Final Symposium
of the DFG Study Group

Functions of Glial Cells

June 7 - 10, 1997

Congress Center Bogensee
Berlin, Germany

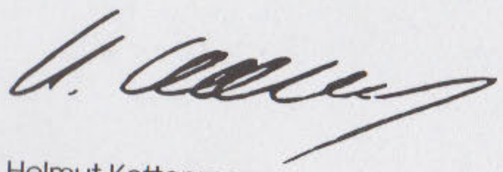
Introductory Remarks

The Symposium will focus on the functions of glial cells in health and disease. The human brain consists of two major cell types: neurons and glial cells, with the latter comprising the majority of cells in the brain. While neurons have been extensively studied, the functional importance of glial cells has been less understood by far. Nevertheless, it has become evident that glial cells are involved in many, if not all, basic functions of the brain, during development, in the adult state, and in disease states.

The impact of nervous system diseases and injury on the society in the Western hemisphere is increasing due to the longevity of the Western populations. Glial cells participate in or even cause some of these central nervous system diseases. This includes multiple sclerosis, Alzheimer's disease, epilepsy, and brain tumors. Furthermore, the extent of repair and recovery after spinal cord and peripheral nerve injury depends largely on the properties of glial cells. Without a knowledge of the basic cellular properties of glial cells none of these pathological states will be fully understood. Thus, basic glial research may give new insights into the mechansims of central and peripheral nervous system diseases. Moreover, such basic knowledge may help to develop novel therapeutic strategies using glial cells as primary targets.

In Germany in the study group *Functions on Glial Cells*, more than forty projects focusing on glial research were financed by the German Research Council (DFG) since 1991. In 1991, glial research had just advanced beyond the early stages leaving behind a long period when neurons dominated the neurosciences. Now, six years later, glial research is a large field, spreading into previously neglected facets of research including clinical aspects. This program is in its last funding period and the combined efforts of this initiative will be presented and evaluated in this Symposium.

I would like to thank the DFG for financially supporting this study group, the reviewing board for evaluating and advising the projects, all project leaders for their input into this initiative and the external speakers for their contribution to this closing symposium.



Helmut Kettenmann

General Information

Registration:	Saturday, June 7, 1997	12.00 - 15.00
Office Hours:	Saturday, June 7, 1997	12.00 - 20.00
	Sunday, June 8, 1997	8.30 - 20.00
	Monday, June 9, 1997	8.30 - 20.00
	Tuesday, June 10, 1997	8.30 - 11.00
Office Phone:	033397 640 0172 715 4022	
Fax:	033397 641 48	
Poster Boards:	height 120 cm width 100 cm Please bring along tape to fix your poster.	
Duration of the oral prestentations:	Project Presentations: 10 + 5 minutes dicussion Presentations of the External Speakers: 15 + 10 minutes discussion	

Organizing Committee

Mathias Bähr, Tübingen
Gerd Bicker, Berlin
Ingolf Blasig, Berlin
Joachim Deitmer, Kaiserslautern
Ulrich Dirnagl, Berlin
Karl Einhäupl, Berlin
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Uwe Heinemann, Berlin
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Fritz Rathjen, Berlin
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Regina Reszka, Berlin
Jutta Schnitzer, Berlin
Wilhelm Stoffel, Köln
Claudia Stürmer, Konstanz
Jacqueline Trotter, Heidelberg

Organizer and Coordinator

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Organization

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Saturday, June 7, 1997

15.00 - 17.00 **Session I: Development**
Chairpersons: J. Trotter & W. Stoffel

15.00 - 15.25 Alain Privat, INSERM, Montpellier, France
Cerebellar defect and impaired coordination in mice lacking vimentin

15.25 - 15.40 Dieter Hartmann, Kiel, Germany
Radial glial and neuronal migration: Mechanisms of cell differentiation and cell adhesion

15.40 - 16.05 Milos Pekny, Göteborg, Sweden
Astrocytes without intermediate filaments. Studies on mice deficient for GFAP and vimentin

16.05 - 16.20 Jürgen Engele, Ulm, Germany
Expression and localization of the GDNF receptor complex in the developing rat brain

16.20 - 16.35 Joachim Urban/ Gerhard M. Technau, Mainz, Germany
Development of glial cells in the embryonic CNS of Drosophila melanogaster

16.35 - 17.00 Christian Klämbt, Köln, Germany
Glial development requires concomitant repression of neuronal differentiation by Tramtrack and activation of glial cell differentiation by Pointed

17.00 - 17.30 **Coffee Break**

17.30 - 19.20 **Session II: Properties of myelin forming cells**
Chairpersons: A. Reichenbach & B. Hamprecht

17.30 - 17.45 Gunnar Jeserich, Osnabrück, Germany
Cells, genes, and proteins of myelin in the CNS of fish

17.45 - 18.00 Wilhelm Stoffel, Köln, Germany
The function of the main CNS myelin proteins proteolipid protein (PLP) and myelin basic protein (MBP) and of oligodendrocyte specific cerebrosides and sulfatides: an analysis in vivo by gene targeting in the mouse

18.00 - 18.15 Jacqueline Trotter, Heidelberg, Germany
Cell-surface glycoproteins expressed by cells of the oligodendrocyte lineage

18.15 - 18.30 Jutta Schnitzer, Berlin, Germany
Analysis of motile oligodendrocyte progenitor cells in brain slices and in culture

18.30 - 18.55 Vittorio Gallo, Bethesda, USA
Regulation of glial progenitor cell proliferation and lineage progression through ligand- and voltage-gated ionic channels

18.55 - 19.20 Rhona Mirsky, London, UK
Schwann cell precursor and signals that control Schwann cell generation

19.20 **Barbecue**

Sunday, June 8, 1997

10.00 - 11.10 **Session III: Physiology and biochemistry of glial cells (part 1)**
Chairpersons: A. Faissner & F. Rathjen

10.00 - 10.25 Bruce Ransom, Seattle, USA
Intracellular Na^+ homeostasis in rat hippocampal astrocytes

10.25 - 10.40 Dieter Leibfritz, Bremen, Germany
Changes in cell volume and organic osmolytes in glial cells during exposure to NH_4Cl studied by NMR spectroscopy

10.40 - 10.55 Bernd Hamprecht, Tübingen, Germany
Functions of astroglial cells in brain energy metabolism

10.55 - 11.10 Jochen Klein, Mainz, Germany
Activation of phospholipase D in astrocytes: Evidence for separate signalling pathways

11.10 - 11.30 **Coffee Break**

11.30 - 12.35 **Session III: Physiology and biochemistry of glial cells (part 2)**

11.30 - 11.55 Joachim R. Wolf, Göttingen, Germany
Many astroglial gap junctions serve autocoupling within one cell rather than heterocoupling between different cells: in vivo and in vitro evidence

11.55 - 12.10 Rolf Dermietzel, Regensburg, Germany
Gap junctions in glial cells

12.10 - 12.35 Marco Tsacopoulos, Geneva, Switzerland
Ammonium and glutamate released by neurons are direct signals regulating the nutritive function of glial cells

12.35 - 13.30 **Lunch**

13.30 - 16.00 **Free Afternoon**

16.00 - 17.25 **Session IV: Microglial cells and interactions with the immune system (part 1)**
Chairpersons: M. Bähr & D. Hartmann

16.00 - 16.15 Peter Gebicke-Haerter, Freiburg, Germany
Regulation of interleukin-3 and IL-3 receptor β in isolated rat microglia and in vivo

16.15 - 16.30 Christiane Nolte/Helmut Kettenmann, Berlin, Germany
Identification of receptors and molecular mechanisms that regulate microglial motile and phagocytic activity

16.30 - 16.45 Christopher Linington, Martinsried, Germany
Differential expression of chemokines and IFNg during MBP and S100β T cell mediated EAE

16.45 - 17.00 Anne Régnier-Vigouroux, Heidelberg, Germany
Specific role of astrocytes and microglia in the brain immune response

17.00 - 17.25 Richard Banati, London, UK
Imaging neuroinflammation and neural connectivity in the human brain

17.25 - 19.00 **Poster Session**

19.00 - 20.00 **Dinner**

20.00 - 21.05 **Session IV: Microglial cells and interactions with the immune system (part 2)**

20.00 - 20.25 Jake Streit, Gainsville, USA
Role of microglia in neuronal injury and death

20.25 - 20.40 Gennadij Ralvich/Georg W. Kreutzberg, Martinsried, Germany
Molecular signals for neuroglial activation in the injured CNS

20.40 - 21.05 Dana Giulian, Houston, USA
Microglial mechanisms of neuron killing

Monday, June 9, 1997

10.00 - 10.45 **Session V: Neuron-glia interaction (part 1)**
Chairpersons: G. W. Kreutzberg & J. Schnitzer

10.00 - 10.15 Gert Brückner, Leipzig, Germany
Perineuronal nets of extracelluar matrix: a complement to the neuron-glia interface

10.15 - 10.30 Andreas Faissner, Heidelberg, Germany
Structural and functional characterization of DSD-1-PG, a chondroitin sulfate proteoglycan of neural tissue

10.30 - 10.45 Fritz Rathjen, Berlin, Germany
Glial cell surface associated components modulating neurite extension

10.45 - 13.00 Poster Session

13.00 - 14.00 Lunch

14.00 - 15.05 Session V: Neuron-glia interaction (part 2)

14.00 - 14.25 Eva Sykova, Prague, Czech Republic
Role of extracellular space volume and geometry in nonsynaptic transmission and neuron-glia interaction

14.25 - 14.40 Hans R. Güttinger, Kaiserslautern, Germany
The influence of the glial neuronal dialogue on brain differentiation in two species of song birds

14.40 - 15.05 Niels Birbaumer, Tübingen, Germany
Slow brain potentials and behavior

15.05 - 16.00 Poster Session

16.00 - 17.35 Session VI: Receptors and intracellular signal cascades
Chairpersons: G. Jeserich & R. Heumann

16.00 - 16.15 Andreas Reichenbach, Leipzig, Germany
Types and modulation of ion channels and transmitter receptors in glial (Müller) cells

16.15 - 16.40 Christian Steinhäuser, Bonn, Germany
Age-dependent changes of AMPA receptor properties in astrocytes of the hippocampus

16.40 - 16.55 Joachim W. Deitmer, Kaiserslautern, Germany
Ca^{2+}-signalling in glial cells

16.55 - 17.10 Peter Hochstrate/Wolf-Rüdiger Schlue, Düsseldorf, Germany
Calcium influx pathways in identified neurones and glial cells of the leech central nervous system

17.10 - 17.35 Ken McCarthy, Chapel Hill, USA
Hippocampal astrocytes in situ respond to neuronal activity with increase in intracellular calcium

17.35 - 17.50 Coffeebreak

17.50 - 19.45 Session III: Glial cells in the de- and regenerating CNS
Chairpersons: J. Urban & G. Raivich

17.50 - 18.15 Hans Lassmann, Vienna, Austria
The spectrum of glial cell pathology in multiple sclerosis

18.15 - 18.30 Rolf Heumann, Bochum, Germany
Impairment of sensory functions in interleukin-6 deficient mice: comparison with mutant mice which are delayed in Wallerian degeneration

18.30 - 18.45 D. M. Lang/Claudia A. O. Stuermer, Konstanz, Germany
Substrate properties and adaptive plasticity of oligodendrocytes: significance for axonal regeneration and repair of fiber tracts in the central nervous system

18.45 - 19.00 Mathias Bähr, Tübingen, Germany
Inhibitory aspects of glial cells in CNS regeneration

19.15 - 19.40 Solon Thanos, Münster, Germany
The role of retinal microglial cells in neural degeneration and repair

19.45 Lunch

Tuesday, June 10, 1997

Breakfast and Departure

1. **Adamczyk, M., Scherrer, R. Kupferberg, A., Malviya, A. N., and Mersel, M.,** Centre de Neurochimie, Strasbourg, France
Inhibition of mitogen activated protein (MAP) kinase by oxysterols in rat astrocytes primary cultures and in C6 glioma cell lines

2. **Avola, R., Spina-Purello, V., Gallo, V., Morale, M. C., Costa, A., Reale, S., Farinella, Z., Ragusa, N., and Marchetti, B.,** University of Catania, Italy
Effect of growth factors and glucocorticoids on nuclear and mitochondrial macromolecular synthesis during astroglial cell proliferation and differentiation in culture

3. **Baltrons, M. A. and Garcia, A.,** Universidad Autonoma de Barcelona, Spain
AMPA receptors are coupled to the nitric oxide / Cyclic GMP pathway in cerebellar astroglial cells

4. **Belachew, S., Rogister, B., Rigo, J. - M., Malgrange, B., Servais-Mazy, C., and Moonen, G.,** University of Liège, Belgium
Molecular and electrophysiological characterization of glycine receptors in cultured cortical oligodendrocyte progenitors

5. **Bogumil, T., Rieckmann, P., Felgenhauer, K. and Brück, W.,** Universität Göttingen, Germany
Microglia-/macrophage-derived calcium-binding proteins MRP-8 and MRP-8/14 in multiple sclerosis. An immunhistochemical and serological analysis.

6. **Burudi, E. M. E. and Régnier-Vigouroux, A.,** Universität Heidelberg, Germany
Immune competence of astrocytes and microglia: Understanding the endocytic and antigen processing pathways

7. **Clement, A., Nadanaka, S., Masayama, K., Ueoka, C., Sugahara, K., Mandl, C., and Faissner, A.,** Universität Heidelberg, Germany
The DSD-1 structure is a chondroitin sulfate modification presented on the dsd-1-proteoglycan and promotes neurite outgrowth of hippocampal neurons

8. **Derouiche, A.,** MPI für Hirnforschung, Frankfurt/M., Germany
Both forms of heme oxygenase (CO-synthase) are predominantly localized in astroglial cells

9. **Distler, C. and Kopatz, K.,** Ruhr-Universität Bochum, Germany
Transience of parafoveal astrocytes in the macaque monkey retina is not caused by selective apoptosis

10. **Dong, Z., Sinanan, A., Parmantier, E., Mirsky, R., and Jessen, K. R.,** University College London, UK
Cell death, survival and maturation in the mouse Schwann cell lineage

11. **Dubovy, P., Jancalek, R., and Svizenska, I.,** Medical Faculty, Brno, Czech Republic
Expression of laminin and alpha 6A integrin in the dorsal root ganglia of the intact rat and following rhizotomy

12. **Eitzen, U. von, Egensperger, R., Kösel, S., Grasbon-Frodl, E. M., Bise, K., Mehraein, P., and Graeber, M. B. ,** Universität München, Germany
Quantitative analysis of activated microglia and spongiform change in Creutzfeldt-Jakob Disease

13. **Engel, S., Isenmann, S., and Bähr, M.,** Neurologische Universitätsklinik Tübingen, Germany
Microglilial activation in the rat retina following adenoviral-mediated gene transfer

14. **Engel, S., Schlüsener, H., and Meyermann, R.,** Universität Tübingen, Germany
Human traumatic brain injury (TBI) as a model for microglial activation

15. **Fansa, H., Keilhoff, G., Frerichs, O., Wolf, G., und Schneider, W.,** Universität Magdeburg, Germany
Effects of predegeneration of peripheral nerves on proliferation of Schwann cells in vitro

16. **Feigenbaum, V., Cartier, N., Aubourg, P., and Dubois-Dalcq, M.,** Institut Pasteur, Paris, France
Adrenoleukodystrophy gene transfer in myelin-forming cells

17. **Gabryel, B., Cieslik, P., Pudelko, A., and Trzeciak, H. I.,** Silesian School of Medicine, Katowice, Poland
Morphometric analysis of in vitro cultured astrocytes treated with nootropics

18. **Gabryel, B., Pudelko, A., Cieslik, P., Malecki, A., and Trzeciak, H. I.,** Silesian School of Medicine, Katowice, Poland
Influence of nootropic drugs on content of high-energy phosphates of in vitro cultured astrocytes

19. **Gallo, F., Spina-Purello, V., Morale, M. C., Tirolo, C., Testa, N., Avola, R., Beaudet, A., and Marchetti, B.,** University of Catania Medical School, Troina, Italy
Neurotrophic effects of astroglia-derived and exogenously applied growth factors in immortalized hypothalamic neurons

20. **Garwood, J., Schnädelbach, O., and Faissner, A.,** Universität Heidelberg, Germany
Structural characterization of DSD-1-proteoglycan

21. **Gimenez y Ribotta, M., Audouy, S., Revah, F., Mallet, J., and Privat, A.,** Université Montpellier II, France
HSV-TK/Ganciclovir system is cytotoxic for proliferating astrocytes in primary culture

22. **Gresser, O. and Régnier-Vigouroux, A.,** Universität Heidelberg, Germany
Functional analysis of the immune comptence of astrocytes and microglia

23. Grosche, J., Möller, T., Kettenmann, H., and Reichenbach, A., Universität Leipzig, Germany
3-D-Reconstruction of dye-injected murine Bergmann glial cell processes from electron microscopical serial sections

24. Guntinas-Lichius, O., Martinez-Portillo, F. Lebek, J., Angelov, D. N., Stennert, E., and Neiss, W. F., HNO-Klinik, Universität Köln, Germany
Nimodipine prolongs the GFAP expression and enhances the astroglial ensheathment of cranial motoneurons following permanent target deprivation in the rat

25. Hähnlein, I., and Bicker, G., Freie Universität Berlin, Germany
Glial patterning during postembryonic development of central neuropiles in the brain of the honeybe

26. Härtig, W., Bringmann, A., Köppe, G., Brauer, K., and Brückner, G., Universität Leipzig, Germany
Experimental modification of perineuronal nets in rat brain

27. Hager, G., Hager, G., and Kreutzberg, G. W., Max Planck Institute of Psychiatry, Martinsried, Germany
Astrocytes express isoforms of agrin lacking the inserts at the Y- and Z-splicing site

28. Hanisch, U., Prinz, M., Lyons, S., Nolte, C., Weber, J. R., Kettenmann, H., and Kirchhoff, F., Max-Delbrück-Centrum für Molekulare Medizin, Berlin, Germany
Interleukin-15 and the components of multimeric IL-15/IL-2 receptors are expressed in mouse brain and by mouse microglia in culture

29. Hartmann, J., Labrakakis, C., Patt, S., and Kettenmann, H., Max-Delbrück-Centrum für Molekulare Medizin, Berlin, Germany
Proliferation of human glioma cells is inhibited by long-term depolarization

30. Janeczko, K., and Pawlinski, R., Jagiellonian University, Krakow, Poland
Inhibitory effect of interferon gamma on the astrocyte proliferation in rat brain injured at the postmitotic stage of postnatal development

31. Jones, L. L., Banati, R., Graeber, M. B., Bonfanti, L., Raivich, G., and Kreutzberg, G. W., MPI for Psychiatry, Martinsried, Germany
Population control of microglia: Does apoptosis play a role?

32. Kann, O., Möller, Th., Prinz, M., Kirchhoff, F., Verkhratsky, A., and Kettenmann, H., Max-Delbrück-Centrum für Molekulare Medizin, Berlin, Germany
Endothelin induced calcium signalling in cultured mouse microglial cells

33. Keihoff, G., Fettkötter, I., Huether, G., and Wolf, G., Universität Magdeburg, Germany
Serotonin - a radical scavenger during the respiratory burst of activated microglia in vitro

34. Keilhoff, G., Seidel, B., and Wolf, G., Universität Magdeburg, Germany
NOS lacks in rat oligodendrocytes and Schwann cells

35. Kerber, G., Grimpe, B., Hager, G., and Kreutzberg, G. W., Max Planck Institut für Psychiatrie, Martinsried, Germany
Laminin and nidogen in the CNS: Expression by astrocytes

36. Kirchhoff, F., and Verkhratsky, A., Max-Delbrück-Centrum für Molekulare Medizin, Berlin, Germany
Expression of TRP store-operated channels in mouse glial cells

37. Kirischuk, S., Kettenmann, H., and Verkhratsky, A., Max-Delbrück-Centrum für Molekulare Medizin, Berlin, Germany
Glutamate transporter-generated currents in cerebellar Bergmann glial cells in situ

38. Kirischuk, S., Kettenmann, H., and Verkhratsky, A., Max-Delbrück-Centrum für Molekulare Medizin, Berlin, Germany
Sodium-calcium exchangers amplify kainate-induced calcium signals in cerebellar Bergmann glial cells

39. Kirischuk, S., Matiash, V., Kirchhoff, F., Kettenmann, H., and Verkhratsky, A., Max-Delbrück-Centrum für Molekulare Medizin, Berlin, Germany
Bergmann glial cells in situ express mGluR1 and mGluR5 metabotropic glutamate receptors

40. Kloss, C., Kreutzberg, G. W., and Raivich G., Max Planck Institut für Psychiatrie, Martinsried, Germany
Proliferation of ramified microglia on an astrocyte monolayer; Characterization of stimulatory and inhibitory cytokines

41. Kötter, K., Eichel-Streiber, C., Löffelholz, K, and Klein, J., Universität Mainz, Germany
Involvement of small G proteins in phopholipase D activation in astrocytes

42. Latzkovits, L., Fónagy, A., and Torday, Cs., A. Szent-Györgyi Medical University, Szeged, Hungary
NO regulates glial cell volume by a cyclic GMP-dependent mechanism

43. Leprince, P., Chanas-Sacre, G., Lewin, M., Pirard, S., Thiry, M., Misson, J. P., Rogister, B., and Moonen, G., University of Liège, Belgium
Labelling of an intermediate-filament-associated protein in cerebellar radial glial cells with the RC2 antibody

44. Leybaert, L., Paemeleire, K. and Sanderson, M. J., University Gent, Belgium
Inositol triphosphate mediates glial-endothelial calcium signaling

45. Lindenau, J., Noack, H., Rothe, F., Asayama, K., and Wolf, Gerald, Universität Magdeburg, Germany
Cell type specific expression of superoxide dismutase isoforms and cellular glutathione peroxidase in the normal rat brain and under conditions of excitotoxically mediated neurodegeneration

46. Lohr, C. and Deitmer, J. W., Univeritat Kaiserslautern, Germany
Ca^{2+} release from intracellular stores in the leech giant glial cell mediated by metabotropic glutamate receptor activation

47. Lyons, S., Prinz, M., Minelli, A., and Kettenmann, H., Max-Delbrück-Centrum für Molekulare Medizin, Berlin, Germany
Rat microglia surviving the exposure to combined hypoxia and hypoglycemia in culture become activated

48. Meier, C., Parmantier, E., Brennan, A., Mirsky, R., and Jessen, K. R., University College London, UK
Developmental regulation of survival in the Schwann cell lineage

49. Melcangi, R. C., Cavarretta, I., Magnaghi, V., Ballabio, M., Motta, M., and Martini, L., University of Milan, Italy
Sex steroid metabolism in astrocytes and in glioma cells: influence of the conditioned medium of neurons and tumoral brain cells

50. Möller, T., Kann, O., Verhkratsky, A., und Kettenmann, H., Max-Delbrück-Centrum für Molekulare Medizin, Berlin, Germany
Purinoreceptor-induced calcium signalling in mouse microglia

51. Monzón-Mayor, M., Yanes, C., Romero-Alemán, M., de Barry, J., and Gombos, G., University of Las Palmas de Gran Canaria, Spain
The „matricial zones" observed during the ontogenesis of Gallotia-galloti could be related with regeneration

52. Neiss, W. F., Mader, K., Andermahr, J., Angelov, D. N., and Stennert, E., Institut für Anatomie, Universität Köln, Germany
Activation of microglia and astroglia in the CNS by electric stimulation or transport block of a peripheral nerve in the rat

53. Nett, W. and Deitmer, J. W., Universität Kaiserslautern, Germany
Intracellular Ca^{2+} regulation in the leech giant glial cell by ATP-driven Ca^{2+} pumps

54. Niehaus, A. and Trotter, J., Universität Heidelberg, Germany
A novel cell-surface glycoprotein expressed by neural precursor cells

55. Nolte, C., Kirchhoff, F., and Kettenmann, H., Max-Delbrück-Centrum für Molekulare Medizin, Berlin, Germany
Epidermal growth factor (EGF) is a motility factor for microglial cells in vitro: evidence for EGF receptor expression

56. Nörenberg, U., Hubert, M., Brümmendorf, T., Tarnok, A., Leuschner, R., and Rathjen, F. G., Max-Delbrück-Centrum für Molekulare Medizin, Berlin, Germany
Structural and functional characterization of tenascin-R (Restrictin)

57. Ohlemeyer, C., Kirchhoff, F., and Kettenmann, H., Max-Delbrück-Centrum für Molekulare Medizin, Berlin, Germany
Stimulation of callosal fibers triggers calcium elevation in the population of glial cells with metabotrobic glutamate receptors

58. Owen, D. D., Rahim, A. T., Brown, F., Roberts, G. W., Stewart, B. R., and Hayes, G. M., SmithKline Beecham Pharmaceuticals, Harlow, UK
The secretion of IL-6 from a human immortalised microglial cell line in the presence and absence of mycoplasma fermentans infection

59. Parkinson, D. B., Harris, B. S., Zoidl, C., Jessen, K. R., Mirsky, R., University College London, UK
Investigation of transcription factors involved in Schwann cell differentiation

60. Parmantier, E., Meier, C., Zoidl, G., Turmaine, M., McMahon, A. P., Jessen, K. R., and Mirsky, R., University College London, UK
Desert Hedgehog is involved in peripheral nerve development

61. Pastor, A., Mülhardt, C., Schmidt, K., Becker, C. M., Kettenmann, H., and Kirchhoff, F., Max-Delbrück-Centrum für Molekulare Medizin, Berlin, Germany
Spinal cord glial cells express glycine-receptors with a truncated ß subunit

62. Perzelova, A., Macikova, I., Mraz, P, Bizik, I, and Steno, J., Comenicus University Bratislava, Slovak Republic
What is the role of protoplasmic astrocytes in adult human brain cortex?

