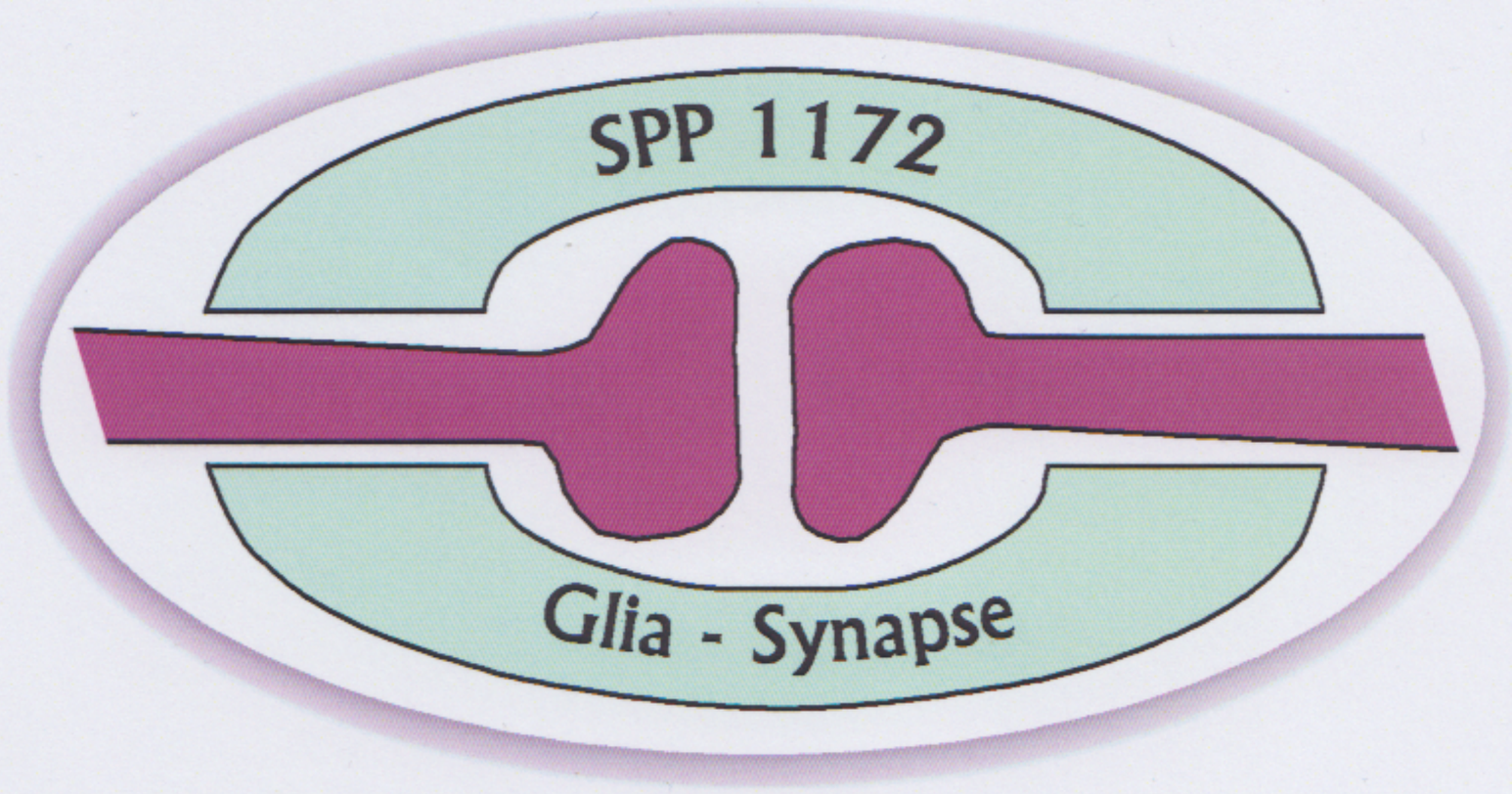
3rd Meeting of the Priority Program SPP 1172

- supported by the Deutsche Forschungsgemeinschaft -

The Significance of Neuroglia for the Formation, Function and Plasticity of Synapses



Poster Presentations

Sept. 28 - 30, 2006

Venue: Life & Brain Center, Medical Faculty, University of Bonn
Sigmund-Freud-Str. 25 (Venusberg), 53105 Bonn, Germany

Preliminary schedule - 3rd Symposium of the Priority Program 1172 „The role of neuroglia for the formation, function and plasticity of synapses“; in Bonn

Thursday, September 28th, 2006

14:00 **Welcome address**

R. Büttner, Dean of the Medical Faculty, University of Bonn

C. Steinhäuser, Co-Coordinator Priority Program

SESSION I

Neuron-glia signalling and synaptic plasticity

Chair: *Helmut Kettenmann*

14:30 ***Heinz Beck (Bonn)***

Synaptic metaplasticity in the normal and epileptic brain

15:00 ***Andreas Draguhn (Heidelberg)***

A novel mechanism of plasticity at inhibitory synapses: modulation of presynaptic GABA content

15:30 ***Joachim W. Deitmer (Kaiserslautern)***

Modulation of synaptic activity in the cerebellar cortex by Bergmann glia and ATP

16:00 *Refreshments and coffee break*

16:30 ***Gerald Seifert, Christian Steinhäuser (Bonn)***

Modulation of signal transmission in the hippocampus by GluR- and GluT-type glial cells

17:00 ***Alexej Verkhratsky (Manchester)***

NMDA-receptors mediated neuronal glia signalling

17:30 **Poster session I** *(Viewing)*

18:45 *Departure to Rolandswerth (dinner at restaurant Rolandsbogen)*

Friday, September 29th, 2006

09:00 **Special Lecture: *Craig E. Jahr (Portland)***

Neural-glial interactions in the cerebellar cortex

9:45 *Refreshments*

SESSION II

Glio- and Neurogenesis, synapse formation

Chair: *Eckart D. Gundelfinger*

10:15 ***Vittorio Gallo (Washington)***

NG2 progenitors and neurogenesis in the hippocampus and olfactory bulb

10:45 ***Tatsuhiro Hisatsune (Kashiwa)***

Nestin+ dividing cells as stem/progenitor cells in adult mammalian brain

11:15 ***Angelique Bordey (New Haven)***

Subventricular zone astrocytes send survival cues to neuroblasts via NMDA receptors

