ANA SALZBURG ANA

16.-18. September

2009

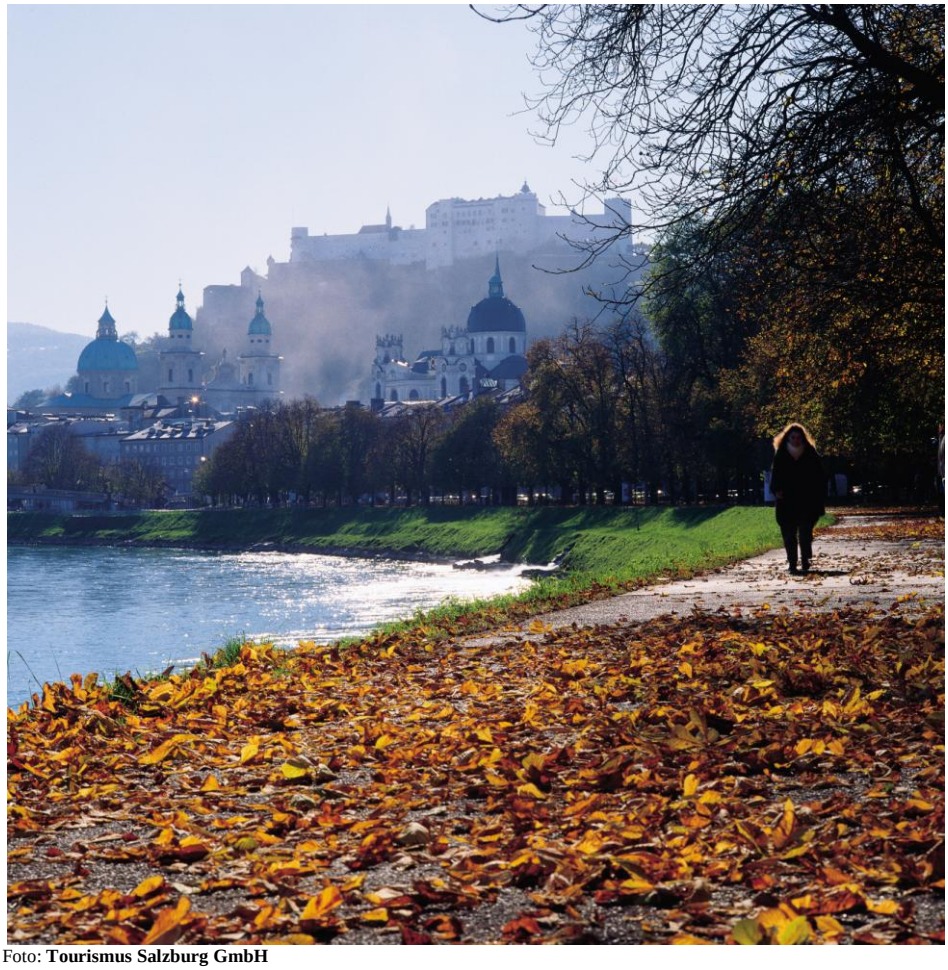
11th Meeting of the Austrian Neuroscience Association

11. Meeting of the „Austrian Neuroscience Association“(ANA)

(ÖSTERREICHISCHE NEUROWISSENSCHAFTLICHE GESELLSCHAFT – ÖGN)

16.- 18. SEPTEMBER, 2009

BILDUNGS- U. KONFERENZZENTRUM ST. VIRGIL, SALZBURG



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Gouverneur Mag. Gabi Burgstaller
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European DANA Alliance London /UK

Red Bull GmbH sponsered the Otto Loewi Prize

General Information

Lecture hall:
All sessions oral as well as poster sessions and meetings will take place in the "Virgil Saal".

Oral presentations: Please upload your presentation in time before your talk on the computer in the main lecture hall. An operator will be available during coffee breaks and 15 minutes before the start of each session. Be aware there is no extra room to check your slides.

Poster presentation: Posters will be presented in two turns please check your poster mounting / demounting and poster presentation time.

Guided Poster tours: There will be guided poster tours for each poster session. Tours start at 20h00 (16.9.2009) for poster session I (posters P1- P32B) and at 16h30 (17.9.2009) for poster session II (posters P33- P70). Please meet with your tourguide at the starting point as outlined in the table below. **Tours will run in parallel!**

The presenting authors should be nearby the poster during this time, explain in 2 -3 minutes their key results and answer questions. Poster tours should be finished after approximately 45-50 minutes to leave space for individual discussions.

Poster#	Tourguide	Starting Point
1-5	Aigner	Poster 1
6-9	Dechant	Poster 6
11-16 & 32A, 32B	Römer	Poster 11
17-23	Huck	Poster 17
24-32	Kubista	Poster 24
33-39	Bauer	Poster 33
40-48	Böhm	Poster 40
49-55	Sieghart	Poster 49
56-62	Flucher	Poster 56
63-70	Ahnelt	Poster 63

Special lunch event 17.9.2009, 12h15: Students and young scientists meet keynote speakers L. Aigner, F. Crews & G. Galizia for lunch. Sign up at the registration desk to reserve your seat (first come first serve).

Breakfast: For those participants who reside in the conference hotel breakfast will be served from 07.00 - 9.00.

Internet access: Each hotel room has an internet access point via cable. Cabels are available from the hotel reception. In addition in the hotel lobby area wireless LAN access is available. To activate your internet access either way please purchase your minutes individually from the hotel reception. To save unused minutes you must log off when finished. Fees are: 3 € for 30 minutes, 5 € for 60 minutes and 13 € for 180 minutes.

Phone calls from private rooms: Calls have to be paid individually at check out.

Check out: **Must not be later then 10h00 at the day of your departure**, or you will be charged an additional night. You can store your luggage in the hotel lobby; please ask the hotel receptionist for the appropriate place. We recommend checking out after breakfast.

Copies: A coin operated copy machine is located in the hotel lobby; alternatively you can buy a copy card from the hotel.

PC & printer: are available in the library, printer paper is handed out by the receptionist of the hotel.

Beverages during lunches and dinners: Due to the low registration fee beverages are not included. Only tap water will be served for free. If you like to drink any other beverage please order and pay individually. Waiters will be around to take your orders.

Bar: The hotel bar next to the dining hall will be open until 23.00 in the evening. During the daytime it operates as a coffee shop. A tip for wine drinkers - try the "Virgil" wines which are produced exclusively for St. Virgil.

ATM: A 24h ATM machine can be found at the Bank "Salzburger Sparkasse" which is located next to the bus stop at the Aigner Strasse (5 minutes walking).

Taxi can be ordered via the hotel reception.

The receptionist of the hotel will help you to organize tickets for concerts in the city or to reserve a table in a restaurant down town.

Legal disclaimer: The organizing committee is not responsible for any loss or damage of personal properties or belongings or any bodily injury during the meeting.

Local Organizing Committee

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WEDNESDAY, SEPTEMBER 16TH, 2009

15.30 – 17.15 **Registration**
15.30 – 16.25 **Mounting of Posters P1 - P32B**

Check in into rooms must not be later than 18.00!

16.30 – 17.15 **ANA board meeting**

17.20 – 17.25 **Welcome address:**
A. Hermann / Salzburg (President of the local organizing committee)

17.25 – 17.30 **Opening ceremony**
C. Bandtlow / Innsbruck (President of the ANA)

17.30 – 18.20_O1 **Opening Lecture I**
***Tobias Bonhoeffer* (MPI / Munich)**
"How experience changes the circuitry of the brain"
Chair: A. Hermann / Salzburg

18.30 **Dinner**

19.45 – 20.00 **Mounting of Posters P1 –P32B**

20.00 – 21.30 **Poster Session I: Posters P1- P32B**

Guided poster tours start at 20h00

Poster#	Tourguide	Starting Point
1-5	Aigner	Poster 1
6-9	Dechant	Poster 6
11-16 & 32A, 32B	Römer	Poster 11
17-23	Huck	Poster 17
24-32	Kubista	Poster 24

THURSDAY, SEPTEMBER 17TH, 2009

08.00 – 17.00 **Registration**

08.00 – 08.50_O2 **Plenary Lecture II**
***Giovanni Galizia* (Univ. Konstanz)**
"Olfactory coding in insects"
Chair: F.G. Barth / Vienna

Oral communications: Session 1

Chair: G. Bernroider / Salzburg & H. Römer / Graz

08.50 – 09.05_O3 Arachnid mechanoreception: I. Micromechanics of spider air flow sensors.
CF. Schaber, F.G. Barth
Vienna

09.05 – 09.20_O4 Arachnid mechanoreception: II. Air flow guiding the spider to its prey.
C. Klopsch, F.G. Barth
Vienna

09.20 – 09.35_O5 Arachnid mechanoreception: III Airflow-sensing in a scorpion.
R. Müllan, F.G. Barth
Vienna

09.35 – 09.50_O6 Matched filters in the auditory system of crickets.
H. Römer, K. Kostarakos
Graz

09.50 – 10.15 **Coffee break**

Oral communications: Session 2

Chair: A. Saria / Innsbruck & L. Klimaschewski / Innsbruck

10.15 – 10.30_O7 Impairment of adult neurogenesis in a psychopathological mouse model of trait anxiety and comorbid depression: effect of antidepressants.
A. Sah, S. Gaburro, C. Schmuckmair, S.B. Sartori, M. Hauschild, L. Czibere, R. Irschick, L. Klimaschewski, R. Landgraf, N. Singewald
Innsbruck / Munich/Germany

10.30 – 10.45_O8 129S1 mice – a novel model for studying impaired extinction in anxiety disorders.
N. Whittle, M. Hauschild, G. Lubec, A. Holmes, N. Singewald
Innsbruck / Vienna / Rockville /USA

10.45 – 11.00_O9 Reorientation from cocaine towards social interaction: Functional brain mapping, immediate early genes, and brain area-targeted pharmacotherapy.
G. Zernig, M. Fritz, R. El Rawas, S. Klement, A. Saria
Innsbruck

11.00 – 11.15_O10 The role of the immune system in a chronobiological model of depression in mice.
D.D. Pollak, F.J. Monje, K.R. Herkner, B.R. Binder
Vienna

Oral communications: Session 3

Chair G.F. Sitdikova / Kazan/Russia & B. Flucher / Innsbruck

11.15 – 11.30_O11 Stable membrane expression of $Ca_V1.2$ calcium channels is independent of activity related rearrangement of post synaptic scaffold proteins.
V. Di Biase, G.J. Obermair, Z. Szabo, C. Altier, J. Sanguesa, E. Bourinet, B.E. Flucher
Innsbruck / Montpellier/France

11.30 – 11.45_O12 L-type voltage-gated calcium channels as anti-epilept(ogen)ic drug targets.
H. Kubista, P. Geier, M. Lagler, S. Böhm
Vienna

11.45 – 12.00_O13 A novel Rab5 guanine nucleotide exchange factor acts as regulator of MuSK signaling at the neuromuscular synapse.
B. Woller, B. Prieler, M. Mutzl, R. Herbst
Vienna

12.00 – 12.15_O14 Role of ryanodine receptors in effects of hydrogen sulfide on transmitter release at frog neuromuscular junction.
G.F. Sitdikova, E.V. Gerasimova, A.L. Zefirov
Kazan/Russia

12.15 – 14.00 **Lunch**
Special event: Students and young scientists meet keynote speakers for lunch
Lunch tables presided by L. Aigner, F. Crews, G. Galizia
(Sign up at the registration desk to reserve your seat)

Change posters during lunch break
(Remove posters P 01- P32B; set up posters P33 – P70)

14.00 – 14.50_O15 **Plenary Lecture III**
***Ludwig Aigner* (PMU Salzburg)**
"Stemness in the Adult Brain: Yes They Can!"
Chair: H. Bauer / Salzburg

Oral communications: Session 4

Chair: C.Bandtlow / Innsbruck & C. Humpl / Innsbruck

14.50 – 15.05_O16 Stem cell quiescence in the hippocampal neurogenic niche is associated with elevated TGF-beta signaling in an animal model of Huntington's disease.
M. Kandasamy, S. Couillard-Despres, K. Raber, M. Stephan, B. Winner, Z. Kohl, F. J. Rivera, O. Riess, U. Bogdahn, J. Winkler, S. v. Hörsten, L. Aigner
Salzburg / Regensburg /Germany

15.05 – 15.20_O17 Modification of fibroblast growth factor receptor1 trafficking promotes axon regeneration.
B. Hausott, B. Schlick, N. Vallant, A. Rietzler, R. Irschick, L. Yang, L. Klimaschewski
Innsbruck

15.20 – 15.35_O18 MRI depiction and quantification of blood-spinal-cord-barrier permeability after Spinal cord contusion injury.
N. Walder, Z. Majdisova, E. Sezemsky, P. Szomolanyi, M. Brejnikow, R. Schmidhammer, S. Trattnig, H. Redl
Vienna

15.35 – 15.50_O19 The role of TLR4 on α-synuclein induced neurodegeneration in a transgenic mouse model of multiple system atrophy.
N. Stefanova, M. Hainzer, S. Stemberger, G.K. Wenning
Innsbruck

15.50 – 16.30 **Coffee break**

16.30 – 18.00 **Poster Session II: Posters P33- P70**

Guided poster tours start at 16h30

Poster#	Tourguide	Starting Point
33-39	Bauer	Poster 33
40-48	Böhm	Poster 40
49-55	Sieghart	Poster 49
56-62	Flucher	Poster 56
63-70	Ahnelt	Poster 63

18.10 – 18.30 **Poster prize committee meeting**
(Bandtlow / Innsbruck, Römer / Graz, Huck / Wien, Weiger / Salzburg)

18.30 **Dinner**

20.00 **Piano Concert with Johannes WILHELM / Salzburg**
(Bach, Beethoven, Mozart, Saint-Saëns)

FRIDAY, SEPTEMBER 18TH, 2009

08.00 – 08.50_O20 **Plenary Lecture IV:**

Sponsored by: Bundesministerium für Wissenschaft und Forschung / Vienna
HIRNFORSCHUNGSSEMINARE
des Projektteams "Hirnforschung in Österreich"

Fulton T. Crews **(UNC-CH Chapel Hill, Bowles Center for Alcohol Studies, USA)**
"Mechanisms of Neurodegeneration and Regeneration during Alcohol Dependence and Recovery"
Chair: T.M. Weiger / Salzburg

Oral communications: Session 5

Chair: N. Singewald / Innsbruck, A. Gbaj / El – Zawai/Lybia

08.50 – 09.05_O21 SNP detection for hereditary neuralgic amyotrophy alleles by target-assembled tandem oligonucleotide systems based on exciplexes.
A. Gbaj, W. Ashati, S. Bahroon, W. Bathni, N. Alfarah
El – Zawai /Lybia

09.05 – 09.20_O22 Pathomechanisms in autism linked to altered function of mutant ribosomal protein rpL10 identified in autistic patients.
J. Wang, A. Chiocchetti, J. Zhou, S. Pfeifenberger, T. Karl, O. Haubenreisser, J. Kellermann, F. Lottspeich, Jaime Martinez, A. Thuer, J. Bauer, H. Hintner, K. Richter, A. Pichova, M. Breitenbach, S. Klauck, M. Ralser, L. Breitenbach-Koller
Salzburg / Heidelberg/Germany / Munich/Germany / Berlin/Germany / Prague/Czech Republic

09.20– 09.35_O23 Modulation of impaired fear extinction by neuropeptides in a psychopathological animal model of high trait anxiety.
S.B. Sartori, M. Hauschild, P. Muigg, D. Slattery, D. Beiderbeck, A. Mathé, G. Wegener, I.D. Neumann, N. Singewald
Innsbruck / Regensburg/Germany / Stockholm/Sweden / Risskov/Denmark

09.35 – 09.50_O24 Peroxisomal localisation of the Proopiomelanocortin derived peptides β-Lipotropin and β-Endorphin.
M. Kunze, R. Hoeftberger, J. Bauer, H. Budka, J. Berger
Vienna

09.50 – 10.15 **Coffee break**

Oral communications: Session 6

Chair: W. Sieghart / Vienna, G. Kastberger / Graz

10.15 – 10.30_O25 Enhanced retinal ganglion cell neurite growth in experimental glaucoma is mediated by activated retinal glia.
B. Lorber, A. Guidi, K. Martin
Cambridge /United Kingdom

10.30 – 10.45_O26 Nicotinic Acetylcholine Receptors (nAChR) in the superior cervical ganglion: A biochemical analysis in wild type mice and mice with deletions of distinct nAChR subunit genes.
Reinhard David, Sigismund Huck, Petra Scholze
Vienna

10.45 – 11.00_O27 A leucine-rich repeat tyrosine kinase regulates neuronal outgrowth and learning-related long-term synaptic plasticity in *Aplysia*.
F.J. Monje, D. D. Pollak
Vienna

11.00 – 11.15_O28 Regulation of monoamine transporters by membrane phosphatidylinositol 4,5-bisphosphate (PIP_2).
K. Schicker, F. Buchmayer, M. Holy, S. Böhm, H. Sitte
Vienna

11.15 – 11.20 **FENS agenda** (reporting A. Saria / Innsbruck)

11.20 – 11.35 **Poster Prize Awards**
presented by the ANA president C. Bandtlow / Innsbruck

11.35 -12.20 **"Otto-Loewi-Award Ceremony"**
The Otto – Loewi Award is sponsored by: Red Bull GmbH, Fuschl am See /Austria, represented by Wings for Life, Spinal Cord Research Foundation / Austria

Presiding the award ceremony & chairing the lecture:
ANA president C. Bandtlow / Innsbruck

Lecture of the Prize Winner

12.20 – 13.00 **General Assembly of ANA**

13.00 **Lunch**

14.00 End of the Meeting

Postersession I

16th of September 2009, 20.00 – 21.30 Posters P1- P32B

P01: S. Luiskandl, C. Melinte, R. Herbst
Cellular trafficking and signaling of the receptor tyrosine kinase MuSK are regulated by dynamin and cholesterol-enriched microdomains.

P02: M. Auer, S. Frauscher, E. Udina, X. Navarro, L. Klimaschewski
Inhibition of RhoA promotes peripheral axon regeneration.

P03: S. Quarta, N. Scherbakov, M. Andratsch, F. Di Cunto, M. Kress
Significance of gp130 signaling in neuronal regeneration.

P04: Z. Puschban, A. Sah, C. Schwarzer, N. Singewald, G. Dechant
Alterations in hippocampal neurogenesis and anxiety behaviour in p75 KO mice".

P04A: B. Loy, R. Dorn, G. Apostolova, G. Dechant
Signaling mechanisms controlling sympathetic cholinergic differentiation

P05: A. Salti, S Neto, Z. Puschban, R. Nat, G. Apostolova, G. Dechant, G. Wenning
Cell Fate Analysis of Embryonal and Adult Stem Sell-Derived Grafts in the 6-OHDA Model of Parkinson´s Disease.

P06: L. Kaya, C.E. Bandtlow
Interaction Partners and Signaling of Reticulons.

P07: B. Meissner, M. Drexel, G. Sperk, C. E. Bandtlow
Reticulon proteins in the epileptic rat brain.

P08: C. McDonald, G. Dechant, C.E. Bandtlow, M. Reindl
The presence and biological role of Nogo receptor in immune cells.

P08A: F. Kern, C.E. Bandtlow, R. Schweigreiter
Identification of Novel Interaction Partners of Nogo-A and NgR2 Using Bait- Proteins Produced in Mammalian Suspension Cell Culture

P09: I. Contarini, T. Stasyk, L. Huber, L. Hengst, C.E. Bandtlow
Cdk-5 dependent phosphorylation of NogoA promotes neurite outgrowth of cultured cerebellar granule cells.

P10: B. E. Bäumer, M. Kress, C. E. Bandtlow
Gene expression analysis in dorsal root ganglia of NgR2-deficient mice.

P11: L. Zopf, A. Schmid
The spectral sensitivity of the spider cupiennius salei: a behavioural test.

P12: L. Fenk, A. Schmid
Spatial acuity of the PM eyes in a hunting spider.

P13: M. Müller, A. Schmid
How do spiders discriminate between vertical and sloped objects?

P14: M. Fritz, R. El Rawas, S. Klement, A. Saria, G. Zernig
Social interaction increases speed of extinction of cocaine-induced place preference and prevents cocaine-induced reconditioning of cocaine seeking.

P15: C. Ullrich, M. Pirchl, C. Humpel
Effects of hypercholesterolemia on learning, cholinergic neurons, vascularization and immunoreactive markers in vivo in rats.

P16: I. Rosskothen, E. Schunk, H. Herzog, C. Schwarzer
Influence of Estrous Cycle on Explorative Behaviour of Wild-type and Prodynorphin Knockout Mice.

P17: I. Milenkovic, G. Sperk, W. Sieghart
GABA-A Receptors in Anxiety and Fear

P18: K. Streil, G.Sperk
GABA-A Receptor Subunit Expression in Temporal Lobe Epilepsy

P19: S. Mazhar, R. Herbst
The role of the receptor tyrosine kinase MuSK during nerve-independent acetylcholine receptor clustering.

P20: A. Ciuraszkiewicz , P. Scholze, S. Huck
Properties of distinct neuronal nicotinic acetylcholine receptors in their native environment.

P21: X. Simeone, P. Scholze, S. Huck
Trans-ganglionic neurotransmission in the sympathetic nervous system of mice with deletions of distinct nicotinic acetylcholine-receptor subunit genes.

P22: B. Harl, H.H. Kerschbaum
Osmoregulation and phagocytosis in murine microglia.

P23: K. Wörndl, M. Sabler, H.H. Kerschbaum
Potassium homeostasis modulates cell volume and cell death in microglia.

P24: P. Subramanyam, G.J. Obermair, S. Baumgartner, M. Gebhart, J. Striessnig, W.A. Kaufmann, S. Geley, B.E. Flucher
Activity and calcium influx regulate nuclear targeting of the calcium channel β_{4b} subunit in nerve and muscle cells.

P25: G.J. Obermair, B. Schlick, V. Di Biase, P. Subramanyam, M. Gebhart, S. Baumgartner, B.E. Flucher
Calcium channel β and α_1 subunit interactions regulate β subunit targeting and $Ca_V1.2$ membrane expression in cultured hippocampal neurons.

P26: B. Nimmervoll, V. Di Biase, B.E. Flucher, G.J. Obermair
Investigating presynaptic calcium channel function by imaging
FM dye unloading in cultured hippocampal neurons.

P27: B. Schlick, B.E. Flucher, G.J. Obermair
High complexity of voltage-activated Ca2+ channel expression patterns in mouse and cultured hippocampal neurons.

P28: C. Bavassano, C. Sailer, B. Sarg, H. Lindner, H.G. Knaus
Protein complexes associated with the pain transduction channel TRPV1

P29: O. Stundner, N. Thon, E. Haschke-Becher, S. Afazel, F.M. Wurst
Immediate changes in drug craving and appetite-regulating hormones – Ghrelin, Leptin, Adiponectin, Resistin, Insulin – in a sample of former heroin addicts receiving opioid maintenance therapy.

*P*30*:* P.K. AHNELT , C. Glittenberg, C. Schubert, D. Meisel, B.Viola, S. Binder
Design of a virtual ground-level perimeter for assessment of the role of facial topographies for human and primate visual field limits.

P31: M. Burgstaller, H. Tichy
Transduction of temporal odor patterns by ON and OFF receptor cells.

P32: N. Jährling, K. Becker, B.M. Wegenast-Braun, S. Grathwohl, D. Eicke, H.U. Dodt
Ultramicroscopy: 3D-reconstruction of Alzheimer´s Disease pathologies in intact mouse brains.

Late Posters

P32A: F. Weihmann, T. Hötzl, G. Kastberger
3-D Patterning of Social Waves in the Giant Honeybee *Apis dorsata*.

P32B: J. Lerchbacher, F. Weihmann, T. Hötzl[1], M.M. Singh, G. Kastberger
Age index defines nest topology regarding division of labour in Giant honeybees (*Apis dorsata*).

Postersession II

17th of September 2009, 16.30 – 18.00 Posters P33- P70

P33: S. Stemberger, N. Stefanova, L. Aigner, G.K. Wenning
Blood brain barrier integrity in a mouse model of MSA: towards mesenchymal stem cell therapy.

P34: S. Couillard-Despres, K. Wagner, P. Rotheneichner, K. Stadler, L. Aigner.
Role of doublecortin mRNA 3'UTR in the regulation of its expression.

P35: S.R. Chirasani, M. Krampert, R. Heuchel, L. Aigner
Role of Smad7 in regulation of adult neural stem/progenitor cell pool.

P36: K. Wagner, S. Couillard-Després, P. Rotheneichner, B. Lehner, K. Stadler, L. Aigner
Regulation of early neural differentiation marker by TGFβ1.

P37: F.J. Rivera, C. Steffenhagen, A-M. Pöhler, F. Dechant, M. Caioni, S. Couillard-Despres, U. Bogdahn, S. Kraus, O. Strauss, L. Aigner
The identity of cells grown in neurospheres: species matters.

P38: F. Hladky, R. Gehwolf, H. Tempfer, A. Hermann, T.M. Weiger, H.C. Bauer
Characterization of neural progenitor cells by means of single cell PCR, whole cell patch clamp and immunocytochemistry.

P39: N. Eberhard, A. Wagner, B. Kofler, H.C. Bauer
Presence of alarin-like immunoreactivity at different stages of murine embryonic development.

P40: S. Gaburro, O. Stiedl, S.B. Sartori, L. Czibere, R.Landgraf, N. Singewald
Altered autonomic reactivity in a psychopathological mouse model of innate trait anxiety and comorbid depression.

P41: S. Gaburro, S. Pellegrini, M. Hauschild, D. Verma, S.B. Sartori, N. Singewald
Automated evaluation of fear response in rodents: TopoWatch.

P42: S.B. Sartori, M. Fink, M. Rederstorf, A. Hüttenhofer, N. Singewald
Spatial and temporal regulation of brain-specific snoRNAs in response to an emotional challenge.