63. Pesheva, R., Angelov, D. N., Walther, M., Streppel, M., Neiss, W. F., and Probstmeier, R., Universität Bonn, Germany
Tenascin-R is repellent for activated microglia which induces downregulation of the protein after facial nerve axotomy - a new role in motoneuron protection

64. Plaza-Peréz, M. L., Monzón-Mayor, M., Romero-Alemán, M. M., Gonzalez, B., Arbelo, J., and Castellano, B., University of Las Palmas de Gran Canaria, Spain
Microglial diversity in the brain of Gallotia-galliotia

65. Prinz, M., Draheim, H., Weber, J. R., Hanisch, U. - K., and Kettenmann, H., Max-Delbrück-Centrum für Molekulare Medizin, Berlin, Germany
Induction of an outward potassium current in rodent microglia by pneumococcal cell wall components

66. Prinz, M., Hanisch, U. - K., Kettenmann, H., and Kirchhoff, F., Max-Delbrück-Centrum für Molekulare Medizin, Berlin, Germany
Subpopulation of mouse microglia express an alternatively spliced mRNA variant of interleukin-15

67. Raivich, G., Jones, L. L., Kloss, C., Werner, A., Neumann, H., Wekerle, H., and Kreutzberg, G. W., Max Planck Institut für Psychiatrie, Martinsried, Germany
Immune surveillance in the injured nervous system: T-lymphocytes invade the axotomized mouse facial motor nucleus and aggregate around sites of neuronal degeneration

68. Reindl, G., David, M., and Dermietzel, R., Universität Regensburg, Germany
Chromatographic purification of connexin43 from bovine brain and detection of its ubiquitination

69. Reuss, B., Pastor, A., Kettenmann, H., and Unsicker, K., Universität Heidelberg, Germany
Regional differences of cultured astroglia in neurotransmitter-evoked calcium responses

70. Reuss, B., Pastor, A., Kettenmann, H., and Unsicker, K., Universität Heidelberg, Germany
FGF-2 modulates neurotransmitter sensitivity and calcium spreading in cultured astroglial cells

71. Schmidt, C., Ohlemeyer, C., Walter, T., and Schnitzer, J., Max-Delbrück-Centrum für Molekulare Medizin, Berlin, Germany
Analysis of motile oligodendrocyte progenitor cells in brain slices and in culture

72. Schmidt, K., Eulitz, D., Veh, R. W., Kettenmann, H., and Kirchhoff, F., Max-Delbrück-Centrum für Molekulare Medizin, Berlin, Germany
Heterogeneous expression of voltage-gated potassium channels of the shaker family in oligodendrocyte progenitors

73. Schnädelbach, O. and Faissner, A., University of Heidelberg, Germany
DSD-1-Proteoglycan modulates the migratory behaviour of the oligodendrocyte cell line oli-neu

74. Schroeter, M., Mertsch, K., and Blasig, I. E., Institut für Molekulare Pharmakologie, Berlin, Germany
Synergistic effects between brain capillary endothelial cells and astrocytes in antioxidative defense during hypoxia/reoxygenation

75. Schumacher, S., Volkmer, H., Buch, F., Otto, A., Tarnok, A., Roth, S., and Rathjen, F. G., Max-Delbrück-Centrum für Molekulare Medizin, Berlin, Germany
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Imaging neuroinflammation and neural connectivity in the human brain

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Detection of microglial activation is presently one of the most sensitive methods to reveal subtle brain pathology without or well before any obvious histopathological abnormalities. While not specific with respect to the cause of the disease, microglial activation is highly localised to the area of on-going disease, hence allowing the distinction between *focal, multifocal, generalised* and *distributed* pathology.

Using high-resolution photoemulsion autoradiography combined with immunocytochemical staining for glial markers, we have recently shown that in areas of intact blood-brain barrier the isoquinoline PK11195 is a marker of activated microglia. This observation was confirmed in a number of animal models, such as peripheral nerve axotomy and other brain lesions and on autopsy material from patients with multiple sclerosis and neurodegenerative diseases.

Labelled with carbon-11, PK 11195 can be used as a ligand in positron emission tomography (PET). In one of our current studies of autoimmune disease (multiple sclerosis), we showed that [^{11}C] PK 11195-binding is found not only at sites of primary lesion but also in areas affected by disease but with no abnormalities in the magnetic resonance imaging (MRI). These latter areas frequently were part of specific neuronal pathways, such as the visual pathway in patients with an optic neuritis. Similarly, patients with a lesion of the facial nerve (Bell's palsy) showed a [^{11}C] PK 11195 signal in the area of the ispilateral facial nucleus as a consequence of a retrograde neuronal reaction accompanied by the expected activation of microglia.

Since PK 11195-binding is almost exclusively localized on activated microglia, [^{11}C] PK 11195 can be used to determine (a) the presence, (b) the location, (c) the spatial extent, and (d) acuity of primary and secondary CNS pathology in vivo. Following the principle that microglial activation occurs along neuronal tracts both in anterograde and retrograde direction, the pattern of [^{11}C] PK 11195 in vivo mirrors neuronal connectivities and can highlight secondarily deafferented cortical areas. The latter may be particularly relevant in combination with functional PET-studies looking at primary lesions that disconnect parts of a distributed neural network. Here, [^{11}C] PK 11195 imaging may help to define the extent of anatomical disconnection.

Inhibitory aspects of glial cells in CNS regeneration

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In the CNS of mammals axonal regeneration is limited by inhibitory influences of the glial and extracellular environment.

We have used in vitro assays where explants from embryonic or adult rat retinae are cultured on various types of glial cells and myelin preparations from PNS and CNS to examine the relative contributions of individual glial cell types and glial-derived factors to growth inhibition and growth promotion of retinal ganglion cell axons.

Our results indicate, that myelin derived from normal and axotomized sciatic nerves is a non-permissive substrate for axonal regrowth. Addition of laminin to either CNS or PNS myelin or freezing of the myelin, however, allows reproducibly axonal growth. Membrane preparations from CNS or PNS tissue on the other hand allow axon outgrowth from retinal explants when adhesive substrates (e.g. Polylysin) are available. Astrocytes prepared from neonatal rat cortex or optic nerves are growth permissive, whereas astrocytes present in cultures from adult rat optic nerves are inhibitory for axonal regrowth in vitro. This might be due to inhibitory proteoglycans experessed by reactive astrocytes. In summary, inhibitory factors for axonal growth are present in peripheral nerves as well as in CNS-fibre tracts of the adult rat. Growth promoting molecules are able to override these inhibitory molecules.

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SLOW BRAIN POTENTIALS AND BEHAVIOR

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Slow brain potentials (SCP) of more than 500ms duration are generated in part from neuroglia in the upper cortical dendritic layers. A series of behavioral studies demonstrate that negative SCP of more than 2μV amplitude exert considerable effects on motor and cognitive processes. Positive SCP usually have a disfacilitating effect on behavior and thought. The local anatomical topography of the SCPs is critical for the type of the behavior change. Local negative changes correlate with cerebral blood flow increase as evidenced by PET and fMRI studies which will be reported. Self-regulation of SCP (instrumental learning) is used as a treatment for intractable epilepsy and in severe motor paralysis. A series of controlled studies demonstrate the applicability of SCP-biofeedback treatment.

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PERINEURONAL NETS OF EXTRACELLULAR MATRIX: A COMPLEMENT TO THE NEURON-GLIA INTERFACE

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The immediate neuronal microenvironment is known to be largely determined by adjacent glial cell processes. However, the neuron-glia interface may be modified by association with extracellular matrix components as shown for nodes of Ranvier and for the perineuronal nets (PNs). We have used immunocytochemistry and staining with N-acetylgalactosamine-binding lectins to analyze the distribution patterns and ultrastructural characteristics of PNs in the mammalian brain[1]. Comparative mapping studies which included marsupials, several placental mammals and human, revealed that aggregating chondroitin sulphate proteoglycans associated with PNs ensheath subpopulations of interneurons and pyramidal cells in the cerebral cortex as well as neurons in many subcortical regions. PNs showed no prevalent correlation with certain excitatory or inhibitory types of transmitter contained in the ensheathed neurons. However, certain cell types, such as the cholinergic basal forebrain neurons, were consistently found devoid of PNs[2]. In the neocortex, high numbers of PNs were characteristic of motor and primary sensory areas. Our developmental studies showed that during postnatal development the PNs in the rat appeared in a sequence from brain stem to cerebral cortex reaching the adult-like state during the fourth week after birth[3]. In adult animals the distribution pattern of PNs obviously remains constant. If experimentally degraded, their matrix components appear to be reconstituted slowly. It is suggested from our results that PNs provide different, but highly active types of neurons with an enlarged, anionic and hydrated microenvironment which may support the functions of glial cells.

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Ca^{2+}- SIGNALLING IN GLIAL CELLS

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Calcium ions have manyfold functions in intra- and intercellular singalling, so in nerve and glial cells in nervous tissue. We have studied the effects of neurotransmitters and membrane depolarization on intracellular Ca^{2+} (Ca^{2+}_i) in cultured rat astrocytes and in the giant glial cell in leech ganglia. The excitatory transmitter glutamate evokes a Ca^{2+}_i transient both via ionotropic and metabotropic receptors in these cells. Ca^{2+}_i transients can also be elicited by neuronal stimulation, which are suppressed by blocking glutamate receptors. In addition, membrane depolarization can induce a Ca^{2+}_i transient by activating voltage-gated Ca^{2+} channels. Distinct Ca^{2+}_i transients can also be recorded in glial cell processes with confocal imaging, indicating a high density of Ca^{2+}-permeable, voltage- and ligand-gated ion channels in the dendritic glial membranes, which may come into close contact with synaptic domains in the ganglionic neuropile.
The role of different modes of Ca^{2+} entry, Ca^{2+}_i release and Ca^{2+}_i extrusion on intracellular homeostasis of, and signalling by Ca^{2+}_i in glial cells will be discussed and in some detail presented on two additional posters (Lohr & Deitmer and Nett & Deitmer).

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Gap Junctions in glial cells.

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In an attempt to identify gap junction mediated intercellular communication between glial cells we have concentrated on diverse tissues studying connexin expression, trafficking and regulation in both classes of macroglial cells. 1) In astrocytes we followed the expression by transfection of an astrocytic cell line with the c-erbB2 oncogene which exhibits constitutive pkC activity. Although expression of Cx43 was on a high level, transfected cells revealed communication incompetence as measured by lack of Lucifer Yellow and Ca^{2+} transfer. However, phosphorylation pattern differed significantly in the transfected cell line with loss of higher phosphorylation isoforms indicative for a functional link between phosphorylation and the establishment of junctional communication in astrocytes. By means of light and electron microscopy immunocytochemistry we studied the transport of Cx43 in reaggregation and freshly dissociated primary astrocytes. The application of a diverse set of anti-connexin43 antibodies allowed us to outline a tentative scheme of connexin43 transport and its re-uptake under in vitro conditions. 2) The complement of glial connexins was further evaluated in cultures of mature oligodendrocytes and in postnatal spinal cords of rodents. We identified an additional connexin, connexin45, in oligodendrocytes which supplements connexin32 expression. In slice preparations from postnatal spinal cords we showed that white matter oligodendrocytes form functional autocellular territories without detectable Lucifer Yellow coupling to neighbouring cells. This is in concert with the data found in myelinating Schwann cells, where gap junctions are considered to be engaged in autocellular coupling rather then in intercellular communication.

EXPRESSION AND LOCALIZATION OF THE GDNF RECEPTOR COMPLEX IN THE DEVELOPING RAT BRAIN

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Glial cell line-derived neurotrophic factor (GDNF) has been recently identified by its ability to promote the survival of midbrain dopaminergic neurons. Other cells presently known to respond to GDNF include spinal cord motor neurons, cerebellar Purkinje cells, and neurons of the locus coeruleus. Interestingly, this relatively limited action is contrasted by the wide expression of GDNF within the developing and adult brain, suggesting other, as yet unknown, functions of this growth factor. To address this issue, the present studies made use of the recent identification of a functional GDNF receptor complex. This complex comprises a novel glycosyl-phosphatidylinositol-anchored protein termed GDNF receptor-α (GDNFR-α) which binds GDNF and enables the activated growth factor to interact with the tyrosine kinase receptor, c-Ret. By using reverse transcriptase-polymerase chain reaction (RT-PCR), we observed that mRNAs encoding GDNFR-α as well as c-Ret are expressed throughout the early embryonic (E15) rat brain. Intriguingly, in all brain areas, the expression of the GDNF receptor complex clearly preceeded that of GDNF, but not that of the GDNF-related protein, neurturin. Attempts to identify GDNF target cells further revealed that in all postnatal brain regions, GDNFR-α and/or c-Ret are predominantly expressed by type-1 astrocytes as well as by cells of the oligodendrocyte-type-2 astrocyte (0-2A) cell lineage.

Together, these findings suggest that neurturin acts as the physiological ligand for the GDNF receptor complex during early brain development. Our studies further provide evidence that, in addition to its effects on distinct neuronal subpopulations, GDNF and related proteins represent paracrine/autocrine growth factors for CNS glia.

STRUCTURAL AND FUNCTIONAL CHARACTERIZATION OF DSD-1-PG, A CHONDROITIN SULFATE PROTEOGLYCAN OF NEURAL TISSUES

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The neural chondroitin sulfate proteoglycan DSD-1-PG displays an apparent Mr of 1000 kD and comprises two core glycoproteins of 250 kD and 400 kD. The monoclonal antibody (mAb) 473HD reacts with the DSD-1 epitope, a glycosaminoglycan modification present on DSD-1-PG. In developing mouse CNS, the proteoglycan is detectable as early as embryonic day 12.5, displays highest expression during the postnatal period and comparatively low levels in the adult. In some regions, for example the somatosensory cortex or the developing rhombomeres of the chicken hindbrain, DSD-1 is distributed in a boundary-like fashion. The proteoglycan is expressed on the surface of immature glial cells in vitro and associated with immature glia in vivo. Upon lesion, DSD-1-PG is significantly upregulated. Using the oligodendrocyte progenitor cell line Oli-neu, it could be shown that TGF-β1 is one of the inducing factors and that DSD-1-PG modulates glial cell motility on laminin, but not merosin or fibronectin substrates. DSD-1-PG has been purified from postnatal mouse brain by a combination of mAb 473HD immunoaffinity with subsequent ion exchange chromatography. The purified molecule promotes neurite outgrowth and accelerates axon maturation from E18 rat hippocampal neurons. The DSD-1 epitope is required and sufficient for this effect. The molecular characterization of the core is being pursued by an integration of peptide sequencing and the screening of cDNA expression libraries with specific polyclonal antibodies.

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REGULATION OF GLIAL PROGENITOR CELL PROLIFERATION AND LINEAGE PROGRESSION THROUGH LIGAND- AND VOLTAGE-GATED IONIC CHANNELS.

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Oligodendrocyte progenitor (O-2A) cells co-express functional non-NMDA glutamate receptors (GluRs). Proliferation and lineage progression (% of $O4^+$ cells) of O-2A cells stimulated with PDGF and/or bFGF is reversibly inhibited by activation of AMPA receptors with glutamate, kainate or AMPA. The anti-proliferative effects of GluR agonists are Ca^{2+}-independent and arise from an increase in intracellular Na^+ and subsequent block of outward K^+ currents. Agents which acted to depolarize O-2A cells or increase intracellular Na^+ (veratridine, high K^+, ouabain) similarly had an anti-proliferative effect, due at least in part to a reduction in voltage-gated K^+ currents. Also these effects were reversible and Ca^{2+}-independent. Chronic treatment with GluR agonists was without any long-term effect on K^+ current function. Cells cultured in elevated K+, however, demonstrated an upregulation of inward rectifier K^+ currents, concomitant with an hyperpolarization of the membrane resting potential. In the same cultures the % of $O4^+$ cells was reduced, when compared to cells maintained in low K^+. The high-K^+ culture condition, therefore, promoted a current phenotype typical of pro-oligodendroblast independently of a developmental transition to a more differentiated stage of the lineage. Finally, cells chronically treated with the mitotic inhibitor retinoic acid displayed a selective down-regulation of outward K^+ currents. In conclusion, signals which affect O-2A cell proliferation do so by regulating K^+ channel function. These data indicate that GluRs and regulation of K^+ currents in cells of the oligodendrocyte lineage play an important role in determining their proliferative potential and demonstrates that O-2A cell K^+ current phenotype can be modified by long-term depolarization of the cell membrane.

Regulation of Interleukin-3 and IL-3 receptor ß in isolated rat microglia and in vivo

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Microglial proliferation is one of the hallmarks of microglial activation. Cytokines like M-CSF, GM-CSF, or IL-3 are very potent mitogenic stimuli. Our previous data confirm these findings. Corresponding receptors on microglia transduce cytokine signals to the cell nucleus and induce cell division. We have shown that cultured microglia expressed measurable amounts of IL-3 receptor ß (transducing subunit) mRNA, whereas this mRNA was absent in normal rat brain. LPS-treatment of cultured cells upregulated receptor mRNA. LPS injections into peripheral blood rapidly induced receptor mRNA in microglia. Comparable results were obtained from brain slices from MCAO-rats. The receptor is considered as an early marker of microglial activation in vivo. Part of receptor cDNA is being subcloned into an expression vector to synthesize antigen for immunizations. Recently, we have cloned and sequenced the rat homologue of human CC-chemokine receptor 5 (CKR5). Evidently, this receptor is essential for docking of HIV-1 gp120 envelope protein and subsequent productive infection of human macrophages. CKR5 mRNA was upregulated in cultured microglia by LPS and down-regulated by its ligands MIP-1α and MIP-1ß. LPS also upregulated these ligands, which suggests feedback down-regulation of CKR5 by MIPs. This, apparently, holds true for LPS-treated rats, where CKR5 upregulation was transient. In MCAO-operated animals, however, receptor mRNA showed a sustained increase in the infarcted hemisphere until 4 d despite transient increases of MIP mRNAs. Further studies are warranted to unravel the corresponding molecular mechanisms.

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Microglial Mechanisms of Neuron Killing

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There is a growing body of evidence to show that microglia release neurotoxic factors during brain disorders. To study immune-mediated pathologies of the CNS, microglia were isolated from rodent or human brain and placed in culture with rodent neurons. Microglia were found to kill neurons by both direct contact and by release of diffusible neurotoxins. The contact killing mechanisms involved short-lived factors which could be stimulated by lipopolysaccharide (LPS); remote killing was stimulated by a variety of signals and depended upon a heat-stable toxin with action involving the NMDA receptor. Purification of the heat-stable killing activity revealed a phenolic amine, **NTox,** while LPS induced nitric oxide production. **NTox** was a highly potent neuron poison found in regions of brain damaged by trauma or stroke whereas nitric oxide production was minimal during these types of brain injury. These observations point to distinct signaling mechanisms to drive neurotoxic microglia. Different strategies were required for drug blockade of these two immune-mediated mechanisms of neuronal cell death.

THE INFLUENCE OF THE GLIAL NEURONAL DIALOGUE ON BRAIN DIFFERENTIATION IN TWO SPECIES OF SONG BIRDS.

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We assess the spatio-temporal glia expressed growth regulating factors in the ontogeny and analyse the morphodynamic effects of these molecules by in vitro assays on cryosections. In the developing CNS of mammals the topography of the expression of growth regulating molecules correlates with key elements, such as cell proliferation, neuronal migration, neurite outgrowth and segregation of neuronal assembly [1]. The plastic vocal control system in the telencephalon of song birds is extremely well suited for studying functional effects of temporal relationship of brain maturation [2]. Here we studied spatio-temporal expression of growth regulating factors (tenascin-C proteoglycan and Ni 35/250) in the plastic telencephalic song system and in the cerebellum of zebrafinch and canary. In addition, we evaluate in vitro the effects of these molecules by the cryoassay. Parasagittal brain sections were cut with 25 µm thickness and used as growth substrate for neuroblasts from embryonal chick cortex (E7). Neurite outgrowth was determined by counting neurons with processes on the tissue substrate and was expressed as a percentage of the total number of neurons attached.

To evaluate the ontogenetic expression of growth regulating molecules, we studied the side-restricted expression of growth regulating molecules at five different age stages. Parallel to the studies with the extracellular matrix markers, astroglial coupling was visualized in slice preparation by iontophoretic injection of Neurobiotin. The following results were obtained: With the major nucleus for vocal control approximately 25 % more astroglial cells were detectable than in the surrounding forebrain tissue. Between the reproductive period in spring and the quiescent period in autumn there is a high decrease in the density of dye coupled astrocytes.

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FUNCTIONS OF ASTROGLIAL CELLS IN BRAIN ENERGY METABOLISM

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The hypothesis underlying the project supported in the Schwerpunktprogramm on Glial Functions was the idea that, in brain, astrocytes and neurons divide tasks not only in the transfer of information but also in energy metabolism. The aspects of brain energy metabolism studied were the metabolism of glycogen and certain amino acids, and the results obtained are compatible with the hypothesis. Thus, by using our monoclonal antibodies against brain glycogen phosphorylase (GP) in immunocytochemical studies have shown that of the several cell types in brain GP is contained only in astrocytes, ventricle-lining ependymal cells and the few sensory neurons in the trigeminal mesencephalic nucleus. Consequently the lactic acid generated by breakdown of glycogen in astroglial cells can be forwarded as fuel to neighboring cells such as neurons and oligodendrocytes.

After generation of antibodies against the 3 purified key enzymes of gluconeogenesis, immunocytochemicstry demonstrated that glycogenogenesis in brain is only possible in astroglial cells, as had already been suggested by the incorporation of gluconeogenic precursors into the glycogen of cultured astroglial cells.

The breakdown of amino acids to the standard fuel molecule lactate requires the presence of malic enzyme. The two isoforms of the enzyme were isolated from bovine brain. Immunocytochemistry with antibodies generated against these isozymes revealed that the cytosolic form was expressed only in astro- and oligodendroglial cells, whereas the mitochondrial isoform was only found in neurons.

Radial Glia and Neuronal Migration: Mechanisms of Cell Differentiation and Cell Adhesion

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Within the mammalian CNS, radial glia cells (RGCs) represent a transient phenotype of the astroglial lineage operative during ontogenesis and disappearing thereafter from most brain regions. In contrast to earlier concepts of cortical development formulated e.g by Rakic, RGCs have been shown to undergo rapid proliferation including de novo differentiation and orientation of radial fibers, which is epigenetically controlled by both neuronal and mesenchymal factors. With the work presented here, we have investigated two different aspects of RGC differentiation, (i) the precise functional role of meningeal cells and (ii) the contribution of neuro-glial contact mechanisms.

Meningeal cells as well as other fibroblasts induce the expression of the radial glia - specific RC 2 antigen by a diffusible factor of about 10-30 kDa acting via a PKC - mediated signal transduction. Meningeal cells also participate in the regulation of (radial) glial morphology by their extracellular matrix and probably also by as yet unidentified membrane components. In addition, meningeal cells secrete a chemotactic factor acting upon migratory neurons.

Likewise, neurons contribute to the regulation of radial glia differentiation both by secreted factors including components of the extracellular matrix and direct cellular contact. As a dramatic example, the failure to express the ECM protein CR-50/reelin by early marginal zone neurons in reeler mutant mice not only alters RGC morphology, but also their ability to sustain neuronal migration. Thereby, a selectin - like cell contact system based upon glycoprotein binding by an ectoenzyme, ß(1,4)galactosyltransferase (ß-GT), seems to be specifically impaired. Our data indicate that this enzyme is localized mostly on neuronal, but less so on glia cell membranes and that the specific blockade of substrate binding by ß-GT in vitro interferes with neuronal migration, but not neuro-glial cell adhesion. This result could reflect the pathological persistence of neuronal cell attachment to RGCs observed in reeler mutants in vivo.

IMPAIRMENT OF SENSORY FUNCTIONS IN INTERLEUKIN-6 DEFICIENT MICE: COMPARISON WITH MUTANT MICE WHICH ARE DELAYED IN WALLERIAN DEGENERATION

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Interleukin-6 (Il-6) is a multifunctional cytokine acting as an inflammatory mediator affecting growth and differentiation. Although, Il-6 and its receptors are found in the peripheral nervous system their neural functions remain to be elucidated. After sciatic nerve lesion Il-6 mRNA is induced in the non-neuronal cells surrounding the axons, displaying a spacial expression pattern identical to that previously found for the initial lesion-induced NGF synthesis (1). Here we investigate the role of Il-6 in the intact and lesioned sciatic nerve using adult Il-6 gene knock-out (IL-$6^{-/-}$) mice. Differential recordings of compound action potentials were optained from either the sensory or the motor branch of the sciatic nerve. There was a 60% reduction in the compound action potential of the sensory branch of adult IL-$6^{-/-}$-mice when compared to the unaffected motor branch. Cross sections of L5 DRG of IL-$6^{-/-}$-mice showed a change in the size distribution of the large diameter neurons. Furthermore, the temperature sensitivity of IL-$6^{-/-}$-mice was significantly reduced.

After sciatic nerve crush the functional recovery was delayed in IL-$6^{-/-}$-mice as estimated from a behavioural test of the limb movements during walking. Measuring compound action potentials 20 days after crush lesion, there was an impaired recovery of the sensory but not of the motor branch of adult IL-$6^{-/-}$-mice. Although, similar results were obtained with mutant mice developing delayed Wallerian degeneration (Wld^s-mice), the mechanisms leading to the sensory deficiencies were distinctly different between IL-$6^{-/-}$- or Wld^s-mice. While in Wld^s-mice there is a delayed invasion of macrophages thus preventing lesion-induced increases of low affinity neurotrophin receptor (p75NTR) synthesis (2), these characteristic effects during Wallerian degeneration were indistinguishable between wild type animals and IL-$6^{-/-}$-mice. The results are compatible with the hypothesis that interleukin-6 cooperates with neurotrophin functions on sensory neurons *in vivo*.

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CALCIUM INFLUX PATHWAYS IN IDENTIFIED NEURONES AND GLIAL CELLS OF THE LEECH CENTRAL NERVOUS SYSTEM

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The cytosolic calcium concentration ($[Ca^{2+}]_i$) controls a multitude of cellular processes. An increase in $[Ca^{2+}]_i$ may occur by an influx of Ca^{2+}across the plasma membrane or by Ca^{2+} release from intracellular stores. In the leech central nervous system, both neurones and glial cells possess voltage-dependent Ca^{2+} channels that hardly inactivate upon prologed depolarization. The Ca^{2+} channels are blocked by various polyvalent cations and menthol, while specific antagonists of vertebrate Ca^{2+} channels such as nifedipine, flunarizine, ω-conotoxin-GVIA or ω-agatoxin IVA are ineffective.[1,2] In leech glial cells, Ca^{2+} may also enter the cytosol via ionotropic receptors for glutamate (AMPA/kainate type) or acetylcholin.[3,4] In some preparations, serotonin or ATP induced a $[Ca^{2+}]_i$ increase which might be mediated by corresponding Ca^{2+}-permeable receptors[5], while dopamine and octopamine had no effect on $[Ca^{2+}]_i$. In leech neurones, however, Ca^{2+}-permeable neurotransmitter receptors seem to be absent. Thus, in the neurones the loci for neurotransmitter action and Ca^{2+} influx may be different, while they are identical in the glial cells. Furthermore, a neurotransmitter-induced Ca^{2+} influx into leech occurs only if the cells were depolarized sufficiently to activate the voltage-dependent Ca^{2+} channels, while in the glial cells any receptor activation is associated with a Ca^{2+}influx. In both neurones and glial cells, the recovery from a $[Ca^{2+}]_i$ increase is virtually independent from extracellular Na^+, suggesting that Ca^{2+} extrusion is mainly mediated by Ca^{2+} pumps. A number of leech neurones possess caffeine-sensitive intracellular Ca^{2+} stores as well as caffeine-sensitive cation channels in their plasma membrane. The cation channels are permeable to various divalent cations including Ca^{2+} and possibly also to Na^+. Both intracellular Ca^{2+} release and cation influx show features of increment detection. The inhibitors of intracellular Ca^{2+} pumps thapsigargin, 2,5-Di-(t-butyl)-1,4-hydroquinone, or cyclopiazonic acid have no effect on the cation influx, while the intracellular Ca^{2+} release is reduced but not abolished. In leech glial cells, caffeine-sensitive Ca^{2+} stores and/or cation channels appear to be absent.