11:45 ***Christian Klämbt (Münster)***

Molecular control of glia differentiation in Drosophila

12:15 *Lunch*

14:00 **Poster session II – Discussion at posters**

16:00 ***Andreas Faissner (Bochum)***

Regulation of synapse formation, plasticity and function by astroglial-derived extracellular matrix

16:30 ***Rüdiger Klein (München)***

Glial control of dentritic spine morphology by Eph/ephrin signalling

17:00 ***Jacqueline Trotter (Mainz)***

NG2-expressing cells in glial-neuronal signalling and synapse formation

18:00 *Business meeting of the members of the Priority Program 1172*

Saturday, September 30th, 2006

SESSION III

Glial receptors and transporters

Chair: *Heinrich Betz*

09:00 ***Sergei Kirischuk (Berlin)***

GABA transporters determine the strength of GABAergic synaptic transmission in layer I of the neonatal visual cortex

09.30 ***Ken McCarthy (North Carolina)***

RASSLING astrocytic GPCRs: Selective activation of astrocytic GPCR – Signalling cascades yields more surprises

10:00 ***Mustapha Bennay (Düsseldorf)***

Glutamate-induced sodium signals as reporters of glutamate uptake in the cerebellum

10:30 *Refreshments*

11:00 ***Rolf Dermietzel (Bochum)***

Hemichannels: Facts and Fictions

11:30 ***Andreas Reichenbach (Leipzig)***

Neuron-to-glia signalling in the mammalian retina

12:00 ***Frank Kirchhoff (Göttingen)***

Analysis of induced deletion of AMPA-type glutamate receptors in Bergmann glia

12:30 *General discussion*
Closing remarks and farewell

P 1 **Effect of different ECM components on synaptic and voltage-activitated current in hippocampal neurons**

K.S. Erlkamp[1] , A.K. Vogt-Eisele[1] , M. Pyka[2], A. Faissner[2], H. Hatt[1]

[1] *Dept. of Cell Physiology, Ruhr-University Bochum, Germany*
[2] *Dept. of Cell Morphology and Molecular Neurobiology, Ruhr-University Bochum*

P 2 **ECM and synaptogenesis: Monitoring the impact of extracellular matrix on synapse formation in hippocampal neurons**

M. Pyka[1], A. Vogt-Eisele[2], K. Erlkamp[2], C. Seidenbecher[3], E. Gundelfinger[3], H. Hatt[2], A. Faissner[1]

[1] *Dept. of Cell Morphology and Molecular Neurobiology, Ruhr-University Bochum*
[2] *Dept. of Cell Physiology, Ruhr-University Bochum, Germany*
[3] *Dept. of Neurochemistry and Molecular Biology, Leibniz-Institute for Neurobiology, Magdeburg, Germany*

P 3 **Local retrograde effects of BDNF on synapse formation after BDNF-transfection into hippocampal neurons from *bdfn*-/-mice**

B. Singh, C. Henneberger, J.C. Meier[3], A. Rodriguez-Tebar[2] and R. Grantyn[1]

[1] *Institute of Neurophysiology, Charite, Berlin, Germany*
[2] *Institute of Neurobiology, Cajal, CSIC, Madrid, Spain*
[3] *Helmholtz Hyperexcitability and RNA Editing Group, Max-Delbrück-Centre for Molecular Medicine, Robert-Rössle-Str. 10, D-13092 Berlin-Buch, Germany*

P 4 **Heterogeneous appearance of perineuronal cells nets of ECM in neural cultures**

R. Frischknecht, N. John, E. Gundelfinger, C. Seidenbecher
Leibniz Institute of Neurobiology, Magdeburg, Germany

P 5 **Activity-induced sodium signals in Bergmann glial cells and Purkinje neurons**

M. Bennay, S. Meier, K. Kafiz, C. Rose
Institute for Neurobiology, Heinrich-Heine-University Duesseldorf, Germany

P 6 **Synaptic activity in the rat cerebellar cortex as modulated by Bergmann glial cells**

M. Ascherl, D. Casel, J. Brockhaus, P. Histel, M. Pottek, J.W. Deitmer
Department of Biology, TU Kaiserslautern, Germany

P 7 **Astrocytes selectively respond to glutamatergic neurotransmission restricted to the innervation domain of L 4 to L 2/3 connections in the barrel cortex**

B. Haas[1], C. Schipke[2] and Helmut Kettenmann[1]

[1] *Cellular Neurosciences, Max-Delbrück-Centre for Molecular Medicine, Berlin, Germany*
[2] *Klinik für Psychiatrie and Psychotherapie, FEM (Forschungseinrichtung Experimentelle Medizin), Charite-Universitätsmedizin Berlin, CBF, Germany*

P 8 **Glia cells listening to the Calyx of Held**

J. Müller, D. Reyes-Haro, H. Kettenmann
Cellular Neurosciences, Max-Delbrück-Centre for Molecular Medicine, Berlin, Germany

P 9 **Analysis of $GABA_A$ receptor currents in hippocampal glial cells**

M. Grauer, G. Seifert, R. Jabs, C. Steinhäuser
Institute of Cellular Neurosciences, University of Bonn, Germany

P 10 **Enhanced nonsynaptic CI conductance in developing hippocampal neurons overexpressing a high affinity glycine receptor results in altered dendrite morphology due to changes in the E/I ratio of synaptic input**

JC. Meier[2], S. Kirischuk[1], R. Grantyn [1]

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[2] *Helmholtz Hyperexcitability and RNA Editing Group, Max-Delbrück-Center for Molecular Medicine, Robert-Rössle-Str. 10, D-13092 Berlin-Buch, Germany*

