UNIVERSITÄT OSNABRÜCK

5. Westerberger Herbsttagung

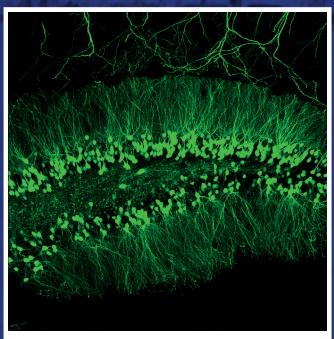
together with the

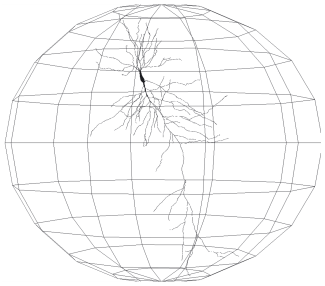
Meeting of the GBM study group "Molecular Neurobiology"

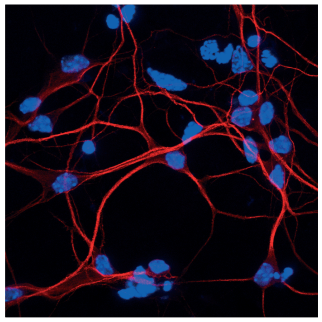
"Molecular Neurobiology: Pathways in Health and Disease"

September 16 - 18, 2010

Osnabrück
Institute of Biology, Barbarastraße 11, Main Lecture Hall

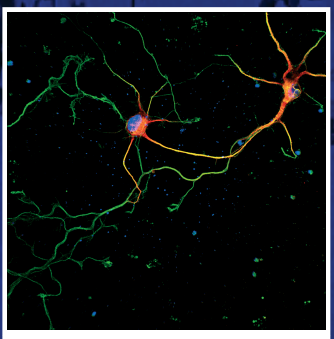


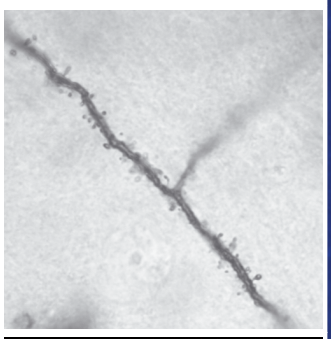


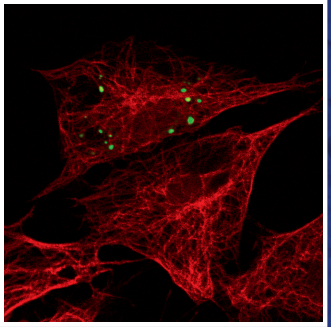


Speakers

T. Arendt (Leipzig)
D. Dieterich (Magdeburg)
P. Gordon-Weeks (London)
T. Hucho (Berlin)
W. Huttner (Dresden)
C. Klämbt (Münster)
S. Lichtenthaler (München)
E.-M. Mandelkow (Hamburg)
H. G. Nothwang (Oldenburg)
C. Richter-Landsberg (Oldenburg)
S. Schilling (Halle)
U. Schweizer (Berlin)
J. Stegmüller (Göttingen)







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This Meeting is supported by the "Sonderforschungsbereich Membranproteine: Funktionelle Dynamik und Kopplung an Reaktionsketten", Gesellschaft für Biochemie und Molekularbiologie, Universitätsgesellschaft Osnabrück, Nikon, Arivis, Sarstedt, Fermentas, Millipore, Dualsystems PAA, Eppendorf, Thermo Scientific, Diagonal, Carl Roth, Dianova, New England Biolabs, Biozol, Ionovation and Buchhandlung Jonscher

Dear Collegues,

it is our pleasure to welcome you to the **5th Westerberg Meeting** ("Westerberger Herbsttagung zu den Perspektiven der molekularen Neurobiologie") on the Science Campus of the University of Osnabrück. This year, the Westerberg Meeting is organized **together with the Study Group "Molecular Neurobiology"** of the GBM (Gesellschaft für Biochemie und Molekularbiologie). The Study Group was founded in 1977 as one of the first and defines itself as a population of scientists with the aim to approach the function of the nervous system on molecular level. The topics are discussed in a frame of biyearly Meetings, which take place at the location of the Speaker of the Study Group.

The Meeting is entitled **"Molecular Neurobiology: Pathways in Health and Disease"** and the aim for the next two days is to present new results as lectures, progress talks or posters, to facilitate contacts between established and young researchers, and to exchange information and material on all levels. A special focus of the Study Group as well as of the biyearly Westerberg Meetings is to promote young researchers. We hope to achieve this by inviting both, established researchers and young scientists, from all over Germany and neighboring countries. In addition, we are putting a special emphasis on Poster presentations including awarding with attractive Poster prizes. In the tradition of our Westerberg Meetings we try our best to provide a familiar and informal atmosphere, which can be also witnessed from the booklets of the previous Meetings. (available at **http://www.biologie.uni-osnabrueck.de/Neurobiologie/ neurobiol/General_info.html**).

In the City of Peace, **Osnabrück**, the history is apparent on every corner, with the remains of the old city wall and its watchtowers, the castle dating from the 17th century and the town hall where the Peace of Westphalia was declared in 1648. The young **University of Osnabrück**, which was founded in 1973, has about 10,000 students with more than 1000 students on the Science Campus, which is located in the "Westerberg" area.

We are delighted to welcome all of you in Osnabrück. We thank you for your contribution and participation and look forward to an exciting Meeting.

Roland Brandt
(on behalf of the organizing committee)

Program: “5. Westerberger Herbsttagung” together with the “Meeting of the GBM Study group ‘Molecular Neurobiology’”

“Molecular Neurobiology: Pathways in Health and Disease”

Thursday, September 16, 2010

From 16:00	Registration
18:30	Buffet
19:30	Opening of the Meeting and welcome addresses (Roland Brandt, Department of Neurobiology, and May-Britt Kallenrode, Vice president for Research, University of Osnabrück)
20:00	**Keynote lecture**: **Wieland Huttner** (Max-Planck-Institute of Molecular Cell Biology and Genetics, Dresden): “Cell biology of neural stem and progenitor cells”
21:00	Get together with drinks and live music (“Sturm und Klang”)

Friday, September 17, 2010

Session I: Neuronal development (Chair: Thomas Arendt)

09:00 - 09:30	Lecture 1: **Phillip Gordon-Weeks** (King's College, London, GB): “Microtubule-Actin Filament Coupling in Axon Guidance”
09:30 - 10:00	Lecture 2: **Daniela Dieterich** (Leibniz Institute for Neurobiology, Magdeburg): “Profiling neuron-glia communication with a 'click'”
10:00 - 10:15	Progress talk 1: **Anna-Lena Hillje** (Center for Molecular Biology of Inflammation, Münster): “Inhibition of TRIM32 induced neural stem cell differentiation by kinase independent function of atypical protein kinase C”
10:15 - 10:45	Coffee break
10:45 - 11:15	Lecture 3: **Christian Klämbt** (University of Münster): “Molecular control of glial migration in the Drosophila PNS”
11:15 - 11:45	Lecture 4: **Judith Stegmüller** (Max-Planck-Institute of Experimental Medicine, Göttingen): “Novel roles for cell cycle regulators in neuronal morphogenesis”
11:45 - 12:00	Progress talk 2: **Eva Bunk** (Center for Molecular Biology of Inflammation, Münster): “Cellular organization of adult neurogenesis in the common marmoset”
12:00 - 15:00	Lunch buffet and Poster viewing (Poster presentation: 13-14 for even numbers; 14-15 for odd numbers)

Session II: Maintenance and plasticity of the nervous system (Chair: Christiane Richter-Landsberg)

15:00 - 15:30	Lecture 5: **Hans Gerd Nothwang** (University of Oldenburg): “Cation chloride cotransporters: reciprocal regulation and structural organization”

15:30 - 16:00	Lecture 6: **Stephan Schilling** (Probiodrug AG, Halle): "Glutaminyl cyclases and pyroglutamate-modified peptides - potential functions in neurophysiology and neurodegenerative diseases"
16:00 - 16:30	Coffee break
16:30 - 17:00	Lecture 7: **Ulrich Schweizer** (Charité Universitätsmedizin, Berlin): "Selenium-containing proteins in neurobiology"
17:00 - 17:30	Lecture 8: **Tim Hucho** (Max-Planck-Institute for Molecular Genetics, Berlin): "Nociceptive signaling modules in pain sensitization"
17:30 - 17:45	Progress talk 3: **Kirsten Oesterwind** (Frankfurt University Medical School): "Participation of hematopoietic cells in Cre mediated recombination of Purkinje neurons"
17:45 - 18:15	Poster viewing (incl. business meeting)
18:15 - 18:30	Poster prize
From 18:30	Dinner buffet and social program (night watch tour starts at 22:00 at the old city hall)

Saturday, September 18, 2010

Session III: Neurodegeneration (Chair: Phillip Gordon-Weeks)

09:00 - 09:30	Lecture 9: **Thomas Arendt** (Paul-Flechsig-Institut für Hirnforschung, Universität Leipzig): "The 'Dr. Jekyll and Mr. Hyde concept' of Alzheimer´s disease - The link between plasticity and neurodegeneration"
09:30 - 10:00	Lecture 10: **Eva-Maria Mandelkow** (Max-Planck-Arbeitsgruppe für strukturelle Molekularbiologie, Hamburg): „Tau Pathology in Alzheimer Disease: Lessons from inducible cell models and transgenic mice"
10:00 - 10:15	Progress talk 4: **Anne Gauthier-Kemper** (University of Osnabrück): "The frontotemporal dementia mutation R406W blocks tau's interaction with the plasma membrane and affects tau distribution in an annexin A2-dependent manner"
10:15 - 10:45	Coffee break
10:45 - 11:15	Lecture 11: **Stefan Lichtenthaler** (Deutsches Zentrum für Neurodegenerative Erkrankungen, München): "When ADAM needs activation - Metalloproteases in Alzheimer's disease"
11:15 - 11:45	Lecture 12: **Christiane Richter-Landsberg** (University of Oldenburg): "Cell stress and cell death: Inclusion body formation in oligodendrocytes"
11:45	Closing remarks

Abstracts:

Oral Presentations

Keynote lecture

Cell biology of neural stem and progenitor cells: lissencephalic versus gyrencephalic cortex

Véronique Dubreuil, Anne-Marie Marzesco, Denis Corbeil, Michaela Wilsch-Braeuninger, Jennifer L. Fish, Federico Calegari, Judith Schenk, Denise Stenzel, Simone A. Fietz, Iva Kelava, Lilla M. Farkas, Christiane Haffner, Yoichi Kosodo, Alessio Attardo, Felipe Mora-Bermudez, Wieland Huttner

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Key words: neural stem cells, neurogenesis, neocortex

Our group studies the cell biological mechanisms of neurogenesis in the context of mammalian brain evolution, specifically the proliferation versus differentiation of neural stem and progenitors cells. In the developing rodent cortex, two principal classes of these cells can be distinguished, (i) progenitors dividing at the ventricular, i.e. apical, surface of the ventricular zone (VZ), called apical progenitors (APs, i.e. neuroepithelial cells and radial glial cells), and (ii) progenitors dividing in a more basal, abventricular location, notably the subventricular zone (SVZ), called basal progenitors (BPs, also called intermediate progenitors). Focusing on the embryonic mouse cortex, we have been studying the following issues:(1) the neurogenic lineage from APs and BPs to neurons, and the underlying molecular machinery, using the marker Tis21;(2) the role and machinery of interkinetic nuclear migration;(3) apical-basal cell polarity, cleavage plane orientation and symmetric versus asymmetric division of APs and BPs;(4) the role of the microcephaly gene Aspm in symmetric AP divisions;(5) apical membrane constituents, notably the cholesterol binding protein prominin-1/CD133;(6) prominin-1–bearing extracellular membrane particles relased into the ventricular fluid from the midbody and primary cilium of APs;(7) the basal process of APs in mitosis;(8) the role of cell cycle length in stem and progenitor cell proliferation versus differentiation.We have recently extended our investigations from the lissencephalic mouse model to species developing a gyrencephalic cortex. We find that progenitors in the outer SVZ (OSVZ) of human and ferret maintain a basal process contacting the basal lamina. This epithelial feature allows integrin-mediated, repeated asymmetric divisions of OSVZ progenitors, which provides a basis for neocortical expansion.

Supported by: DFG(SPP 1109, HU 275/7-3; SPP 1111, HU 275/8-3, SFB/TR 13, B1!SFB 655 A2!CRTD!Fonds der Chemischen Industrie!BMBF!NGFN-2, SMO RNAi, 01GR0402, PRI-S08T05!

