Final report of the Priority Programme of the Deutsche Forschungsgemeinschaft (SPP 1172) on

The role of neuroglia for the formation, function and plasticity of synapses

(2004 - 2010)

I. Background, general information and meetings

The Priority Programme (PP) on "The role of neuroglia for the formation, function and plasticity of synapses" started its work in August 2004 with 16 groups, which were selected from 26 groups, which had applied for funding within the PP following the call by the DFG.
In the last funding period (2008-2010), five young principal investigators - most of them had just started to set up a new group ("Nachwuchsgruppe") – were encouraged to submit a proposal within the PP, and four of them have newly been accepted to the PP during the course of the reviewing process. These were

- Daniela Dieterich, Magdeburg
- Johannes Hirrlinger, Leipzig
- Swen Hülsmann, Göttingen
- Martin Theis, Bonn

The coordinator created a homepage for the PP (http://www.uni-kl.de/FB-Biologie/AG-Deitmer/neuroglia/), where the aims and the scope of the PP and a news page were presented together with the list of PP members (PIs) and their affiliations. In addition, 2-3 info letters per year have been circulated regularly to the PP members. By means of the coordination funds, a number of student/postdoc exchanges between laboratories of the PP members and some meetings among collaborating PIs have been financially supported with minimal bureaucratic effort.
The following meetings have been organized by the PP:

- October 2004 in Kaiserslautern (organized by coordinator)
- September 2005 in Kaiserslautern (organized by coordinator)
- September 2006 in Bonn (organized by co-coordinator C. Steinhäuser)
- September 2007 in Bad Dürkheim / Pfalz (Internal meeting organized by coordinator)
- September 2008 in Berlin (Organized by Helmut Kettenmann and coordinator)
- 2009 in Frankfurt (Internal Meeting organized by Heinrich Betz and coordinator)
- August 2010 Final Symposium in Kaiserslautern (organized by coordinator)

The final symposium took place from 26. - 29. August 2010 at the Fraunhofer Center in Kaiserslautern. Speakers were all but one principal investigator of the Priority Programme and the following 16 guest speakers, including some scientists who served as reviewers for the PP in the last years:

- Nils Brose, Göttingen
- Mitchell Chesler, U.S.A.
- Shumin Duan, China
- Philip G. Haydon, U.S.A.
- Schuichi Koizumi, Japan
- Christian Lohr, Hamburg
- Yoshi Moriyama, Japan
- Martin Oheim, France
- Vladimir Papura, U.S.A.
- Elena B. Pasquale, U.S.A.
- Frank Pfrieger, France
- Nathalie Rouach, France
- Eliana Scemes, U.S.A.
- Arne Schousboe, Denmark
- Alexej Verkhratsky, U.K.
- Robert Zorec, Slovenia

This three-day meeting with 32 presentations, of each 30 min, was very intense and interesting, and a worthy final gathering of the PP in the spirit that the topic of the PP should be continued and extended in the future.

II. Some highlights of the Priority Programme

All PP projects will deliver their own report as usual; therefore an extensive overview of all projects will not be covered here. In contrast, only a few, selected, highlights of our PP are indicated to give evidence for the very successful efforts of the PP, often achieved in collaborative work between PP members:

- *In vitro* systems that permit to investigate the selective and specific influence of astrocytes on the synaptogenesis of hippocampal neurons were designed (Faissner in collaboration with Gundelfinger, Seidenbecher and Götz). They could show that astrocytes support the survival of embryonic hippocampal neurons and the formation of structurally intact synapses, as documented by the co-localisation of bassoon- and proSAP1-positive puncta. The development of synapses was paralleled by the emergence of perineuronal net (PNN) like structures that contained the 473HD-epitope and tenascin-C. (Sirko et al., 2010, Stem Cells 28, 775-787; Pyka et al., 2011, Synapse 65, 41-53).
  Related studies were concerned with the functional properties of neurons derived from astroglia of the early postnatal cerebral cortex by reprogramming with neurogenic fate determinants (Berninger/Götz). Expression via these retroviral vectors resulted in a more complete reprogramming with astroglia-derived neurons, now establishing fully functional synapses between themselves. Most importantly, it was shown that different transcription factors can induce distinct neuronal subtypes from the same type of astroglia. They could also show that such subtype specific reprogramming is not restricted to postnatal astroglia, but can in principle also be achieved by adult astroglia. These data provide proof of principle that astroglia of distinct maturation state can be instructed to functional neurogenesis by neurogenic fate determinants, thus opening new avenues towards cell-based therapies for neurodegenerative disorders (Heinrich et al., 2010, PLoS One 8;8(5):e1000373; Blum et al., 2010 Cereb. Cortex, epub).