P 11 **Selective permeability of different connexin channels to the second messenger cyclic AMP**

P. Bedner[1,,3], H. Niessen[1], B. Odermatt[1], M. Kretz[1], K. Willecke[1], H. Harz[2]

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[2] *BioImaging Centre of the Ludwig-Maximilians-University of Munich, Martinsried, Germany*
[3] *Institute of Cellular Neurosciences, University of Bonn, Germany*

P 12 **The developmental expression of K^+ channels in retinal glial cells is associated with a decrease of osmotic cell swelling**

A. Wurm[1,2], Th. Pannicke[1], I. Iandiev[2,3], P. Wiedemann[2], A. Reichenbach[1], A. Bringmann[2]

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[3] *Interdisziplinäres Zentrum für Klinische Forschung (IZKF) at the Medical Faculty University of Leipzig, Germany*

P 13 **Viscoelastic properties of glial cells and neurons in the CNS**

Y.-B. Lu[1,2,3,*], K. Franze[2,3,*], G. Seifert[4], C. Steinhäuser[4], F. Kirchhoff[5], H. Wolburg[6], J. Guck[3], P. Janmey[7], E-Q. Wei[1], J. Käs[3], A. Reichenbach[2]

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[6] *Institute of Pathology, University of Tübingen, Germany*
[7] *Institute for Medicine and Engineering, University of Pennsylvania, Philadelphia, USA*

* Y.B. Lu and K. Franze contributed equally to this work.

P 14 **Ca^{2+} responses of Müller cells induced by light stimulation of photoreceptor cells**

K. Rillich[1], J. Gentsch[1], M. Weick[1], A. Bringmann[2], A. Reichenbach[1]

[1] *Paul Flechsig Institute of Brain Research, University of Leipzig Medical Faculty, Leipzig*
[2] *Dept. of Ophtalmology and Eye Clinic, University of Leipzig Medical Faculty, Leipzig*

P 15 **Genetic approaches towards the understanding of GlyT1 functions in vivo**

V. Eulenburg, M. Retiounskaia, C. Shimizu-Okabe, H. Betz
Max-Planck-Institute for Brain Research, Dept. Neurochemistry, Frankfurt/Main, Germany

P 16 **Functional properties of neurons derived from in-vitro reprogrammed astroglia**

B. Berninger[1,2], R. Blum[1], B. Sutor[1], M. Götz[1,2]

[1] *Department of Physiological Genomics, Institute of Physiology, LMU Munich, Germany*
[2] *Institute for Stem Cell Research, National Research Center for Environment and Health, Neuherberg, Germany*

P 17 **New transgenic mouse models to study the role of astrocytes in synaptogenesis *in vivo***

M. Slezak[1,2], D. Metzger[3], P. Chambon[3], F.W. Pfrieger[1]

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[2] *Dept. of Neuroanatomy, Jagiellonian University, Krakow, Poland*
[3] *IGBMC and ICS, Illkirch, France*

P 18 **Functional expression of genetically-encoded calcium sensors in neurons and astrocytes of transgenic mice**

M. Mank, M. Friedrich, O. Griesbeck
Max-Planck-Institute for Neurobiology, Martinsried, Germany

P 19 **Generation of a NG2-EYFP mouse for studying the role of NG2-expressing cells in synaptic function**

K. Karram[1], S. Goebbels[2], M. Schwab[2], F. Kirchhoff[2], K.A. Nave[2], J. Trotter[1]

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Effect of different ECM components on synaptic and voltage-activated currents in hippocampal neurons

K.S. Erlkamp[1], A.K. Vogt-Eisele[1], M. Pyka[2], A. Faissner[2] and H. Hatt[1]

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[2] *Lehrstuhl für Zellmorphologie und Molekulare Neurobiologie, RUB*

The presence of astrocytes is crucial for the development of synapses in central neurons[1], but the factors mediating their effects on the formation and maintenance of synapses are only partially known. In order to investigate the involvement of astrocyte-secreted ECM molecules, we established astrocyte-neuron cocultures and removed several ECM components by treatment with appropriate degrading enzymes. Electrophysiological characterization of enzyme-treated and control cultures of rat astrocytes and hippocampal neurons showed that not only synaptic activity but also voltage-dependent currents were affected by the removal of certain matrix components. In future, we will do further measurements to complete our data, especially evoked autaptic currents, for which we have established single-neuron microcultures, and we will look for interaction partners and signalling pathways involved in the observed effects.

[1] Pfrieger FW, Barres BA (1997) Synaptic efficacy enhanced by glial cells in vitro. Science 277: 1684-1687

ECM and synaptogenesis: Monitoring the impact of extracellular matrix on synapse formation in hippocampal neurons

Martin Pyka[1], Angela Vogt-Eisele[2], Katja Erlkamp[2], Constanze Seidenbecher[3], Eckart Gundelfinger[3], Hanns Hatt[2] and Andreas Faissner[1]

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Synapses represent specialized cell-cell contact sites between nerve cells. These structures mediate the rapid and efficient transmission of signals between neurons and are surrounded by glial cells. Former investigations have shown that astrocytes and astrocyte-derived extracellular matrix (ECM) components are important for formation, maintenance and function of synapses in the CNS. In order to study the effects of glial-derived ECM on synaptogenesis, we have established an *in-vitro* co-culture system for E18 rat hippocampal neurons and various glial cell types. Neurons were cultured without direct contact to glial cells and after 10 days in culture a concordant expression of pre-and postsynaptic proteins could be documented. Moreover, the colocalization of bassoon and proSAP1 indicated the formation of structurally intact synapses. Furthermore we have developed a technique that permits the semi-automated quantitative determination of the number of synaptic puncta per neuron. Therefore significant differences of the efficacy of cell types for and the effects of defined treatments on synaptogenesis could be documented. Thus, primary astrocytes proved the most efficient cell type in fostering synaptogenesis. Our present studies focus on maturation of synapses in the presence or absence of enzymes, which are able to degrade astrocyte-released ECM components.