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O-01

Microtubule-Actin Filament Coupling in Axon Guidance

Philip R. Gordon-Weeks

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A diverse range of cellular processes including cell division, directed cell motility, neuritogenesis and growth cone pathfinding depend on the regulated interaction between dynamic microtubules and actin filaments. The molecular mechanisms mediating this interaction, the proteins involved and how they are regulated, are now being discovered. In growth cones, dynamic microtubules interact with the actin filaments within filopodia and this interaction depends on the direct binding of the +TIP protein EB3, located on the plus-end of microtubules, and the F-actin-binding protein drebrin, bound to the proximal ends of filopodial actin filaments. Disruption of this interaction impairs growth cone formation and the extension of neurites but its role in growth cone pathfinding has yet to be determined. Domain mapping analysis of drebrin has revealed the presence of two F-actin-binding domains whose properties explain the specific location of drebrin to parallel actin filament bundles. In the adult nervous system drebrin regulates F-actin dynamics in dendritic spines and loss of drebrin from spines is causal to the loss of dendritic spines that underlies cognitive impairment in diseases such as Alzheimer's. Future work will focus on the regulation of drebrin in these different cellular contexts.

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O-02

Profiling neuron-glia communication with a 'click'

Anke Müller (1), Oliver Kobler (2), Ines Erdmann (1), Ulrich Thomas (2), Daniela C. Dieterich (1)

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Key words: metabolic labeling, proteomics, astrocytes, *Drosophila melanogaster*, *in situ* visualization

Dynamic protein synthesis is a common feature of cells to react to changes in their environment and is, therefore, of particular interest. To enable labeling of newly synthesized proteins, we use a recently introduced technique, which is called BONCAT (bioorthogonal non-canonical amino acid tagging). Here, the use of the azide-carrying methionine surrogate azidohomoalanine (AHA), which can be integrated into proteins by the endogenous protein synthesis machinery, allows coupling to affinity or fluorescent alkyne-tags via copper-catalyzed click chemistry, and, thus, facilitates the isolation or in situ detection of newly synthesized proteins. To identify changes in protein synthesis in mammalian heterologous cell culture systems, we developed GINCAT (genetically introduced non-canonical amino acid tagging). Different amino acid residues of the methionyl-tRNA-Synthethase (MetRS) were exchanged to enable the incorporation of the modified amino acid azidonorleucine (ANL) and the subsequent tagging by click chemistry. A single amino acid substitution within the binding pocket for methionine of MetRS was found to be most effective for ANL incorporation into proteins, and was further used to analyze any cellular and morphological consequences of ANL integration into proteins. Incorporation of ANL is specific for cells carrying the mutated enzyme. These results prompt the idea to use GINCAT and BONCAT for the analyses of cell specific proteomes in a complex cellular environment such as glia-neuron co-cultures, and, therefore, may improve our understanding of neuron-glia communication and interaction both *in vitro* and *in vivo*. As a demonstration of the suitability of this approach we use mammalian neuron-glia primary cocultures as well as the Gal4-UAS expression system in *Drosophila melanogaster* to express the mutant LtoG-MetRS fused in astrocytes, or in neurons, glia and in muscle cells, respectively.

Supported by: DFG Emmy Noether Program and SPP1172 to DCD

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O-03

Molecular control of glial migration in the Drosophila PNS

Marion Silies and Christian Klämbt

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Coordinated cell migration is a key feature during the development of multicellular organisms. This is of particular importance within the peripheral nervous system where migrating glial cells follow growing axons into the periphery. Axonal outgrowth and glial migration thus have to be well coordinated, but the underlying molecular mechanisms are unknown. Here we demonstrate that a subcellular, axonal gradient of the Ig-superfamily cell adhesion protein Fasciclin 2 (Fas2) allows glial migration in the Drosophila PNS. The homophilic adhesion protein Fas2 interacts with a GPI-linked glial Fas2 isoform. Glial migration is initiated along axonal segments that present low Fas2 levels but stalls in axonal domains decorated with high levels of Fas2. We further demonstrate that the graded distribution of Fas2 in axons is brought about by a novel function to the anaphase promoting complex / cyclosome (APC/C) co-activator Fizzy-related/Cdh1 (Fzr/Cdh1). In fzr mutants the graded distribution of Fas2 is lost. Fzr/Cdh1 is required in postmitotic motor neurons Fzr/Cdh1 and acts through the APC/C to non-autonomously allow glial migration. Our data suggest an elegant mechanism in which modulation of adhesion protein expression through Fzr/Cdh1 creates a subcellular gradient of adhesiveness to coordinate glial migration with axonal growth.

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O-04

Novel roles for cell cycle regulators in neuronal morphogenesis

Judith Stegmüller

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Axon growth is a crucial event that ensures correct wiring in the brain. Growing evidence implicates the ubiquitin proteasome system (UPS) in neurodevelopment. We found that the E3 ubiquitin ligase Cdh1-Anaphase Promoting Complex (APC) plays an important role in axon growth control. By targeting the transcriptional regulator SnoN for proteasomal degradation, Cdh1-APC suppresses axon growth. Since Cdh1-APC activity and regulation has been well studied in mitotic cells, where the E3 ligase acts as a pivotal cell cycle regulator, we reasoned that regulatory mechanisms of Cdh1-APC might be conserved in neurons. Here, we examined the mutual regulation of two cell cycle regulators in axonal morphogenesis, Cdh1-APC and the Cullin1-based SCF complex FBXO31-SCF (Skp1/Cullin1/F-box protein complex). We found that Cdh1 associates with FBXO31 and that FBXO31 is degraded in a proteasome dependent manner. Using an RNAi approach, we acutely knocked down FBXO31 in neurons and determined axonal length. While we did not find a difference between a non-functional RNAi plasmid and control, we found a significant decrease in axonal length with two functional FBXO31 RNAi plasmids. In addition, we found in gain-of-function analyses that FBXO31 promotes axon growth. Also, an FBXO31 form that lacks the F-box does not stimulate axon growth, suggesting that E3 ligase activity is required for FXBO31's axon growth control. In epistasis analyses, we determined that FBXO31 acts downstream of Cdh1-APC. Taken together, we have identified FBXO31-SCF as a novel component of the Cdh1-APC pathway of axonal morphogenesis.

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O-05

Cation chloride cotransporters: reciprocal regulation and structural organisation

Hans Gerd Nothwang

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Cation-chloride cotransporters (CCCs) play pivotal roles in the nervous system and sensory organs. The two main branches consist of the Cl^- extruders KCC1 to KCC4 and of the Cl^- inward transporters NKCC1, NKCC2, and NCC. NKCC1 and KCC2 are the most important members of CCCs in the central nervous system, as they set the $[Cl^-]i$ in neurons. Their activity is therefore crucial for the action of GABA and Glycine. Because of their opposite effect on intracellular Cl^- concentration, the activity of these transporters requires a precise and coordinated regulation. In this work, we investigated cellular mechanisms that contribute to their regulation. Furthermore, mutational analyses of KCCs were performed to gain insight into their structural organization. Biochemical analyses revealed that membrane-rafts regulate the activity of NKCC1 and KCC2 in a reciprocal way. NKCC1 is mainly localized in membrane-rafts. In contrast, KCC2 is localized both in membrane-rafts and non-membrane rafts in the mature brain. Disruption of membrane rafts activated KCC2, whereas NKCC1 was mainly inactivated. Transport inactive KCC2 in the immature brain is exclusively localized in membrane-rafts. Structure-function analyses of the large extracellular loop (LEL) revealed different roles of evolutionary conserved cysteines in KCC2 and KCC4. Mutation of all four cysteines abolished KCC2 transport activity, whereas the same mutation in the paralogous KCC4 had no effect. Analyses of chimera demonstrated that the LEL of KCC2 requires the parental backbone for proper function, whereas the LEL of KCC4 acts autonomously.

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O-06

Glutaminyl cyclases and pyroglutamate-modified peptides – potential functions in neurophysiology and neurodegenerative diseases

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Pyroglutamate (pGlu)-modified Aβ peptides are found in amyloid deposits in sporadic and inherited Alzheimer's disease. Similar to AD, the inherited neurodegenerative disorders Familial British and Danish dementia are also characterized by accumulation of pGlu-modified amyloid in plaques. We could show, that N-terminal truncation and formation of pGlu at the N-Terminus increases the toxicity of the peptides and speeds up Aβ aggregate formation. An enhanced hydrophobicity of N-truncated Aβ most likely accounts for a rapid oligomer formation and increased potency of N-truncated Aβ to interfere with hippocampal LTP. Our *in vitro* and *in vivo* studies provided evidence for a slow Glutaminyl cyclase (QC, EC 2.3.2.5) catalyzed cyclization of N-terminal glutamic acid, suggesting a crucial role for generation of pGlu-Aβ peptides. To further substantiate the role of pGlu-Aβ in mouse models, we treated novel mouse models of AD with QC-inhibitors and generated QC-transgenic and knock-out mice for cross-breeding and evaluation of potential target-related side effects. The data suggest that QC-catalyzed pGlu-formation can be suppressed without expectation of severe side effects, which provides potential for development of a treatment paradigm to prevent Aβ- and inflammation-driven pathophysiology in AD, FDD and FBD.

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O-07

Selenium-containing proteins in neurobiology

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Key words: selenoproteins, epilepsy, interneurons, gaba, parvalbumin

Proteins containing the rare amino acid selenocysteine (Sec) are called selenoproteins. Sec is incorporated into proteins co-translationally, and is encoded by the UGA codon. Hence, Sec is the 21st proteinogenic amino acid in mammals. A decicated metabolic pathway consisting of re-coding factors and biosynthetic factors for Sec have been identified. Among selenoenzymes, glutathione peroxidases (GPx), thioredoxin reductases (TrxR), and iodothyronine deiodinases (Dio) are the best known. We have inactivated the gene encoding selenoprotein P (SePP), the major Se transport protein, and discovered a novel role of Se for brain function. Cerebral Se deficiency is associated with neurological phenotypes including seizures and ataxia. We wanted to define (i) whether neurons require selenoprotein expression, (ii) which selenoprotein(s) is/are most important, and (iii) explore the possible pathomechanism. Therefore, we abrogated the expression of all selenoproteins in neurons by genetic inactivation of the tRNA[Ser]Sec gene. Cerebral expression of selenoproteins was significantly diminished in the mutants and histological analysis revealed progressive neurodegeneration. Developing interneurons failed to specifically express parvalbumin (PV) in the mutants. Electrophysiological recordings, before overt cell death, showed normal excitatory transmission, but revealed spontaneous epileptiform activity consistent with seizures in the mutants. We then analyzed mice lacking neuronal expression of the Se-dependent enzyme GPx4. Again, the number of PV+ interneurons was reduced. A similar phenotype is found in Sepp$^{-/-}$ mice. A selective loss of PV+ neurons is observed in schizophrenic patients and rodent models of schizophrenia. Interestingly, one animal model involves administration of an inhibitor of GSH synthesis – the cofactor of GPx4. We are thus following the hypothesis that metabolic insufficiency in selenoprotein expression may contribute to neuropsyciatric disease in humans.

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O-08

Nociceptive signaling modules in pain sensitization

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Progress in identifying novel extracellular mediators and ion channels involved in pain sensitization is tramendous. In contrast the understanding of the underlying signaling network establishing and maintaining sensitization is still rather sparse. We now attempted to identify biochemically and cell biologically novel aspects of sensitization signaling. Recently we characterized the binding site of tubulin with the C-terminus of TRPV1. Now, molecular modeling identified, that the phosphorylation of S800 at TRPV1 by PKCe should interfere with binding. Indeed, phosphorylation and binding appear as exclusive events. In the cellular context activation of the PKCe signaling pathway by e.g. Estrogen ort he GPR30-selective derivate G-1 results in rapid morphological change. This change is based on a strong destabilization of the microtubular network. Destabilization was restricted purely to TRPV1 expressing cells and even further to the phosphoratability of TRPV1-S800. We therefore suggest the dynamic regulation of the microtubule-TRPV1 interaction as novel functionality of sensitization signalling.

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O-09

The 'Dr. Jekyll and Mr Hyde concept' of Alzheimer´s disease – The link between plasticity and neurodegeneration

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Key words: Alzheimer´s disease, neurodegeneration, neuroplasticity, cell cycle, cell death

Higher cerebral functions are based upon a dynamic organization of neuronal networks. To form synaptic connections and to continuously re-shape them in a process of ongoing structural adaptation, neurons must permanently withdraw from the cell cycle. In other words, synaptic plasticity can only occur on the expense of the ability to proliferate. This mechanism of synaptic plasticity, i.e. of structural stabilization and labilization underlaying a life-long synaptic remodelling are largely based on external morphoregulatory cues and internal signaling pathways that non-neuronal cells have phylogenetically been acquired to sense their relationship to the local neighbourhood and to control after development is completed proliferation and differentiation in the process of tissue repair and regeneration. Here, we put forward the hypothesis that differentiated neurons after having withdrawn from the cell cycle are able to use those molecular mechanisms primarily developed to control proliferation alternatively to control synaptic plasticity. The existence of these alternative effector pathways within a neuron, puts it on the risk to erroneously convert signals derived from plastic synaptic changes into positional cues that will activate the cell cycle. This cell cycle activation potentially links synaptic plasticity to cell death. Preventing cell cycle activation by locking neurons in a differentiated but still highly plastic phenotype will, thus, be crucial to prevent neurodegeneration.