P43: M. Hauschild, S.B. Sartori, S. Gaburro, J-C. Yen, I. Milenkovic, G. Sperk, W. Sieghart, R. Landgraf, N. Singewald
Circadian modulation of fear conditioning in a mouse model of trait anxiety.

P44: D. Verma, R.O. Tasan, H. Herzog G. Sperk
Fear and anxiety after NPY deletion.

P45: M. Drexel, A.P. Preidt, E. Kirchmair, G. Sperk
Plastic changes in parahippocampal regions of the rat after kainic acid-induced epilepsy.

P46: A.P. Preidt, M. Drexel, G. Sperk
Status epilepticus induced epileptogenesis in the rat traced by video-supported EEG-telemetry.

P47: M. Hartbauer, G. Radspieler, H. Römer
Sensory coding of predator cues by insect prey under high background noise levels**.**

P48: S. Hirtenlehner, H. Römer,
Neuroethology of background-masking in acoustic.

P49: Y. Kasugai, W. Sieghart, R. Shigemoto, F. Ferraguti
Structural features and subunit composition of GABAergic synapses in basolateral neurons of the amygdale.

P50: B. Kerschner, K. Harvey, L. Saiepour, C. Fuchs, W. Sieghart, S. Moss, V. Tretter
Characterization of $GABA_A$ receptor alpha3 subunit interaction with gephyrin.

P51: A. Deréky, I. Sarto-Jackson, J. Ramerstorfer, M. Ernst, W. Sieghart
Engineering of a benzodiazepine binding site into $GABA_A$ receptors composed of αβ subunits.

P52: S. Radner, I. Sarto-Jackson, W. Sieghart
Expression and purification of $GABA_A$ receptor extracellular domains.

P53: J. Ramerstorfer, R. Furtmüller, I. Sarto-Jackson, W. Sieghart, M. Ernst
Identification of a novel binding site at $GABA_A$ receptors.

P54: R. Irschick, T. Trost, L. Klimaschewski
Fibroblast growth factor receptor 1 trafficking in glial cells.

P55: Z. Muneer, J. Berger, H. Werner, K-A. Nave, S. Forss-Petter
Trangenic overexpression of ABCD2 in microglia / macrophages in X-linked adrenoleukodystrophy mice.

P56: R. Cervenka, T. Zarrabi, P. Lukacs, X. Koenig, K-H. Hilber, H. Todt
In the voltage-gated Na channel double mutant gating perturbation analysis reveals a high conformational stability of the domain IV S6 segment.

P57: P. Geier, M. Lagler, S. Böhm, H. Kubista
Role of L-type voltage-gated calcium channels in the excitability of primary hippocampal neurons - Part I (modulation of depolarisations).

P58: M. Lagler, P. Geier, S. Böhm, H. Kubista
Role of L-type voltage-gated calcium channels in the excitability of primary hippocampal neurons - Part II (afterpotentials).

P59: S. Krahwinkler, T.M. Weiger, A. Hermann
Effect of hydrogen sulfide (H_2S) on ion currents of *Helix pomatia* neurons.

P60: G.F. Sitdikova, T.M. Weiger, A. Hermann
Effect of hydrogen sulfide on the activity of calcium-activated potassium channels of rat pituitary tumor cells.

P61: A.G. Handlechner, T.M. Weiger, V. Kainz, A. Hermann
Acetaldehyde countervails the augmenting action of ethanol on calcium-activated potassium (BK) channel activity in pituitary (GH3) cells.

P62: C. Brunnemann , T.M. Weiger, C. Langelüddecke, V. Kainz, M. Jakab, S. Schmid, M. Ritter, A. Hermann
Ethanol induces cell volume changes and depolarizes the membrane potential of pituitary tumor cells (GH3).

P63: B. Kofler, R. Lang, I. Rauch, O. Odusanwo, D. Bovell
The galanin-receptor subtype 3 mediates chloride secretion in the human sweat glandcell line NCL-SG3.

P64: S. Wintersteller, I. Rauch, S. Holzmeister, J. Hahnhaußen, G. Kollarz, E.S. Fernandes, S.D. Brain, W. Sperl, K. Emmanuel, B. Kofler
The expression of galanin and substance P is dramatically increased in the lung of septic mice.

P65: B. Pletzer Belinda, M. Kronbichler, H-C. Nuerk, H.H. Kerschbaum
Gender-dependent differences in the BOLD-response to arithmetic tasks.

P66: M. Pirchl, C. Ullrich, C. Humpel
Effects of hyperhomocysteinemia on learning, cholinergic neurons, vascularization and immunoreactive markers in vivo in rats**.**

P67: B. Thauerer, S. z. Nedden, V. Podhraski, G. Baier-Bitterlich
p42/44 MAPK supports adenosine receptor-mediated signaling in purine nucleoside-induced neuroprotection of hypoxic neuronal cells.

P68: J. Marksteiner , G. Kemmler, E.M. Weiss, G. Knaus, C. Ullrich, S. Mechteriakov, H. Oberbauer, S. Auffinger, J. Hinterhölzl, H. Hinterhuber, C. Humpel
Five out of 16 plasma signaling proteins are enhanced in plasma of patients with mild cognitive impairment and Alzheimer´s disease.

P69: M.L. Pretterklieber, R. Pflug, A. Frank
Target of efferent neurons of the superior laryngeal nerve in the rat.

P70: C. Schwarzer, W. Wittmann, E. Schunk, I. Rosskothen, S. Gaburro, N. Singewald, H. Herzog
Endogenous dynorphin in emotional control and stress response.

ABSTRACTS 1

ORAL

PRESENTATIONS

O1: **Opening lecture I**

How Experience Changes the Circuitry of the Brain

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Abstract:

One of the most fundamental properties of the brain is its ability to adapt rapidly to environmental changes. This is achieved mainly by changes in the connectivity between individual nerve cells. Synapses can be modulated in their strength by a variety of different mechanisms. We have used traditional *in vitro* systems to investigate a number of these mechanisms, ranging from homeostatic control of synaptic efficacy to morphological manifestations of synaptic strengthening or weakening, on both excitatory and inhibitory cells. Yet, while we are beginning to understand the cellular mechanisms underlying synaptic changes, it is important to consider the functional implications of synaptic plasticity in the intact brain. We are therefore applying new imaging methods to investigate the effects of experience on synaptic changes in cortical circuits. In particular, *in vivo* two-photon microscopy has enabled us to study morphological as well as functional plasticity at the level of individual neurons in the intact neocortex. Experiments, in which we quite literally observe how sensory deprivation alters cortical circuitry, allow us to make detailed statements on how information about the environment is reflected in brain circuits. Such studies are beginning to close the gap between traditional cellular and systems studies, and they will enable us to obtain a much more comprehensive understanding of the phenomenon of synaptic plasticity and its role in cortical function and ultimately behaviour.

O2: **Plenary Lecture II**

Olfactory coding in insects

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Odors are coded in combinatorial patterns of neural activity. At a first glance, the system impresses with its simplicity: a family of olfactory receptors (approx. 50 in the fruit fly) converges onto the same number of olfactory glomeruli. This arrangement creates a virtually infinite number of possible activity combinations, which are relayed to higher-order processing centers via uniglomerular projection neurons (corresponding to the vertebrate mitral/tufted cells). At a second glance, however, the picture becomes more complex: a family of diverse local neurons creates an intricate network of connectivity across glomeruli. Thus, the response of a projection neuron depends not only on the receptor neuron input to its glomerulus, but also on the input to other glomeruli. As a result, the "transfer function" of a glomerulus (i.e. the response properties of projection neurons as a function of receptor neuron input) are diverse: for a given odor, projection neurons might follow the receptor neuron input (linear transfer function) or respond less (narrowing function), for another odor they might respond even without receptor neuron input (broadening function), and for yet another odor the relationship may be complex.

I will review recent data from fruit fly and honeybee neurophysiology that investigates the information flow along this olfactory network. "Transfer functions" are measured using calcium imaging on receptor neurons, local neurons and projection neurons in *Drosophila* using genetically encoded calcium probes.

Next, I will ask how this information is processed at the level of the next brain structure, i.e. the mushroom bodies. Here, coding becomes increasingly sparse and temporally more distinct, i.e. activity is strongest at odor pulse onset and at odor pulse offset.

The data answers questions and raises new ones about how odor concentration and odor identity is encoded in the nervous system, and how an animal can navigate efficiently in a turbulent environment.

O3: **Arachnid mechanoreception: I. Micromechanics of spider air flow sensors**

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Apart from using their substrate vibration sense, spiders orient towards prey and avoid predators by detecting air flows with extreme sensitivity. Torques of 10^{-15} Nm suffice to deflect their hair-like air flow sensors above firing threshold (0.1°–0.01° (1)).

The outstanding sensitivity of the trichobothria is to a considerable degree based on their mechanical properties: tiny mass, elaborate lever system (scaling up stimulus force by a factor of 245 ± 36 (N=5)), mechanical tuning related to boundary layer thickness, and a delicate suspension of the sensory hair.

To examine the dynamics of their suspension trichobothria were deflected at angular velocities from 0.0004 to 0.26 rad s^{-1} using the cantilever in an atomic force microscope. Surprisingly, the torque needed to deflect the hair shaft dropped significantly at deflection velocities below 0.05 rad s^{-1} indicating a strong viscoelasticity of the hair suspension. A three-parameter viscoelastic standard solid model derived from the experimental data yielded two spring parameters $S_1=2.9\times10^{-11}$ Nm rad^{-1} and $S_2=2.8\times10^{-11}$ Nm rad^{-1}, and a damping parameter $R=1.5\times10^{-12}$ Nm s rad^{-1} (2).

The viscoelasticity of the trichobothrium´s suspension facilitates the start of hair motion and therefore the detection of the rapid changes characteristic of biologically relevant air flow signals. It thus works to promote the electrophysiologically measured highly phasic response character of the sensory cells.

Supported by the program BioSenSE of DARPA to FGB.

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O4: **Arachnid mechanoreception: II. Air flow guiding the spider to its prey**

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Spiders use highly sensitive hairs (trichobothria) on all of their eight legs to detect air motions generated by flying prey. They successfully jump towards a flying fly which they are obviously able to detect and to localize (Barth 2002). We analyzed the flow field generated by a freely flying blowfly (Calliphora erythrocephala) close to the air flow sensors of Cupiennius salei using digital particle image velocimetry in order to learn about its potential use for prey localization.

When approaching, the blowfly induces an exponentially increasing airflow signal (flow velocity) with comparatively little fluctuation (phase I) detected by the spider while the fly is still some 4 cm away. The degree of fluctuation of the phase I airflow increases linearly with the fly's altitude. Differences in the time of arrival of the fly signal at its different legs likely inform the spider about the direction to the prey. Phase I of the airflow is followed by the much more fluctuating phase II which corresponds to the wake below and behind the fly indicating to the spider that its prey is now within reach and actually triggering the jump. In theory then the spider should be able to derive information on the fly's position and the proper timing of its jump from the clues contained in the airflow. Behavioral experiments in which different components and characteristic features of the air flow were used as stimuli strongly support this view. The horizontal velocity of the approaching fly is reflected by the time of arrival differences of the signal at different legs and also by the steepness of the exponential increase of velocity in phase I.

Supported by DARPA BioSenSE AFOSR Grant # FA9550-05-1-0459 to FGB.

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O5: Arachnid mechanoreception: III Airflow-sensing in a scorpion

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Like in crickets and spiders (Barth, 2002) airflow stimuli play an important role in the prey capture, orientation (Linsenmair, 1968) and defence behavior of scorpions (*Smeringurus mesaensis*). Blinded scorpions are still able to detect and capture insect prey (*Galleria*) in flight, tethered by a thread and about 2 cm above the substrate (of 27 individuals, 17 showed prey-capture movements, including 13 successive trials). According to preliminary behavioral experiments, the distance at which the grasping movement started decreased from an average of 36 +/- 9 to 20 +/- 8 mm, after ablation of all trichobothria; likewise seemingly involuntary contacts between scorpion and prey increased from 31% to 86% of all prey capture attempts.

The main question now is how the scorpion manages to detect, recognize and localize airflows of different origin in a noisy environment and how single sensors and arrays of them are adapted to this task. We expect that a signal's frequency contents are used for signal recognition and that differences in the temporal and spatial stimulation of sensor arrays contain the information needed for the detection of stimulus direction.

More than half of the 48 trichobothria on each pedipalp of *S. mesaensis* are located on external surfaces facing the approaching airflow. In adult scorpions short trichobothria (length: 313 +/- 22 µm) with small basal diameter (6.6 +/- 0.9 µm) can be distinguished from long ones (1259 +/- 104 µm) with larger basal diameter (9.8 +/- 0.6 µm). Mechanical frequency tuning is broad in both cases with a peak velocity response at 600 Hz for short and at 300 Hz for long trichobothria. According to detailed measurements of the s/d ratio (s distance between trichobothria, d diameter of the hairshaft) the sensors in an array function independently from each other without being mechanically coupled due to the air's viscosity.

Supported by the program BioSenSE of DARPA to FGB.

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O6: Matched filters in the auditory system of crickets.

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Many acoustic communication systems involve the production and transmission of specific sound frequencies. The "matched filter hypothesis" (1) argues that receivers should gain an advantage from being selectively tuned to these frequencies, since the match between the sensitivity of their auditory system and the energy spectrum of the sender´s vocalisation would maximize the signal-to-noise-ratio for reception. Highly tuned receivers, however, are a challenge for senders, since any deviation from the best frequency in tuning would render their signal less less attractive. We studied such a system in crickets, where males call at a characteristic carrier frequency (CF), and females orient to the male call. The filter is represented by an identified auditory interneurone (AN1-neuron), tuned to a particular frequency. Indeed, when we studied the phonotactic behaviour (in a two-choice paradigm; 5 kHz vs. alternative CFs) and the tuning of this neuron in the same females, we found a strong correlation between behavioural preference and tuning of the interneurone.

Due to biophysical reasons, directionality in crickets is also sharply frequency tuned, so that reasonable interaural intensity differences (IIDs) for localization are provided for only a narrow range of frequencies (2). By examining both filters in the same individuals we show that the frequency providing strongest stimulation for the auditory system may provide only poor directional cues. On average there is a discrepancy of 400 Hz between the two frequency optima; these two optima are significantly different (Whitney rank sum test, $p<0{,}001$; $N=20$). In a comparative approach with four species of field crickets we further show that the mismatch between both filters exists in three of the four species, and can amount to about 1 kHz in single individuals. We discuss these findings in relation to the separate evolution of both filters.

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O7: Impairment of adult neurogenesis in a psychopathological mouse model of trait anxiety and comorbid depression: effect of antidepressants

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Evidence has been provided linking neurogenesis to mood disorders. Notably, it has been shown that chronic experimental stress resulting in enhanced depression-like behavior decreases neurogenesis in the dentate gyrus (DG), while antidepressants reverse these stress-induced effects. However, in most studies "normal" animals reflecting physiology rather than pathophysiology are used. Therefore, we aimed to investigate neurogenesis in the DG of a mouse model of high-trait anxiety and comorbid depression (HAB), which mimics important features of human psychopathology, and their normal anxiety/depression (NAB) controls. Compared to NABs, HAB mice displayed enhanced depression-like behavior in the forced swim test and showed a lower number of BrdU-positive cells (5-bromo-2'-deoxyuridine) in their DG. This was observed both at 15 and 42 days after BrdU injections, indicating reduced cell proliferation and survival in HABs, respectively. Interestingly, in HABs compared to NABs more newborn cells migrated to the outer zone of the granular cell layer. Using double-labeling of BrdU and c-Fos, a marker for neuronal activation, we found evidence that some of the newborn cells in the survival paradigm were functionally integrated in NABs, while no such evidence was so far found in HABs. To gain insight into altered neurochemistry, possibly contributing to the reduced neurogenesis in HABs, we performed gene expression studies of substrates known to be involved in cell maturation. First results point towards a lower abundance of cyclin-dependent kinase 5 (Cdk5) and brain-derived neurotrophic factor (BDNF) in the DG of HAB compared to NAB mice. Finally, although chronic treatment with the selective-serotonin reuptake inhibitor (SSRI) fluoxetine reduced the depression-like behavior exclusively in HABs, it did not alter cell survival or functional integration of new born neurons in the DG. Taken together, the enhanced depression-like behavior in a psychopathological animal model is accompanied by decreased hippocampal neurogenesis as well as BDNF and CdK5 expression. Since the antidepressant effect of fluoxetine is discerned from neurogenesis, it is suggested that mechanisms other than adult neurogenesis underlie the therapeutic action of SSRIs in the HAB model, which, however, has to be confirmed by using additional SSRIs. Thus, the present data do not support the idea that neurogenesis is a prerequisite for therapeutic action common to all antidepressants. Moreover, this finding highlights the need of psychopathological animal models in order to investigate molecular mechanism of current effective antidepressants. The present work is supported by FWF DK SPIN W1206-B05.

O8: 129S1 mice – a novel model for studying impaired extinction in anxiety disorders

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Mechanisms of improving the impaired extinction of learned fear responses in patients with phobias, panic or post-traumatic stress disorder are not well understood. We have recently identified that 129S1/SvImJ (129S1) mice display greatly impaired fear extinction which provides an ideal tool to study mechanisms involved in reversal of impaired fear extinction in detail. 129S1 mice were subjected to cued fear conditioning and subsequent extinction learning and retrieval using C57BL/6N mice as a reference strain. Zinc (Zn)-deficiency was investigated as a treatment tool to reverse the impaired extinction learning in 129S1 mice. Quantification of immediate-early gene (IEG) expression following extinction recall was assessed to gain insight into neural correlates associated with successful improvement of impaired extinction. Results showed that non-extinguishing 129S1 displayed enhanced IEG expression in the central amygdala and medial paracapsular intercalated cell mass and reduced IEG expression in the infralimbic cortex, lateral amygdala, basolateral amygdala and intercalated cell mass nucleus following extinction recall. Zn-deficiency greatly facilitated within-session extinction learning in 129S1 mice and enhanced extinction retention when assessed 24 hr later. Successful extinction learning in 129S1 mice was associated with enhanced IEG response in the infralimbic cortex and amygdala regions including basolateral, lateral central nucleus and intercalated cell mass nucleus. Furthermore, reduction in IEG expression was observed in pre-limbic and dorsal insular cortices as well as amygdala regions including medial paracapsular intercalated cell mass and the medial subdivision of the central nucleus. No modulation of IEG responses by Zn was observed in motor, cingulate, perirhinal and entorhinal cortices, lateral paracapsular intercalated cell mass and cortical amygdala regions in mice indicating the regional specificity of the observed changes. In conclusion, we have demonstrated that Zn-deficiency is a novel and effective tool to induce extinction learning in an animal model of greatly impaired fear extinction. Furthermore, successful extinction learning in 129S1 mice was associated with normalisation of dysfunctional neuronal activity changes in specific regions of a cortico-limbic circuit important in fear extinction mechanisms.

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O9: **Reorientation from cocaine towards social interaction: Functional brain mapping, immediate early genes, and brain area-targeted pharmacotherapy**

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In a conditioned place preference paradigm in Sprague Dawley rats, a single 15 min social interaction during extinction was able to reverse place preference induced by 15 mg/kg intraperitoneal cocaine (see Fritz et al. 2009, poster presentation at the ANA 2009 meeting). Additionally, social interaction during extinction fully prevented cocaine-induced reconditioning of cocaine CPP (one exposure to 15 mg/kg i.p. cocaine in the previously cocaine-paired chamber, CPP test 24 h later). Finally, even if cocaine conditioning was continued after acquisition of cocaine CPP, the compartment associated with social interaction was preferred over the cocaine-associated compartment. Immunohistochemistry of zif268, an immediate early gene the activation of which is considered important in animal models of addiction, indicate that social interaction during cocaine extinction leads to differential activation of several brain areas known to play critical roles in motivated behaviors and conditioning of drug-associated stimuli. Thus, our novel experimental paradigm yields an impact of social interaction on drug seeking and salience of drug-associated contextual stimuli previously thought unlikely and suggests that social interaction may be a fundamental factor in preventing relapse to cocaine addiction. We intend to investigate which brain-area targeted pharmacotherapies can further shift the behavior of the recovering addict away from the abused drug towards therapeutically beneficial social interaction such as psychotherapy or sociopsychiatric interventions. Supported by FWF P18787-B05, MFF 154, Swarovski-Foundation, and the VEPPP.

O10: **THE ROLE OF THE IMMUNE SYSTEM IN A CHRONOBIOLOGICAL MODEL OF DEPRESSION IN MICE**

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Increasing evidence proposes an involvement of the circadian system in mood disorders Sleep disturbances represent a prominent feature of depression and depressed patients show marked diurnal mood swings with the most severe symptoms occurring typically in the morning. Although these associations are well known and documented, the biological mechanisms mediating this connection are only beginning to be understood. Alterations of the biological clock at the molecular level could induce neurobiological disturbances leading to or presenting the depressed condition such as the preponderance of an inflammatory state known as a prominent feature in depression. It has been proposed that proinflammatory cytokines might act as neuromodulators and as such be at the core of the behavioral and biochemical features of depressive disorders.

It has recently been shown that several weeks of constant darkness (DD) lead to dysregulation of the circadian sleep-waking rhythm and induce depression-like behavior in rats. Here we use this model in mice and investigate the effect of the chronobiologically induced depressive state on the expression of proinflammatory markers (circulating and locally in the hippocampus) and compare it to the behavioral and neurochemical phenotype of a model of jet-lag (8 hours phase advance). We find that DD, but not jet-lag, induces depression-like behavior and that this is associated with increased levels of IL-6 in the plasma and the hippocampus and IL-1R1 in the hippocampus whereas circulating IL-1beta is significantly elevated in the jet-lag model. Moreover we find that DD lowers neurogenesis in the dentate gyrus of the hippocampus and that this is not due to reduced neurotrophic support through BDNF.

In summary, we describe that in mice a depressive state can be induced by prolonged periods in DD and that this is paralleled by activation of specific elements of the immune system and reduced hippocampal neurogenesis. We for the first time propose activation of the immune system in a chronobiological model of depression.

O11: Stable membrane expression of $Ca_V1.2$ calcium channels is independent of activity related rearrangement of post synaptic scaffold proteins

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In neurons L-type calcium currents contribute to synaptic plasticity and to activity-dependent gene regulation. The subcellular localization and incorporation of $Ca_V1.2$ into macromolecular signaling complexes is crucial for specificity and efficacy of these processes. Here we examined the role of postsynaptic scaffolds including A-kinase anchoring proteins (AKAPs) or PDZ-proteins in targeting and anchoring of $Ca_V1.2$ in cultured hippocampal neurons. Double-immunofluorescence labeling of hippocampal neurons transfected with external HA epitope-tagged $Ca_V1.2$ demonstrated that clusters of membrane-incorporated $Ca_V1.2$-HA were colocalized with AKAP79/150 but not with PSD-95 in the spines and shafts of dendrites. To disrupt the interactions with these scaffold proteins, we mutated known binding sequences for AKAP79/150 and PDZ proteins in the C-terminus of $Ca_V1.2$-HA. Unexpectedly, the distribution pattern, the density, and the fluorescence intensity of clusters were similar for wild-type and mutant $Ca_V1.2$-HA, indicating that targeting is independent of protein-protein interactions with the C-terminal leucine zipper and VSNL. In agreement, brief treatment with NMDA (a chemical LTD paradigm) caused the degradation of PSD-95 and the redistribution of AKAP79/150 and alpha-actinin from dendritic spines into the shaft, without a concurrent loss or redistribution of $Ca_V1.2$-HA clusters. Thus, in the postsynaptic compartment of hippocampal neurons $Ca_V1.2$ calcium channels form signaling complexes apart from those of glutamate receptors and PSD-95. Their number and distribution in dendritic spines is not altered upon NMDA-induced disruption of the glutamate receptor signaling complex, and targeting and anchoring of $Ca_V1.2$ is independent of its interactions with AKAP79/150 and PDZ proteins.

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O12: **L-type voltage-gated calcium channels as anti-epilept(ogen)ic drug targets**

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L-type voltage gated calcium channels (LTCCs) play several crucial roles in the nervous system. For example, LTCCs were shown to be involved in the control of neuronal excitability, synaptic plasticity and gene expression. These mechanisms are altered in epileptic tissue and are thought to contribute to epileptogenesis. Hence, LTCCs are interesting targets for epileptic and anti-epileptic therapy. However, their role in epilepsy, whether LTCCs enhance or reduce epileptiform/epileptogenic activity, remained unclear. To address this issue, electrophysiological experiments were performed on neurons of primary hippocampal networks and the dihydropyridines BayK 8644 (LTCC agonist) and isradipine (antagonist) were applied to determine the role of LTCCs in epileptiform activity (EA). In the majority of the neurons, EA was enhanced by BayK and reduced by isradipine. However, in a subset of neurons, the LTCC agonist lead to a reduction of EA. Interictal-like discharge activity, so called paroxysmal depolarisation shifts (PDS), was not seen under any of these conditions under control (solvent only), but were repeatedly induced by BayK and blocked by isradipine. We used incremental current injections of various length in the presence of TTX to investigate the direct and indirect effects of LTCC-mediated Ca^{2+}-influx on membrane voltage. Various de- and hyperpolarising responses were observed. This diversity in LTCC-mediated effects was analysed in detail and is presented in the contributions by Geier et al. and Lagler et al. Despite the apparent heterogeneity in LTCC coupling to Ca^{2+} dependent channels, the early effect of LTCC activation on depolarising events was always an enhancement, irrespective of whether hyperpolarisation, oscillation or depolarisation predominated in the later part of the response. Hence, the role of LTCCs in short depolarisations appears rather uniform, whereas opposite effects of LTCC activity occur in the course of longer lasting neuronal excitation. Our results provide an explanation for the controversy surrounding LTCCs as useful anti-epileptic drug targets. Moreover, PDS were recently implied as important elements in epileptogenesis, appearing prior to actual seizures, probably altering neuronal circuits by causing repetitive, synchronised pulsative cytosolic Ca^{2+} rises. In the framework of this hypothesis our data point to a potential use of LTCC inhibitors to counteract PDS, and hence epileptogenesis (supported by FWF grant P19710).