Local retrograde effects of BDNF on synapse formation after BDNF-transfection into hippocampal neurons from bdnf-/- mice.

B. Singh, C. Henneberger, J. Meier, A. Rodriguez-Tebar[2] and R. Grantyn*[1]

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[2] *Institute of Neurobiology, Cajal, CSIC, Madrid, Spain.*

Extracellular concentration gradients of neurotrophins are thought to facilitate attraction and/or stabilization of excitatory inputs. The respective mechanisms are not yet clear. Here we used low-density neuronal cultures from the hippocampus of E18 bdnf-/- mice to create a defined localisation of BDNF by transfection of a BDNF::EGFP chimera plasmid into solitary neurons. Neurons expressing the transfected BDNF (tBDNF) were compared with wild-type neurons and bdnf-/- neurons expressing only EGFP (controls). After an expression time of 16 h the cultures were fixed and processed for double or triple immunostaining applying antibodies against synaptophysin (Syp), the vesicular glutamate transporter (VGluT) and the vesicular inhibitory amino acid transporter (VIAAT). Under control conditions, on day in vitro 5, an average bdnf-/- hippocampal neuron in culture (n>300) possessed ~5 primary dendrites, with a total of ~11 branch points per neuron (within a circular view field of r=50 µm, centred to the soma), and it carried ~8 excitatory and ~4 inhibitory synaptic contacts. Neurons exhibiting expression of EGFP-tagged BDNF carried a significantly larger number of glutamatergic synaptic terminals than EGFP-labelled controls (~+100%). The tBDNF-induced increase in the number of glutamatergic synaptic contacts was sensitive to block of TrkB signalling (with a blocking TrkB-Ab or with K252a, a tyrosin kinase inhibitor), but resistant to a blocking p75(NTR) antibody.

The tBDNF-induced change in glutamatergic synaptogenesis was acompanied by a reduction in the length growth of the dendrites, while the branching of dendrites strongly increased. Experiments with receptor antagonists (block of TrkB, p75 or GluRs) revealed that the arrest of dendritic elongation was probably due to the tBDNF-induced increase in glutamatergic synaptic activity, whereas enhanced dendritic branching displayed little dependence on synaptic input, being mediated by p75(NTR).

Finally, we observed that the up-regulation of glutamatergic synaptic terminal number was always associated with a down-regulation of inhibitory synaptic terminal number (~-50%). Recording of miniature PSCs revealed a corresponding change in the E/I ratio of synaptic input. The experiments with receptor blockers are consistent with the idea that not only the reduced length growth of the dendrites, but also the down-regulation of inhibitory synaptogenesis were consequences of the tBDNF-stimulated enhancement of glutamatergic synaptic activity.

Together, these experiments on solitary BDNF::EGFP expressing neurons in bdnf-/- hippocampal cultures identify a strong, presumably retrograde, TrkB-mediated up-regulation of glutamatergic synaptic input which resulted in a significant increase in the E/I ratio of synaptic input and a suppression of dendrite elongation.

It is now intended to apply this approach to study the contribution of individual nonneuronal cells to the neurotrophinergic regulation of dendritic growth and synapse formation.

Heterogeneous appearance of perineuronal nets of ECM in neural cultures

Renato Frischknecht, Nora John, Eckart Eckart, Constanze Seidenbecher

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Perineuronal nets (PNN) are specialized extracellular matrix structures enwrapping CNS neurons. They are important regulators for neuronal and synaptic functions. Here, we have classified the appearance of these structures in hippocampal primary cultures. We used brevican, a chondroitin sulfate proteoglycan that is an integral component of PNN as molecular marker. It is primarily synthesized by co-cultured glial fibrilary acidic protein- (GFAP-) positive astrocytes and co-assembles with its interaction partners in PNN-like structures on neuronal somata and neurites as identified by counterstaining with the PNN marker *wisteria floribunda* agglutinin (WFA). We found three major classes of brevican-containing PNN-like structures in dissociated neurons. Type 1 is the most abundant PNN appearance observed in cultured neurons. It is formed around somata of excitatory cells and is typically wrapped around the axon initial segment. Type 2 is formed on a different excitatory cell type and shows very little immunoreactivity for brevican and a punctate staining pattern with WFA. As type 3 we classified the most pronounced net formed around inhibitory GAD65-positive cells. It shows strong immunoreactivity for brevican and tenascin-R and is strongly labeled with WFA. It is, like type-2 nets, expressed only on approx. 1% of hippocampal neurons in culture. Co-cultures of wild-type mouse neurons with brevican-deficient mouse glial cells and *vice versa* reveal glial vs. neuronal contributions to the brevican nets. Furthermore, we found that some but not all axon initial segments appear to be heavily labeled. Both excitatory and inhibitory synapses are embedded into PNN. Altogether, we show that mature primary cultures can form different types of PNN, and that basic features of these extracellular matrix structures described in the brain may also be studied *in vitro*.
Funding: DFG SPP 1172/2 (Gu230/5-2)

Activity-induced sodium signals in Bergmann glial cells and Purkinje neurons

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Glial glutamate transporters are central for the termination of synaptic transmission and protect neurons from excitotoxicity induced by elevated levels of extracellular glutamate. They are electrogenic and use the electrochemical gradient of sodium to move glutamate into the cell. Electrophysiolo gical recordings at the somata of cerebellar Purkinje neurons indicated that glutamate uptake results in a fast decline of glutamate in the synaptic cleft and shapes the time course of synaptic conductance. Such recordings, however, only provide an indirect look at the sites of synaptic transmission. Because both glutamate receptor activation and glutamate transport involve movement of sodium, glutamatergic transmission is likely to result in sodium increases in cellular processes. Sodium accumulation will cause a reduction in the biochemical driving force for glutamate uptake at active synapses and might result in increased extracellular glutamate concentration and excitation of neurons.