Supported by: Supported by the European Commission, the German Federal Ministry of Education and Research (ERA-Net Neuron; ProGen; Neuron-48-038_01EW0907) and the Sächsisches Staatsministerium für Wissenschaft und Kunst (4-7531.50-02-0361-07/2 and BBZ "Theranostik"-WI803-14494).

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O-10

Tau toxicity in cell and animal models

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Key words: Tau, neurofibrillary tangles, microtubules, Alzheimer's disease, neurodegeneration

Tau pathology is a hallmark of AD and other tauopathies. In AD, Tau is changed in several ways (e.g. phosphorylation, missorting, aggregation), but the causes of neuronal degeneration are uncertain. Several pathways of Tau-induced toxicity have been proposed: (1) Detachment of Tau from microtubules with subsequent breakdown of microtubules, the tracks of axonal transport. (2) Aggregation into paired helical filaments. (3) Clustering of Tau on microtubules and inhibition of axonal transport. (4) Cleavage of Tau into toxic fragments. (5) Toxic functions of modified Tau. (6) Over- or understabilization of microtubules. We generated several cell and transgenic mouse models, based on Tau mutants derived from FTDP17 mutants as well as mutants inducing structural changes, to distinguish between these possibilities. Some cell models display traffic deficits upon expression of Tau, due to reduced activity of motor proteins moving along microtubules. Other cell models show that toxicity of Tau is induced by enhancing the beta-propensity of Tau, which leads to proteolysis and aggregation, whereas anti-aggregation Tau variants are not toxic. Aggregation of Tau is reversible by inhibitor compounds or by switching off Tau expression. Analogous observations are made with inducible transgenic mice, where Tau variants with high beta-propensity lead to Tau aggregation, toxicity, and decay of neurons. The exogenous Tau species can even poison endogenous mouse Tau and lead to co-aggregation of both species. Here, too, the process is partly reversible by switching off the expression of exogenous Tau.

Supported by: MPG, DFG, KNDD, Metlife Award, EU/Memosad.

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O-11

When ADAM needs activation - metalloproteases in Alzheimer's disease

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The amyloid precursor protein (APP) undergoes constitutive shedding by a protease activity called α-secretase. This is considered a key mechanism preventing the generation of the Alzheimer's disease amyloid-β peptide (Aβ). α-secretase appears to be a metalloprotease of the ADAM family, but its identity remains to be established. Using a novel α-secretase-cleavage site-specific antibody we found that RNAi-mediated knock-down of ADAM10, but surprisingly not of ADAM9 or ADAM17, completely suppressed APP α-secretase cleavage in different cell lines and in primary murine neurons. Other proteases were not able to compensate for this loss of α-cleavage. This finding was further confirmed by mass-spectrometric detection of APP cleavage fragments. Surprisingly, in different cell lines the reduction of α-secretase cleavage was not paralleled by a corresponding increase in the Aβ-generating β-secretase cleavage, revealing that both proteases do not always compete for APP as a substrate. Instead our data suggest a novel pathway for APP processing, in which ADAM10 can partially compete with γ-secretase for the cleavage of a C-terminal APP-fragment generated by β-secretase. We conclude that ADAM10 is the physiologically relevant, constitutive α-secretase of APP.

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O-12

Cell stress and cell death: Inclusion body formation in glial cells

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Key words: oligodendrocyte, astrocyte, proteasome, apoptosis, heat shock proteins

Proteasomal dysfunction has been implicated in neurodegenerative disorders and during aging processes. In frontotemporal dementias (FTD), e.g. corticobasal degeneration (CBD) and progressive supranuclear palsy (PSP), oligodendrocytes, the myelin forming cells of the CNS, are specifically damaged. Characteristic pathological features are filamentous tau inclusions containing heat shock proteins (HSPs) and ubiquitin. Also, tau-positive tufted astrocytes and astrocytic plaques are typical for PSP and CBD, respectively. The present studies were undertaken to elucidate the molecular mechanisms underlying inclusion body formation in glial cells. Proteolytic stress in cultured rat brain oligodendrocytes, applied by the proteasome inhibitor MG-132, causes heat shock protein induction, tau-ubiquitination and the recruitment of ubiquitin to protein aggregates in oligodendrocytes, and leads to the induction of apoptotic cell death. Activation of the mitochondrial pathway is involved in the apoptotic process, which can be prevented by the broad caspase inhibitor zVAD-fmk. In contrast to oligodendrocytes, cultured astrocytes show resistance to the treatment with proteasomal inhibitors and did not reveal severe cytotoxic responses. In astrocytes, protein aggregates, containing the small HSPs (αB-crystallin and HSP25) are formed and the microfilament network appeared disorganized, without affecting mitochondrial integrity. This effect was reversible after removing MG-132 from the culture medium, indicating that aggregate formation is not toxic in astrocytes but provides a rescue mechanism. Astrocytes, in contrast to oligodendrocytes, constitutively express a high level of HSP25, which exerts protective effects against proteasomal stress. Furthermore, macroautophagy is activated by Mg-132 and involved in aggresome clearance in cultured astrocytes. Hence, cell type specific stress responses contribute to the varying cellular pathological features observed in tauopathies.

Supported by: Deutsche Forschungsgemeinschaft

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Abstracts:

Poster Presentations

The organizers ask the poster presenters to be at their poster on:

Friday, September 17, 2010

13-14 for even numbers

14-15 for odd numbers

P-01

Asymmetric cell division of human neuroepithelial-like stem cells

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One important aspect in the field of stem cell biology is to elucidate the mechanisms which control the decision whether progenies of somatic stem cells are maintained as stem cells or whether they become committed to differentiate. In principal two different settings have been identified that can control such decisions, the process of asymmetric cell division and the stem cell niches. For the most investigated mammalian stem cells, the hematopoietic stem and progenitor cells (HSPCs), it has been elaborated that they reside in special niches, which are required for their maintenance. In addition, due to the identification of asymmetrically segregating proteins, we confirmed that HSPCs also have the ability to divide asymmetrically. Other asymmetrically dividing stem cells are found within the developing neural tube. In contrast to early stages of CNS development, during which neuroepithelial stem cells expand by symmetrical cell division, they switch to asymmetrical cell division during later stages. Since we can recapitulate certain aspects of neural development, expansion and differentiation in induced pluripotent stem cell (iPSC)-derived neuroepithelial-like stem cells, we evaluate whether these cells also perform asymmetric cell divisions *in vitro*. To this end, we use similar methods as we previously applied to detect asymmetrically segregating proteins in dividing HSPCs. The strategy and our ongoing results will be presented.

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P-02

Amyloid beta antibody treatment protects from spine loss and tau-dependent cell death in organotypic slice cultures from ArcAbeta mice

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Key words: Alzheimer's disease, Abeta, Tau, immunotherapy, dendritic spines

Alzheimer's disease (AD) is the most common age-related dementia characterized by the presence of extracellular aggregates of the amyloid beta peptide (Aβ) and intracellular accumulated hyperphosphorylated tau protein. Anti Aβ immunotherapy is one of the most promising disease modifying therapies for AD. Here we analyze the effect of Aβ antibody administration in organotypic hippocampal slice cultures from transgenic mice expressing human APP with the Swedish (KM595/596NL) and Arctic (E693G, "ArcAβ") mutation. Using ELISA we show that Aβ levels are strongly increased in the medium of hippocampal slice cultures from ArcAβ mice. Treatment of cultures with Aβ antibody 6E10 reduced the ELISA signal to control levels confirming the binding of the antibody to Aβ. In ArcAβ cultures the density of dendritic spines is strongly reduced. However, the transgenic produced ArcAβ itself is not neurotoxic but induces toxicity after a virus-mediated expression of human tau protein confirming previous results in slice cultures from a different AD mouse model (Tackenberg and Brandt, 2009). Both, spine loss and induction of tau-dependant cell death is prevented when cultures are treated with Aβ antibody 6E10. These results could be verified using a second Aβ specific antibody. We further aim to identify the mechanism of antibody-mediated protection. Is the binding of the antibody sufficient to inactivate toxic Abeta species or does its binding further induce Fc receptor-mediated phagocytosis of Aβ by activated microglial cells? We also analyzed hippocampal slice cultures from mice expressing mutated human APP with the Swedish and Japanese (E693Delta, "JapAβ") mutation. Interestingly, no spine loss is observed in these cultures. This is remarkable since JapAβ cultures produce similar amounts of Aβ compared to cultures from ArcAβ mice and both mutations are located at the same position within the Aβ sequence. The different behaviour of Arc- and JapAβ will further be analyzed.

Supported by: This work is supported by the Deutsche Forschungsgemeinschaft (DFG, TA 762/1-1).

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P-03

Partial recovery of genetically down-regulated adult hippocampal neurogenesis and short-term memory impairment: role of Tis21

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Key words: neurogenesis, learning, Ras

We have recently shown that transgenic activation of Ras in neurons in mice named synRas leads to a down regulation of adult neurogenesis in the dentate gyrus of the hippocampus. These synRas mice suffer from poor performance in hippocampus-dependent learning tasks, i.e. object recognition and radial maze assays (Manns et al., 2010, Lafenetre et al., 2010). In order to gain insight into the mechanism of the reduced hippocampal cell proliferation in synRas versus wild type mice we investigated the formation of newborn cells in defined stages of neurogenesis (type 2a, 2b, 3) 24 h and 72 h after BrdU injection. Co-labelling of BrdU positive cells with markers Sox2 and doublecortin (DCX) revealed that 72 h after BrdU injection, synRas mice show an increased share of cells in the further advanced stage of DCX expression (type 3 cells), yet the total number of DCX positive cells was diminished. These data are compatible with the assumption that in synRas mice the velocity of newborn cells progressing through the neurogenic lineage was enhanced. The protein Tis21 is an antiproliferative gene involved in the control of cell cycle progression and neuronal differentiation. Here we used Tis21 knock-in mice expressing green fluorescent protein under control of the Tis21 promoter (Haubensak et al., 2004) but lacking endogenous Tis21 protein. We cross-bred synRas mice with Tis21 knock-in mice and found that the reduced numbers of cells in the 3 stages of neurogenesis (type 2a, 2b, 3) were partially reversed by the absence of Tis21 protein. Furthermore, the accelerated progression through the cell lineage in synRas animals was attenuated. This partial recovery of subtype specific cell formation in double transgenic animals was correlated with a restored learning capacity in the 8-arm radial maze assay suggesting that short term spatial memory processes are dependent on the regulation of newborn cells progressing through the neurogenic lineage.

Supported by: International Graduate School of Neuroscience

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P-04

Antidepressant-like features of mice with transgenic activation of Ras in differentiated neurons

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Key words: synRas mice, clinical depression, adult neurogenesis, BDNF

Brain-derived neurotrophic factor (BDNF) is implicated in clinical depression and its treatment. Administrations of antidepressants have been shown to enhance BDNF expression and phosphorylation of its cognate TrkB receptor. In contrast, stress exposure and depression are associated with down regulation of BDNF expression. Furthermore, an up regulation of adult neurogenesis in the hippocampus has been proposed to be correlated with drugs effective in the treatment of depression. The Ras-mediated extracellular signal-regulated cascade (ERK) pathway is considered as a major BDNF/TrkB intracellular signalling pathway. Here, we test its possible contribution on antidepressant activity by utilizing a synRas transgenic mouse model expressing constitutively activated human Ha-Ras in differentiated neurons [Heumann et al., 2000 J Cell Biol 151: 1537]. SynRas mice showed elevated levels of activated Ras and activating phosphorylation levels of ERK in the cortex and hippocampus. Immunoblotting analysis revealed that chronic fluoxetine administration to wild type mice led to an increased Ras activation followed with subsequent elevation of ERK phosphorylation thus mimicking the synRas phenotype. Consistently, our results obtained in depression-associated animal models showed an antidepressant-like behavior of synRas transgenic mice compared to their wild type littermates. Furthermore, synRas mice exhibited a normal basal HPA-axis activity, but a suppression of corticosterone release in response to acute restraint stress. Interestingly, synRas mice displayed a reduction of the number of newborn cells within the dentate gyrus of the hippocampus, indicating that the antidepressive-like behavior is not linked to increased neural progenitor proliferation. Taken together, an antidepressant state was established in a genetic model of enhanced neuronal Ras signalling without correlation to hippocampal precursor cell proliferation.

