarrays. The most interesting non-coding RNAs will be selected for further functional analysis, to investigate their roles in neural differentiation. The non-coding microarray chip developed will serve as a diagnostic tool for neurodegenerative diseases.

P 28

Influence of different ambient light conditions on the serotonergic system after tryptophan depletion

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Introduction: The central serotonergic system plays a crucial role in the regulation of mood and emotions. The production of serotonin in the brain is mainly influenced by the activation of the nucleus suprachiasmaticus by light and the availability of its precursor tryptophan. Tryptophan depletion (TD) is an established method to reduce serotonin production in the brain through administration of a tryptophan-free mixture of large neutral amino acids (LNAA´s). The purpose of this study was to examine the effect of different ambient light conditions on the effect of tryptophan depletion in healthy female subjects measured by different plasma levels and a mood questionnaire.
Methods: Eight healthy female participants carrying one or two copies of the s-allele of the serotonin transporter promoter polymorphism (5-HTTLPR) spent three testdays (duration 8 hours each) at our clinic. On the beginning of each testday a TD was administered and the probands were exposed to one of three different light conditions (A=80lx, B=300lx, C=1500lx). At baseline and 3, 5 and 7 hours after TD blood samples were taken to analyze levels of serotonin, 5-HIAA, tryptophan (tr) and tryptophan/LNAA ratio and mood was assessed by a visual analogue scale (VAS).
Results: TD induced a significant reduction of plasma tryptophan from baseline to 5h after TD in all three light conditions with no additional change after 7h. A decrease of the tr/LNAA ratio was seen in all light conditions after 3h. Except of the 1500lx group the tr/LNAA ratio decreased between 3 and 5 hours, followed by a significant increase 7h after TD. Plasma levels of 5-HIAA decreased in all light conditions between baseline and 3 hours after TD. A significant increase of 5-HIAA was detected after 5h in condition C and after 7h in conditions B and C but not in condition A. Results of the VAS showed no change of mood in condition A and B. Condition C (the one with the highest illuminance level) showed a significant improvement in mood reflected by an increase of VAS ratings after 5h.
Conclusion: Results of our study showed an influence of ambient light on the progression of TD. High illuminance levels affected the subjective mood as well as plasma levels of tryptophan, Tr/LNAA ratio and 5-HIAA. Further studies are needed to assess if there is a dose dependent interrelation between light and the serotonergic system.

P 29

The central and basolateral amygdala are critical sites of NPY/Y2 receptor-mediated regulation of anxiety and depression

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Anxiety is integrated in the amygdaloid nuclei and involves the interplay of the amygdala and various other areas of the brain. Neuropeptides play a critical role in regulating this process. Neuropeptide Y (NPY), a 36 amino acid peptide, is highly expressed in the amygdala. It exerts potent anxiolytic effects through cognate postsynaptic Y1 receptors, but augments anxiety through presynaptic Y2 receptors. To identify the precise anatomical site(s) of Y2-mediated anxiogenic action, we investigated the effect of site-specific deletion of the Y2 gene in amygdaloid nuclei on anxiety and depression-related behaviors in mice. Ablating the Y2 gene in the basolateral and central amygdala resulted in an anxiolytic phenotype, whereas deletion in the medial amygdala or in the bed nucleus of the stria terminalis had no obvious effect on emotion-related behavior. Deleting the Y2 receptor gene in the central amygdala, but not in any other amygdaloid nucleus, resulted in an added antidepressant-like effect. It was associated with a reduction of presumably presynaptic Y2 receptors in the stria terminalis/bed nucleus of the stria terminalis, the nucleus accumbens and the locus coeruleus. Our results are evidence of the highly site-specific nature of the Y2-mediated function of NPY in the modulation of anxiety- and depression-related behavior. The activity of NPY is likely mediated by the presynaptic inhibition of GABA and/or NPY release from interneurons and/or efferent projection neurons of the basolateral and central amygdala.
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P 30