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O13: **A novel Rab5 guanine nucleotide exchange factor acts as regulator of MuSK signaling at the neuromuscular synapse**

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The neuromuscular synapse is a vital structure and serves as model system for synapse formation in general, due to its bigger size, simplicity and easier accessibility compared to CNS synapses. It is formed by a motor axon terminal contacting a single muscle fiber and covered by a Schwann cell. During formation of the neuromuscular synapse the nerve-derived factor Agrin binds to its receptor Lrp4. This in turn activates the muscle specific kinase MuSK and elicits a downstream signaling cascade leading to the clustering of acetylcholine receptors (AChRs) to synaptic sites.

With the aim to resolve mechanisms that regulate MuSK signaling we screened for new interaction partners of MuSK. We have isolated a novel protein - currently termed Cl6 - as a specific interaction partner of MuSK. Cl6 is closely related to the family of RIN proteins that have been shown to play a role in early endocytosis. Most importantly, RIN proteins act as guanine nucleotide exchange factors (GEFs) for Rab5 via their Vps9 domain.

We confirmed the specific interaction of MuSK and Cl6 by co-immunoprecipitation and GST-pulldown assays. In addition, we found that Cl6 preferably interacts with dominant-negative Rab5 via its Vps9 domain supporting the notion that Cl6 functions as Rab5 GEF. Ongoing in vitro experiments concentrate on the functional analysis of Cl6 with particular emphasis on its potential role as a protein involved in MuSK trafficking. Using a muscle cell culture system we can model postsynaptic differentiation that occurs during synapse formation in vivo. We studied changes in postsynaptic structures upon down regulation of Cl6 expression in these cells. A Cl6 knock-down by RNA interference disturbs Agrin-induced AChR clustering as the overall cluster size is markedly reduced and the number of microclusters increases. These results prompt us to propose a model whereby Cl6 as novel Rab5 effector modulates MuSK signaling by regulating its endocytosis.

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O14: **Role of ryanodine receptors in effects of hydrogen sulfide on transmitter release at frog neuromuscular junction**

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Hydrogen sulfide (H_2S) is well known as a toxic gas can be generated in many types of mammalian cells. Two pyridoxal-5-phosphate-dependent enzymes - cystathionine β-synthase or CBS and cystathionine γ-lyase or CSE - are responsible for endogenous production of H_2S in mammalian tissues that use L-cysteine as the main substrate. Functionally, H_2S has been implicated in the induction of hippocampal long-term potentiation, brain development and blood pressure regulation. It is hypothesized that H_2S is the third endogenous signaling gasotransmitter, besides nitric oxide and carbon monoxide. Mechanisms of H_2S action in neuromuscular synapses are unknown.

In the experiments at the frog neuromuscular preparations using extracellular recordings of motor nerve ending responses and end-plate currents (EPCs) the role of intracellular Ca^{2+}-channels in the effects of H_2S on evoked transmitter release have been investigated. Sodium hydrogen sulphide (NaHS), donor of H_2S, in the concentration 1 μM increased the evoked transmitter release from the motor nerve endings. The amplitude of EPCs rose up to 123.94±3.43% ($n=6$; $p<0.05$) of control by 10 minutes of application. Caffein - activator of ryanodine receptors in concentration 3 mM induced the increase of EPC amplitude by 151.76±6.14 ($n=8$; $p<0.05$) of control. NaHS in this condition did not change transmitter release. It is known that ryanodine in small concentrations activates ryanodine receptors. Application of ryanodine in concentration 0.5 μM on neuromuscular junction induced the increase of EPC amplitude by 116.01±6.88% ($n=4$; $p<0.05$) of control and prevented NaHS effect on transmitter release.Dantrolen – inhibitor of ryanodine receptors in concentration 25 μM decreased the EPC amplitude by 71.96±5.95 % ($n=5$; $p<0.05$) of control. NaHS did not induce any significant changes in transmitter release in these conditions.

Thus, activation or inhibition of intracellular Ca-channels prevented the increase of transmitter release by NaHS. It was concluded that H2S increase the evoked transmitter release in frog neuromuscular junction by activation of ryanodine receptors of endoplasmic reticulum of motor nerve ending and increasing of intracellular calcium concentration.

This study was funded from the Russian Foundation of basic research N 09-04-00748 and by the Russian Ministry of Education N 2.1.1/786.

O15: Plenary Lecture III

Stemness in the Adult Brain: Yes They Can!

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The last two decades of neuroscience were revolutionized by the fact that stem and progenitor cells exist in the adult brain throughout lifetime of mammals including humans. At least within the two classical neurogenic regions, the dentate gyrus in the hippocampus and the subventricular zone / olfactory bulb system, the stem and progenitor cells generate new neurons spontaneously and thus provide a continuous source for cellular homeostasis. In addition, it becomes more and more evident that progenitors outside of the typical neurogenic regions contribute to the generation of new cells in the brain. Hippocampal neurogenesis is tightly linked to memory function, the functional role of subventricular zone neurogenesis is less clear. The fact that neurogenesis can be modulated by molecular and cellular means, makes it an attractive target for neuro-regenerative approaches. This presentation will review the current knowledge on neural stem cells of the adult brain. It will touch basic science and clinical aspects of adult neurogenesis.

O16: **Stem cell quiescence in the hippocampal neurogenic niche is associated with elevated TGF-beta signaling in an animal model of Huntington's disease**

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The adult neural stem cell niche responds to pathological stimuli with alterations in cellular proliferation, differentiation, integration and survival. The molecular cues regulating the niche in neurodegenerative diseases are mostly unknown. Here, we addressed the hippocampal neural stem cell niche in a transgenic rat model of Huntington's Disease (tgHD). This HD model develops cognitive deficits during the period of 8 months and 12 months of age implying hippocampal dysfunction. We encountered a disease progression-associated impairment in hippocampal progenitor proliferation that was paralleled by a gain in the pool of BrdU-label retaining, Sox-2 positive but PCNA negative quiescent stem cells. Gain of quiescent stem cells was at the expense of CREB-mediated neuronal differentiation and survival. As we recently demonstrated that elevated levels of TGF-beta1 impair neural progenitor proliferation, we investigated TGF-beta signaling in the tgHD hippocampus. Phospho-Smad2, an indicator of TGF-beta signaling, was present in Sox2/GFAP expressing neural stem cells of the subgranular zone in tgHD rats and in TGF-beta1 infused WT animals, while it was absent in WT subgranular stem cells. In summary, we present stage-specific alterations in neurogenesis in the tgHD rats and associate these changes with elevated TGF-beta signaling. TGF-beta1 might thus be crucial modulator of neurogenesis in HD pathology.

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O17: MODIFICATION OF FIBROBLAST GROWTH FACTOR RECEPTOR 1 TRAFFICKING PROMOTES AXON REGENERATION

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Fibroblast growth factors (FGFs) and their cognate receptors (FGFRs) are involved in a variety of biological processes in the nervous system including axon growth. FGFR1, -2 and -4 are expressed in adult dorsal root ganglion neurons with FGFR1 being expressed at the highest level. FGF-2 is up-regulated in response to a nerve lesion and promotes axonal elongation of pre-lesioned neurons via binding to FGFR1 which activates the Ras/ERK and the PI3K/Akt pathway.

We analyzed the effects of FGFR1-overexpression combined with inhibition of receptor endocytosis or lysosomal receptor degradation on adult axon growth. In sensory neurons, FGFR1 overexpression enhanced FGF-2-induced axon growth which was further increased by inhibition of endocytosis or by lysosomal inhibition of receptor degradation. The lysosomal inhibitor leupeptin increased co-localization of the receptor with lysosomal markers and enhanced receptor membrane levels similar to an inhibitor of endocytosis (MßCD). Moreover, FGFR1 overexpression enhanced FGF-2-induced activation of ERK and Akt, which was further increased by inhibition of endocytosis.

Our data indicate that inhibitors of endocytosis or of lysosomal protein degradation promote growth factor dependent axon regeneration by adult peripheral neurons in vitro. The positive effects of FGFR1 overexpression and interference with receptor trafficking demonstrate the significance of FGFR signaling not only for developmental but also for adult regenerative axonal growth.

O18: MRI Depiction and Quantification of Blood-Spinal-Cord-Barrier Permeability after Spinal Cord Contusion Injury

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Objectives: The Blood-Spinal-Cord-Barrier (BSCB) represents a selective physiologic barrier that provides a stable microenviroment within the neuronal tissue. Spinal cord injury (SCI) causes a BSCB breakdown, which results in increased capillary-permeability for plasma molecules that increase neuronal damage as well as for intravenously administered neuroprotective drugs. Here we used contrast enhanced MRI to determine evolution and duration of BSCB-breakdown after standardized SCI and define the "therapeutic window" for this injury model.

Materials/Methods: In male Sprague-Dawley rats, a laminectomy was carried out at TH11 and a contusion injury was inflicted, using the IH® Impactor with a force of 150kdyn. Rats were divided into groups with different observation times: 0h (n=8); 24h, 72h, 4d, 5d, 6d, 10d (n=5 each). At the end of the observation time each rat received an intravenous injection of 0.8ml/kg gadopentetate dimeglumine (Magnevist®, Bayer HealthCare). Rats were euthanized 10 minutes (0h group, n=3) or 1 hour after contrast agent administration and MRI was performed on a 3Tesla scanner.

Results: Pronounced signal enhancement at the injury epicenter was measured after observation periods up to 5 days, gradually decreasing in cranial and caudal direction. After 6 days or later, no signal enhancement was visible. When euthanization and MRI were performed 10 minutes after contrast agent application, the measurement revealed lacking signal enhancement at the lesion site, whereas adequate distribution of contrast agent within the neuronal tissue and strong signal enhancement could be observed 1 hour after contrast agent application.

Conclusion: The inflicted SCI increases BSCB-permeability for 5 days. Delayed dispersion of the contrast agent within the neuronal tissue has to be considered.

O19: **The role of TLR4 on α-synuclein induced neurodegeneration in a transgenic mouse model of multiple system atrophy.**

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Multiple system atrophy (MSA) is a neurodegenerative movement disorder manifesting with parkinsonism, cerebellar, autonomic, and pyramidal dysfunction in any combination. Its pathogenesis remains largely unknown. Oligodendroglial cytoplasmic inclusions (GCIs) are observed throughout the cortico-striato-pallido-cortical loops and may contribute to neuronal loss. Several efforts have been made in the last decade to model the neuropathology of MSA. We have recently described a transgenic mouse model that reproduces key features of glial and neuronal pathology present in human MSA. More recently, we observed toll-like receptor 4 (TLR4) up-regulation in microglia of transgenic MSA mice as well as in human MSA. The aim of the present study was to establish the role of TLR4 for the neurodegeneration induced by oligodendroglial α-synucleinopathy.

For this experiment we used (PLP)-αSYN mice with normal TLR4 expression (αSYN,$TLR4^{+/+}$) and (PLP)-αSYN mice with deletion of all 3 exons of the TLR4 gene (αSYN,$TLR4^{-/-}$). Genotype was determined by tail clip PCR. Mice at the age of 6 months were utilized. The animals were randomized into groups and underwent motor behaviour evaluation by flex field activity test and pole test. Finally brains were fixed and neuropathological analysis was performed.

Locomotor performance was more severely impaired in αSYN,$TLR4^{-/-}$ compared to αSYN,$TLR4^{+/+}$ mice. The more pronounced locomotor deficits in αSYN,$TLR4^{-/-}$ mice correlated with augmented loss of dopaminergic SNc neurons, accompanied by reduced microglial and astroglial activation but increased density of GCI-like aggregates. The effects of TLR4 deficiency on αSYN induced degeneration were specific for SNc and not observed in striatum. Further studies will be required to define the pathogenic cascade underlying these effects and their implication for the development of novel therapeutic approaches for MSA.

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O20: **Plenary Lecture IV**

Mechanisms of Neurodegeneration and Regeneration During Alcohol Dependence and Recovery.

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Human alcoholics have difficulty controlling their drinking and have smaller brain volumes than moderate drinkers. Binge drinking models in rats and mice were used to investigate the impact of high alcohol intake on adolescent and adult brain. Binge drinking doses of alcohol were found to persistently increase neuroinflammatory gene expression, to increase oxidative stress, to cause neuronal death and loss of neurogenesis. Adolescent brain was found to have high levels of neurogenesis and to be more sensitive to frontal cortical neurodegeneration and loss of hippocampal and frontal neurogenesis. The P rat, a genetic model of alcoholism, was found to have greater binge neurodegeneration suggesting genetic sensitivity to neurodegeneration. Neuronal death and loss of neurogenesis correspond with increased glial NFκB transcription and decreased pCREB. Post-Mortem human alcoholic brain studies support the validity of the animal models. Binge drinking models in rats and mice cause persistent deficits in reversal learning, consistent with frontal cortical dysfunction that could contribute to loss of control over drinking. Chronic alcohol consumption and loss of neurogenesis are also associated with increasing negative affect, e.g. depression-like behavior, a comorbidiy often found in alcoholism.

In human alcoholics, abstinence and recovery are associated with brain growth including shrinkage of the ventricles and expanded grey matter as well as a return of cognition. Models of alcohol dependence followed by abstinence find bursts of neurogenesis and glial genesis that could underlie regrowth of the brain. Brain regrowth during abstinence could contribute to the return of brain function. Exercise, as modeled by mice with a running wheel, increases neurogenesis and blocks alcohol inhibition of neurogenesis.

These studies suggest that alcohol induced neurotoxicity causes dysfunctional degeneration contributing to the progressive loss of control in alcohol dependence as well as changes in mood. Alcohol inflammatory neurodegeneration may contribute to other neurodegenerative diseases. Exercise and abstinence stimulate brain growth, likely through enhanced CREB transcription and blunted NFκB transcription. (Supported by the National Institutes of Health, USA).

O21: SNP Detection for Hereditary Neuralgic Amyotrophy Alleles by Target-assembled Tandem Oligonucleotide Systems Based on Exciplexes

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Hereditary Neuralgic Amyotrophy (HNA), also known as Brachial Plexus Neuropathy, is a rare hereditary disease that is characterized by recurrent episodes of severe arm and shoulder pain accompanied by muscle weakness, nerve deterioration, and sensory impairment of the nerves in the arm. Linkage studies have shown that HNA is an autosomal dominant disorder caused by a genetic defect localized to the long arm of the seventeenth chromosome between the 24th and 25th band (17q24-17q25)[1]. Detection of specific target nucleic acid sequences is commonly achieved by hybridization of fluorescently labeled oligonucleotide probe(s) to a complementary target sequence[2,3]. We report the first use of exciplex-based split-probes for detection of the wild type and mutant alleles of Hereditary Neuralgic Amyotrophy. A tandem 12-mer split DNA oligonucleotide probe system was designed that allows detection of the complementary target DNA sequence. This exciplex-based fluorescence detector system operates by means of a contiguous hybridization of two oligonucleotide exciplex split-probes to a complementary target nucleic acid target. Each probe oligonucleotide is chemically modified at one of its termini by a potential exciplex-forming partner, each of which is fluorescently silent at the wavelength of detection. Under conditions that ensure correct three-dimensional assembly, the chemical moieties on suitable photoexcitation form an exciplex that fluoresces with a large Stokes shift (in this case 130 nm). Target sequences tested were in the form of (i) synthetic oligonucleotides, (ii) embedded in short PCR products (150 bp), or (iii) inserted into plasmid DNA (~ 3 Kbp). The exciplex system was able to differentiate wild type and mutated SNP (835 A→G) alleles, based on fluorescence emission spectra and DNA melting curves, indicating promise for future applications in genetic testing and molecular diagnostics.

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O22: **Pathomechanisms in autism linked to altered function of mutant ribosomal protein rpL10 identified in autistic patients**

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Autism is comprised of a clinically heterogenous group of neurological disorders which present with impaired social relationship, impaired language and restricted mental capacities. A promising route to investigate the pathomechanisms on cellular level is offered by investigating single gene disorders identified in autistic patients.

Here, we report on the functional analysis of yeast and human alleles of the autism associated ribosomal protein rpL10. We have identified human mutant alleles in autistic patients (Klauck et al., 2006), rpL10_L206M and rpL10_ H213Q and we have shown that these human mutant alleles confere a severe translation defect when analyzed in the yeast model system. We now have extended these studies in functional analysis in yeast. First, we found, that gene dosage reduction of yeast rpL10 results in reduction of protein synthesis and increase in replicative life span (Chiocchetti et al., 2007). Second, we used 2DIGE analysis to monitor differential protein expression in a strain with gene dosage reduction in rpL10. We identified several isoforms of a phosphoprotein, which show increased expression in the RPL10/delRPL10 strain. Fourth, two hybrid analysis identified a neuronal signal transduction component as a rpL10 interaction partner. Interestingly, the human mutant autism specific alleles L206M and H213Q show increased affinity to this interaction partner..We speculate, that these novel interaction partners may be also components of the molecular network affected in autism.

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O23: **Modulation of impaired fear extinction by neuropeptides in a psychopathological animal model of high trait anxiety**

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Current procedures to cure anxiety disorders such as post-traumatic stress disorder and specific phobias involve psychotherapy including exposure-based therapy. They are effective, but an improvement of their therapeutic efficacy is desired. Using a classical fear conditioning paradigm, we have recently reported that a rat line characterized by extremely high levels of trait anxiety (HAB) displays an impaired ability to inhibit learned fear responses which is a hallmark of the human psychopathology. Moreover, similar to what has been observed in specific human anxiety disorders, the impaired extinction of HABs was shown to be improved by a single administration of d-cycloserine before extinction training (Singewald et al., 2007 SFN meeting, nr. 170.17). Here, we tested the effect of a selective vasopressin V1a receptor antagonist d(CH(2))(5)Tyr(Me)AVP and neuropeptide S (NPS) on the impaired fear extinction observed in HAB rats. Intracerebral application of NPS prior to extinction training seemed to accelerate fear extinction learning in HABs as indicated by a faster decrease in freezing behavior which, however, did not reach statistical significance. 24 hours later retrieval of fear extinction was performed. NPS-treated HABs displayed lower freezing levels in the extinction retrieval test pointing towards enhanced consolidation of extinction memory by NPS. Although treatment with the V1a receptor antagonist did not affect extinction training, it also dose-dependently caused lower fear responses in the extinction retrieval test on the next day. This result reflects what we have previously observed in d-cyclsoserine-treated HABs; i.e. no effect on extinction training, but reduced freezing responses during retrieval of extinction. To gain insight into the neural basis of the positive effects of NPS and the V1a receptor antagonist expression patterns of different immediate early genes as markers of neuronal activation were assessed in cortico-limbic regions after retrieval of extinction. Impaired extinction in HAB rats was associated with a hypoactivation of the medial prefrontal cortex, in particular the infralimbic cortex, which is considered an important area in extinction circuits. It remains to be shown whether and how the two neuropeptide ligands modulate this and other aberrant activation patterns in HABs. Taken together, the present results indicate that HAB rats represent a clinically relevant model to study neuropeptidergic drug targets with potential clinical benefit in exposure-based therapy. Supported by the FWF NFN-S-102 (NS).

O24: **Peroxisomal localisation of the Proopiomelanocortin derived peptides β-Lipotropin and β-Endorphin**

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Corticotroph cells of the pituitary gland produce peptide hormones derived from the precursor molecule proopiomelanocortin (POMC), including adrenocorticotropin (ACTH), melanocyte-stimulating hormones (MSHs), β-lipotropin (β-LPH), and β-endorphin. We demonstrate by confocal laser microscopy and immunoelectron microscopy in human pituitary gland that β-LPH and β-endorphin were not only present in secretory vesicles, but also colocalized with peroxisomes, whereas ACTH was not detectable in peroxisomes. Moreover, β-LPH and β-endorphin were also found in ALDP-positive peroxisomes in many other human tissues, including dorsal root ganglia, adrenal gland, testis, kidney, and skin. Moreover, in HeLa cells, ectopic expression of a POMC variant, which is able to release β-LPH within the secretory apparatus, also resulted in a peroxisomal targeting of the hormone. However, in cells of the adrenal medulla, which primarily secrete β-endorphin and do not contain ALDP, β-endorphin was undetectable in peroxisomes. Our findings suggest either a role of peroxisomes in the intracellular fate of POMC derived peptides or a regulatory role of these peptides on peroxisomal function.

This work was supported by the EU project “Peroxisomes” LSHG-C/2004-512018

O25: **Enhanced retinal ganglion cell neurite growth in experimental glaucoma is mediated by activated retinal glia**

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Elevation of intraocular pressure (IOP) as an experimental model of glaucoma is generally associated with a detrimental effect on retinal ganglion cell (RGC) survival but overall retinal cell responses are not fully understood. We used a retinal cell culture model to dissect out these cellular responses, and in particular to explore the interactions between RGC and activated retinal glia.

Experimental glaucoma was induced in adult rats by using a well established trabecular laser model. Adult RGC from experimental glaucoma eyes showed significantly enhanced neurite outgrowth in culture compared to RGC derived from contralateral eyes or control animals. This enhanced neurite outgrowth potential was correlated with significantly enhanced numbers of activated glia in retinal cultures from laser-treated eyes. Interestingly, use of a glial-specific toxin, aminoadipic acid, which reduced the numbers of activated glia by 70%, without affecting RGC survival, significantly reduced the neurite outgrowth potential of RGC in retinal cultures from experimental glaucoma eyes.

These results show that elevation of IOP leads to enhanced RGC neurite outgrowth potential, an effect that appears to be mediated by activated retinal glia. Future studies will investigate the mechanisms of this glial-dependent RGC neurite outgrowth, potentially revealing novel growth factors for the treatment of retinal injury and disease.

O26: Nicotinic Acetylcholine Receptors (nAChR) in the superior cervical ganglion: A biochemical analysis in wild type mice and mice with deletions of distinct nAChR subunit genes

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The biophysical and pharmacological properties of neuronal nicotinic acetylcholine receptors (nAChRs) are critically determined by their subunit composition. nAChRs can either assemble as homopentamers (e.g. five α7 subunits) or heteropentamers (2 or more different subunits). Heteropentameric nAChRs in the central nervous system are primarily made up by the subunits α4 and β2, whereas such receptors in the peripheral nervous system (PNS) contain α3 and β4 as major and α5 and β2 as accessory constituents. We have previously reported that Superior Cervical Ganglia (SCG) of mice express mRNA coding for the nAChR subunits α3, α4 (transiently after birth), α5, α7, β2, and β4 (Putz et al., 2008). Our current project aimed at investigating the subunit composition of nAChRs in the SCG of wild type (WT) mice and how deletion of distinct subunits affects receptor assembly in our various mouse knockout (KO) models.

Subunit-specific antibodies were generated in rabbits and extensively characterized in HEK 293 cells transfected to express appropriate nAChRs, and on nervous materials dissected from WT, α5β2-double KO, or α5β4-double KO animals. Receptors were solubilized by Triton X-100, incubated with both the nicotinic agonist [^{3}H]-Epibatidine and our subunit-specific antibodies, and precipitated with immobilized Protein A.

We find that the overall number of receptors in the SCG of 17 to 19 day old C57/BL6 is comparable in WT, α5 KO, β2 KO, and α5β2-double KO mice. In contrast, β4-single and α5β4-double KO animals alike express significantly less receptors. Though all receptors in WT SCGs contain the subunits α3 and β4, a fraction also contains either the α5 (20%) or the β2 (18 %) subunit. α5 and β2 are thus not assembled into the same receptor. Deletion of α5 does not cause up-regulation of β2 and vice versa. However, α5 does not assemble into α3β2 nAChRs of β4 KO animals, indicating an absolute requirement of β4 for the expression of α5-containing receptors in the PNS.

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O27: **A Leucine-Rich Repeat Tyrosine Kinase regulates neuronal outgrowth and learning-related long-term synaptic plasticity in Aplysia.**

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Vertebrate tyrosine kinase receptors containing Leucine-Rich Repeat motifs (LRR) regulate neuronal outgrowth and morphology, synaptic connectivity and long-term synaptic plasticity. The roles of invertebrate LRR tyrosine kinase receptors remain however poorly understood. We here studied ALERT, a structurally unique transmembrane tyrosine kinase from *Aplysia* with numerous extracellular LRR motifs and homologous to growth factor receptors. Using live-cell microscopy, molecular biological and electrophysiological techniques we examined the effects of ALERT overexpression or inhibition on synaptic plasticity in Aplysia sensory-motor synapses. Presynaptic overexpression of ALERT induced a pronounced outgrowth of neuritic branches capable of targeting the postsynaptic cell and containing synaptic-vesicle proteins involved in synaptic transmission. ALERT also enhanced neurotransmitter-induced long-term synaptic plasticity, whereas its inhibition impaired neurotransmitter-induced growth and long-term -but not short-term- synaptic plasticity. These results reveal a previously unknown invertebrate LRR tyrosine kinase as a molecular component critical for the modulation of neuronal outgrowth and synaptic plasticity.

O28: **Regulation of monoamine transporters by membrane phosphatidylinositol 4,5-bisphosphate (PIP_2)**

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By rapidly removing neurotransmitters from the extracellular space, monoamine transporters control their extracellular concentrations and thereby determine the function of the whole nervous system[1]. Thus, understanding the regulation of these proteins is of utmost importance. A variety of ion channels, including Kv7, Cav2, Kir, TRP, or P2X receptors, require membrane phosphatidylinositol-4,5-bisphosphate (PIP_2) for their function[2]. Neurotransmitter transporters also display ion channel functions[3], but their regulation by PIP_2 has not been reported.