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P-05

Proteomics reveals the native phosphorylation pattern of plasma membrane proteins from mouse cerebellum

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Key words: phosphoproteomics, plasma membrane proteins, brain, proteome

Neuronal processing strongly depends on plasma membrane proteins. The activities of plasma membrane proteins are therefore tightly controlled. One of the key mechanisms to modulate protein function is protein phosphorylation. So far, most studies concerning this modification focused on single proteins and only few addressed the entire phosphorylation pattern of a given brain region and/or a cellular compartment. High throughput studies were impaired by the lack of appropriate protocols for the enrichment of plasma membranes and by the low abundance of phosphorylated amino acids in proteins. To this end, we implemented a strategy that combines efficient enrichment of plasma membranes from small brain regions with techniques for enrichment of phosphopeptides and the power of modern mass spectrometric analyses of phosphopeptides. Starting with a single cerebellum of a 30 days old mouse, we identified 1609 different native phosphorylation sites, which belong to 507 different proteins. Based on gene ontology, 396 of these proteins (78%) were located in membranes, and 230 proteins (45%) were assigned to the plasma membrane. Almost a quarter of all phosphorylation sites have not been previously reported. Examples of proteins with multiple identified phosphorylation sites are the voltage gated calcium channel Cav2.1, the hyperpolarization activated cyclic nucleotide gated channel HCN2 and the GABA-B receptor subunit 2. Regarding GABA-BR2, we identified 7 native phosphorylation sites, of which 5 were novel. A bioinformatic screen identified various kinase consensus sequences among the identified native phosphorylation sites. Taken together, these data provide a valuable and rich resource for further functional analyses in the cerebellum, aiming at the identification of regulatory mechanisms.

Supported by: DFG NO428/3-1

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P-06

Curcumin protects oligodendroglial OLN-93 cells against oxidative stress

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Key words: curcumin, oxidative stress, OLN-93 cells, mitochondria, heat shock response

Oxidative stress induced cell death and tissue degeneration are associated with pathological conditions occurring during aging and neurodegenerative diseases. Hence antioxidants are promising therapeutic agents. Curcumin, a natural compound with anti-inflammatory, antiviral and antioxidant capacities, extracted from the herb *Curcuma longa*, has been used in traditional asian medicine for thousands of years. In the present study, we have examined the antioxidative effects of curcumin in OLN-93 cells, a cell line derived from primary rat brain glial cells that resemble immature oligodendrocytes. Cells were incubated with hydrogen peroxide either in the absence or presence of curcumin, and cell morphology, cytotoxicity and mitochondrial integrity were monitored. Furthermore, immunoblot analysis was carried out to assess heat shock protein induction and the activation of mitogen-activated protein kinases ERK1 and ERK2, which have been implicated in the regulation of cell death and survival. Treatment with hydrogen peroxide (100µM;26h) resulted in cell death with apoptotic features, the induction of HSP32 and HSP70, and caused the activation of ERK1 and ERK2. Fluorescence staining of mitochondria with anti-HSP60 antibodies and MitotrackerRed indicated mitochondrial fission and loss of mitochondrial membrane integrity. Pretreatment of the cells with curcumin (10µM) for 2 hours prior to the induction of oxidative stress showed a significant protective effect. Cell viability was increased and the heat shock response was amplified. Mitochondria stayed intact and mitochondrial fission was prevented. Furthermore, curcumin attenuated the activation of ERK1 and ERK2. Hence, curcumin protects OLN-93 cells against oxidative stress by upregulation of the heat shock response and preserving mitochondrial activity. Our data support evidence that curcumin is a powerful and potent protective substance and may be used to combat neurodegenerative processes associated with oxidative stress.

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P-07

Differences in the large extracellular loop between the K^+-Cl^- cotransporters KCC2 and KCC4

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Key words: cation chloride cotransporter, structure, function, extracellular loop, transport activity

Cation chloride cotransporter (CCCs) plays a pivotal role in chloride homeostasis, transepithelial salt transport and cell volume regulation. They are divided into the Cl^--inward transporters NCC, NKCC1 and NKCC2, and the Cl^--extruders KCC1 to KCC4. CCCs consist of 12 transmembrane domains (TDM), a large extracellular loop (LEL), and the two cytoplasmic termini. The TMDs are involved in ion translocation and the termini likely regulate conformational changes of the transporters by mediating protein interactions or being the target of posttranslational modifications (Isenring and Forbush 2001, Rinehart et al. 2009). Here we analyzed the structure-function relationship of the LEL of the closely related KCC2 and KCC4 (protein identity 72%), that is located between the TMD5 and 6. Mutation of all four evolutionary conserved cysteines of the LEL strongly reduced transport activity of KCC2, whereas the activity of KCC4 was unaffected. Analyses of chimera supported this difference in structural requirements of the LEL. Swapping the LEL of KCC4 to KCC2 had no effect on the transport activity, whereas the reciprocal chimera lost transport activity. Immuncytochemistry demonstrated that mutants were expressed to the same level as the respective wild-type clone. Cell surface labeling revealed that most mutants were trafficked normally to the plasma membrane. This suggests conformational changes as the underlying cause for the reduced transport activity. Finally, sensitivity to the loop diuretica furosemide was in part a function of the extracellular loop. Taken together, our results identified surprising differences in the sequence requirements of the LEL between KCC2 and KCC4. Furthermore, they demonstrate that evolutionary highly conserved amino acids can have different functions within closely related KCC members.

Supported by: DFG-No428/2-3 to H.G.N. and National Institute of Health DK57708 to D.B.M

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P-08

Caldendrin is intracellularly translocated by interaction with recoverin

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Key words: caldendrin, recoverin, calcium signaling, retina

Caldendrin and recoverin are Ca^{2+}-sensor proteins operating in neuronal systems. Recoverin is known to regulate its target rhodopsin kinase in photoreceptor cells of the vertebrate retina in a Ca^{2+}-dependent manner. A light induced translocation of recoverin from the photoreceptor cell outer segments to the synapses was observed, while rhodopsin kinase did not translocate, indicating the existence of binding partners of recoverin different from rhodopsin kinase. In search of novel interaction partners of recoverin, we identified the neuronal Ca^{2+}-sensor protein caldendrin by employing a recoverin-affinity column. Caldendrin and recoverin are co-localized in a subset of bipolar cells in the retina and in the pineal gland as revealed by immunofluorescence studies. Pull-down-assays and surface plasmon resonance studies indicated that the recoverin-caldendrin-complex was formed in the presence of Ca^{2+} with low to moderate affinity. Importantly, caldendrin *in vitro* built a Ca^{2+}-independent homodimer. To study the cellular distribution, COS-7 cells were co-transfected with fluorescently labelled recoverin and caldendrin. Time lapse fluorescence microscopy revealed that the increase of intracellular Ca^{2+} facilitated the translocation of fluorescently labelled caldendrin to intracellular membranes. This process was apparently attributed to the complex formation with recoverin.
Reference: Fries, R. et al (2010) J.Neurochem. 113: 1150-1162

Supported by: DFG (SFB 779/TPB8,SFB 845/TP7,KO948/7-1,KO948/7-2), the Land Saxony-Anhalt (C1/TP4) and the BMBF/DBT

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P-09

The Ca^{2+} relay model in vertebrate cone vision

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Key words: cone vision, light adaptation, Guanylate cyclase, GCAPs, zebrafish

Guanylate cyclase-activating proteins (GCAPs) belong to the subfamily of neuronal Ca^{2+} sensor proteins. They are considered to be retina specific and play a crucial role in photoreceptor cells' adaptation to different background light intensities. In vertebrate rod photoreceptors two GCAP isoforms are expressed. Although these two isoforms adress to the same target molecules - Guanylate cyclases (GCs) – they possess different properties and Ca^{2+} sensitivities. The expression of two GCAP isoforms in the same cell has led us to propose a Ca^{2+} relay model, where the GCAP isoforms consecutively step into action, due to increasing light intensities and according to decreasing $[Ca^{2+}]i$.While rod photoreceptors operate at low light intensities, cone photoreceptors are responsible for daylight vision and exhibit more dynamic responses over a huge range of ambient illumination. Recent studies suggest that Ca^{2+} homeostasis in cones is more complex than in rods due to the different requirements of both cell types for adaptation. How do these results fit in our Ca^{2+} relay model? Are the differences in fluctuating Ca^{2+}-homeostasis in rods and cones mirrored in different sets of GCAPs with slightly different biochemical properties? To adress these questions the cone dominated retina of zebrafishes, where six GCAP isoforms (zGCAPs) are expressed, is an excellent model. We heterologously expressed the six zGCAP isoforms and compared the biochemical properties of the purified proteins: All zGCAPs exhibit properties suitable for their role as Ca^{2+} sensors like Ca^{2+}-induced conformational changes. Furthermore, all six isoforms were activators of membrane bound guanylate cyclases from bovine retina. The halfmaximal activation (IC_{50}-values) range from 30 nM to approx. 500 nM $[Ca^{2+}]$free. Given the different expression pattern of zGCAPs in the retina, these results support a role for GCAPs with fine-tuned Ca^{2+}-sensing properties operating consecutively in rods and cones.

Supported by: Deutsche Forschungsgemeinschaft (KO948/7-1 & KO948/7-2)

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P-10

Signaling components of the mGluR6 pathway in on-bipolar cells of the bovine retina

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Key words: mGluR6, PLCβ4, Gα0, OPL

In on-bipolar cells, the activation of the metabotrophic glutamate receptor 6 (mGluR6) signal transduction pathway culminates in the closing of a non-selective cation channel. Meanwhile evidence is strong that the Gα subunit of the G protein coupled to mGluR6 is Gα0. Recent findings show, that phospholipase C β isoforms (PLCβ) were Gαq-protein regulated and the transient receptor potential (TRP)M1 cation channel is the endpoint of the mGluR6 signal transduction pathway. The aim of the project was to identify PLC and/or TRPM1 as a direct interaction partner of Gα0. For this, we isolated and purified outer plexiform layer (OPL) preparations, which were not contaminated by abundant photoreceptor outer segment proteins. Specific antibodies against Gα0, mGluR6, PLCβ4 and TRPM1 were chosen for their identification in the OPL. Immunoblotting assays give evidence that cytoskeletal proteins were co-immunoprecipitated. For co-immunoprecipitation a specific mouse monoclonal antibody against Gα0 as an agarose conjugate of Gα0 was used. GTPγS was taken for activation of the G-coupled transduction pathway. PLC enzyme activity measurements were performed with OPL and co-immunoprecipitates as crude enzyme fractions. p-nitrophenylphosphorylcholine (NPPC) was chosen as non-natural substrate and released p-nitrophenol (PNP) quantified photometrically at 405 nm. For the direct interaction studies of TRPM1 with Gα0 methods of co-immunoprecipitation and final immunoblotting analysis were combined with the same monoclonal mouse antibody. The results of this study provide evidence that Gα0 is co-localized with a cytoskeletal scaffolding complex. We also demonstrate by direct biochemical analysis that Gα0 acts downstream from mGluR6 in the signal transduction cascade of on-bipolar cells but is not directly involved in activating the PLC and in binding to the TRPM1 cation channel.

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P-11

Cell-selective detection of newly synthesized proteomes in *Drosophila melanogaster*

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Key words: *Drosophila melanogaster*, metabolic labeling, proteome dynamics, imaging

Dynamic protein synthesis is a common feature of cells and cellular networks to respond to changes in their environment, or to alterations in molecular communication itself. Here we introduce an approach to track newly synthesized proteins in selected cells in larvae of *Drosophila melanogaster*, extending the previously reported technologies BONCAT (bioorthogonal amino acid tagging) and FUNCAT (fluorescent non-canonical amino acid tagging). Metabolic labeling of newly synthesized proteins is based on the non-canonical amino acid Azidonorleucine (ANL), which is incorporated into proteins upon expression of a mutant methionine tRNA synthetase (MetRS). Replacement of a single leucine residue to glycine in the binding pocket of the enzyme enables the efficient activation of ANL and subsequent incorporation into proteins of cells expressing the mutant LtoG-MetRS, thus endowing the proteins with a novel azide functionality. Employing copper-catalyzed 3+2-azide-alkyne-cycloaddition ('click chemistry'), the azide group of ANL is covalently coupled either to a fluorescent alkyne-tag (FUNCAT) or to an alkyne-bearing affinity tag (BONCAT). While FUNCAT is aimed at visualizing ANL incorporation, BONCAT provides a means to identify labeled proteins, e.g. by Western Blot– or mass spectrometry analyses. By using the well established Gal4-UAS expression system in *Drosophila melanogaster*, we are able to express the mutant LtoG-MetRS fused to GFP in neurons, glia or in muscle cells. Only cells bearing the mutant are able to use ANL as a surrogate for methionine during translation, and show extensive signals for newly synthesized proteins in imaging approaches and on Western Blot level. Using this cell-selective labeling we are able to visualize for the first time neuronal (and glial) proteome dynamics in a native cellular context.