Subpopulations of NK1 receptor expressing interneurons in the rat lateral amygdala display a differential pattern of innervation from distinct glutamatergic afferents

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Abstract

Neurokinin 1 receptor (NK1) expressing neurons in the amygdaloid complex play an important role in anxiety and fear learning, however little is known about their detailed cellular localization in this region. We used immunohistochemical methods at light and electron microscopic level, to study the co-localization of NK1 receptors with various calcium binding proteins, and their synaptic relationship to glutamatergic axonal inputs in the lateral amygdala (LA). Light microscopic observations revealed intense NK1 immunoreactivity (NK1-IR) in scattered sparsely spiny neurons. Double and triple immunofluorescence microscopy revealed that 35% of NK1-IR neurons also expressed parvalbumin, 25% contained calbindin whereas 5% were immunopositive for both calbindin and parvalbumin. Dual localization of NK1-IR and calcium calmodulin protein kinase II alpha-IR revealed that NK1 expression is restricted to GABAergic neurons. By using a combined immunoperoxidase/immunogold-silver procedure at the ultrastructural level, we found that 54% of asymmetrical synapses on NK1-IR neurons contained the vesicular glutamate transporter 1 (VGluT1). On the other hand, asymmetrical synapses containing the vesicular glutamate transporter 2 (VGluT2) accounted for 18%. In the subpopulation of NK1/parvalbumin-IR neurons asymmetrical synapses were could never detect VGluT2-IR in presynaptic boutons. These findings indicate that NK1-IR interneurons are strongly innervated by cortical/hippocampal/amygdala axonal inputs (VGluT1-IR boutons) compared to thalamic/hypothalamic/parabrachial axonal inputs (VGluT2-IR boutons) and that the relatively large population (40%) of NK1-IR interneurons expressing parvalbumin is not regulated by the latter inputs.

P 31

The biological role of nogo receptor in immune cells

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Nogo receptor (NgR) is the common receptor for several myelin-associated inhibitory factors (MAIFs). Binding of ligand to NgR results in RhoA activation, which alters actin cytoskeleton dynamics and inhibits axonal outgrowth or adhesion and spreading of cells. After axonal injury, an inflammatory response is induced, resulting in the presence of peripheral immune cells in the degenerating lesion. We hypothesise that NgR expressed on peripheral immune cells could have an effect on adhesion and migration of these cells on myelin debris, such as that found in inflammatory lesions of the central nervous system (CNS).

Human monocytes were isolated from full blood and differentiated into immature monocyte-derived dendritic cells (moDCs) or mature moDCs. Using Real Time PCR, it was found that NgR1 mRNA is expressed to a higher extent in immature 7-day moDCs compared to monocytes. This expression returns to the baseline level after maturation of the cells. Human immature moDCs expressing NgR1 are inhibited from adhering to a myelin substrate. To determine if NgR mediates this inhibition of adhesion to myelin, DCs were isolated from NgR1/2 double knock out mice (NgR1/2 -/-). When bone marrow-derived DCs (bmDCs) from NgR1/2 -/- mice were plated on myelin, it was found to have no effect on adhesion. Wild type bmDCs were found to express NgR1 mRNA and were inhibited from adhering to myelin in the same way as human DCs.

Thus, in mouse and human DCs expressing NgR, adhesion to myelin is inhibited. We demonstrate that NgR may mediate this inhibition by showing that mouse DCs lacking NgR can adhere to a myelin substrate. The proposed interaction between peripheral DCs and myelin could help us better understand the action of immune cells during neuroinflammatory events, in which DCs probably come into contact with myelin debris.