In perforated patch clamp measurements, activation of phospholipase C (PLC) reduced median amphetamine-evoked currents through human serotonin transporters (SERT) from -32.3 pA to 0.72 pA (n=9; p=0.039 Mann-Whitney u test). Likewise, amphetamine induced reverse transport was clearly diminished, but transporter mediated uptake was not altered. The same effects were observed when PIP_2 levels were lowered by sequestration via a PIP_2 scavenging peptide. Mutation of putative PIP_2 binding domains on the SERT led to the loss of amphetamine induced currents (median amplitude =-1.4 pA; n=6) and greatly reduced the release of labeled substrate, but left its uptake unchanged. With this mutation, depletion of PIP_2 did not cause any effect.

These data show for the first time that PIP_2 is required for channel function and reverse transport, but not for transmitter uptake, in monoamine transporters.

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ABSTRACTS 2

POSTER

PRESENTATIONS

P01: **Cellular trafficking and signaling of the receptor tyrosine kinase MuSK are regulated by dynamin and cholesterol-enriched microdomains**

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The development of the neuromuscular junction (NMJ) requires a complex exchange of signals between a motor neuron, a muscle fiber and a Schwann cell leading to the aggregation of acetylcholine receptors (AChRs) and other postsynaptic components at postsynaptic sites. The localization of AChR clusters is regulated by the release of the motor neuron-derived protein Agrin. Agrin binds to its receptor LRP4, followed by the activation of the muscle-specific receptor tyrosine kinase (RTK) MuSK. Mice lacking MuSK, LRP4 or Agrin fail to form NMJs and die shortly after birth due to respiratory failure. Downstream signaling is essential for the signal transduction pathway and therefore crucial for the synaptic development. Mechanisms that regulate expression of cell surface MuSK, its stability and localization are largely unknown. Accumulating evidence support the notion that trafficking of receptors can modulate their signaling events. We therefore postulate that MuSK trafficking and signaling are closely linked. For this purpose, we aim to study the pathways and mechanisms of MuSK endocytosis and post-endocytic sorting for a better understanding of its crucial role in MuSK signaling and consequently in NMJ formation and maintenance.

To characterize the endocytic pathway in more detail, including the internalization machinery and the endosomal pathway, a live-cell staining approach is used. MuSK shows a partial overlap with the lipid raft marker cholera toxin B, caveolin-1 and flotillin-1 and MuSK endocytosis is blocked by cholesterol-depletion. Current results suggest that MuSK internalization also requires the large GTPase dynamin. Beyond that, blocking lipid raft- or dynamin-dependent endocytosis interferes with Agrin-induced AChR clustering. Transportation of internalized MuSK occurs via nonclassical, Rab5/EEA1-independent early endosomes. This is in contrast to other RTKs, whose endocytosis is mostly Rab5-dependent. Additional results provide evidence that MuSK recycling occurs via Rab4-positive vesicles and its degradation via Rab7-positive late endosomes. Taken together, we propose that MuSK uses a unique, lipid raft-dependent internalization pathway.

This project is funded by the Austrian Science Fund (FWF).

P02: **Inhibition of RhoA promotes peripheral axon regeneration**

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The small GTPase RhoA is an inhibitor of axonal regeneration. Activation of Rho and its effectors mediates growth cone collapse and retraction via phosphorylation of myosin light chain and interference with actin turnover. In contrast, inhibition of RhoA or expression of a dominant negative construct result in process outgrowth by rat pheochromocytoma cells and cultured hippocampal neurons. Increased RhoA activation was observed in sensory neurons following peripheral nerve lesion. The aim of this study was to assess possible effects of Rho inhibition and Rho down-regulation on peripheral axon regeneration in primary sensory neuron cultures and in the sciatic nerve lesion model.

Dissociated dorsal root ganglia (DRG) neurons obtained from adult rats were transfected with RhoA siRNA plasmids also encoding EGFP. Pharmacologically, Rho activation was inhibited by Clostridium botulinum toxin C3 [10 ng/ml], which inactivates Rho by ADP-ribosylation. Axon growth of cultured neurons was determined by measurement of the total axonal length. For the in vivo experiments the right sciatic nerve of adult rats was transected and a 12 mm-silicon tubule bridging the nerve stumps was implanted. It was filled with a collagen solution containing C3 [0.5 mg/ml or 0.25 mg/ml]. Controls received collagen or an enzymatically inactive mutant version of C3. Functional sciatic nerve regeneration was assessed monthly using the walking track (testing locomotor function), thermal algesimetry (assessing pain sensation), and electrophysiological recording of digital sensory nerve action potentials (assessing regenerated nerve conduction).

RhoA siRNA transfected or C3 treated DRG neurons exhibited a significant increase in total axonal length within a 24h culture period. After C3 application in vivo, locomotor function improved in the group that received C3 [0.5 mg/ml] at 60 days post operation (dpo). Animals treated with either C3 concentration regained normal sensation at 90 dpo. Sciatic nerve action potentials at the digital level could be detected first in the C3 [0.5 mg/ml] treated group.

Taken together, our results indicate a growth promoting effect of Rho down-regulation or Rho inactivation in adult primary neuron culture and a functional improvement in response to Rho inactivation following peripheral nerve injury.

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P03: **Significance of gp130 signaling in neuronal regeneration**

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Background and aims: The cytokine interleukin-6 (IL-6) activates target genes involved in differentiation, survival, apoptosis and proliferation as well as in inflammatory processes. It acts by binding to plasma membrane receptor complexes containing the common signal transducing receptor subunit gp130. Subsequently and by activation of JAK, STAT and MAPK signaling pathways, IL-6 regulates various target genes leading to its broad effects. In the present work, we have investigated the role of gp130 in the regeneration of peripheral sensory neurons using specific knock-out mice (SNS-gp130).

Results: Dorsal root ganglion neurons (DRGs) from SNS-gp130 mice showed a significantly reduced neurite extension in culture as compared to flox/flox and wild-type animals. The number of neurite bearing neurons was reduced in SNS-gp130 mice. After nerve crush injury regeneration of the sciatic nerve was monitored by determining mechanical and heat thresholds and motor capabilities in control and knock-out mice. SNS-gp130 mice showed slower sensory and motor recovery as compared to wt mice.
In order to elucidate the effects of IL-6 and gp130 on regulation of peripheral regeneration and to find potential signaling partners we used Affymetrix® gene expression analysis of DRG neurons from SNS-gp130 mice and controls. We found up-regulated expression of Citron mRNA, a small GTPase effector that has previously been associated with inhibition of neuronal proliferation and differentiation. Moreover, we found down-regulation of Atf3 and Sprr1a mRNAs, two regeneration-associated genes. Qualitative PCR revealed that Citron and its upstream partner, TTC3, are expressed in the DRG neurons and we demonstrate that Citron and TTC3 immunoreactivity is present in small $IB4^+$ DRG neurons.

Conclusion: Our data suggest that the nerve regeneration of neurons is significantly inhibited in SNS-gp130 in vitro and in vivo. We have shown that genes that are associated with the nerve injury or neurite outgrowth regulation are modulated in SNS-gp130 mice. Further experiments will be required to address the functional importance of TTC3 and Citron in sensory neurons.

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P04: **Alterations in hippocampal neurogenesis and anxiety behaviour in p75 KO mice"**

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The subventricular zone of hippocampal dendate gyrus (DG) is one of few brain areas associated with neurogenesis in the adult brain. Brain derived neurotrophic factor (BDNF) and the expression of p75 neurotrophin receptor have been linked to changes in hippocampal neurogenesis. In addition, alterations in hippocampal neurogenesis have been correlated with changes in learning and depressive-like behaviour. We now analysed anxiety- associated behaviour in long and short p75 deficient mice (p75KO) and wild-type (WT) controls. Neurogenesis was analysed by applying Ki67 immunohistochemistry on horizontal sections of the ventral DG and on coronar sections of the dorsal DG. Neuronal numbers of hippocampal principal cells were counted from DAPI stained sections using an optical grid.

Female p75KO mice showed an anxiolytic-like phenotype in the elevated plus maze test and enhanced exploration in a novel open field, while spontaneous locomotor activity in the home cage did not differ between the genotypes in the light phase, where behavioral testing was pursued. Locomotor activity of p75KO mice was reduced in the dark phase as compared to WTs. These data demonstrate that p75 receptor may play a role in anxiety-like behavior.

Histochemical analysis revealed markedly reduced numbers of Ki67 immunopositive cells in the dorsal, but not ventral DG of p75KO mice. This is in line with a reduced volume of the dorsal DG in p75KO mice. Noteworthy, no changes were observed in the entire CA1 region and the ventral DG.

Our data demonstrate that p75 receptors may play a role in anxiety-like behaviour. Interestingly, germ-line deletion of p75 differentially effects dorsal and ventral DG formation.

P04A: **Signaling mechanisms controlling sympathetic cholinergic differentiation**

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Our experiments aim at providing novel synoptic insight into integrative molecular mechanisms that orchestrate signal processing in primary neurons. Specifically we intent to identify intracellular signaling pathways that regulate neurotransmitter differentiation in sympathetic neurons. To this end we study the molecules and processes that regulate the coordinated up-regulation of cholinergic and the concomitant down-regulation of noradrenergic markers in sympathetic neurons in vitro.

The acquisition of the cholinergic transmitter phenotype of primary rat sympathetic neurons can be triggered by growth factors under defined, serum-free conditions. In our cultures the cholinergic signaling is acutely initiated by switching from nerve growth factor (NGF) to ciliary neuronotrophic factor (CNTF). The signaling pathways downstream of the CNTF receptor are then studied employing specific pharmacological inhibitors. The effect of these substances is monitored by determining expression levels of neurotransmitter marker genes by real-time RT-PCR. Further we investigate activation of signaling proteins using antibodies which selectively recognize activated forms on Western Blot.

The analysis revealed that the signalling mechanisms controlling the upregulation of cholinergic marker genes after CNTF treatment are distinct from those responsible for the downregulation of the noradrenergic markers. Moreover we found that p38 MAP kinase is activated downstream of the CNTF receptor and its sustained activity is required for cholinergic differentiation in vitro. Blockade of the p38-pathway has no effect on the activation of STAT3 or STAT1 activation by CNTF. Since this activation is considered the canonical form of CNTF signalling our findings reveal a novel non-canonical CNTF signaling mechanism in primary neurons that depends on p38 signaling.

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P05: **Cell Fate Analysis of Embryonal and Adult Stem Sell-Derived Grafts in the 6-OHDA Model of Parkinson´s Disease**

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Parkinson's disease (PD) is a neurodegenerative disorder characterized by a progressive loss of dopaminergic neurons in the pars compacta region of the substantia nigra whit a subsequent reduction in striatal dopamine content.

Levodopa is effective in alleviating motor symptoms of PD in early stages of the disease but chronic treatment is often limited by disease progression and therapy-related complications.

Intrastriatal grafts of mouse embryonal Ventral Mesencephalon (VM) DA neurons represent the "gold standard" for neurotransplantation of PD. However, duo to ethical and logistical concerns there is a necessity to look for alternative sources for the replacement of degenerating population of DA neurons. Therefore, embryonic and adult stem cells are regarded as an alternative and promising source of dopaminergic cells for neurotransplantation in PD.

Further progress is hindered in particular by the lack of two sets of crucial information. First, a direct comparison of the efficacy of the different stem cell types *in vivo* is still missing. Second, a strictly quantitative analysis of the cellular phenotypes at various stages before and after transplantation is needed. In this context, we will briefly go through the aims of this project.

The primary goal of this study is to directly compare the neurorestorative potential of mesencephalic precursor cells, embryonic stem cells (ESC) and adult mesenchymal stem cells (MSC) in the PD 6-OHDA rat model. The second aim of the project is to refine cell culture protocols in order to stimulate differentiation into neuron-like cells and the expression of DA markers. Finally, we will conduct a quantitative molecular characterization of cell fate and cell lineage decisions that occur in the grafted cells before and after transplantation in-OHDA rat model. To this end we will quantify the presence of a spectrum of markers for different neuronal cell types, glial populations, immune cell and mesenchymal derivatives.

P06: **Interaction Partners and Signaling of Reticulons**

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Reticulons (RTNs) are endomembrane bound proteins with a uniquely conserved C-terminal domain named Reticulon homology domain (RHD) [1,2]. RTN-4/Nogo proteins are well characterized members of the RTN protein family and shown to impair axonal regeneration after brain insult and to restrict neuronal plasticity in the intact adult mammalian nervous system. Recent studies, mainly on non-neuronal cells, suggest however, a role of Nogo in shaping the ER membrane and in endocytic and exocytic vesicle-traffic [3,4]. To further support this hypothesis and to unravel the molecular mechanisms by which Nogo proteins participate in membrane-trafficking, we sought to identify interaction partners of Nogo using pull-down and co-immunoprecipitation assays. By sequence comparison, at least three structural domains of NogoA can be recognized. A putative proline-rich domain (PRD) at its N-terminus encompassing aa 1-172, a long unstructured middle domain and the RHD. The PRD is characterized by several binding sites for SH3 domains (RXPXXP and PXXPXR) which possibly mediate the interaction of Nogo protein with SH3-containing proteins. Recombinant and immobilized domain proteins were used as bait to interact with proteins from mouse brain lysate. This pull-down approach identified several synaptic vesicle-associated, and endo/exocytic pathway-related proteins by Mass Spectrometry (MS). Further structure function analysis will be performed to validate and characterize the physiological relevance of these NogoA binding partners.

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P07: **Reticulon Proteins in the Epileptic Rat Brain**

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Activity-dependent synaptic plasticity in the nervous system is not only important during brain development but also for storing information. Various CNS disorders, such as epilepsy, employ mechanisms of functional and morphological changes. Possible candidate molecules important for regulation of structural plasticity include the so-called reticulons, a family of evolutionary conserved proteins mainly associated with the endoplasmatic reticulum. One member - RTN4A, also named Nogo-A - is best known for its inhibitory effect on neurite outgrowth after nerve injury when being expressed on the cell surface of myelinating oligodendrocytes. However, high levels of Nogo-A can be detected in certain neurons of the CNS - not only during development, but also in the adult rodent brain. The intracellular localization of the protein – displaying a reticular pattern – suggests that Nogo-A might have additional functions as a signaling molecule.(1) In a study of human(2) and experimental(3) temporal lobe epilepsy it was shown that Nogo-A mRNA and immunoreactivity were clearly up-regulated in hippocampal neurons and their processes. As it is believed that axonal and synaptic re-organizations in the hippocampal formation are promoting epileptogenesis, our group wants to test the hypothesis that silencing Nogo-A leads to a change in the pathology of seizures in a rat model of temporal lobe epilepsy. Therefore, a recombinant adeno-associated virus, containing shRNA targeted against Nogo-A, is injected unilaterally into the ventral aspect of the rat hippocampus. After the system has reached peak expression, seizures are induced by administering Kainic acid intra-peritoneally. The severity of the status epilepticus gets rated and continuous observation via video monitoring gives insight into seizure frequency and duration. Finally, the animals get sacrificed in order to evaluate morphological changes via immunohistochemical techniques.

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P08: **The Presence and Biological Role of Nogo Receptor in Immune Cells.**

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Nogo-A (RTN4-A) is one of the major myelin-associated factors contributing to the growth inhibitory environment of the central nervous system (CNS) after neuronal injury. The receptor for Nogo-A (Nogo-66 receptor/NgR) was subsequently found to act as the common receptor for several other myelin-associated inhibitory factors (MAIFs). Due to the coincidence of neurodegeneration and neuroinflammation, and the finding that Nogo-A and NgR are upregulated in multiple sclerosis lesions, MAIFs and their receptor may also play a role in immune modulation during CNS injury. As such, the current aim is to determine the expression and possible function of NgR in immune cells.

Human primary monocytes were isolated from full blood and cultured, or differentiated into monocyte-derived dendritic cells (moDCs). Using Real Time PCR and Western Blot, it was found that NgR is expressed in immature 7 day moDCs. This expression returns to the baseline level after maturation of the cells. To investigate the role of up-regulated NgR in immature DCs, the differences in adhesion and migration between mature and immature DCs will be determined. Surface NgR expression should result in less cell adhesion in the presence of the MAIFs and migration away from MAIFs.

The expression of NgR in immune cells has important implications for all neurodegenerative disorders which encompass a neuroinflammatory element. This could include the protection of healthy tissue by preventing migration of activated immune cells. Furthermore, with the proposed block of MAIFs as a therapeutic response, the role of these factors in the immune response needs to be further investigated.

P08A: **Identification of Novel Interaction Partners of Nogo-A and NgR2 Using Bait- Proteins Produced in Mammalian Suspension Cell Culture**

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Specific and efficient protein-protein interaction of membrane bound proteins in mammalian cells often depends on correct folding and /or posttranslational modifications (PTMs) of binding partners. For this reason, we use bait-proteins produced in mammalian cells which, other than bacteria or yeast, ensure mammalian-type modifications of proteins, including glycosylation and phosphorylation.

Two different systems are employed for bait-protein production. First, we have established an inducible expression system (Hek 293 cells) based on tetracyline/doxycycline which allows for production of proteins, which would be toxic for the cells if a non induceable system would be used. Second, we pursue a transient gene expression approach relying on Amaxa electroporation. To overcome shortcomings of low production rates we have adopted suspension cell culture giving us the possibility for easy scale up.

For proof of principle, we show here preliminary results with bait proteins derived from the myelin associated nerve growth inhibitor Nogo-A and NgR2, a member of the Nogo66 receptor family, both of which exhibit PTM dependent interactions [1,2]. For better separation of proteins 2D gels are used. The spots unique for the pull-down are picked and analysed by mass spectrometry.

Once this system is established it is easy to adjust for any other recombinant bait protein. Although it is not a high-throughput system, as for example yeast-two-hybrid, it is of advantage when focusing on interactions that depend on PTMs and correct folding.

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P09: **Cdk-5 dependent phosphorylation of NogoA promotes neurite outgrowth of cultured cerebellar granule cells**

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NogoA/RTN-4A is a member of the Reticulon protein family and well known to inhibit axonal regeneration after injury and to restrict neuronal plasticity in the intact mammalian nervous system. In the cerebellum, Nogo-A was shown to be transiently expressed in post-mitotic neurons during neuronal migration and axogenesis (1) but its biological function remains unclear. A key regulator of neuronal migration during CNS development is the cyclin-dependent kinase 5 (Cdk5). Absence of Cdk5 leads to lamination defects in the cerebral cortex and retardation of Purkinje and granule cells migration in the cerebellum (2). Since *in silico* analysis of the Nogo-A/RTN-4A protein sequence revealed the existence of several potential Cdk phosphorylation sites, we addressed the question whether Nogo-A/RTN-4A is a target of Cdk5 and if its phosphorylation affects neurite outgrowth of cerebellar granule neurons. Our results show that in HEK293T cells, coexpression of Cdk5/p35 and RTN-4A leads to phosphorylation of Nogo-A/RTN-4A within the first 172 aa of the protein, in the NiR region. Using different recombinant mutant forms of Nogo-A as substrates in an *in vitro* kinase assay indicate Cdk5-dependent phosphorylation at NogoA residues S16 and T160. Phosphorylation of Nogo-A promotes neurite growth of cultured cerebellar granule neurons. Moreover, mutation of S16 blocks Nogo-A mediated neurite extension in a fashion similar to silencing effects of Cdk5. These results identify Nogo-A as a novel substrate of Cdk5 and suggest that specific phosphorylation of Nogo-A by Cdk5 regulates its action on neurite growth of cerebellar granule neurons.

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P10: **Gene expression analysis in dorsal root ganglia of NgR2-deficient mice**

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NgR2 is a homolog of the previously identified Nogo-66 receptor (NgR1) a GPI-linked neuronal high affinity binding receptor for a number of myelin-associated neurite growth inhibitors, notably Nogo-A (RTN4), oligodendrocyte-myelin glycoprotein (OMgp) and myelin-associated glycoprotein (MAG) (Venkatesh et al., 2005). While NgR1 mediates axonal growth inhibition and may play a role in regulating axonal regeneration and plasticity in the adult central nervous system, the biological relevance of NgR2 in the nervous system is largely unknown. To address this question in more detail, we have created a NgR2-deficient mouse. Similar to *ngr1* deletion mutants (Zheng *et al*., 2005), homozygous adult NgR2$^{-/-}$ mice appeared healthy, and first histological examination showed no obvious abnormalities in the brain, liver, kidney, or heart. Because NgR2 is abundantly expressed on sensory dorsal root ganglion (DRG) neurons, we performed gene expression analyses to facilitate the identification of regulated genes in the NgR2-/- mice. Interestingly, a significant up-regulation of specific axon-injury induced transcripts, such as ATF3, NPY, SPRR1A, and CAR1 were identified. These changes were validated by semi-quantitative RT-PCR and by immunohistochemical analysis on the protein level on DRG sections. Our data suggest that the deletion of NgR2 triggers a signal cascade in DRG neurons, which results in an up-regulation of transcription factors like ATF3 ending up with neuroprotective and regeneration related proteins.

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P11: **The spectral sensitivity of the spider *cupiennius salei*: a behavioural test**

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The spectral sensitivity of adult male *Cupiennius salei*, a noctural hunting spider, was studied in a behavioural test. As known from earlier behavioural tests, *Cupiennius salei* walks towards a black target presented in front of a white backround (Schmid 1996). In this study a black target (size 43 x 70cm) was presented in a white arena which was illuminated with monochromatic light in the range of 365 to 695nm using 19 monochromatic filters (HW in the range of 6 – 10nm). In the first trial, the transmission of the optical filters was between 40% and 80%, in a second trial the transmission was reduced to 5%, using a neutral density filter. At high intensity the spiders showed a spectral sensivity in the range from 380m to 670nm. In the second trial the animals only showed directed walks if the illumination was in the range of 449 of 599nm. In previous intracellular recordings (Walla et al. 1996), the measured spectral sensitivity was between 320 and 620nm. Interestingly, these results do not match with the behavioural testet spectral sensitivity of the photoreceptors, which sensitivity is shifted to longer wavelengths.

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P12: **Spatial acuity of the PM eyes in a hunting spider**

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Cupiennius salei is a night active hunting spider from Central America. Like most spiders it has four pairs of camera type eyes. The anterior median eyes (principle eyes) are equipped with two muscles each that move the retina. All other pairs (secondary eyes) have a reflecting tapetum that increases the effective length of the photoreceptors. In these eyes the photoreceptors are arranged in rows on the tapetum strips. The interreceptor angle is smaller along such rows than between the rows and thus the spatial resolution should be different for the two orientations (Land and Barth, 1992).

Motion detection in the secondary eyes enhances the eye muscle activity in the principal eyes (Kaps and Schmid, 1996). We used moving sinusoidally modulated black and white bars of variable width on a computer screen as stimuli and recorded the eye muscle activity to monitor motion detection in the secondary eyes.

The spiders showed a significant response for vertical gratings at bar widths of 1° visual angle, and for horizontal gratings at bar widths of 1.3° visual angle. For vertical gratings the visual angle of a bar matches the interreceptor angle on the retina reported by Land and Barth (1992). Interestingly the spiders responded to horizontal gratings whose bars had visual angles smaller than the corresponding interreceptor angle. This can be explained with a very simple model taking the intensity change in single photoreceptors into account.

Our results suggest that this night active spider makes full use of its theoretical anatomical resolution and spatial pooling seems improbable.

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P13: **How do spiders discriminate between vertical and sloped objects?**

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The Central American wandering-spider *Cupiennius salei*, a nocturnal hunter, is known to have very good mechanosensory systems. Recent studies showed that also their visual capabilities are highly developed. In a twofold simultaneous-choice experiment the animals showed no preference between two identical targets (100cm high, 48cm width). But if the animals have to choose between two targets that differ only in their vertical orientation, they strongly preferred a vertical to a sloped bar (Schmid 1998). The aim of this study was to examine how the visual system processes "verticality". Therefore it was tested whether the spiders use either gravity or a visible horizon as a possible reference. The results showed that an artificial horizon had no influence on the animals choice. In experiments with an inclined arena the animals significantly preferred the vertical bar in reference to gravity at 12° and 22° but interestingly not at 32°. These results suggest that the alignment of the spider's body posture on an inclined arena could be a crucial intrinsic factor for calculating verticality. Therefore photos of spiders sitting on an arena with different inclinations were taken and their body posture with respect to the ground was measured. The results show that the spiders compensate an inclination in reference to gravity but the amount of the compensation decreases with increasing inclination and reaches a kind of saturation.

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P14: **Social interaction increases speed of extinction of cocaine-induced place preference and prevents cocaine-induced reconditioning of cocaine seeking**

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A single 15 min social interaction during extinction was able to completely reverse 15 mg/kg i.p. cocaine-induced place preference in rats. After one saline extinction trial each, the mean time spent in the previously cocaine-paired vs the saline-paired chamber was 377 ± 34 s vs 262 ± 29 s (means ± SEM of N=17 rats; 2-sided t-test $p<0.0001$; total CPP test time, 900 s) in absence of social interaction and 341 ± 37 s ($p=0.582$; cocaine) vs 315 ± 31 s (saline+social;N=18) after social interaction with a same-sized single-housed male playfellow. After four extinction trials each, the respective dwell times were 299 ± 20 s (cocaine) vs 303 ± 21 s (saline only) and 244 ± 17 s (cocaine) vs 408 ± 16 s (saline+social). More importantly, social interaction during extinction fully prevented cocaine-induced reconditioning of cocaine CPP (one exposure to 15 mg/kg i.p. cocaine in the previously cocaine-paired chamber, CPP test 24 h later): In the absence of social interaction, mean dwell times were 393 ± 17 s (cocaine) vs 208 ± 17 s (saline only; $p<0.0001$), whereas social interaction changed the respective dwell times to 242 ± 18 s (cocaine) vs 333 ± 25 s (saline+social; $p=0.007$). In addition, even if cocaine conditioning was continued after acquisition of cocaine CPP, social interaction was preferred over cocaine (407 ± 34 s vs 233 ± 21 s; $p=0.0003$). Finally, using immunohistochemistry for zif268, an immediate early gene considered as a marker of neuronal activation, we also showed that social interaction during extinction prevented cocaine-induced reconditioning of cocaine CPP by reducing the activation of several brain areas known to play critical roles in motivated behaviors and conditioning of drug-associated stimuli.