Supported by: DFG, Emmy Noether Program to D.C.D. (DI1512/1-1)

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P-12

Protease-activated receptor 2 and α-crystallin are involved in protection of astrocytes

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Key words: Protease-activated receptor-2, α-crystallin, astrocytes, phosphorylation, neuroprotection

α-Crystallin (comprising αA-crystallin and αB-crystallin), is a small heat shock protein functioning as lens structural protein and chaperone. Highly expressed α-crystallin observed in neurodegenerative diseases suggests its participation in neurodegeneration. Protease-activated receptor 2 (PAR-2), activated by trypsin and trypsin-like serine proteases, is a G-protein-coupled receptor and regulates inflammatory responses in many tissues. Our previous report demonstrated that PAR-2 activation and over-expression of α-crystallin rescue astrocytes from C2-ceramide and staurosporine treatment. In the current study, we investigated the mechanism of cytoprotection of astrocytes by PAR-2 and α-crystallin. Our data indicated that PAR-2 activation increases the expression of αA-crystallin and phosphorylation level of αB-crystallin at Ser59. Further experiments by mimicking phosphorylation or unphosphyrylation of α-crystallin showed that the phosphorylation of αA-crystallin at Ser122 and Ser148 and αB-crystallin at Ser45 and Ser59 is required for the protection. In addition, our results revealed that application of p38 and ERK inhibitors blocked the protection of astrocytes by PAR-2 activation and over-expression of α-crystallin. These data suggest that PAR-2 and α-crystallin are involved in astrocytes survival by regulation of the expression and the phosphorylation status of α-crystallin which is mediated by p38 and ERK.

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P-13

Chronic neuroinflammation inhibits the neurogenic potential of neural stem cells

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Key words: neurogenesis, neuroinflammation

Neuroinflammation is an accompanying factor in many brain diseases or injuries. Depending on the type of brain damage, the neuroinflammatory response can vary in its chronicity, i.e. it can be acute, like in stroke, or chronic, like in Parkinson's disease. Although even the aged mammalian brain contains adult neural stem cells with the potential to produce new neurons, regeneration during neurodegenerative diseases is extremely limited. Therefore, it is conceivable that inhibition of adult neurogenesis is part of the neurodegeneration associated pathology. Here we show for a homogenous *in vitro* Neural Stem Cell (NSC) system that the pro-inflammatory cytokine TNF-α modulates the cell cycle of NSCs depending on the chronicity of the application, with a positive effect under acute and a negative effect under chronic conditions. Unaffected, however, is their potential to differentiate into the three possible types of progeny: neurons, oligodendrocytes and astrocytes, though TNF-α acts by increasing the differentiation threshold of NSCs. Our data suggest that short term neuroinflammation acts in a pro-neurogenic way, while chronic inflammation inhibits neurogenesis.

Supported by: Emmy Noether Programm, Kompetenznetzwerk Stammzellforschung NRW, Eurosystem, SFB 629, Medizinische Fakultät WWU Münster, Fonds der chemischen Industrie

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P-14

Identification of miRNAs and miRNA-target genes regulating neural stem cell fate specification

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Key words: miRNA , miRNA-target gene, neural stem cell, fate specification

Neural stem cells are somatic stem cells with the ability to self-renew (maintenance) or to differentiate in either the neuronal lineage or the glial lineage. miRNAs are a large family of approximately 21 nucleotides in size, which are involved in numerous cellular processes, including development, proliferation, and differentiation. At present, the contribution of miRNAs to maintenance or lineage specific differentiation is poorly understood at the systems level. In order to identify differentially expressed miRNAs, we isolated the total RNA from neural stem cells that were grown under conditions for maintenance or for lineage specific differentiation. These miRNAs were then subjected to microarray hybridization, followed by bioinformatic data analysis. According to this analysis we identified distinct groups of miRNAs that involved in maintenance of neural stem cell identity or in the lineage specific differentiation of neural stem cells. Further investigations allowed predicting target genes of those miRNAs and the analysis of those target-genes at the systems level.

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P-15

Systemic analysis of self-renewal and differentiation in mouse neural stem cells

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Key words: neural stem cells, maintenance, differentiation, systemic analysis, molecular profiling

Neurogenesis involves the co-ordination of multiple pathways and control elements that govern processes like cell proliferation, DNA replication, cell cycle arrest, apoptosis and differentiation. Recent insights into the functional aspects of neural stem cells (NSCs) already helped to better understand the molecular mechanisms implicated in NSC fate decisions; however, the “molecular concert”, describing the interaction between the various control elements, is not yet fully understood. To address this question, we established the mRNA-microarray based expression profiles of NSCs, neurons and glial cells, and isolated specifically expressed transcripts. Analysis by the Gene Set Enrichment Analyser (GSEA) software allowed the identification of differentially regulated signalling pathways and transcription factors, with potential regulator effects on stem cell maintenance (e.g. E2F1 transcription factor and activated cell cycle in proliferating cells) and differentiation (e.g. FOXO4 transcription factor in differentiating cells). Additionally, by associating the gene/protein interactions of transcripts highly expressed in NSCs, we established a functional network highlighting the molecular mechanisms playing a major role in the maintenance of stemness characteristics. Deciphering the framework of molecular elements controlling NSC fate decisions generates a thorough understanding of neurogenetic events and unravels the underlying regulation complexity. This is of prime importance to better understand the functioning of neurogenesis.

Supported by: DFG, Emmy Noether Programm

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P-16

Inhibition of TRIM32 induced neural stem cell differentiation by kinase independent function of atypical protein kinase C

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Key words: neurogenesis, neural stem cells, asymmetric cell division, neural differentiation

In the developing mouse neocortex progenitors give rise to differentiating neurons and daughter cells that maintain progenitor properties. The TRIM-NHL protein TRIM32 is polarized during mitosis and accumulates in the daughter cell that differentiates into a neuron. TRIM32 regulates protein degradation by ubiquitinating the transcription factor c-Myc. As progenitors express TRIM32 but do not differentiate, TRIM32 has to be regulated in these cells. We provide data suggesting that TRIM32 is inhibited by aPKCζ in a kinase independent manner. aPKC is an apically enriched polarity component which was recently shown to function as a nuclear determinant in enhancing proliferation and regulation of cell fate during primary neurogenesis. Data from *in vitro* and *in vivo* interaction experiments and kinase assays indicate that TRIM32 and aPKCζ form a complex without TRIM32 being phosphorylated. While expression of TRIM32 in neuronal progenitors induces degradation of c-Myc and triggers neuronal differentiation, co-expression with PKCζ blocks these effects. Mechanistically, interaction of TRIM32 with aPKCζ results in a lower binding affinity of TRIM32 for c-Myc leading to an inhibition of c-Myc degradation. Localization studies indicate that TRIM32 and aPKCζ colocalize in the cytoplasm of neural stem cells under proliferative conditions but not under conditions inducing neuronal differentiation. Translocation of TRIM32 to the nucleus of differentiating neural progenitors prevents interaction with PKCζ and allows this cell fate determinant to become active. Altogether, these data provide an explanation why neural progenitor cells do not differentiate although they express fate determinants that induce neuronal differentiation. During neurogenic cell divisions the asymmetric segregation of the inhibitor of neuronal differentiation (PKCζ - apical) and the neuronal fate determinant (TRIM32 - basal) allows TRIM32 to become active.

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Cellular organization of adult neurogenesis in the Common Marmoset

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Key words: Common marmoset (*Callithrix jacchus*), neurogenesis, aging

Adult neurogenesis has been well characterized in at least two specific areas of the brain, the subgranular zone (SGZ) of the hippocampal dentate gyrus (DG) and the subventricular zone (SVZ) of the lateral ventricle (LV). Here, neural stem cells (NSCs/type B cells) give rise to transient-amplifying progenitor cells (type C cells) which in turn will develop migrating neuroblasts (type A cells) and mature into neurons. Newly generated cells of the DG mature into granule neurons of the granular cell layer (GCL) while cells generated in the SVZ migrate longer distances into the olfactory bulb (OB) where they give rise to interneurons. The processes involved in neurogenesis were most intensely studied within the brains of rodents such as mice and rats, however, little is known about adult neurogenesis within our closer relatives; primates such as the common marmoset (*Callithrix jacchus*). The common marmoset is a New World monkey that reaches sexual maturity at 1.5 years of age and has a life span that ranges from 8 – 16 years. The onset of senescence, a stage of life that is associated with the development of neurodegenerative diseases, begins around 8-10 years. Here, we set out to characterize the anatomy and cell type-specific gene expression of the neurogenic niches in the common marmoset brain using immunohistochemical and autogradiographic analysis. Moreover, we demonstrate ongoing proliferation of neuroblasts within both the SGZ and SVZ of the adult brain and provide further evidence that the age-dependent decline of neurogenesis in the hippocampus is associated with a decrease in DCX-positive, transiently amplifying progenitor cells.

Supported by: DFG Emmy Noether Fellowship

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Formation of pyroglutamic acid in amyloid peptides – Influence on biophysical and biochemical properties

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Key words: Alzheimer's disease, amyloid, pyroglutamate, aggregation

Deposition of N-terminally truncated and pyroglutamate (pGlu)-modified amyloid peptides is a characteristic feature not only of sporadic and inherited Alzheimer's Disease (AD) but also of familial Danish dementia and familial British dementia (FDD and FBD). Formation of pGlu at the N-terminus of amyloid peptides confers resistance against cleavage by most aminopeptidases, increases the cytotoxicity of the peptides and might seed Aβ aggregate formation. Our previous studies revealed a prominent influence of the N-terminal pGlu residue on the pH-dependent solubility of amyloid peptides, rendering modified peptides less soluble and more prone to aggregation. Moreover, differences are also observed in structure and morphology of mature fibrils. Modified peptides form very short fibrils, which are frequently arranged in bundles, thus contradicting in shape to the long fibrils of unmodified peptides. Here, we show that pGlu-modified and unmodified Aβ peptides form mixed fibrils using a combination of cross-seeding experiments followed by immuno-gold labeling and electron microscopy, providing first evidence for an ordered, mixed fibril formation of N-truncated and full-length peptides. In addition, N-truncated and modified peptides displayed a fast formation of low molecular weight oligomers as revealed by photo-induced cross linking experiments. These differences apparently provoke functional differences of the Aβ forms. Hippocampal long-term potentiation (LTP) was inhibited by pGlu-modified Aβ3-40, but not by the unmodified or non-truncated forms. Thus, the data provide evidence for an important role of the N-terminus of Aβ not only for fibril formation, but also for formation of (mixed) oligomers with potentially higher potency to affect neuronal physiology and function.

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Distinct role of glutaminyl cyclases (QCs) in inflammation/neuroinflammation: First results from QC and isoQC knock out mice

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Key words: inflammation, chemokine, disease

Neuroinflammation caused, e.g., by deposition of toxic peptides in Alzheimer's disease leads to a profound activation of astrocytes and microglia cells in affected tissues. The proinflammatory chemokine CCL2 (MCP-1) plays a major role in mediating inflammatory activation and microglia migration. In this regard, CCL2 has been found to deteriorate plaque pathology in transgenic Alzheimer's disease mouse models. In addition, CCL2 is upregulated in early stages of Alzheimer's disease. We have shown, that posttranslational modification of CCL2 is mediated by Glutaminyl Cyclase (QC) activity leading to a proteolytically stable phenotype of CCL2 possessing a pyroglutamyl residue at the N-terminus (pGlu-CCL2). For further target validation in Alzheimer's disease (pGlu-Abeta) and inflammation/neuroinflammation (pGlu-CCL2), we have isolated an isoenzyme of secreted mammalian QCs sharing a similar substrate specificity but possessing a different subcellular localization as resident enzyme of the Golgi complex. To gain further insights into the substrate conversion and regulation of QC and isoQC *in vivo*, we have challenged QC and isoQC knock out animals with inflammatory stimuli. First results point to a differential role of QC/isoQC in distinct inflammatory conditions. Furthermore, although both enzymes are located within the secretory compartment, evidence will be presented, that both enzymes convert different subsets of substrates *in vivo*. Future studies will focus on the role of QC and isoQC in different disease conditions, explaining, e.g. the upregulation of QC in Alzheimer's disease brains.

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P-20

Effect of different Aβ species on primary neuronal cells

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Key words: glutaminyl cyclase, CCL2, amyloid-beta

One pathological hallmark of Alzheimer's disease (AD) is the presence of neuritic plaques. These extracellular amyloid-beta (Aβ)-aggregations consist primarily of Aβ peptides, which are truncated at their N- and C-termini. In addition, N-terminal modifications of Aβ have been described like isoaspartate or pyroglutamate (pGlu) formation. The enzyme glutaminyl cyclase (QC) was identified to catalyze the pGlu-modification from N-terminal glutamine or glutamate precursors. The analysis of human neocortical brain samples from AD-patients showed an increase of QC, which coincides with accumulation of pGlu-Aβ. Next to the Aβ-species (3-40/42) or (11-40/42), there are other pGlu-proteins that are increased in the AD pathology such as the chemokine CCL2 (MCP-1). This led to the hypothesis that an increased expression of the QC substrates induces an increase of QC itself, i.e. during inflammatory signal responses. Hence, we established the culture of primary neuronal cells to study the effect of different N-truncated Aβ species on cytokine secretion. Analysis of the cytokine secretion revealed prominent expression of the QC substrate CCL2 upon stimulation with Aβ. In ongoing studies, we will compare the potency of different Aβ species for the stimulation of CCL2 expression. Furthermore, the expression of QC and isoQC in microglia, astrocytes and neurons will be investigated.

