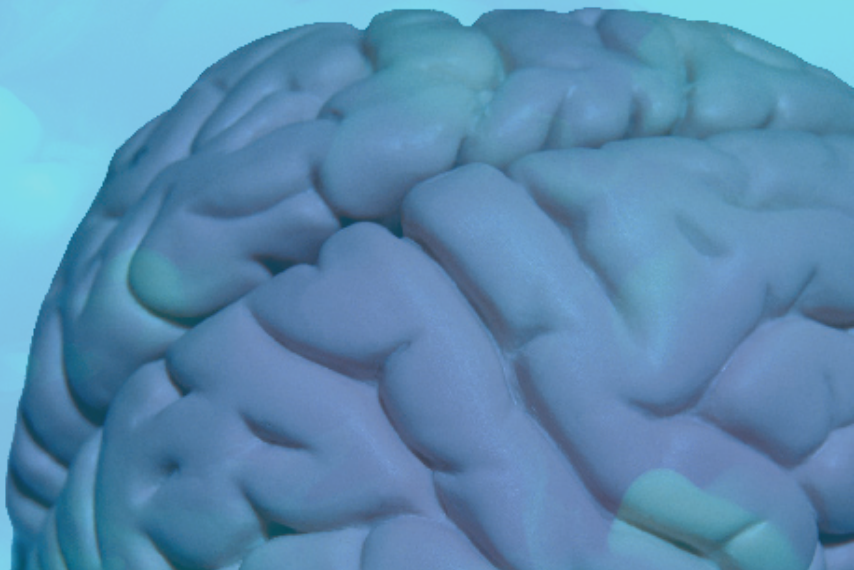
INNSBRUCK MEDICAL UNIVERSITY &
UNIVERSITY OF INNSBRUCK - NEUROSCIENCE

Neuroscience Day 2011

Friday, May 20th 2011. Grillhof Vill.

Final Program and Abstract Book



08:15 - 09:00 Registration and Poster Set-Up

09:00 - 09:15 **Georg Dechant**
Introduction and Opening

KEYNOTE LECTURE
Chair: Jörg Striessnig

09:15 - 10:15 **Carsten Wotjak**
Max-Planck-Institute of Psychiatry
Munich, Germany
„Traces of a trauma - lessons from a mouse model of posttraumatic stress disorder."

PRESENTATIONS: SESSION 1
Chair: Bernhard Flucher

10:15 - 10:30 **Serena Quarta**
Division of Physiology and Medical Physics, IMU
„Significance of the IL-6 signal transducer gp130 for neuronal regeneration."

10:30 - 10:45 **Petronel Tuluc**
Division of Physiology and Medical Physics, IMU
„Intramolecular CaV1.1 chimeras reveal the molecular mechanism determining the characteristic gating behaviour of the skeletal muscle calcium channel."

10:45 - 11:00 **Dilip Verma**
Department of Pharmacology, IMU
„Fear reversal after NPY vector administration in the BLA during fear acquisition."

Lectures

11:00 - 12:30 Coffee and POSTER SESSION 1 - odd numbers

12:30 - 13:30 Lunch

13:30 - 15:00 POSTER SESSION 2 - even numbers

PRESENTATIONS: SESSION 2
Chair: Georg Dechant

15:00 - 15:15 **Lisa Fellner**
Clinical Department of Neurology, IMU
„The role of toll-like receptor 4 on glial function in alpha-synucleinopathies."

15:15 - 15:30 **Bastian E. Bäumer**
Division of Neurobiochemistry, IMU
„The Nogo receptor homolog NgR2 is crucial for proper sensory innervation of the skin."

15:30 - 15:45 **Ahmad Salti**
Institute for Neuroscience, IMU
„Cell fate analysis of mouse embryonic stem cells during in vitro differentiation on telencephalic and mesencephalic neurons as compared with in vivo development."

15:45 - 16:00 **Sébastien Couillard-Després**
Institute of Molecular Regenerative Medicine, Paracelsus Medical University Salzburg
„TGF-beta signaling in the adult neurogenic niche.

16:00 - 16:15 **Anupam Sah**
Department of Pharmacology and Toxicology, IMU
„Adult neurogenesis in a mouse model of affective behavior pharmacological and non-pharmacological interventions."

Science

Events

NEUROSCIENCE DAY 2011

General Informations:

This year the Neuroscience Day will be held at the "***Bildungsinstitut Grillhof***" in Vill near Innsbruck. You can reach it either by car (follow the road to Igls and turn left in Vill, follow the signs to Grillhof) or by bus using line J (get off in Vill and walk up to Grillhof).

In order to keep costs low, we would like to ask all participants to bring their own name badges (recycle those of the last ANA meeting, last years NSD or else) and print your own copy of this program, if needed.

Short talks are 10 min plus a 5 min discussion. Please keep strictly to these limitations. Authors who give a talk are asked to visit the lecture hall between 8:30 and 8:45 to upload their powerpoint presentations.

Posterboards are 120 x 150 cm portrait. You will find numbers on the boards corresponding to the number of your abstract in the abstract book, indicating the place where you should mount your poster. Pins are available at Grillhof. Presenters of posters with odd numbers should be present at their poster during the pre-lunch poster session, those with even numbers should present their poster in the after-lunch session.

T 1

Significance of the IL-6 Signal Transducer gp130 for Neuronal Regeneration

S. Quarta[1], N. Scherbakov[1], M. Andratsch[1], S. Geley[2], M. Kress[1]
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[2]*Division of Molecular Pathophysiology, Innsbruck Medical University, Austria*

The interleukin-6 signal transducing receptor gp130 is involved in diverse biological processes as inflammation and pain. We have shown previously that gp130 expressed in the nociceptive membrane is essential for the maintenance of heat hypersensitivity in mouse models of pathological pain. This is mediated by activation of PKC-δ via Gab1/2/PI3K and subsequent regulation of TRPV1. In pathological conditions, the signal transducer gp130 seems to play a role in mechanical hypersensitivity as well. At present, the role of IL6/gp130 interaction in injured peripheral neurons is poorly understood. Here, 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).
The number of free nerve endings in the skin was similar in non-injured control and SNS-gp130$^{-/-}$ mice. In contrast, dorsal root ganglion (DRG) neuron cultures from SNS-gp130$^{-/-}$ mice showed significantly reduced neurite extension and numbers of neurite bearing neurons as compared to controls. We found lower expression levels of BDNF gene in explants and acutely dissociated SNS-gp130$^{-/-}$ DRG neurons. However, the substitution of neurotrophins like NGF or BDNF did not rescue neurite outgrowth in SNS-gp130$^{-/-}$ cultures. Preliminary evidence suggests that adenoviral re-expression of gp130 in cultured gp130-deficient neurons restored neurite extension.
Mechanical and heat sensitivity and motor capabilities were monitored for 25 days after sciatic nerve crush injury in *vivo*. Recovery of sensitivity was similar in wild type and gp130$^{fl/fl}$ control mice but significantly delayed in SNS-gp130$^{-/-}$ mice. To elucidate potential signaling pathways of gp130-dependent regeneration we used Affymetrix® gene chip expression analysis of mRNA harvested from DRG explants. We found down-regulation of the two regeneration-associated genes Atf3 and Sprr1a in SNS-gp130$^{-/-}$ DRG explants and this was confirmed by quantitative PCR. mRNA of both Atf3 and Sprr1A was lower in non-injured SNS-gp130$^{-/-}$ compared to gp130$^{fl/fl}$ DRG but increased after sciatic nerve injury at 3 and 7 days in both mice strains suggesting that Atf3 and Sprr1a may not be essential for regeneration in peripheral neurons.
Our data show that regeneration of neurons was significantly impaired in SNS-gp130$^{-/-}$ *in vitro* and *in vivo*. We therefore suggest that gp130 is an important regulator of regeneration in peripheral neurons.

Acknowledgement: Supported by FWF project P18444 and FWF DK SPIN W1206.

T 2

Intramolecular $Ca_V1.1$ chimeras reveal the molecular mechanism determining the characteristic gating behaviour of the skeletal muscle calcium channel

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The Ca^{2+} channel $Ca_V1.1$ is the voltage sensor of skeletal muscle excitation-contraction coupling. The classical skeletal muscle $Ca_V1.1$ isoform has poor voltage sensitivity and conducts a small, slowly activating Ca^{2+} current. In contrast, a splice variant lacking exon 29 (α_{1S}-ΔE29) (Tuluc *et al*., 2009) has an 8-fold higher current amplitude, fast activation-kinetics, and a 30mV left-shifted voltage-dependence of activation. Therefore, the extracellular loop in repeat IV (IVS3-IVS4) mainly coded by exon 29 is a critical determinant of the characteristic gating properties of $Ca_V1.1$. Here we used intramolecular chimeras between repeats I and IV to characterize the structural basis of the gating properties of $Ca_V1.1$.

Inserting exon 29 (alone or in combination with IVS3) into the corresponding region of repeat I was ineffective. However, in combination with the voltage sensor (IVS4) it fully restored α_{1S}-like amplitude and voltage-sensitivity to α_{1S}-ΔE29. Interestingly, all three chimeras exhibit faster activation kinetics. Secondary structure predictions showed that the long IVS3-IVS4 loop contains a beta-sheet while the short loop forms a coil. Point mutations in exon 29 which abolish the beta-sheet fully mimic the effects of deleting exon 29 regarding the kinetic properties and increase the current amplitude by 3-fold and left-shift the voltage dependence by -15mV. Together with previous findings (Nakai *et al*., 1994) our data suggest that the S3-S4 loop of the first repeat determines activation kinetics, while the corresponding loop plus voltage sensor in the fourth repeat with its unique secondary structure dictate the voltage-dependence, amplitude, and kinetics of skeletal muscle Ca^{2+} currents.

Grants: PT (MFI-2007-417), BEF (FWF-P20059-B05).

T 3

Fear reversal after NPY vector administration in to BLA during fear acquisition

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Neuropeptide Y (NPY) is a 36 amino acid peptide that is abundantly expressed in the central nervous system. NPY and its receptors are involved in various physiological and pathophysiological processes including energy homeostasis, pain and epilepsy. NPY also seems to play an important role in psychiatric disorders, such as anxiety and depression. Consistent findings across different rodent models have demonstrated an anxiolytic effect of NPY. The presence of different NPY receptors in the amygdala and the effects of NPY on anxiety raise the question, weather, NPY and its receptors may influence acquisition and extinction of conditioned fear. Therefore, we investigated NPY and NPY receptor knockout (KO) mouse models in *Pavlovian* fear conditioning.
In cued fear conditioning Y1 KO mice show faster conditioning and delayed extinction, whereas Y2KO mice are similar to wildtype mice. Interestingly Y4 KO mice show normal fear conditioning but exhibit impaired extinction. NPY knockout mice acquire higher freezing levels during fear conditioning and show increased expression and impaired extinction of conditioned fear.
AAV-Vector mediated re-expression of NPY in the basolateral amygdala (BLA) of NPY KO mice significantly reduced the increased fear acquisition of NPY KO mice. Fear expression determined 24 h later, however, was still high. In addition extinction was significantly improved after re-expression of NPY in the BLA of NPY KO mice. No change was observed, however, after re-expression of NPY in the central amygdala.
Our data indicate that NPY has an inhibitory role in the acquisition of fear and facilitates extinction of conditioned fear. These effects seem to be mediated predominantly in the BLA, as shown by AAV-NPY vector injections. Results from Y receptor KO mice suggest that the Y1 receptor is the most likely candidate for modulating the acquisition of fear, whereas for extinction a concerted action of Y1 and Y4 receptors seems to be conceivable.