In conclusion, our novel experimental paradigm yields an impact of social interaction on drug seeking and salience of drug-associated contextual stimuli previously thought unlikely and suggests that social interaction may be a fundamental factor in preventing relapse to cocaine addiction. Supported by FWF P18787-B05, MFF 154, Swarovski-Foundation, and the VEPPP.

P15: **Effects of hypercholesterolemia on learning, cholinergic neurons, vascularization and immunoreactive markers in vivo in rats**

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Alzheimer´s disease (AD) is a chronic brain disorder and the decline in cognitive functions, implications of the cholinergic system, as well as beta-amyloid deposition are the hallmarks of this disease. Since recent studies indicate that cholesterol may play a role in the pathogenesis of AD, the aim of the present study was to examine the effects of a cholesterol-enriched diet in vivo in rats. Sprague Dawley rats were fed with or without a 5% cholesterol-enriched diet for 5 months, then tested in an 8-arm radial maze, and finally rats were perfused or the brains were fresh frozen. In addition levels for plasma cholesterol were measured. Immunohistochemical staining was performed for cholineacetyl transferase (ChAT), rat IgG, beta-amyloid, laminin, glial fibrillary acidic protein (GFAP) and CD11b (OX-42) and different inflammatory markers. Rats fed with a high cholesterol diet had significantly enhanced plasma cholesterol levels. In the 8-arm radial maze a significantly diminished ability in learning and storage of long-term memory was found. The number of cholinergic neurons in the basal nucleus of Meynert (nBM) and septum, but not in the striatum was decreased in cholesterol treated rats and cortical acetylcholine levels were declined. No changes in cortical vascularization were seen for laminin between controls and cholesterol-treated rats. However, an enhanced immunoreactivity against rat IgG in cholesterol-fed rats was found in the cortex, but no changes in cortical beta-amyloid and NGF levels were seen. However, amyloid precursor protein (APP) mRNA, Tau and phospho-Tau181 levels were increased in the cortex. The density of astrocytes (GFAP) or microglia (CD11b) was not different between controls or cholesterol-fed rats. However, different inflammatory markers (IL-1α, IL-6, TNFα, MIP3α, MCP1) were significantly increased in cholesterol-treated animals in the cortex. In summary, hypercholesterolemia in rats causes memory impairment and cholinergic dysfuntion, inflammation and tau pathology, and locally enhanced rat IgG blood-brain barrier disruptions, which partly resembles some AD-like pathologies.

P16: **Influence of Estrous Cycle on Explorative Behaviour of Wild-type and Prodynorphin Knockout Mice**

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Since several years dynorphin, a member of the opioid peptide family was suggested to play a regulatory role in numerous functional pathways of the brain. In line with its localization in hippocampus, amygdala, hypothalamus and striatum these functions resemble learning and memory, emotional control and stress response. Male prodynorphin deficient (Dyn KO) mice display an anxiolytic phenotype (Wittmann et al, 2008). However, emotional control and stress response depend on the hormonal state and differ between sexes. We now analysed anxiety and stress related behaviour in correlation to the estrous cycle in female wild-type (WT) and Dyn KO mice. In the elevated plus maze test Dyn KO mice showed a significant anxiolytic phenotype with about double time spent, distance travelled and entries in the open arm at all estrous stages compared to WT mice. In addition, WT mice showed a significant increase in anxiety related parameters during proestrous and estrous. This increased anxiety was markedly attenuated by prodynorphin deficiency without interference on total activity. In the open field test WT mice showed significantly increased anxiety during the estrous stage. This effect was abolished in Dyn KO mice. In the light dark test WT mice showed a decrease in time spent and distance travelled in the lit area during proestrous. In contrast, the behaviour of KO mice was not altered throughout the stages. In the tail suspension test Dyn KO mice spent comparable times immobile throughout the estrous stages. WT mice showed a significant increase (by ca. 40%) in immobility during diestrous. In the forced swim test proestrous WT mice showed similar decreases in time spent immobile, during the initial and final 4 minutes of a 15 minutes trial. At the beginning of the trial, when anxiety induces escape behaviour, Dyn KO mice showed less activity. In contrast, in the last four minutes, which reflects stress induced depression like behaviour WT mice were more immobile. Our data support the influence of estrous stage on anxiety in female mice. Of note is, that the influence of the estrous stage appears to be abolished by the prodynorphin deficiency. This is in line with altered stress responses during the estrous cycle in WT, but not in Dyn KO mice. The functional and pharmacological background of the interplay of hormones and dynorphin will be investigated in further experiments.

P17: **GABA-A Receptors in Anxiety and Fear**

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Anxiety and fear are adaptive components of an overall response to dangerous situations. These emotional reactions are, therefore, crucial for life and even survival of an individual, but they also threaten to perturb homeostasis. Recent studies have shown, that corticolimbic structures like the amygdala and the hippocampus play a central role in regulating anxiety and fear. These structures contain major networks of GABAergic interneurons as well as, in certain cases, GABAergic projection neurons. The pathophysiologic mechanisms underlying anxiety as well the role of GABA-A receptors within the neuronal circuit remain relatively obscure. This research project aims to identify differences in the expression of GABA-A receptors in genetic mouse models of high (HAB), normal (NAB) and low (LAB) anxiety behavior, as well as in a mouse model (129S1) exhibiting impaired fear extinction. In addition, changes in the expression of GABA-A receptors during generation of fear and anxiety, fear extinction and in mediating anxiolytic drug actions will be investigated primarily using receptor autoradiography and immunohistochemistry (IHC), to define possible differences in the GABAergic system and in the plasticity of GABA-A receptors in these mouse models. Potential changes in different emotional states would be of great clinical importance for the future treatment of patients, as it would define potential drug targets for anxiety related disorders.

In the first few months of my thesis, GABA-A receptor subtype-selective antibodies suitable for IHC were selected and the necessary immunohistochemical techniques were established. In addition, receptor autoradiography was established. IHC and receptor autoradiography were used to study the distribution of GABA-A receptors in wild-type mice.

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P18: **GABA-A Receptor Subunit Expression in Temporal Lobe Epilepsy**

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Temporal lobe epilepsy (TLE) is the most common seizure disorder in adults and, when associated with hippocampal sclerosis (HS), is highly refractory to pharmacotherapy. Evidence from human studies suggests that postsynaptic $GABA_A$ receptors contribute to the pathophysiology of TLE. $GABA_A$ receptors are pentameric chloride channels formed by many different combinations of subunits resulting in functional heterogeneity of $GABA_A$ receptors. Previous results demonstrated pronounced changes in the expression of several $GABA_A$ receptor subunits in animal models of TLE and in the hippocampus of TLE patients indicating functional changes induced by recurrent seizures. In several animal models of TLE, subunits contributing to extrasynaptic receptors mediating tonic inhibition were altered; expression of subunits δ and α_5 were decreased in the dentate gyrus and sector CA3, respectively, indicating a decrease in tonic inhibition. Possible changes in these subunits have not been investigated in human TLE so far.

Using immunohistochemistry and *in situ* hybridization we now investigated the expression of $GABA_A$ receptor subunits α_4, α_5, γ_2, and δ in hippocampal specimens obtained at surgery from TLE patients and in autopsy controls. Increased mRNA levels were observed in the granule cell layer of the dentate gyrus (DG) for: α_4 250% ± 61.1 of control; α_5 140% ± 6.9 of control; γ_2 481% ± 70.0 of control; δ 235% ± 28.3 of control. Similarly, increases in the immunoreactivity of all these subunits were detected in the DG (155 – 249 % of control) and in the subiculum (154 – 230 % of control). Our data indicate that $GABA_A$ receptors may be rearranged in TLE reflecting alterations in subunit expression. In contrast to animals models, upregulation of the $GABA_A$ receptor subunits α_5 and δ could be detected in our specimens of TLE patients, indicating unchanged or even increased tonic inhibition by GABA.

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P19: **The role of the receptor tyrosine kinase MuSK during nerve-independent acetylcholine receptor clustering**

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Synaptic development of functional neuromuscular junctions requires high density aggregates of acetylcholine receptors (AChRs) to be precisely aligned with the motor axon terminal. Exchange of signals between muscle and nerve has been thought to be responsible for the transformation of the AChR-rich postsynaptic apparatus from an oval plaque into a pretzel shaped array of branches that precisely mirrors the branching pattern of the motor nerve terminal. Recently, this model has been put into question since the process of in vivo neuromuscular junction maturation has been reproduced in vitro by culturing myotubes aneurally on laminin coated substrate. Thereby in the absence of nerve derived factors, plaque shaped AChR clusters are transformed into complex AChR pretzels similar to those seen in vivo. These morphological changes require the muscle-specific receptor tyrosine kinase MuSK, as myotubes lacking MuSK expression form no AChR aggregates. These data suggest that neuromuscular junction maturation is controlled by a muscle intrinsic programme that requires MuSK function.

The aim of this project is to study the molecular mechanisms involved in the nerve-independent formation of AChR pretzels, in particularly by dissecting the role of MuSK. For this, we have generated different MuSK mutant and chimeric constructs. Muscle cell lines expressing these MuSK mutant proteins have been generated from MuSK knockout myoblasts. The established laminin-induced AChR clustering assay will be used to test the ability of MuSK mutant proteins to induce AChR pretzels without nerve-derived signals. The analysis will focus on the AChR cluster morphology, size and number as well as on the characterization of the temporal events. This study will allow us to determine whether MuSK functions as signalling or/and scaffolding molecule.

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P20: **Properties of distinct neuronal nicotinic acetylcholine receptors in their native environment**

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The pharmacological and biophysical properties of nicotinic acetylcholine receptors (nAChRs) are critically determined by their subunit composition. We have recently analyzed nAChRs in the mouse superior cervical ganglion (SCG) and found 3 types of heteropenta-meric receptors: α3β4, α3α5β4, and α3β2β4 (see abstract by David et al.). Hence, whole cell or single channel currents in response to a nAChR agonist will reflect a mixed image due to the activation of all these receptor types. To date, distinct receptors have only been investigated in heterologous expression systems. However, properties of recombinant receptors depend on the host cell type and can thus not directly be related to nAChRs found *in vivo*.

We used patch clamp electrophysiology to study nAChR in cultured neurons of the superior cervical ganglion (SCG) and analyzed wild type (WT) mice, mice with single deletions of the α5, β2 and β4 subunit genes, and double KO of either α5/β2 or α5/β4. These double knockouts (KO) leave neurons with a single population of heteropentameric receptors consisting of either α3/β4 or α3/β2, respectively.

The application of nAChR agonists induced currents that greatly differed between the two genotypes. On one hand, currents were significantly smaller in the α5/β4 compared to the α5/β2 KO. These data are in line with our observation that the overall number of receptors, as determined by radioactive ligand binding, is significantly reduced in the α5/β4 KO (see abstract by David et al.). Interestingly, the lesser number of receptors does not overtly impair transganglionic transmission (see poster by Simeone et al.). On the other hand, α3/β2 receptors in the α5/β4 KO are significantly more susceptible to desensitization than α3/β4 receptors in α5/β2 KO animals. When current traces in response to 300 µM ACh were fitted to a double-exponential function, the time constants of both the fast and the slow components were significantly larger for α3/β4 than for α3/β2 receptors. Likewise, 1 µM nicotine desensitized about 50 % of α3/β4 receptors, compared to just 0.1 µM nicotine required to desensitize about 50 % of α3/β2 receptors. These are the first observations on “pure” α3/β4 and α3/β2 receptors not expressed in a heterologous system.

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P21: **Trans-ganglionic neurotransmission in the sympathetic nervous system of mice with deletions of distinct nicotinic acetylcholine-receptor subunit genes**

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Neuronal nicotinic acetylcholine receptors (nAChRs) consist of five identical (homo-pentamers) or different (hetero-pentamers) subunits that critically determine their biophysical and pharmacological properties. We study the function of nAChRs in the superior cervical ganglion (SCG), the uppermost ganglion of the sympathetic trunk, where receptors mediate fast excitatory neurotransmission.

Adult wild type (WT) mice express three heteropentameric nAChRs in the SCG: α3β4, α3α5β4, and α3β2β4 (see poster by David et al.). Targeted deletions of the α5 and the β2, or the α5 and the β4 subunits, leave just one type of heteropentameric receptors in the SCG (α3β4 or α3β2, respectively). Our patch clamp recordings from cultured SCG neurons revealed that receptor properties do not differ significantly between WT and α3β2 knockout (KO) animals. However, α3β2 receptors in the SCG of α5β4-double KO mice are significantly different in two aspects: The overall number of receptors is reduced by about 80 %, and α3β2 receptors are notably more susceptible to desensitization (see poster by Ciuraszkiewicz et al.).

We now wanted to know how these distinct receptor properties affect the synaptic transmission in the intact SCG. Mice at the age of 4-6 weeks were put under deep anaesthesia and decapitated. The two SCGs with their pre-and post-ganglionic nerves attached were removed and kept in gassed Tyrode's solution for the entire experiments. Trans-ganglionic synaptic transmission was tested using suction electrodes for stimulation of the afferent and recordings from the efferent nerves, respectively. Brief (50 µsec) supra-maximal electrical pulses applied to the pre-ganglionic nerve induced compound action potentials (CAPs) at the postganglionic nerve that were compared between the genotypes. Surprisingly, no differences were found in the amplitude between CAPs of WT, α5β2-KO, and α5β4-KO mice. Thus, with stimuli at supra-maximal intensity, the density of postsynaptic nAChRs in the SCG of α5β4-double KO mice seems sufficient to mediate EPSPs up to the threshold for the generation of action potentials.

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P22: **Osmoregulation and phagocytosis in murine microglia**

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Phagocytosis is the uptake of solid particles of at least 0.1-1 µm in diameter, such as microbial pathogens, dead cells, and cellular debris. The uptake mechanism is a highly coordinated process involving the following successive steps: I) particle recognition, II) receptor-mediated particle attachment to the phagocyte, III) uptake of the particle through englulfment pseudopodia, IV) internalization via fusion of the protrusion and building of a phagosome, and V) intracellular digestion of the particle. Within the central nervous system (CNS), phagocytosis is mainly accomplished by microglial cells, a cell type well-known for its role in immune defense. Microglial phagocytosis plays pivotal roles in the developing CNS, the maintenance of tissue homeostasis, acute demyelinating diseases, chronic neurodegenerative diseases, and in aging. As the phagocytosis process is combined with cellular volume regulation, we investigated the impact of osmoregulation on particle uptake. Specifically, we quantified the impact of swelling-sensitive anion channels and different osmotic challenges on the formation of engulfment pseudopodia and internalization of microspheres (Ø 4 µm) in the microglial cell line, BV-2. Using scanning electron microscopy and confocal laser scanning microscopy we investigated the phagocytosis degree of BV-2 cells in the presence and absence of anion-channel inhibitors (NPPB, FFA and DCPIB), the anion-channel inhibitor and K^+Cl^- -cotransport-inhibitor DIOA, Cl^- -free solution, and hypertonic solutions. We found that particle uptake decreased in microglial cells incubated with chloride-channel inhibitors for 15 minutes and one hour, respectively. Dose-dependent inhibition of phagocytosis was measured using the most efficient channel-inhibitors (DIOA and NPPB). Incubation with Cl^- -free solution and hypertonic solutions revealed a decline in particle engulfment too. Incubation with chloride-channel inhibitors prevent the extension of protrusions, attached to microspheres. In summary, our findings indicate that osmoregulation participate in particle uptake.

P23: **Potassium homeostasis modulates cell volume and cell death in microglia**

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Cells exposed to anisotonic conditions change their cell volume and ion conductance. They react to these extracellular osmotic challenges by activating cell-volume regulatory responses in order to maintain their cell volume. During apoptosis cell shrinkage occurs in isotonic conditions and is referred as apoptotic volume decrease (AVD). This characteristic feature of apoptosis is found in every cell type irrespective of the apoptotic stimuli.

Deregulation of the intracellular ionic balance, especially potassium, sodium and chloride, activates caspases and nucelases. For example, high intracellular K^+ concentration inhibits, whereas low K^+ concentration promotes caspase 3 activity. The activity of the Na^+ /K^+ -ATPase, other transporters and ion channels are responsible for maintaining an ion concentration gradient. During apoptosis the intracellular potassium concentration decreases due to the efflux of K^+ and through the inhibition of the Na^+ /K^+ -ATPase. This cation movement is accompanied by loss of intracellular chloride in order to keep electroneutrality.

In our study, the cellular loss of potassium was achieved with the K^+ ionophore valinomycin and, furthermore, the K^+ concentration of the extracellular solution was varied (5mM or 135 mM K^+). The cell phenotype was observed with a scanning electron microscope, the DAPI labelling with a confocal laser microscope (LSM 510), and the cell volume was quantified via a cell sorter.

We found that three hours of treatment with valinomycin leads to shrinkage of cells, which is inhibited by higher extracellular K^+ concentration. DAPI staining showed chromatin condensation in cells treated with valinomycin as well as in the presence of a high extracellular K^+ concentration. Electron microscopy revealed apoptotic changes in valinomycin-exposed cells, including cell blebbing. These findings indicate that disturbance of intracellular K^+ homeostasis promotes apoptosis in microglial cells.

P24: **Activity and Calcium Influx Regulate Nuclear Targeting of the Calcium Channel β_{4b} Subunit in Nerve and Muscle Cells**

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Auxiliary β subunits are critical determinants of membrane expression and gating properties of voltage-gated calcium channels. Mutations in the β_4 subunit gene cause ataxia and epilepsy. However, the specific function of β_4 in neurons and its causal relation to neurological diseases are unknown. Here we report the localization of the β_4 subunit in the nuclei of cerebellar granule and Purkinje cells. β_{4b} was the only β isoform showing nuclear targeting when expressed in cultured hippocampal neurons and dysgenic skeletal muscle cells. Its specific nuclear targeting property was mapped to an N-terminal double-arginine motif, which was necessary and sufficient for targeting β subunits into the nucleus. Spontaneous electrical activity and calcium influx negatively regulated β_{4b} nuclear localization by a CRM-1-dependent nuclear export mechanism. The activity-dependent shuttling of β_{4b} into and out of the nucleus indicates a specific role of this β subunit in neurons, in communicating the activity of calcium channels to the nucleus.

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P25: **Calcium channel β and α_1 subunit interactions regulate β subunit targeting and $Ca_V1.2$ membrane expression in cultured hippocampal neurons**

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Auxiliary Ca_V β subunits modulate current properties and mediate the functional membrane expression when coexpressed with an α_1 subunit in heterologous cells. In brain all four β isoforms are widely expressed, yet little is known about their specific roles in neuronal functions. Here we investigated the expression and targeting properties of β subunits and their role in membrane expression of $Ca_V1.2$ in cultured hippocampal neurons. Quantitative RT-PCR and immunofluorescence showed equal levels and similar distribution patterns of all endogenous β subunits. High-resolution microscopy of hippocampal neurons transfected with six different V5 epitope-tagged β subunits revealed a comparable clustered distribution of all βs in dendrites and axons. All β subunits were able to accumulate in synaptic terminals and to colocalize with postsynaptic $Ca_V1.2$, indicating a great promiscuity in α_1-β interactions. In contrast restricted axonal targeting of β_1 and weak colocalization of β_{4b} with $Ca_V1.2$ indicated isoform specific differences in local channel complex formation. Membrane expression of external HA epitope-tagged $Ca_V1.2$ was strongly enhanced by all β subunits in an isoform specific manner. Conversely, mutating the alpha interaction domain of $Ca_V1.2$ (W440A) abolished membrane expression and targeting into dendritic spines. This demonstrates that in neurons the interaction of a β subunit with the AID is absolutely essential for membrane expression of α_1 subunits, as well as for the subcellular localization of β subunits, which by themselves possess little or no targeting properties.

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P26: **Investigating Presynaptic Calcium Channel Function by Imaging FM Dye Unloading in Cultured Hippocampal Neurons**

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Neuronal voltage-gated calcium channels (VGCCs) consist of a pore-forming α_1 and auxiliary $\alpha_2\delta$ and β subunits and are involved in numerous neuronal functions. The presynaptic α_{1A} ($Ca_v2.1$) and α_{1B} ($Ca_v2.2$) isoforms trigger neurotransmitter release. However, to date little is known about the contribution of their associated $\alpha_2\delta$ and β subunits to vesicle fusion. To address this question, we established a quantitative assay to analyze changes in neurotransmitter release using live-cell-imaging of FM dye loading and unloading of synaptic vesicles. FM1-43 and FM4-64 are fluorescent styryl membrane dyes. Upon depolarization-induced exocytosis FM dye laterally diffuses into the fused vesicles and becomes endocytosed into the synaptic vesicle pool. Upon a second depolarizing stimulus, experimentally induced by a high extracellular K^+, vesicles fuse and release the FM dye. The resulting decline of fluorescence provides a quantitative measure of synaptic release. Therefore, if a given calcium channel subunit is important for the function of the synaptic release apparatus in hippocampal neurons, impeding its function – for instance pharmacologically or by RNAi techniques – is expected to result in altered FM dye release rates. First we confirmed the dependence of vesicle release on calcium influx by showing complete ablation of release by the general VGCC blockers Cd^{2+}/La^{3+}. Surprisingly, with strong depolarizing stimuli (60 mM K^+) the specific α_{1A} and α_{1B} blockers agatoxin and conotoxin failed to hinder release, indicating a contribution of other channel types (e.g. L-type channels) to synaptic release. Studying the dependence of the fusion rate on the degree of depolarization we first observed synaptic release at 30 mM K^+ and full release at 50 mM K^+. At 40 mM K^+ agatoxin and conotoxin successfully blocked presynaptic function, indicating that with moderate depolarizing stimuli synaptic release primarily depends on P/Q- (α_{1A}) and N-type (α_{1B}) calcium channels. Next we will determine the fractional contribution of α_{1A} and α_{1B} and the effects of $\alpha_2\delta$-siRNA knock-down and β subunit overexpression on synaptic release.

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P27: **High complexity of voltage-activated Ca^{2+} channel expression patterns in mouse and cultured hippocampal neurons**

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The importance and diversity of Ca^{2+} signaling in the CNS is mirrored by the expression of a multitude of Ca^{2+} channels. Whereas the general patterns of voltage-gated Ca^{2+} channel expression in the CNS are well established, the expression of individual channel isoforms and their subunit composition in specific types of neurons are still incompletely understood. Moreover, it is unknown whether differentiation and neuronal activity are accompanied by changes of the Ca^{2+} channel subunit composition. Therefore, we established quantitative TaqMan RT-PCR to determine the expression profile of voltage-gated Ca^{2+} channels by combining absolute quantification based on standard curves with relative quantification based on a set of endogenous reference genes. The expression profile of α_1, β, and $\alpha_2\delta$ Ca^{2+} channel subunits in adult mouse hippocampus, cortex and cerebellum shows that all three tissues express the same isoforms. As expected, $Ca_V2.1$, β_4, and $\alpha_2\delta$-2 are the most abundant isoforms in cerebellum. Remarkably, $Ca_V2.3$ is the dominant Ca^{2+} channel in hippocampus. To investigate, whether expression patterns change during development, Ca^{2+} channel subunit expression was determined in embryonic (E16.5), 1-day-old, 2 weeks-old and 8 weeks-old mice. In cortex, expression levels of $Ca_V1.2$, $Ca_V2.2$, $Ca_V2.3$, β_1, and $\alpha_2\delta$-2 decline significantly within the first 2 weeks of postnatal development, whereas β_3 levels drop after birth. Similar developmental changes of $Ca_V1.2$, β_2 and β_3 are found in hippocampus; however, the majority of the subunits is stably expressed. We also analyzed preparations from differentiated low-density hippocampal neuron cultures, representing >90% glutamatergic pyramidal cells. Surprisingly, in this homogenous neuronal culture the same spectrum of channel isoforms was detected as in whole hippocampus. The expression levels of most α_1 subunits are similar to adult tissue, whereas mRNA of $Ca_V2.3$, β_1, β_2, β_4 and $\alpha_2\delta$-1 is reduced in cultured neurons. During differentiation (5DIV to 24DIV) expression of $Ca_V2.1$ and $\alpha_2\delta$-2 increases significantly. Interestingly, blocking spontaneous electrical activity with TTX did not alter the expression profile, indicating that the observed developmental changes were not triggered by the onset of electrical activity. This comparative qRT-PCR approach now allows a broad correlation analysis of specific Ca^{2+} channel isoforms in preparations from different tissues and cells at various developmental stages and activity states. As this analysis confirmed known subunit compositions like $Ca_V2.1$, $\alpha_2\delta$-2, β_4 in cerebellum, it will be useful to identify hitherto unknown interaction partners in other neurons.

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P28: **Protein complexes associated with the pain transduction channel TRPV1**

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Abstract. TRPV1 ion channels are exceptional in their ability to detect and transduce a vast array of pain-producing stimuli. As such, they are in a strategic position to determine specificity, speed and modulation of sensory and nociceptive neurotransmission. Emerging evidence demonstrated that ion channel regulation is critically dependent on protein-protein interactions. Especially proteomic research supported the idea that ion channels do not operate as isolated molecular devices but rather within tissue specific microdomain signalling complexes (so-called supercomplexes). Therefore the specificity of TRPV1 signalling is also expected to result form proteins or molecules associated with the channel.

In order to investigate TRPV1 channel complexes from both mouse and rat dorsal root ganglia (DRG) a panel of selective sequence-directed antibodies against TRPV1 protein was raised and characterized. Western blots using these antibodies demonstrated the presence of a single TRPV1 band with an apparent molecular weight of 90 kDa, which was missing in KO animals.

Immunohistochemical distribution experiments in section derived from rat/mouse DRG and spinal cord, detected TRPV1 protein in small diameter DRG neurons as well as lamina 1/2 of spinal cord.