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CELLS, GENES, AND PROTEINS OF MYELIN IN THE CNS OF FISH

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The myelin-forming cells in the CNS of bony fish are exceptional, since in their structural phenotype and their cell lineage relationship they coincide with mammalian oligodendrocytes while regarding their biochemical features and their capacity for regeneration they strikingly resemble Schwann cells. Molecular cloning and sequencing of the cDNAs encoding two major myelin glycoproteins of trout CNS myelin (IP1, IP2) revealed two highly homologous isoforms (splice variants), which in their extracellular domain exhibited more than 50% amino acid sequence identity with rat Po, while in their cytoplasmic region sequence homology was rather low. As a first step towards a functional characterization of these Po-like components the IP1 cDNA was subcloned into a eukaryotic expression vector and transiently transfected into a rat oligodendroglial cell line (OLN 93). One day after transfection the cells showed bright immunostaining with polyclonal and monoclonal antibodies against trout IP1, thus allowing to verify the correct membrane topology of the recombinant protein. The molecular weight determined by western blot analysis was identical to those of the glycosylated native myelin protein. Studies are now in progress to generate stable IP1 transfectants and to monitor changes in their adhesive properties.

Furthermore the gene coding for the two splice variants of trout IP was isolated from a trout genomic library and partially sequenced. The transcription initiation site was mapped by primer extension analysis and the first 600 bp of the 5'-flanking region were isolated. A detailed comparison between the promotor regions of the mammalian myelin genes Po and PLP on the one side and those of trout IP on the other revealed striking similarities with the proximal PLP promotor only, which might explain the expression of Po-like peptides by oligodendroglial cells in the fish CNS. Work is in progress to functionally characterize the trout IP promotor, to identify *cis*-acting elements potentially involved in gene regulation during development and regeneration of myelinated fiber tracts in the fish CNS.

GLIAL DEVELOPMENT REQUIRES CONCOMITANT REPRESSION OF NEURONAL DIFFERENTIATION BY *TRAMTRACK* AND ACTIVATION OF GLIAL CELL DIFFERENTIATION BY *POINTED*.

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To unravel genes required for glial cell development we have conducted a saturating F2 EMS mutagenesis. Following antibody staining we have more than 600 mutations, which disrupt the organisation of the axonal scaffold in the embryonic nervous system in a way indicative for glial cell defects. Among these mutations, we have so far identified 12 complementation groups, required for midline glia development.

One of these complementation group corresponds the *tramtrack* (*ttk*) gene. *tttk* encodes two protein forms, ttkp69 and ttkp88. Only ttkp69 is expressed in the CNS, where it is found exclusively in all non neuronal cells. To further analyse the function of *ttk* we have used the GAL4 system to ectopically direct expression of different ttk protein forms in the CNS. Expression of ttkp88 in the CNS does not lead to dramatic phenotypes, expression of ttkp69 however blocks neuronal differentiation.

Our results indicate that glial cell differentiation is controlled in a dual process. Activation of glial differentiation mediated by transcription factors such as POINTED and REPO occurs in concert with repression of neuronal differentiation mediated by TRAMTRACK.

ACTIVATION OF PHOSPHOLIPASE D IN ASTROCYTES: EVIDENCE FOR SEPARATE SIGNALLING PATHWAYS

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The hydrolysis of choline-containing phospholipids such as phosphatidylcholine has recently attracted interest as a receptor-regulated process which contributes to cellular signal transduction (1). The hydrolysis of phosphatidylcholine is catalyzed by phospholipase D (PLD) and yields phosphatidic acid and choline. Phosphatidic acid and its hydrolysis product, diacylglycerol, are important second messengers for the activation of certain isoforms of protein kinase C (PKC). In the present study, we investigated the activation of PLD in primary astrocytes prepared from newborn rat cortex using the transphosphatidylation assay in the presence of ethanol. Noradrenaline led to a rapid, threefold activation of PLD activity which was mediated by α1-adrenoceptors and completely inhibited in the presence of prazosin (IC_{50}: 0.2μM). 4ß-Phorbol-12ß,13α-dibutyrate (PDB) caused a more than tenfold activation of PLD in a more delayed fashion; this effect was attenuated by the PKC inhibitor Ro 31-8220 in a concentration-dependent manner (0.01-1μM). Fetal calf serum caused a twofold stimulation of PLD activity which was partially (by 35%) inhibited by genistein (100 μM), a tyrosine kinase inhibitor. Genistein did not affect the PLD activation seen after noradrenalin or phorbol ester, while Ro 31-8220 did not affect stimulation by noradrenaline or serum. We conclude that separate signalling pathways are linked to PLD activation in astrocytes.

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Substrate properties and adaptive plasticity of oligodendrocytes: significance for axonal regeneration and repair of fiber tracts in the central nervous system

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Permissive substrate properties of oligodendrocytes as well as their ability to remyelinate axons are crucial for success of axonal regeneration and repair of lesioned central nervous system (CNS) fiber tracts.

Our analysis in *Xenopus* (Anura, Amphibia) has demonstrated a difference in substrate properties between oligodendrocytes derived from the optic nerve (ON) and spinal cord (SC) (1). Upon transection of the frog ON, retinal axons regenerate. Here, differentiated oligodendrocytes are permissive for growing axons. Moreover, these cells dedifferentiate and acquire outgrowth promoting properties while regrowing axons pass through the nerve. Subsequently, they reexpress advanced myelin marker proteins and remyelinate the regenerated axons (2). However, severed fiber tracts fail to regrow in the frog spinal cord (SC). Here, oligodendrocytes carry neurite growth inhibitory (NI) proteins - much like their mammalian counterparts (3) -, fail to dedifferentiate and block axonal regrowth. The factors or signals that are responsible for these differences in oligodendrocyte properties are presently unknown.

One factor, platelet-derived growth factor (PDGF), was found to modulate the inhibitory substrate properties of oligodendrocytes derived from the mammalian (rat) CNS (4). When exposed to PDGF for several days *in vitro*, the cells are freely crossed by axons and show reduced immunofluorescence with MAb IN-1 raised against NI proteins, thus indicating a down-regulation of these neurite growth inhibitors on the oligodendrocyte surface. Upon withdrawal of PDGF, the oligodendrocytes re-acquire their inhibitory substrate properties and cause growth cone collapse. Exposure of oligodendrocytes to PDGF appears to induce phosphorylation of various proteins; however, the signal transduction pathways underlying the regulation of NI proteins remain to be explored.

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THE SPECTRUM OF GLIA CELL PATHOLOGY IN MULTIPLE SCLEROSIS

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Multiple Sclerosis is an inflammatory demyelinating disease of the central nervous system. During the last decades much emphasis has been laid on the immunological mechanisms of T-cell mediated inflammation in this disease. However, experimental evidence suggests that T-cell mediated inflammation alone leads to only very limited permanent tissue damage. In contrast the multiple sclerosis lesion is characterized by widespread destruction of myelin together with pronounced changes in astroglia cells and microglia. Recent observations in multiple sclerosis suggest, that the patterns and immunopathogenic mechanisms of demyelination are heterogenous in multiple sclerosis patients. In one group of patients demyelination is associated with destruction of mature oligodendrocytes, which is followed by recruitment of oligodendrocyte progenitor cells and rapid remyelination. This pattern of disease in general is associated with deposition of immunoglobulins and complement in the lesions in a similar way as shown in the model of myelin oligodendroglia glycoprotein induced autoimmune encephalomyelitis. In another group of patients a similar pattern of acute demyelination is associated with a progressive and complete loss of oligodendrocytes and their progenitor cells, resulting in demyelinated plaques that are incapable of remyelination. In this pattern it is likely that additional immune responses are directed against oligodendrocyte progenitor cells. A third type of lesions is characterized by extensive microglia activation, which is associated by apoptotic cell death of oligodendrocytes. In these lesions recruitment of progenitor cells and remyelination is limited, too. In a fourth group of patients primary. degeneration of oligodendrocytes is noted which may even progress on the background of very minor or absent inflammatory reaction. In these cases the primary pathogenetic target may be the oligodendrocyte itself. Astrocytes within multiple sclerosis lesions in general react by scar formation in a similar way as in non-inflammatory brain lesions. In some cases, however, astrocytes may also be destroyed in the demyelinating process. In such lesions remyelination by Schwann cells may be prominent. At present the mechanisms responsible for the wide spectrum of glia pathology in multiple sclerosis lesions are poorly understood.

Changes in Cell Volume and Organic Osmolytes in Glial Cells During Exposure to NH_4Cl Studied by NMR Spectroscopy

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Hyperammonemia and hepatic encephalopathy are often accompanied by brain edema resulting primarily from ammonia-induced swelling of astrocytes. It is suggested, that the intracellular accumulation of the osmotically active glutamine is responsible. Therefore, primary astrocyte cultures were incubated
with 10 mM NH_4Cl in the presence of C-13 labelled glucose to measure the effect on the steady state concentrations of organic osmolytes as well as on the *de novo* synthesis of cytosolic metabolites. Proton, carbon and phos-phorous NMR spectra are taken from PCA extracts. Immobilized astrocytes in basal membrane gel(BMG) threads are used to measure the cell volume after the ammonia load. After a transient shrinkage of 10 % in the first 10 minuets the cells swell within 3 hours to 125% of control. The swelling is very different compared to a hypotonic swelling. Regulatory volume decrease (RVD) mechanism are not operative in the presence of ammonia. The energy charge is only minor affected, i.e. PCr decreases by 20-25%. The cells loose large amounts of osmolytes, in particular taurine and myo-inositol. The cell swelling and the osmolyte loss is reduced considerably, but not prevented, if MSO is added to inhibit glutamine synthetase(GS). On the other hand, inhibition of GS increases the concentration of alanine, glutamate and aspartate generated via transaminase activity. The latter is largely inhibited again in the presence of AOAA, a transaminase inhibitor. This inhibitor also impairs glycolysis and TCA cycle activity.
As astrocytes are unable to regulate their volume during hyperammonemia the
glutamine dependent regulation of total brain water will be affected, which will then spoil various transport processes to and from the brain. Additionally, the impairment of the glutamine/glutamate cycle will deteriorate the brain function.

DIFFERENTIAL EXPRESSION OF CHEMOKINES AND IFNγ DURING MBP AND S100ß T CELL MEDIATED EAE.

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In EAE induced by the adoptive transfer (tEAE) of MBP-specific T cells the CNS parenchyma is invaded by large numbers of T cells and activated macrophages which disrupt neurological function. In contrast, T cells specific for S100ß mediate a sub-clinical inflammatory response in which large numbers of T cells, but very few macrophages enter the CNS[1]. In this study we analyzed the relationship between these different pathologies and mRNA expression for IFNγ, a marker for T cell activation, and the chemokines MCP-1 and RANTES in the CNS by semi-quantitative RT-PCR/blot hybridization assays. Immunohistochemical analysis and RT-PCR for the T cell marker CD2 revealed that T cell infiltration into the CNS was similar in both models. However, despite the large numbers of T cells present in the CNS of rats injected with S100ß-specific T cells, T cell activation as determined by RT-PCR for IFNγ RNA was substantially less than in those animals with MBP-mediatedtEAE. In MBP-mediated tEAE the appearance of IFNγ mRNA coincided with a massive increase in the signal for both MCP-1 and RANTES in the CNS, concordant with the invasion of this tissue by both T cells and macrophages. However, whereas MCP-1 expression then decreased, expression of RANTES remained moderately elevated until day 6 when the MBP experiment was terminated. In striking contrast, no substantial increase in MCP-1 mRNA expression was observed during this period in S100ß tEAE, although RANTES expression increased 3 to 6 fold by day 6 and remained elevated until day 10. These results indicate that the inability of S100ß T cells to mediate the infiltration of the CNS by activated macrophages is related to a failure to induce the local expression of appropriate chemokines, in particular MCP-1.

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HIPPOCAMPAL ASTROCYTES IN SITU RESPOND TO NEURONAL ACTIVITY WITH INCREASES IN INTRACELLULAR CALCIUM.

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A large number of reports indicate that astrocytes *in vitro* and *in vivo* express neuroligand receptors. The goal of the experiments described here was to determine if astrocytes *in situ* respond to synaptic activity with increases in intracellular calcium levels. Transverse hippocampal brain slices were prepared from 10-16 day old rats and loaded with a calcium indicator dye. Slices were then placed into a perfusion chamber and loaded cells within CA1 stratum radiatum visualized using a confocal microscope. Cells analyzed were subsequently identified using immunocytochemical markers. Two different experimental approaches were used to stimulate synaptic activity in CA1 stratum radiatum: electrical stimulation of the Schaffer collateral pathway and 4-AP induced-seizure activity. Stimulating Schaffer collateral axons under conditions known to induce LTP led to a rapid rise in astrocytic calcium levels. Increases in astrocytic calcium levels were blocked by either 1) lesions of the Schaffer collateral pathway, 2) conotoxin MVIIC or 3) TTX. Astrocytic calcium responses were blocked by MCPG, a metabotropic glutamate receptor antagonist. However, when 4-AP was included in the perfusion buffer to increase the duration of neurotransmitter release, both ionotropic and metabotropic glutamatergic receptor antagonists were required to block astrocytic responses. Seizure activity developing in CA3 and propagating through Schaffer collaterals into CA1 also led to increases in astrocytic calcium levels. However, these increases were inhibited by voltage-gated calcium L-channel blockers without blocking neuronal seizure activity. Overall, these findings demonstrate that astrocytes *in situ* respond to neuronal activity.

SCHWANN CELL PRECURSORS AND SIGNALS THAT CONTROL SCHWANN CELL GENERATION.

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In recent years cellular events involved in the development of Schwann cells from neural crest cells to myelination have become much clearer. As a result, a major challenge in this field is to identify the molecules (growth factors, growth factor receptors and transcription factors) that regulate the multiple developmental steps that have been defined.

Recently, we identified a distinct intermediate cell type in the Schwann cell lineage, the Schwann cell precursor, which differs in molecular, morphological, mitogenic and survival properties from an immature Schwann cell. These cells are the major cell type in emergent rat and mouse limb nerves at embryo day (E) 14/15 (rat) and E12/13 (mouse). Precursors are highly dependent on neuronal signals for survival and maturation to Schwann cells, whereas Schwann cells possess independent autocrine survival mechanisms. We have identified ß neuregulins as the axonal signals that support survival of Schwann cell precursors and their progression to Schwann cells, a conclusion backed up by neuregulin expression data in vivo and studies on neuregulin null mice from other laboratories. We can demonstrate that one of the important intracellular signalling pathways used by ß neuregulins involves sustained high-level phosphorylation of the transcription factor CREB.

The irreversible conversion of precursors to Schwann cells takes place in vivo between E15/17 (rat) and E13/15 (mouse). An important objective now is to identify genes that regulate this key developmental transition. In this context we are studying known transcription factors, including Krox-20, Oct-6 and Pax-3 and are in the process of identifying novel molecules that are sharply regulated at the transition point using differential mRNA fingerprinting methods.

IDENTIFICATION OF RECEPTORS AND MOLECULAR MECHANISMS THAT REGULATE MICROGLIAL MOTILE AND PHAGOCYTIC ACTIVITY

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Microglia, the resident tissue macrophages of the brain, are rapidly activated in response to insults of the CNS, including trauma, infection, tumorigenesis and immune-mediated disorders. It is well documented that microglial cells become ameboid and accumulate at sites of injury where they eventually become phagocytes. However, the signals that serve to recruit microgial cells to the lesion site and that control their phagocytic activity are incompletely understood. We used physiological and Ca^{2+}-imaging techniques to identify receptors for immunoregulators and growth factors on microglial cells. Applying different *in vitro* assays (video-timelapse microscopy combined with computer assisted-motitliy analysis, microchemotaxis, and flow-cytometry) we analysed which of these receptors control motile and phagocytic activity of microglial cells.

Epidermal growth factor and the complement components C5a and C3a were identified as important motility signals for cultured microglia. C5a controls motility and chemotaxis via an inhibitory G-protein and rearrangement of the actin cytoskeleton. Microglial Ca^{2+}-transients and K^+-outward currents are concomitantly triggered by both complement factors. However, the intracellular signaling cascades mediating motility, Ca^{2+}-release and K^+-currents diverge downstream the G-protein and are not causally interconnected. Searching for receptor systems that modulate microglial phagocytosis, tumor necrosis factor-α and granulocyte/macrophage colony stimulating factor were shown to enhance, whereas both transforming growth factor-β1 and interleukin-4 significantly reduced phagocytotic activity in vitro. In summary, microglial cells are highly flexible cells equipped with a large repertoire of cytokine-receptors controlling their different activation parameters. According to the pathological situation, there is a need for a variability in microglial responses to modulate their distinct activation stages. The fine-modulation of microglial response to injury might not only be governed by the composition of activating/deactivating factors in the microglial micro-environment but is furthermore dependent on the actual receptor diversity of the microglia, thereby fine-tuning the physiological properties of these cells.

CEREBELLAR DEFECT AND IMPAIRED COORDINATION IN MICE LACKING VIMENTIN.

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Vimentin belongs to the family of intermediate filament (IF) proteins. During nervous system development of mammals, it is transiently expressed in precursor cells of neuronal and glial lineages. In adults, Bergmann glia (BG) of the cerebellum continue to express vimentin together with glial fibrillary acidic protein (GFAP). Surprisingly, mice devoid of vimentin develop and reproduce without obvious defects[1]. However, the analysis of cerebellar morphology and ultrastructure showed poorly developed and highly abnormal BG, whereas the migration of granular neurons proceeded normally. Moreover, many Purkinje cells (PC) appeared stunted with a loss of spiny branchlets, and some of them were necrotic. Finally, impaired motor coordination was evidenced by behavioral tests. These observations demonstrate a role for vimentin in contributing to the normal development and morphology of BG and reveal an hitherto unreported functional relationship between BG and PC.

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INTRACELLULAR Na^+ HOMEOSTASIS IN RAT HIPPOCAMPAL ASTROCYTES

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We determined the intracellular Na^+ concentration ($[Na^+]_i$) and mechanisms of its regulation in cultured rat hippocampal astrocytes using fluorescence ratio imaging of the dye SBFI-AM (acetoxymethylester of sodium-binding benzofuran isophthalate, 10 μM).[1] Intracellular dye signal calibration was performed after each experiment. Baseline $[Na^+]_i$ of astrocytes was 14.6 ± 4.9 mM in CO_2/HCO^-_3-containing saline with 3 mM K^+. Blocking Na^+, K^+-ATPase activity by ouabain (1 mM) or removal of extracellular K^+ led to an increase in $[Na^+]_i$ by 2 and 4 mM/min, respectively. Changing from CO_2/HCO^-_3-free to $C0_2/HCO^-_3$-buffered saline resulted in a transient increase in $[Na^+]_i$ by 5 mM, suggesting activation of inward Na^+-HCO^-_3-cotransport by CO_2/HCO^-_3. During bumetanide (50 μM) application, a decrease in $[Na^+]_i$ by 2 mM was observed, indicating a steady inward transport of Na^+ via Na^+, K^+-$2Cl^-$ cotransport under control conditions. Glial cells in culture rapidly developed different levels of baseline $[Na^+]_i$ when they were isolated from one another by uncoupling agents. Gap junctions, therefore, pass Na^+ and equalize $[Na^+]_i$ among coupled astrocytes. Elevation of $[K^+]_0$ by as little as 1 mM resulted in a rapid and reversible decrease in $[Na^+]_i$ indicating that the external cation site of glial Na^+, K^+-ATPase is not saturated at an $[K^+]_0$ of 3 mM. The K^+-induced decrease in $[Na^+]_i$ was significantly prolonged during perfusion with bumetanide. Compared to astrocytes, $[Na^+]_i$ in hippocampal neurons was not sensitive to small changes in $[K^+]_o$.[2]

These results implied that in hippocampal astrocytes low baseline $[Na^+]_i$ is determined by the actions of Na^+, K^+-ATPase, Na^+-HCO_3^--cotransport and Na^+-K^+-$2Cl^-$-cotransport, and that both Na^+,K^+-ATPase and inward Na^+-K^+-$2Cl^-$-cotransport are activated by small, physiologically relevant increases in $[K^+]_0$, helping to buffer increases in $[K^+]_0$ associated with neural activity.

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MOLECULAR SIGNALS FOR NEUROGLIAL ACTIVATION IN THE INJURED CNS

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Growth factors and cytokines play a crucial role in regulating cellular activation, growth and differentiation. To understand the molecular mechanisms operating in the process of nerve regeneration, we are analysing the regulation of these signalling molecules and their contribution to posttraumatic growth and repair after injury. Previous studies have shown that injury causes massive, local and transient expression of many different growth factors in the lesioned nervous system, like NGF, TGF-ß, IL6, MCSF, GMCSF and their receptors.

Here, particularly striking effects are observed for macrophage colony stimulating factor (MCSF) and interleukin-6 (IL6) deficiency. As a model we have concentrated on the axotomized mouse facial motor nucleus, where peripheral injury leads to a well documented neuroglial activation in the affected part of the central nervous system. Absence of MCSF led to an almost complete abrogation of posttraumatic microglial proliferation and a clear reduction in their activitation markers such as aMb2-integrin, thrombospondin and the receptor for MCSF. It did not interfere with the astrocytic or neuronal response to facial nerve axotomy. Deficiency for IL6 led to a massive reduction in the activation of GFAP-positive astrocytes and moderate inhibition of microglial proliferation and activation. Surprisingly, absence of IL6 also slightly enhanced the neuronal response to injury.

In summary, current data help to define the physiological role of growth factors like MCSF and IL6 in the injured neural tissue. This combination of cytokine knockouts and nervous injury models provides a new approach to gain insight into the cellular and molecular activation cascade which regulates plasticity and regeneration in the lesioned nervous system.

Glial cell surface associated components modulating neurite extension

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A challenging and long-standing goal of developmental neurobiology is to characterize cellular and molecular mechanisms underlying the formation of synaptic connections between neurons. Molecules providing guidance cues include cell surface and extracellular matrix glycoproteins which might be differentially localized. They might contain permissive and/or non-permissive activities for axonal extension. In our studies we have characterized two proteins associated both with glial and neuronal surfaces: tenascin-R (TN-R) and CALEB.

TN-R is a homotrimeric extracellular matrix glycoprotein that binds to the axonally expressed IgSF member F11. The interaction of TN-R with F11 in in vitro cultures enhanced F11 mediated neurite outgrowth and results in an enlargement of the surface area of growth cones suggesting that TN-R might modulate F11 mediated axon extension.

In order to identify proteins interacting with TN-R we have combined the sensitivity of binding detection in ELISAs with immunological screens. The application of these steps resulted in the identification of a novel member of the EGF family of differentiation factors which we termed CALEB and that was found to be implicated in neurite extension.

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Specific role of astrocytes and microglia in the brain immune response.

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The definitive and respective function of microglia and astrocytes in the initiation and/or regulation of the immune response that takes place in the brain is not yet known. The immune competence of these glial cells has been mainly evaluated with regard to their capacities to activate specific T lymphocytes and to secrete or respond to various cytokines. But we know nothing about some basic features of these cells despite their high relevance to immune competence of cells, such as the membrane traffic that underlies the processing of antigen and its association with MHC II class molecules.

We have undertaken a comparative analysis at the biochemical, ultrastructural and functional levels of the endocytic and exocytic pathways in mouse microglia and astrocytes, at both their resting and immune (cytokines)-activated state. Using primary cultures, we are currently characterizing the endocytic/recycling pathway of these cells as well as the biosynthetic route and intracellular fate of MHC class II molecules, using cell labelling, subcellular fractionation and electron microscopy. We are analysing the capacity of microglia and astrocytes to generate a peptidic repertoire from a given antigen by comparing their capacity to stimulate a panel of antigen-specific T cells.

We have recently extended our study of the immune competence of glial cells by exploring the putative role of the CD1 molecule in the brain immune response. Moreover, we further investigate the relationship between immune activation and regulation of membrane traffic by analysing the pathway(s) of the amyloid precursor protein processing in both resting and immune competent cells.

TYPES AND MODULATION OF ION CHANNELS AND TRANSMITTER RECEPTORS IN GLIAL (MÜLLER) CELLS

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The vertebrate retina has been established as a suitable model of the central nervous system. This tissue contains one dominant form of macroglia, the Müller cells. It has been suggested that the phenotype of Müller cells develops by optimal adaptation to neuron-glia interactions. Recent studies have shown that Müller cells undergo considerable morphological and functional changes during the period of postnatal retinal network maturation. Furthermore, Müller cells are the primary targets of hepatic retinopathy, and respond to various forms of retinal (neuronal) degeneration by a characteristic repertoire of morphological and biochemical changes.

We have studied the physiological properties of Müller cells from various mammalian species including primates and man. Our studies have shown that Müller cells express at least four different types of voltage-dependent K^+ channels (inward-rectifiers, transient A type channels, delayed rectifiers, and large conductance, Ca^{2+}-activated channels), neuron-type Na^+ channels (observed in some but not all mammalian species), and $GABA_A$ receptors (found only in primates). We have observed that the pattern of channel expression changes dramatically during postnatal development, as well as in cases of retinal pathology. In early postnatal stages, inward rectifier-mediated currents are very small whereas large conductance, Ca^{2+}-activated channel-mediated currents dominate. The opposite is found in cells from normal adult retinae. When cells were isolated from pathologically altered retinae, a current pattern was observed that was similar to that of cells from young postnatal stages. These findings may be related to clinically important events of retinal development and pathophysiology. In particular, enhancement of currents through Ca^{2+}-activated K^+ channels seems to be related to an initiation of Müller cell proliferation. The aim of further studies is to define the signals mediating (i) the development of optimal "symbiotic" neuron-glia interactions, and (ii) the pathogenetic events disrupting these interactions.

ANALYSIS OF MOTILE OLIGODENDROCYTE PROGENITOR CELLS IN BRAIN SLICES AND IN CULTURE

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Video-time-lapse analysis was performed on isolated precursor cells in living brain slices, in co-culture with cerebellar microexplants and in single cell cultures. Motility analysis of individual cells was combined with electrophysiological, immunological, and morphological characterizations. In slices of the corpus callosum from neonatal rat, glial progenitor cells did not strictly migrate along the main direction of the axonal fibers of the corpus callosum but rather traversed the fibers with an overall direction towards the cortex. All motile cells had a simple morphology and migrated with a velocity of 10-100 µm/h. After Lucifer Yellow filling of the motile progenitor cells *in situ,* we could demonstrate that they were dye-coupled to yet unidentified cells of the corpus callosum. Patch clamp analysis of the motile cells confirmed that they belonged to the O-2A lineage. In co-culture experiments of oligodendrocyte progenitor cells with cerebellar microexplants, O-2A progenitor cells did also not migrate strictly along neurite fascicles which were radially projected from the microexplant cores, although O-2A progenitors preferably attached to the neurite fascicles shortly after addition. Their overall velocities were not influenced by the presence of cerebellar microexplants. The velocities of single cell cultures highly enriched in O-2A progenitor cells was analyzed using a specifically designed computer-based analysis system. As has been seen *in situ*, all motile cells exhibited a simple morphology and most, but not all, of them expressed the A2B5 epitope *in vitro*. Besides growth factors (PDGF and bFGF) which are known to stimulate O-2A progenitor cell migration we could demonstrate that the extracellular matrix component laminin did also enhance the motility of these cells, as the percentage of motile cells, as well as their velocities were increased compared to the control on substrate-coated poly-L-lysine. Translocation of the cell bodies was not continuous, but occurred in waves comparable to the situation in the brain slices.