Supported by the Austrian Science Fund (FWF, projects 102040, 194640)

T 4

The Role of Toll-like Receptor 4 on Glial Function in Alpha-synucleinopathies

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Background: Alpha-synucleinopathies are characterised by alpha-synuclein-positive cytoplasmic inclusions spread throughout the neurodegenerating central nervous system. Neurodegeneration is commonly accompanied by microgliosis and astrogliosis in these diseases. The inflammation process is considered as possible mediator of the disease progression. In human MSA and PD/DLB brains and in murine transgenic models of neuronal and oligodendroglial alpha-synucleinopathy an upregulation of TLR4/CD14 has been evidenced, however the role of innate immunity in these disorders remains unexplored. The current study addressed the role of TLR4 on microglial function in response to alpha-synuclein.
Methods: Phagocytosis of microglia, activation and ROS production of microglia under the influence of alpha-synuclein were examined in vitro. Primary murine microglia (P1-3) were used for the experiments. The role of TLR4 was assessed by preparing cell culture from TLR4 deficient mice. FACS analysis, immunocytochemistry and fluorescence microscopy were applied to characterise microglial activation and phagocytosis in response to alpha-synuclein. For analyzing ROS production nitro blue tetrazolium chloride was added to the treated cells. The role of TLR4 was further studied in vivo in TLR4 knock-out mice over-expressing alpha-synuclein in oligodendroglia to model MSA pathology.
Results and Conclusions: Our in vitro results suggest effects of alpha-synuclein on microglial activation and phagocytosis which is partly mediated through TLR4. Furthermore, ROS production is enhanced after alpha-synuclein treatment in wild type, but not in TLR4 deficient microglia. Ablation of TLR4 in a transgenic mouse model of MSA with oligodendroglial alpha-synuclein over-expression augmented motor disability and enhanced loss of nigrostriatal dopaminergic neurons. Our data suggest that TLR4 ablation impairs the phagocytic response of microglia to alpha-synuclein and enhances neurodegeneration in a transgenic MSA mouse model. The current studies provide new insights into the role of innate immunity in alpha-synucleinopathies.
Acknowledgements: This study is supported by grants of the Austrian Science Funds P19989-B05 and SFB F44-B19.

T 5

The Nogo Receptor Homolog NgR2 is Crucial for Proper Sensory Innervation of the Skin

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The **Nogo**-66 **receptor** homolog **NgR2** is a member of the Nogo-receptor family and is suggested to impair axonal regeneration of injured nerve fibers and to restrict structural plasticity in the uninjured brain. In the PNS, NgR2 is expressed in dorsal root ganglia (DRG) neurons, but its physiological relevance remains unknown. In this study we examined how absence of NgR2 affects anatomy, gene expression and nociceptive behavior of cutaneous DRG sensory afferents.
In comparison to wt mice, no change in the percent of nociceptive (IB4-binding and P2X3-positive) and non-nociceptive (NF200-positive) DRG neurons was found in NgR2 knock-out mice, consistent with normal number and caliber of unmyelinated and myelinated axons in a cutaneous nerve. Strikingly, nonpeptidergic (CGRP-negative) free nerve endings in footpad epidermis were about 50% higher in NgR2-KO mice than in their wild-type littermates. In contrast, the density of CGRP-positive epidermal innervation remained unaffected. Interestingly, NgR2-KO mice were markedly hypersensitive to mechanical but not to noxious heat stimuli. The cutaneous hyperinnervation in NgR2-KO mice was not associated with changed expression levels of neurotrophins in the skin or their cognate receptors in DRG neurons. However, dissociated NgR2-/- DRG neurons revealed more extensive branching patterns than wt neurons and were significantly less inhibited by chondroitin-sulfate proteoglycans such as versican, which is abundantly present in glaborous skin. These data demonstrate that NgR2 is required for proper cutaneous innervation and suggest that it limits axonal outgrowth and branching of IB4-positive neurons.

T 6

Cell fate analysis of mouse embryonic stem cells during *in vitro* differentiation to telencephalic and mesencephalic neurons as compared with *in vivo* development

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Embryonic stem (ES) cells are a powerful source for generating potentially unlimited numbers of specialized cells. Previous studies have reported the directed in vitro differentiation of mouse ES cells into specific neuron types in response to patterning factors defining anterior/posterior and dorso/ventral central nervous system identity *in vivo*. We have tested the patterning role of hedgehog signaling to generate telencephalic and mesencephalic cell types. Cell differentiation was monitored using a profile of neural markers and region specific transcription factors linked to telencephalic and mesencephalic development *in vivo*. For ventral patterning, we applied purmorphamine, a pharmacological modulator of the hedgehog signaling pathway, which acts as an agonist on the Smoothened receptor.
In the first model we studied differentiation of telencephalic neurons *in vitro*. Neural induction, neural patterning and neural specification were temporary aligned in an attempt to mimic the developmental progression *in vivo*. Cell cultures were compared in the absence or presence of purmorphamine. The results show that purmorphamine efficiently stimulated the ventral patterning of mouse neural progenitors by up-regulating a set of ventrally expressed telencephalic transcription factors reflecting the process of ventral patterning *in vivo*.
In the second model, we employed a 5-stage protocol to generate mesencephalic dopaminergic neurons *in vitro*. A molecular comparison with embryonic brain regions revealed that the neural population generated *in vitro* displays the typical molecular profile of embryonic day 11 ventral mesencephalon and presents, in addition, markers for ventral telencephalon. Moreover, the analysis of the expression of a wide variety of markers during each stage of the protocol helped to generate milestones of key markers that lead to generation of ventral midbrain and ventral forebrain phenotypes.
Our direct comparison with *in vivo* development is useful to guide ES cell derivatives through cell fate decisions occurring *in vitro*. The successful application of this approach will give access to unlimited numbers of specific neurons *in vitro* and holds considerable potential for cell based therapies in neurodegenerative disorders such as Alzheimer and Parkinson's disease.

T 7

TGF-beta signaling in the adult neurogenic niche

Couillard-Després, Sébastien[1] and Aigner, Ludwig[1]

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This abstract will be available at the meeting.

T 8

Adult neurogenesis in a mouse model of affective behavior: pharmacological and non-pharmacological interventions

Anupam Sah[1,2], Patrick Markt[2], Sergey Sotnikov[2], Carina Koehl[1], Rainer Landgraf[2], Nicolas Singewald[1]

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Earlier studies have shown that chronic experimental stress resulting in enhanced anxiety/depression-like behavior decreases neurogenesis in the dentate gyrus (DG), while antidepressants (ADs) reverse these stress-induced effects. However, in most studies animals with "normal" anxiety/depression-like behavior have been used, although it is well known that the behavioral and neurochemical effects of ADs can differ in disturbed (pathophysiological) versus intact systems. Therefore, we aimed to investigate the action of ADs in a mouse model of high-trait anxiety and comorbid depression (HAB), which mimics important human psychopathological features, and their normal anxiety/depression (NAB) controls. A range of ADs of different pharmacological classes attenuated the depression-related behavior selectively in HABs without affecting this behavior in NABs. We subsequently investigated if there is a difference in the basal rate and/or AD-induced increase in adult neurogenesis between these lines. We observed that indeed HABs had a lower rate of neurogenesis compared to NABs and that reduced neurogenesis was associated with enhanced anxiety, rather than depression-related behavior. Next we used a non-pharmacological approach, namely environmental manipulations. Enriched environment induced an anxiolytic, but not an antidepressant-like effect in HABs together with an increase in neurogenesis. In parallel, low anxiety mice (LABs) when exposed to unpredictable chronic mild stress showed a shift towards enhanced anxiety and a reduction in neurogenesis. Taken together, our data indicate that 1) the phenotype of high and low anxiety mice can be bidirectionally modulated towards 'normal' anxiety using pharmacological and non-pharmacological approaches 2) neurogenesis seems to be associated with anxiety- rather than depression-related behavior.

Acknowledgement: Supported by FWF DK SPIN (W1206).

P 1

Extending the phenotype of transgenic MSA: presence of h-α-syn in PNS

Daniela Kuzdas, DI[1], Michaela Kress, Dr.[2], Lars Klimaschewski, Dr.[3], Nadia Stefanova, MD, PhD[1] and Gregor K Wenning, MD, PhD[1].

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Objective: To evaluate the relevance of h-α-syn positive aggregates in the peripheral nervous system (PNS) of an h-α-syn overexpressing mouse model of multiple system atrophy (MSA) using different behavioral tests as well as extensive morphological characterization.
Background: The CNS of the h-α-syn overexpressing transgenic MSA mouse model has been well-characterized in previous studies. This mouse model works under the control of a PLP promoter, which leads to the specific expression of h-α-syn restricted to oligodendrocytes. Findings in an α-syn-overexpressing PD mouse model showed reduced myelination and increased axonal degeneration after peripheral nerve lesion.
Methods: Immunohistochemistry has been used to detect h-α-syn positive aggregates in sciatic nerve sections of our MSA model. Behavioral tests to investigate potential motor impairment via the rotarod test, as well as pain and sensitivity tests like the Hargreaves and Von Frey test will be applied to further analyze whether the peripheral α-syn pathology of this MSA mouse model also leads to decreased sensitivity or increased pain sensation. Extensive morphological analysis will be performed comparing peripheral nerve sections from the h-α-syn MSA model with age-matched wildtype controls.
Results: Our preliminary findings comparing peripheral sciatic nerve sections of a h-α-syn overexpressing transgenic MSA mouse model with age-matched wildtype controls, indicate the presence of α-syn immunoreactivity in transgenic tissue slices, whereas the wildtype control tissue appears to be devoid of α-syn aggregates.
Conclusions: According to our results, h-α-syn aggregates in the PNS of tg MSA mice with established CNS pathology. The presence of h-α-syn in the PNS may have an impact on the functional integrity of peripheral nerves, myelination and axonal degeneration.
Acknowledgement: Supported by FWF DK SPIN (W1206).

P 2

Neuregulin 1 – a molecular link between Schizophrenia and Epilepsy

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Since more than five decades, a potentially strong association between epilepsy (especially temporal lobe epilepsy; TLE) and schizophrenia or schizophrenia-like psychoses was proposed. Morphological studies were conducted identifying the hippocampus and the extended amygdala as potential areas of overlap of the two diseases. Functionally both diseases share imbalances of excitatory and inhibitory neurotransmission as important feature. In schizophrenia several genetic components where identified, while TLE is mostly triggered by non-genetic factors, although components of predisposition exist. Therefore schizophrenia risk genes, which influence development and excitability of neuronal circuits of the forebrain may be of particular interest to understand the neurochemical background of TLE. The schizophrenia risk gene *neuregulin 1* (*Nrg1*) was shown to influence hippocampal development and formation of LTP. We now have conducted experiments investigating seizure behavior and neurochemistry of neuregulin 1 hypomorphic mice.
Pentylenetetrazole (PTZ) kindling was performed by repeated injections of 20 or 25 mg⁄kg PTZ (i.p.). PTZ treatment progressively increased the seizure score in wild-type mice from no response to forelimb cloni and severe jerks with both doses. No generalized tonic-clonic seizures were observed in wild-type mice. In contrast, five out to seven Nrg 1 hypomorphic mice treated with 25 mg/kg PTZ displayed severe generalized seizures and four actually died. Upon kindling with 20 mg/kg PTZ one out of seven Nrg1 hypomorphic mice died in seizures, while the behavior of the others did not differ from that of wild-type mice. These data suggest that from a certain level of stimulation, Nrg1 hypomorphic mice are no more able to control neuronal activity. Below this threshold, they behave normal. This could be due to imbalances within the inhibitory system. However, we did not observe any differences in the expression of GAD 65 or 67, GABA transporters, or major $GABA_A$ receptor subunits.
Our data are in line with a couple of recent papers suggesting that Nrg1 may be involved in the formation of excitatory synapses in inhibitory Chandelier cells. Therefore, activation of these cells through cortical projections may be reduced in Nrg1 hypomorphic mice, thereby reducing the inhibitory drive. This model would allow normal brain functioning at low levels of excitation, but would lack an inhibitory component at high levels of network activity.

Acknowledgement: Supported by FWF DK SPIN (W1206).