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Molecular background of mechanosensitivity in primary afferent neurones

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The mammalian somatosensory system detects a wide variety of stimuli matched by a diverse array of somatosensory neurones in the dorsal root (DRG) and trigeminal ganglia (TG). Mechanical stimuli can range from innocuous stimuli such as texture, shape, vibration, or pressure to noxious, potentially harmful stimuli. Although it is widely understood which class of fibre detects what kind of mechanical stimulation, the molecular basis underlying mechanotransduction is only beginning to be uncovered. It is mainly thought that ion channels in the membranes of the nerve endings directly or indirectly open upon mechanical stimulation giving rise to changes in ionic conductance, and a consecutive depolarization of the cellular membrane and action potential firing.
Electrical events in the peripheral nerve can be measured extracellularly by single-fibre recordings. However, the very small size of the mechanosensitive peripheral endings have precluded direct intracellular recording, so far. Primary cultures of DRG neurones can, to a certain extent, serve as a model of their afferent terminals detecting noxious stimuli *in vivo*. Therefore a promising attempt is to monitor the membrane currents using the whole-cell voltage clamp configuration of the patch-clamp technique while stimulating the DRG soma or neurite with heat-polished glass-pipettes.
Stimulation of the soma or the neurite has led to the characterisation of three distinct types of mechanosensitive inward currents, which can be differentiated into rapidly adapting (RA), intermediate adapting (IA) and slowly adapting (SA) currents according to their inactivation kinetics. These currents show a distinct distribution among subsets of sensory neurones and IV-plots suggest different underlying ion channels. The aim of this study is to investigate the regulation of mechanosensitivity by cytokines and growth factors on the cellular level using the aforementioned method.

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Novel treatments to rescue impaired extinctionlearning in 129S1 mice
N. Whittle, M. Hauschild, S.B. Sartori, G. Lubec, A. Holmes, N. Singewald

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Influence of diurnal rhythm and trait anxiety on short and long-term memory after cued fear conditioning
M. Hauschild, S.B. Sartori, S. Gaburro, F. Hetzenauer, R. Landgraf, N. Singewald

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Loss of peripheral sensory projection in *islet1/isl1* mutant zebrafish

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The islet1/isl1 gene encodes a LIM homeobox transcription factor with various conserved embryonic functions. Throughout the animal kingdom homologues of isl1 are expressed in various postmitotic neurons and genetic studies in fly, fish and mouse demonstrated essential roles in regulating motoneuron survival and behavior. Analysis *isl1* knock out mice revealed additional *isl1* functions during formation of the dorsal aorta, the early morphogenesis of the pancreas, the differentiation of endocrine cells in the islet of Langerhans and the regulation of proliferation of stem cells in the heart. Two recent studies that used conditional knock out approaches to overcome early embryonic lethality (E10) provided first hints on late embryonic roles of isl1 in regulating differentiation, survival and axon outgrowth behavior in retinal ganglion cells (RGC) and in spinal sensory neurons.
Here we report on the primary analyses of a novel zebrafish *isl1 -/-* mutant, which was identified as part of the ‚open source' EU project ZF-Models (http://www.zf-models.org/). Unlike the *isl1* mutant mice, the zebrafish *isl1* mutants show a normal dorsal aorta and they survive embryogenesis. Due to the obvious differences in isl1-requirement in fish and mice, we first asked if *isl1* zebrafish mutants develop defects in the in the peripheral nervous system, that are similar to those described in the conditional knock out mice. We find that primary sensory cells of the fish, the Rohon Beard cells (RBC), are formed in normal numbers but with major projection defects. Further, we find that loss of *isl1* does not induce increased Apoptosis but leads to the loss of *isl2a* and *isl2b* mRNA expression in RBCs. We also find that RGCs are formed in *isl1* mutants but that they lack *isl2b* expression. As in mouse a single *isl2* gene in also regulated by *isl1*, our data show that the is*l1-isl2* interaction is conserved but in zebrafish has diverged in a way that *isl1* regulated two different *isl2* genes in cell-type specific manner. Our preliminary analyses reveal different requirements of fish and mouse *isl1* in preventing neuronal apoptosis they suggest a conserved requirements in regulating differentiation not only in motoneurons but also in sensory neurons.