In order to characterize TRPV1 complexes by means of immunoprecipitation and mass spectrometry sequencing, a high yield solubilisation protocol was established, and about 70% of the channels present in the membrane were brought into solution. Immunoprecipitation of the TRPV1 protein was confirmed both by Western blotting and LC-ESI-MS/MS sequencing. A number of additional proteins were sequenced after precipitation by two different anti-TRPV1 antibodies, which are not present in DRG membranes derived from KO animals.

Further experiments will determine the functional and structural relationship of the newly detected proteins with respect to the TRPV1 channel complexes.

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P29: **Immediate changes in drug craving and appetite-regulating hormones – Ghrelin, Leptin, Adiponectin, Resistin, Insulin – in a sample of former heroin addicts receiving opioid maintenance therapy.**

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Craving is believed to be a major incitement for drug seeking, consumption and relapse. Several modulators - hormones, paracrines and neural structures - have been reported to supposedly underly the complex biochemical response, including appetite-regulating hormones like Ghrelin, Leptin, Adiponectin, Resistin and Insulin. We decided to assemble our study focusing on near term alterations of repeated craving and hormone level measures in the context of an opioid substitution therapy setting.

A sample of 17 heroin addicts (4 female, 13 male; median age: 30 years) consented to participate in this study. Craving was assessed using the general craving scale (GCS) and heroin craving questionnaire (HCQ). The craving scores and blood hormone levels were determined before and three hours after administration of the substitution substance.

Results: All psychological craving scores showed a highly significant decrease ($r = 0,885$; $p < 0,01$) after intake of the substitution opioid. Leptin levels also decreased significantly between the two sampling periods ($r = 0,989$; $p < 0,05$). Initially, Insulin levels and craving for heroin presented with a marked negative correlation ($r = -0,535$; $p < 0,05$). Ghrelin and Resistin exhibited a clear, yet not significant trend to inversely correlating with all craving scores both before and after substitution. Regarding psychiatric comorbidities, 70% of the participants (4 female, 8 male) had a score > 11 in the Beck depression inventory (BDI).

Our results most importantly support the assumption that opioid substitution decreases craving for illicit drugs, even over a very short course of time. Pathways regulating hunger (Insulin, Ghrelin, Leptin) apparently seem to influence craving.

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P30: **Design of a virtual ground-level perimeter for assessment of the role of facial topographies for human and primate visual field limits**

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Purpose: Establish a virtual perimetric system to study variations of anatomical constraints on visual field limits in humans and primates. Methods: Virtual masks were obtained from human subjects and chimpanzee and baboon taxidermy specimens. were positioned at The masks' pupils were aligned with upright head orientation to the center of a virtual perimetric dome (radius = 50 m) developed with *Cinema 4D*. For human subjects the optimum positions of virtual floodlights (180°) along the mask's pupillary axis were identified by maximum congruence to Goldmann visual field limits. This allowed to demarcate the unobstructed rays at a) the perimetric surface, and b) on a virtual ground floor related to eye level as well as to c) „paint" facial elements obstructing the light path. Results and Conclusion: For humans the resulting virtual contours (demarcating areas of potential visibility) largely concur with large-stimulus isopters and display the limiting roles of anatomical features, in particular the nasal ridge, as well as the relatively extended ventral and temporal limits. In contrast, the facial design of chimpanzees and baboons obstructs significant portions of the ventral foreground putting them at risk during bipedal locomotion. On the other hand the lack of nasal wings in both primates leads to larger binocular overlaps (125° in chimp versus 90° in human). Thus the virtual perimeter is able to compare monocular and binocular visual field extensions and its projection on a virtual floor or other virtually (re-) constructed spaces. This may allow differentiation of visual field impairments from anatomical constraints as well as assessment of intra- and interspecific variability.

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P31: **Transduction of temporal odor patterns by ON and OFF receptor cells**

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The cockroach's olfactory system for fruit odors is subdivided, right at the level of the antenna, into antagonistically responding ON and OFF cells. The generation of a dual system of ON and OFF responses is a key process in promoting the detection of slight changes in odor concentration and in enhancing the temporal information contained in fluctuating odor signals (Tichy et al. 2005). Another, less investigated aspect concerns the accurate perception of the durations of both the odor pulses and the gaps between subsequent odor pulses. In natural environments, a wide range of size and time scales characterize the pulses and gaps in odor plumes formed downwind from an odor source. This is due to variations in the size of the odor source, the release rate, the velocity of the surrounding medium, the size of the turbulence relative to the plume diameter, and the structure of the habitat. Odor signals have on and off durations ranging from hundreds of milliseconds to several seconds, and how the nervous systems could measure such long times is unclear. We present here data that obviate the difficulties of identifying the relative timing of on and off durations by detecting temporal odor patterns without explicit knowledge of time. This is due to a direct transduction of the durations of both odor pulses and odor gaps by the excitatory signals of the ON and OFF cells evoked by them. In judging odor pulses and odor gaps by the continuous discharge of the ON and OFF cells, the information about pulse durations and time intervals is transmitted in real time; its timing, therefore, need not be encoded at all. The ON and OFF cells produce steady discharge rates when stimulated by steady fruit odor concentration. In the ON cell, the steady discharge increases with rising pulse concentration, and in the OFF cell, with falling gap concentration. The steady discharge rates of each cell can distinguish three levels of steady odor concentration, high concentration levels from moderate levels and moderate levels from low levels. Thus, the ON and OFF cells not only provide excitatory responses to prolonged pulses and gaps of fruit odor concentrations, but also the concentration is indicated continuously by the flow of activity of both types of cells. *Supported by the Austrian Science Found (P21777).*

Tichy H, Hinterwirth A, Gingl E (2005) Eur J Neurosci 22:3147-3160

P32: **Ultramicroscopy: 3D-reconstruction of Alzheimer´s Disease pathologies in intact mouse brains**

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Alzheimer´s disease (AD) is characterized by a variety of neuropathological abnormalities such as amyloid deposition in the parenchyma and the vasculature, neurofibrillary tangles, and loss of neurons and synapses (Vickers et al., 2000). Various transgenic mice have given new insights to AD´s pathology. Ultramicroscopy (UM) (Dodt et al., 2007; Becker, Jährling et al., 2008) allows us to visualize the spatial distribution of amyloid plaques in whole mouse brains.

We labelled amyloid deposits in two different mouse lines (APPPS1 (Radde et al., 2006) and APPDutch (Herzig et al., 2004)) with the dye Methoxy-X04 (Klunk et al., 2002). As UM requires specimen to be translucent, the mouse brains were rendered transparent by chemical clearing. In an ongoing study we analyse the amyloid plaque distribution in APPPS1 mice expressing amyloid plaques in dependence of age in the brain´s parenchmya.

The novel approach of double-labelling (Methoxy-X04 & Lectin-FITC staining) allows us to visualize the amyloid deposits along the cortical vasculature in APPDutch mice using UM.

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*Dodt et al, Nature Methods (2007), **4**, 331-336*
*Becker, Jährling et al., Journal of Biophotonics (2008), **1**, 36-42*
*Radde et al., EMBO reports (2006), **7**, 954-960*
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P32A: **3-D Patterning of Social Waves in the Giant Honeybee *Apis dorsata***

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This project investigates the phenomenon of ´shimmering behaviour´ in Giant Honeybees which is a main component of collective defence (Kastberger et al 2008, PLoS ONE 2008). Shimmering occurs in the bee curtain that makes up the changeable and reactive multi-layer cover of the central comb of the honeybee nests. Shimmering is evoked by visual stimuli of mainly predatory impact and may align hundreds of colony members to flip their abdomens. It is generated at distinct spots on Giant Honeybee nests and spreads in a wave-like, highly coordinated reaction within a fraction of a second over the nest. For external addressees shimmering provides dynamic visual cues which may confuse, misguide and repel potential predators.

The knowledge of how the curtain members are coupled together is fundamental for understanding of proximate causes (HOW shimmering is generated and distributed over the nest) and ultimate causes (WHY shimmering has been evolved?). In this paper we analyze one of the key attributes of shimmering-making, and investigated the time course of the movement of curtain bees in the x- (horizontal),y- (vertical) and z- (towards and off the nest surface) -direction of the nest. The data base regarded to HDTV video sequences, (to assess the x- and y-components of the movement of surface bees by image analysis, and to provide the complete information about the wave pattern from the whole nest), and to laser vibrometry (to extract the z-component of the movement of single surface bees).

We evoked shimmering by automated dummy-wasp stimulation which provided standardised velocity and direction of visual cues. Manually controlled presentations produced more forceful and complex shimmering responses; here the dummy wasp had been moved 10 cm in front of the nest surface in different directions.

Preliminary results showed that the z-components of shimmering arose to more than 1 mm off the nest surface. This shimmering strength occurred during wave sequences, and is likely caused by a summarized response of surface and sub-surface bees. Similarly, during a sequence of shimmering waves the nest portions of the bee curtain massively shifted aside. The findings let us hypothesize that shimmering would provide mechanoceptive cues to code information for sub-surface bees and thus has the capacity to alert colony members of all layers of the bee curtain at least at these spots where it occurs.

P32B: **Age index defines nest topology regarding division of labour in Giant honeybees (*Apis dorsata*)**

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Eusociality is expressed in a society by nesting traits such as brood, stored food, shared defence and the division of labour (Lindauer M, Z vergl Physiol 1952). In Giant honeybees the functional architecture of the nest reflects this principle, in particular as the 'bee curtain' displays functional regions in its 'mouth', quiescent, attachment and rim zones, as well as surface and subsurface layers.

This paper investigates age-aspects in the topology of Giant honeybee nests (*A. dorsata*). In a breeding experiment at the Bharatpur campus (Nepal) we labelled freshly hatched bees with day-specific colour pads and introduced them to a small queen-right colony. Thus we obtained the colour pattern of specimens of identified age up to 16 days. For older bees we took forager bees as reference, they turn the more black-striped the older they get (Woyke et al. Asian Bee J. 2000). Their 'age level' was interpolated between the labelled cohorts and the most black-striped foragers. This allowed us to index the age according to ten categories of abdominal colour patterns.

The findings show that in the mouth zone forager bees occurred of 'age levels' 8-10. From the mouth region off to the peripheral rims of the nest there was an age gradient towards younger bees of 6-10 days age. Defence waving (Kastberger et al., PLoS ONE 2008) occurs in the quiescent parts of the nest and is therefore up to mid age bees. Flying defenders recruited from the nest interior and mobilised to counter-attack enemies are of the same age.

P33: **Blood brain barrier integrity in a mouse model of MSA: towards mesenchymal stem cell therapy**

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Background: Multiple system atrophy (MSA) is a fatal rapidly progressive neurodegenerative disorder associated with prominent alpha-synuclein oligodendroglial inclusions. Since symptomatic therapies are only partially effective there is a growing interest in disease modifying strategies. Within the last years, the therapeutic application of mesenchymal stem cells (MSCs) in animal models of stroke, amyotrophic lateral sclerosis, experimental autoimmune encephalomyelitis and Parkinson`s disease appeared to be effective. MSCs are said to play a role in the modulation of deleterious immune responses, as well as neuronal survival by secretion of neurotrophic factors. Furthermore MSCs can be obtained from autologous tissue, thus providing an ethically justified source for transplantation. In 2007, Lee et al. reported preliminary efficacy and safety data in an open label trial of autologous MSCs delivered intravenously and intraarterially in 11 patients with MSA-C. However up to now, there is no pre-clinical experimental evidence supporting the use of MSCs as a cell therapeutic intervention in MSA. In this experimental study we therefore examine whether autologous MSCs induce functional improvement and modify neurodegeneration.

Methods: Our first step towards MSC transplantation was to analyze blood brain barrier integrity in a transgenic mouse overexpressing human alpha synuclein in oligodendrocytes. Exposure to oxidative stress by 3-nitropropionic acid (3-NP) leads to MSA-like glial and neuronal pathology in this model (Stefanova 2005). Follwing intracardiac perfusion of Evans Blue and Hoechst, blood brain barrier integrity can be determined.

Results: Our results indicate that the blood brain barrier integrity is disrupted in the combined MSA mouse model allowing systemically infused MSCs to reach MSA-like glial and neuronal lesion sites.

Conclusion: Our findings imply that systemic MSC delivery is feasible to evaluate their therapeutic efficacy in MSA mice. Our present studies are aimed at determining MSC migration in affected brain areas and elucidating their functional role.

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P34: **Role of doublecortin mRNA 3'UTR in the regulation of its expression.**

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Doublecortin (DCX) is a microtubule-associated protein present in neuronal precursors and young neurons. Expression DCX is being induced as neural stem cells adopt a neuronal phenotype and is maintained until newly generated neurons mature and get functionally integrated. Hence, DCX is an early and specific marker of neuronal differentiation.

The tight regulatory control of DCX expression is not only exerted at the transcriptional level, but at the translational level as well. Evidence for the translational regulation is provided by the observation that DCX mRNA can be find in almost every regions of the adult CNS, although expression of the DCX protein is virtually limited to neurogenic regions. Examination of the DCX mRNA revealed an unusually long 3' untranslated region (3'UTR) that might contain translational regulatory elements. In particular, 3'UTR are known to be targeted by microRNAs, resulting in silencing of gene expression. In silico comparative analysis of the DCX 3'UTR from different species revealed conserved putative miRNA binding sites. Using luciferase reporter gene constructs, we addressed the functionality of these binding sites in transfected cell culture models. Considering that a single miRNA can regulate whole group of genes simultaneously, the identification of miRNAs regulating the expression of DCX will shed light on the key genes responsible for an orchestrated neuronal differentiation and integration program.

P35: **Role of Smad7 in regulation of adult neural stem/progenitor cell pool**

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Members of the transforming growth factor (TGF)-β family of proteins modulate the proliferation, differentiation and survival of many different cell types. Neural stem and progenitor cells (NPCs) in the adult brain are inhibited in their proliferation by TGF-β and by bone morphogenetic proteins (BMPs). Recently we showed that TGF-beta suppresses the proliferation of adult neural progenitor cells both in vivo and in neurosphere cultures. TGF-beta acted by arresting the cells in the cell cycle in a reversible manner. However, it is not yet known if endogenous TGF-beta under non-disease conditions is involved in regulating neural stem cell proliferation and /or differentiation. We therefore asked whether the deletion of an inhibitor of TGF-beta signalling, Smad7, also causes reduced neurogenesis, which would be an indirect prove of TGF-beta action under physiological conditions. Here, we investigated neurogenesis in a hypomorphic mouse model for the TGF-β and BMP inhibitor Smad7, with the hypothesis that NPC proliferation might be reduced due to increased TGF-β and BMP signalling. Unexpectedly, we found enhanced NPC proliferation as well as an increased number of label-retaining cells in vivo. The enhanced proliferation potential of mutant cells was retained in vitro in neurosphere cultures. We observed both a higher sphere-forming capacity as well as faster growth and cell cycle progression. Use of specific inhibitors revealed that these effects were independent of TGF-β and BMP signalling. The enhanced proliferation might be at least partially mediated by elevated signalling via epidermal growth factor (EGF) receptor, as mutant cells showed higher expression and activation levels of the EGF-receptor. Conversely, an EGF receptor inhibitor reduced the proliferation of these cells. Our data indicate that endogenous Smad7 regulates neural stem/progenitor cell proliferation in a TGF-beta and BMP-independent manner.

P36: **Regulation of early neural differentiation marker by TGFβ1**

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The cytokine transforming growth factor β 1 (TGFβ1), a well known regulator of immune response and wound healing, plays a significant role within the central nervous system under physiological and pathological conditions. For instance, TGFβ1 was shown to promote neuronal survival and we recently demonstrated that it also strongly inhibits proliferation of adult neural stem/progenitor cells *in vitro*, as well as *in vivo* (Wachs et al. 2006). Furthermore, TGFβ1 levels were reported to be elevated under neuropathological conditions such as Huntington's or Parkinson's disease.

In a gene expression array analysis of adult neural progenitor cells isolated from the rat hippocampus, we observed that TGFβ1 treatments induced a strong upregulation of doublecortin (DCX) mRNA expression. DCX is a microtubule-binding protein involved in neuronal migration, which is specifically expressed in neuronal precursors and young neurons. Interestingly, *in silico* analysis of the human DCX promoter revealed a putative binding site for Smad3, a downstream effector of TGFβ1 signaling, suggesting that TGFβ1 directly modulates the expression of DCX.

In this study, we addressed the functionality of the putative Smad3 binding site in the human DCX promoter. The goal was to verify whether TGFβ1 can directly modulate DCX expression, or alternatively, if additional indirect pathways contributed, in parts or in totality, to the TGFβ1-associated upregulation of DCX expression. To this end, we made use of directed mutagenesis of the putative Smad3 binding site and using Luciferase-reporter assays, Smad3-associated chromatin immunoprecipitation (Smad3-ChIP) and as well as direct Smad3 inhibitor Sis3.

Wachs FP, Winner B, Couillard-Despres S, Schiller T, Aigner R, Winkler J, Bogdahn U, Aigner L, Transforming growth factor-beta1 is a negative modulator of adult neurogenesis. J Neuropathol Exp Neurol. 2006 Apr;65(4):358-70.

P37: **The Identity of Cells Grown in Neurospheres: Species Matters**

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Adult neural stem/progenitor cells (NSPCs) are currently explored for their potential therapeutic use in degenerative brain diseases. NSPCs can be obtained from different CNS regions and in vitro expanded for several passages as neurospheres (NS). NS cultures are widely used in the field and considered as in vitro model for NSPCs and for neurogenesis. Starting out with the notions that NS cultures do not consist of a homogenous cell population and display species depending differences, we aimed to characterize the cell identity of NS derived from adult brains of different species. NS were characterized regarding: 1) phenotype (markers expression); 2) cell fate potential; 3) differentiation potential; 4) electrophysiology and 5) growth factors response. Results showed that NS cells derived from rat brains highly expressed the oligodendrocyte precursor marker O4 and spontaneously differentiate into oligodendrocytes after growth factor removal. Mouse NS cells, in contrast, highly co-express GFAP/Nestin in both situations, suggesting an astrocyte/stem cell phenotype. Moreover, mouse and rat NS exhibited a very distinct response pattern to neurogenic, astrogenic and oligodendrogenic stimuli. In summary, our data indicate that rat NS display "oligodendroglial progenitor" properties, while mouse NS demonstrate more of an "astrocyte/stem cell-like" identity. Our findings might explain some inconsistencies among studies using NS cultures from different rodent species and might be relevant for future studies on adult human derived NS cultures.

P38: **Characterization of neural progenitor cells by means of single cell PCR, whole cell patch clamp and immunocytochemistry**

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In addition to the possibility of developing new medical approaches towards the therapy of diseases of the central nervous system (CNS) the study of neural stem / progenitor cells offers insights into the complex mechanisms of cell differentiation and maturation. The phenotypic changes in this process occur on the one hand on the level of the expression of transcription factors (TFs) and on the other hand on the functional level.

In the present work immunocytochemistry was carried out to reveal the cell phenotype on the level of TFs and whole cell patch clamp was performed to characterize the cells with respect to their electrical properties. Single cell PCR was employed after electrophysiological measurements in order to further describe the neural progenitor cells phenotypes in more detail. In addition various cell culture conditions were used to analyze their effect in the cell phenotype.

Electrophysiological measurements showed that the membrane resting potential (MRP) of the progenitor cells was more depolarized (-28 mV) in comparison to mature neurons of the CNS (-70 mV). On the level of membrane currents two populations of progenitor cells have been distinguished according to their sensitivity towards the maxi calcium-activated potassium channel blockers tetraethylamonium (1 mM) and iberiotoxin (50 nM). This difference of outward currents was also detected among cells which were cultured in various culture conditions.

With immunocytochemistry and single cell PCR the expression of several markers among the progenitor cells such as neuro D, glial fibrillary acidic protein and nestin have been found depending on the various culture conditions. Both, single cell PCR and electrophysiology revealed the cells phenotype mostly consisting of neuro D positive cells with a depolarized MRP.

Taken together these results demonstrate that the combination of phenotypic and functional analysis is a valuable approach to characterize neural progenitor cells in vitro.

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P39: **Presence of alarin-like immunoreactivity at different stages of murine embryonic development**

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The galanin family of neuropeptides consists of 3 members: galanin, galanin-like peptide (GALP) and a splice variant of GALP called alarin. Recently, galanin was identified in undifferentiated mouse embryonic stem cells as one of the most abundant transcripts (1) and galanin-like immunoreactivity was observed in mesenchyme, heart, bone and neural crest derivates at embryonic day E10 to E15 (2).
The aim of the present study was to determine the distribution of alarin during the most important developmental stages in murine embryogenesis. Immunohistochemistry (IHC) was performed on paraffin embedded tissue of NMRI mice at stage E14.5 and E17 using an affinity purified polyclonal anti-alarin antibody. Strong alarin-like staining at both developmental stages was detected in the intermediate zone of the cortex, choroid plexus, epidermis and cartilage of the developing bones. Further staining was observed in the eye lens at stage E14.5 and in the leptomeninges as well as in neurons of the spinal cord at E17.
These data indicate a function of alarin in morphogenesis and a developmental role of this peptide in ectodermal an neural crest origin tissues in the mouse embryo. Whether Alarin has a growth and/or differentiative role, remains to be demonstrated.

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P40: **Altered autonomic reactivity in a psychopathological mouse model of innate trait anxiety and comorbid depression**

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There is a lack of diagnostic biomarkers in anxiety and depression research that can be used as reliable indicators of beneficial drug treatment. Markers of autonomic regulation such as heart rate (HR), HR variability (HRV) and blood pressure (BP) have been widely studied revealing that HRV is consistently reduced in anxious and depressed patients, whereas HR and BP show rather inconsistent results. To date, the vast majority of animal studies investigating autonomic function in anxiety and depression involve "non-pathological" models, and there is still a need to study autonomic markers in these models that more closely resemble the human pathology. Thus, the aim of the present study was to investigate parameters of autonomic regulation such as body temperature, HR and HRV in mouse lines selectively bred for high innate anxiety (HAB) and normal anxiety-like behavior (NAB), respectively.

Basal (home cage) conditions did not reveal any difference in HR and body temperature between HABs versus NABs. However, during classical cued fear conditioning, in which an initial innocuous conditioned stimulus is associated with an aversive unconditioned stimulus, an enhanced tachycardic response and increased freezing were observed in HABs compared to NABs during initial extinction training. The difference in HR but not freezing between HABs and NABs disappeared when the extinction training was performed in the home cage, suggesting that HR does not seem to be a reliable autonomic biomarker, i.e. with sufficient discriminatory sensitivity. HRV analysis revealed reduced HRV in HABs during extinction training, independent of the paradigm used. The reduced HRV was accompanied by increased fear expression in HABs. The finding of reduced HRV in this psychopathological mouse model parallels symptoms observed in patients suffering from anxiety and depressive disorders. In order to determine whether and how successful drug treatment will affect HRV, the NK1 receptor antagonist L-7822429, known to elicit both anxiolytic and antidepressant properties in HABs, was chronically applied via the drinking water. Treated HAB mice showed reduced fear expression and normalization of HRV during extinction training when compared with untreated HABs. Finally, a gene array study indicated an upregulation of the choline transporter gene (CHT1) in HABs. CHT1 has been associated with reduced HRV and increased susceptibility for depression in human subjects. These findings demonstrate that HRV serves as useful diagnostic biomarker that is sensitive to chronic antidepressant treatment in HABs.

P41: **Automated evaluation of fear response in rodents: TopoWatch**

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In a fear conditioning (FC) paradigm animals learn to fear an initially neutral stimulus (CS; either cue such as a tone or context) by pairing it with an aversive unconditioned stimulus (US; e.g. mild footshock). Subsequently, characteristic behavioral and autonomic responses are displayed indicating fear in the presence of the CS. In rodents, fear can be recognized by freezing behavior which is defined by the complete absence of movements except those associated with breathing. Although the manual analysis of freezing behavior by a well-trained observer blinded to experimental groups usually gives reliable results, there may be inter-personal differences in the absolute values and the procedure is very time consuming. Therefore, we aimed to design and validate a software which detects freezing behavior of rats and mice in a sensitive, objective, automatic and quick manner. An algorithm was developed that calculates the pixel to pixel difference between two consecutive time frame intervals with high time resolution (33 frames/s) and scores freezing behavior when the number of altered pixels is below a certain threshold (freezing threshold). The ability of the new software named TopoWatch to accurately detect freezing behavior of rodents was tested by comparing with manually quantified data from diverse cued and contextual conditioned fear experiments. By adjusting the freezing threshold, data obtained by TopoWatch highly correlated with the data of a manual evaluator in experiments involving different species (e.g. rats and mice) and strains (e.g. C57Bl6 and CD1 mice). Furthermore, TopoWatch was compared with a commercially available automated system using infrared beams for detecting freezing behavior. It was found that the commercial system detected considerably high levels of "freezing" e.g. in low fear periods of experiments, while both the observer and TopoWatch revealed very low or no freezing on these occasions. This discrepancy may be due to small movements of the animals that cannot be sensitively detected by infrared beams. Using TopoWatch the analysis of a 40 min experiment takes approximately 10 min and it is possible to run four analyses in parallel. Taken together, we present a novel software which appears to be highly sensitive, objective, reliable and fast in scoring freezing behavior of rodents. This software may also be applied to other behavioral tests for automated analysis of specific behavioral readouts.