P 3

The negative feedback inhibitor, Sprouty2, inhibits axon outgrowth of adult DRG neurons

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Sprouty2 (Spry2) is a negative feedback inhibitor of growth factor-dependent ERK-signaling in neurons. Mammals exhibit four Sprouty isoforms which are evolutionary conserved. Spry2 is highly expressed in sensory dorsal root ganglia (DRG). The down-regulation of Spry2 by short hairpin RNAs (shRNAs) has been shown to promote axon outgrowth in peripheral and central neuron cultures (Hausott et al., 2009). Here, we investigated the involvement of Spry2 in growth factor-dependent axon outgrowth of sensory neurons in $Spry2^{+/-}$ and $Spry2^{-/-}$ knockout mice (on a mixed genetic background of C57BL/6 and 129).
Adult sensory neurons dissociated from dorsal root ganglia of $Spry2^{+/-}$ mice were plated on a poly-L-lysin/laminin substrate. A significant enhancement of the length of the longest axon (maximal distance, MD) was observed as compared to wildtype littermates. After treatment for 24 hours with NGF or FGF-2 the parameters for total axon length (TAL), MD and number of branch points (BPs) significantly increased as well. Subpopulations of sensory neurons were identified by antibodies against CGRP and IB4 lectin in vitro. We observed that in $Spry2^{+/-}$ mice the CGRP-positive neuron population responded to NGF with significant increases in the TAL, MD and BPs. IB4-positive neurons of heterozygous mice revealed significant axon outgrowth in the presence of FGF-2 with regard to the MD, but not for TAL and BPs. In homozygous $Spry2^{-/-}$ mice we observed differences in the TAL and in BPs. In these cultures NGF, but not FGF-2, promoted MD, TAL and BPs. Taken together, the results demonstrate that $Spry2^{+/-}$ DRG neurons exhibit enhanced axonal elongation, whereas homozygous $Spr2^{-/-}$ neurons show a tendency for increased axonal branching in the presence of growth factors. These differences are probably dependent on different activites of the Ras/Raf/ERK pathway which is currently under investigation.

Acknowledgement: Supported by FWF DK SPIN (W1206).

P 4

Deep Brain Stimulation in a Psychopathological Animal Model of Affective Behavior

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[3] Max Planck Institute of Psychiatry, Munich, Germany

Very recent evidence from clinical studies indicates that high frequency electrical stimulation via deep brain stimulation (DBS) in the nucleus accumbens (NAcb) reduces depression and anxiety in individuals suffering from treatment-resistant depression (TRD), and therefore may represent a novel therapeutic strategy for this group of patients. However, the underlying mechanisms of DBS e.g. interference with neuronal circuits and neurobiochemical changes, remain largely unknown. To elucidate these mechanisms, we used an animal model for high-trait anxiety and comorbid depression (HAB), which has been shown to be insensitive to the clinically effective antidepressant and anxiolytic drug fluoxetine, and thus is suggested to reflect a core condition of TRD in humans. DBS electrodes were implanted into the NAcb of male HAB mice. After a 3 days recovery period, mice received daily high frequency stimulations of 1h duration for seven days. Sham-stimulated animals were used as controls. Anxiety- and depression-related behaviors were assessed by performing established tests with predictive validity. In DBS-treated HAB mice, the enhanced depression-like behavior was decreased following seven, but not one day of stimulation, indicating that chronic stimulation is required to elicit antidepressant effects in HABs. Furthermore, we observed a reduction in anxiety-like behavior already after 3 days of DBS. Supporting data of TRD patients, these experiments have successfully demonstrated anxiolytic and antidepressant effectiveness of DBS in male HAB mice indicating the translational value of this approach. Using this mouse model, exploration of mechanisms underlying the effect of DBS in the psychopathological brain is under way.

Supported by Hope for Depression Research Foundation (HDRF/ISAN).
Acknowledgement: Supported by FWF DK SPIN (W1206).

P 5

Investigating gene deregulation in the CNS of the NgR2 knockout mouse

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[2]Department of Pharmacology and Toxicology, Institute of Pharmacy & Center for Molecular Biosciences Innsbruck, University of Innsbruck.

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 of this line show 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 a decrease in depressive-like behavior was observed in decreased immobility time in the tail suspension test. Affymetrix microarray analysis of the hippocampus identified genes differentially expressed in Ngr2KO. These did not include genes classically involved in depression and anxiety such as NPY. Instead, a number of the differentially expressed genes have also been reported in other recent microarray studies of anti-depressant models, suggesting they could be novel depression-related genes. Further investigation of these genes is ongoing. Alongside this, studies of well-known genes involved in depression and anxiety demonstrated no significant changes in NPY, BDNF or nNOS in the hippocampus or amygdala. However in the dentate gyrus of the hippocampus a significant increase of activating transcription factor 3 (ATF3), an adaptive response gene of the ATF/CREB transcription factor family, was seen in NgR2-/- mice compared to wildtype controls. ATF3 is known to be induced under stress conditions but is also reported to have a neuroprotective function, and is downstream of CREB and various intracellular signaling pathways, which are now under investigation.

Acknowledgement: Supported by FWF DK SPIN (W1206).

P 6

Molecular Background of Reduced Mechanical Hypersensitivity in Conditional IL-6 Signal Transducer gp130 Knock-Out Mice

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The mammalian somatosensory system detects mechanical stimuli at the peripheral endings of somatosensory neurones in the dorsal root (DRG) and trigeminal ganglia (TG). The molecular basis underlying mechanotransduction is only beginning to be uncovered. It is thought that specific ion channels in the membrane of the sensory nerve terminal directly or indirectly open upon mechanical stimulation. Under inflammatory or neuropathic conditions the mechanical threshold can be lowered leading to mechanical hypersensitivity. Our lab has generated a conditional glycoprotein 130 knock-out mouse strain lacking the transducer molecule specifically in nociceptors (SNS-gp130$^{-/-}$). Behavioural tests reveal a reduced mechanical hypersensitivity of these mice under inflammatory and neuropathic conditions.
Primary cultures of DRG neurones can serve as a model of their afferent terminals detecting noxious stimuli *in vivo*. We used the whole-cell voltage clamp configuration of the patch-clamp technique to monitor mechanosensitive ionic currents while applying pressure to the DRG soma with heat-polished glass-pipettes to simulate mechanical stimulation. Alternatively, calcium microfluorimetric measurements were performed. Affmetrix® gene chip analysis was used to identify deregulated genes in DRG explants of gp130$^{fl/fl}$ and SNS-gp130$^{-/-}$ mice. Differential expression of possible candidate genes involved in mechanotransduction was confirmed with quantitative RT-PCR.
mRNA expression assays revealed a downregulation of the mechanosensitive ion channel TRPA1 in SNS-gp130$^{-/-}$ vs. gp130$^{fl/fl}$ mice. Furthermore, a significantly smaller number of neurones responded to the selective TRPA1 agonist NPPB with calcium transients in neurones from SNS-gp130$^{-/-}$ vs. controls.
Our data show downregulation of TRPA1 in SNS-gp130$^{-/-}$ mice. We hypothesise that this could explain the reduced mechanosensitivity of these mice under pathological conditions.

Acknowledgement: Supported by FWF DK SPIN (W1206).

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HIF-1 alpha is an essential regulator for purine nucleoside-mediated neuroprotection against hypoxia

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Acute neurological conditions massively damage the central nervous system, which leads to cell death by necrosis and apoptosis. A central role for functional recovery is the reduction of apoptosis. It is known that purine nucleosides are critical regulators under hypoxic stress and that physiological responses are mediated by the hypoxia-inducible factor-1 alpha (HIF-1 alpha) [1]. However the signaling mechanisms have not yet been fully elucidated. Based on our earlier data, showing the positive impact of purine nucleosides on chemical-induced cell death [2, 3], I have established a physiological hypoxia model for neuronal PC12 cells and primary cerebellar granule neurons [4, 5]. We could show that adenosine and inosine efficiently rescued rat pheochromocytoma (PC12) cells (up to 43.6%) as well as primary cerebellar granule neurons (up to 25.1%) from hypoxic insult. Hypoxia resulted in a heightened accumulation (up to 2.5 fold induction) of HIF-1 alpha, which was further enhanced by purine nucleosides up to 3.1 fold in cell lysates derived from cerebellar granule neurons. Concomitant with these results, siRNA-mediated reduction of HIF-1 alpha completely abolished adenosine- and inosine-mediated protection in PC12 cells and severely hampered purine nucleoside-mediated protection in primary neurons (up to 94.2%). Taken together, these results clearly demonstrate the key role of HIF-1 alpha in purine nucleoside-mediated protection of neuronal cells under hypoxic conditions. In my recent work I investigated the adenosine receptor- and p42/44 MAPK-mediated activation of HIF-1alpha during hypoxic injury and preliminary new data showed that pharmacological inhibition of A2A adenosine receptor-mediated signaling not only inhibited the p42/44 MAPK phosphorylation but also HIF-1alpha protein levels in PC12 cells.

This work has been supported by the Austrian FWF P19578-B05 and T421-B18.

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Interaction Partners of Reticulons

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Reticulons (RTNs), membrane bound proteins with a uniquely conserved C-terminal domain named Reticulon Homology Domain (RHD), gained increasing attention in the recent years due to their involvement in neurodegenerative diseases. In particular, RTN-4/Nogo proteins, members of the RTN protein family were shown to impair axonal regeneration after brain insult and to restrict neuronal plasticity in the intact adult mammalian nervous system. However, the intracellular function of Nogo and other RTNs in neurons, primarily localized in endoplasmic reticulum (ER), remained an intriguing question. Recent *in vitro* studies with non-neuronal cells suggest that RTN1/RTN4 proteins may have additional roles in induction of membrane curvature; especially pivotal in ER shaping and maintenance, and vesicle trafficking.

To further investigate this hypothesis and to shed light on the molecular mechanisms by which Nogo and RTN1A participate in neurons, we aimed to identify interaction partners of Nogo and RTN1A. For this purpose, with the purified NIR-GST (N-terminal domain of Nogo A/B) and GST-RTN1A$_{spec}$-His fusion proteins, we had performed the pull-down experiments via bringing into contact with adult mouse brain extract. N-terminal domain of Nogo, NIR, harbors a proline-rich domain encompassing 68-160 aa, which notably invloves several SH3 binding epitopes. Pull-down products were resolved by SDS-PAGE and analyzed by MS/MS, leading to identification of a set of proteins as potential interaction partners of RTN1A and Nogo. Among these potential interaction partners from our panel, we had chosen a number of proteins to further characterize the interaction with Nogo and RTN1A. Within the potential Nogo interaction partners, site specificity of Nogo binding was investigated for selected candidates, via screening of pulldown products of various NogoA/B domain fusion proteins. The physiological interaction of potential Nogo/RTN1A binding partners was examined by co-immunoprecipitation of endogenous proteins from mouse/rat brain extracts. Currently, in-detail biochemical characterization of these potential interactions, as well as colocalization studies by advanced microscopy techniques are performed, and functional significance of the validated interactions are being addressed.

Acknowledgements:
This study is supported by FWF, within graduate program Signal Processing in Neurons (SPIN, W1206). We thank Prof. H. Lindner, head of Protein Core Facility and Dr. B. Sarg for the advices and service provided for Mass Spectometry analyses. We extend our thanks to Prof. Dr. H. G. Knaus and Prof. Dr. G. Dechant, for helps and advices.