AGE-DEPENDENT CHANGES OF AMPA RECEPTOR PROPERTIES IN ASTROCYTES OF THE HIPPOCAMPUS.

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Glial cells in the hippocampus express different types of glutamate receptors[1]. By using single cell RT-PCR we have previously identified the subunit composition of AMPA receptors expressed by astrocytes in the CA1 subregion[2]. To better understand their significance, here we investigated functional properties of AMPA receptor channels in astrocytes of 3-40 days old mice. A fast concentration clamp technique was applied to acutely isolated cells to detect rapidly activating and desensitizing receptor responses.

The overall efficacy of glutamate and kainate responses differed between postnatal days (P) 5 and 10. While the efficacy of kainate was almost the same at all developmental stages, a steep rise in the glutamate efficacy was observed in the same time range. Between P5 and P10, there was an increase in the potentiation of AMPA receptor currents by cyclothiazide (CTZ) while the dissociation kinetics of CTZ was delayed. These changes in the pharmacological properties suggested a variation in splice variant expression. The degree of desensitization of glutamate- and AMPA-induced receptor currents increased during maturation. Thus, in addition to a switch in splice variants, our results possibly suggested a developmental regulation by reducing RNA editing in the R/G site.

The overall Ca^{2+} permeability remained constant during development. The shift of the reversal potential caused by Joro spider toxin in high Ca^{2+} solutions, however, pointed to the expression of a mosaic of Ca^{2+} permeable and Ca^{2+} impermeable receptor complexes early in development (P5). With further maturation, the cells probably expressed heteromeric receptors assembled from almost the same subunit combinations, including the GluR2 subunit.

Altogether, the present results demonstrate changes in astrocytic AMPA receptor functioning early in postnatal development, while after P10 the receptor properties remained almost constant.

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THE FUNCTION OF THE MAIN CNS MYELIN PROTEINS PROTEOLIPID PROTEIN (PLP) AND MYELIN BASIC PROTEIN (MBP) AND OF OLIGODENDROCYTE SPECIFIC CEREBROSIDES AND SULFATIDES: AN ANALYSIS *in vivo* BY GENE TARGETING IN THE MOUSE.

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Oligodendroglia synthesizes during the short period of myelination (p10-30 in rodents) specific myelin proteins and the complex spectrum of myelin lipids in a coordinated fashion. We have disentangled this process in the mouse as a model for the human CNS myelination regarding the function of the two main myelin proteins PLP and MBP by gene ablation through gene targeting by homologous recombination. These studies led to two discoveries: 1. PLP is responsible for the compaction of the spirally wrapping plasmamembrane processes at the extracytoplasmic surface and stabilizes the compact structurte (1,2). 2. Expression of oligodendrocyte specific expression of genes responsible for the synthesis of constituents of the myelin membrane structure is severely inhibited, if the stoichiometry of MBP and PLP expression is impaired (e.g.*shiverer* mouse).

We have adressed the question for the function of oligodendrocyte specific lipids cerebrosides and sulfatides and isolated the key enzyme *UDP-Galactose Ceramid eGalactosyltransferase (CGT)* , cloned the enzyme (3), analyzed the gene organization of the human and mouse genes *(4,5)* and generated the *cgt* "knock out" mouse (*cgt-/-*) (6).The CGT deficiency causes a severe dysmyelinosis. The vital function of these two lipid classes is to provide the insulation properties of the myelin multilayer membrane system. The deficiency leads to a breakdown of the saltatory rapid conduction velocity, whole body tremor, seizures, death.It does not cause a pleiotropy due to oligodendrocyte death.

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ROLE OF MICROGLIA IN NEURONAL INJURY AND DEATH.

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Microglial activation, or reactive microgliosis, is an intrinsic response of the CNS to injury. It occurs in virtually all kinds of CNS lesions. *In vivo*, microgliosis has been characterized primarily in terms of morphological and phenotypical changes, and these studies collectively suggest a beneficial role for activated microglial cells. By contrast, *in vitro* studies have examined microglial activation mainly with regard to the cells' ability of producing cytokines and growth factors. These studies have indicated potentially adverse and cytotoxic actions of activated microglia. In order to reconcile a developing controversy over the functional significance of reactive microgliosis, we have embarked on a series of studies designed to establish microglial cytokine gene expression profiles *in vivo*. The results from these studies suggest that lesions involving a break in the blood brain barrier, such as spinal cord contusion injury, result in a transient and short-lived gene expression of proinflammatory cytokines, like IL-1 β and TNF-α, within hours after spinal cord injury. The cellular sources responsible for producing these cytokines remain to be determined conclusively, but are likely to include activated microglia which are prevalent early during lesion development. Thus, we propose that acute traumatic CNS injury results in transitory expression of proinflammatory cytokines by activated microglia. As a counterpart to investigating microgliosis in acute injury paradigms, we have also examined microglial activation in the aging human brain, and in age-matched sets of patients with and without heart disease. Our results confirm data from rodents that there is increased microglial activation occurring with normal aging. In addition, the brains of patients with heart disease show a higher level of microglial activation than non-heart disease control subjects. This enhanced activation of microglia is coincident with increased presence of intra-and extracellular β-amyloid. We have also found increased microglial activation in hypercholesterolemic rabbits suggesting a link between hypercholesterolemia/heart disease and accelerated aging. These findings have led to speculations about how slowly progressive inflammation of the CNS may be a fundamental mechanism in aging. Such chronic inflammation could contribute significantly to the development of Alzheimer's disease in genetically susceptible individuals.

ROLE OF EXTRACELLULAR SPACE VOLUME AND GEOMETRY IN NONSYNAPTIC TRANSMISSION AND NEURON-GLIA INTERACTION.

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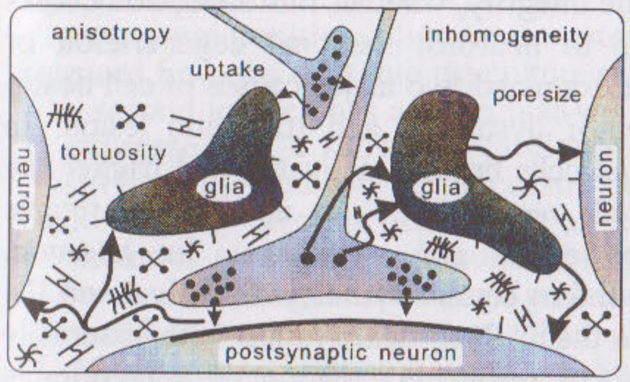
Nonsynaptic transmission plays an important role in short- and long-distance communication between neurons, axons and glia. It is mediated by the diffusion of neuroactive substances, including ions and transmitters, through the extracellular space (ECS) (1). In this way, transmitters can reach high-affinity receptors located outside synapses (often coupled to G-proteins) and on glial cells. The structure of cellular aggregates and/or the extracellular matrix can channel the migration of molecules in the ECS, so that diffusion in certain regions is facilitated in one direction rather than in another (see Fig.). This so-called diffusion anisotropy has been found e.g. in white matter, cerebellum and hippocampus. Changes in ECS composition and geometry are a consequence of neuronal activity and of glial K^+, pH and amino acid homeostasis. They accompany the phenomena of repetitive neuronal activity, seizures, anoxia, injury, gliosis, demyelination and many other pathological states in the CNS and may significantly affect signal transmission. Activity-related or CNS damage-related ionic changes and amino acid release result in fast and pulsatile or long-term cellular (particularly glial) swelling. Cellular swelling is compensated for by ECS volume shrinkage and by a decrease in the apparent diffusion coefficients of neuroactive substances diffusing in the ECS. The movement of substances is hindered in the narrower clefts, but presumably also due to the crowding of molecules of the ECS matrix and/or by the swelling of the fine glial processes which form diffusional barriers. This can either increase the efficacy of synaptic as well as non-synaptic transmission, by an increased accumulation of substances, or induce damage to nerve cells if these substances reach toxic concentrations.



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THE ROLE OF RETINAL MICROGLIAL CELLS IN NEURAL DEGENERATION AND REPAIR.

S. Thanos.

In a relatively short period of few decades, the retinal microglial cell has gone form a strongly contested component in the 60ies, to become a favoured model cell in studies of neural de- and regeneration. Microglial cells are thought to arise from cells of haematopoetic origin, and to enter the retinal tissue during the period of programmed cell death. The uniformity in distribution of dying neurons results in a uniform scattering of immigrating microglia. The unique ability of these cells to carry out phagocytosis while integrated within the tissue enables their labelling and monitoring in vivo. One of the main roles of microglia cells is to immediately respond to disruption of tissue integrity, whether this disruption comes from direct damage to neurons, neuronal degeneration or through disease. We examined two major causes of cell death: inherited photoreceptor dystrophy and traumatic lesion. In these cases microglial cells become activated and trigger the viability of affected neurons by a number secretable substances like proteases and cytokines. In addition, microglial cells respond to certain cytokines like IL-4 and IL-10 which deactivate them. Such deactivation with externally supplied IL-4 or IL-10 results in creation of a favourable environment for prolonged nerve survival and regeneration. In short, microglial cells may be seen as key components of the cascade of nerve cell response to diseases and some therapeutic strategies will be directed to microglial cells.

CELL-SURFACE GLYCOPROTEINS EXPRESSED BY CELLS OF THE OLIGODENDROCYTE LINEAGE

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In a search for molecules regulating the early events of myelination such as migration and ensheathment of axons, we have generated monoclonal antibodies against lectin-binding glycoproteins isolated from an immortalised murine line of oligodendroglial precursor cells. Two different cell-surface antigens recognised by different monoclonal antibodies have been studied in detail. The first antigen has a molecular weight of 140 kD and is expressed by a subpopulation of O4- and myelin-associated glycoprotein-positive oligodendrocytes. The antigen is released from the cells into the culture supernatant.
The second antigen has a molecular weight of 330 kD and is expressed by a subset of proliferative, GD3- and O4-positive oligodendrocyte precursor cells. Biosynthetic radiolabelling experiments, as well as enzymatic digestion of material isolated by immunoaffinity chromatography show that the antigen has a large protein core (approx. 300 kD) and in some instances attached glycosaminoglycan (GAG) chains. Subpopulations of Schwann cells also express the antigen and in this instance the protein core carries a substantial number of of GAG chains. In Western Blots of mouse brain, expression of the protein is first seen at E13, peaks at P10 and is down-regulated in the adult. Using the affinity-purified protein as substrate, we have shown that astrocytes express a receptor for the antigen. The existence of the antigen in man and a possible role in neural diseases are under investigation.

DEVELOPMENT OF GLIAL CELLS IN THE EMBRYONIC CNS OF *DROSOPHILA MELANOGASTER*

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The late embryonic CNS of *Drosophila* consists of a highly ordered array of neurons and glia that are produced by about 30 stem cells per hemineuromere. To be able to investigate glial cell determination and differentiation we first described the whole population of glial cells within the embryonic ventral nerve cord [1]. Lineage tracing with DiI revealed, that more than 90% of these cells derive from the lateral neuroectoderm. This suggests the existence of a lateral "gliogenic region" which might be specified via dorsoventral patterning genes.

In collaboration with A. Travers (Cambridge, UK) we cloned and characterized the gliaspecific homeoboxgene *reversed polarity* (*repo*) [2]. The functional analysis showed, that Repo is an essential factor for gliogenesis in the embryonic CNS. *repo* loss of function mutants exhibit a block in glial differentiation leading to a reduction of cell number and lack of glial differentiation markers at the end of embryogenesis. The cloning of the *repo* homologue of *Drosophila virilis* revealed a strong conservation of potential binding sites for *glial cells missing (gcm)*, a recently characterized gene conferring a genetic switch towards glial cell fate [3,4]. This suggests that *repo* is a direct target of *gcm*.

While *gcm* seems to be a general switch gene involved in the determination of all glial cells except the midline glia, the activation of *gcm* might be lineage specific. The analysis of the NB 1-1 lineage development in the *huckebein (hkb)* loss of function mutant showed, that *hkb* is necessary to activate *gcm* in this lineage. All other glial cells not derived from NB 1-1 seem to develop normal in this mutant.

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MANY ASTROGLIAL GAP JUNCTIONS SERVE AUTOCOUPLING WITHIN ONE CELL RATHER THAN HETEROCOUPLING BETWEEN DIFFERENT CELLS: IN-VIVO AND IN-VITRO EVIDENCE.

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Astrocytes are distributed at similar densities (15.000-30.000 cells/mm^3 of tissue) throughout the CNS. Their plasma membranes contain molecular receptors and carriers for synaptic transmitters, receptors for hormones, growth factors etc. and ion channels, which enable these cells to sense or monitor and counteract local variations in extracellular fluid composition around individual neuronal components of the neuropil. It is still unclear, how local changes interact between different parts of the same astrocyte. At present the view is held, that local changes, e.g. in [Ca++]i, spread within the astroglial cytosol and is transfered into neighboring astrocytes through gap junctions (GJ). This interpretation disregards characteristic features of astroglial structure:

i.) A large part of the astroglial cell volume may not be available to free diffusion of cytosolic components. Astroglial processes and cell bodies are studded with numerous lamellipodia and filopodial extensions, which collectively form 50% of the astroglial volume, but individually each contains a narrow cytosolic space (width: 5-100nm) devoid of organelles. **Lamellae** carry about 80% of the astroglial cell surface, penetrate the neuropil, surround synapses, form extensive contacts with various neuronal surfaces and are heavily involved in GJ-formation.

ii.) Many GJ do not connect cell membranes of different cells, but different processes of the same cell: **In situ**, astrocytic cell bodies give rise to radially extending, branched processes (2-10 stem processes/cell). Since astrocytes show a rather even "contact spacing" in CNS-tissue, each cell penetrates with all its processes a "**private territory**" of considerable size (statistically: 30.000-70.000μm^3/cell; diameter ~ 50μm). In contrast, morphometric analysis revealed that the numerical density of GJ is much higher (5-10*10^8 gap junctions/mm^3; appr. 30.000 GJ/cell; avarage spatial distance 1-3μm).

From various brain parts, ultrastructural and immunocytochemical evidence will demonstrate the tendency of astrocytes to form GJ (containing Connexin 43) between different parts of the same cell. In addition, isolated astrocytes **in vitro** will be shown to form GJ even in the absence of any contact to other astrocytes. Results suggest (1.) that mechanism of GJ-formation obviously can not distinguish between Connexons in membranes of different cells or different parts of the same cell; and (2.) conditions for GJ-coupling may be established between parts of one astrocyte.

INHIBITION OF MITOGEN ACTIVATED PROTEIN (MAP) KINASES BY OXYSTEROLS IN RAT ASTROCYTE PRIMARY CULTURES AND IN C6 GLIOMA CELL LINES.

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We have previously demonstrated that oxysterols inhibit the extent of astrogliosis[1] (reactive astrocyte proliferation) and the growth of glioblastoma[2] induced in the rat brain cortex. Mechanism of action of these compounds remain obscure. In this study, we have investigated the effect of 7ß-hydroxycholesterol (7ß-OHCH) and 7 ketocholesterol (7k-CH) on the growth pattern of three biological models *in vitro:* rat astrocyte primary cultures (normal cells), normal cells treated with dibutyryl-cAMP (reactive astrocytes) and C6 glioma cell line. The effect of oxysterols on MAP kinase was determined by measurement of kinase activity in total cellular lysate (kit assay) and by quantification of ERK-1 and ERK-2 activity in Myelin-Basic-Protein (MBP)-containing gel. Both oxysterols are not lethal to normal cells, even if MAP kinase activity is decreased and this particularly for 7k-CH treatment (36%). 7ß-OHCH and 7k-CH are toxic to reactive astrocytes. In comparison to normal cells, the decrease of MAP kinase activity (gel assay) is amplified by 2,5 and 1,3 for 7ß-OHCH and 7k-CH, respectively. In C6 cells there is a correlation between 7ß-OHCH antiproliferative and toxic effect and the decrease of MAP kinase activity.

Whole these observations indicate that: (1) the MAP kinase activity is not required for normal cell survival, (2) the effect of 7ß-OHCH is linked to MAP kinase activity in highly proliferating C6 cells, (3) the action of oxysterols on the astrogliosis model passes through an additional/other mechanism(s) at the cellular messenger traffic level.

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EFFECT OF GROWTH FACTORS AND GLUCOCORTICOIDS ON NUCLEAR AND MITOCHONDRIAL MACROMOLECULAR SYNTHESIS DURING ASTROGLIAL CELL PROLIFERATION AND DIFFERENTIATION IN CULTURE

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Functioning of the nervous system depends upon the extensive and intimate coupling between neuronal cells and the glial cells. The growth factors (GFs) have emerged as crucial intercellular signaling agents that coordinate the developmental and adult physiological processes of astrocytes and neurons. Epidermal growth factor (EGF), basic fibroblast growth factor (bFGF), Insulin-like growth factor I (IGF-I) and Insulin (INS) are known to exert profound effects in the astroglial cell compartment. Besides other hormones, adrenal corticosteroids are known to markedly influence astroglia, and are key hormones participating in neurodegenerative changes during brain aging. In previous studies, we have shown a marked increase in nuclear poly-ADP ribose polymerase (pADPR) activity in bFGF or IGF-I treated fetal brain tissue slices. In primary astroglial cell cultures, nuclear pADPRP and mitochondrial ADP ribosyltransferase activities showed different degrees of stimulation as a function of astroglial "age" and the presence or absence of a specific GF. The aim of the present study was to investigate the interaction between GFs (EGF,bFGF,IGF-I,INS) and glucocorticoids (dexamethazone, Dex) on the development of glucocorticoid receptor gene expression (GR), nuclear and mitochondrial DNA and RNA labeling as a function of glial "age". Expression of GR in developing glial cells is characterized by low levels of GR mRNA at 8 and 12 DIV, and a sharp stimulation at 20, 30 and 40 DIV. The morphologic and proliferative response of astroglia to GFs and DEX varies not only as a function of astroglia age, but also depending on the GF applied, the timing of application and the concomitant presence of DEX. Moreover, glucocorticoids may either act in concert with or abrogate GFs effects at specific stages of glia differentiation, supporting a prominent role of GFs and glucocorticoids in maintaining cell homeostasis and growth regulation of the astroglial compartment.

AMPA RECEPTORS ARE COUPLED TO THE NITRIC OXIDE / CYCLIC GMP PATHWAY IN CEREBELLAR ASTROGLIAL CELLS

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In the cerebellum, the brain region with the highest NOS-I activity, the enzyme is present in granule and basket cells but not in Purkinje cells and is also expressed in astrocytes and Bergmann glia[1,2,3]. In primary cultures of granular neurons, the excitatory aminoacids (EAAs) are the major activators of NO and cyclic GMP (cGMP) formation mainly through NMDA receptors[4,5]. However, in cerebellar astroglia, we[6] and others[4] could not detect any effect of GLU. In these cells, we shown here that kainate (KA) stimulates cyclic GMP (cGMP) formation with low potency (EC_{50} = 310 μM). The KA (1 mM) effect is completely inhibited by the AMPA/KA receptor antagonist CNQX (50 μM). In the presence of cyclothiazide (100 μM), a blocker of AMPA-receptor desensitization, the effect of KA is potentiated and glutamate (1 mM) and AMPA (100 μM) elicited large increases (over 100-fold) in cGMP levels. The response to all three agonists is abolished by the NO synthase inhibitor N^G-nitro-L-arginine (10 μM) and requires extracellular calcium. In some astroglial cells Co^{2+} uptake is stimulated by AMPA alone and to a higher extend in the presence of ciclothiazide. Thus, these results indicate that calcium-permeable AMPA receptors mediate stimulation of nitric oxide formation in cerebellar astroglia.

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MOLECULAR AND ELECTROPHYSIOLOGICAL CHARACTERIZATION OF GLYCINE RECEPTORS IN CULTURED CORTICAL OLIGODENDROCYTE PROGENITORS.

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Within the central nervous system (CNS), glycine is the main inhibitory neurotransmitter of the spinal cord and the brainstem but very little information is known about the description of a glial glycine receptor (GlyR). Using oligosphere-derived oligodendrocyte progenitor (OP) cell cultures, we demonstrated and characterized glycine-induced responses in rat cortical oligodendroglial cells. With whole-cell patch-clamp experiments, we showed maximum glycine-elicited inward currents at an intermediate stage of the oligodendrocyte maturation suggesting a developmental regulation of (GlyR) expression.

In our recording conditions, glycine-gated currents in OP reversed around 0 mV and were reversibly inhibited by the glycine competitive antagonist strychnine and the Cl^- channel blocker picrotoxin. Similar inward currents were also triggered by β-alanine and taurine, two specific GlyR agonists. Cortical OP GlyR shares many features with neuronal GlyR but differs in terms of glycine and especially strychnine sensitivity. RT-PCR analysis of GlyR subunit expression detected α_2, α_N and β with total RNA isolated from OP cultures.

The combination of our electrophysiological and biochemical data suggests a prevalent α_2 homo-oligomeric stoichiometry for this cortical OP GlyR. This is the first description of GlyR expression in oligodendroglial cells derived from cortex. This provides evidence that this receptor is ubiquitous in the oligodendroglial lineage and thus could have a functional relevance in the proliferation and differentiation events leading to CNS myelin-forming cells.

MICROGLIA-/MACROPHAGE-DERIVED CALCIUM-BINDING PROTEINS MRP-8 AND MRP-8/14 IN MULTIPLE SCLEROSIS. AN IMMUNOHISTOCHEMICAL AND SEROLOGICAL ANALYSIS.

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The macrophage-related-proteins (MRPs) form a group of calcium-binding S100-proteins which are expressed by activated macrophages under inflammatory conditions depending on their stage of activation and differentiation. These proteins are shedded into body fluids, i.e. serum or cerebrospinal fluid. The serological determination of MRP levels was shown to differentiate between acute and chronic inflammatory disease activity in several inflammatory autoimmune disorders.

The immunohistochemical examination of biopsy samples of multiple sclerosis (MS) lesions revealed a high expression of the acute stage inflammatory macrophage marker MRP-8/14 in early and late active lesions whereas the chronic stage marker MRP-8 was also expressed in lesions with decreasing demyelinating activity[1].

The analysis of serum samples of 22 patients with an acute relapse of a relapsing-remitting form of MS showed statistically significant higher serum levels of MRP-8/14 (7177 ± 4091 ng/ml) in comparison to patients in stable remission of disease (3337 ± 1579 ng/ml). The serum levels of MRP-8 were not different between these two groups.

In two patients serum samples were obtained during an acute relapse as well as later in remission. Examination of these samples showed in both patients higher levels of MRP-8/14 (and to a lower extent of MRP-8) during the acute relapse in comparison to remission.

The data obtained indicate a differentiated activation of macrophages/ microglia in acute MS lesions. This activation is probably reflected in the serum levels of the calcium-binding proteins MRP-8 and MRP-8/14. This rises hope to the possible development of a serological paraclinical disease activity parameter in multiple sclerosis.

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Immune Competence of Astrocytes and Microglia: Understanding the Endocytic and Antigen Processing pathways.

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Immune functions within the Central Nervous System are thought to be carried out mainly by Astrocytes and Microglia. This, not withstanding the role played by other inflammatory cells of hematopoietic origin.

The role of Astrocytes and Microglia as antigen presenting cells is supported by in vitro studies showing their ability to internalise, process and present antigen to antigen specific $CD4^+$ T cells. This is yet to be confirmed in vivo. Moreover, the capacity to internalise and the pathways taken by antigen in these cells under different inflamatory conditions is yet to be unravelled. Using primary glial cultures from Balb C mice, we have determined the cumulative capacity to internalise a fluid phase marker over time, and the role of inflammatory cytokines on these properties.

Studies are underway to dissect the intracellular routes of internalisation under varying immunological conditions.

THE DSD-1 STRUCTURE IS A CHONDROITIN SULFATE MODIFICATION PRESENTED ON THE DSD-1- PROTEOGLYCAN AND PROMOTES NEURITE OUTGROWTH OF HIPPOCAMPAL NEURONS

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The chondroitin sulfate (CS) proteoglycan (PG) DSD-1-PG has been identified with the monoclonal antibody (mAb) 473HD. The results of digestion experiments with glycosaminoglycan (Gag) lyases support the interpretation that the epitope of the mAb 473HD is composed of a dermatan (DS)/CS C hybrid (designated DSD-1 for dermatan sulfate dependent). DSD-1-PG is expressd by immature glial cell in a developmentally regulated fashion and Western blot analysis of mouse brain extracts documented maximal expression during early postnatal stages. When coated as substrate, DSD-1-PG promotes neurite outgrowth of embryonic hippocampal neurons *in vitro*. This supportive effect is partially blocked by mAb 473HD, which suggests functional relevance of the DSD-1 Gag in neuronal process outgrowth. Further structural analysis indicated that the 473HD epitope requires the sulfation of the Gag. Thus, pharmacological agents, which block sulfation, abolished the epitope in the DSD-1-PG expressing oligodendroglial Oli-neu precursor cell line, as shown by immunoprecipitation and immunocytochemistry. This conclusion is consistent with competition ELISAs using various CS of similar structure, but distinct sulfate composition. In order to obtain more detailed information about the DSD-1 structure , CS D was loaded on a 473HD affinity column. The eluat was tested in a neurite outgrowth test. The results indicate that the neurite outgrowth promoting fraction is enriched in the eluat but not in the unbound material.
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EXPRESSION OF LAMININS AND α6A INTEGRIN IN THE DORSAL ROOT GANGLIA OF THE INTACT RAT AND FOLLOWING RHIZOTOMY

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Laminins, a large and complex glycoprotein group, are present as a major component of the extracellular matrix, predominantly in the basal laminae. The cell contacts to the extracellular matrix components including laminins are mediated by integrins, a membrane receptor family. In the case of laminins mainly α6β1 integrin is a candidate contributing to a link between the cells and laminins[1]. In the dorsal root ganglia (DRG) the satellite cells envelope the bodies of the primary sensory neurons and their external surface is covered by the basal lamina. In the present paper we have studied an immuno-histochemical localization of laminins and its α6 integrin in the rat intact dorsal root ganglia (DRG), and the ganglia 2 and 3 weeks after rhizotomy. The following antibodies were used: rabbit polyclonal anti-laminin (Gibco); mouse monoclonal anti-laminin-S (D7, Hybridoma Bank, The University of Iowa); mouse monoclonal VLA-α6A (Immunotech, a.s., Praha) that specifically recognized the intracellular compartment of rat α6 integrin subunit (α6A). Intact DRG expressed laminins (recognized by polyclonal antibody) on the surface of satellite cells but the capsule was free of the distinct immunoreactivity. The laminin-S was identified on the satellite cells as well as the capsule. A weak diffuse immunostaining was observed in the perikarya of the primary sensory neurons of both the small and large diameters. A significant decrease in the laminin immunoreactivity (recognized by both used antisera) was observed in the satellite cells two and tree weeks after rhizotomy. The immunostaining for α6A integrin chain corresponded with the expression of laminin-S both in intact DRG as well as following their rhizotomy. These findings suggest a fine reaction of the satellite cells to changes in the primary sensory neurons following a loss of their central axonal processes.