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P42: **Spatial and temporal regulation of brain-specific snoRNAs in response to an emotional challenge**

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Brain-specific small nucleolar RNAs (b-snoRNA) represent a group of non-coding RNAs exclusively expressed in the central nervous system of various species, including man. Up till now, their functions are poorly understood, but the murine homologues MBII-52, MBII-85 and MBI-36 have been associated with regulation of serotonin 5-HT2C receptor expression. Thereby, the 5-HT2C serotonin receptor is known to be involved in learning and memory and diverse stress-related psychopathologies, thus, suggesting a role of b-snoRNA in higher brain functions. Therefore, we aimed to investigate whether and how a single exposure to an emotional challenge would affect b-snoRNA expression in the murine brain and, thus, also might influence 5-HT2C levels. Mice were subjected to a forced swim test for 6 min and gene expression of MBII-48, MBII-52, MBII-85 and MBI-36 was determined 2, 6, 12 and 24 h later by northern blot analysis in specific brain areas known to be involved in stress and emotional processing. Forced swim stress did not modulate b-snoRNA expression in the striatum and cerebellum. In contrast, compared to unstressed conditions, levels of MBII-52 and/or MBII-85 were increased in the olfactory bulb, prefrontal cortex, amygdala and hindbrain as well as of MBI-36 in thalamic/hypothalamic regions. Moreover, expression levels of MBII-48 were down-regulated in the prefrontal cortex, while in the hippocampus reduced levels of MBII-85 could be observed. A similar reduction of expression levels was found in the hindbrain for MBI-36. These effects were observed either 2, 6, 12 h or 24 h after the stressor indicating transient, central induction of b-snoRNAs by an aversive emotional stimulus. Specifically, 24 h after the challenge, the expression of MBII-52 and/or MBII-85 was modulated in the prefrontal cortex, hippocampus and amygdala. Experimental groups did not differ in the times spent floating, swimming or struggling during the forced swim test excluding the possibility that the observed regulation of b-snoRNAs may be due to behavioural inter-group differences. Taken together, the present findings provide first evidence of a spatial and temporal regulation of specific b-snoRNAs, in particular of MBII-52 and MBII-85, in response to an emotional challenge suggesting functional significance of these b-snoRNAs in stress-processing. Changes observed 24 h later in the prefrontal cortex and amygdala, two key brain regions in anxiety processing, may reflect long-lasting, functional consequences to stressful experiences and, thus, a possible relation to stress-related psychopathologies such as anxiety disorders or depression. Yet, a causal role of b-snoRNAs in stress-related mechanisms remains to be demonstrated.
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P43: **Circadian modulation of fear conditioning in a mouse model of trait anxiety.**

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A number of anxiety disorders are characterised by an impaired ability to inhibit learned fear responses. In line with human psychopathology, we recently described impaired fear extinction in a rat model of high trait anxiety. Here, we aimed to characterise fear-related behaviour in the corresponding mouse line. Male and female CD-1 mice selectively bred for either high (HAB) or normal (NAB) anxiety-related behaviour underwent a classical cued fear conditioning paradigm during both day (inactivity phase) and night (activity phase). Irrespective of circadian cycle or gender, both HAB and NAB mice learned to fear an initial neutral stimulus (CS) coupled to a mild footshock on day 1 as indicated by enhanced freezing behaviour. However, on the next day (day 2), HAB but not NAB mice displayed high freezing levels in response to the CS suggesting increased consolidation of fearful memories in HAB mice. High CS-induced freezing levels were still present on day 3, 24 h after extinction training. Investigation into possible neurochemical differences between HABs and NABs revealed differential α1, α3 and β3 GABA-A receptor subunit distribution in the central amygdala, a main fear output region. The behavioural results suggest that HAB mice may be useful to test novel fear-inhibiting drugs. Indeed, chronic treatment with a selective neurokinin-1 receptor antagonist during the inactive phase decreased fear expression to CS assessed 24 hours after conditioning. A similar effect was observed following acute intracerebral application of neuropeptide S prior CS-only exposure. These findings suggest that both treatments exert an anxiolytic-like effect on fear expression in HAB mice. To conclude, HAB mice seem to be a potential animal model to study behavioural and neurochemical aberrations of enhanced fear as well as its pharmacological reversal. Although within session extinction was observed during the active but not inactive phase in HAB mice, fear expression during extinction retrieval 24 h later was at a similar level, independent of the light-dark phase. Thus, training in the active phase may alter learning but not consolidation of extinction in HABs. Since genetic approaches are limited in the rat, the HAB mouse line may be particularly powerful for the functional analysis of candidate genes and gene products underlying both trait anxiety and fear. Supported by the Austrian Science Fund FWF NFN-S-102 (NS)

P44: **Fear and Anxiety after NPY deletion**

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Neuropeptide Y (NPY) is a 36 amino acid peptide. NPY and its receptors are involved in numerous physiological and pathophysiological processes including processing of emotions like anxiety and stress-coping (depression).

Here we used NPY knockout (NPY KO) mice for investigating changes in motor activity (home cage activity - HCA, open field test - OFT), anxiety (elevated plus maze - EPM, light/dark test - LDT, open field test - OFT, stress-induced hyperthermia - SIH) and depression-like behavior (tail suspension test -TST, force swim test - FST, novelty suppressed feeding - NSF) as well as fear conditioning and extinction.

NPY KO mice show less motor activity in the dark phase of the light/dark cycle during Home Cage activity. In the EPM and LDT, the time of NPY KO mice spent on the open arms and in the light box was reduced respectively and during stressed-induced hyperthermia (motor activity independent) the increase in rectal temperature was higher in NPY KO mice indicating increased anxiety. NPY KO mice exhibited reduced stress coping ability during TST and FST and in the NSF the latency to food consumption was prolonged, suggesting increased depression-like behavior of NPY KO mice. The role of NPY in expression and extinction of conditioned fear was investigated by the fear conditioning paradigm (0.7mA, 68dB 10 kHz, 5 times). NPY knockout mice acquire higher freezing levels during fear conditioning and exhibit impaired extinction of conditioned fear in comparison to WT.

Our present findings using NPY KO mice indicate an anxiolytic and anti-depressant action of endogenous NPY. In addition NPY inhibits expression and facilitates extinction of conditioned fear. Supported by FWF (NFN, S10204)

P45: **Plastic changes in parahippocampal regions of the rat after kainic acid-induced epilepsy**

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The subiculum - the main output region of the hippocampus - remains largely preserved in temporal lobe epilepsy (TLE) and therefore may be importantly involved in the generation of epileptic activity arising from the hippocampus. It receives input from hippocampal sector CA1 and projects to deep layers of the entorhinal cortex (EC), septum, mamillary nuclei, and the amygdala. To characterize possible functional changes in the subiculum and EC of KA-injected rats, we investigated histopathological and neurochemical alterations in these regions by immunohistochemistry and *in situ* hybridization using neuropeptides and Ca^{2+} binding proteins as markers for different neuronal subpopulations.

The number of parvalbumin-ir GABAergic interneurons was reduced by 30% in the subiculum and by 50% in the deep entorhinal layers, respectively. Increased (or even *de novo*) expression of NKB mRNA was observed in pyramidal neurons of sector CA1 and the subiculum. In these regions and in the deep EC, increased fiber labeling for NKB was measured. NPY mRNA expression and immunolabeling of fibers was present in principal neurons throughout the hippocampal formation at late intervals after KA-induced seizures.

Our data indicate activation of pro-epileptogenic and also of antiepileptic mechanisms. Loss of parvalbumin-ir GABAergic basket- and axo-axonic cells may result in decreased inhibition of pyramidal neurons. Expression of the prokonvulsive neuropeptide NKB in principal neurons of sector CA1 and the subiculum may contribute to the generation of epileptic seizures. On the other hand, increased expression of NPY in axon terminals of pyramidal neurons and interneurons indicates neurochemical and morphological plasticity resulting in activation of endogenous anticonvulsive mechanisms.

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P46: **Status epilepticus induced epileptogenesis in the rat traced by video-supported EEG-telemetry**

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Temporal lobe epilepsy (TLE) is the most common focal epilepsy in humans and represents the most frequent type of epilepsy in adults. It is often associated with severe neuronal damage in the hippocampus including sectors CA1 and CA3 and interneurons of the dentate gyrus. In contrast, the subiculum, the major out-put area of the hippocampus, is mostly preserved. It has therefore been proposed that the subiculum may critically contribute to seizure generation. We now established a video-supported EEG-telemetry system in freely moving rats to evaluate the relationship between the occurrence of spontaneous seizures and neurochemical changes in the kainic acid (KA) model of temporal lobe epilepsy.

Male Sprague-Dawley rats (ca. 250 g) were implanted with reusable biopotential transmitters (EA-F20, DSI) connected to an EEG-electrode placed supra-durally above the hippocampus. After one week, rats were injected with KA (10 mg/kg, i.p.) and behavioral seizures were rated during the initial status epilepticus (SE). At the same time monitoring by video-supported EEG (Dataquest A.R.T. analysis software, DSI) was started and continued 24 hrs/day for 3 months.

Onset, frequency and severity of generalized seizures in SE varied between KA-injected rats and ranged from rating 1-4: total number of rats was 37 rats. Two rats responded to KA with rating 1, three with rating 2, seventeen with rating 3 and fifteen rats with rating 4. Two hours after the first generalized seizure diazepam i.p. was applied as anticonvulsant and interrupted EEG- and behavioral seizures. SE recurred 4 ± 1.1 hrs later and lasted again for several hours (12 ± 2.4 hrs). All KA-injected rats that sustained generalized seizures in SE became epileptic (rats with initial SE rating of 2 and more): mean onset of spontaneous seizures was 13 ± 2.3 days after KA-induced SE. Frequency and severity (in seizure rating) of spontaneous seizures increased during the first two of three months after KA-induced SE from 1 to 12 seizures/week in rats with an initial SE rating of 4. In conclusion, our data indicate that a KA-induced SE leads to fast progression of epilepsy characterized by high frequencies of spontaneous seizures.

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P47: **Sensory coding of predator cues by insect prey under high background noise levels**

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The detection of predators is crucial for survival and lifetime reproductive success. However, such detection is seriously affected by background noise when insects communicate by sound, and at the same time listen to echolocation calls of one of their main predators, i.e. insectivorous bats. The noise can amount to 70 dB SPL in the nocturnal rainforest where our study was performed. Here we present results of neurophysiological studies performed both in the field and laboratory demonstrating the coding of bat-like sound by a local auditory neuron (omega cell) of a tropical katydid. Echolocation calls of *Saccopteryx bilineata*, a bat species abundant in Central America, were broadcast at three different rates (10, 18 and 24 Hz) to insect preparations together with natural background noise pre-recorded at different rainforest locations on BCI (Panama). At physiological sound levels of the echolocation call, the omega cell fired brief bursts of action potentials at high rates in response to each bat sound pulse. Repetition rates of 18 and 24 Hz resulted in a moderate response decrement with successive bursts, but at the same time in a suppressed response to background noise, leaving the maximum firing rate in the bat-associated bursts unaffected. Inter-spike-interevals (ISIs) calculated from omega cell firing demonstrated a characteristic pattern in response to bat calls. This pattern was rarely found in outdoor recordings of the same cell in a bat-free habitat, whereas it dominated outdoor recordings performed in rainforest gaps where aerial hunting bats were active, strongly suggesting that katydids are able to detect echolocating bats despite high background noise levels.

This hypothesis was tested by developing a spike-interval-based bat detector that was optimized by using recordings of omega cells from insects being exposed to a forest clearing with high bat activity and to rainforest noise alone. This neuronal “bat detector” detected the presence of bats even in noise with high reliability (high rate of hits, low false-alarm rate).

P48: **Neuroethology of Background-Masking in Acoustic Communication of Crickets**

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An animals´ ability to detect predators or conspecifics acoustically, and to localize them is strongly reduced by masking background noise (1). This is true in particular in environments with high species diversity and strong overlap of signals in time and frequency. For example, in the tropical rainforest of Panama about 50 nocturnal species of cricket compete for the acoustic communication channel, resulting in a background noise level of 70 dB SPL. Here we studied in a combined behavioural and neurophysiological approach the ability of female crickets to detect and localize a calling song in masking noise. We used two noise-paradigms to investigate the masking: 1) a mixture of nine calling songs of rainforest crickets, differing in carrier frequency (CF) from 3 to 6.8 kHz and 2) a single heterospecific calling song, with a CF identical to the best frequency of the females´ hearing system. The duty cycle (% signal on-time) of this masker was varied from 10 – 90%.

With the mixed-species masker the ability of females to detect and orient towards the conspecific signal was only reduced significantly, when the signal-to-noise-ratio (S/N-ratio) was -10. By contrast, the single-species masker was more effective in masking, since a significant reduction in phonotactic performance occurred at S/N-ratio of 0. As expected, this was dependent on duty cycle: for the 10% and 50% duty cycle, the S/N-ratios were -15 and -5, respectively. The described reduction in phonotactic performance was correlated neurophysiologically with a decline in the spike pattern representation in the response of the studied interneuron (AN1), which is of prime importance for this behaviour (2). The temporal pattern of the stimulus was surprisingly well preserved up to S/N-ratios of about 0, most probably as a result of the neuronal mechanism of selective attention, previously described in crickets and katydids (3).

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P49: **Structural features and subunit composition of GABAergic synapses in basolateral neurons of the amygdala**

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γ-Aminobutyric acid (GABA), acting through $GABA_A$ receptors, is the primary inhibitory neurotransmitter in the central nervous system. $GABA_A$ pentameric receptors mediate fast inhibitory neurotransmission in the brain. The receptors are composed of two α, two β and one γ subunits and 16 $GABA_A$ receptor subunits have been identified (α1-α6, β1-β3, γ1-γ3, δ, ε, θ and π) in mammals. This multiple composition confers a huge variety of biophysical and pharmacological properties to these receptors. The amygdala is critically involved in emotional behavior and it plays a major role in conditioned fear as well as in physiological and pathological anxiety. The amygdala can be divided into several distinct nuclei and cortical areas based on the respective connectivity and chemo-architectonics. The basolateral nucleus of amygdala (BA) includes mainly includes glutamatergic principal neurons (85-90 %), whose activities are powerfully controlled by interneurons (McDonald, 1992). Recently it was suggested that the anxiolytic action of benzodiazepines is mediated by $GABA_A$ receptors containing the α2 and/or α3 subunit.

Although the physiological properties of different $GABA_A$ receptors in the BA have been intensely investigated, very little is known about their subunit composition and distribution in this area. In order to analyse the detailed subcellular localization of $GABA_A$ receptors in the BA, we have exploited the novel freeze fracture replica immunolabelling technique (SDS-FRL). Immunogold particles for α2 subunit tend to concentrate in clusters of intramembrane particles (IMP) on the protoplasmic face (P-face) of the plasma membrane, indicating that these IMP clusters represent α2 positive GABAergic synapses. The average size of labelled IMP clusters was 0.041μm^2, however, there was a large variability in the synaptic size (0.003 to 0.149 μm^2). The labelling density in the IMP clusters corresponding to the postsynaptic density of GABAergic synapses was 501 gold particles/μm^2.

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P50: **Characterization of $GABA_A$ receptor alpha3 subunit interaction with gephyrin**

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Synaptic $GABA_A$ receptors are frequently associated with submembraneous aggregates of gephyrin. A gephyrin interaction site within the $GABA_A$ receptor alpha2 subunit intracellular loop has recently been characterized by using hippocampal cultures and biochemical methods. The presence of a linker protein between the $GABA_A$ receptor and gephyrin could be ruled out by overlay assays, where only the two interaction partners were present in the biochemical experiment (Tretter et al 2008). Here we report that similar overlay assays and the yeast two-hybrid system also reveal a strong interaction of the $GABA_A$ receptor alpha3 subunit with gephyrin. Only a minor population of $GABA_A$ receptors contains the alpha3 subunit, mostly in monoaminergic neurons and in the nucleus reticularis thalami (nRT). In $GABA_A$ receptor alpha3 subunit knockout mice there is no up-regulation of other alpha subunits in nRT neurons, while the clustering of the gamma2 subunit is disrupted. Gephyrin forms large aggregates in the nRT neurons of these mice, that in most cases are not associated with synaptic sites (Studer et al 2006). These results are again an example of a dominant role of an alpha subunit in synaptic $GABA_A$ receptor and gephyrin clustering.
Here we determine the interaction site between the alpha3 subunit and gephyrin.

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P51: **Engineering of a benzodiazepine binding site into $GABA_A$ receptors composed of αβ subunits**

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$GABA_A$ receptors belong to the family of the Cys-loop pentameric ligand gated ion channel receptors (pLGIC). Members of this superfamily use similar sequences and functional domains to establish receptor structure and functionality. Two alpha (α), two beta (β) and one gamma (γ) subunit form the functional heteropentameric receptor. GABA and benzodiazepines bind at subunit interfaces of the predominantly β-stranded extracellular domains, which are formed by a plus side of one and a minus side of another subunit. The two GABA binding sites are located each at a β+/α- interface, and high affinity binding of classical benzodiazepines requires the minus side of the γ2 together with the plus side of one of the α1, 2, 3, or 5 subunits. Here, we investigate whether it is possible to engineer a benzodiazepine binding site into α1β3 receptors. To achieve this task, a γ2- side has to be engineered into the β3- side. For that, we introduce structurally homologous amino acids of the γ2- side, that are important for benzodiazepine binding, into the β3- site of an α1β3 $GABA_A$ receptor. The mutated recombinant receptors are then investigated by the two electrode voltage clamp method in Xenopus laevis oocytes. First results indicate that zolpidem, a benzodiazepine which binds with high affinity at the α+/γ- interface, can potentiate GABA currents of the mutated, but not of the wild-type α1β3 receptors.

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P52: **Expression and purification of $GABA_A$ receptor extracellular domains**

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$GABA_A$ receptors are the major inhibitory neurotransmitter receptors in the central nervous system and are composed of five subunits that can belong to different homologous subunit classes. 19 subunits have so far been identified, but their exact molecular structure is still unknown. Structural analysis of the $GABA_A$ receptor by crystallization or NMR techniques requires milligram amounts of pure receptors. The extracellular domains (ECDs) of $GABA_A$ receptor subunits are especially interesting for structural analysis because they form the benzodiazepine and the GABA binding sites of these receptors. In my thesis, the ECDs of several $GABA_A$ receptor subunits will be cloned and over-expressed in *E.coli*, attached to a poly-histidine-tag or to fusion proteins. My aim is to generate mg amounts of pure extracellular domains of selected subunits of the $GABA_A$ receptor as monomer, homo- or hetero-oligomer for subsequent structural analysis. To achieve this goal, large-scale expression of these domains as soluble proteins in *E.coli* and appropriate multi-step purification strategies, including Immobilized Metal-ion Affinity Chromatography (IMAC), Size Exclusion Chromatography (SEC), etc., will be employed. Crystallization and structure determination by X-ray crystallography or solution NMR studies will then be accomplished in collaboration with international partners. By now, small-scale expression and IMAC purification of alpha1, beta3, and gamma2 subunit ECDs have successfully been performed. We are currently in the process of up-scaling protein expression and optimizing purification. Also, co-expression experiments of alpha1 and gamma2 ECDs have been done and a possible hetero-oligomerization of the proteins was demonstrated by IMAC-based co-purification of poly-histidine tagged and untagged constructs, respectively. Results so far obtained will be presented. Financial support by the EC FP7 integrated project grant HEALTH-F4-2008-202088 is gratefully acknowledged.

P53: **Identification of a novel binding site at $GABA_A$ receptors**

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The $GABA_A$ receptor is the site of action of a variety of pharmacologically and clinically important drugs such as benzodiazepines, barbiturates, steroids, anesthetics and convulsants. For most of these compounds the exact site of interaction with $GABA_A$ receptors is not known. The majority of $GABA_A$ receptors are composed of two alpha (α), two beta (β) and one gamma (γ) subunit. So far only three binding sites have been unambiguously identified in the extracellular domain. The two GABA binding sites are located at the two $\beta+/\alpha-$ interfaces and the benzodiazepine binding site is located at the $\alpha+/\gamma-$ interface. So far, no compounds are known that interact with the $\alpha+/\beta-$ interface. Such compounds would interact with receptor subtypes that so far cannot be specifically modulated by drugs and have the potential for being receptor subtype-selective. Here we focus on the $\alpha+/\beta-$ interface of recombinant $\alpha1\beta3\gamma2$ or $\alpha1\beta3$ receptors. We investigate which compounds are able to bind to this interface, and which amino acid residues are important for drug binding and the drug effect. To achieve our goals, we have established a steric hindrance strategy. We mutate single amino acid residues to cysteines located at the $\alpha+/\beta-$ interface. The mutated recombinant receptors are investigated by the two electrode voltage clamp method in Xenopus Oocytes in the absence or presence of various compounds. A reduced drug effect on the receptor would indicate that the mutated residue might be near the drug binding site. Covalent labeling of the cysteines with bulky cysteine reactive reagents would then block the accessibility of the drug and further reduce the drug effect. Using this technique we were able to identify ligands that specifically interact with this novel binding site at the $\alpha+/\beta-$ interface of the $GABA_A$ receptor.

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P54: **Fibroblast growth factor receptor 1 trafficking in glial cells**

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Fibroblast growth factor receptors (FGFRs) are among the most important membrane-bound tyrosine kinases promoting tumor growth. Down-regulation of these receptors represents one treatment strategy for multiple tumors, including glioblastoma. Signal attenuation of receptor tyrosine kinases may be achieved by promoting receptor endocytosis with subsequent transfer of the receptor to lysosomes for degradation.

We are investigating the degradation pathway of FGFR1 from the membrane to the lysosome in the human glioma cell line U373. In order to analyze the trafficking pathway of the receptor, U373 cells were transfected with FGFR1-constructs fused to the fluorescent marker protein eGFP. The cells were subsequently treated with FGF and/or the lysosomal inhibitor leupeptin followed by imaging with a confocal microscope (Leica SP5, Zeiss AxioObserver with ApoTome). Receptor internalization and trafficking in different endosomal compartments (early/late endosomes and recycling endosomes) was shown in fixed and living cells.

FGF-2 treatment of U373 cells leads to a decrease in cell size and an increase in BrdU incorporation, demonstrating the proliferation-stimulating effect of the FGFR-ligand FGF on U373 cells. The application of FGF-2 or leupeptin alone and the combination of both leads to an increase of receptor internalization followed by an accumulation of FGFR1-containing lysosomal vesicles, shown with the lysosomal marker LysoTracker as well as with the cotransfected Lamp1-DsRed-fused plasmid.

Visualization of FGFR internalization and degradation and its interference by various biomolecular substances leads to a new understanding of receptor trafficking.

P55: **Transgenic overexpression of ABCD2 in microglia/ macrophages in X-linked adrenoleukodystrophy mice**

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X-linked adrenoleukodystrophy (X-ALD) is an inherited neurodegenerative disorder with different phenotypes including highly progressive childhood cerebral ALD with lethal inflammatory demyelination and slowly progressive adrenomyeloneuropathy with adult onset. Mutations in the *ABCD1* gene encoding Adrenoleukodystrophy protein (ALDP), a member of a peroxisomal subfamily of membrane-spanning transporters, cause the disease. ALD-related protein (ALDRP) encoded by *ABCD2*, another member of this subfamily, can compensate for *ABCD1*-deficiency in experimental models. Ubiquitous transgenic overexpression of ALDRP corrected the phenotype of *Abcd1*-deficient mice. To determine if overexpression of *ABCD2* in microglia/macrophages would be sufficient to compensate for loss of *Abcd1,* we generated transgenic mice designed to express ALDRP under control of the Ionized calcium-binding adaptor molecule 1 (Iba1) promoter. The Iba1-hALDR transgene construct was assembled and confirmed by sequence analysis. Microinjection of the construct into C57BL/6 oocytes resulted in two transgene positive founder animals among offspring, which were bred with wild type C57BL/6 mice to secure the transgenic lines. Integration status of the transgene was studied by Southern blot analysis. The expression pattern of the transgene is currently being evaluated by RT-PCR and northern blot analyses; *in situ* hybridization is planned to establish the cell-type specificity. Transgenic males were bred with *Abcd1*(+/-) females to generate Iba1-hALDRP transgenic/*Abcd1*(y/-) double mutants, Iba1-hALDRP transgenic and *Abcd1*(y/-) single mutants, respectively, as well as wild type mice to determine the effect of microglia/macrophage-specific overexpression of ALDRP on the *Abcd1*-deficient phenotype (accumulation of very long-chain fatty acids in tissues, late onset microglia activation and axonopathy in the spinal cord). The results of these studies should help to establish which of the X-ALD symptoms can be cured by microglia/macrophage-specific overexpression of ABCD2 in the mouse model.

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P56: **In the voltage-gated Na channel double mutant gating perturbation analysis reveals a high conformational stability of the domain IV S6 segment**

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The S6 segment of domain IV (DIV-S6) of voltage-gated Na channels is considered to be a key player in gating and local anesthetic drug block. Thus, mutations at several sites of DIV-S6 are known to substantially alter the channel's inactivation properties. For a comprehensive analysis of the kinetic role of DIV-S6 in fast inactivation we performed a cysteine scanning analysis of sites 1575-1591 in the DIV-S6 of the rNav1.4 channel. These mutations were engineered into the wild-type channel and into rNav1.4 carrying the mutation K1237E. K1237 is located in the P-loop of domain III and mutations at this site have dramatic effects both on permeation and gating properties. Hence, K1237E most likely causes a complex conformational change of the channel. We sought to explore whether K1237E changes the pattern of gating perturbations produced by the serial cysteine replacements in DIV-S6. The constructs were expressed in Xenopus laevis oocytes and studied by means of two electrode voltage-clamp. The half-point of availability following a 50 ms conditioning prepulse (V05) was -44 ± 1 mV and -51 ± 1 mV in wild-type and K1237E, respectively ($P < 0.001$) . Most serial amino acid replacements by cysteines in DIV S6 produced shifts in V05, both in the background of wild-type and in the background of K1237E, ranging from +17 ± 1 mV to -9 ± 2 mV. A plot of the shifts in V05 by single DIV-S6 mutants relative to wild-type versus the shifts in V05 by double mutants relative to K1237E showed a significant positive correlation ($R= 0.72$, $P=0.002$). This indicates that the general pattern of gating perturbations in DIV-S6 is not affected by K1237E, suggesting a high conformational stability of the DIV-S6 segment during the fast inactivated state.