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Protein interactions of voltage-gated calcium channels

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Voltage-gated calcium channels mediate cellular responses of excitable cells by transducing membrane depolarization into calcium influx. They consist of a pore-forming alpha1 subunit and of beta and alpha2delta auxiliary subunits, which modulate channel membrane trafficking and current properties. The aim of this study is to develop new approaches to detect hitherto unnoticed VCGG interaction partners and to study the dynamics of the different subunits in the calcium channel complex.
Recent findings suggested a function of alpha2delta in synapse formation and in nociception. In order to analyse the proteome of native membrane-expressed alpha2delta subunits in the brain, and therefore identify alpha2delta signalling partners, we plan to use a proteomics approach. Because available antibodies against endogenous alpha2delta fail to immunoprecipitate the native protein, constructs with extracellular N-terminal HA and GFP-derivative SEP tags were generated. The two constructs were characterized, indicating that the recombinat proteins are correctly targeted, interact with the alpha1 subunit, are post-translationally processed, and can be immunoprecipitated with the tag-specific antibody. Proteomic analysis will be performed on differentiated hippocampal neurons and immortalized skeletal muscle cells (C_2C_{12}) tranfected with 2HA-a_2d-1, in collaboration with B. Fakler (Univ. Freiburg).
In order to study the dynamics of different subunits in the calcium channel complex, we established a live imaging method, FRAP, in dysgenic skeletal muscle cells (GLTs). Both the homologous alpha1S and the heterologous cardiac alpha1C subunits are immotile in clusters. Since in skeletal muscle the alpha1S II-III loop interacts with the ryanodine receptor, while the cardiac alpha1C II-III loop does not, we can conclude that this interaction has no impact on alpha1S mobility. The skeletal muscle beta1a auxiliary subunit co-expressed with the homologous alpha1S subunit showed a recovery of fluorescence up to 30%, indicating a more rapid turnover in comparison to the alpha1 subunit. However, when bleached areas outside of clusters were analyzed, the same recovery was observed, indicating that, in addition to the immobile beta associated in clusters, there is also a free component outside the clusters. When beta1a was co-expressed with the heterologous alpha1C subunit, it showed the same behaviour. Together these data suggest that in the muscle the interaction of alpha1S and beta1a with the ryanodine receptor is not necessary for the assembling of the calcium channel subunits and the stability observed. Currently we are investigating the mobility of different beta subunits and extending the analysis to the alpha2delta subunit.

Support: FWF, P20059-B05 and W1101-B12.

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Modulatory interactions with the I-II linker of L-type calcium channels

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Calcium influx through L-type voltage-gated Calcium channels (LTCCs) underlies important physiological processes like muscle contraction, neuronal excitability, modulation of gene expression and release of hormones. In the multisubunit complex of a LTCC, the β-subunit binds with high affinity to the cytoplasmic I-II loop of the pore forming α1 -subunit and thereby promotes plasma membrane targeting and modulates channel gating. We have recently discovered that the $Ca_v1.3$ I-II loop peptide is targeted to the plasma membrane without β-subunit and also in complex with β-subunits and other proteins (β, β+ RIM).
The aim of this study is to identify potential binding partner(s) of LTCC I-II loop in the plasma membrane and localize structural features of LTCC l-ll loops involved in channel function and/or plasma membrane targeting. We produced FLAG-tagged I-II loops from different L-type ($Ca_v1.1$, $Ca_v1.2$, $Ca_v1.3$ and $Ca_v1.4$) calcium channels, transiently expressed them in HEK-293 cells and monitored their subcellular localization by high resolution immunofluorescence microscopy. All L-type I-II loops were localized in the plasma membrane. As expected, β-subunits (β_3, non-palmitoylated β_2) were evenly distributed in the cytoplasm but were targeted to the plasma membrane by co-expressed L-type I-II loops. Mutation of a single residue (W441A) in the β-subunit binding motif of the I-II loop disrupted β-subunit interaction but not plasma membrane targeting. This shows that independent structural motifs determine β-subunit binding and targeting of LTCC I-II loops. Deletion of amino acids 91-119 on the I-II loop prevented plasma membrane targeting suggesting that this region contains structural motifs required for plasma membrane targeting. This region is predicted (PSIPRED) to form an alpha helix with positive charges located on one side of the helix which forms a possible interaction site for binding partners. This interaction could be important for the channel function and/or plasma membrane targeting of the whole channel complex.
We are currently identifying essential amino acids of this motif by site directed mutagenesis. This will allow us to study the relevance of this domain for channel function using patch-clamp experiments and for channel and I-II loop targeting using immunofluorescence. A co-immunoprecipitation assay to identify protein binding partners has already been established.

Support: FWF (P20670, W11, University of Innsbruck)

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An Animal Model for C-terminal Deregulation of $Ca_v1.3$ Channels

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Intramolecular protein interaction within the C-terminus of the voltage gated L-type Ca^{2+} channel (LTTC) $Ca_V1.3$ α_1 subunit modulates $Ca_V1.3$ channel function [1]. This C-terminal modulatory mechanism (CTM) is present in a long ($Ca_V1.3_{42}$) and absent in a short ($Ca_V1.3_{42A}$) splice variant. In the absence of CTM, activation of the channel occurs at a more negative voltage range and Ca^{2+}-dependent inactivation (CDI) is faster. The physiological role of the CTM is unknown. However, pharmacological interference with the CTM automodulatory mechanism may present a novel concept of Calcium channel modulation. We found a higher relative amount of the short splice variant $Ca_V1.3_{42A}$ in substantia nigra (SN), which leads us to suggest that alternative splicing may serve as an important mechanism to fine tune Ca^{2+} signaling in dopaminergic neurons.

To assess the physiological relevance of $Ca_V1.3$ CTM, we are currently developing a mouse model in which the CTM is disrupted by an HA-tag. To establish the mutation, we have generated a targeting construct, containing about 7.5 kb of homologous region, two selection markers and the mutation, by standard cloning technique. The construct was recombined successfully in murine embryonic stem cells, as confirmed by PCR screening. Four clones were selected for microinjection in mouse blastocyst to raise chimeric mice.

The $CTM^{-/-}$ mouse model will be useful in investigating the roles of CTM *in vivo* in different tissues; it will mimic pharmacological inhibition of CTM and allow predictions about the potential of such inhibitors and the suitability of CTM as a new drug target. Furthermore, in absence of specific antibodies, the expression of $Ca_v1.3_{42}$ can be quantified by immunostaining with anti-HA antibodies.

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Expression of BK α and β subunits in mouse brain

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High- conductance Ca^{2+} activated (BK) potassium channels are widely expressed through the CNS, e.g. hippocampus, cerebellum, interpeduncular nucleus and amygdala. Their functional role has been established in hippocampus or cerebellum, however, little is known regarding their precise function in the amygdala. The use of BK-channel alpha subunit deficient mice was not permissive as these mice exhibit a strong cerebellar phenotype (ataxia) precluding their investigation in amygdala-specific tasks. In order to address these issues, mice floxed for the alpha subunit of BK-channel will be injected with an adeno-associated virus mediating cre induced regional specific KO. As a first step, the distribution of the BK subunits in the mouse brain were investigated.
The neuronal channel is believed to be formed by association of alpha with beta4 subunits, however, there exist other beta subunits and alpha homomeres that may be functional as well. The precise distribution of the individual BK channel subunits in mouse brain is currently not available. While some data on the distribution of the alpha subunit were published, detailed knowledge regarding the expression of beta4 subunits is required as this subunit strongly influences the functional and pharmacological properties of the channel complex. Therefore, *in situ* hybridization, immunohistochemical as well as toxin binding experiments are performed. In brief, the neocortex and principal neurons of the hippocampus do express both, alpha and beta4 mRNA. In contrast, certain brain regions (e.g. amygdala and some thalamic nuclei) do express BK-channel alpha, but not beta4 subunits, which is in line with binding data obtained with radioiodinated iberiotoxin, which bind exclusively to BK channels devoid of beta4. To our surprise, we also identified regions where high levels of beta4 mRNA were detected. However, the respective alpha subunit mRNA appears to be missing. These data are currently confirmed using alpha or beta4-specific antibodies. Our data identify neurons of the lateral and basolateral amygdala as interesting candidates to study the function of BK channels which do not associate with beta4 subunits.

Acknowledgement: Supported by FWF DK SPIN (W1206).

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

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Previous work showed that Pavlovian fear conditioning induces a dramatic downregulation of benzodiazepine binding sites and transcripts for gephyrin and some $GABA_A$ receptor subunits in the basal nucleus of amygdala (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. 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.

Acknowledgements: We thank Sabine Schönherr for excellent technical help. This work was supported by the FWF grant S10720 to F.F.

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FGFR1 endocytosis and trafficking in human glioma cells

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Fibroblast growth factor receptors (FGFRs) belong to the receptor tyrosine kinase (RTK) family. RTKs are transmembrane molecules which trigger intracellular signaling pathways through extracellular binding of their ligands. Of the four different FGFRs (FGFR1-4), FGFR1 is commonly expressed in glial and neuronal cells. FGFR1 activation is followed by rapid endocytosis and sorting of the receptor to either the late endosome for degradation in the lysosome or to the recycling endosome for recycling back to the cell surface.

FGFR1 is commonly over-expressed in many types of cancer, including human glioma and glioblastoma, and many mutations of this receptor have been found which are leading to ligand-independent and self-sustained signaling and tumor growth. A better understanding of FGFR1 trafficking could help to develop novel treatment strategies for tumor growth inhibition.

We are studying the internalization, trafficking, recycling and degradation of FGFR1 in the human glioma cell line U373. For this purpose, cultured cells are transfected with FGFR1 constructs fused to fluorescent marker proteins. Stably overexpressing cells are used for imaging. The distribution of the receptor in intracellular compartments is visualized via different fluorescent markers (EEA1 for early endosomes, Lamp1 for late endosomes and Transferrin for recycling endosomes as well as LysoTracker and Lamp1 for lysosomes) and analyzed with confocal microscopy and structured illumination techniques.

FGF-2 enhances colocalization of FGFR1 with early, late and recycling endosomes as well as with lysosomes. The lysosomal inhibitor leupeptin leads to receptor accumulation in late endosomes and lysosomes. The distribution of endosomes, lysosomes and receptor containing vesicles throughout the cell was analyzed with three-dimensional reconstruction of a confocal image stack. Results show that the recycling endosomes are specifically localized in a circumscribed region within the cell (the so-called ‘recycling compartment’), whereas early endosomes, late endosomes and lysosomes as well as FGFR1-containing vesicles are homogeneously distributed throughout the cell.

Our results contribute to a better understanding of FGFR1 trafficking in human glioma cells and provide a basis for manipulating FGFR1-trafficking as a means to develop new treatment strategies to reduce RTK-mediated cell proliferation.

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Surface traffic of dendritic CaV1.2 calcium channels in hippocampal neurons

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In neurons L-type calcium currents function in gene regulation and synaptic plasticity, while excessive calcium influx leads to excitotoxicity and neurodegeneration. The major neuronal L-type channels CaV1.2 are localized in clusters in dendritic shafts and spines. Whereas CaV1.2 clusters remain stable during NMDA-induced synaptic depression, L-type calcium currents are down-regulated during strong excitatory stimulation. Here we tested the hypothesis that in dendrites of differentiated hippocampal neurons a stably clustered and a dynamic population of CaV1.2 channels coexist and give rise to this diverging behavior. We examined this hypothesis using fluorescence microscopy techniques in neurons transfected with superecliptic pHluorin and HA tagged CaV1.2 (CaV1.2-SEP and CaV1.2-HA, respectively). Fluorescence recovery after photobleaching (FRAP) of CaV1.2-SEP showed ~20% recovery within 20min without reappearance of clusters, revealing the coexistence of a major stably clustered and a minor diffusive non-clustered CaV1.2 population. To estimate the dwell time of CaV1.2 in clusters, we labeled membrane expressed CaV1.2-HA for 10min and analyzed clusters size and density 0, 5, 15, 30, and 60min thereafter. The values at all time points were indistinguishable, indicating little turnover within one hour. To exclude that stability was maintained through a dynamic steady-state mechanism, we blocked dynamin-dependent endocytosis with dynasore. Whereas clusters size remained unaffected, their density increased after 30min, suggesting a turnover rate of clustered CaV1.2s on the minutes-to-hour time scale. Single particle tracking (SPT) of single CaV1.2s in the membrane demonstrated immobile and strongly confined mobile channels, with a ~30% population reversibly exchanging between these two states. Depolarization with high potassium did not alter the recovery rates in FRAP, or the diffusion coefficients in SPT analyses. Thus, an equilibrium of clustered and dynamic CaV1.2 may maintain stable calcium channel signaling complexes while simultaneously allowing for activity-dependent adaptations to protect neurons from damage due to excessive calcium influx. *Support: FWF FT004430 to VDB and 20059 to BEF.*

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Involvement of intra-coerulear galanin in enhanced depression-related behaviour and antidepressant pharmacotherapy

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Increasing evidence proposes a possible role of the neuropeptide galanin (Gal) in the pathophysiology and treatment of depression. Here, we investigated whether transcriptional processes in the Gal system may be involved in depression-like behaviour. The abundance of Gal mRNA was increased in the paraventricular hypothalamus (PVN), the central amygdala and the locus coeruleus (LC), but not in the dorsal raphe of HAB rats characterised by high trait anxiety- and depression-like behaviour compared to their low anxiety/depression (LAB) counterparts. Conversely, long-term (42 days, p.o.) treatment with either desipramine, paroxetine or tranylcypromine caused a general reduction in Gal mRNA expression in the locus coeruleus (LC) in unselected rats indicating a common response to antidepressant drug treatment while in the PVN Gal mRNA was increased by tranylcypromine only. The common effect of the antidepressants on Gal mRNA in the LC is opposite of the finding in the HAB model raising the exciting possibility that altered coerulear Gal mRNA expression may be associated with depression-related behaviour. To provide functional evidence of a role of intra-LC Gal in trait anxiety/depression, Gal ligands were intra-cerebrally applied to HAB and LAB animals and behavioural responses were investigated in the forced swim test. Intra-LC galanin caused a pronounced increase in the immobility of LAB rats indicating enhanced depression-like behaviour while a Gal receptor antagonist reduced immobility in HAB rats. Collectively, the present data suggest that Gal is of relevance in depression-like behaviour. In particular, its modulation in the LC where Gal highly coexists with noradrenaline appears to be critical.