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Participation of hematopoietic cells in Cre mediated recombination of Purkinje neurons

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Key words: Purkinje neuron, inflammation, cell fusion, Cre/LoxP system, horizontal gene / protein transfer

In the recent past several studies reported the potential of hematopoietic cells to contribute to the generation of non-hematopoietic tissues including Purkinje neurons. This apparent plasticity of hematopoietic cells was initially attributed to transdifferentiation, but was lately explained by cell fusion that could be further increased by inflammation. In most previous studies however, the question of hematopoietic plasticity has been addressed with the help of a murine cell transplantation model that requires initial lethal irradiation of the recipient mice, which is a strong pathological stimulus. To address the question whether endogenous hematopoietic cells also contribute to certain tissues we were using a transgenic mouse reporter system instead of the highly invasive combination of irradiation and bone marrow transplantation. By this means we could show a Cre mediated reporter gene induction in Purkinje neurons without any evidence of cell fusion. Additionally, this recombination increases dramatically after an induced inflammation and further suggests that a putative fusion of hematopoietic cells with Purkinje neurons is only transient and does not lead to stable heterokaryon formation under noninvasive conditions. To further investigate the exact mechanism of interaction between a hematopoietic cell and a Purkinje neuron, we were interested whether genetic material or proteins can be transferred horizontally between these two cell types. The possibility of a direct, lateral transfer from hematopoietic to neural cells presents a previously unrecognized way of communication between the immune system and the brain.

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Functions of the cannabinoid receptor 1 (CB1) on adult neurogenesis

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Key words: adult neurogenesis, endocannabinoid system, CB1 receptor, conditional knock-out mice

Adult neurogenesis in the mammalian brain has been clearly demonstrated to occur at two locations under normal conditions: the subventricular zone (SVZ) of the lateral ventricles and the subgranular zone (SGZ) of the dentate gyrus in the hippocampus. A variety of physiological and pathological factors are known to modulate neurogenesis in these distinct regions. However the exact molecular mechanisms which drive adult neural stem cells (NSCs) proliferation and differentiation into the neuronal and glial lineage are still poorly understood. Endocannabinoids (eCBs) exert a neuromodulatory role by controlling neurotransmitter release via presynaptic CB1 receptors and have been implicated in neuroprotection. In addition, recent studies suggest an important role in adult neurogenesis. Adult NSCs express a functional eCB system, and studies using adult CB1 knock-out (KO) mice showed reduced NSC proliferation and impaired differentiation into the astroglial lineage in the hippocampus of adult mice. To determine which neuronal population expressing the CB1 receptor is mandatory for adult neurogenesis, we used two conditional CB1 knockout mouse lines (CB1-KO on glutamatergic and CB1-KO on GABAergic neurons) to investigate changes in the proliferation and differentiation of NSCs. We performed BrdU injections into 2-3 month old animals and subsequent immunofluorescence experiments on hippocampal sections with neuronal and astroglial differentiation markers. As evaluated by confocal microscopy, we observe that reduced proliferation is only present in mice where the CB1 receptor was knocked-out on GABAergic neurons. This effect is even more drastic if animals are 24 month old. In contrast, proliferation remained unaffected in mice which were KO for CB1 on glutamatergic neurons. Astroglial differentiation was slightly reduced in both conditional knock-out mice, whereas neuronal cell fate determination seemed to stay unaffected.

Supported by: DFG

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Fluglotse is required for synapse maturation at the Drosophila neuromuscular junction

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Key words: Drosophila, synapse

Synapse formation involves bidirectional signaling between pre- and postsynaptic cells leading to the development of specialized structures for release and detection of neurotransmitters. Following formation and initial maturation more than 95% of all individual synapses will be stabilized at *Drosophila melanogaster* neuromuscular junctions. We thus used the presence of pre- and postsynaptic marker proteins - Bruchpilot and GluRIII- to screen for mutants in which synapses are not correctly stabilized. Here, we describe one of the identified mutants: fluglotse. Homozygous fluglotse mutant larvae are characterized by impairments in axonal transport, defects in synaptic structure and function as well as by impaired locomotion. About 30% of all synapses on muscle 6/7 are devoid of presynaptic Bruchpilot staining. Evoked excitatory junctional currents are reduced in amplitude and show an increase in rise time. Behavioral assays indicate that defects are caused by impaired synapse assembly rather than by defects in the stabilization of synapses. The temporal sequence of molecular and cellular events that lead to the formation of these “defective” synapses was clarified using *in vivo* imaging.

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Formaldehyde stimulates glutathione efflux from cultured astrocytes

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Key words: astrocytes, formaldehyde, glutathione

Formaldehyde is an environmental toxin that has been linked to neurotoxicity. Since the tripeptide glutathione (GSH) plays an important role in the detoxification of xenobiotics by brain cells, we tested for the consequences of a formaldehyde exposure on the GSH metabolism of brain cells using astrocyte-rich primary cultures as model system. Treatment of these cells with formaldehyde resulted in a rapid depletion of the cellular total glutathione content (GSx = GSH + 2 x GSSG) in a time- and concentration-dependent manner. Exposure of astrocytes to 1 mM formaldehyde for 3 h almost completely deprived the cells of GSx. The decrease in cellular GSx levels on exposure to formaldehyde was accompanied by a matching increase in the extracellular GSx content, although the viability of the cells was not compromised. Analysis of the oxidation state of GSx in both cells and media following formaldehyde treatment revealed that predominantly GSH contributed to the GSx values determined. Deprivation of cellular GSx appears to be rather specific for formaldehyde, since its metabolites methanol and formate as well as acetaldehyde did not affect cellular GSx levels. Both cellular GSx deprivation and the increase in extracellular GSx content after formaldehyde exposure were completely prevented by the presence of MK571, an inhibitor of the multidrug resistant protein 1 (Mrp1) which is known to mediate GSH efflux from cultured astrocytes. These data suggest that formaldehyde deprives astrocytes of GSH by stimulating Mrp1-mediated GSH export.

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Role of Cdh1-APC/RhoA pathway in the regulation of axon growth of cerebellar granule neurons

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Key words: Cdh1-APC, ubiquitination, RhoGTPases, cerebellar granule neurons, axon growth

Regulation of axon growth is a fundamental event in the developing nervous system. The E3 ubiquitin ligase, Cdh1-Anaphase Promoting Complex (APC) exerts a cell-intrinsic control on axon growth. Cdh1-APC has been shown to inhibit axon outgrowth both in primary neuronal cultures. A cell-autonomous role for Cdh1 has also been described in the regulation of cerebellar patterning *in vivo*. Apart from cell-intrinsic mechanisms, axon growth is regulated by extrinsic factors including growth factors and guidance cues which trigger well-established signaling cascades. The small Rho GTPases, RhoA, Rac1/2/3 and Cdc42 control the dynamics of the actin cytoskeleton and integrate signals from extrinsic factors to directly regulate neurite growth and modulate the steering of the growth cone. In this study, we examined the role of small Rho GTPase, RhoA in the Cdh1-APC pathway of axon growth control. Preliminary results indicate that RhoA operates in a linear pathway downstream of Cdh1-APC in the control of axon outgrowth and that RhoA activity is inhibited when the APC is inactivated. Over-expression of wild-type or constitutively active RhoA in Cdh1-knockdown neurons significantly reduces axonal length while dominant negative RhoA maintains the long axon phenotype of Cdh1-knockdown. Since Cdh1-APC is an E3 ubiquitin ligase involved in ubiquitin-proteasome pathway of protein degradation, we reasoned that RhoA is not likely to be a direct target of Cdh1 as both Cdh1-APC and RhoA inhibit axon growth. In a candidate approach, we screened Rho-GTPase activating proteins (GAPs), which are negative regulatory proteins of the Rho GTPases. We identified p250GAP, a brain-abundant RhoA/Cdc42 GAP as a novel interacting partner of Cdh1. We propose a working model whereby Cdh1-mediated degradation of p250GAP increases the activity of RhoA and thereby exerts control on axon outgrowth.

Supported by: Deutsche Forschungsgemeinschaft (DFG)

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The role of Fbxo41 in mammalian brain development and disease

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Key words: brain development, F-box proteins, Fbxo41, DISC1, schizophrenia

The development of the brain requires a well-orchestrated sequence of events including neuronal differentiation, migration, the formation of dendrites and axons to ultimately create a network with other neurons. These processes require precise spatial and temporal regulation of gene expression and equally important the controlled degradation of unwanted proteins. The latter is ensured by the ubiquitin-proteasome system, which targets polyubiquitynated proteins to the 26S proteasome. Attachment of ubiquitin molecules involves three sequential steps that are mediated by different classes of enzymes. Among those, E3 ubiquitin ligases are most numerous as they confer substrate specificity. F-box proteins, interchangeable subunits of the Skp1/Cullin-1 F-box protein (SCF) complex, serve as substrate recognition elements. Fbxo41 is a novel F-box protein, which is exclusively expressed in the brain. On the subcellular level Fbxo41 localizes to the centrosome, which is essential e.g. for neuronal differentiation and migration. To determine the role of Fbxo41 in neurons we carried out gain- and loss-of function experiments in vitro, which suggest that Fbxo41 promotes axonal growth but has little or no effect on dendrites. In further experiments we found that while Fbxo41 associates with Skp1, it fails to form a complex with Cullin-1, suggesting that Fbxo41 acts independently of an SCF complex. Owing to Fbxo41`s centrosomal localization, we performed a candidate approach and identified DISC1 (disrupted in schizophrenia-1) as an interaction partner of Fbxo41. The DISC1 gene is considered a susceptibility gene for schizophrenia and related mental diseases and it will be important to determine if Fbxo41 regulates axonal growth in a DISC1 dependent manner. Future morphological analyses and an Fbxo41 knockout model should give deeper insight into the regulatory pathway by which Fbxo41 exerts its function in neural development and reveal a possible role in schizophrenia.

Supported by: DFG

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Crosstalk between APC/C and SCFFbxo31 in the development of cerebellar granule neuron

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Key words: Fbxo31, Cdh1-APC, cerebellar granule neuron, neuronal morphogenesis, E3 ubiquitin ligase

The Ubiquitin-Proteasome System (UPS) plays a pivotal role in brain development. E3-ubiquitin ligases, crucial components of the UPS, have been shown to regulate important events during neuronal development including progenitor proliferation, neuronal differentiation and morphogenesis. A recently identified E3 ubiquitin ligase F-box protein 31 (Fbxo31), a component of the Skp1-Cullin1-F-box (SCF) protein complex, has been implicated in cell cycle regulation and DNA repair. While Fbxo31 is highly enriched in the brain, its neuronal functions remain to be elucidated. Here, we demonstrate that Fbxo31 over-expression in cerebellar granule neuron (CGN) promotes axon and dendrite growth. Consistently, knockdown of Fbxo31 in CGNs using RNA interference, leads to a reduction in axon and dendrite growth. We identify that the association of Fbxo31 protein with Skp1-Cullin1 complex is required for its axonal and dendritic growth control since over-expression of F-box deletion mutant does not result in enhanced axon and dendrite growth. Moreover, we find that Fbxo31 interacts with C-terminal of cell-division cycle 20 related 1 (Cdh1), an activator protein of another E3-ubiquitin ligase Anaphase-Promoting Complex (APC), which has an inhibitory effect on axonal growth. Collectively, our results demonstrate that Fbxo31, a putative target of Cdh1, acts downstream of Cdh1-mediated axon growth control and we identify Cdh1-Fbxo31 pathway as one of the novel mechanisms of regulation of axonal growth in post-mitotic neurons.