References: (1) Reichardt, Tomaselli (1991) Annu. Rev. Neurosci. **14***:531-570.*

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QUANTITATIVE ANALYSIS OF ACTIVATED MICROGLIA AND SPONGIFORM CHANGE IN CREUTZFELDT-JAKOB DISEASE

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The role of microglia in spongiform encephalopathies remains speculative. Recent *in vitro* experiments have revealed a close link between the neurotoxicity of a prion protein fragment and microglia (Nature 380:345, 1996). We have now studied 11 cases of Creutzfeldt-Jakob disease (CJD) in order to assess the spatial relationship between activated microglia and spongiform change *in situ* employing computerized image analysis. Brain tissue was obtained at autopsy from 10 patients with CJD and 5 age-matched controls. In addition, biopsy material from one patient with CJD was available. Subjects ranged from 54 to 81 years of age. Serial sections of the frontal lobe and, in selected cases, of the parietal and occipital lobe were utilized for the quantitative assessment of MHC class II positive microglia (CR3/43 immunostaining, DAKO) and spongiform degeneration (H&E). The percent area ± standard deviation of the cortical area occupied by activated microglia was significantly greater in CJD cortex (10.57 % ± 6.40) than in the control group (0.02 % ± 0.01) ($p < 0{,}002$). Early stages of spongiform change, characterized by a selective involvement of lamina II, III and V, were closely associated with the presence of MHC class II positive microglia. At more advanced stages, spongiform change affected the full cortical thickness and was most pronounced in the deeper cortical laminae. III to V. Similarly, the density of microglia was highest in laminae III to V where many cells displayed atypical, tortuous cell processes suggesting that microglia themselves may become affected by the disease process. Our observations indicate that microglial activation occurs early in CJD and increases dramatically during disease progression. The concept of „sick" microglia is discussed.

MICROGLIAL ACTIVATION IN THE RAT RETINA FOLLOWING ADENOVIRAL-MEDIATED GENE TRANSFER

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Adenoviral vectors are used as vehicles for gene transfer into cells of the nervous system and are of interest as promising tools for the treatment of human neurological diseases. We used the paradigm of optic nerve crush to investigate the effects of adenovirus-mediated gene transfer on survival of retinal ganglion cells.
In the present, study a nonreplicating adenovirus encoding the *lacZ* gene was injected intravitreously. Several retinal cells expressed ß-Galactosidase for 4 weeks but thereafter expression decreased rapidly. Lightmicroscopy showed cell infiltrates suggesting that the limiting factor for transgene expression could be the local immune response. Microglial cells are known to be activated rapidly by inflammatory agents. We therefore used a panel of antibodies against microglial cells to investigate microglial activation.
OX1, OX6, OX18, OX22 and OX42 positive cells were detected in the retinal ganglion cell layer and in the internal nuclear layer within two days. Infiltration of monocytes, lymphocytes and macrophages was delayed and started at days 3 and 4. Upregulation of antigens, e.g., against MHC class II and macrophage related proteins also labeled microglia in the colliculus contralateral to the optic nerve lesion.
Presence of microglial cells was increased over 21 days resulting in a massive infiltration (day 8) of the retina that partly disrupted the integrity of the layers. Understanding the functional heterogenity of microglial cells could advance our understanding of the local immune response to gene transfer. In addition, strategies to suppress or modulate immune responses might reduce cytotoxicity of microglial cells and enhance the efficacy of adenovirus-mediated gene transfer.

HUMAN TRAUMATIC BRAIN INJURY (TBI) AS A MODEL FOR MICROGLIAL ACTIVATION.

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The time course of microglial activation is extensively described in different experimental models. Similar data from pathological conditions in man, however, are not available. In most human diseases the onset is not known. In TBI the onset of lesion formation is precisely defined by the accident. Therefore TBI is a suitable paradigma to investigate the kinetics of microglial activation in human brain.
We examined 17 brains of patients who died after a survival time of between 6 hours and 6 months. Brains obtained from autopsy were formalin fixed. Samples of different traumatic and nontraumatic areas were studied with antibodies against microglial cells. Our results showed a delayed expression of the macrophage related proteins, MRP8 and 14, which belong to the S-100 protein family and which reflect the very early phase of activation. Expression of the leucocyte chemotactic factor, LCF, a natural ligand to CD4, is expressed in numerous microglial cells in the whole brain in a time independent manner. CD4, however, could only be detected on perivascular cells and some microglia near the injury site. CD4+ leukocytes were rarely found. We conclude a delayed microglial activation in human TBI which is strictly different from human ischemia (1), and which is confirmed by experimental studies (2). Interestingly, ischemic mechanisms can be detected within TBI by double immunolabeling with MRP8 and IL16. Distinct microglia in the vicinity of ischamically injured neurons express both antigens despite delayed general microglial activation.

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(2) Aihara (1995) J Neurotrauma, **12**:53-63

Effects of predegeneration of peripheral nerves on proliferation of Schwann cells in vitro

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Predegeneration of peripheral nerve grafts is known to improve axonal regeneration in the rat sciatic nerve. It is suggested that proliferated Schwann cells (SC) are responsible for this enhanced regeneration. Aim of this study was to evaluate the influence of predegeneration of peripheral nerves in vivo on cultivated SC.
The rat sciatic nerve was transected distally to the spinal ganglion. Predegeneration was allowed to take place for 1, 2, 3 and 4 days and 1, 2 and 3 weeks, respectively. The nerve was then resected and prepared for SC cultivation. SC cultivated from the contralateral untreated nerve served as control. S100 immunostaining, nerve growth factor (NGF) and N-cadherin were used to characterize SC. Viability was assessed by fluoresceine fluoroscence staining. SC proliferation index was determined by BrdU DNA incorporation.
After 7 days in vitro the SC from the predegenerated nerves revealed an increased proliferation compared to the control. Best proliferation and optimal development of cultured SC were seen after 1 week of predegeneration. Although an increased proliferation was evident, a similar expression of NGF and N-cadherin could be noted.
These results indicate that predegeneration may support the enhanced axonal regeneration in predegenerated nerve grafts due to a better proliferation of SC. Similar expression of growth factors and adhesion molecules in predegenerated and non-predegenerated groups determine a regular maturation of SC in culture despite the markedly increased proliferation.
Besides, the phenomenon of predegeneration induces an increased cell yield which may become relevant for future therapeutic approach in repair of peripheral nerve lesions.

Adrenoleukodystrophy gene transfer in myelin-forming cells

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X-linked adrenoleukodystrophy (ALD) is a neurodegenerative disease characterized by progressive demyelination of the central nervous system (CNS) and adrenal insuffiency. The disease is caused by mutation in the ALD gene, encoding a 75kDa peroxisomal protein of the ABC transporter family. Impairment of peroxisomal ß-oxidation results in accumulation of very long chain fatty acids in the patient's serum and cells.
We investigate whether transfer of the normal gene in mutated myelin-forming cells can correct the demyelination observed in the disease. To this goal, we purified populations of newborn rat Schwann cells (SC) after successive immunopannings, and maintained them in rhGGF-2 (8ng/ml) and forskoline (10µM) up to 70 days. We are also culturing adult rat Schwann cells according to the method of Morrissey & al (J. Neurosci.,11,2433,1991) because adult Schwann cells could be used in autografts. For gene transfer, we are using two different retroviral vectors : the M48-ALD construct has been shown to correct the ß-oxidation defect in fibroblasts of ALD patients (Cartier & al, PNAS, 92, 1674, 1995) . The second vector expresses the human ALD gene as well as the neomycin resistance and ß-galactosidase genes.
A mouse oligodendrocyte precursor (OP) cell line, N20.1 (gift of Dr.A.Campagnoni) and primary rat Schwann cells were infected by the M48-ALD virus. Forty-eight hours after infection, 45% of OP and 20% of SC expressed both human and mouse ALDP in overlapping peroxisomal populations, as detected by double labelling with antibodies specific for the mouse and human ALDP. Southern and western blots analysis of OP confirmed the integration of the human gene and its expression. Peroxisomal ß-oxidation was measured in the mouse OP line with or without the human ALD gene and revealed no significant change, suggesting that expression of human ALDP in addition to the endogenous mouse ALDP does not alter normal peroxisomal function. Thus, we can now perform similar gene transfer experiments in myelin-forming cells with mutations in the ALD gene in order to compare the remyelinating potential to those expressing the transfected ALD gene.

We thank the European Leukodystrophy Association for its support and M.O. Rolland for the biochemical assays.

MORPHOMETRIC ANALYSIS OF IN VITRO CULTURED ASTROCYTES TREATED WITH NOOTROPICS

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Glia constitutes a heterogenous family of cells in the central nervous system. Astrocytes subjected in vivo to such pathological factor as hypoxic injury exhibit morphological abnormalites. Similar effects are observed by culturing astrocytes in the presence of dibutyryl-cAMP (dBcAMP), which transforms elongated cell into stellate ones, with numerous long processes. This process is accompanied by substantial biochemical changes (e.g. decreased content of glycogen and changes in the levels of ATP and PCr) (1). There are no reports with regard to of morphology of glial cells treated with nootropics.

The aim of the study was to evaluate the effects of nootropics on the following morphological parameters of astrocytes: area, perimeter and form factor.

Astrocytes were isolated and cultured by method of Hertz et al.(2). 14-day-old cells were cultured with or without dBcAMP (0.25 mM) and treated with nootropics at $1x10^{-7}$M final concentration. The following drugs were examined: piracetam (PIR), oxiracetam (OXI) and nebracetam (NEB). Morphometric parameters were determined in 28-day-old cultures stained with 1% toluidine blue.The area, perimeter and form factor were established in 80 single cells with the image analysis system VIDS IV.

Analysis has shown that the effects of nootropics on the morphometric parameters of astrocytes are variable. In the cell cultures grown without dBcAMP the most marked effects were induced by OXI which caused a considerable increase in the area (by 245%) and perimeter of astrocytes (by 33%) and decreased their form factor (by 11%). The area of astrocytes decreased upon treatment with all the nootropics in the presence dBcAMP. The above-mentioned alternations may be attributable to the action of nootropics on the phosphorylation or synthesis of cytoskeletal proteins.

References : (1) Fedoroff (1987) Neuroscience **22**:255-66; *(2) Hertz (1985) Handbook of Neurochemistry Ed. Lajtha.*

INFLUENCE OF NOOTROPIC DRUGS ON CONTENT OF HIGH-ENERGY PHOSPHATES OF IN VITRO CULTURED ASTROCYTES.

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Nootropics are thought to directly infuence energetic processes in brain cells and therefore they are supposed to improve memory, cognition and orientation by more efficient utilization of cellular energy stores (1).

The aim of the study was to evaluate the effects of nootropics on the intracellular levels of adenosine triphosphate (ATP) and phosphocreatine (PCr) in astrocytes cultured with or without dibutyryl-cAMP (dBcAMP). DBcAMP added to cell culture medium induces substantial metabolic and biochemical changes (e.g. ion-channel and cell surface receptors expression, enzyme activities).

Astrocytes were isolated and seeded by method of Hertz et al. (2). 14-day-old cells were grown with or without dBcAMP (0,25 mM) and treated with nootropics at 1×10^{-7} M final concentration. The following drugs were used: piracetam (PIR), oxiracetam (OXI) and nebracetam (NEB). In 28-day-old cultures ATP and PCr were extracted with 0,4 M $HClO_4$ dissolved in 70% (v/v) ethanol according to method of Lowry and Passonneau (3). ATP concentration was measured by bioluminescence assay according to the method of Sonderhoff et al.(4).

Astrocytes treated with either PIR or NEB exhibited decreased intracellular ATP and PCr levels by 37% and 21%, respectively. Of all the nootropics studied only OXI increased the content of ATP (by 26%), while it did not change the intracellular content of PCr.

In dBcAMP-treated astrocytes only OXI increased ATP concentration (by 104%) and only NEB increased PCr concentration (by 63%).

The results indicate involvement of nootropic drugs in energetic metabolism pathways.

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STRUCTURAL CHARACTERISATION OF DSD-1-PROTEOGLYCAN.

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DSD-1-proteoglycan is a secreted protein which was first isolated from the PBS soluble homogenate of postnatal mouse brain (P7-14) using an affinity chromatography column to which was coupled the 473HD monoclonal antibody, which specifically recognises a chondroitin sulphate epitope on the GAG chains. Digestion of the DSD-1-proteoglycan with chondroitinase ABC reduces the apparent molecular weight of the molecule from greater than 500kD to around 400kD. A polyclonal antibody, KAF13(5), has been raised against this core glycoprotein and has been used to screen several mouse brain cDNA phage expression libraries, resulting to date in the isolation of a number of clones coding for both known and apparently new proteins. Meanwhile further improvements in the purification protocol have resulted in higher yields of the DSD-1-proteoglycan, revealing an additional 250kD core glycoprotein following chondroitinase ABC digestion. More extensive biochemical analysis has also shown that there is additional O- and N-linked glycosylation of the core which can be removed with keratanase, hyaluronidases, and N-glycosidase F. We are currently seeking to obtain peptide sequence data from the resulting protein.
Co-purifying with the DSD-1-proteoglycan following affinity chromatography are a number of known extracellular matrix proteins as identified by Western blot analysis, including F3, appican, and tenascin-C, suggesting the possibility of binding interactions with these proteins.
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HSV-TK/GANCICLOVIR SYSTEM IS CYTOTOXIC FOR PROLIFERATING ASTROCYTES IN PRIMARY CULTURE.

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Astrocytic proliferation is one element of CNS post-traumatic gliosis, which constitutes an obstacle to axonal regeneration. In order to overcome this obstacle, we have used an adenoviral vector (ADV/RSV) to evaluate the ability of herpes simplex virus thymidine kinase (HSV-TK) gene coupled with the prodrug ganciclovir (GCV) to reduce the proliferation of cultured primary astrocytes, taken as a model of reactive gliosis.
Primary cultures of astrocytes were obtained from the cerebral cortex of 2-days postnatal rats. After mechanical dissociation a cell suspension was obtained and adjusted to 10^6 cells/ml. The Bromodeoxyuridine (BrdU) labeling index, measured by double immunocytochemical detection of BrdU/GFAP, was used to determine the time in culture at which the peak percentage of phase S-cells was achieved. Transfection efficiency of adenovirus was demonstrated by using ADV/RSV-βgal.
A peak percentage of BrdU+ cells is reached at 6 days in vitro and an optimal transfection efficiency at a multiplicity of infection (MOI) =16. One day after transfection with ADV/RSV-TK at MOI values of 8 or 16, the cultures were treated with either culture medium or GCV (10 or 50 µg/ml). Two or four days later, the surviving cells were counted. The maximum effect (88% of death) was obtained for a GCV concentration of 10 µg/ml and a survival of 4 days.
In conclusion, the HSV-TK/GCV system may constitute an efficient tool to investigate the influence of the elimination of proliferating cells on the regrowth of severed axons following traumatic injury of the CNS in vivo.

The ADV/RSV-TK and -βgal were kindly provided by Rhône-Poulenc Rorer (RPR).
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Functional Analysis of the Immune Competence of Astrocytes and Microglia

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Astrocytes and microglia possess the capacities to function as antigen presenting cells in the brain. Several lines of evidence from *in vitro* studies point to the key role of these cells during brain injury and repair.
We analyse the immune competence of these cells by investigating the putative antigenic peptide repertoire that these cells can generate from a given antigen. For this purpose we use apamin, a neurotoxin of the bee venom, and a panel of apamin-specific MHC class II restricted T cell lines which show different fine specificities for apamin recognition. Experiments are carried out on primary glial cultures from BalbC mice. After activation by cytokines, astrocytes and microglia are presented to apamin-specific T cells with the antigen or analogs of the antigen. The T cell response to the antigenic stimulation is measured by the quantitation of IL-2 production in a CTL-L assay. The analysis of this peptidic repertoire is compared to the ones of a B lymphocyte cell line and a macrophage cell line.
We want to further investigate the kinetics of antigenic processing and presentation and to characterize the enzymes involved in the processing of apamin in astrocytes and microglia.

3-D Reconstruction of dye-injected murine Bergmann glial cell processes from electron microscopical serial sections

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In cerebellar embryogenesis, the radially aligned Bergmann glia cell processes play an important role as guidelines for the migration of young granule cells away from the external granular layer. During this period, the glial cell processes are rather thin and smooth, with the very exception of some mitochondria that cause local bulges. This figure changes dramatically after cessation of the neuronal migration. Then, the glial stem processes become thicker, and a massive outgrow of lateral branches is observed. Previous two-dimensional light- and electron microscopical observations have caused the impression that these glial appendages are irregular, random-shaped structures.
To study the architecture of these apendages in greater detail, Lucifer Yellow was injected into Bergmann glial cells from murine cerebellar slices. Thereafter, the dye was converted, and the tissue was prepared for transmission electron microscopy. Serial ultrathin sections were used for 3-D reconstruction of a part of one cell, and, particularly, in 450 consecutive sections of one radial cell process and its appendages.
It was found that processes of cells from young adult cerebella are covered with two types of outgrowths, short simple thorns and longer complex appendages. The latter consist of singular or repetitive units, formed by a stalk and a cabbage-like head. These 'head' elements have an extremely large surface-to-volume ratio (up to more than 20 μm^{-1}), contain one or several mitochondria, and form complex sheaths around (groups of) synapses.
The surface membranes of these excrescenses constitute the principal sites of glial-neuronal interaction in the cerebellar molecular layer. This peculiar geometry may have significant functional consequences for cerebellar information processing.

NIMODIPINE PROLONGS THE GFAP EXPRESSION AND ENHANCES THE ASTROGLIAL ENSHEATHMENT OF CRANIAL MOTONEURONS FOLLOWING PERMANENT TARGET DEPRIVATION IN THE RAT

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After axotomy astrocytes first upregulate the GFAP synthesis. Later, the astrocytes form thin, GFAP-free lamellar processes which cover the surface of the perikarya of lesioned neurons. These processes are thought to have a neuroprotective function[1]. We studied the effect of the Ca^{2+} channel blocker nimodipine on the GFAP immunoreactivity and the lamellar formation of astrocytes after permant target deprivation.

Unilateral resection of facial and hypoglossal nerve was performed in 108 female adult Wistar rats. Half of the animals was fed with standard food (placebo), half with food pellets containing 1000 ppm nimodipine. Using immunocytochemistry the GFAP expression of astrocytes and by electron microscopy the number of thin cytoplasmic lamellae of astrocytes ensheathing perikarya of lesioned motoneurons was compared 7 to 112 days post operation (dpo).

No differences in GFAP reaction were observed up to 28 dpo. At 42 to 56 dpo a stronger GFAP reaction was obvious in the nimodipine than in the placebo group. At 112 dpo GFAP positive astrocytes were found only in nimodipine-treated animals. In both groups astrocytes covered neuronal surfaces with thin lamellar processes at 28 dpo: In the nimodipine group, facial and hypoglossal neurons were covered by 2.9 ± 0.2 (mean ± SD) respectively 2.6 ± 0.3 processes. In the placebo group the number of astrocytic processes was significantly lower with 1.7 ± 0.3 processes in the facial and with 1.7 ± 0.2 in the hypoglossal nucleus. Whereas nimodipine maintained the higher number of lamellar sheets covering the neurons at 56 and 112 dpo, the number decreased significantly in the placebo group.

Thus, nimodipine *in vivo* enhances lamellar formation and may facilitate the protective role of activated astrocytes on surviving motoneurons after permanent target deprivation.

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GLIAL PATTERNING DURING POSTEMBRYONIC DEVELOPMENT OF CENTRAL NEUROPILES IN THE BRAIN OF THE HONEYBEE

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Glial cells are involved in several functions during the development of the nervous system. The anatomical organization of insect glial cells has been reconstructed from ethyl gallate stained serial sections [1]. Histochemical stainings for lectin binding suggested that a glial derived extracellular matrix is elaborated during pupal development of the insect brain [2]. To understand potential glial contributions to neuropile formation we examined the cellular pattern of glia during the development of the mushroom body, antennal lobe, and central complex in the brain of the honeybee. Using an antibody against the glial specific repo-protein of *Drosophila* the location of the glial somata was detected in the larval and pupal brain of the bee. In the early larva, a continuous layer of glial cell bodies defines the boundaries of all growing neuropiles. Initially, the neuropiles develop in the absence of any intrinsic glial somata. In a secondary process, glia cells migrate into defined locations of the neuropiles. The corresponding increase in the number of the neuropile associated glial cells is most likely due to massive immigrations of glial cells from the cell body rind using neuronal fibers as guidance cues. The combined data from the three brain regions suggest that glial cells can prepattern the neuropilar boundaries.

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EXPERIMENTAL MODIFICATION OF PERINEURONAL NETS IN RAT BRAIN

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In previous lectin-histochemical and immunocytochemical investigations using fixed tissue perineuronal nets (PNs) were revealed as lattice-like accumulations of extracellular matrix proteoglycans at the surface of several types of neurons[1,2]. The associated matrix components were shown to be sensitive to enzymatic treatments[3]. In the first part of the present study, PNs in the rat brain were labelled *in vivo* by stereotaxic injections of biotinylated *Wisteria floribunda* agglutinin (Bio-WFA). Six days after Bio-WFA injections into the parietal cortex, medial septum, reticular thalamic nucleus and red nucleus, the lectin remaining bound to PNs was detected by streptavidin/biotinylated peroxidase complexes or red fluorescent Cy3-streptavidin, respectively. Double-fluorescence staining showed that Bio-WFA applied *in vivo* reacted with the chondroitin sulphate proteoglycan (CSPG) immunoreactive PNs in the injection zone. The *in vivo* labelling confirmed the region-dependent distribution patterns, chemical heterogeneity and structural characteristics of PNs known from histochemical studies. In parallel experiments, the associated extracellular matrix components were degraded by intracerebral injection of chondroitinase ABC resulting in spatially limited, but long-lasting changes. Differently altered staining patterns were shown e.g. by Bio-WFA lectin cytochemistry and CSPG immunocytochemistry.

In vivo attachment and degradation experiments may be used for the functional characterization of PNs as variable components at the neuron-glia interface.

References: (1) Härtig et al. (1994) *Brain Res* **635**, 307-311; (2) Brückner et al. (1993) *Glia* **8**, 183-200; (3) Köppe et al. (1997) *Histochem J* **29**, 11-20.

ASTROCYTES EXPRESS ISOFORMS OF AGRIN LACKING THE INSERTS AT THE Y- AND Z- SPLICING SITE.

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The heparan sulphate proteoglycan agrin was first isolated from the electric organ of Torpedo. It became interesting because it promoted the clustering of acetylcholine receptors at the neuromuscular junction. A similar function for agrin is proposed for the CNS where it is expressed in many types of neurons. Recent experiments give evidence for a non-neuronal expression of agrin in the CNS.

The agrin gene codes for the full-length agrin protein and for several isoforms resulting from alternative splicing of the mRNA. Four different splicing sites which modify the protein function have been identified. Alternative splicing at the C-terminal end of the molecule influences the acetylcholine receptor clustering activity.The binding activity for dystroglycan and heparin might also be regulated by alternative splicing. The analysis of the functions of the different splicing products is, however, presently at an early stage.

We analysed the expression of agrin and its splicing variants in astrocytes. In order to do this we applied immunohistochemistry and polymerase chain reaction (PCR) techniques to brains of adult rats and primary astrocytic cell cultures. In addition we studied changes in astrocytic agrin production after CNS lesion by analyzing sections of the facial nucleus seven days after transection of the facial nerve.

Antibodies raised against agrin labelled both basement membranes and brain capillaries in the adult brain. Weak labelling of the *glia limitans* was observed. The antibodies failed to stain astrocytes in other brain areas. In contrast, after facial nerve axotomy, labelled astrocytes were observed in the facial nucleus. Astrocytes in primary cell cultures of rat brain showed a positive immunoreaction for antibodies raised against the agrin protein. PCR analysis of whole rat brain cDNA revealed that all splicing variants described so far for the x-, y- and z-splicing site are present. We could not identify the N-terminal splicing variants recently described in chicken. In contrast, astrocytes in primary cell culture expressed only the short isoforms of agrin, lacking the inserts of the y and z-splicing site. For the x-splicing site variants with and without the inserts were found.

Our observations show that detectable levels of agrin production by astrocytes *in vivo* are restricted to situations of brain lesion. Antibodies raised against agrin may therefore serve as a marker for lesion induced activation of astrocytes *in vivo*. Agrin may be involved in the rearrangement of receptor systems or in the reassembly of the extracellular matrix after brain lesion. Agrin may also serve as a binding partner for extracellular matrix molecules such as laminin, secreted by neurons in lesioned brain regions.

INTERLEUKIN-15 AND THE COMPONENTS OF MULTIMERIC IL-15/IL-2 RECEPTORS ARE EXPRESSED IN MOUSE BRAIN AND BY MOUSE MICROGLIA IN CULTURE

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The novel cytokine, interleukin-15 (IL-15), and the T cell growth factor, IL-2, exhibit a similar spectrum of immune effects and share the IL-2 receptor (IL-2R) subunits IL-2Rβ and IL-2Rγ for signaling in hematopoietic cells. Experimental and clinical observations additionally suggest numerous potent neuroregulatory activities for IL-2, although its expression in the CNS is apparently very low and regionally restricted. In the present study, we show by RNA and protein detection that IL-15, its specific receptor molecule, IL-15Rα, and the IL-2/IL-15 signal-transducing receptor subunits, IL-2Rβ and IL-2Rγ, are constitutively present in various regions of the developing and adult mouse brain. We further demonstrate — also at the single-cell level — that IL-15 and IL-15Rα/IL-2Rβγ receptors are expressed by microglia. Furthermore, stimulation of microglial cells with IL-15 is shown to result in Tyr phosphorylation of the Janus kinase, JAK1, suggesting the activation of a JAK/STAT pathway in these cells. Using various assays, e.g., based on the detection of LDH activity and nitrite in culture supernatants, the incorporation of BrdU in cultured cells or the MTT cell viability test, IL-15 in the dose range of 0.1 to 10 ng/ml is also shown to support the survival of microglial cells in culture, to stimulate their proliferation and to modulate microglial NO production, as it can be induced by lipopolysaccharide. Taken together, IL-15 may thus represent an autocrine survival/growth factor of microglia. Moreover, the brain-intrinsic IL-15 could potentially serve several CNS functions that have previously been ascribed to IL-2. Considering that virtually any disturbance in CNS homeostasis leads to microglial activation and that IL-15 is a T cell chemoattractant, microglial IL-15 could crucially participate in mechanisms of CNS inflammation and processes following brain tissue injury. Moreover, the constitutive expression of IL-2/IL-15 receptors in neural cells is relevant to the use of these cytokines in the immunotherapies of cancer and especially the treatment of brain tumors.