Supported by the HDRF.

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Morphological characterization of large intercalated neurons provide novel insight on intrinsic networks of the amygdala

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Although extinction-based therapies are effective treatments for anxiety disorders, the neural bases of fear extinction remains still largely unclear. Recent evidence suggests that the intercalated cell masses of the amygdala (ITCs) are critical structures for fear expression and extinction. They consist of clusters of densely packed medium spiny GABA-ergic neurons surrounding the basolateral complex of the amygdala (BLA). Previous studies have also identified large ITC cells mostly present near the borders of the clusters and corresponding to only ~5% of ITC neurons. So far, no information was available regarding the neurochemical features, neuronal input and axonal projections of large ITC cells, preventing any elucidation of their functional role within the amygdala. We recently discovered that large ITC neurons encircle ITC clusters with their extensive dendritic arborization and display immunoreactivity for either neurokinin 1 or metabotropic glutamate 1α (mGlu1α) receptors. In addition, we found that dendrites of large ITC neurons receive inhibitory inputs from medial capsular projecting-ITC neurons located in the medial paracapsular ITC cluster. Three large ITC neurons have been recorded and filled with neurobiotin by means of the juxtacellular technique *in vivo*, in anesthetized rats. Immunofluorescent experiments revealed that these mGlu1α receptor positive cells express also the α1 subunit of $GABA_A$ receptor and that their axon terminals contain the vesicular GABA transporter, thus revealing their GABAergic nature. Confocal and pre-embedding electron microscopy demonstrated that dendrites of the large mGlu1a receptor immunopositive ITC neurons receive dense neuronal inputs from axonal terminals enriched in presynaptic mGlu7 and/or mGlu8 receptors. The tridimensional reconstruction of the full dendritic and axonal arborizations of the *in vivo* filled large ITC neurons, performed by Neurolucida, showed that, unlike small ITC cells, the axon of these neurons extends widely in the rostro-caudal direction and predominantly targets the BLA and, to a smaller extent, the central nucleus of the amygdala or other ITC clusters. In addition, immunofluorescence analysis indicates that, within the BLA, large ITC neurons appear to preferentially target distinct interneurons subtypes rather then principle neurons. These findings elucidate for the first time the anatomical features of large ITC neurons and shed new light on intrinsic microcircuits of the amygdala.

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Reversal of cocaine conditioned place preference by social interaction

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A main challenge in the therapy of drug dependent individuals is to help them reactivate interest in non-drug-associated activities. Among these activities, social interaction is doubly important because treatment adherence itself depends on it. We developed a rat animal experimental model based on the conditioned place preference (CPP) paradigm in which only four 15-min episodes of social interaction with a gender- and weight-matched male conspecific (i) reversed CPP from cocaine to social interaction despite continuing cocaine training and (ii) prevented the reinstatement of cocaine CPP (Fritz et al. 2011, Addiction Biology). The reversal of CPP from cocaine to social interaction was enhanced by the sigma1 receptor antagonist BD1047 with an ED50 of 0.0036 mg/kg (i.p.) (Fritz et al. 2011, Pharmacology 87:45-48). Social interaction also reversed cocaine CPP-induced expression of the immediate-early gene zif268 in the nucleus accumbens shell, the central and basolateral amygdala and the ventral tegmental area (Fritz et al. 2011, Addiction Biology). These findings suggest that social interaction, if offered in a context that is clearly distinct from the previously drug-associated ones, may profoundly decrease the incentive salience of drug-associated contextual stimuli. In the present study, we investigated if the two subregions of the nucleus accumbens (Acb), the core (AcbC) and shell (AcbSh) would differentially affect CPP for cocaine vs social interaction. Animals were concurrently trained for CPP to cocaine and social interaction (mutually exclusive stimulus presentation during training).
We are currently investigating which type of neuron in the AcbC is affected by this reversal.

This project is supported by FWF grants P18787-B05, W1206-B18, and M1169-B18, and by the Verein zur Förderung der Experimentellen Psychiatrie, Psychotherapie und Pharmakologie (VEPPP).

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Role of IL-6 Signal Transducer gp130 in Mechanonociception

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Glycoprotein 130 (gp130) is a transmembrane protein required for signal transduction of interleukin-6 like cytokines. Mice with a conditional deletion of gp130 in nociceptive primary afferents (*SNS-gp130*$^{-/-}$) have been shown to develop less thermal[1] and mechanical hypersensitivity in tumour, inflammation and neuropathic pain models.
We are aiming to clarify the mechanisms underlying the difference in mechanonociception in the maintenance phase of pathological pain states. As a fist step, we address the question at which level (e.g. nerve endings, somatosensory neuron populations or synaptic transmission) alterations take place.
Neuropathic state is induced by means of the spared nerve injury (SNI) model[2] which is characterised by robust mechanical hypersensitivity in the sural nerve innervation territory. *Von Frey* mechanical threshold testing is used in *SNS-gp130*$^{-/-}$ and gp130$^{fl/fl}$ control mice before and after surgical intervention. Single fibre recordings from unmyelinated nociceptive fibres (C-fibres) are performed *in vitro* to determine C-fibre function following nerve lesion in both mice strains. Dorsal root ganglia (DRG) L3-L6 are harvested and mRNA expression analysis is performed to detect differential expression. Focus is placed on ion channels potentially involved in mechanotransduction like the transient receptor potential channel TRPA1.
Baseline behavioural measurements yielded significantly lower mechanical withdrawal thresholds for control *vs. SNS-gp130*$^{-/-}$ mice. These results correspond with preceding single fibre recordings from the saphenous nerve where C-fibres had significantly higher mechanical thresholds in *SNS*-gp130$^{-/-}$ mice *vs*. controls. After induction of neuropathy mechanical hypersensitivity developed in both mouse strains, however, was significantly less severe in *SNS-gp130*$^{-/-}$ mice *vs*. controls.
Investigation of mRNA levels of transient receptor potential channel A1 (TRPA1) in explants is ongoing. *SNS-gp130*$^{-/-}$ show a significant downregulation compared to *gp130*$^{fl/fl}$. First analysis of SNI-injured mice indicates a less pronounced upregulation of TRPA1 mRNA in *SNS-gp130*$^{-/-}$ mice *vs. gp130*$^{fl/fl}$.
Together, our data obtained from the SNI neuropathic pain model are in line with previous results. They suggest a pivotal role for gp130 in chronic pain states with mechanical hypersensitivity and a potential - gp130 dependent - upregulation of the mechanotransducer channel TRPA1.

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P 20

Structural determinants of $Ca_v1.3$ L-type calcium channel gating

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Mice lacking functional $Ca_v1.3$ L-type Ca^{2+} channels show sinoatrial node dysfunction and are congenitally deaf. We have recently discovered a homozygous in frame glycine insertion mutation in position 403 (MUT) in the pore-forming IS6 helix of Cav1.3 α1 -subunits that causes bradycardia and deafness in humans. This mutation prevents channel opening despite the presence of capacitive gating currents (Q_{on}) resulting from S4 voltage sensor movement.We further characterized the gating properties of MUT channels after expression in tsA-201 cells together with accessory subunits (β_3, $\alpha_2\delta_1$) using the patch-clamp technique. Half maximal activation voltage (V_h) of MUT Q_{on} was significantly shifted by 16.2±2.8 mV (n=6-8, p<0.0001) to more negative voltages as compared to wildtype (WT). Inward I_{Ca} of WT activated 11.5±2.9 mV more positive than WT Q_{on} (n=7-8; p=0.0016). In addition, MUT Q_{on} kinetics were significantly faster than for WT evident as shorter time constants for gating current decay over a large voltage range(-10 to +50mV).
In addition, we identified pronounced differences in the calcium-dependent inactivation (CDI) and activation voltage range of rat Cav1.3 α_1 subunit cDNA clones isolated from superior cervical ganglion (SCG) cells (AF370010; $rCav1.3_{scg}$) versus pancreatic islets (D38101,$rCav1.3_{pan}$) (mean difference in half-maximal activation voltage, V_h, 17.2 mV). $rCav1.3_{scg}$ differs from $rCav1.3_{pan}$ at three amino acid positions (S244G, V1104A, A2073V) and one alternatively spliced locus (absence of exon 31). Alternative splicing did not explain the functional differences between the two rCav1.3 α1 subunits. The amino acid difference A2073V is located within a recently identified distal part (DCRD) of a C-terminal modulatory domain. Mutation of A2073 in $rCav1.3_{scg}$ to the corresponding valine (A2073V) in $rCav1.3_{pan}$ fully restores the slower CDI of $rCav1.3_{pan}$. In contrast, A2073V only weakly affected the activation voltage range (rescue of only 5.3 mV of the 17.2. mV difference). Additional mutation of S244 to 244G in the $rCaV1.3_{scg}$ S4-S5 linker of domain I caused a further shift but the double mutant still activated with a V_h 7.7 mV more negative than $CaV1.3_{pan}$. We currently investigate an additional contribution of V1104 in this process.
Our data identify residues at proposed interfaces between voltage-sensors and the intracellular channel gate controlling the voltage-dependence of Cav1.3 activation. We also show that the DCRD domain can moderate CDI independent of its effect on V_h, suggesting that its control of voltage-dependence requires interaction with additional structural features such as G244 and A1104.
Support: Austrian Science Fund (P20670, W11)

P 21

Neuropeptide Y Y2 receptors modulate trace fear conditioning in the dorsal hippocampus

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Neuropeptide Y (NPY), a highly conserved 36 amino acid peptide is widely distributed in the central nervous system. Besides its functions in various metabolic processes NPY has attracted considerable attention in modulating emotional-affective behavior. NPY exerts a pronounced anxiolytic effect most likely mediated by Y1 receptors, whereas stimulation of predominantly pre-synaptic Y2 receptors results in increased anxiety. The role of NPY Y2 receptors in the processing of fear, however, remains still elusive. The current study aims to investigate the role of NPY Y2 receptors in *Pavlovian* fear conditioning, a simple form of associative learning.

Y2KO mice were subjected to *delay* (amygdala dependent) and *trace* (hippocampus dependent) fear conditioning paradigms. While in *delay* fear conditioning Y2KO mice performed similar to wildtype controls, recall of a *trace* fear memory, was significantly increased in Y2KO mice. Trace fear conditioning is predominantly mediated by the dorsal hippocampus. For investigating the specific contribution of Y2 receptors in the adult dorsal hippocampus in *trace* fear conditioning we now locally deleted hippocampal Y2 receptors in *conditional* Y2KO mice by injection of an rAAV-CreGFP vector. Moreover we over-expressed NPY_{3-36}, a Y2 receptor preferring agonist, at the same brain sites.