- Many synapses in the mature CNS are wrapped by a dense extracellular matrix (ECM). Using single-particle tracking and fluorescence recovery after photobleaching, Gundelfinger, Frischknecht and coworkers found that this net-like ECM formed surface compartments on rat primary neurons that acted as lateral diffusion barriers for AMPA-type glutamate receptors. Enzymatic removal of the ECM increased extrasynaptic receptor diffusion and the exchange of synaptic AMPA receptors. Whole-cell patch-clamp recording revealed an increased paired-pulse ratio as a functional consequence of ECM removal. These results suggest that the surface compartments formed by the ECM hinder lateral diffusion of AMPA receptors and may therefore modulate short-term synaptic plasticity (Frischknecht et al., 2009, Nature Neurosci. 12, 897-904).

- AMPA-mediated responses in NG2 cells of the calyx of Held, which is a giant nerve terminal that serves as a model system to analyse basic mechanisms of synaptic transmission, were studied by the Kettenmann group. The NG2 glia, as the principal postsynaptic cells, establish synapse-like structures with the calyx of Held terminal. In contrast to the principal neurons, which are known to receive excitatory as well as inhibitory inputs, the NG2 glia receive mostly, if not exclusively, AMPA receptor-mediated, evoked and spontaneous synaptic input. Simultaneous recordings from neurons and NG2 glia indicate that they partially receive synchronized spontaneous input. This shows that a NG2-positive glial cell

and a postsynaptic neuron share presynaptic terminals (Müller et al., 2009, J. Gen. Physiol. 134, 115-127; Reyes-Haro et al., 2010, J. Gen. Physiol. 135, 583-594.

- The role of cells expressing the proteoglycan NG2 in glial-neuronal signalling and synapse formation has also been studied using transgenic mice where NG2 cells have been fluorescently labeled (Trotter in collaboration with Steinhäuser and Seifert). The results show that nearly all NG2-EYFP cells express antigens and transcription factors typical for immature oligodendrocytes (Olig 2, Sox 10, PDGF-A receptor) but do not express antigens expressed by microglia, mature astrocytes or neurons. In contrast to several papers in the published literature, we see no overlap with any neuronal markers (NeuN, Doublecortin, ß-III tubulin) in any brain region and at any age studied. However, the NG2-EYFP cells are a heterogeneous population when studied for expression of distinct proteins (such as glutamine synthetase) as well as in their electrophysiology (Karram et al., 2008, Genesis 46, 743-757).

- The Eph/ephrin signaling system in trans-endocytosis events was studied at the neuron-to-glia interface (Klein). Using time-lapse microscopy, hippocampal neurons exogenously expressing EphB2 receptors were shown to release or pinch-off EphB2-containing vesicles from the neuron at sites of neuron-to-glia contact. Our analysis revealed that tyrosine phosphorylation sites in ephrinB2 are required to mediate normal hippocampal LTP, but are not needed not for LTD. Conversely, ephrinB2 lacking the C-terminal PDZ interaction site, but competent to undergo tyrosine phosphorylation, cannot mediate either form of long-term plasticity. These results provided the first evidence for phosphotyrosine-dependent ephrinB reverse signaling in a neuronal network and for differential ephrinB2 reverse signaling in two forms of synaptic plasticity. The results also suggest a novel mechanism by which astrocytes modulate neuronal plasticity in the CA1 region of the hippocampus (Klein in collaboration with Rose). Astrocytes receive a signal from dendritic EphA4 receptors via ephrinA3, which prevents them from upregulating glial glutamate transporter expression to non-physiologically high levels. Dendritic EphA4 and ephrinA3 in astrocytes thereby control glutamate concentrations near synapses and promote LTP. In the absence of either dendritic/postsynaptic EphA4 or ephrinA3, glutamate transporter levels are increased and glutamate is more efficiently removed during high frequency stimulation. As a consequence, peri- and extrasynaptic glutamate receptors might not be sufficiently activated, resulting in insufficient depolarization of the postsynaptic neuron and partial impairment of LTP (Filosa et al. (2009) Nature Neurosci. 12, 1285-1292.