To analyse activity-induced sodium transients in processes of Bergmann glial cells and Purkinje neurons, we use ratiometric imaging with the fluorescent dye SBFI combined with whole-cell patch-clamp recordings in slices of mouse cerebellum. Our experiments show that exogenous application of glutamate as well as short bursts of parallel or climbing fibres activation induce intracellular sodium transients in both cell types that amount to up to 6-8 mM in fine processes. Glutamate-induced sodium transients in Bergmann glial cells are independent of neuronal activity because the y persist in the presence of TTX. The amplitude of activity-induced glial sodium transients is only slightly diminished by the ionotropic receptor blocker CNQX, but largely reduced by the glutamate transport antagonist TBOA.

Thus, our data demonstrate that synaptic activity in the cerebellum causes significant intracellular sodium signals in Bergmann glial cells due to activation of sodium-dependent glutamate uptake. The determination of activity-induced changes of potassium ions and protons together with the reported sodium transients will allow to determine the consequences of these changes for the efficacy of glutamate uptake and, thus, glutamatergic synaptic transmission at Purkinje cell synapses.

Synaptic activity in the rat cerebellar cortex as modulated by Bergmann glial cells

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We have shown that depolarising stimuli in a Bergmann glial (BG) cell in acute rat cerebellar slices modifies the frequency of spontaneous postsynaptic currents (sPSCs) in the neighbouring Purkinje neuron by activating ionotropic glutamate receptors (Brockhaus & Deitmer, 2002, J. Physiol.545.2, 581-593). This effect is long-lasting (>1 h), and is primarily due to suppression of sPSCs originating from the inhibitory interneurons. Injection of D-aspartate or D,L-threo-ß-hydroxy-aspartate into the BG cell suppressed this effect, suggesting that glutamate was released by reversed glutamate uptake. The ionotropic receptors involved were identified to be AMPA receptors, presumably located in inhibitory interneurons. The mechanism, by which BG stimulation reduced the frequency of sPSCs in Purkinje neurons appears to involve interference of purinergic signalling in the cerebellum by glutamate via AMPA receptors. Application of AMPA indeed reduced the synaptic response to ATP. Blocking purinergic P2 receptors with PPADS (10 μM) reduced the frequency of sPSCs itself and prevented further modulation by BG stimulation. Our results suggest that activation of AMPA receptors by glutamate, released from a single BG cell, reduces the frequency of synaptic events in Purkinje neurons. This reduction appears to be due to impairment of the effect induced by endogenous ATP released in the cerebellar cortex, as observed from the third postnatal week onwards (Casel et al., 2005; J. Physiol. 568.1, 111-122).

Astrocytes selectively respond to glutamatergic neurotransmission restricted to the innervation domain of L 4 to L 2/3 connections in the barrel cortex

Brigitte Haas[1], Carola Schipke[2] and Helmut Kettenmann[1]

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The sensory information from single whiskers in rodents projects to defined morphological units in the cortex, the barrel fields. We studied astrocyte Ca^{2+} signalling in response to neuronal activity in acute slices of the mouse barrel cortex. Electrical stimulation in layer 4 triggered distinct tetrodotoxin-sensitive Ca^{2+} responses in two cell populations: astrocytes and neurons. The astrocyte population was identified by their sulforhodamine 101 positive labelling, while we were able to record action potentials from the other cell population. The neuronal signal was observed immediately with the onset of the stimulation and had a duration of 1-2 s. Responding cells were observed across barrel borders, mainly in layer 2/3. The astrocytic signal was recorded with a delay of about 1 second to the stimulus. This Ca^{2+} response was significantly higher in amplitude, lasted for 5 to 7 seconds and was confined to the barrel field. We also observed synchronized spontaneous activity in the same population of cells which responded immediately with the onset of the stimulation, but this activity was not followed by a Ca^{2+} response in the astrocytes. The astrocyte response after stimulation was strongly attenuated by a cocktail of glutamate receptor antagonists indicating that astrocytes are activated by excitatory, glutamatergic neuronal signal transmission within a barrel column. In contrast, in the presence of the $GABA_A$ receptor antagonist bicuculline or the purinergic antagonist suramin, the stimulation activated a Ca^{2+} response in a much larger population of astrocytes, no longer restricted to the barrel field. Our data show that astrocytes respond to the activation of glutamatergic neurons within the innervation domain of layer 4 to layer 2/3 connections in the barrel cortex. We assume that bicuculline and suramin change the balance towards excitation over inhibition and thereby lead to a larger astrocyte response.

Glial cells listening to the Calyx of Held

Jochen Müller, Daniel Reyes-Haro, Helmut Kettenmann

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The Calyx of Held is a giant presynaptic terminal in the superior olivary complex of the mammalian brain stem. It plays an important role in sound location and is designed for rapid signal transmission with high fidelity. In the last years this preparation has been used to study mechanisms of synaptic transmission and plasticity, because it offers the advantage to study both, the pre- and postsynaptic element simultaneously.

In the present study we characterized the glial cells associated with Calyces of Held in the medial nucleus of the trapezoid body (MNTB). We identified astrocytes in acute slices from young (P8-P10) mice by loading slices with fluo-4. To test if astrocytes respond to Calyx synapse activity, we stimulated the afferent fibres crossing the midline of the brainstem and imaged calcium activity in astrocytes. We selected for astrocytes in close proximity to principal neurons. Afferent fiber stimulation triggered a transient increase in astrocyte Ca^{2+} and this response was completely blocked by tetrodotoxin. Combined application of gabazine and strychnine (antagonists of $GABA_A$ and glycine receptors) reduced the response to 67% of control, the kainate/AMPA antagonist CNQX to 73%. Combined application of all three antagonists reduced the response to 47%.

To test for the presence of functional transmitter receptors on the astrocytes, we applied glycine and found that a small population of astrocytes responded with an increase in Ca^{2+}. This response was strychnine-sensitive. In a first set of experiments kainate induced Ca^{2+} responses that where blocked by the antagonist NBQX. The expression of glycine and kainate/AMPA receptors were confirmed by patch-clamp recordings. We also obtained evidence for the expression of metabotropic glutamate receptors. t-ACPD (agonist of mGluR I and II) triggered a Ca^{2+} response which was partially blocked by AIDA (antagonist of the metabotropic glutamate receptor I). Our data indicate that astrocytes can sense the activity of the Calyx synapse and that this form of neuron-glia interaction involves several transmitter systems.