Our data indicate that while Y2 receptors are not involved in amygdala dependent delay fear conditioning, they seem to play an inhibitory role on the acquisition of trace fear memories. This action is probably mediated by inhibition of glutamate release in dorsal hippocampal circuitries.

This work was funded by the Austrian Science fund (S10204 and P 22830-B18)

P 22

Nuclear targeting of the calcium channel β4b subunit in wildtype and lethargic cultured cerebellar granule cells

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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) from wild type and β_4-null (lethargic) mice to further analyze the function of β_4 nuclear targeting. 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 with anti-tau and anti-MAP2 indicated elaborate axonal networks and few short dendrites, respectively. Observing calcium transients with the fluorescent calcium indicator Fluo4-AM demonstrated the spontaneous activity of the differentiated CGC cultures. Immunolabeling with synapsin and vGlUT1 revealed a high density of presynaptic specializations. However the great majority of these did not colocalize with the postsynaptic proteins PSD95, NMDA receptor and $GABA_A$ receptor, indicating that CGCs in culture very rarely form synapses. Nevertheless, depolarization-induced uptake and release of FM 1-43 dye demonstrated that the presynaptic specializations correspond to functional nerve terminals**.** 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. Whereas the available β_4 antibody was inefficient in demonstrating the nuclear localization of endogenous or heterologous β_4 subunits, nuclear targeting was revealed by labeling V5- and GFP-tagged β_{4b} expressed in wild type and lethargic CGCs. Reconstitution of lethargic CGCs with either β_{4a} or β_{4b} showed isoform-specific differences in nuclear targeting. Depolarization or blocking spontaneous activity with TTX decreased or increased nuclear targeting, respectively. Thus, the properties of β_4 nuclear targeting that have previously been shown in heterologous cells could now be reproduced in the native neuronal system.
Support: FWF, P20059-B05 and W1101-B12.

P 23

L-type calcium channels control age-dependent desensitization and cocaine-induced plasticity of D2-autoreceptor function in substantia nigra dopamine midbrain neurons

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Somatodendritic dopamine D2-autoreceptors (D2-ARs) modulate spontaneous electrical activity (*in vivo* and *in vitro)* of most adult dopamine (DA) midbrain neurons in a negative feedback loop and thereby dampen dopamine release. Differences in functional D2-AR expression have been associated with traits like novelty seeking and vulnerability to drug addiction. DA neurons in the *substantia nigra* (SN) that project to dorsolateral striatum are necessary for development of compulsive drug seeking and taking. Given the age dependency of addiction, we were interested in the maturation of the tonic D2-AR function in DA neurons and its modification by cocaine. In SN DA neurons of adult (3-month) C57BL/6 mice, D2-ARs were tonically active *in vivo,* as indicated by increased mean firing rates (134±7.8%, n=6, p=0.01) and enhanced burst firing (207±25%, n=6; p=0.02) induced by their selective inhibition (0.5mg/kg eticlopride i.p.). To mimic tonic DA signaling *in vitro*, we recorded neuronal activity from brain slices in perforated patch configuration and challenged DA SN neurons of adult mice with prolonged bath applications of 100µM dopamine (15 min), which led to a D2-AR-mediated complete inhibition of pacemaker firing without desensitization (n=5). In contrast, we observed significant desensitization of the D2-AR-mediated pacemaker inhibition in immature DA SN neurons from juvenile mice (P12-14), where neurons discharged with about 40% of their control frequencies still in the presence of dopamine. Furthermore, three days after a single cocaine injection *in vivo* (15mg/kg i.p.), the D2-AR-desensitization was significantly less prominent and dopamine-induced pacemaker-inhibition was significantly more robust compared to saline injected controls (control: 63.9±9.3%, n=14; cocaine: 91.8±6.4%, n=12; p=0.03) and resembled the adult phenotype (control: 100±0%, n=7, cocaine: 99,9±0,1%, n=7; p=1.00). This cocaine-induced plasticity of D2-AR function was selective for juvenile DA SN neurons and was not observed in neighboring ventral tegmental area DA neurons (n=5). Thus, a single cocaine challenge appears to selectively hasten the maturation of D2-AR function in DA SN neurons, which might be a protective response to prevent habit formation. Selective inhibition of voltage-gated L-type calcium channels by 300nM isradipine as well as analyzing Cav1.3 KO and Cav1.2 DHP KO mice pointed to a crucial role of these channels both in the age-dependent maturation of D2-AR desensitization and its modulation by cocaine. We currently aim to define the signal transduction of D2-AR desensitization in juvenile SN DA neurons, which might lead to new ways to boost D2-AR function to protect against development of addiction.

P 24

Selective vulnerability of dopaminergic midbrain neurons in Parkinson's disease: focus on ion channels and genomic integrity.

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Parkinson's disease (PD) is a progressive neurodegenerative disorder, characterized by loss of dopamine (DA) containing midbrain neurons, in particular within the *substantia nigra* (SN). The cause for this selective and progressive neurodegeneration of DA midbrain neurons in PD still remains unclear. However, genetic and environmental factors, leading to impaired DNA integrity and mitochondrial dysfunction, as well as altered ion channel activity in DA midbrain neurons, especially of ATP-sensitive K^+-channels (K_{ATP}) and L-type calcium channels (LTCCs) have been identified as important factors in PD.
Insights from mice lacking Cav1.3 L-type Ca^{2+} channels indicated protection of DA neurons from degeneration in a MPTP mouse model of PD. Recent clinical studies also suggest that LTCC blockers (dihydropyridines) might have neuroprotective effects in PD. A direct link between altered genomic and mitochondrial DNA integrity and mitochondrial dysfunction as well as LTCC activity (spontaneous pacemaker generation) in context of DA degeneration and PD has been proposed. Although described as key players in the complex degenerative process of PD, the exact molecular composition and the distinct physiological role of LTCCs in highly vulnerable SN DA neurons still remains unclear.
Therefore, our aim was to characterize the cell specific functional roles of specific LTCC-subunit-complexes in SN DA neurons, and linking them - at the level of individual neurons - to genomic and mitochondrial DNA integrity, mitochondrial dysfunction and selective vulnerability in PD and its mouse models.
Functional analysis of basal electrophysiological properties that we performed on wild type and CaV1.3 KO mice revealed that AHPs and sag-components are significantly different in SN DA from juvenile / adult WT and Cav1.3 KO mice, indicating an age-dependent role of Cav1.3 channels. Additionally, block of hyperpolarisation activated cyclic nucleotide gated cation (HCN) channels inhibited activity of most SN DA neurons from juvenile Cav1.3 KO but not wild type mice, implying a flexible pacemaker mechanism. Our real-time qPCR data further showed a different expression ratio of Cav1.3 and Cav1.2 α_1-subunits in highly vulnerable SN DA and more resistant VTA DA neurons of juvenile WT mice. We have also established a reliable method for qualitative and quantitative mitochondrial DNA analysis which revealed high levels of mitochondrial DNA deletions in single SN neurons of PD patients and age-matched controls.

P 25

Progression of clinical milestones in multiple system atrophy – an analysis of the EMSA study registry

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Objective: To prospectively assess the progression of clinically relevant milestones in multiple system atrophy (MSA).
Background: MSA is a disabling neurodegenerative disorder progressing relentlessly until death which occurs at median nine years after disease-onset. This fatal disorder is characterized clinically by any combination of autonomic failure, Parkinsonism, cerebellar dysfunction and pyramidal sings. Previous studies revealed a rapid succession of disability milestones within a short period of time.
Methods: 144 patients were included in a natural history study conducted by the European MSA study group (EMSA-SG). Patients were followed up for two years having a complete neurological examination every six months. The unified MSA Rating Scale (UMSARS) was used to assess disease progression and to gather information on clinical milestones, namely feeding by nasogastric tube or gastrostomy due to severe dysphagia, falls at least once a day, unintelligible speech, and inability to walk. The interval to reach clinical milestones was calculated using Kaplan-Meier analysis considering data from all patients having experienced the emergence of these milestones during the study period.
Results: During the study period, patients tended to deteriorate to more severe impairment in the observed milestones. Furthermore, statistical analysis revealed a rapid succession of clinical milestones emerging at median 5.0 (falls at least once a day) to 6.5 (feeding by nasogastric tube or gastrostomy) years after disease-onset.
Conclusion: In line with previous studies, we observed an accumulation of clinical milestones over a short period of time. Additionally, the present work provides useful information for planning future interventional trials.

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Erythropoietin is Neuroprotective in a Transgenic Mouse Model of Multiple System Atrophy

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Objective: To assess the neuroprotective efficacy of Erythropoietin (EPO) in a transgenic mouse model of Multiple System Atrophy (MSA).
Background: MSA is a rapidly progressive neurodegenerative disorder of unknown aetiopathogenesis accomplished with a markedly reduced life expectancy. Clinical characteristics include parkinsonian, cerebellar, and autonomic symptoms. The histopathological hallmarks are widespred α-synuclein positive glial cytoplasmic inclusions (GCI) associated with distinctive neuronal multisystem degeneration. EPO is known for its effects on stimulation of erythropoiesis. Moreover, EPO and EPO-receptor are expressed in human brains throughout development. In MSA, EPO is known to be related to orthostatic hypotension and anemia.
Materials & Methods: A total of 40 homozygous transgenic mice with targeted overexpression of α-synuclein were randomized into four experimental groups. Thirty mice were exposed to mitochondrial stress by 3-nitroproprionic acid (3-NP) intoxication. Intoxicated animals were treated with saline, early EPO and late EPO with treatment starting before (early EPO) and after (late EPO) intoxication. Non-intoxicated animals receiving EPO from the beginning and intoxicated animals which were treated with saline served as controls. Behavioral tests included motor behavior scale (MBS), pole test and spontaneous locomotor activity. Animals were sacrificed at day 29 under at least threefold thiopental overdose. Serial coronal sections were cut on a freezing microtome and stained with antibodies against tyrosine hydroxylase (TH) and dopamine and cyclic adenosine monophosphate (cAMP)-regulated phosphoprotein (DARPP-32). Cell numbers were estimated using the optical dissector method. Statistical analysis was performed using SPSS 15.0 (SPSS). All data are expressed as mean ± standard deviation.
Results: Animals receiving EPO before and after 3-NP intoxication scored significantly lower on the MBS and they performed better in the pole test than controls with no significant difference between early and late EPO administration. Immunohistochemistry revealed significant attenuation of 3-NP induced loss of TH and DARPP-32 positive neurons in substantia nigra pars compacta and striatum, respectively, in both EPO-treated groups compared to untreated controls. At striatal level, a significant difference between early and late EPO administration was observed.
Conclusion: EPO improves motor deficits in an established transgenic mouse model of MSA. Additionally, EPO preserves dopaminergic cells in the substantia nigra and gabaergic neurons at striatal level. Our finding of EPO-derived effects within the striatonigral pathway suggest that further studies to explore the underlying mechanisms and to define the preclinical rationale of EPO-based therapies in MSA are highly warranted.

P 27

Parvalbumin neurons and calretinin immunoreactive fibers degenerate in the subiculum after kainate-induced seizures in the rat

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The subiculum, the major output area of the hippocampus, is closely interconnected with the entorhinal cortex (EC) and other parahippocampal areas. In animal models of temporal lobe epilepsy (TLE) and in TLE patients it exerts increased network excitability and may crucially contribute to the propagation of limbic seizures.
Using immunohistochemistry and *in-situ* hybridization we now investigated neuropathological changes affecting parvalbumin (PV) and calretinin (CR) containing neurons in the subiculum and EC after kainic acid (KA)-induced status epilepticus.
We observed prominent losses in PV containing interneurons in the subiculum and EC. Degeneration of PV-positive neurons was associated with significant precipitation of PV-immunoreactive debris 24 hrs after KA-injection. In the subiculum the superficial part of the pyramidal cell layer was more severely affected than its deep part. In the EC, mainly the deep layers were affected. The decrease in number of PV-positive neurons in the subiculum correlated with the number of spontaneous seizures subsequently experienced by the rats. CR-positive fibers terminating in the molecular layer of the subiculum, in sector CA1 of the hippocampus proper and in the EC degenerated together with their presumed perikarya in the thalamic nucleus reuniens.
Notably, the loss in PV-positive neurons in the subiculum equaled that in human TLE. It may result in marked impairment of feed-forward inhibition of the temporo-ammonic pathway and may significantly contribute to epileptogenesis. Similarly, the loss of CR-positive fiber tracts originating from the nucleus reuniens thalami significantly contributes to the rearrangement of neuronal circuitries in the subiculum and EC during epileptogenesis.