- In their study on viscoelastic and optical properties of glial cells, Reichenbach & Bringmann 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 because of 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) intriguingly, glial cells are even softer than their neighboring neurons. The results indicate that glial cells can neither serve as structural support cells (as they are too soft) nor as glue (because restoring forces are dominant) for neurons. Nevertheless, from a structural perspective they might act as soft, compliant

embedding for neurons, protecting them in case of mechanical trauma, and also as a soft substrate required for neurite growth and facilitating neuronal plasticity (in collaboration with Steinhäuser & Seifert). Intact retinal tissue and individual Müller cells, which are radial glial cells spanning the entire retinal thickness were also studied. Müller cells have an extended funnel shape, a higher refractive index than their surrounding tissue, and are oriented along the direction of light propagation. Transmission and reflection confocal microscopy of retinal tissue *in vitro* and *in vivo* showed that these cells provide a low-scattering passage for light from the retinal surface to the photoreceptor cells. Using a modified dual-beam laser trap, it was demonstrated that individual Müller cells act as optical fibers. Furthermore, their parallel array in the retina is reminiscent of fiber-optic plates used for low-distortion image transfer. Thus, Müller cells seem to mediate the image transfer through the vertebrate retina with minimal distortion and low loss. This finding elucidates a fundamental feature of the inverted retina as an optical system and ascribes a new function to glial cells (Lu et al., 2006, Proc. Natl. Acad. Sci. U.S.A. 103, 17759-17764; Franze et al., 2007, Proc. Natl. Acad. Sci. U.S.A. 104, 8287-8292).

- Astrocytes with a radial glia (RG)-like morphology are considered stem cells in the adult brain. Combining patch-clamp techniques with different genetic approaches, Steinhäuser, Seifert and coworkers (together with Theis) could demonstrate that a significant proportion of RG-like cells in the dentate gyrus (DG) are coupled through gap junctions and predominantly express connexin43 and connexin30. Ablation of connexin expression in RG-like cells led to a dramatic decrease of proliferation and reduced numbers of RG-like cells and granule neurons in the adult DG. It was thus shown that connexin expression by radial glia-like cells in the subgranular zone of the DG is essential for proper adult neurogenesis.

  Astrocytes play a key role in the uptake of K^+ and neurotransmitters, thereby modulating synaptic and neurovascular signalling. Inwardly rectifying K^+ (Kir) channels support the redistribution of K^+ across glial membranes, and were found mainly on astroglial processes ensheathing synapses and blood vessels. These studies identified Kir4.1 as the major subunit mediating resting currents in hippocampal astrocytes. Upregulation of Kir4.1 transcripts and protein during the first 10 postnatal days was accompanied by fourfold increase in astrocyte inward current density. Hippocampal astrocytes from Kir4.1-/- mice lacked Ba^{2+}-sensitive currents. In addition, functional expression of K2P channels of the TREK subfamily (TREK1, TREK2), which mediate astroglial outward currents, were demonstrated. These findings demonstrate that Kir4.1 constitutes the pivotal K^+ channel subunit and that superposition of currents through Kir4.1 and TREK channels underlies the ‘passive’ current pattern of hippocampal astrocytes (Kunze et al., 2009, Proc. Natl. Acad. Sci. U.S.A. 106, 11336-11341; Seifert et al., J. Neurosci. 29, 7474-7488).