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Novel role of Hedgehog signaling in motoaxon guidance

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Spinal motoneurons in zebrafish form axon projections along a small set of stereotyped pathways to connect with specific muscles. The formation of the spinal motoneuron system is highly dependent on hedgehog signaling, which is required for the induction of motoneurons and for the specification of various cells fates including adaxial muscles that provide essential guidance cues for outgrowing motoaxon. Here we describe a novel function of hedgehog signaling on post mitotic motoneurons that defines outgrowth behavior.
Characterization of a novel nonsense mutation in the intracellular the hh-signaling component *dzip1/iguana* revealed previously not described projection defects in hh-signaling mutants. Transplantation experiments using wild type and *dzip1/igu* or *gli1/dtr* mutants, show that motoaxon outgrowth depends on cell-autonomous and non-cell-autonomous functions of intracellular hh-signaling components. While *dzip1/igu* and *gli1/dtr* mutants form motoneurons in normal numbers, we now show that expression of the lim-homeobox gene *isl2* is strongly reduced in these mutants. A similar reduction of *isl2* expression was observed when wild type embryos where treated with the hegdehog antagonist cyclopamine, independent if this treatment was started at the time of motoneuron birth or later at the onset of axon formation. This shows that motoneurons depend on continued hh-signaling to establish and maintain an appropriate expression profile. Finally, we show that loss of *isl*-gene expression in *igu* and *dtr* mutants and in *isl*-morphants correlates with a cell-autonomous loss of *nrp1a*-mRNA, which encodes a co-receptor for semaphorin axon guidance factors. In summary these data reveal a novel role of hh-signaling in maintaining motoneuron fates and in regulation intrinsic properties of axon guidance.

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Reticulon Proteins in the Epileptic Rat Brain

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The most common histopathological findings of mesial temporal lobe epilepsy (TLE) include neuronal cell loss as well as gliosis. Another characteristic phenomenon in TLE is termed mossy fiber sprouting – relating to the axons of granule cells – which is believed to enhance excitability. RTN-4A – better known as Nogo-A – is a possible candidate molecule which might be implicated in the regulation of activity-dependent plasticity. This protein is a member of the conserved family of endoplasmic reticulum-associated reticulon proteins and is best studied for its neurite outgrowth inhibitory effect when being expressed on the surface of myelinating oligodendrocytes after nerve injury.
High levels of Nogo-A can be detected in certain neurons of the CNS - not only during development, but also in the adult rodent brain. The intracellular localization of the protein – displaying a reticular pattern – suggests that Nogo-A might exert additional functions, such as a signaling molecule.(1) In studies of human(2) and experimental(3) temporal lobe epilepsy it was shown that Nogo-A mRNA and immunoreactivity were clearly up-regulated in hippocampal neurons and their processes. As it is believed that axonal and synaptic re-organizations in the hippocampal formation are promoting epileptogenesis, our group wants to test the hypothesis that knocking down Nogo-A leads to a change in the pathology of seizures in a rat model of temporal lobe epilepsy. Recombinant adeno-associated viral particles, containing shRNA targeted against Nogo-A, are injected into the rat hippocampus. After the system has reached peak expression, Kainic acid is administered intra-peritoneally. This algae-derived acid is a specific agonist of the kainate receptor, mimicking the effects of glutamate and thereby eliciting seizures. The severity of the status epilepticus gets rated and continuous observation via video monitoring gives insight into seizure frequency and duration. Immunohistochemical techniques will be used to further characterize the plastic changes within the epileptic rat hippocampus.

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References:
(1) Oertle & Schwab (2003) Nogo and its paRTNers. Trends Cell Biol. Vol.13 No. 4, 187-94
(2) Bandtlow et al. (2004) Increased expression of Nogo-A in hippocampal neurons of patients with temporal lobe epilepsy. Eur J Neurosci. Vol. 20, 195-206
(3) Meier et al. (2003) Molecular analysis of Nogo expression in the hippocampus during development and following lesion and seizure. Faseb J. Vol.17 No.9, 1153-5

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The Nogo Receptor 2 (NgR2) is Required for Proper Innervation of the Skin