PROLIFERATION OF HUMAN GLIOMA CELLS IS INHIBITED BY LONG-TERM DEPOLARIZATION

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Gliomas are the most common of primary brain tumors and account for more than 40 % of all CNS neoplasms. They are classified either according to the presumed cell of origin as astrocytomas or oligodendrogliomas or according to their grade of malignancy which increases from I to IV.

Patch-clamp investigations were performed on acutely prepared slices as well as on primary cultures of human glioma material obtained from neurosurgery. In nearly 40% of the tumor cells we observed TTX-sensitive fast inward currents indicating the presence of voltage-gated sodium channels. Their density was sufficient to generate action potentials in response to depolarizing current pulses. Large sodium currents are present in similar incidence in cells from different glioma types independent of their malignancy grade.[1] Increase of sodium channel expression therefore seems to be involved at a rather early stage of neoplastic transformation since normal glial cells have few if any sodium channels.

In order to clarify the role of upregulated sodium channel expression for unlimited growth typical for malignant tumors we measured the influence of sodium channel ligands on glioma cell proliferation by means of bromo-deoxy-uridine incorporation. It was inhibited in the presence of the channel activator veratridine (VTN) whereas TTX slightly increased the proliferation of glioma cells. The proportion of cells in the S-phase of the cell cycle compared to control conditions was 80 % with veratridine and 108 % with TTX. Incubation of glioma cultures with 50 mM potassium in the culture medium had the same inhibitory effect on cell proliferation. Under these conditions cells are depolarized, as we proved in current clamp measurements.

These data suggest that long-term activation of sodium channels could inhibit glioma cell proliferation probably due to a depolarization-linked mechanism.

Reference: (1) Labrakakis, C. et al. (1997) *J. Neuropathol. Exp. Neurol.* **56**: 243-254

INHIBITORY EFFECT OF INTERFERON GAMMA ON THE ASTROCYTE PROLIFERATION IN RAT BRAIN INJURED AT THE POSTMITOTIC STAGE OF POSTNATAL DEVELOPMENT

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Effects of interferon gamma (IFNγ) on astrocytes depend not only on the presence of other factors but also on the species from which the astrocytes are derived. Therefore, results of *in vivo* action of this cytokine cannot be predicted on the basis of *in vitro* studies. For the above reasons the present study examines the influence of IFNγ on the astrocyte proliferation in the injured rat brain.

Thirty-day-old male rats received a mechanical lesion in the left cerebral hemisphere which was followed by immediate injection of 1μl of recombinant murine interferon gamma (IFNγ) into the lesion site. IFNγ was administered at three concentrations: 5, 50 or 500 U/μl. One or 2 days after the injury the rats were injected with ^{3}H-thymidine and sacrificed 4 hours after the injection. Thereafter, brain sections were immunostained for glial fibrillary acidic protein (GFAP), subjected to autoradiography and examined microscopically to record numbers of GFAP-immunopositive astrocytes labeled with ^{3}H-thymidine. In the IFNγ-injected rats, a statistically significant decrease in the intensity of reactive astrocyte proliferation was revealed on day 2 following injury. The effect, however, did not vary in a dose-dependent manner. Relations between the astrocyte reactivity and the developmental stage when the brain was injured and IFNγ-injected are discussed. The results represent the first *in vivo* evidence of IFNγ influence on the astrocyte proliferation in response to injury.

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Population Control of Microglia: Does Apoptosis play a Role?

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Brain lesions, even of the most subtle type, are accompanied by the activation of microglia, the main immune cells of the brain. Microglial cells dramatically increase in number through proliferation and adhere to the injured neurons, where they displace the synaptic input. After proliferation, microglia gradually migrate into the nearby parenchyma and appear to decrease in number.

Here we examined the possible involvement of apoptosis in the regulation of the microglial cell number using Terminal transferase mediated d-UTP Nick End-Labelling (TUNEL). *In vitro*, cell death is a common phenomenon in microglial cell cultures, which was enhanced by the withdrawal of the mitogen, granulocyte-macrophage colony stimulating factor. *In vivo*, application of the TUNEL-reaction revealed TUNEL-positive microglia beginning at day 4, with a peak 7 days after transection of the facial nerve. Surprisingly, TUNEL-labelling *in vivo* was localized on the outer side of the nuclear membrane and in the microglial cytoplasm, with very little staining within the nucleus itself. These TUNEL-labelled cells also lacked other classic morphological signs of apoptosis, like membrane blebbing, chromatin condensation and apoptotic bodies.

In summary, massive microglial proliferation after CNS injury is followed by the appearance of TUNEL-positive microglia at later stages after injury. Unlike the *in vitro* situation, these labelled microglia lack the classic signs of apoptosis *in vivo*. These data suggest that the regulation of post-mitotic microglia is not mediated by the classic pathways of apoptosis.

ENDOTHELIN INDUCED CALCIUM SIGNALING IN CULTURED MOUSE MICROGLIAL CELLS

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Endothelins (ETs) are a family of three related peptides which were originally isolated from cultured porcine aortic endothelial cells. They are known as very potent vasoconstrictors. In the present study ET isoforms triggered transient fluctuations of the intracellular calcium concentration ($[Ca^{2+}]_i$) in cultured mouse microglial cells. $[Ca^{2+}]_i$ was monitored by using fluo-3 based calcium imaging. In more detail we determined the pharmacological properties of the endothelin receptor. In 13 % of the cells both, endothelin-1 and endothelin-3 in a 100 nM concentration, caused intracellular calcium signals. These $[Ca^{2+}]_i$ transients could also be triggered in Ca^{2+}-free solution, but were reduced in duration. They were mimicked by BQ 3020, an ET_B receptor agonist, and blocked by the selective ET_B receptor antagonist BQ 788, respectively. Our microfluorimetric $[Ca^{2+}]_i$ recordings indicating an expression of a functional ET_B receptor were substantiated by employment of single-cell RT-PCR. With this technique transcripts encoding the ET_B receptor were detected in 9 % of electrophysiological characterised mouse microglial cells. We conclude that cultured mouse microglial cells express the ET_B subtyp of endothelin receptors which might play a role in the activation of microglial cells under pathological conditions.

Serotonin - a radical scavenger during the respiratory burst of activated microglia in vitro

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Activated microglia and macrophages invade sites of CNS trauma soon after injury. They produce large amounts of reactive oxygen species (ROS), which have been attributed a causative role in brain edema and ischemia-induced secondary nerve cell damage. Activated microglia has also been shown to be present and suggested to play a role in neurodegenerative disorders such as Alzheimer's, Parkinson's and Pick's disease, amyotrophic lateral sclerosis, and multiple sclerosis. Little is presently known about the mechanisms involved in the modulation of microglia-derived ROS-release and ROS-mediated tissue damage in the brain.

From activated monocytes and granulocytes it is known that serotonin (5-HT) is oxidized and acts, thus, as a scavenger of ROS during the respiratory burst. One of the oxidation products, the 5-HT dimer 5,5'-dihydroxy-4,4'-bitryptamine (DHBT), is also formed during the incubation of 5-HT in the presence of H_2O_2 and peroxidase, and was suggested to be a potential neurotoxin.

In the present study the release of ROS during the respiratory burst of cultured microglial cells from newborn rat cortex was measured by luminol enhanced chemiluminescence (CL) in the absence and in the presence of serotonin. Both amplitude and integral of the CL signal produced after stimulation of microglial respiratory burst activity by addition of phorbol myristate acetate were dose-dependently suppressed by 5-HT. During this process 5-HT is oxidized to DHBT. The assumed neurotoxic potential of DHBT, however, could neither be confirmed by in vivo nor by in vitro studies.

It is concluded that 5-HT locally released from thrombocytes and 5-HT nerve endings at sites of brain damage and inflammation may contribute to attenuate secondary tissue damage in the CNS via scavening of ROS produced during the respiratory burst of activated microglia.

NOS lacks in rat oligodendrocytes and Schwann cells

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Nitric oxide (NO) has been revealed to act as an inter-/intracellular messenger molecule in both the central and peripheral nervous system of mammals. NO is generated enzymatically from L-arginine by molecular isoforms of NO synthase (NOS): in neurons by the constitutively expressed NOS I and in microglial cells by an inducible form, NOS II. Astroglial cells were discussed mainly as potential reservoir of L-arginine, but they display both NOS I and, following activation, NOS III activity. The NO-producing capacity of oligodendrocytes is not yet known. More attention was paid on oligodendroglia as a highly sensitive NO target. NO or NO-releasing chemicals induce necrotic death of oligodendrocytes, and reactive oxygen species have been suggested to be a „myelin-killer" by lipid peroxidation. Consequently, a key role of NO in the pathogenesis of inflammatory demyelinating disorders was postulated.
In the present study we carried out a detailed characterization of rat oligodendrocytes and Schwann cells in situ and in vitro by means of NOS immunocytochemistry, NADPH-diaphorase (NADPH-d) histochemistry and in situ hybridization. A highly sensitive electron microscopic NADPH-d staining, basing on the production of osmiophilic formazan by the NADPH-d catalyzed reduction of BSPT, was used to demonstrate even traces of NADPH-d.
NADPH-d activity is delicately expressed in the myelinating cells in vivo and in vitro. Only the highly sensitive electron microscopic technique revealed traces of NADPH-d at membranes of the nuclear envelope and the endoplasmic reticulum. Sometimes NOS immunolabeling was found in the perikaryal cytoplasm up to the processes.By in situ hybridization S^{35}-labeled cRNA probe of NOS I was detected in some neurons and astrocytes, but has never been seen in oligodendrocytes.
The failure of NOS immunostaining emphasized the probability that this NADPH-d activity is not due to NOS but rather to some other NADPH-dependent enzymes. The absence of NOS is discussed as a weak point of myelin producing cells in the defense line against pathogenic free radicals that may be scavenged by NO to form less reactive compounds.

Laminin and Nidogen in the CNS: Expression by Astrocytes

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Laminins are glycoproteins of the extracellular matrix (ECM), composed of three different chains (α, β, γ) forming a cruciform structure. Presently ten different chains (5 α-, 3 β- and 2 γ-chains) coding for eleven laminin isoforms are known. Laminin is a major component of basement membranes were it forms tight complexes with the 150 kD glycoprotein nidogen (entactin).

Applying immunohistochemistry techniques we have shown that neurons and astrocytes express laminin in the CNS. Antibodies directed against the γ1-chain and native laminin-1 revealed that after transection of the facial nerve laminin is upregulated on astrocytes in the facial motor nucleus. We applied polymerase chain reaction (PCR) techniques to identify laminin isoforms expressed by astrocytes. With specific primers for the five α-chains we detected α1-3 and α 5, but not α 4 in cDNA from astrocytic cultures. Using the same techniques we could demonstrate that astrocytes in primary culture also express nidogen.

Our results suggest that astrocytes express multiple isoforms of laminin. Laminin and nidogen might be secreted into the extracellular space to form there a laminin-nidogen complex as part of the ECM.

EXPRESSION OF *TRP* STORE-OPERATED CHANNELS IN MOUSE GLIAL CELLS

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Glial cells respond to a broad variety of stimuli by generating intracellular calcium signals. $[Ca^{2+}]_i$ responses display complex kinetics, being often comprized of an initial peak response followed by a long-lasting plateau phase or even oscillations of $[Ca^{2+}]_i$. The common pathway for Ca^{2+} signalling in glial cells is associated with Ca^{2+} release from intracellular stores via $InsP_3$-gated channels. In a variety of non-excitable cells, this Ca^{2+} release is followed by a secondary Ca^{2+} influx from the extracellular space. The Ca^{2+} influx is triggered by a so far unknown factor produced by the depletion of Ca^{2+} stores. Recent experiments demonstrated that this depletion activates a specific set of plasmalemmal Ca^{2+} permeable channels of the *TRP* family. Ca^{2+} influx through these channels delivers Ca^{2+} for the replenishment of Ca^{2+} pools, thereby shaping Ca^{2+} signals during the long-lasting plateau outlasting the period of agonist action.

We observed the Ca^{2+} influx following the depletion of Ca^{2+} stores in cultured microglial cells, cultured cortical astrocytes and in Bergmann glial cells in cerebellar slices. To investigate the molecular identity of *TRP* channels in glial cells, we employed the RT-PCR technique to cDNAs prepared from purified microglial and astrocyte cultures. Using subtype-specific primers, cDNA fragments corresponding to the 6 subtypes of mouse *TRP* genes, *mTRP*1-6, could be amplified. Transcript expression of *mTRP*3 and 5 could be detected in astrocytes, whereas *mTRP*3, 5, and 6 were found in microglia.

Based on our observations, we conclude that glial cells from CNS can express several members of the *TRP* family which can serve as a substrate for the generation of store-operated Ca^{2+} influx.

GLUTAMATE TRANSPORTER-GENERATED CURRENTS IN CEREBELLAR BERGMANN GLIAL CELLS IN SITU

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The effects of glutamate and kainate on Bergmann glial cells were investigated in mice cerebellar slices using the whole-cell configuration of patch-clamp technique combined with SBFI-based Na^+ microfluorimetry. Kainate (KA) and L-glutamate induced an inward current in Bergmann glial cells voltage-clamped at -70 mV. The currents were accompanied by an increase in $[Na^+]_i$ from the average resting level of 5.2 mM to 33 and 26 mM, respectively. Kainate-evoked signals were completely blocked in the presence CNQX. On the contrary, CNQX had minor influence on both glutamate-induced current and $[Na^+]_i$ elevation (10-15% inhibition). In standard external solution, the amplitude of both KA- and L-glutamate-induced currents were a linear function of the membrane potential. The KA-induced current showed a reversal potential around 0 mV, whereas L-glutamate-activated response did not reverse even when the neurotransmitter was applied to a cell voltage-clamped at +50 mV. Removal of Na^+ from extracellular medium suppressed currents evoked by both substances. In the case of L-glutamate this inhibition was not dependent on the substitute used - N-methyl-D-glucamine or Li^+, whereas KA-induced current remained almost unaffected in Na^+-free, Li^+-containing solution. Insensitivity to CNQX, reversal potential more positive than +50 mV and blocking effect of Li^+ support the idea that an activation of a Na^+-dependent glutamate transport rather than ionotropic receptors underlied the measured current. To substantiate this suggestion, D,L-threo-3-hydroxyaspartate (THA), dihydrokainate (DHK) and L-α-aminoadipiate (L-AA), the blockers of L-glutamate uptake, were tested. At the concentrations up to 1 mM they did not dramatically affect the L-glutamate-induced current. Moreover, two of them, THA and L-AA, evoked also an inward current presumably via activating the same mechanism. These results show that L-glutamate effects are mainly mediated by a Na^+-dependent glutamate transport. The latter may be involved in the regulation of synaptic transmission in the central nervous system.

SODIUM-CALCIUM EXCHANGER AMPLIFIES KAINATE-INDUCED CALCIUM SIGNALS IN CEREBELLAR BERGMANN GLIAL CELLS

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The role of sodium-calcium exchanger in calcium homeostasis of Bergmann glial cells *in situ* was investigated by monitoring cytoplasmic calcium ($[Ca^{2+}]_i$) and sodium ($[Na^+]_i$) concentration. The $[Ca^{2+}]_i$ or $[Na^+]_i$ transients were measured either separately by using fluorescent indicators fura-2 or SBFI, or simultaneously using the indicators fluo-3 and SBFI. Substitution of extracellular Na^+ with Li^+ or NMDG induced only a small elevation of resting $[Ca^{2+}]_i$. In contrast, lowering the extracellular Na^+ concentration significantly suppressed $[Ca^{2+}]_i$ increase triggered by kainate. The mean amplitude of $[Ca^{2+}]_i$ elevation was reduced from 355 nM under control conditions to 136 nM in Na^+-deficient media. In parallel with $[Ca^{2+}]_i$ rise, kainate triggered significant elevation in $[Na^+]_i$. The 10 second application of kainate raised $[Na^+]_i$ from 5.2 mM to 33 mM, when the cells were voltage-clamped at -70 mV. The $[Na^+]_i$ increase was blocked by CNQX, or by removal of extracellular Na^+, pointing out that the $[Na^+]_i$ elevation resulted from the Na^+ influx through the receptors. This increase in $[Na^+]_i$ is presumably capable to turn the Na^+/Ca^{2+} exchanger in the reverse mode providing thus additional Ca^{2+} influx pathway. In Na^+-deficient external solution this Ca^{2+} influx component does not occur, which results in smaller $[Ca^{2+}]_i$ elevation. When extracellular Na^+ was removed immediately after kainate application $[Ca^{2+}]_i$ decayed towards the resting level much slower, indicating that Na^+/Ca^{2+} exchanger regulates the recovery phase of Ca^{2+} signals. Our results support the hypothesis that transmembrane Na^+ gradient controls the amplitude and the shape of $[Ca^{2+}]_i$ signals associated with activation of ionotropic glutamate receptors. The Ca^{2+} transport through Na^+/Ca^{2+} exchanger contribute only modestly in regulation of resting $[Ca^{2+}]_i$, however, under conditions of $[Na^+]_i$ overload this mechanism could provide a significant Ca^{2+} influx. These results imply that Na^+-Ca^{2+} exchanger is an important component of neurotransmitter-induced $[Ca^{2+}]_i$ signalling in Bergmann glial cells.

BERGMANN GLIAL CELLS IN SITU EXPRESS MGLUR1 AND MGLUR5 METABOTROPIC GLUTAMATE RECEPTORS

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Bergmann glial cells form close contacts with Purkinje neurones by creating complex membrane structures which ensheathe synapses at the dendrites of Purkinje neurones. Glutamate, released from the parallel and climbing fiber synapses, might affect Bergmann glial via activation of specific receptors. Previous experiments demonstrated that Bergmann glial cells in mouse cerebellar slices express high Ca^{2+}-permeable AMPA/kainate receptors which while being activated by specific ligand, kainate, can generate large Ca^{2+} signals in these cells. In the present study we used the same preparation to investigate the Ca^{2+} signals in Bergmann glial cells triggered by physiological ligand, glutamate itself. We found that neither removal of Ca^{2+} from extracellular solution, nor incubation of cerebellar slices with CNQX, a selective inhibitor of AMPA/kainate receptor, significantly affected the glutamate-induced $[Ca^{2+}]_i$ transinets. In contrast, kainate-triggered $[Ca^{2+}]_i$ elevation was completely blocked by both Ca^{2+} removal from the extracellular milieu and by CNQX. Thus, we conclude that kainate triggered $[Ca^{2+}]_i$ transients in the Bergmann glial cells exclusively due to the activation of Ca^{2+} influx, which can be prevented by either pharmacological blockade or by Ca^{2+} removal from the external solutions. However, Ca^{2+} influx accounts for only a minor part of the glutamate-triggered $[Ca^{2+}]_i$ response. The latter is mostly independent of the extracellular Ca^{2+} and is barely sensitive to CNQX, suggesting a major role of Ca^{2+} release from internal stores, activated presumably via metabotropic GluR/$InsP_3$ pathways. To substantiate this conclusion, we tested for the ability of pharmacological agents, known to interact specifically with intracellular Ca^{2+} stores, to interfere with glutamate-driven Ca^{2+} signals. We found that they were inhibited by slice incubation with thapsigargin or by intracellular perfusion with heparin. Single-cell RT-PCR analysis revealed that Bergmann glial cells express both mGluR1 and mGluR5 specific mRNA. It can be concluded that in Bergmann glia the mGluR1 and/or mGluR5 mediated Ca^{2+} release from the $InsP_3$-sensitive intracellular stores plays the major role in glutamate-induced Ca^{2+} signals, despite the fact that these glial cells possess Ca^{2+} permeable AMPA/KA receptors.

Proliferation Of Ramified Microglia On An Astrocyte Monolayer Characterization Of Stimulatory And Inhibitory Cytokines

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Proliferation of microglia is a common phenomenon in brain pathology. *In vitro* studies have identified a growing number of mitogens for microglia-derived macrophages. However, most of these studies were performed on rounded macrophages, which show numerous differences to ramified microglia, the main proliferating, monocyte-related cell in the developing or lesioned central nervous system. In the current study, we circumvent this problem by examining the effect of different cytokines on the proliferation of cultured ramified microglia.

Ramified microglia were obtained using a 10-day co-culture on top of a confluent astrocyte monolayer. The addition of the classical macrophage-mitogens, macrophage colony-stimulating factor (MCSF), granulocyte-macrophage colony-stimulating factor (GMCSF) and interleukin-3 (IL3), strongly stimulated the proliferation of ramified microglia. Of all the other cytokines tested (IL1, IL2, IL4, IL6, IL10, IFNγ, LIF and TNFα) only IL1 strongly enhanced proliferation. This effect, however, was indirect and neutralized by antibodies against GMCSF and MCSF. IL2, IL4, IL10, TNFα and IFNγ inhibited microglial proliferation. IL6 and LIF had a slight, but non-significant, stimulatory influence.

In summary, the current study describes the stimulatory and/or inhibitory effects of various cytokines on ramified microglia in culture. Surprisingly, only the classical macrophage-mitogens (MCSF, GMCSF, IL3) promoted proliferation directly. The redundancy of inhibitory cytokines underlines the importance of inhibition of microglial proliferation in vivo.

Involvement of small G Proteins in Phospholipase D Activation in Astrocytes

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Phospholipase D (PLD) catalyzes the hydrolysis of phosphatidyl-choline to yield phosphatidic acid, a lipid second messenger, and choline. The enzymatic activity of PLD is regulated by a variety of extracellular signals acting via G-protein coupled receptors, tyrosine kinases, or protein kinase C (PKC). Recent results have identified small G proteins of the Ras superfamily as potent PLD activators. In the present study, we investigated the possible involvement of small G proteins of the ARF and Rho families in the activation of PLD in primary astrocytes which were stimulated by noradrenaline, phorbol ester and fetal calf serum (see (1) and abstract by Klein et al., this volume). For this purpose, we used brefeldin A, a fungal metabolite which inhibits ADP-ribosylating factor (ARF)-dependent processes, and *Clostridium difficile* toxin B (TcdB) which glucosylates and inhibits Rho proteins. Brefeldin A inhibited PLD stimulation by noradrenaline and by phorbol ester by more than 50% in a concentration-dependent manner (1-100 µg/ml); it also attenuated serum-stimulated PLD activity. TcdB pretreatment (10-1000 pg/ml) caused morphological changes of the astrocytes due to a rearrangement of the actin cytoskeleton. However, PLD activity stimulated by noradrenaline or serum was not affected, and phorbol ester activation was only slightly attenuated. We conclude that ARF proteins, but not Rho proteins, seem to be required for PLD activation in primary astrocytes.

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NO• REGULATES GLIAL CELL VOLUME BY A CYCLIC GMP-DEPENDENT MECHANISM

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It is well established that high level of NO• is formed in brain ischemia by excited neurons. Evidence show that the effect of the increased concentration of NO• exerts a double edged effect: on the one hand, it is directly neurotoxic and on the second hand it is beneficial for it leads to an increase of the local cerebral microcirculation. The mechanism of the latter effect is ambiguous. NO• molecules would have to reach first the smooth muscle cells of arteriolas to produce vazodilatation, however, NO• has a too short life time for that. An other theoretical possibility is that NO• may advance the diffusion of other vazodilatator molecules, e.g. adenosine, by reducing glial cell volume. We undertook our work to test this hypothesis. Primary cultures of Type-I rat astroglial cells were exposed to the NO•-donor compound sodium-nitroprusside (SNP) or isosorbide-dinitrate (ISDN). Concentration of cGMP, changes in cell volume (assay was performed by application of ^{14}C-labelled 3-O-methyl-D-glucose) and that in transport of $^{36}Cl^-$, $^{22}Na^+$ and $^{86}Rb^+$ were studied as a function of time and SNP or ISDN levels. Both compounds significantly increased cGMP formation and decreased cell volume of the cultured astroglial cells in a concentration-dependent manner. The effect could be mimicked by the cGMP-analog dibutyryl-cGMP (dB-cGMP) and 8-bromo-cGMP (8Br-cGMP). SNP and ISDN also significantly inhibited $^{22}Na^+$ uptake by the cells but either $^{36}Cl^-$ or $^{86}Rb^+$ uptake were not affected. All of the effects of SNP, ISDN and cGMP-analog compounds could be inhibited by anion transport inhibitors and acetazolamide indicating that effects of NO• released from SNP and ISDN are brought about by a significant inhibition of Na^+/HCO_3^- cotransport. This conclusion was further supported by the dependence of the effects on HCO_3^- concentration in the medium.

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LABELLING OF AN INTERMEDIATE-FILAMENT-ASSOCIATED PROTEIN IN CEREBELLAR RADIAL GLIAL CELLS WITH THE RC2 ANTIBODY.

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We have used anti-GFAP and the RC2 antibody, a marker of mouse radial glia, to characterize cerebellar glial cells *in vitro* and *in vivo* and document their transformation from the radial glia to the astrocytic phenotype. Observations on cryostat sections of the developing cerebellum show the existence of an outward-directed gradient of glial development. This is exemplified by the pattern of change of glial cells which loose their radial morphology and become astrocyte-like first in the periventricular region and then in the white matter. Also there is a loss of RC2 staining and gain of expression of GFAP occurring first in the subcortical region and then in the Bergmann glia layer. In cultures, the expression of the antigen recognized by the RC2 antibody is seen only when the cells have been obtained from embryonic cerebella before the formation of the Bergmann glia layer. In these cultures, a change of phenotype occurs that parallels that seen in the intact cerebellum, i.e. acquisition of the stellate astrocytic morphology, loss of RC2- and increase in GFAP-staining, suggesting that *in vitro* an autonomous glial differentiation program can be completed. Neurons added to these cerebellar cultures release a soluble signal that induces a change of the GFAP-positive epithelioid glia into stellate forms and the accelerated suppression of the RC2 labeling. At the ultrastructural level, EM observations show that the RC2 antibody recognizes a cytoskeleton-associated proteinaceous material located along the cell intermediate filaments (IF) suggesting that the antigen is an intermediate-filament-associated protein (IFAP). By Western blotting, we find that in IF preparations obtained from cultured cerebellar glia, the RC2 antibody recognizes a 295 kDa protein whose expression is strongly decreased in cells that have been cocultivated with neurons. Thus cell autonomous as well as epigenetic mechanisms appear to regulate the morphology of cerebellar radial glial cells and astrocytes and the expression of a component of their cytoskeleton.