  In another study on the Kir4.1 channel in astrocytes (Deitmer and Hülsmann) it was shown that this channel becomes permeable to divalent cations at low external K^+ concentrations. This e.g. allows a calcium influx into astrocytes under conditions of low K^+ concentration, which was sensitive to Ba^{2+}, and which were suppressed in Kir4.1 knock-out mice (Härtel et al., 2007, Cell Calcium 42, 271-280).

- ***Final Review Volume.*** At our annual meeting in Berlin 2008 we discussed a final joint publication on the topic of the PP. It was common sense that the publication should not be a monography, but a review series in an ISI-listed journal with acknowledged impact factor. The coordinator, with the help of the co-coordinator, made an outline of a review series including nearly all P.I.s of the PP, several of their co-workers, and more than twenty additional, international experts in the field, according to suggestions from PP members. Finally, German and international experts formed a group of co-authors for each identified sub-topic. The coordinator submitted a proposal for a volume of BRAIN RESEARCH REVIEWS (BBR), to which the editors of BBR readily agreed. The BBR volume appeared in May 2010 with 19 review articles grouped in 5 sub-topics, which had also been the main topics of the PP:
    - Morphology of astrocytes and synaptogenesis
    - Generation of neurons and glia
    - Synaptic transmission and plasticity
    - Metabolic interactions and networks
    - Neurodegeneration and diseases

  This volume has since received wide attention by the scientific community, and it is being very well quoted in the field.

  Brain Research Reviews - Special Issue: Synaptic processes – the role of glial cells. Guest Editors: Joachim W. Deitmer, Christian Steinhäuser; Elsevier; Vol. 63 (1-2), 1-232. (An issue of this volume is attached to this report).

III. Collaborations within the Priority Programme

Collaborations between groups of the PP have been intensified, increased in number, and most groups have an on-going collaboration with one or more other groups within the priority programme. In particular the use of transgenic mouse models has gained much interest, and some groups have jointly created, or have planned to create, new transgenic mice. A particularly interesting model e.g. is the Bergmann glia GluR1$^{-/-}$, GluR4$^{-/-}$ mouse produced by Frank Kirchhoff, and several cell type-specific fluorescent knock-in mice such as the Cx43kiECFP mouse line by M. Theis for identifying astrocytes. A joint force within the priority programme (Eulenburg, Griesbeck, and Kirchhoff) is currently generating novel transgenic mice with glia-specific expression of genetically encoded calcium indicators or light-gated ion channels, which is of great interest for several groups within the PP.

A list of some, selected, publications as the result of collaborations within the PP of the last two years are given below (IV). The joint effort in various fields of glia-synapse biology is going to be continued vigorously beyond the duration of the PP, and more significant publications from PP projects are in progress.

IV. Selection of joint publications of members of the Priority Programme in last two years (PP members marked bold)

1. Blum R, Heinrich C, Sanchez R, Lepier A, **Gundelfinger** ED**, Berninger** B**, Götz** M (2010) Neuronal Network Formation from Reprogrammed Early Postnatal Rat Cortical Glial Cells. Cereb Cortex, in press (Epub ahead of print).

2. **Deitmer** JW, **Rose** CR (2010) Ion changes and signalling in perisynaptic glia. Brain Res Reviews 63(1-2):113-29.

3. Dityatev A, **Seidenbecher** CI, **Schachner** M (2010) Compartmentalization from the outside: the extracellular matrix and functional microdomains in the brain. TINS 33:503-512.

4. **Faissner** A, Pyka M, Geissler M, Sobik T, **Frischknecht** R, **Gundelfinger** ED, **Seidenbecher** C (2010) Contributions of astrocytes to synapse formation and maturation – Potential functions of the perisynaptic extracellular matrix. Brain Res Rev 63:26-38.