Enhanced nonsynaptic Cl^- conductance in developing hippocampal neurons overexpressing a high affinity glycine receptor results in altered dendrite morphology due to changes in the E/I ratio of synaptic input

J.C. Meier, S. Kirischuk, R. Grantyn

Dev Neurophysiol, University Medicine - Charite, Berlin, Germany

Nonsynaptic actions of ambient GABA and glycine are believed to influence the survival, growth and connectivity of developing neurons. Drug-induced enhancement of Cl^- conductances in the immature brain is known to have deleterious effects on the cognitive abilities at older age. By inducing overexpression of a high affinity GlyR a3 subunit in developing hippocampal neurons in culture we aimed at clarifying the possible consequences of persistent strong Cl^- conductances on dendrite morphology and synapse development. To this end, hippocampal neurons were transfected with an isoform of GlyR a3 (a3P185L) that has previously been demonstrated in the neonatal and adult brain as a result of posttranscriptional editing (Meier et al 2005). In mature neurons a3P185L was shown to produce tonic inhibition. Here we report that the expression of a3P185L resulted in reduced dendritic length, enhanced dendritic branching and a higher ratio of excitatory/inhibitory (E/I) synaptic terminal numbers, if compared with controls expressing the unedited isoform. As hippocampal neurons display an inverse relationship between the number of glutamatergic contacts and dendritic length (Singh et al 2006) we investigated the possibility that the suppression of dendrite elongation was due to the enhancement of the glutamatergic synaptic input in neurons overexpressing a3P185L. Indeed, chronic treatment with a cocktail of GluR antagonists abolished the suppression of dendrite growth although the number of glutamatergic contacts was even higher than in untreated controls. These results suggest that a persistent increase of nonsynaptic Cl^- conductance in developing hippocampal neurons alters the E/I ratio of synaptic input and this imbalance may lead to pathological dendrite morphology.

The role of astrocytes in the activation of these persistent high Cl^- conductance need to be analyzed to fully understand the nonsynaptic effects of inhibitory neurotransmitters in the developing brain.

Analysis of $GABA_A$ receptor currents in hippocampal glial cells

Michael Grauer, Gerald Seifert, Ronald Jabs, Christian Steinhäuser

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Recent work identified two different types of glial cells with hGFAP promoter activity coexisting in the hippocampus, possessing either glutamate receptors or glutamate transporters (GluR and GluT cells). The GluR-type is equipped with functional AMPA and $GABA_A$ receptors and receives direct synaptic input from glutamatergic and GABAergic neurons. The functional impact of synaptic innervation of GluR cells is still unclear. Here, we combined functional and molecular techniques to analyse properties of $GABA_A$ receptors in GluR cells.

GABA activated slowly desensitizing receptor responses in GluR cells (tau about 2 s). The $GABA_A$ receptor agonist, muscimol, mimicked GABA-induced responses. Currents were sensitive to the $GABA_A$ receptor antagonist, bicuculline. To elucidate the $GABA_A$ receptor subunit composition, we tested the Zn^{2+} sensitivity of receptor responses as well as their modulation by benzodiazepines. Micromolar concentrations of Zn^{2+} blocked GABA responses effectively. Preincubation of benzodiazepines, modulators of $GABA_A$ receptors, did not increase the receptor responses. To identify receptor subunits expressed by GluR cells, single cell transcript analysis was performed subsequent to functional characterization. The subunits alpha 2 and 4, beta 2/3, and gamma 2 were most abundant, matching the pharmacological properties.

To determine the effect of $GABA_A$ receptor activation on membrane potential, perforated patches were obtained from GluR cells *in situ*. In the current-clamp mode, maximal activation of the GABA-mediated Cl^- conductance depolarized the cells to -20 ± 6 mV ($n = 6$). Comparison of reversal potentials obtained with different $[Cl^-]_i$ (whole-cell mode) revealed a physiological $[Cl^-]_i$ of GluR cells of about 60 mM. Current experiments aim at elucidating the impact of GABA-mediated depolarization of GluR cells on intra- and intercellular signalling pathways.

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Selective permeability of different connexin channels to the second messenger cyclic AMP

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Gap junctions are intercellular conduits that are formed in vertebrates by connexin proteins and allow diffusional exchange of intracellular ions and small molecules. At least 20 different connexin genes in the human and mouse genome are cell type specifically expressed with overlapping expression patterns. A possible explanation for this diversity could be different permeability of biologically important molecules, such as second messenger molecules. We have recently demonstrated that cyclic nucleotide-gated channels can be used to quantify gap junction-mediated diffusion of cyclic AMP. Using this method, we have compared the relative permeability of gap junction channels composed of connexin26, -32, -36, -43, -45 or -47 proteins towards the second messenger cAMP. Here we show that cAMP permeates through the investigated connexin channels with up to 30fold different efficacy. Our results suggest that intercellular cAMP signalling in different cell types can be affected by the connexin expression pattern.

The developmental expression of K^+ channels in retinal glial cells is associated with a decrease of osmotic cell swelling

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A major function of glial cells is the control of osmotical and ion homeostasis, mediated predominantly by K^+ and water movements through inwardly rectifying K^+ (Kir) and aquaporin water channels. During the postnatal differentiation, retinal glial cells increase the expression of Kir channels. Here, we investigated whether the developmental expression of Kir channels is associated with an increased expression of aquaporins and by an alteration of the osmotic swelling characteristics of glial (Müller) cells in the rat retina. Around the time of eye opening at postnatal day (P) 15, developing glial cells fully alters their membrane conductances, from a current pattern with prominant fast transient K^+ and Na^+ currents to a pattern which displays only non-inactivating currents mediated by Kir and delayed rectifier K^+ channels. Concomitantly with the expression of Kir channels in glial cells, aquaporins-1 and -4 are expressed in the developing retina. During the retinal development, the glial cells alter their swelling characteristics; somata of immature cells in early postnatal retinas (P5 to 15) swell upon hypotonic challenge which is not observed in mature cells at P20 and 25. However, glial cells at all developmental stages swell when the Kir channels were blocked by Ba^{2+} ions. The data suggest that Kir channel-mediated release of K^+ ions normally prevents glial cell swelling during anisoosmotic conditions, and that the low expression level of K^+ channels in immature glial cells supports osmotic glial cell swelling. It is suggested that the expression of Kir channels by mature glial cells is a precondition for the homeostasis of the extracellular space volume in the neural tissue.