Supported by the Austrian Research Funds (P 19464) and the European Union Grant FP6 EPICURE (LSH-CT-2006-037315).

P 28

Participation of large-conductance Ca^{2+}-activated K^+ channels in the formation of PLasmERosomes in central principal neurons

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The endoplasmic reticulum (ER) forms a continuous network of tubules and cisterns in neurons. However, recent evidence suggests that neuronal ER does not represent a uniform Ca^{2+} pool but rather a spatially heterogeneous system organized into subcompartments. These ER domains, or *calciosomes*, are usually enriched in certain isoforms of Ca^{2+} pumps, Ca^{2+} binding proteins and Ca^{2+} permeant channels. Two families of Ca^{2+} permeant channels are known in neuronal ER, namely the inositol 1,4,5-triphosphate receptors (IP3-Rs) and the ryanodine receptors (RyRs). Areas of the plasma membrane overlying calciosomes also form specialized microdomains that contain unique sets of proteins. These plasma membrane domains together with the underlying calciosomes are proposed to build a functional unit, termed *PLasmERosomes*.

This study was undertaken (i) to unravel the exact morphological parameters of subsurface cisterns (SSCs), representing particular types of calciosomes, in cerebellar Purkinje cells (PCs) and (ii) to analyze the molecular composition of SSCs as well as overlying plasma membrane domains with respect to Ca^{2+} release channels (IP3-Rs, RyRs) and large-conductance Ca^{2+} activated K^+ (BK_{Ca}) channels. The morphological parameters of SSCs are established by 3D-reconstruction plane-by-plane from series of ultrathin sections by using the software CAR (Contour Alignment Reconstruction). The molecular composition is studied by means of post-embedding immunogold electron microscopy and SDS-digested freeze-fracture replica immunolabeling.

SSCs are discoid flattened cisterns, 0.4-1.5 µ m wide, with a luminal depth of 4-5 nm (widening at their lateral edges), situated beneath the inner leaflet of the plasma membrane at a regular distance of 10-13 nm. IP3-Rs and RyRs are both localized to SSCs indicating that these Ca^{2+} release channels share a common Ca^{2+} pool and dispose SSCs to the generation of both Ca^{2+} puffs and sparks. Clustered BK_{Ca} channels are always associated with plasma membrane domains overlying SSCs and facilitate the generation of small transient outward currents. These findings indicate that functional units exist in cerebellar PCs resembling PLasmERosomes in myocytes, and these units may contribute significantly to spatial signalling in central principal neurons.

Acknowledgements:
This work was supported by a grant from the Innsbruck Medical University, MFI-4305 to W.A.K. The excellent technical assistance of Sabine Schoenherr and Dr. Barbara Kapelari are gratefully acknowledged. I thank Dr. Francesco Ferraguti at the Dept. Pharmacology for continuous support.

P 29

Differential activation of nucleus accumbens shell vs. core during acquisition of cocaine- vs. social interaction conditioned place preference (CPP)

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Is social interaction as a non-drug ("alternative") reinforcer able to help addicts reorient their behavior away from the drug of abuse? We could previously show (Fritz et al. 2011, Addiction Biology, in press) that social interaction is able to speed up extinction of cocaine conditioned place preference (CPP), reverses place preference even if cocaine CPP training is continued, and can prevent reacquisition of cocaine CPP. In the present study, we investigated the contribution of the core (AcbC) and the shell (AcbSh) subregions of the nucleus accumbens, and of the basolateral amygdala (BLA) to the acquisition/expression of social interaction CPP vs cocaine CPP.

Male Sprague-Dawley rats were single-housed and CPP-trained for cocaine (15 mg/kg i.p.) alone, for an i.p. saline injection paired with social interaction (weight- and gender-matched partner within the confines of the CPP chamber) alone, or concurrently for cocaine- vs social interaction CPP. We performed excitotoxic lesions of the AcbC, the BLA, or the AcbSh. All groups were tested for CPP expression and Zif268 immunocytochemistry.

Lesioning the AcbSh before the **concurrent** acquisition and expression of social interaction CPP vs cocaine CPP shifted the preference toward cocaine whereas AcbC and BLA lesions shifted the preference toward social interaction. These findings suggest that the inactivation of the AcbC or the BLA is sufficient to inhibit the incentive salience of drug-associated stimuli, and increases the motivation for the non-drug stimulus social interaction. In addition, different brain areas seem to be engaged during the **acquisition and expression of social interaction** CPP as compared to the **reversal of reacquisition of cocaine** CPP by social interaction.

Supported by FWF grant P18787-B05, FWF Lise-Meitner Fellowship M1169-B18 to R.E., and by the Verein zur Förderung der Experimentellen Psychiatrie, Psychotherapie und Pharmakologie (VEPPP).

P 30

Social interaction prevents cocaine relapse in a rat model: Correlation with functional brain mapping

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The worsening of drug abuse by drug-associated social interaction is a well studied phenomenon. In contrast, the molecular mechanisms of the beneficial effect of social interaction, if offered as a mutually exclusive choice to drugs of abuse, are underinvestigated. In a rat place preference conditioning (CPP) paradigm, four 15 min episodes of social interaction with a gender-matched adult conspecific inhibited cocaine-induced reacquisition, a model of relapse, in extinguished rats. These protective effects of social interaction were paralleled by a reduced activation and by an alteration of FosB/Delta FosB expression in brain areas known to play critical roles in drug-seeking behavior.These findings suggest that social interaction, if offered in a context that is clearly distinct from the previously drug-associated ones, may profoundly prevent relapse to cocaine addiction.

Keywords : Substance dependence, social interaction, cocaine, brain activation, FosB/Delta FosB, conditioned stimulus, relapse

P 31

Application of embryonic stem cell-derived neuronal network activity

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Similar to *in vivo* neuronal assembly also *in vitro* cultivated neuronal populations are able to generate spontaneous synchronous burst activity. Such neuronal network activity can be assessed by the microelectrode array (MEA) technology that allows simultaneous extracellular recording of the electrical activity exhibited by entire populations of neurons over several weeks or months *in vitro*. Combination of MEAs and neurons – called neurochip – are used as model systems to understand the principles of brain activity as well as serves as functional assay for drug discovery. In particular, neuroactive candidates, which have been identified in animal-based neuronal *in vitro* systems, could be analysed in human-based *in vitro* systems prior clinical phases. However, the generation of functional neuronal assembly from human primary tissue or neuronal cell line is not possible.
We and other demonstrated that murine and human embryonic stem cell derived neurons are able to generate synchronous active neuronal network on MEAs. We present here developmental and pharmacological properties of embryonic stem cell-derived neuronal networks. Furthermore, we applied CSF specimens of human controls or patients suffering from severe traumatic brain injury (TBI) to these neurochips. We found that pathologically altered CSF from acute traumatic brain injury (TBI) patients suppresses *in vitro*-neuronal network function. Functionally relevant substances within pathologically altered CSF could be biochemically encircled and *in vitro* network activity could partially be recovered by pharmacological intervention.
Thus, the fusion of MEA technology and embryonic stem cell-derived neuronal populations represents a powerful tool to describe the temporal progression of human stem cell-derived neural populations towards mature, functioning neuronal networks. Furthermore such *in vitro* human cell-based model systems can be applied to investigate the influence of human CSF-derived neuroactive components on neuronal network activity.

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Generation and Neural Differentiation of Induced Pluripotent Stem Cells from Human Fibroblasts

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Induced pluripotent stem cells (IPS cells) are somatic cells that have been reprogrammed to a pluripotent state by the introduction of specific transcription factors. We have generated IPS cells from human fibroblasts using a single polycistronic lentiviral vector comprised of the transcription factors Oct-4, Klf4, Sox-2 and c-Myc (OKSM), separated by the self-cleaving 2A peptide and IRES sequences. Here, we present the validation of two IPS cell lines that are similar to embryonic stem cells in several aspects including morphology, expression of pluripotency markers and the capacity to differentiate to cells of all three germ layers. These IPS cells were differentiated into neuronal lineages. In future experiments patient-specific IPS cells from donors suffering from neurological disorders will be differentiated into the specific neuronal cell type affected by the disease. These new human cell-based models can be used to study pathophysiological processes in vitro and as systems to screen new drugs. Moreover, human IPS cells promise to be good candidates for regenerative medicine.

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Intracellular sorting of fibroblast growth factor receptor 1 controls axon elongation and branching of adult sensory neurons

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Fibroblast growth factors (FGFs) are involved in a variety of developmental and regenerative processes in the nervous system including axon regeneration. FGFs mediate their intracellular responses by activation of four types of high affinity tyrosine kinase receptors (FGFR1-4). The Ras/ERK and the PI3K/Akt pathway represent the two main FGF signaling pathways required for axon growth. The Ras/ERK pathway is mainly involved in elongative axon growth, while activation of PI3K/Akt results in enhanced axon caliber and branching. FGFR1 is highly expressed in adult sensory neurons of dorsal root ganglia. Activation of FGFR1 is followed by endocytosis and rapid degradation in lysosomes, rendering receptor signaling incompetent.

In this study, we modulated intracellular sorting of FGFR1 in adult sensory neurons. Overexpression of FGFR1 stimulated FGF-2-induced elongative axon growth and this effect was strongly enhanced by the lysosomal inhibitor leupeptin. Furthermore, leupeptin enhanced plasma membrane localization of FGFR1 by increased receptor recycling. In addition, a mutant of FGFR1, which is preferentially sorted to recycling back to the cell surface, promoted elongative axon growth. By contrast, inhibition of FGFR1 endocytosis by methyl-β-cyclodextrin or chlorpromazine promoted FGF-2-induced axonal branching through increased phosphorylation of ERK and Akt.

Together our data indicate that trafficking of FGFR1 controls axon regeneration of adult sensory neurons in vitro. Inhibition of FGFR1 endocytosis promotes axonal branching, whereas enhanced recycling of FGFR1 promotes elongative axon growth.

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Distribution of large-conductance voltage- and Ca^{2+}-activated K^+-channels (BK-channels) in mouse and rat pituitary

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Large-conductance Ca^{2+}-activated K^+-channels (BK channels) play an important role in neurosecretion and hormone release; they are involved in repolarization and fast afterhyperpolarization and thereby influence hormone release.
We used double-immunofluorescence labelling to investigate BK-channel expression in hormone releasing structures of mouse and rat anterior and posterior pituitary, using BK-deficient mice as controls.
BK channels are highly expressed in the termini of oxytocin- and vasopressin-releasing neurons in the posterior pituitary; we were also able to demonstrate the expression of BK channels in cells secreting somatotropin and corticotropin in anterior pituitary.
The close localization of BK channels to hormone containing vesicles indicates the important role of BK-channels in the pituitary gland.