Supported by: DFG

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Neurobeachin-A novel regulator of functional receptors

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Key words: AKAP, Beach protein family, trafficking, autapses, neurotransmitter receptors

In the neuronal circuitry information is passed mainly via chemical synapses. Typically a neuron receives thousands of input and this information is transmitted via activation of large number of receptors present at the postsynapses. Therefore, the integrity of synapses is heavily dependent on the expression and function of the receptors present at the postsynapse, which receive this information and allow signal transduction. In this study we have identified Neurobeachin (Nbea) as a novel protein regulating the trafficking/transporting of neurotransmitter receptors to the plasma membrane. Nbea deficient neurons show a dramatic reduction in the expression and function of postsynaptic receptors on the synapse without any morphological alterations at the synapses or total expression levels of proteins. Hence Nbea is essential in maintaining the synaptic strength in CNS neuronal network

Supported by: International Max Planck Stipend

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The Role of RNF157 in Central Nervous System Development

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Key words: Ubiquitin-proteasom system (UPS), E3 ubiquitin ligase, RING finger protein (RNF), RNF157, neurodegeneration

The ubiquitin-proteasom system (UPS) is a pivotal regulatory process for protein turnover to ensure the maintenance of cellular homeostasis. In addition, the UPS is essential for brain development. Here, the UPS is instrumental to formation and maintenance of axons, dendrites and synapses. Beyond the developing and adult CNS, the UPS is also implicated in a broad array of neurological diseases including Alzheimer's disease, Parkinson's disease or Huntington's disease. The RING (Really Interesting New Gene) E3 ubiquitin ligases represent the most numerous components of the UPS. While hundreds of RING finger proteins have been identified, the role of only a few RING finger proteins (RNFs) has been characterized. Among those, the E3 ubiquitin ligase Mahogunin 1 (Mgrn 1) provided strong evidence linking aberrant ubiquitination to disease. Loss of Mgrn 1 in knockout animals results in an autosomal recessive form of spongiform neurodegeneration. Interestingly, the putative E3 ligase RNF157 shares sequence homologies with the N-terminus of Mgrn 1. However, its function in the brain remains to be elucidated. In this study, we show that RNF157 is a brain-dominant protein that localizes to the cytoplasm in neurons. In addition, we identified the Amyloid precursor protein (APP) adapter protein Fe65 as an interaction partner of RNF157 and found that shRNA based knockdown of RNF157 in cultured granule neurons led to significant increase of apoptotic neurons. Interestingly, morphological analyses of RNF157 knockdown neurons revealed impaired dendrite development and maintenance. Collectively, these results identify RNF157 as an important neuronal survival factor that appears to be involved in a dendrite growth regulation program.

Supported by: DFG

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P-30

Analysis of functional domains regulating tau's microtubule interaction in living cells

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Key words: Tau, live imaging, mathematical modeling

The microtubule-associated protein tau is subject of research for some time, not least because of its role in Alzheimer's disease, where aggregates of hyperphosphorylated tau proteins lead to neurofibrilliary lesions. Aim of this work is to get insight into the functional role of tau domains with respect to mediating the mobility of human tau in living neural cells. For this purpose htau441 containing four microtubule-binding repeats and exons 2 and 3 was tagged with photoactivatable GFP. The influence of functional domains was tested using a panel of carboxyterminal deletion constructs with different numbers of repeats. Binding to microtubules and intracellular mobility was determined using live cell imaging after focal activation and mathematical modeling of tau distribution. Our results show that the apparent diffusion coefficient correlates with the number of repeats of the respective constructs. From a minimum number of two repeats, an increase of the diffusion coefficient with decreasing number of repeat domains is observed. Calculations of the percentage of microtubule-bound tau from the effective diffusion coefficients reveal that the ratio of bound to free tau increases from 30% (2-repeats) to 95% (wildtype protein). Removal of the R'-domain, which carboxyterminally flanks the repeat region (residues 369-401) leads to an increase in diffusion similar to the contribution of a repeat. The data indicate that fluorescence photoactivation provides a useful method to analyze the interaction of functional domains of tau with microtubules in living neuronal cells. Co-transfection with full-length constructs could provide further insight into the mechanism of tau mobility in the cells.

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P-31

Region specific effects of tau and Aβ on neuron morphology in the hippocampus of an Alzheimer's disease mouse model

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Neurons are remarkable with respect to their size and their highly polarized structure. While the axon serves as neuronal output, dendrites function as the main receptive field of the neuron. They arise from the cell body and ramify to a different, however distinct degree of grey matter. To determine a potential influence of Alzheimer's disease relevant factors on neuronal connectivity, organotypic hippocampal slices were prepared from APP_{SDL} transgenic mice and non-transgenic littermates, and the morphology of the neurons compared after virus-mediated expression of fluorescent proteins. While the transgenic background allowed analysis of the role of mutated APP, virus-expressed human wild-type tau tagged to EGFP provided the possibility to analyze the effect of tau alone or in combination with APP transgenic background. EGFP expression alone served as a control. Infected pyramidal neurons from CA1 and CA3 regions of the hippocampus and granule cells from DG were imaged using confocal laserscanning microscopy. The z-stacks were stitched using VIAS software. 3D reconstruction and analysis of the neurons was done using Neuromantic software. Results show that neither presence of APP transgene nor wild-type tau alone alter gross neuronal morphology in either region of the hippocampus. However, presence of APP induces a tendency to reduce the complexity of the apical part of the dendritic tree of pyramidal neurons. In contrast, wild-type tau on APP background reduces the complexity of neurons in the CA1 region. With respect to CA3 pyramidal neurons, this effect is region dependent and the complexity is reduced in the basal part of the dendritic tree. To determine whether the effect of APP_{SDL} was due to production of Aβ, the non-transition state γ-secretase inhibitor DAPT was used. We observed that the changes in the neuronal morphology were abolished in the presence of DAPT. Our data indicate that presence of Aβ in combination with tau affects neuronal connectivity in the hippocampus in a region specific manner.

Supported by: Supported by the Deutsche Forschungsgemeinschaft (DFG BR1192/11-1)

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Age-dependent changes in spine density and morphology in a transgenic mouse model of Alzheimer's disease

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Alzheimer's disease (AD) is a progressive neurodegenerative disorder of the central nervous system and is characterized by changes in synaptic connectivity and progressive neuronal degeneration. To analyze the contribution of Aβ to the pathology, we used APP695 transgenic mice carrying the three familial APP mutations Swedish (S), Dutch (D) and London (L). To visualize dendritic spines, which represent the postsynaptic site of glutamatergic input, mice were crossed with the Thy1-GFP M line, in which EGFP is expressed in few neurons (Feng et al., 2000). Brain slices were prepared at different ages of the mice, high resolution image stacks were prepared by confocal laserscanning microscopy (LSM), and spine density and morphology were evaluated using a computer-assisted approach. We report that spine densities were lower in CA1 and CA3 hippocampal subregions of APP_{SDL} transgenic mice compared to control mice. Spine densities were decreased by about 22% at all ages. Mean spine lengths showed a slight decrease in transgenic compared to control mice in both regions. Spine volume strongly decreased with age with no consistent difference between transgenic and control mice. In parallel, the ratio of thin spines increased with age. At 6 months of age, the ratio of thin and mushroom spines was decreased in APP transgenic mice, while the percentage of stubby spines increased. We demonstrate that computer-assisted image analysis of confocal micrographs of EGFP-expressing mice allows determination of changes in spine number and morphology. Our data indicate that spine densities are reduced in hippocampal subregions of APP_{SDL}-transgenic mice and suggest the development of morphological changes of the remaining spines in a transgenic mouse model of AD.

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Different Aβ species have distinct effects on the mobility of the microtubule-associated protein tau and neuronal plasma membrane fluidity

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Alzheimer´s Disease (AD) is characterized by the formation of extracellular amyloid plaques, composed of aggregated amyloid beta (Aβ) peptide and intracellular neurofibrillary tangles with hyperphosphorylated tau protein as the major component. According to the amyloid cascade hypothesis, Aβ is central in the pathogenesis of AD, but requires tau for neuronal degeneration. However, how and which species of Aβ affect tau pathology and whether they have additional effects remains unclear. To determine the effect of different Aβ species on tau and membrane properties in neurons, we constructed lentiviral vectors coding for photoactivatable GFP (PAGFP)-tagged tau fusion protein or farnesylated PAGFP (fPA), as a probe for plasma membrane properties. Primary cortical mouse cultures were infected and protein distribution was recorded after focal activation in axons and dendrites. To test the effect of different Aβ species, synthetic Aβ40 and Aβ42 and N-terminally truncated variants were prepared as soluble or fibrillar material and added to the cells prior to analysis. We report that treatment with Aβ40 and Aβ42 monomers and fibrils leads to a retention of tau in axons and dendrites. The retention is increased, when using N-terminally truncated Aβ peptides (Aβ3-40, Aβ3-42 and pyroglutamate-modified variants). Membrane fluidity, as determined with fPAGFP differs between axons and dendrites with the mobility of fPAGFP being significantly lower in dendrites. Treatment with Aβ leads to a higher mobility of fPA, which was more pronounced in dendrites. The data indicate that Aβ influences the intracellular behavior of tau protein, with aminoterminally truncated Aβ species being most active. In addition to its effect on tau, Aβ influences also membrane properties. Interestingly, the axonal and dendritic plasma membrane showed characteristic differences both with and without Aβ, suggesting that Aβ specifically affects the plasma membrane in a compartment-specific manner.

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Live imaging of tau mRNA-binding proteins in neural cells

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mRNA-binding proteins play a crucial role in different developmental processes and may also be important during neuronal development. Biochemical studies revealed that specific mRNA-binding proteins interact with the tau mRNA and assemble into so-called ribonucleoparticles (RNPs). Here we studied the two mRNA-binding proteins, G3BP-1 and IMP-1, that have previously been shown to interact with the tau mRNA in neurons. To study the morphology of G3BP-1 and IMP-1 containing RNPs, fusion constructs with fluorescent proteins were prepared. The constructs were transfected into PC12 cells and the volume of the particles was determined using an algorithm-based approach of 3D image stacks. G3BP-1 and IMP-1 containing particles had approximately the same volume (~ 0,007 µm³ – 0,009 µm³), suggesting that they represent the same class of RNPs. However, tracking experiments revealed that G3BP-1 including particles are much more dynamic than IMP-1 containing particles indicating that this have distinct properties with respect to intracellular movement. Colocalization experiments provided evidence that the particles were associated with the microtubule skeleton in the cell. The data indicate that tau mRNA-binding proteins are present in different RNPs with distinct properties. Further experiments will be performed to characterize the structure and function of tau mRNA containing RNA granules in more detail.

Supported by: Supported by the Interdisciplinary Graduate School of the University of Osnabrück "Cell and Tissue Differentiation" and the "Niedersächsisches Promotionsprogramm Membranen und zelluläre Kommunikation"

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P-35

The frontotemporal dementia mutation R406W blocks tau´s interaction with the plasma membrane and affects tau distribution in an annexin A2-dependent manner

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Pathological changes in the distribution and function of the microtubule-associated protein tau comprise histopathological hallmarks of tauopathies such as Alzheimer's disease (AD) and Frontotemporal dementia and Parkinsonism linked to chromosome 17 (FTDP-17). In AD, increased phosphorylation contributes to tau pathology, whereas the functional consequence of FTDP-17 tau mutations is not known. To scrutinize the effect of the frontotemporal dementia R406W mutation, which causes a tauopathy closely reflecting AD, we expressed human wild type and R406W-mutated tau in neuronally differentiated PC12 cells and analyzed their distribution and interaction by subcellular fractionation and live cell imaging. We report that the R406W mutation does not influence short-term diffusion of tau indicating unchanged microtubule interaction. However, the R406W mutation causes a complete loss of tau's ability to interact with the neuronal plasma membrane. Loss of membrane association is associated with a decreased trapping of R406W tau in the tip of neurites as evidenced by long-term imaging. TAP-tag mass spectroscopy identified the calcium-regulated plasma membrane-binding protein annexin A2 as a potential tau interacting protein. Knockdown of annexin A2 by shRNA or sequestration of intracellular Ca^{2+} by BAPTA/AM abolishes the differences in trapping of wt tau and R406W tau, consistent with an involvement of annexin A2 in mediating the membrane interaction of wt tau. However, yeast two hybrid screens and pull down assays do not support a direct and strong interaction between annexin A2 and tau. The data indicate that tau´s membrane association contributes to its subcellular distribution and the enrichment of tau in the distal axon. They suggest that the pathological effect of the R406W mutation is caused by impaired membrane interaction, which involves complex interaction including annexin A2 as a membrane-cytoskeleton linker.

Supported by: supported by the DFG graduate college 612

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Expression and phosphorylation of heat shock protein 90 is affected by Alzheimer's disease-like modified tau protein

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Key words: heat shock protein, microtubule associated protein, tau, hyperphosphorylation

Alzheimer's disease is associated with an increased phosphorylation (hyperphosphorylation) of the microtubule-associated protein tau. In different culture models it has been shown that hyperphosphorylation may convert tau to a toxic species however the downstream cascades, which mediate neuronal cell death, remain elusive. To identify potential candidate proteins that are affected by tau modification, we employed cells that express pseudohyperphosphorylated (PHP) tau to simulate a permanent disease-like modification of tau protein. As a control, human wildtype (wt) tau expressing cells were used. We had previously shown that PHP tau establishes key structural and functional changes of hyperphosphorylated tau and induces cell death in cultured cells and organotypic slices in contrast to wt tau. We performed a phosphoproteomic approach, where we used a combination of stable isotope labeling, phosphoprotein affinity chromatography and mass spectroscopy to identify proteins that are differentially synthesized or phosphorylated in PHP tau versus wt tau expressing PC12 cells. We identified heat shock protein 90 (Hsp90) as a candidate protein. Quantitative Western blotting showed that the protein amount was not affected. In contrast, Hsp90 exhibited a lower phosphorylation in PHP tau compared to wt tau expressing cells, as detected by 2D gel electrophoresis. In PHP-tau expressing mice, the amount of Hsp90 was reduced in old age. The data indicate that tau hyperphosphorylation induces a dephosphorylation of Hsp90 and may reduce Hsp90 expression after long term exposure. It remains to be shown whether changes in Hsp90 contribute to tau-induced cell death in neurons.