Viscoelastic properties of glial cells and neurons in the CNS

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150 years ago glial cells were discovered as a second, non-neuronal, cell type in the central nervous system (CNS). In order to ascribe a function to these new, enigmatic cells, it was suggested that they either glue the neurons together (the Greek word "γλια" means "glue"), or provide a robust scaffold for them ("support cells"). While both speculations are still widely accepted, they would actually require quite different mechanical cell properties, and neither one has ever been confirmed or rejected experimentally.

In our study, we investigated the biomechanics of CNS tissue and acutely isolated individual neurons and glial cells from mammalian brain (hippocampus) and retina. Scanning force microscopy, bulk-rheology, and optically induced deformation were used to determine their viscoelastic characteristics. We found that (i) in all CNS cells the elastic behavior dominates over the viscous behavior, (ii) in distinct cell compartments, such as soma and cell processes, the mechanical properties differ, most likely due to the unequal local distribution of cell organelles, (iii) in comparison to most other eukaryotic cells, both neurons and glial cells are very soft ('rubber elastic'), and (iv), finally, glial cells are even softer than their neighboring neurons. Our results indicate that glial cells can neither serve as structural support cells (as they are too soft) nor as glue (since restoring forces are dominant) for neurons. Rather, they may act as shock absorbing compliant embedding around neurons, protecting them in case of mechanical trauma. Intriguingly, others have shown that in culture, neurites grow better on soft than on stiff substrates. This means that the softness of glial cells may facilitate neurite growth and synapse formation, and may even be a precondition of these processes during ontogenetic development as well as in the course of physiological (i.e., activity-dependent) and pathophysiological neuronal plasticity (e.g., during neuronal regeneration).

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Ca^{2+} responses of Müller cells induced by light stimulation of photoreceptor cells

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Müller glial cells respond with an intracellular calcium rise to light stimulation of the retina. Under dark adapted conditions this is a very slow calcium rise, which applies to all Müller cells and starts right after the beginning of the light stimulation. After further strong light stimulation Müller glial cells react with a second calcium response which is faster and starts at the level of the ganglion cell layer, similar to that published by Newman (2005). Pharmacological experiments so far showed for the slow calcium rise, that about two third of the signal transfer from neurons to Müller cells occurs at the level of the photoreceptor cells. Glutamate seems to be involved in the signal transduction, but neither via ionotropic nor via metabotropic glutamate receptors, but rather via glutamate transporters. Blocking of the glutamate transporters by TBOA reduces the signal by some 30%. The glutamate dependence of the slow calcium rise might also be due to a feedback inhibition mediated by the mGluR8 receptor, which is presynaptically located on photoreceptors (Koulen et. al., 1999). Chloride channels seem also to be involved in the generation of the slow calcium rise, since it can be blocked by the chloride channel blockers NPPB, Flufenamic acid and Niflumic acid.

The fast calcium rise starts after a delay of about 2.5 minutes at the level of the ganglion cell layer. It originates in the smooth endoplasmatic reticulum of the Müller glial cells, which could be shown by the application of cyclopiazonic acid. Cyclopiazonic acid reduces the number of Müller cells displaying fast calcium rises to zero, but leaves the first slow calcium rise of the cells unaltered.

Genetic approaches towards the understanding of GlyT1 functions in vivo.

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Glycine acts as inhibitory neurotransmitter in many interneurons of the spinal cord and brain stem by activating strychnine glycine receptors. In addition, it is an essential co-agonist of the NMDA subtype of the ionotropic glutamate receptors at excitatory synapses. At both receptors, the concentration of glycine in the synaptic cleft must be precisely regulated. This is accomplished by two high-affinity Na^+-dependent glycine transporters located in the plasma membranes of glial (GlyT1) and neuronal (GlyT2) cells.

GlyT1 is widely expressed in glial cells of all major regions of the CNS and in addition in a subset of presumably glutamatergic neurons, whereas GlyT2 is found predominantly in brain stem and spinal cord interneurons and concentrated in the plasma membrane of axonal boutons directly opposed to glycine receptors. The analysis of GlyT deficient mice has shown that, in the neonatal mouse, GlyT1 is essential for the removal of glycine from the extracellular space, whereas GlyT2 replenishes glycinergic nerve terminal with glycine for neurotransmitter loading of synaptic vesicle.

To investigate the role of GlyT1 in the adult at both excitatory and inhibitory synapses, we generated mice allowing the conditional inactivation of the GlyT1 gene using the Cre-LoxP-system. Mice carrying homozygously a floxed GlyT1 allele do not display any apparent phenotype thus confirming the functionality of the modified gene. The predominantly glial inactivation of the GlyT1 allel using mouse-GFAP driven Cre expression resulted in perinatal death, as seen with conventional GlyT1 -/- mice. In contrast, mice carrying a neuronally inactivated GlyT1 gene are normal at birth and do not show obvious abnormalities at later developmental stages. The functional properties of glutamatergic synapses in these animals are currently being investigated.

In conclusion, our data show that the phenotype seen in conventional GlyT1 deficient mice is caused by glial expressed GlyT1. Further analysis of the conditional GlyT1 deficient mice will allow to investigate GlyT1 function at later developmental stages and in a cell type-specific manner.