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While the first identified receptor of the Nogo Receptor family (NgR1) signals axonal growth inhibition via its coreceptors P75NTR and Lingo and the interactors Nogo, MAG or OMgp for NgR2 just MAG as binding partner was identified and its biological relevance is largely unknown. Therefore we obtained a mouseline deficient in NgR2 to investigate its function in more detail with respect to the molecular and histological effects of the receptor deletion. Surprisingly these mice show a significant increase of sensitivity to mechanical stimuli in behavioural experiments and *in vitro* single cell assays respectively but no other obvious abnormalities.
First we could show that the deletion of NgR2-/- results not in a shift or loss of neuron subpopulations in the dorsal root ganglia (DRG) what could led to the hypersensitivity phenotype. In this study we investigate weather the deletion of NgR2 effects the nerve innervation of the epidermis as the first source of tactile recognition. In the process the total number of free nerve endings in glabrous skin of the hindpaw from NgR2 deficient mice in comparison to the wildtype situation was significantly increased. Since the most dens innervation of the epidermis is observed at post natal day five and decreases drastically afterwards we addressed the question at which developmental stage a defect occurs and if this is restricted to a specific subpopulation of nerve endings namely $CGRP^+$ or $Mrgprd^+$ fibres.
Finally these data identify NgR2 as a receptor required for proper cutaneous innervation and could give a deeper insight in the largely unknown signal transduction and procession of mechanical stimuli. Additional cell culture based *in vitro* assays could show if the defect in axonal outgrowth is DRG-autonomous or dependent on factors secreted e.g. from keratinocytes of the epidermis.

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The reticulon protein Nogo-B modulates glial - neuronal crosstalk in the peripheral nervous system

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Reticulons (RTNs) are prominent endoplasmic reticulum (ER) associated proteins with a conserved C-terminal and variable N-terminal architecture that seems to reflect a dual-mode functionality. We are interested in Nogo/RTN4 proteins, in particular in Nogo-B which we found to be highly enriched in Schwann cells of the peripheral nervous system (PNS). We have shown previously that Nogo-B is a caspase target and that cleavage is markedly increased after axotomy in the PNS (Schweigreiter R. et al., 2007, Proteomics 7: 4457-67). Here we demonstrate that, in a co-culture of Schwann cells and sensory neurons of the mouse, Schwann cell expressed Nogo-B reduces sprouting of sensory neurons. This finding is of potential clinical relevance because it might contribute to the design of novel therapeutic strategies that tackle the sprouting problem of regenerating nerve fibres in the injured PNS.

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Lentivirus based knockdown of Rtn1A in cultured cerebellar granular neurons

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Rtn1A is a member of the reticulon protein family which also contains the neurite growth inhibitor Nogo (Rtn4). Reticulon proteins are associated with the ER membrane, and in particular Rtn1A is thought to be involved in trafficking and secretion of vesicles.

In preliminary experiments we observed enhanced neurite length following siRNA mediated downregulation of Rtn1A in cerebellar granular neurons (CGN). Interestingly, this effect was accompanied by a slight increase in BDNF secretion into the growth medium implying that Rtn1A negatively modulates secretion of the neurite growth promoter BDNF. However, in those experiments only a small fraction of the CGN appeared to be transfected by siRNAs. We aim to achieve highly efficient downregulation of Rtn1A by taking advantage of lentivirus mediated gene transfer. This technique has been reported to be superior to alternative transfer systems due to its high infectivity for postmitotic cells including neurons.

We succeeded in the construction of vesicular stomatitis virus glycoprotein G pseudotyped lentiviruses expressing shRNAs targeting either all isoforms of Rtn1 or Rtn1A specifically. Two types of vectors were constructed: One set of vectors allowing for constitutive bidirectional expression of shRNAs and red fluorescent protein (RFP), the other set enabling tetracycline inducible expression of shRNAs and EGFP.

We have achieved highly efficient infection of HeLa cells as well as of CGN isolated from P7 mice demonstrating the validity of our shRNA delivery approach.

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