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Dynamic phosphorylation-dependent association with the plasma membrane is required for trafficking of GAP-43 to the neuronal growth cone

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GAP-43 is an intracellular growth-associated protein thought to regulate the growth of axons during neuronal development and regeneration. It is synthesized in the neuronal cell body and travels in a developmentally regulated fashion to the growth cone, where it becomes highly concentrated. To scrutinize the determinants of GAP-43 motion and enrichment in neural cells, we constructed a fusion protein of GAP-43 coupled to photoactivatable GFP (PAGFP) and analyzed its distribution after focal activation in living cells. We report that GAP-43 wild type protein quickly accumulates in the growth cones of neuronally differentiated PC12 cells after photoactivation in the cell body. To test the effect of phosphorylation at Ser41, a potentially important regulatory site, which can be phosphorylated by PKC, we constructed phosphorylation-mimicking (S41D) and non-phosphorylatable GAP-43 mutant (S41A). Neither S41D nor S41A exhibit a comparable accumulation. About 53% of wt GAP-43 is membrane associated as determined by subcellular distribution. In contrast, phosphorylation-mimicking S41D is almost completely associated with the plasma membrane, while S41A is predominantly cytosolic, suggesting a requirement of dynamic plasma membrane association for effective enrichment. To modulate the plasma membrane association of wt GAP-43, cells were treated with DMSO or the temperature was lowered to RT. Both treatments reduce accumulation in the growth cone and mobility in the shaft. To model the distribution of GAP-43, parameters of the cellular morphology were determined by atomic force microscopy and used to simulate GAP-43 behavior in a virtual neuron. The simulation shows that the motion of GAP-43 in the neurite is compatible with diffusion/reaction as opposed to active transport/reaction. The data indicate that dynamic association of a cytosolic protein with the neuronal plasma membrane suffices to locating it to the growth cone by a diffusion/reaction mechanism. Affecting membrane association by phosphorylation may provide a mechanism to regulate trafficking.

Supported by: supported by the DFG graduate college 612.

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A novel computer-assisted approach for determination of spine dynamics of 2-photon microscopy time-lapse images

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Key words: computational image processing, 2P-microscopy, spine

Dendritic spines are abundantly present mainly on principal neurons of the mammalian CNS. They are specialized subcellular compartments for receiving synaptic excitatory inputs. Spines have different shapes and are motile and plastic structures. Changes in spine number and shape are correlated with alterations in behavior occurring in variety of circumstances, ranging from developmental adaptation through learning paradigms as well as hormonal status. However spine loss or altered plasticity can be a causative for impaired brain activity during neurodegenerative diseases. To address this question individual hippocampal pyramidal spines had been followed up over 2h with 2-photon microscopy within organotypic slices taken from an Alzheimer's disease mouse model. Since live imaging of slices over longer period compromises the image quality due to settings used to minimize phototoxicity, a novel, algorithm-based approach was developed to determine the spine length. Plugins assisted the manual thresholding and determination of the base of the spine neck and spine head. For further processing algorithms were utilized to asses the voxels belonging to the spine head and the determination of the longest distance between the midpoint of the spine base and head surface. The changes in distance, as determinant of the length of the spine were followed in 20 min intervals. By this approach the level of dynamicity of individual spines could be seen, which was not in correlation with spine length or direction of length change (e.g. whether a spine is in shrinking or elongating phase). Surprisingly the percentage of the analyzed elongating and shrinking spines were equal, however the degree of the slope of the whole population was slightly shifting towards negative values, suggesting a potential mechanism by which Aβ acts on spines. Implementation of series of algorithms was successful in analyzing the spine length change as a descriptor of spine dynamics.

Supported by: Deutsche Forschungsgemeinschaft (DFG BR1192/11-1) and the Lichtenberg-Fellowship for Doctoral Students "Membranes and cellular communication" (FS).

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Systematic Mapping Of Integral Membrane Protein Interactions Using The Membrane Split-Ubiquitin System (M-SUS)

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Key words: membrane proteins, signal transduction, protein-protein interaction, split-ubiquitin system, cDNA library screening

Integral membrane proteins and membrane-associated proteins represent approximately 30% of the entire human proteome and constitute important drug targets. Yet, very little information is available about interactions involving membrane proteins, mainly due to a lack of suitable methods. Here, we present a simple and powerful method to screen membrane proteins for interaction partners, the membrane split-ubiquitin system (M-SUS). M-SUS is a yeast-based genetic selection system which identifies protein interactions directly at the membrane. Full-length integral membrane proteins are fused to two small reporter modules. An interaction between the two proteins results in reporter module reconstitution and subsequent transcriptional activation of genomic reporter genes. Thus, a protein interaction at the membrane is converted into a genetic readout, selecting all cells expressing an interacting protein pair. Substitution of one defined partner by a pool of candidates expressed from a cDNA library of choice allows simultaneous screening of millions of potential interactors and the discovery of novel interaction partners for a membrane protein of interest. We demonstrate the versatility of the system by mapping the subunit topology of the heterotrimeric sodium channel ENaC and by screening the beta-ENaC subunit against a human lung cDNA library to identify novel interaction partners. Follow-up assays verify the identified interactors and also demonstrate a role for several interactors in regulating ENaC activity. In summary, M-SUS is a powerful tool to map protein interactions involving integral membrane proteins and can also be used at high throughput to systematically map interactions of different membrane protein classes, such as GPCRs, RTKs or ion channels.

Supported by: EU FP6 Projekt

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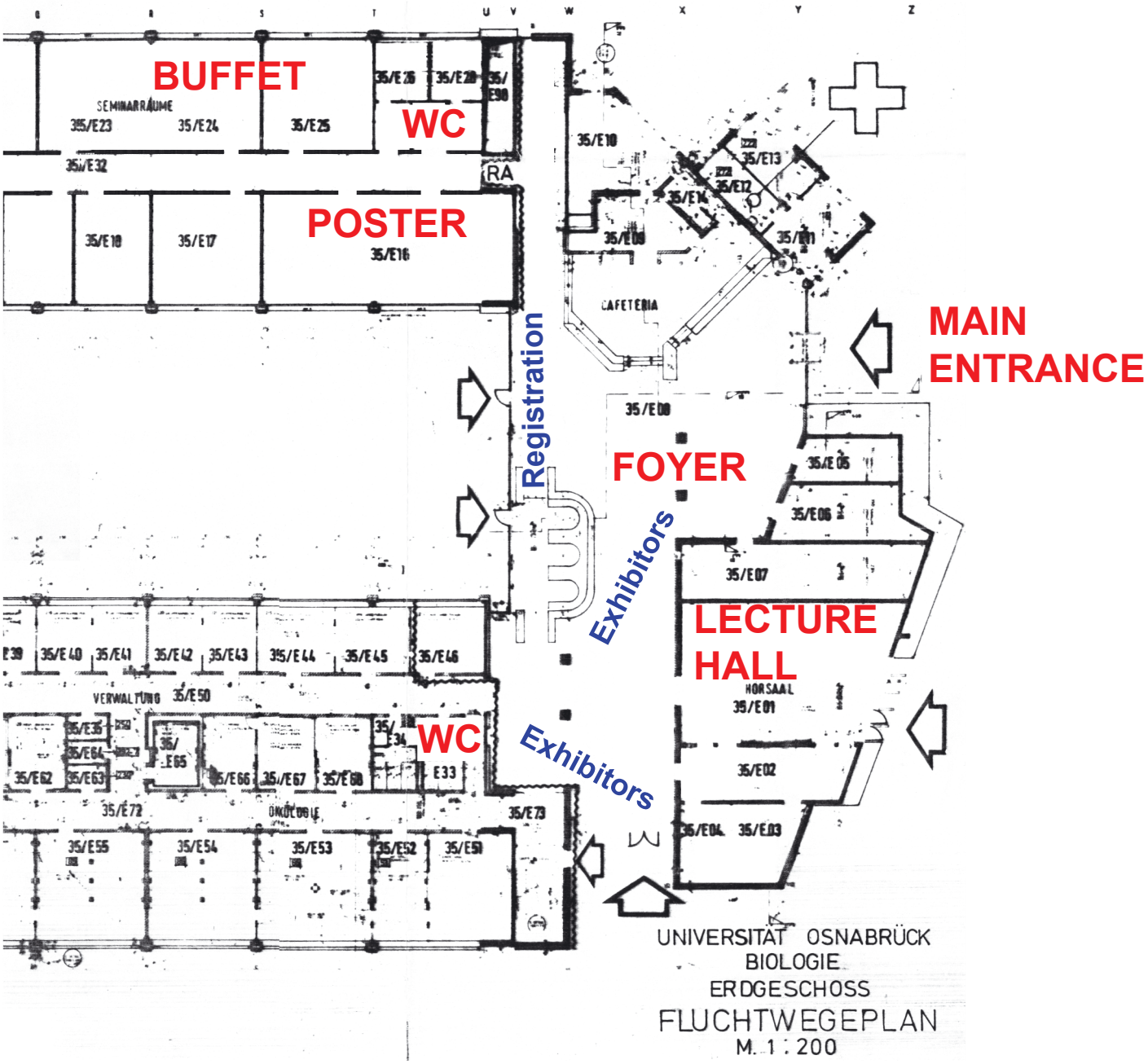

Free WLAN and internet access is available in the Foyer and the buffet room. Please ask for the code at the registration desk.

Nr. 259/2010
Osnabrück, 2010-09-06

Im Kampf gegen Krankheiten des Nervensystems

Universität Osnabrück lädt zur 5. Westerberger Herbsttagung ein

Die »Westerberger Herbsttagung« ist inzwischen gute Tradition: Bereits zum fünften Mal veranstaltet die Universität Osnabrück, diesmal zusammen mit der Studiengruppe »Molekulare Neurobiologie« der Gesellschaft für Biochemie und Molekularbiologie (GBM), von Donnerstag, 16. bis Samstag,18. September ein international besetztes Symposium zu den Perspektiven der molekularen Neurobiologie mit einem besonderen Fokus auf neurodegenerative Erkrankungen. Den Festvortrag zur Stammzellthematik hält am Donnerstagabend Prof. Dr. Wieland Huttner, Direktor des Max-Planck-Instituts für Zellbiologie und Genetik (Dresden) und Vorsitzender des Wissenschaftlichen Rates der Max-Planck Gesellschaft. Die öffentliche Veranstaltung beginnt um 19.30 Uhr im großen Hörsaal der Biologie, Barbarastraße 11.

Unter dem Titel »Molecular Neurobiology: Pathways in Health and Disease« werden während der Herbsttagung neue Resultate der Forschung in Form von Vorträgen und Postern präsentiert. Dabei liegt der Fokus auch in der Förderung junger Forscher und deren Austausch mit etablierten Wissenschaftlern. Die besten Poster werden mit Posterpreisen ausgezeichnet.

»Ziel dieses Symposiums ist es nicht nur, neue Forschungsergebnisse auszutauschen, sondern darüber hinaus auch Kontakte zwischen renommierten Wissenschaftlern und Nachwuchsforschern zu knüpfen«, so der Tagungsleiter Prof. Dr. Roland Brandt von der Universität Osnabrück. Seit 2002 findet die Tagung alle zwei Jahre in der Biologie auf dem Westerberg statt. Im Vordergrund steht das Ziel, grundlegende Mechanismen verschiedener degenerativer Hirnerkrankungen wie der Alzheimer-Erkrankung besser verstehen zu lernen. Brandt: »Dabei sind vor allem in den letzten Jahren immer stärker die molekularen Mechanismen der Krankheit in den Mittelpunkt der Forschung geraten. Hierin sehen wir auch eine Chance, langfristig neue Ansätze für grundlegende therapeutische Interventionen zu entwickeln.«

Unter anderem referieren Prof. Dr. Phillip Gordon-Weeks (London), Prof. Dr. Thomas Arendt (Leipzig), Dr. Tim Hucho (Berlin), Dr. Eva-Maria Mandelkow (Hamburg), Dr. Stefan Lichtenthaler (München) und Dr. Daniela Dieterich (Magdeburg). Zu der Tagung sind alle Interessierten herzlich eingeladen.

Erstmalig in diesem Jahr beteiligt sich die GBM an der Westerberger Herbsttagung. Die GBM e. V. ist die größte biowissenschaftliche Fachgesellschaft in Deutschland. Ihr gehören rund 5500 Mitglieder aus Hochschulen, Forschungsinstituten und der Industrie an. Ziel der Vereinigung ist die Förderung von Forschung und Lehre der Biochemie und molekularen Biowissenschaften, die Umsetzung wissenschaftlicher Erkenntnisse in Biotechnologie und Medizin und deren Verbreitung in der Öffentlichkeit.

Die Tagung ist als Fortbildungsveranstaltung für Ärztinnen und Ärzte anerkannt.

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