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Functional properties of neurons derived from in-vitro reprogrammed astroglia

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In neurogenic regions of the adult brain astrocytes were identified as stem cells that give rise to neurons and oligodendrocytes. However, this capability to generate neurons is restricted to a subpopulation of astrocytes residing within specific neurogenic niches such as the subependymal zone or the subgranular zone in the hippocampus. Albeit generally non-neurogenic, previous studies have shown that early postnatal neocortical astrocytes can be reprogrammed under the influence of the paired box transcription factor (TF) Pax6 to adopt a neuronal fate. Here we addressed the question of which degree of functionality can be achieved by these cells. Neurons derived from glial cells expressing Pax6 or the pro-neural basic-helix-loop-helix TFs Neurogenin-2 (Ngn2) or Mash1 were found to acquire active conductances and were capable of firing action potentials. Moreover, when co-cultured with neurons from embryonic cortex the cells received synaptic input. Despite this remarkable degree of functionality glia-derived neurons failed to give rise to functional synaptic output in sharp contrast to neurons derived from embryonic cerebral cortex or postnatal cortical neurospheres. Failure of synapse formation was also corroborated by lack of significant expression of key molecules underlying synapse assembly such as the presynaptic cytomatrix protein bassoon or postsynaptic molecule Shank3. Moreover, while neurons derived from astroglial cells apparently form a dendritic compartment as shown by MAP2 staining, we have preliminary evidence for a failure of establishing a fully mature axonal compartment. These results suggest that the reprogramming induced by neurogenic TFs may remain incomplete indicating that further steps are required for achieving the goal of obtaining fully functional neurons from a-priori non-neurogenic glial cells. The project was funded by the Deutsche Forschungsgemeinschaft within the SPP 1172.

New transgenic mouse models to study the role of astrocytes in synaptogenesis *in vivo*.

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The relevance of astrocytes for synapse formation and function is well documented *in vitro*, but evidence for such interactions in vivo is missing due to a lack of animal models to target astrocyte function *in vivo*. We decided to establish transgenic mice that enable temporally-controlled targeted mutagenesis in defined subpopulations of astrocytes based on the Cre-ER^{T2} system (Metzger & Chambon 2001). To drive expression of Cre-ER^{T2} in astrocytes, we generated lines carrying Cre-ER^{T2} under the control of large genomic DNA fragments of astrocyte-specific promoters (aquaporin-4, apolipoprotein E, connexin-30 and GLAST) contained in bacterial artificial chromosomes (BACs). Characterization of these lines revealed that a) Cre-ER^{T2} expression patterns across different organs and brain regions matched the activity pattern of the driving promoter, b) transgenic lines derived from a given promoter construct differed in the level of Cre-ER^{T2} expression, but not in its regional distribution and c) Cre-ER^{T2} activity was strictly tamoxifen-dependent. Out of the four promoters chosen, GLAST and Cx30 induced strong Cre-ER^{T2} activity in brain cells with non-overlapping regional distribution, whereas ApoE-CreER^{T2} and Aqp4-Cre-ER^{T2} lines generated much lower levels of Cre-ER^{T2} activity in the CNS. In GLAST-Cre-ER^{T2} lines, Cre-ER^{T2}-mediated recombination reached the highest level specifically in cerebellar Bergmann glia, retinal Mueller cells, as well as in the olfactory bulb, cortex, hippoccampal dentate gyrus and subventricular zone. In contrast, in Cx30-CreER^{T2} lines the highest level of recombination occurred in astrocytes of mesencephalon, thalamus, hypothalamus, pons and brain stem. These transgenic models will be important tool to test the role of different subpopulations of astrocytes in the formation, function and plasticity of synapses as well as their degeneration under pathologic conditions *in vivo*.

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Functional expression of genetically-encoded calcium sensors in neurons and astrocytes of transgenic mice

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Because glial cells are electrically non-excitable, fluorescent imaging of intracellular calcium signals has proven an invaluable tool in the study of neuron-glia communication. One problem with such measurements is, however, that specific loading of astrocyte assemblies within tissues with synthetic calcium dyes is difficult to achieve. We have recently developed a novel family of genetically-encoded calcium sensors based on mutants of the Green Fluorescent Protein (GFP) and Troponin C. These sensors allow for specific and functional labeling of neuronal populations in transgenic mice that are suitable for two-photon imaging. We are currently extending this work to astrocytes using the hGFAP promoter system.

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Generation of a NG2-EYFP mouse for studying the role of NG2-expressing cells in synaptic function.

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The NG2 glycoprotein is a type I membrane protein expressed by many different types of immature cells including oligodendroglial progenitor cells in the developing and adult CNS. However, NG2 is also expressed by many cells in the grey matter. As shown to date in the hippocampus and cerebellum by the group of Dwight Bergles, subpopulations of neurons in both developing and adult CNS form synapses on NG2+ cells and neuronal stimulation leads to glutamate- and GABA-mediated signals in the glia. The NG2 transmembrane protein has a large extracellular region with 2 *LNS* (Laminin G/Neurexin/Sex-hormone binding globulin) domains near the NH_2 terminus, and a short intracellular region containing a PDZ binding motif at the C terminus. We identified GRIP (binds to GluRB and GluRC of AMPA receptors) as an intracellular binding partner of NG2+. We hypothesise that NG2 may bind to neurons and thus position the AMPA receptors towards sites of neuronal glutamate release at such glial-neuron synapses. In order to facilitate studies on NG2 cells including such synaptic interactions, we generated an *enhanced yellow fluorescent protein* (EYFP) "knock-in mouse". A targeting vector was generated where an EYFP gene followed by a loxP flanked neo resistance cassette was fused with the start codon of exon 1 of the NG2 gene. Labelling of EYFP-expressing cells with antibodies to NG2, PDGFα-R, Olig2, Sox 10 and O4 showed an overlap with these markers in the neonatal and adult mouse brain. Cells expressing EYFP did not label with markers for mature glia, neurones or microglia. NG2+ cells represent a unique, but heterogeneous population within the developing mouse CNS.

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