INOSITOL TRISPHOSPHATE MEDIATES GLIAL-ENDOTHELIAL CALCIUM SIGNALING.

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Interactions of glial and endothelial cells are believed to play an important role in the control of blood-brain barrier permeability and transport. Glial and endothelial cells respond to a variety of stimuli with an increase of intracellular free calcium ($[Ca^{2+}]_i$) that is propagated to adjacent cells as an intercellular Ca^{2+} wave. The mechanism of wave propagation is believed to be based on the intercellular diffusion of inositol trisphosphate (IP_3) via gap junctions. We hypothesized that intercellular Ca^{2+} signaling also occurs between glial and endothelial cells, and we investigated this question in a glial-endothelial co-culture model making use of the caged intracellular messengers IP_3 and Ca^{2+}. Co-cultures were prepared from primary astrocytes and an immortalized endothelial cell line (ECV304). $[Ca^{2+}]_i$ was measured with fluo-3 epifluorescence videomicroscopy. Ca^{2+} waves induced by mechanical stimulation of a single cell often showed propagation that crossed the astrocyte-endothelial interface. Intercellular Ca^{2+} waves could also be induced, most easily in astrocytes, by flash photolysis of pressure-injected caged-IP_3, but these waves did not cross the interface. Interestingly, flashing an astrocyte remotely located from the injected astrocyte also initiated an intercellular Ca^{2+} wave. Flashing an endothelial cell remotely located from the injected astrocyte caused an endothelial $[Ca^{2+}]_i$ increase that often propagated to the astrocytes. Flashing cells without any previous caged-IP_3 injection did not cause any change in $[Ca^{2+}]_i$. Flashing cells loaded with caged Ca^{2+} (nitrophenyl-EGTA) caused a substantial increase of $[Ca^{2+}]_i$ not followed by any propagation to adjacent cells. These results indicate that the diffusion of IP_3 (and not Ca^{2+}) through gap junctions, mediates intercellular Ca^{2+} wave propagation in astrocytes, endothelial cells and also between these two cell types. This mode of communication may be important in glial-endothelial interactions at the level of the blood-brain barrier.

CELL TYPE SPECIFIC EXPRESSION OF SUPEROXIDE DISMUTASE ISOFORMS AND CELLULAR GLUTATHIONE PEROXIDASE IN THE NORMAL RAT BRAIN AND UNDER CONDITIONS OF EXCITOTOXICALLY MEDIATED NEURODEGENERATION

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The formation of reactive oxygen and nitrogen species seems to play a critical role in processes of excitotoxin-mediated neurodegeneration. A first line in the defense against oxygen-derived radicals and their metabolites represents the scavenger enzymes superoxide dismutase (SOD) and cellular glutathione peroxidase (GPX). SOD is present in brain as a cytosolic Cu/Zn-dependent isoform and a mitochondrial Mn-dependent isoform. This enzyme protects against direct and indirect effects of superoxide and prevents the formation of peroxynitrite by competing with NO for superoxide. Cellular GPX detoxifies peroxides including H_2O_2.
In an attempt to closer characterize changes in the defense systems against reactive oxygen and nitrergic species during excitotoxic processes in brain tissue the cellular localization of GPX and SOD isoforms (Cu/Zn- and Mn-SOD) in the normal brain was compared with that of excitotoxically lesioned brain tissue. Intrastriatal injection of the NMDA-receptor agonist quinolinic acid (Quin) was used as an in-vivo model for glutamate-mediated excitotoxicity.
Immunohistochemical studies of the normal rat brain revealed a neuronal localization of Mn-SOD, whereas Cu/Zn-immunoreactivity was homogeneously distributed and only slightly enriched in astrocytes. GPX was found to be enriched in microglia cells. In contrast to that, a massive increase in Cu/Zn- and Mn-SOD immunoreactivity was detected in the lesioned tissue 3 days after Quin application. Colocalization with cell type specific markers (GFAP for astroglia, isolectin B4 for microglia) and subcellular neuronal markers (microtubule associated protein and neurofilament) demonstrates that Cu/Zn SOD is upregulated in activated astroglia cells, whereas Mn-SOD is further enriched in dendritic processes of neurons. GPX immunoreactivity is strongly upregulated in activated microglia cells invading the lesioned tissue.
Our results suggest a differential protective response in the Quin lesioned striatum in that Mn-SOD is upregulated in neurons, Cu/Zn-SOD in astroglia and GPX in microglia. These enzymes are assumed to be induced to prevent oxidative and nitric oxide/peroxynitrite-mediated damage. In the border zone of the lesion core this strategy may contribute to resist the noxious stimulus.

Ca^{2+} RELEASE FROM INTRACELLULAR STORES IN THE LEECH GIANT GLIAL CELL MEDIATED BY METABOTROPIC GLUTAMATE RECEPTOR ACTIVATION.

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Changes in the intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) and the membrane potential (E_m) induced by ligand- or voltage-gated ion channel activation were measured in the giant neuropile glial cell of the leech *Hirudo medicinalis*. Application of 200 or 500 µM glutamate (1 min) elicited transient increases in $[Ca^{2+}]_i$ of 24.1 +/-1.3 nM (n=55) and 43.2 +/-5.2 nM (n=11), respectively, accompanied by small biphasic E_m shifts of a few mV. Application of the ionotropic glutamate receptor agonist kainate (10 µM, 1 min) or elevation of the external potassium concentration from 4 to 20 mM produced $[Ca^{2+}]_i$ transients of 84.0 +/-10.8 nM (n=11) and 620.3 +/-144.3 nM (n=4), respectively. In nominally Ca^{2+}-free saline, $[Ca^{2+}]_i$ increases induced by kainate or high potassium were reduced to less than 10% of the control, whereas the transients induced by glutamate were reduced by only 40%, suggesting that Ca^{2+} release from intracellular stores contribute to the glutamate-induced $[Ca^{2+}]_i$ increase. The non-NMDA receptor antagonist CNQX (Fig. 1), but not the NMDA receptor antagonist AP5, reduced the $[Ca^{2+}]_i$ transients elicited by glutamate by about 50%. Among the tested compounds known to act at metabotropic glutamate receptors, quisqualate, trans-ACPD and L-AP3 induced a $[Ca^{2+}]_i$ rise with the following rank of potency: quisqualate > glutamate > AP3 > ACPD. The metabotropic glutamate receptor antagonist MCPG significantly reduced the glutamate-induced $[Ca^{2+}]_i$ transients by about 20%. In addition, depletion of the intracellular Ca^{2+} stores by CPA, an inhibitor of the endoplasmic Ca^{2+}-ATPase, led to a decrease of 50% of the glutamate-induced transients. The effects of CPA and CNQX were additive. We conclude that glutamate elicit $[Ca^{2+}]_i$ increases due to Ca^{2+} influx through non-NMDA receptor channels and to Ca^{2+} release from intracellular stores mediated by metabotropic glutamate receptors.

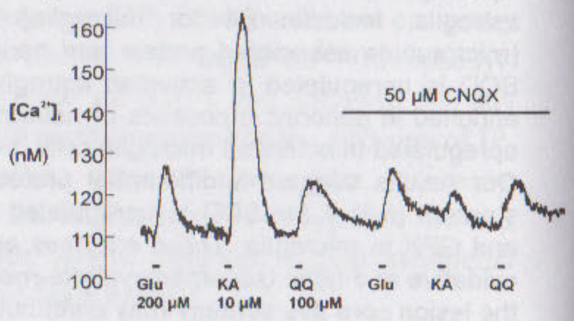

Fig. 1: $[Ca^{2+}]_i$ transients induced by glutamate (Glu), kainate (KA) and quisqualate (QQ) before and during application of the non-NMDA receptor antagonist CNQX.

Rat microglia surviving the exposure to combined hypoxia and hypoglycemia in culture become activated.

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We are interested in the response of microglia to combined hypoxia and hypoglycemia. As a model, we used purified cultures of microglial cells and exposed them to N_2/CO_2 in a sealed glass chamber at 37°C. Cells were plated onto coverslips, rinsed with gassed buffers before exposure to combined hypoxia and hypoglycemia. Glucose was replaced by nonmetabolized sugars, 2-deoxyglucose or mannitol. Control cells were also maintained at 37°C, but in glucose-containing buffer gassed with O_2/CO_2. After exposure time, supernatant was removed for lactate dehydrogenase (LDH) determination and after 30 min reperfusion in medium containing glucose, microglia were stained with ethidium bromide for dead cells and immunostained with microglia-specific isolectin B_4-FITC at specified time points and counted. Six hours of exposure in the presence of 2-deoxyglucose resulted in 1% survival whereas in the presence of mannitol about 30% of the microglia survived. Mannitol is known to act as a radical scavenger and this could explain its protective property. At 4h exposure, about 60% of the microglia survived in the mannitol buffer compared to about 30% survival in the 2- deoxyglucose buffer. Cultured oligodendrocytes from the same 4h and 6h experiments exhibited 5-10% survival while astrocytes showed nearly 100% survival as previously observed by others.

Activation of cultured microglia by inflammatory agents such as LPS is accompanied by the induction of K^+ outward channels which are not observed in unstimulated conditions. After exposure to combined hypoxia and hypoglycemia, the membrane currents of surviving microglial cells were investigated by using the whole cell mode of the patch clamp technique. Microglial cells tested immediately after the 4 h exposure exhibited only inward rectifying K^+ currents, the same as unstimulated cultured microglia. However, after 24 hour recovery period, 85% (11/13) of the microglia tested exhibited the additional K^+ outward current in comparison to 13% (1/8) of the controls. In conclusion, we demonstrate that microglia surviving a combined hypoxia and hypoglycemia insult express outward currents as an activation marker similarly as after exposure to LPS. The new current appears with a delay consistent with a *de novo* expression of a channel protein.

DEVELOPMENTAL REGULATION OF SURVIVAL IN THE SCHWANN CELL LINEAGE

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In the peripheral nervous system, it has recently been shown that Schwann cell precursors, obtained from embryonic day 14 rat embryos, undergo rapid apoptotic death when removed from contact with axons and plated in vitro[1]. This death can be prevented by application of neuregulins or neuron-conditioned medium. The death of precursor cells in vitro contrasted with the excellent survival of neonatal Schwann cells when plated under same conditions, i.e. at moderate to high density[2].

In the present study we have investigated survival regulation in Schwann cells from nerves of newborn rats and compared it with survival regulation in Schwann cell precursors.

We find that the death rate of newborn Schwann cells in vitro is density dependent and that Schwann cells die by apoptosis, like their precursors, if they are plated sparsely enough. Furthermore, we show that low density cultures can be rescued by conditioned medium derived from high density cultures. The survival promoting activity may therefore be derived from Schwann cells themselves, secreted into the medium and acting in an autocrine mode.

Since neuregulins are potent survival factors for precursor cells and can be made by Schwann cells under certain conditions, they are obvious candidates for such an autocrine survival signal in neonatal Schwann cells. In our culture system, NDFβ2 clearly shows survival promoting but also mitogenic activity on neonatal Schwann cells. This finding contrasts the effects of conditioned medium, which does not cause proliferation. Also, neuregulins, but not Schwann cell conditioned medium, promote the survival of Schwann cell precursors. Thus, NDFβ2 is unlikely to be the autocrine survival factor.

In summary, the difference in survival regulation between precursor and Schwann cells relates to the developmental emergence of an autocrine survival loop. This important signal is unlikely to be neuregulin and its identity is under investigation.

References: Jessen et al. (1994) *Neuron* **12**: 509-527; Dong et al. (1995) *Neuron* **15**: 585-596

SEX STEROID METABOLISM IN ASTROCYTES AND IN GLIOMA CELLS: INFLUENCE OF THE CONDITIONED MEDIUM OF NEURONS AND TUMORAL BRAIN CELLS.

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We have investigated the possibility that secretory products of normal and tumoral brain cells might influence the metabolism of steroid hormones in fetal rat neurons, in neonatal rat type 1 astrocytes, as well as in two tumoral glial cell lines (the rat C6 glioma and the human 1321N1 astrocytoma). The activities of the enzymes 5α-reductase (5α-R) and 3α-hydroxysteroid dehydrogenase (3α-HSD) have been evaluated, in the controls and in the experimental groups, using testosterone (T) and progesterone (P) for the 5α-R, and dihydrotestosterone (DHT) and dihydroprogesterone (DHP) for 3α-HSD. Cultures of the different cellular types have been exposed to the conditioned media (CM) in which fetal rat neurons, neonatal rat astrocytes, C6 glioma and 1321 astrocytoma cells had been grown. The results indicated that the CMs of: 1) neurons, but not of type 1 astrocytes, are able to stimulate, in a statistically significant fashion, the formation of DHT, 3α-diol and tetrahydroprogesterone in C6 cells; 2) C6 cells do not influence the metabolism of T and P in neurons; 3) C6 and 1321N1 do not modify the formation of DHT, but decrease that of DHP in type 1 astrocytes. In conclusion, the present observations show that normal as well as tumoral cells derived from the brain are able to secrete "in vitro" principle(s) which may modify the metabolism of T and P in a series of CNS derived cells. Differences (stimulation vs. inhibition) have been found depending the type of cells considered. Surprisingly, the effects on T and P metabolism have been not found to run always in parallel. Work is presently in progress to identify the principle(s) responsible for the effects here described. (Grants from CNR through FATMA, n° 95.000868.PF41; ACRO, n° 95.00395.PF39; and by MURST)

PURINORECEPTOR-INDUCED CALCIUM SIGNALLING IN MOUSE MICROGLIA

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The effects of purine nucleotides on the intracellular calcium concentration ($[Ca^{2+}]_i$) in mouse microglial cells were investigated using fluo-3 microfluorimetry and confocal laser scanning microscopy. The extracellular application of ATP (1 - 300 μM) induced $[Ca^{2+}]_i$ elevation in both, microglial cells *in situ*, namely in corpus callosum slices (25%, n = 32), and in microglial cells in primary culture (84,9%, n = 602). The removal of extracellular Ca^{2+} reduced the amplitude and duration of ATP-evoked $[Ca^{2+}]_i$ transients. The incubation of microglial cells with thapsigargin (500 nM, 5 min) completely inhibited ATP-induced $[Ca^{2+}]_i$ elevation in 53% of cell tested; however in the remaining cell subpopulation thapsigargin failed to inhibit $[Ca^{2+}]_i$ responses. To characterize the pharmacological profile of microglial purinoreceptors we investigated the effects of various purinergic agonists on $[Ca^{2+}]_i$ in cultured microglial cells. The extracellular application of 100 μM βγATP (P_{2X} receptor agonist) induced $[Ca^{2+}]_i$ elevation in 29.4% of 994 cells tested; ADPβS (P_{2Y} agonist) triggered $[Ca^{2+}]_i$ increase in 73.8% (n = 670) of the cells; UTP (P_{2U} agonist) evoked $[Ca^{2+}]_i$ elevation in 74,0% (n = 946) of cells; 2MeSADP (P_{2T} agonist) elevated $[Ca^{2+}]_i$ in 65.6% (n = 1031) of microglial cells and finally Ap_4A (P_{2D} agonist) failed to induce $[Ca^{2+}]_i$ responses in all microglial cells tested (n = 104). The P_2 receptors expression pattern in individual microglial cells tested by consecutively applied subtype-specific ligands revealed a large heterogeneity among different cells. We suggest that mouse microglial cells express both ionotropic (P_{2X}) and metabotropic (P_{2Y}, P_{2U} and P_{2T}) purinergic receptors which are linked to the generation of cytoplasmic Ca^{2+} signals.

THE "MATRICIAL ZONES" OBSERVED DURING THE ONTOGENESIS OF Gallotia galloti COULD BE RELATED WITH REGENERATION

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Most of the studies on regeneration involve mammals, fish and anphibians, however the studies on reptiles are very scarce. Our objective is to demonstrate that reptiles could be involved in CNS regeneration like other lower vertebrates. Using immunohistochemistry, Golgi and EM techniques during the ontogeneny of Gallotia galloti, the "matricial zones" or "sulci" are Vim, GFAP, GS and PCNA immunopositives from E.40 to adult specimens. Also with EM we observed undiferentiated, mitotic cells and glioblasts. These cells apparently "migrating" out of the ventricular zone[1,2,3,4].

To conclude, in Gallotia galloti the presence of immature cells in the sulci and a large proportion of radial glia is present throughout lizard lifetime. During ontogeny, cells are formed not only in the ventricular zone, but in the sulci and migrate out them. Probably the immature cells in sulci of the brain of Gallotia galloti is related to the regenerative capacity of reptilian CNS.

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ACTIVATION OF MICROGLIA AND ASTROGLIA IN THE CNS BY ELECTRIC STIMULATION OR TRANSPORT BLOCK OF A PERIPHERAL NERVE IN RATS

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Axotomy of motoneurons, e.g. by transection of the peripheral hypoglossal nerve, affects the perikarya of motoneurons and the surrounding microglia and astroglia in two separate ways. First, axotomy interrupts the bidirectional axoplasmic transport, notably that of trophic factors from muscle to neuron. Second, it severely disturbs the electric membrane potential and activity of lesioned nerve fibers and cell bodies. In summary, these two factors induce directly the chromatolysis of neurons and indirectly the GFAP-upregulation of astroglia as well as the activation and proliferation of microglia. We wanted to know, whether the lack of target-derived trophic factors or the altered neuronal membrane electrophysiology triggers the transneuronal activation of glia. For this aim we compared axotomy to colchicine block (interrupts axoplasmic transport but not electric conductivity) and to antidromic electric stimulation (does not impede transport) of the peripheral hypoglossal nerve.
After preloading of the hypoglossal neurons with Fluorogold, we performed in Wistar rats either resection, colchicine block or brief antidromic electrostimulation (one session of 15 min, 4 Hz, 5 mA) of the hypoglossal nerve. The rats were fixed by perfusion 1, 3, 5, 7, 10, 15, 25, 35 or 50 days post operation (DPO) and microglia (OX-42, OX-18, OX-6, ED1, ED2), astroglia (anti-GFAP) and neurons (anti-CGRP) were immunostained in the lower brainstem.
Electrically stimulated animals showed microglial activation 1-7 DPO, CGRP was upregulated for 1-7 DPO and astroglial GFAP-expression was elevated 3-15 DPO. Colchicine block caused strong activation of microglia 5-25 DPO, CGRP was upregulated for 3-15 DPO, GFAP expression was elevated 3-35 days. Nerve resection caused microglial activation 2- 35 DPO, CGRP expression above baseline 2-15 DPO and elevated GFAP expression 3-35 DPO. Double labelling of ED2 and Fluorogold in perivascular microgliocytes as sign of neurophagocytosis occured in axotomized and colchicine-treated animals, but not after electrostimulation.
In summary, rat microglia and astroglia can both be activated to an axotomy-like reaction by either transport block or antidromic electric stimulation of motoric axons. A single 15 min session of peripheral electrostimulation that neither impedes axoplasmic transport nor kills any neurons transcellularly triggers a fast and intense answer of microglia and astroglia.

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INTRACELLULAR Ca^{2+} REGULATION IN THE LEECH GIANT GLIAL CELL BY ATP-DRIVEN CA^{2+} PUMPS

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In most cells, a low free intracellular Ca^{2+} concentration, $[Ca^{2+}]_i$, is maintained by Ca^{2+} extrusion mechanisms, e.g. Ca^{2+} pumps and/or Na^+/Ca^{2+} exchange. We have shown that in the giant neuropile glial cells of the medicinal leech the latter contributes to the regulation of $[Ca^{2+}]_i$ (1). In this study we have investigated the role of Ca^{2+} sequestration into intracellular stores, and of a plasma membrane Ca^{2+} ATPase for the $[Ca^{2+}]_i$ regulation in these cells. We have measured $[Ca^{2+}]_i$ with the fluorescent dyes fura-2 and bis-fura-2 during application of different ATPase inhibitors. To investigate the recovery from intracellular Ca^{2+} loads, the cells were depolarized in high K^+ saline, which induced a Ca^{2+} influx through voltage-gated Ca^{2+} channels (2). 1 µM thapsigargin, a selective inhibitor of intracellular store Ca^{2+} ATPases had no significant effect on baseline $[Ca^{2+}]_i$ or on the recovery from Ca^{2+} loads. 10 µM cyclopiazonic acid, which also inhibits intracellular store Ca^{2+} ATPases, induced a transient increase in baseline $[Ca^{2+}]_i$ of 44.5 ± 24.2 nM (n=5), but had also no effect on the $[Ca^{2+}]_i$ recovery. Iontophoretical injection of orthovanadate, a non-selective ATPase inhibitor, induced a large increase in baseline $[Ca^{2+}]_i$ of 264 ± 191 nM (n=5), and significantly increased the time constant of the recovery after a Ca^{2+} load from 16.2 ± 5.3 s to 49 ± 33.7 s (n=9, $p<0.05$). Removal of external Na^+ during orthovanadate injection evoked an additional $[Ca^{2+}]_i$ increase of 438 ± 148 nM (n=3), but had no further effect on the time course of the recovery after a Ca^{2+} load. The results suggest that the maintenance of $[Ca^{2+}]_i$ and the recovery after intracellular Ca^{2+} loads in these glial cells is predominantly achieved by a plasma membrane Ca^{2+} ATPase.

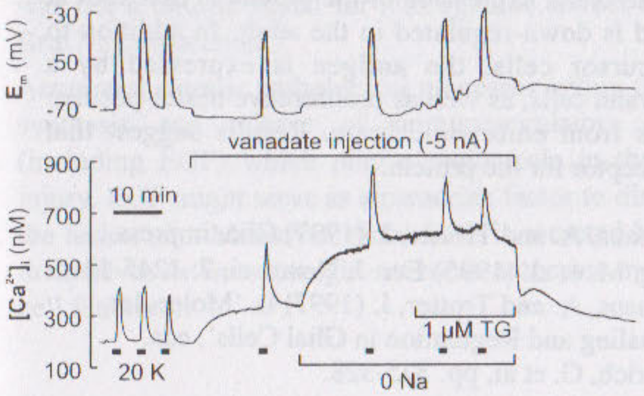

Fig. 1: Membrane potential (upper trace) and intracellular free Ca^{2+} concentration (lower trace) during high K^+-induced de-polarizations and injection of orthovanadate in normal, Na^+-free, and thapsigargin (TG) containing saline.

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A NOVEL CELL-SURFACE GLYCOPROTEIN EXPRESSED BY NEURAL PRECURSOR CELLS.

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Cell-surface molecules regulate early events of myelination such as precursor cell migration and recognition of axons. Lectins can be used to isolate cell-surface glycoproteins. We have studied the lectin-binding properties of oligodendrocyte-lineage cells and observed that peanut lectin binds specifically to oligodendrocyte precursor cells and stimulates their proliferation[1].
Subsequently we have generated monoclonal antibodies against lectin binding glycoproteins isolated from an immortalised murine line of oligodendroglial precursor cells that ensheathes axons upon *in vivo* transplantation[2]. A new cell-surface glycoprotein of 330kD expressed by a subset of proliferative oligodendrocyte precursor cells has been identified[3].
Expression of the antigen shows a partial overlap with O4 and GD3 antigens. It is down-regulated as the cells mature and express the myelin-associated glycoprotein and is not in myelin. Neurons and astrocytes do not express the antigen. The protein expresses the HNK-1 carbohydrate epitope and binds peanut lectin. Metabolic radiolabelling experiments with ^{35}S sulphate as well as enzymatic digestion of the isolated antigen indicate that a subpopulation of the molecules carries glycosaminoglycan chains and is thus a part-time proteoglycan. In Western Blots of mouse brain, expression of the protein is first seen at E13, peaks at P10 and is down-regulated in the adult. In addition to oligodendrocyte precursor cells, the antigen is expressed by a subpopulation of Schwann cells, as well as proliferative nestin-positive neural precursor cells from embryonic brain. Results suggest that astrocytes express a receptor for the protein.

References: (1) Niehaus, A. and Trotter, J. (1997) Glia, in press.
(2) Jung, M. et al. (1995) Eur. J. Neurosci. 7: 1245-1265.
(3) Niehaus, A. and Trotter, J. (1997) In 'Molecular Signaling and Regulation in Glial Cells', eds. Jeserich, G. et al, pp. 325-328.

EPIDERMAL GROWTH FACTOR (EGF) IS A MOTILITY FACTOR FOR MICROGLIAL CELLS IN VITRO: EVIDENCE FOR EGF RECEPTOR EXPRESSION

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Epidermal growth factor (EGF) and its receptor are present in the central nervous system and modulate a variety of neural functions. Here, we show that microglial cells, the brain-intrinsic macrophages, express the receptor for EGF and migrate in response to EGF. Transcripts encoding the EGF receptor could be detected in purified microglial cultures obtained from newborn mouse cortex. More specifically, cDNA fragments derived from EGF receptor mRNA could be amplified from 21% of electrophysiologically characterized microglial cells by the use of single-cell RT-PCR. Expression of the protein was confirmed on rat microglia by flow-cytometry. EGF dose-dependently stimulated chemotactic migration as revealed with a micro-chemotaxis assay. The dose response curve peaked at 10 ng/ml EGF reaching a 3 fold increase of migration over the unstimulated control and was about half of that induced by complement 5a (10 nM), a previously described microglial chemoattractant. Checkerboard-analysis showed that EGF-induced motility was composed of both, chemotaxis and chemokinesis. In contrast to its pronounced effect on cell motility, EGF (0.01 - 10 ng/ml) was not a mitotic signal for microglia as shown by lack of increase in BrdU-incorporation.

Acute and chronic pathological processes within the brain stimulate the synthesis and release of immunoregulators and growth factors (including EGF) which play a major role in the brain´s response to injury. EGF might serve as a paracrine factor to direct microglial cells to the lesion site. Moreover, since EGF is secreted by activated microglia themselves *in vivo*, it might act as an autocrine modulator of microglial cell function.

STRUCTURAL AND FUNCTIONAL CHARACTERIZATION OF TENASCIN-R (RESTRICTIN)

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Tenascin-R is a chick extracellular matrix glycoprotein expressed by neurons and glial cells.

cDNA cloning revealed that TN-R is a large multidomain protein composed of four structural motifs. At the N-terminus TN-R contains a cysteine-rich segment of about 140 aa that might link TN-R monomers into oligomers. This region is followed by four and a half epidermal growth factor (EGF)- like repeats and then by nine consecutive motifs that are similar to fibronectin typ III (FNIII) motifs. At the C-terminus TN-R is related to the β -and γ-chains of fibrinogen including similarity to a calcium-binding segment.

To analyze the structure to function relationship of the different domains of TN-R several recombinant TN-R fragments were expressed in bacterial cells. Two distinct binding regions were localized on the TN-R polypeptide: a region binding the axon associated immunoglobulin (Ig)-like F11 protein and a cell attachment site. The binding region of the GPI anchored F11 was allocated to the second and third FNIII-like domain within TN-R. By using a mutant polypeptide of F11 containing only Ig-like domains a direct interaction between the Ig-like domains of F11 and FNIII-like domains two to three of TN-R was demonstrated. The interaction of TN-R with F11 in in vitro cultures enhanced F11 mediated neurite outgrowth and results in an enlargement of the surface area of growth cones. These results suggest that the combined action of F11 and TN-R might be of regulatorial influence on axon extension. A cell attachment region was identified in the FNIII-like domain eight of TN-R .