Acknowledgement: Our work was supported by the Legerlotz foundation

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Tolerogenic effect of apoptotic cells

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Background: Multiple sclerosis (MS) is a chronic inflammatory demyelinating disease of the central nervous system. It was shown that dendritic cells (DCs) are recruited to MS lesions, where they mature and might contribute to the local activation and expansion of T cells. Uptake of apoptotic cells by DCs has been involved in tolerogenesis as well as in immune activation. Treatment of cells with the apoptosis-inducing, chemical cross-linker ethylcarbodiimide (ECDI) was shown to promote tolerance in several animal models of autoimmune diseases. In the current study we analyze whether uptake of apoptotic autologous blood mononuclear cells (PBMCs) affects the activation of antigen-presenting-cells (APC) - monocyte-derived DCs and monocytes.
Methods: DCs were generated from monocytes in the presence of IL-4 and GM-CSF. Cells were co-cultured either with ECDI-treated-, UV-irradiated-, γ-irradiated- or untreated autologous PBMCs. The next day, cells were further stimulated with LPS or a maturation cocktail. Cell activation was analyzed by the expression of cell surface markers and the secretion of various cytokines in cell culture supernatants. The immunostimulatory capacity of apoptotic-cell loaded DCs was assessed in allogeneic mixed leukocyte reactions (MLRs). Furthermore, monocytes were pre-incubated with γ-irradiated- or ECDI-treated antigen-coupled cells (tetanustoxoid or myelin-proteins), washed and further co-cultured with autologous T cells and tetanustoxoid/myelin-proteins. The proliferation of T cells was measured via dilution of carboxyfluorescein-succinimidyl-ester (CFSE).
Results: We found that different strategies to induce apoptosis had no profound influence on the maturation and activation of DCs and monocytes. The uptake of apoptotic cells by DCs did not affect their immunostimulatory capacity in allogeneic MLRs. However, in autologous MLRs, T cells which were co-cultured with monocytes - pre-incubated with ECDI-treated cells - did not proliferate in response to antigens.
Conclusion: Our results indicate a potent immunomodulatory effect of ECDI-treated cells on APCs, exclusively in an autologous manner.
These results provide further evidence for the importance of APCs in antigen specific tolerization with ECDI-treated antigen-coupled cells, a novel therapeutic approach in MS.
Acknowledgements: The study is supported by a research grant of the Cumming Foundation (WY, USA).

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Complement activating antibodies to MOG and AQP4 in CNS demyelinating diseases

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Autoantibodies targeting aquaporin-4 (AQP4) are important biological markers and pathogenic factors in Neuromyelitis optica spectrum disorders (NMOSD). Recent studies indicated a role for anti-myelin oligodendrocyte glycoprotein (MOG) autoimmune responses in animal models of NMOSD. Although high-titer autoantibodies to human native MOG were detected in pediatric acute disseminated encephalomyelitis (ADEM) and multiple sclerosis (MS) patients, the role of anti-MOG antibodies in NMOSD remains unresolved. Therefore, we analyzed serum samples from patients with NMOSD (n=69), ADEM (n=33), clinically isolated syndrome (16), MS (65), other neurological diseases (OND, n=24), systemic lupus erythematosus (SLE, n=26) and 47 healthy controls for antibodies to MOG and AQP4 via immunofluorescence assay. Furthermore, we investigated their ability to induce complement mediated cytotoxicity (CDC). AQP4-IgG were detected in 54 patients (78%) with NMOSD, but not in other diseases and controls. In contrast, we detected high titer anti-MOG antibodies in 14 patients with ADEM (42%, median titer 1:2,560, range 1:160-1:20,480), MS (n=2, both 1:160), CIS (n=2, 1:640 and 1: 5,120), SLE (n=2, 1:160 and 1:320), one OND (1:640) and 4 of 15 NMO-IgG seronegative NMOSD patients (median titer 1:2,560, range 1:1,280-1:5,120), but not in anti-AQP4 antibody positive patients. Antibodies to MOG and AQP4 were predominantly IgG1 and activated the complement cascade. Human antibodies to MOG and AQP4 might provide new insights in the pathogenesis of NMO.

Acknowledgements: The study is supported by a research grant of the Austrian Multiple Sclerosis Society.

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Nogo-B is associated with cytoskeletal structures in human monocyte-derived macrophages

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Background: The reticulon Nogo-B participates in cellular and immunological processes in murine macrophages. Since leukocytes are an essential part of the immune system in health and disease, we decided to investigate the expression of Nogo-A, Nogo-B and Nogo-C in different human immune cell subpopulations. Furthermore, we analyzed the localization of Nogo-B in human monocyte-derived macrophages by indirect immunofluorescence stainings to gain further insight into its possible function.
Findings: We describe an association of Nogo-B with cytoskeletal structures and the base of filopodia, but not with focal or podosomal adhesion sites of monocyte-derived macrophages. Nogo-B positive structures are partially co-localized with RhoA staining and Rac1 positive membrane ruffles. Furthermore, Nogo-B is associated with the tubulin network, but not accumulated in the Golgi region. Although Nogo-B is present in the endoplasmic reticulum, it can also be translocated to large cell protrusions or the trailing end of migratory cells, where it is homogenously distributed.
Conclusions: Two different Nogo-B staining patterns can be distinguished in macrophages: firstly we observed ER-independent Nogo-B localization in cell protrusions and at the trailing end of migrating cells. Secondly, the localization of Nogo-B in actin/RhoA/Rac1 positive regions supports an influence on cytoskeletal organization. To our knowledge this is the first report on Nogo-B expression at the base of filopodia, thus providing further insight into the distribution of this protein.
Acknowledgements: The study was supported by a research grant of the Wings for Life foundation (Salzburg, Austria).

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Synaptic release and synapse formation in neuronal calcium channel $\alpha_2\delta$ 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, e.g. by knockdown of $\alpha_2\delta$, 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 47% 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 (time constants: 23.4±2.2 s [mean±SEM] vs 28.8±2.8 s in control neurons [p=0.14; n=17]). In order 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. 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 (time constants: 19.4±1.4 s [mean±SEM] vs 23.0±2.4 s in control neurons [p=0.21; n=11]). 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 21-29 DIV old $\alpha_2\delta$-1 knockdown/ $\alpha_2\delta$-3 knockout neurons; both in efferent (axonal) and in afferent (dendritic) synapses of the transfected neuron. Together these results suggest that synapse formation and the primarily P/Q-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. Currently we are investigating the contribution of N-type channels to synaptic release in $\alpha_2\delta$-1 knockdown neurons as well as the effects of pharmacological inhibition of $\alpha_2\delta$-1 and $\alpha_2\delta$-2 in $\alpha_2\delta$-3 knockout neurons.
Support: FWF P20059-B05, W1101-B12, T 443-B18.

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Transient decline of cholinergic neurons of the basal nucleus of Meynert in organotypic rat brain slices after ethanol exposure

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The cholinergic system plays a major role in learning and cognition and cholinergic neurons appear to be particularly vulnerable to ethanol exposure. Nerve growth factor (NGF) is so far the most potent trophic molecule for cholinergic neurons, but also mitogen activated protein kinase (MAPK) p38 and nitric oxide (NO)-synthase pathways, may also play a role in ethanol-induced effects. The aim of the present study was to investigate the effect of different ethanol concentrations on cholinergic neurons in organotypic brain slices of the nucleus basalis of Meynert (nbM) and to investigate if NGF or inhibitors of MAPK p38 or NO-synthase pathways may counteract the ethanol effect. Two-week old organotypic rat brain slices of the nbM were exposed to different ethanol concentrations (1 mM to 500 mM) for 7 days. NGF (10 ng/ml), MAPK p38 inhibitor SB 203580 (10 µM) and NO-synthase inhibitor L-thiocitrulline (10 µM) were added to slices and incubated with 50 mM or 100 mM ethanol. Our data show that ethanol significantly reduced the number of choline acetyltransferase (ChAT)-positive neurons with the most potent effect at a concentration of 50 mM ethanol (54±5 neurons per slice, n=46***), compared to control slices (120±13 neurons per slice, n=19). Inhibition of MAPK p38 and NO-synthase counteracted the ethanol (50 mM)-induced decline of cholinergic neurons (80±9 neurons per slice, n=27** MAPK p38 inhibitor; 134±10 neurons per slice, n=18*** NO-inhibitor). NGF protected cholinergic neurons against ethanol (100 mM)-effect (97±8 detectable neurons, n=19*). Withdrawal of ethanol for 7 days resulted in a reversal of cholinergic neurons to nearly controls (108±22 neurons per slice, n=18***). In conclusion, ethanol caused a transient decline of cholinergic neurons, possibly involving MAPK p38 and NO-synthase pathways. The ethanol-induced decline of cholinergic neurons may possibly be caused by a transient downregulation of the enzyme ChAT, which may have significant effects on cholinergic neurons and cognition.

Acknowledgements: This study was supported by the Austrian Science Funds (P191220-B05).

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Homocysteine enhances transmigration of rat monocytes through a brain capillary endothelial cell monolayer via ICAM-1

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Increased homocysteine (Hcy) levels contribute to a variety of cardiovascular and cerebrovascular diseases including stroke and Alzheimer's disease. Recent data has shown that elevated levels of Hcy can lead to blood-brain barrier (BBB) dysfunction and activation. However, the mechanism for Hcy-mediated dysfunction remains unclear. The aim of this study is to characterize the effects of moderate Hcy administration in rat brain capillary endothelial cells (BCECs), which serve as a simple model to study blood-brain barrier functions. This present study shows that addition of 20 µ M Hcy for 6 days does not significantly effect BCEC survival, as measured by acridine orange staining, propidium iodide staining, and nitrite content. However, addition of 20 µM Hcy for 6 days does elevate lactate dehydrogenase (LDH) activity released into the supernatant of BCECs, as well as significantly enhances the transmigration of monocytes across the BCEC in a time-dependent manner. In addition, TNFα levels in BCEC are also elevated by Hcy, whereas inflammatory markers MIP3α and RANTES are significantly reduced. Finally, this study shows that intercellular adhesion molecule-1 (ICAM-1) expression is significantly enhanced by 20 µM Hcy treatment compared to control conditions. These results suggest that moderate levels of homocysteine can affect proinflammatory patterns expressed by BCEC, ultimately leading to BBB activation and dysfunction through enhanced monocyte transmigration and ICAM-1 expression.

Acknowledgement: This study was supported by the Austrian Science Funds (P191220-B05) and the Tyrolean Science Funds. We thank Ursula Kirzenberger-Winkler for excellent technical support. L.A.H. was supported in part by a U.S. Fulbright Student Research grant, sponsored by the Austrian-American Educational Commission.

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Analyses of *islet1/isl1* mutant zebrafish reveal similarities and differences in vertebrate isl1 functions

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The *islet1/isl1* gene encodes a LIM homeobox transcription factor with highly conserved early embryonic expression in the developing pancreas, heart and nervous system. In mouse, *Isl1* knock out analyses revealed essential roles for differentiation and survival of motoneuron, morphogenesis and cell differentiation in the pancreatic, formation of the dorsal aorta, and proliferation of cardiac cell. However, analyses of *isl1* knock out mutant mouse only provided limited information on *isl1* functions due to the early lethality of the mutants at embryonic day 10. Only recently conditional knock out approaches provided additional hints on embryonic functions of *isl1* in the retina, sensory neurons and on maturation and survival of endocrine cells in the pancreas. Here we report the primary analysis of neuronal and pancreatic phenotypes in an *islet1* mutant zebrafish. As *isl1* mutant zebrafish die after embryogenesis, these fish enable a detailed analyses of all embryonic *isl1* functions without the requirement for conditional loss of function approaches. We show that the *isl1* mutant zebrafish display defects in all tissues that have been reported to require *isl1* function in mouse. Further we show that the neuronal and pancreatic phenotypes are slightly different in mouse and fish. Our data reveal that conserved expression does not always correlate with a conserved cellular function, while they also show that the *isl1* mutant zebrafish is an ideal model to identify novel sites of *isl1* function.

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Cell-autonomous 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.
During the characterization of a novel nonsense mutation *dzip1/iguana* gene we had previously identified a novel postmitotic role of hh-signaling pathway in regulating axon outgrowth in motoneurons. We could further show that projection defects in motoneurons correlate with the loss of expression. *isl2a* and *nrp1a*-mRNA encodes a lim-homeobox transcription factor nd co-receptor for semaphorin axon guidance factors. Our data suggest a role of hh-signaling in maintaining motoneuron fates and in regulation intrinsic properties of axon guidance. Recent data will be presented.