P57: **Role of L-type voltage-gated calcium channels in the excitability of primary hippocampal neurons - Part I (modulation of depolarisations)**

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L-type voltage-gated calcium channels (LTCCs) come in four different isoforms ($Ca_v1.1$-$Ca_v1.4$) and are broadly distributed in various tissues. Studies especially from the last decade demonstrate important roles in skeletal and smooth muscle contraction and cardiac function, insulin secretion, as well as acoustical and optical sensation. In the CNS, only $Ca_v1.2$ and $Ca_v1.3$ are expressed abundantly. These two LTCC isoforms differ in localisation and biophysical properties (e.g. a more negative activation threshold for $Ca_v1.3$ than for $Ca_v1.2$). The aim of this study was to identify, if and in which manner LTCCs contribute to neuronal voltage changes. Therefore, current-clamp experiments were performed on hippocampal neurons in culture using the perforated patch-clamp method to record membrane voltage. The neurons were continuously superfused and LTCC activity was modulated by application of the dihydropyridines BayK 8644 (LTCC agonist) and isradipine (LTCC antagonist), all in the presence of TTX. Activation of LTCCs was provoked by incremental current injections to depolarise the neurons experimentally beyond the LTCC activation threshold. Doing so (n=86), LTCC mediated effects appeared as enhanced depolarisations (21 % of the neurons), hyperpolarising sags (17 %), or oscillatory activity (49 %). No LTCC effect was observed in the remainder of cells (13 %). In 21 % of the responsive neurons, a contribution of LTCCs in the depolarisations was already present under control conditions and was further enhanced by BayK, whereas in 79 % of these neurons LTCC contributions became only evident after administration of BayK. The recordings were further analysed and frequency distributions of the onset of LTCC effects indicate two peaks of activation thresholds. This was seen under control conditions (n=34) with peaks at -41 mV and ~-33 mV versus -53 mV and -41 mV in the presence of BayK (n=53; note that BayK shifts channel activation to more hyperpolarised potentials). Our data suggest that both $Ca_v1.2$ and $Ca_v1.3$ channels operate in primary hippocampal neurons, and indicate diverse modality of coupling of LTCC-mediated Ca^{2+}-influx to both hyperpolarising and depolarising conductances. These results provide the basis for studies using $Cav1.2^{-/-}$ and $Cav1.3^{-/-}$ mice, which should allow allocation of the channel subtypes to the observed effects of LTCC activation (supported by FWF grant P19710).

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P58: **Role of L-type voltage-gated calcium channels in the excitability of primary hippocampal neurons - Part II (afterpotentials)**

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L-type voltage-gated calcium channels (LTCCs) were shown to have several distinct roles in excitable cells. For example, Ca^{2+}-influx via LTCCs was shown to be an important component of the excitation-transcription coupling. Additionally, LTCC-mediated Ca^{2+} influx can also affect membrane voltage and discharge properties, either directly or via activation of Ca^{2+}-dependent conductances (coupling).

Here we evaluated the modality of LTCC coupling in the hippocampus. The methodological details of this study are given in the abstract of Geier et al. In brief, neurons were depolarized by pulse shaped, 8 s-long incremental current injections in the presence of TTX. BayK 3 µM was applied to enhance, isradipine 3 µM to block LTCC activity, and the effects on afterpotentials, occurring after the current pulse, were analysed. After-hyperpolarisations (AHPs) or after-depolarisations (ADPs) were typically seen already under control conditions, and increased with BayK. In the majority of neurons, the afterpotential was an AHP. AHPs could be blocked by apamin 100 nM or UCL1684 30 nM, which indicates the involvement of SK channels. SK channel blockade also revealed that the hyperpolarising afterpotentials can represent pure AHPs (n=10, BayK: -1.37, 1.30; BayK + apamin: 0.11, 1.00) or an overlap of AHPs and ADPs (n=5, BayK: -3.25, 8.05; BayK + apamin: 18.99, 22.72). In neurons which showed a depolarising afterpotential, co-application of BayK and apamin as compared to BayK alone revealed a further enhancement of the ADP and therefore a concomitantly occurring AHP. Reduction of external [Na^{+}] to 1.5 mM led to a significant diminishment of the ADP-area and -amplitude, suggesting that Ca^{2+}-activated non selective cation (CAN) channels mediate the primary excitatory LTCC-coupling (n=7, BayK + apamin = 2.22, 3.99; low Na^{+}/BayK + apamin = 0.34, 0.68; all values are given as median afterevent area normalized to control). Variation of the current pulse duration (t = 0.1 to 8 s) and evidence obtained with close-to-LTCC-threshold depolarisations indicate that less LTCC-activation may be required to induce ADPs than to evoke AHPs. Our data suggest that in primary hippocampal neurons (i) LTCCs couple to both SK and CAN channels, (ii) that coupling efficiency differs among the two Ca^{2+}-dependent conductances, and (iii) that both coupling modes can operate in parallel (supported by FWF grant P19710).

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P59: **Effect of hydrogensulfide (H_2S) on ion currents of *Helix pomatia* neurons**

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Hydrogen sulfide (H_2S) known as a very toxic gas has been found to be produced endogenously in living cells and to exert physiological functions. In brain tissue H_2S has been found in relatively high concentrations from 40 to 150 µM and therefore is expected to play a role in modulating electrical activity and synaptic transmission. Production of H_2S has also been detected in invertebrate tissue (Julian et al., 2002; Gainey & Greenberg, 2005; Watanabe et al., 2006) but its role in the modulation of nervous activity has not been investigated. In the present work the effect of H_2S on ion currents from snail neurons (U-cells, *Helix pomatia*) was studied.

NaHS (sodium hydrosulfide) was used as H_2S donor in the extra cellular bath solution. Membrane currents were measured using two-electrode voltage clamp techniques.

NaHS increased total potassium (K^+) outward currents in a dose-dependent manner with a half-maxium increase at 50 µM. The outward currents at a holding potential of -60 mV primarily consists of two components – the voltage-dependent delayed- and the calcium-activated K^+ current – both or only one of these currents may be affected. From our experimental design it appeared that the Ca^{2+} activated K^+ current was increased by H_2S in time dependent manner at potentials around +20 mV. Current-voltage plots revealed that outward currents were shifted to the left on the voltage axis. For further investigations the K^+ outward current was measured in Ca^{2+}-free solution and dibromo-BAPTA as a calcium chelator was injected into the cells to prevent the Ca^{2+} activated current component. The residual delayed K^+ outward current still increased during NaHS (100 µM) perfusion.

To examine the H_2S effect on the Ca^{2+} inward current, K^+ channels were blocked by tetraethylammonium (TEA, 45 mM) and 4-aminopyridine (4-AP, 5 mM) and measurements were carried out in sodium free solution. At a holding potential of -40 mV only the high voltage activated Ca^{2+} current was activated (KITS et al., 1996). H_2S (100 µM) decreased the Ca^{2+} inward current in a time dependent manner.

In current clamp experiments H_2S decreased the amplitude of action potentials, probably by activating K^+ outward currents and/or by inhibiting the Ca^{2+} inward current, both are expected to decrease the action potential amplitude.

In conclusion our experiments indicate that H_2S increases both, the calcium-activated- and the voltage-dependent K^+ outward currents and decreases the Ca^{2+} inward current which alters action potentials.

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P60: **Effect of hydrogen sulfide on the activity of calcium-activated potassium channels of rat pituitary tumor cells**

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Hydrogen sulfide (H_2S) is the third gasotransmitter found to be produced endogenously in living cells and to exert physiological functions (Wang R., 2002). The aim of present study was to evaluate the effects of H_2S on maxi calcium-activated potassium **(**BK) channels from rat GH_3 pituitary tumor cells using patch clamp techniques (whole cell, exercised single channel recordings). Application of sodium hydrogen sulfide (NaHS, 300 μM) as H_2S donor in whole cell experiments hyperpolarized the membrane potential of GH_3 cells associated with a decrease of membrane resistance and truncated action potentials. The addition of NaHS caused a significant and reversible increase of membrane outward currents elicited in response to a series of voltage pulses. Extracellular application of tetraethylammonium (1 mM) or iberiotoxin (100 nM) decreased outward currents and suppressed the effect of NaHS. In addition, after inhibition of Ca^{2+}-channels with nifedipine or applying an extracellular solution, containing EGTA (5 mM) but no Ca^{2+}, we still observed the increase of outward currents by NaHS. These data indicate a direct increase of calcium-activated potassium currents by NaHS.

In single channel recordings NaHS induced a reversible increase in channel open probability by 244% of control value. Single channel conductance were not affected by NaHS. It is known that H_2S is a reducing agent and is able to modulate ion channels by a redox reaction of cysteine residue(s) in the channel protein (Zhao W., 2001, Kawabata A., et al., 2007). Using reducing (dithiothreitol, 1 mM) or oxidizing (thimerosal, 0.1 mM) agents it was found that the action of H_2S could be linked to reducing action of H_2S on sulfhydryl groups of the channel protein. We hypothesize that the stimulatory effect of H_2S on BK channels leading to truncation of action potentials may in turn cause a change of growth hormone secretion in these cells.

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P61: **Acetaldehyde countervails the augmenting action of ethanol on calcium-activated potassium (BK) channel activity in pituitary (GH3) cells**

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Acetaldehyde (ACA), the primary metabolite of ethanol (EtOH) oxidation is considered to be responsible for some of the effects on the brain that have been attributed to ethanol. Evidence of catalase activity within the nervous system supports the notion of ACA production in the brain. However, the actual ACA concentrations in brain after ethanol consumption and the action of ACA on electrical activity are not established. Maxi calcium-activated potassium (BK) channels, a prominent target of EtOH, exhibit various functions like action potential repolarisation, hormone secretion, transmitter release or regulation of blood pressure. EtOH is well documented to increase BK channel activity in a dose dependent manner. We investigated the BK channel response to EtOH in the presence of ACA which reflects the physiological situation in the brain after drinking. BK single channel activity was recorded both from outside out and inside out patches and analysed using pClamp software. Cells were exposed to 30 mM EtOH and/or to ACA in concentrations from 100 µM to 10 mM from the extracellular side. Intracellular ACA (0.03 – 100 µM) was administered in the simultaneous presence of 30 mM extracellular EtOH. BK channel amplitude, open probability and mean channel open time were analysed. Extracellular ACA did not alter channel activity, whereas extracellular EtOH enhanced BK channel activity. The EtOH-related increase in channel activity was impeded by intracellular ACA in a concentration dependent manner. The half maximal ACA concentration was 435 nM. Furthermore, the augmenting effect of a 30% hypotonic solution on BK channel open probability was not blocked in the presence of ACA indicating that osmotically induced changes in BK activity are not modulated by ACA. Neither EtOH nor ACA affected BK channel amplitudes from either side of the cell membrane. Mean channel open time on the other hand was reduced by intracellular ACA.

Our results suggest an inhibitory impact of ACA on BK-activation by EtOH. Furthermore, ACA interacts specifically with EtOH at BK channels, since intracellular ACA had no effect related to hypotonic conditions. Our study shows that ACA interferes with BK activity and implies that ACA effects have to be considered carefully in the context of EtOH actions.

P62: **Ethanol induces cell volume changes and depolarizes the membrane potential of pituitary tumor cells (GH3)**

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Ethanol (EtOH) is known to affect a number of different ion channels. A prominent member targeted by EtOH is the maxi calcium activated potassium channel (BK). In the present investigation we asked whether the membrane potential of pituitary tumor GH3 cells is modulated by EtOH, which role BK channels play in this context and how EtOH changes the cell volume.

The action of EtOH on the membrane potential was examined using the whole cell configuration of the patch-clamp technique. Cell swelling was examined by measuring circumferences from entire cells by images taken every minute. 30 mM EtOH (~ 1.3 ‰) was applied in an isoosmotic or hyperosmotic fashion.

After EtOH application the membrane potential depolarized within 5 minutes by 17.1 mV from an average membrane resting potential of -44 mV under isoosmolar conditions and by 10 mV under hyperosmolar EtOH. These effects were completely reversible. Spontaneous action potentials were found in approximately 50 % of the cells and EtOH abolished them irreversibly. Cell volume changes were observed under isoosmotic EtOH conditions as cell swelling during the first 4 minutes of application followed by a regulatory volume decrease. In contrast hyperosmolar EtOH induced cell shrinking. In the presence of the BK channel blocker tetraethylammonium (1 mM) and isoomolar EtOH the membrane depolarization was more pronounced then for EtOH alone. A block of EtOH degrading catalases modulated the time course of the depolarizing EtOH effect indicating an involvement of acetaldehyde. DIDS a chloride channel blocker reduced the membrane depolarizing effect of EtOH profoundly.

Our data indicate that EtOH activates chloride as well as BK channels in GH3 cells, which may in part be related to cell volumes changes. Beside the cell volume related modulation of the membrane potentials there is an EtOH specific action on the cell membrane potential which is dependent on the presence of acetaldehyde.

P63: **The galanin-receptor subtype 3 mediates chloride secretion in the human sweat gland cell line NCL-SG3**

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The galanin family currently consists of four members, namely galanin, galanin-message-associated peptide (GMAP), galanin-like peptide (GALP) and alarin, which exert their biological effects via the hitherto three known galanin-receptor subtypes GalR1, GalR2 and GalR3. Recent studies show that this family of peptides modulates microvascular activity in the skin in accordance with their vascular localisation. In human dermal sweat glands, galanin-like immunoreactivity and galanin binding sites have been detected suggesting a putative role in sweat gland function.

In this study, we determined possible effects of galanin, GALP and alarin on ionic secretion in the human sweat gland cell line NCL-SG3 in a Ussing chamber assay. The application of GALP the basolateral side of the cells caused a dose-dependent increase of the short circuit current (Isc). A significant increase of Isc was also observed with the application of ⟨5 µM galanin, whereas alarin had no effect on the short circuit-current. These effects were not changed by the presence of the sodium channel blocker amiloride (10 µM) in the bath solution. However, in chloride-depleted solutions both galanin and GALP failed to elicit any change in Isc.

Analysis of mRNA expression by RT-PCR suggests the involment of GalR2 and GalR3 only in mediating the observed effects. Moreover, the application of SNAP 37889, a nonpeptidergic selective antagonist of GalR3 abolished the effect of galanin and GALP on Isc indicating that transepithelial chloride ion transport is mediated via GalR3 in NCL-SG3 cells.

In summary, our results show that the galanin can regulate transepithelial ion transport and fluid secretion from the eccrine secretory coil through stimulation of GalR3 in NCL-SG3 cells and demonstrate a possible important extraneural function in sweat gland physiology.

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P64: **The expression of galanin and substance P is dramatically increased in the lung of septic mice**

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Substance P (SP) has been shown to be an important mediator in lung injury during polymicrobial sepsis. SP exhibits its proinflammatory effects via increased capillary permeability and neutrophil extravasation. For the neuropeptide galanin so far no data concerning septic conditions are available. However, in murine skin, galanin has been shown to inhibit plasma extravasation induced by SP and calcitonin-gene related peptide (CGRP). This suggests galanin as an opponent of SP and CGRP in neurogenic inflammation. To study a possible role of galanin also during systemic inflammation we investigated the expression of galanin and SP mRNA in two different animal models of sepsis (bacterial lipopolysaccharide (LPS) induced sepsis and the colon ascendens stent peritonitis (CASP)). Sepsis was induced in NMRI mice by implantation of a stent in the colon ascendens (CASP) or intraperitoneal injection of LPS. Quantitative real time PCR was performed to determine the relative expression of galanin and SP mRNA. We were able to detect upregulation of the expression of galanin (34-fold) and SP (23-fold) in lungs of CASP mice compared to healthy animals. The increase of galanin expression was not found in the LPS model. A significant increased expression of both peptides was also observed in liver, spleen and kidney of CASP mice. Our data indicate differences of the two mouse models of sepsis, although, we were not able to compare the severity of the septic conditions directly. However, CASP has been reported to resemble more closely human septic conditions than the LPS model. Our results implicate that galanin plays a role in septic conditions and may have an anti-inflammatory activity in systemic inflammation similar to its functions in the skin.

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P65: **Gender-dependent differences in the BOLD-response to arithmetic tasks**

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Gender- as well as menstrual cycle-dependent differences have been observed in several cognitive tasks, and related to differences in brain activation patterns. We investigated the influence of gender and female cycle on brain activation patterns during arithmetic tasks. 16 healthy male and 16 healthy female subjects have been examined by functional magnetic resonance imaging during a two-digit number comparison task. Female subjects had a regular menstrual cycle and were scanned twice, once during the follicular and once during the luteal phase. Stimuli were varied for decade distance and unit-decade compatibility (Nuerk et al., Cognition 82: B25-B33, 2001). In incompatible items the smaller number contains the larger unit-figure. We found stronger activation of the left postcentral gyrus, inferior parietal cortex including the intraparietal sulcus bilaterally, supplementary motor area, and right inferior frontal regions for women in their follicular compared to the luteal phase. Compared to men, women during their follicular phase additionally show stronger occipital activation. Activation patterns do not differ between men and women during their luteal phase. Furthermore, we found that the compatibility effect in the default mode network was more pronounced for women during follicular compared to luteal phase and men. Thus, deactivation was stronger when solving the difficult incompatible items compared to the easier compatible items during follicular, but not during luteal phase or in men. Comparably, gender and cycle phase interact with unit-decade compatibility in analysis of reaction times and error rates. Women during their follicular phase exhibit the highest error rates compared to their luteal phase and men for compatible items, but the lowest for incompatible items. However, overall performance does not differ between men and women in different cycle phases. These findings point to a strategy shift during follicular phase.

P66: **Effects of hyperhomocysteinemia on learning, cholinergic neurons, vascularization and immunoreactive markers in vivo in rats**

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Alzheimers disease (AD) and cerebrovascular diseases share common risk factors, such as e.g. high plasma levels of cholsterol or homocysteine (Hcy). The aim of the present study was to observe the effects of hyperhomocysteinemia on cholinergic neurons, NGF levels, vascular integrity, immunological markers and spatial memory in rats. Sprague Dawley rat were fed with a special diet (+3 g/kg DL-Homocystine without folic acid) for 5 months (short) or 15 months (long), then tested in an 8-arm radial maze. Subsequently rats were perfused or brains were fresh frozen. Plasma levels of Hcy were increased in 6 and 15 month-treated rats. Learning performance was reduced in short but not in long-term Hcy-treated rats, while long-term memory performance was reduced after both short and long-term treatments. The number of cholinergic basal nucleus of Meynert neurons was decreased after short but not long-term Hcy treatment but no effects were observed on septal or striatal cholinergic neurons. Cortical acetylcholine was reduced after short and long-term Hcy treatment and cortical NGF levels were increased only after long-term Hcy treatment. Vascular integrity (laminin and RECA) was not changed after short or long-term Hcy treatment. No changes in different inflammatory markers (IL-1α, IL-6, IL-10, MIP1α, TNF-α) were observed, whereas a marked increase of rat-IgG immunoreactivity was seen after long-term Hcy treatment only. In summary, hyperhomocysteinemia caused cognitive impairment, possibly resulting from cholinergic dysfunction, while the vascular integrity was not changed. No inflammatory processes were seen, however, the increase of rat-IgG immunoreactivity in the cortex may reflect local disruption of the blood-brain barrier. While short-term hyperhomocysteinemia causes severe learning impairments and cholinergic downregulation, it seems that after long-term hyperhomocysteinemia these dysfunctions may be partly compensated. However, the impaired memory storage after long-term hyperhomocysteinemia may be accompanied by local severe damage of the blood-brain barrier.

P67: p42/44 MAPK SUPPORTS ADENOSINE RECEPTOR-MEDIATED SIGNALING IN PURINE NUCLEOSIDE-INDUCED NEUROPROTECTION OF HYPOXIC NEURONAL CELLS

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Purine nucleosides are critical mediators of physiological responses to acute and chronic hypoxia [1], however the signaling mechanisms have not yet been fully elucidated. Based on our earlier data, showing the positive impact of adenosine, guanosine and inosine 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]. In cells, which were incubated in a special cell incubator designed for maintaining low oxygen (1%), I detected increased loss of viability. Adenosine and inosine however, significantly reduced this hypoxia-evoked cell death and augmented neurite outgrowth of neuronal cells. Accompanying morphological changes, I observed an increase of p42/44 MAPK activation. Vice versa, pharmacological inhibition of the MAPK pathway severely hampered adenosine-mediated induction of cell viability and neurite outgrowth. Consistently, siRNA-mediated knockdown of p42/44 MAPK completely blocked adenosine-mediated rescue of hypoxic PC12 cells. I also studied the role of MAPK activation in primary cerebellar granule neurons. Again, siRNA-mediated knockdown severely affected purine-mediated rescue of neuronal viability after hypoxic insult. Thus, my results point to the fundamental role of p42/44 MAPK for adenosine receptor-mediated neuroprotection. Current data support the future detailed investigation of purine nucleoside-mediated MAPK activation in order to exploit the potential use of these signaling mechanisms for future ischemia/reperfusion drug therapies.

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P68: **Five out of 16 plasma signaling proteins are enhanced in plasma of patients with mild cognitive impairment and Alzheimer´s disease**

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Alzheimer´s disease (AD) is a progressive neurodegenerative disorder with characteristic neuropathological changes of the brain. Great efforts have been undertaken to determine the progression of the disease and to monitor therapeutic interventions. Especially, the analysis of blood plasma had yielded incongruent results. Recently, Ray et al. (Nature Med. 13, 2007,1359f) identified changes of 18 signaling proteins leading to an accuracy of 90% in the diagnosis of AD. The aim of the present study was to examine 16 of these signaling proteins by quantitative Searchlight multiplex ELISA in order to determine their sensitivity and specificity in our plasma samples from AD, mild cognitive impairment (MCI), depression with and without cognitive impairment and healthy subjects. Quantitative analysis revealed an increased concentration in Biocoll isolated plasma of 5 out of these 16 proteins in MCI and AD patients compared to healthy subjects: EGF, GDNF and MIP1δ (in AD), MIP4 (in MCI) and RANTES (in MCI and AD). ROC analysis predicted a sensitivity of 65-75% and a specificity of 52-63% when comparing healthy controls versus MCI or AD**.** Depression without any significant cognitive deficits did not cause any significant changes. Depressed patients with significant cognitive impairment were not different from MCI patients**.** conclusion, we detected a number of altered proteins that may be related to a disease specific pathophysiology. However, the overall expression pattern of plasma proteins could not be established as a biomarker to differentiate MCI, from AD or from depression.

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P69: **Target of Efferent Neurons of the Superior Laryngeal Nerve in the Rat**

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Purpose: Standard anatomical textbooks describe the internal branch of the superior laryngeal nerve (SLN) as an exclusively sensory nerve implying the motor innervation of the larynx being based on the external branch of the SLN (M. cricothyroideus) and the inferior laryngeal nerve (ILN, all other intrinsic laryngeal muscles). Since this was already doubted by Exner (1884) and Schwarzacher (1991) we reevaluated the target of the efferent (motor) fibers running within the internal branch of the superior laryngeal nerve by electromyography.

*Methods:*The internal branch of the right SLN of five male Wistar-rats (Harlan Laboratories, Udine, Italy) as well as five male albino rabbits (Department of Biomedical Sciences, Himberg) was identified and placed on a bipolar stimulation electrode. The SLN at its commencement from the vagus nerve and the Ansa Galeni (the anastomosis between the SLN and ILN) were cut to exclude collateral signal pathways. For EMG recordings a coaxial needle electrode was inserted into the ventrolateral part of the posterior crico-arytaenoid muscle (VLP). *Results*: In all animals distinct EMG signals were obtained from the VLP following stimulation of the internal branch of the SLN. As a negative control the recording electrode was inserted into the omohyoid muscle and stimuli were again sent via the internal branch of the SLN or EMG recording was tested from VLP after stimulation of the ansa cervicalis profunda. Electrophysiological tests together with previously performed glycogen depletion experiments as well as immunohistochemistry proved that the ventrolateral part of the posterior cricoarytaenoid muscle receives an exclusively unilateral motor innervation by the internal branch of the superior laryngeal nerve.

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P70: ENDOGENOUS DYNORPHIN IN EMOTIONAL CONTROL AND STRESS RESPONSE

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Cerebral control of stress and anxiety involves several neurotransmitter systems. Beside serotonin, noradrenaline or catecholamines, also several neuropeptide systems are considered to be involved in generating symptoms of anxiety and stress. These systems act in a circuit connecting amygdalar and hypothalamic nuclei, the pituitary and adrenal glands, regulating the physiological response via ACTH and corticosterone release.
In this study, we investigated anxiety and stress related behaviour of germ-line prodynorphin knockout (dyn-KO) mice. Male dynKO exhibited about 2-fold ambulation in the open field center. DynKO mice showed also more visits (2-fold) and more time (3-fold) spent on open arms of elevated plus maze test. Significantly higher numbers of entries, distance and time spent in open lit area (ca. 30% higher values) in light-dark test were observed in dynKO as compared to wild type mice (WT). The anxiolytic phenotype of dynKO could be mimicked by injection of the selective kappa antagonists norBNI (10 mg/kg ip) or GNTI (3 nmoles, ic) in WT. Applying the specific kappa agonist U50488H (2.5 mg/kg, ip) entirely reversed the anxiolytic phenotype of dynKO. These data are in line with reduced CRH expression in the hypothalamic paraventricular and central amygdaloid nuclei and attenuated basal corticosteron serum levels. Also stress-induced increases in corticosterone levels were less pronounced in dyn-KO mice, however did not translate into marked differences in stress-induced immobility.
In female mice, the anxiolytic phenotype is much less pronounced. Only in the elevated plus maze, exploration of open arms was increased about 2-fold. Of note is the fact, that dynKO mice lacked the drop in exploratory drive, which was evident in WT mice during proestrous und estrous. In addition, significant alterations in the forced swim test and tail suspension test were observed, which argue also for increased anxiety during proestrous and estrous in WT but not dynKO mice.

Taking together our data suggest anxiogenic effects of endogenous dynorphin. These effects are mediated by kappa opioid receptors, however not in an immediate manner. Therefore, we propose a higher order controlling level for the action of dynorphin, like regulating the expression of CRH and serum corticosterone levels, which in turn influence the behaviour of mice.

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