5. Filosa A, Paixão S, Honsek SD, Carmona M, Becker L, Feddersen B, Gaitanos L, Rudhard Y, Schoepfer R, Klopstock T, Kullander K, **Rose CR**, Pasquale EB, **Klein** R (2009) Neuron-glia communication via EphA4/ephrinA3 modulates glial glutamate transporter levels and synaptic plasticity. Nature Neurosci, 12:1285-1292.

6. Kunze A, Congreso MR, Hartmann C. Wallraff-Beck A, Hüttmann K, Bedner P, Requardt R, **Seifert** G, Redecker C, Willecke K, Hofmann A, Pfeifer A, **Theis** M, **Steinhäuser** C (2009) Connexin expression by radial glia-like cells is required for neurogenesis in the adult dentate gyrus. Proc. Natl. Acad. Sci. U.S.A. 106:11336-11341.

7. Maglione M, Tress O, Haas B, Karram K, **Trotter** J, Willecke K, **Kettenmann** H (2010) Oligodendrocytes in mouse corpus callosum are coupled via gap junction channels formed by connexin47 and connexin32. Glia 58(9):1104-17.

8. Pyka M, Busse C, **Seidenbecher** C, **Gundelfinger** ED, **Faissner** A (2010) Astrocytes are crucial for survival and maturation of embryonic hippocampal neurons in a neuron-glia cellinsert co-culture assay. Synapse, in press (Epub ahead of print).

9. **Reichenbach** A, Derouiche A, **Kirchhoff** F (2010). Morphology and dynamics of perisynaptic glia. Brain Res Rev. 63:11-25.

10. Requardt RP, Kaczmarczyk L, Dublin P, Wallraff-Beck A, Mikeska T, Degen J, Waha A, **Steinhäuser** C, Willecke K, **Theis** M (2009). Quality control of astrocyte-directed Cre transgenic mice: the benefits of a direct link between loss of gene expression and reporter activation. Glia. 57:680-692.

11. Sirko S, von Holst A, Weber A, Wizenmann A, Theocharidis U, **Götz M, Faissner A** (2010). Chondroitin sulfates are required for fibroblast growth factor-2-dependent proliferation and maintenance in neural stem cells and for epidermal growth factordependent migration of their progeny. Stem Cells 28:775-787.

V. Conclusions and Perspectives

In the past six years, the PP consisted of 16 – 19 groups, and in each funding period there was a turnover of 2-5 groups, with mainly junior groups joining (e.g. Dietrich, Hirrlinger,Theis), and senior groups leaving (e.g. Schachner), or junior partners continuing the project of leaving senior partners (e.g. Kirischuk following Grantyn, Eulenburg following Betz), while a core of about 12 groups were part of the PP over the whole duration of the PP. This allowed continuity, but also supplied new projects and young PIs. In the end, the six years appeared too short for most PP members, and the desire to continue was reiterated frequently. Therefore, five younger members will get together to discuss the possibility of a new PP, in which glial cells play a major role, in view of the fact that we still have to learn much more about glial cells to catch up with our knowledge on neurons.

In summary, it is the distinct opinion of the PP members and coordinators that this PP was extremely useful and instrumental to promote the field of glia-neurobiology and the role of glial cells for supporting synapses in the nervous system. The collaboration within the PP has steadily increased, as indicated by the rising number of joint publications, of which some will still follow, and has been very satisfying with respect to both the projects and the education of young scientists. Doctoral dissertations and postdoctoral training have been important aspects in the PP, and has drawn quite a number of young scientists into this interesting field.

We, the PP members, and the coordinators of the PP in particular, are very grateful to the international reviewers, who have inspired and promoted the PP with their constructive criticism over the entire six years. After all, the excellent support by the DFG, and in particular by Dr. T. Hogenkamp and Dr. J. Kunze and their teams, as well as the generous funds have been instrumental for the success of this PP. We do hope that others share our strong opinion that the efforts and funds have been matched by the success of the PP.

Prof. Dr. Joachim W. Deitmer

- *Coordinator of SPP 1172* -