Ref.: Nörenberg et al. (1995) J.Cell Biol.130:473-484

Nörenberg et al. (1996)Int.J.Devl.Neuroscience,14:217-231

Stimulation of callosal fibers triggers calcium elevation in the population of glial cells with metabotrobic glutamate receptors

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We used a brain slice preparation to study signaling from neurons to glial cells. Axons in the corpus callosum of young mice (3 to 7 days after birth) were stimulated while recording levels of intracellular [Ca^{2+}] in adjacent glial cells. Slices were bulk loaded with fluo-3 acetoxymethyl ester and changes in [Ca^{2+}] levels were analysed by confocal microscopy imaging. Neuronal fibers were stimulated by low resistance glass pipetts connected to a computer controlled stimulator. Glial cells in the corpus callosum showed a rise in calcium concentration after fiber tracts were stimulated. This response was also observed in calcium free solution and was blocked by thapsigargine indicating that Ca^{2+} is released from intracellular IP_3-sensitive stores in response to neuronal activity. Receptor antagonists such as CNQX (for kainate/AMPA type of glutamate receptors), bicuculline (for $GABA_A$ receptors), suramine (for ATP receptors) or MCPG (for metabotropic glutamate receptor mGluR1) were not able to pharmacologically block this response.

Next we tried to correlate individual cells which reponded to stimulation of the fiber tracts with those that responded toreceptor activation by specific ligands. The best match was found for class I metabotropic glutamate receptors mGluR1 and mGluR5, which are linked to PI hydrolysis and subsequent rise in $[Ca^{2+}]_i$. About 90% of the cells responding with a calcium elevation to stimulation also responded to glutamate (n=70) and more than 80% to t-ACPD (n=221), a specific agonist for metabotropic glutamate receptors. The portion of cells responding to GABA or kainate were lower (both about 50%). MCPG, a selective antagonist of mGluR1, did not block the t-ACPD induced response suggesting the involvement of mGluR5.

Our data indicate an important role for metabotropic glutamate receptors (mGluR5) in neuron-glia interaction via glutamate release from electrically stimulated fiber tracts in the corpus callosum.

THE SECRETION OF IL-6 FROM A HUMAN IMMORTALISED MICROGLIAL CELL LINE IN THE PRESENCE AND ABSENCE OF MYCOPLASMA FERMENTANS INFECTION.

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A number of immortalised human microglial cell lines generated by transformation of foetal microglial by SV40 were described by Janabi et al.[1] The microglia had the characteristics of macrophages, but lacked the monocytic markers for CD14, CD4, CD68/Ki-M6 and CD11c. In addition, the constitutive production of interleukin -6 (IL-6) could be increased by interleukin-1a (IL-1α) stimulation; however, the cells did not produce Tumour Necrosis Factor-alpha (TNF-α) or IL-6 in response to lipopolysaccharide. We evaluated the microglial cells from Clone 3 and found that their cytokine responsiveness was as described by Janabi et al; however, routine screening by PCR showed that the cell line was infected with mycoplasma fermentans (classified by Mycoplasma Experience, U.K.). Following the removal of the mycoplasma infection, the consititutive secretion of IL-6 was reduced from approximately 1500pg/ml to < 20pg/ml, although the pattern of macrophage marker expression was largely unaltered. These data suggest that the constitutive high expression of IL-6 from Clone 3 microglial cells was due to the presence of a mycoplasma infection. This finding highlights the need to rule out exogenous factors when assessing the effect of inflammatory stimuli on cytokine release from microglia.

References: Janabi, N. et al. (1995) Neurosci. Lett. 195, 105-108

INVESTIGATION OF TRANSCRIPTION FACTORS INVOLVED IN SCHWANN CELL DIFFERENTIATION.

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Analysis of differentiation of Schwann cells from the neural crest through various stages of gliogenesis have characterised several distinct cell types (Jessen et al, 1994, Dong et al 1995) in terms of their survival, growth, and antigenic phenotypes through development. Whilst these characteristics have thus been well documented, relatively little is known as to the transcription factors controlling the development of these cell types from the neural crest through to the myelinating or non-myelinating Schwann cell of the adult nerve.

Using a modified differential display procedure on total RNA from mouse sciatic nerve (see abstract by Georg Zoidl, this meeting), we have recently identified several novel members of the zinc finger transcription family, which are differentially expressed between an early stage of mouse development (embyonic day 12, the Schwann cell precursor) and birth (P0). A preliminary characterisation of several of these novel trancription factors in Schwann cell differentiation will be presented.

Jessen KR *et al*, Neuron 12: 509-527 (1994
Dong Z *et al*, Neuron 15: 585-596 (1995).

DESERT HEDGEHOG IS INVOLVED IN PERIPHERAL NERVE DEVELOPMENT.

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The mouse *Hedgehog* family consists of three members: *Sonic* (*Shh*), *Desert* (*Dhh*) and *Indian hedgehog* (*Ihh*) which share a striking homology with the *Drosophila* segment polarity gene *hedgehog* (*hh*), a key regulator of pattern formation in the embryonic and adult fly. All of these genes encode secreted proteins and *Shh* is already established as an important signaling molecule in vertebrate development. In contrast, very little is known about the roles of *Dhh and Ihh.* In view of our earlier studies that *Dhh* is expressed in Schwann cell precursors[1], we have now set out to determine whether *Dhh* is involved in peripheral nerve development. For this purpose, we have studied in detail the cellular localization of *Dhh* and one of its main target genes, *Patched* (*Ptc*), in normal developing nerves and examined developing nerves in a *Dhh* knockout mouse[2]. We find 1) that *Dhh* mRNA is expressed in Schwann cell lineage during embryonic development from E13.5 (precursor) to birth (Schwann cell) and in myelinating Schwann cells from birth to P10. 2) that *Ptc* is expressed in cells surrounding the developing nerve and 3) that perineurial development is highly abnormal in *Dhh* null mutants.

Taking together, our results suggest that the connective tissue that surrounds embryonic nerves is induced to organize into peri- and epineurial sheaths by glial signals involving *Dhh*.

References: (1) Bitgood, M.J. and McMahon A.P. (1995) *Dev. Biol.* **172**:126-138; (2) Bitgood, M.J., Shen, L. and McMahon A.P (1996) *Current Biology* **6**:298-304.

SPINAL CORD GLIAL CELLS EXPRESS GLYCINE RECEPTORS WITH A TRUNCATED β SUBUNIT.

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The expression of the inhibitory glycine receptor in glial cells from rat spinal cord was investigated by the use of the patch-clamp technique in combination with single-cell RT-PCR.

Subunit-specific transcript expression was analyzed in individual glial cells of rats aged 3 to 18 days. Employing the patch-clamp technique in the whole-cell configuration, glial cells were identified by their membrane current pattern. After recording of glycine-evoked chloride currents, cytoplasm was harvested followed by reverse transcription of total mRNA. Subunit specific cDNA fragments were amplified and analyzed by agarose gel electrophoresis, Southern blotting and sequencing. In all glial cell types investigated, transcripts of the α1 subunit, but not of α2 or α3 subunits, were detected. In addition, about half of the glial cells analyzed contained β subunit mRNA. These results illustrate that glial cells of rat spinal cord express functional glycine receptors.

Sequencing of GlyRβ transcripts revealed a novel alternative splice variant of the GlyRβ subunit. Due to the omission of exon 7 in the splicing process, this particular variant lacks the first transmembrane domain. Electrophysiological properties of GlyRβΔ7 have been characterized in co-transfection experiments with the GlyR α1 subunit in HEK293 cells. GlyRα1/βΔ7 heteromeric channels were found to be functionally expressed in transfected cells. Δ7-mediated whole-cell currents had a picrotoxin sensitivity intermediate to those determined for GlyRα1 homomeric and GluRα1/β heteromeric channels. By use of single-channel recordings, a single-channel conductance of 56 pS was determined for GlyRα1/βΔ7 heteromeric channels, a value intermediate to 76 ps for α1 homomeric and 45 ps for α1/β heteromeric channels.

To investigate the physiological role of the truncated GlyRβ subunit in glial cells, we have to establish techniques allowing subunit-specific GlyR modulation.

WHAT IS THE ROLE OF PROTOPLASMIC ASTROCYTES IN ADULT HUMAN BRAIN CORTEX.

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It is generally accepted that astrocytes are divided into protoplasmic and fibrous types based on their morphologic appearance. Fibrous astrocytes are most common in the white matter and protoplasmic astrocytes in the gray matter. At present, the immunochemical and morphologic findings indicate the further classification of astrocytes. Type 1 (GFAP+/A2B5-) and type 2 (GFAP+/A2B5+) astrocytes are proposed to be analogous to protoplasmic and fibrous astrocytes, respectively [1]. However the latter classification is investigated mainly on rat optic nerve and on the embryonic rodent brain tissue and cultures. The purpose of this study is to demonstrate the problems with immunohistochemical detection of protoplasmic astrocytes in adult human brain tissue.

Paraffin and frozen sections were prepared from ten apparently normal adult human brain biopsies. They were processed for GFAP and A2B5 immunohistochemistry. The staining for A2B5 antigen was negative in all biopsies. The positively stained cells for GFAP were located in the subpial area and in the white matter. No positive staining was found in the cortical gray matter (layer II-VI). In two biopsies with gliosis in the white matter we observed rare appearance of GFAP-positive stained astrocytes in the cortical gray matter (layer II-VI). The GFAP-positive cells showed different morphologic features.

Our results indicate that GFAP which is considered to be the best marker for astroglia is not present in astrocytes in the cortical gray matter except subpial area. However, the rare appearance of GFAP-positive astrocytes in the cortex is due to brain gliosis. These findings suggest that the role of protoplasmic astrocytes is not clear. In addition, is not known the reliable marker for the detection of protoplasmic astrocytes in the normal human brain cortex.

References: [(1)] Montgomery (1994) *Vet Pathol* **31**:145-167.

TENASCIN-R IS REPELLENT FOR ACTIVATED MICROGLIA WHICH INDUCES DOWNREGULATION OF THE PROTEIN AFTER FACIAL NERVE AXOTOMY - A NEW ROLE IN MOTONEURON PROTECTION

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Tenascin-R (TN-R), a member of the TN family of ECM proteins, acts as (i) inhibitor of neurite outgrowth[1,2] and (ii) oligodendrocyte differentiation factor[3] in vitro suggesting that under pathological conditions, TN-R may contribute to the poor regenerative capacity of the CNS. Here we have examined the expression pattern and functional implication of the protein in the facial nucleus after peripheral nerve injury (postoperative day (POD) 3 to 112) in Wistar rats. Studies at light microscopic and ultrastructural level demonstrate that TN-R is a constituent of the perineuronal network of motoneurons and about 7 days after peripheral axotomy of N. facialis, becomes downregulated in the corresponding facial nucleus. Under regenerative conditions (nerve suture), TN-R expression in the ECM reaches original levels at POD 14 while under nonregenerative conditions (nerve resection), the protein is stably downregulated at the operated site. Functional studies in vitro give evidence for the participation of (1) TN-R in motoneuron protection against microglial attack and (2) activated microglia/macrophages in the downregulation of TN-R after peripheral nerve injury. First, we show that activated microglial cells readily adhere to the operated site of cryosections containing the appropriate brain stem nuclei of unilaterally operated animals, while their adhesion to the unoperated site is strongly inhibited in a TN-R-dependent manner. The nonpermissive properties of the intact, TN-R-containing tissue are almost neutralized by a monoclonal antibody to TN-R. Second, activated microglia seem to be the main cellular source for injury-associated soluble factors inducing the downregulation of the protein under pathological conditions as microglia-conditioned media induce the downregulation of both TN-R protein and mRNA by cultured oligodendrocytes.

Our findings suggest a new role for TN-R in neuronal protection against activated microglia and the participation of the latter in perineuronal network destruction, e.g. downregulation of TN-R.

References: (1) Pesheva et al. (1993) Neuron **10**:69-82; (2) Pesheva et al. (1994) J. Cell Sci. **107**:2323-2333; (3) Pesheva et al. (1997) J. Neurosci.: in press.

MICROGLIAL DIVERSITY IN THE BRAIN OF *Gallotia-galloti.*

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Microglial cells in layered zones such as optic tectum and telencephalic cortex have been poorly studied. During development there is no study available about lizard microglia[1]. The aim of this work is to characterize lizard microglia under normal conditions since we are involved in knowing its role in regeneration processes.

Paraffin, cryostat and vibratome sections of embryos (E.31-E.40) and adults (2 years old) were processed using tomato lectin like microglial marker. Our results show three different types of microglia during development, namely ameboid, pseudopodic and primary microglial cells. These three main types firstly appear in the vecinity of ventricular areas. On the other hand, in adults, most microglial cells show ramified morphology although some ameboid cells appear, ocassionally, near blood vessels.

Microglial distribution presents a heterogeneous pattern during development. However, in adult specimens microglial cells seem to show a specific pattern being situated in fibrous and cellular layers in optic tectum and telencephalic cortex.

In conclusion, our results show that tomato lectin is a good microglial marker during development of CNS in lizard. The lizard microglial tipology is similar to that of mamals. However, its timecourse of development is anterior to that mammals and is maintained all along the adult life. Besides, the discovered pattern of distribution could be related to microglial function.

Ref.:(1) Castellano et al. (1991) J. Comp. Neurol. 311:434-444.

INDUCTION OF AN OUTWARD POTASSIUM CURRENT IN RODENT MICROGLIA BY PNEUMOCOCCAL CELL WALL COMPONENTS.

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The Gram-positive bacterium *Streptococcus pneumoniae* is a major cause of meningitis in human adults with a surprising high mortality rate of up to 30% and preparations of pneumococcal cell wall (PCW) can evoke the characteristic symptoms of meningitis in rodent models. Microglia are likely an early cellular response element in bacterial infection and become rapidly activated by many CNS insults. Induction of new currents appears to be one of the physiological changes associated with microglial activation. In the present work, we compared the changes in the microglial current pattern as a response to exposure to either PCW or lipopolysaccharide (LPS), an established model for Gram-negative infection. We applied the whole cell mode of patch clamp-technique to cultured mouse microglial cells of developmental stages E14 and P1. The cells were incubated with a PCW preparation (equivalent of 10^7 cfu/ml) or LPS (100 ng/ml), each with or without 10% fetal calf serum (FCS) supplement, respectively. Both, PCW and LPS induced outward currents within 6 h of incubation. The properties of the current indicates that it is due to the activation of outward rectifying K^+ channels: (1) the reversal potential of the PCW-induced outward current was also near the K^+ equilibrium potential; (2) the current was abolished by 10 mM 4-aminopyridine, and (3) was depressed by extracellular 45 mM tetraethylammonium. In microglia from newborn mice, addition of FCS facilitated the induction of outward currents by both PCW and LPS from 20% and 43% (without FCS) to 100% and 95% (with FCS), respectively. In contrast to LPS, this facilitation was not be observed in PCW stimulated embryonic microglia. Our results indicate that the full action of both Gram-positive and -negative bacteria on mouse microglia is strongly dependent on serum factors and the stage of maturation.

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SUBPOPULATIONS OF MOUSE MICROGLIA EXPRESS AN ALTERNATIVELY SPLICED MRNA VARIANT OF INTERLEUKIN-15

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Interleukin-15 (IL-15) is a pleiotropic cytokine modulating growth and differentiation of several hematopoietic cell types. However, its expression and function in the central nervous system is mostly unknown. Recently, we have demonstrated that microglial cells, the brain macrophages, do not only express the mRNA encoding IL-15 but also secrete the protein. In human hemapoeitic cells and different cell lines, IL-15 precursor proteins with two different leader sequences have been shown to be derived from two different messengers of the IL-15 gene arising from alternative splicing of the designated exon A.

To investigate IL-15 transcript expression and splicing in mouse microglia, we applied the single-cell RT-PCR technique. First, cultured microglial cells derived from mouse brain were characterized electrophysiologically by whole-cell patch-clamp recordings. Subsequently, cytoplasmic RNA was harvested and subjected to reverse transcription and PCR amplification allowing the analysis of alternative splicing of IL-15 transcripts.

In a first series of experiments, 14 % of microglial cells (6 out of 43) with amplified IL-15 encoding transcripts were found to express a larger transcript. PCR cycle sequencing revealed the new sequence of the alternatively spliced exon A of the mouse IL-15 gene. The mouse exon A has a length of 136 nt (17 nt longer than the human exon A), contains two stop codons within the reading frame at its 5´end and a new start codon at its 3´end. Thus, this splice variant encodes an IL-15 polypeptide with a shortened leader sequence of 26 amino acids compared to the known 48 amino acid leader sequence encoded by transcripts lacking exon A. In general, mouse exon A displays similar sequence properties as its human counterpart.

A second series of experiments was performed, to investigate whether the alternative splicing of exon A might be indicative of the microglial activation status. Upon stimulation by lipopolysaccharide for 12 to 24 h, a threefold increase of microglial cell number expressing transcripts with exon A was observed (40 % – 8 out of 20 – compared to 14 % under control, non-stimulating conditions). It can be concluded that the change in the expression frequency of the two alternatively spliced IL-15 mRNAs is indicative for microglial stimulation.

IMMUNE SURVEILLANCE IN THE INJURED NERVOUS SYSTEM: T-LYMPHOCYTES INVADE THE AXOTOMIZED MOUSE FACIAL MOTOR NUCLEUS AND AGGREGATE AROUND SITES OF NEURONAL DEGENERATION.

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Although the central nervous system (CNS) is an established immuno-privileged site, it appears to be under surveillance by the immune system, particularly under pathological conditions. At present, little is known about how this is regulated. In the current study we examined changes in lymphocyte infiltration, a key component of the neuroimmune surveillance, into the axotomized facial motor nucleus using immunohistochemistry at light and electron-microscopic (EM) level. We also studied possible changes in the synthesis of proinflammatory cytokines and in the blood-brain barrier.

Peripheral nerve transection lead to a biphasic increase in the number of CD3, CD44 and CD11a-immunoreactive T-cells in the axotomized mouse facial motor nucleus, reaching a first, low-level plateau 2-4 days after injury (2-4 DAI), and a second, much stronger increase 7-21 DAI, with a maximum at 14 days. These CD3-positive T-cells frequently formed aggregates, exhibited typical cleaved lymphocyte nuclei at the EM-level and were completely absent in mice with *severe combined immunodeficiency* (*scid*), which suffer from a congenital block of T- and B-cell differentiation. Immunohistochemical colocalization with thrombospondin, a marker for phagocytotic microglia, revealed aggregation of these T-cells around microglia removing neuronal debris, which is particularly frequent 2 weeks after injury. This was accompanied by a late, strong induction of mRNA for proinflammatory cytokines IL1b, TNFa and IFNg. In contrast, there was no infiltration by the neutrophil granulocytes; intravenous injection of horseradish peroxidase also showed an intact blood brain barrier.

In summary, the current study shows a selective infiltration of the CNS by activated T-cells during the retrograde reaction to axotomy. The striking aggregation of these lymphocytes around neuronal debris and phagocytosing microglia suggests an important role for the immune surveillance during neuronal cell death in the injured nervous system.

CHROMATOGRAPHIC PURIFICATION OF CONNEXIN43 FROM BOVINE BRAIN AND DETECTION OF ITS UBIQUITINATION

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Connexin43 (Cx43) is the most dominant gap junction protein in brain tissues and is preferentially expressed in astroglial cells. Recent studies of our group indicate that neurons may also utilize Cx43 as a major gap junction protein [1]. Although the cell specific and functional expression of Cx43 has been extensively described, little is known about the biosynthesis and trafficking of this protein under in situ conditions. In order to unravel the biochemistry of native Cx43 we designed a multi-step chromatographic method which allows the isolation of Cx43 from different subcellular fractions of bovine brain. The isolation protocol includes a four-step purification process starting with adsorption chromatography - cation exchange chromatography - reversed phase chromatography and a final step which exploits reversed phase chromatography with bound cyano-nitril-chains.
The final step yields more than 90% pure Cx43. The method allows the purification of Cx43 from plasma membranes as well as from microsomal fractions. This technique can thus be applied for further identification of posttranslational modifications of Cx43 during the process of trafficking from the cytoplasmic compartment to the plasma membrane.
A first outcome of this preparative approach was the detection of a ubiquitinated form of Cx43. By applying a Cx43 specific antibody we were able to detect a 52 kDa-Cx43 form in microsomal preparations which was absent in plasmalemmal fractions. This 52 kDa form could be identified as a monoubiquitinated Cx43 isoform, indicating a processing of Cx43 which involves helper proteins during its biosynthesis or degradation events in the CNS.

References: (1) Simburger, E., Stang, A., Kremer, M., Dermietzel, R.; Expression of connexin43 in adult rodent brain; Histochem Cell Biol (1997) 107: 127-137

REGIONAL DIFFERENCES OF CULTURED ASTROGLIA IN NEUROTRANSMITTER-EVOKED CALCIUM RESPONSES

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Astroglial cells express receptors for different neurotransmitters, as e.g. dopamine (DA) and glutamate (GLU). Treatment of astroglial cells with DA or GLU results in a transient increase of intracellular calcium. Information on putative regional differences of astroglial cells in the expression of neurotransmitter receptors is scarce. We have investigated the calcium responses of rat cortical, striatal and mesencephalic astroglial cultures following treatment with DA, GABA, and GLU. Cells were loaded with the calcium sensitive fluorescent dye FLUO-3-AM and calcium dependent changes in fluorescence intensity were imaged with a scanning laser microscope.
Our results reveal that cortical and striatal astroglial cells respond to both DA and GLU with transient increases in intracellular calcium. In contrast, mesencephalic astrocytes respond to DA only. As expected, astroglial cells did not respond to GABA. To further characterize the effect, cortical astroglial cutures were treated with kynurenic acid and Fluphenazine, both potent antagonists to GLU and DA, respectively. Both substances blocked the responses partially. In addition, the response pattern to GLU is apparently heterogeneous, including both monophasic and biphasic responses. This may suggest expression of different receptor subclasses. To test whether the same astroglial cells express receptors for both transmitters, or whether receptors are expressed by different subsets of astrocytes, cells were uncoupled by octanol to prevent passive spread of the calcium signals to cells not expressing the receptor. These experiments revealed the existence of different subsets of astroglial cells expressing either both, DA receptors and GLU receptors, or GLU receptors alone. In conclusion, these results demonstrate regional heterogeneity in the expression of neurotransmitter receptors on astroglial cells in vitro. In addition, there is apparent heterogeneity in the receptor pattern expressed by distinct cells even from one region.

FGF-2 MODULATES NEUROTRANSMITTER SENSITIVITY AND CALCIUM SPREADING IN CULTURED ASTROGLIAL CELLS

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Treatment of cultured rat cortical and striatal astroglial cells with FGF-2 results in a decrease of cell coupling (Reuss et al., submitted). To further characterize this effect, we investigated the calcium responses in FGF-2-treated versus control cultures upon exposure to dopamine (DA) or glutamate (GLU). To distinguish cells that actively expressed the neurotransmitter-receptors from cells receiving the signals by passive spreading, they were transiently uncoupled with octanol.

Our results show that in coupled control cultures of cortical astroglia 89% of the cells were responsive to DA. Uncoupling caused a reduction to 59%, which was reversible after washing out of octanol. In FGF-2-treated cortical astroglia cultures, 93% of the cells were responsive to DA, and there was a slight, fully reversible 5% decrease following uncoupling with octanol. FGF-2 did not interfere with responses to GLU by coupled or uncoupled cells, respectively. Similar results were obtained for striatal cultures. In the coupled stage 83% of the control cells were responsive to DA, dropping to 38% upon octanol treatment, and finally recovered to 70% when octanol was removed. In FGF-2-treated striatal cultures uncoupling resulted in a 10% decrease, with complete recovery when coupling was reestablished. As in cortical cultures, GLU responses dropped only by 10% upon uncoupling, and no differences with regard to FGF-2 treatment could be detected. Mesencephalic cultures were responsive to DA only, and FGF-2 failed to alter sensitivity to DA.

Treatment of astroglial cells with FGF-2 therefore seems to modulate their sensitivity to DA, leading to a significant increase of cells expressing the dopamine receptor. In contrast, cells passively receiving the calcium signal via gap junctions are greatly reduced in number. This is the first documentation that FGF-2 interferes with astroglial neurotransmitter receptors. Its in vivo relevance remains to be elucidated.

ANALYSIS OF MOTILE OLIGODENDROCYTE PROGENITOR CELLS IN BRAIN SLICES AND IN CULTURE

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Video-time-lapse analysis was performed on isolated precursor cells in living brain slices, in co-culture with cerebellar microexplants and in single cell cultures. Motility analysis of individual cells was combined with electrophysiological, immunological, and morphological characterizations. In slices of the corpus callosum from neonatal rat, glial progenitor cells did not strictly migrate along the main direction of the axonal fibers of the corpus callosum but rather traversed the fibers with an overall direction towards the cortex. All motile cells had a simple morphology and migrated with a velocity of 10-100 µm/h. After Lucifer Yellow filling of the motile progenitor cells *in situ,* we could demonstrate that they were dye-coupled to yet unidentified cells of the corpus callosum. Patch clamp analysis of the motile cells confirmed that they belonged to the O-2A lineage. In co-culture experiments of oligodendrocyte progenitor cells with cerebellar microexplants, O-2A progenitor cells did also not migrate strictly along neurite fascicles which were radially projected from the microexplant cores, although O-2A progenitors preferably attached to the neurite fascicles shortly after addition. Their overall velocities were not influenced by the presence of cerebellar microexplants. The velocities of single cell cultures highly enriched in O-2A progenitor cells was analyzed using a specifically designed computer-based analysis system. As has been seen *in situ*, all motile cells exhibited a simple morphology and most, but not all, of them expressed the A2B5 epitope *in vitro*. Besides growth factors (PDGF and bFGF) which are known to stimulate O-2A progenitor cell migration we could demonstrate that the extracellular matrix component laminin did also enhance the motility of these cells, as the percentage of motile cells, as well as their velocities were increased compared to the control on substrate-coated poly-L-lysine. Translocation of the cell bodies was not continuous, but occurred in waves comparable to the situation in the brain slices.

HETEROGENEOUS EXPRESSION OF VOLTAGE-GATED POTASSIUM CHANNELS OF THE *SHAKER* FAMILY IN OLIGODENDROCYTE PROGENITORS.

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The membrane conductance of young oligodendrocytes and their progenitors is dominated by voltage-gated, outwardly rectifying K currents. By combined application of immunocytochemistry using K^+ channel-specific antibodies, patch-clamp recording and single-cell RT-PCR, we characterized the subunit expression of voltage-gated K^+ channels of the *shaker* family in individual oligodendrocytes obtained from embryonic mouse brain.

First, cultures of young oligodendrocytes and progenitors were electrophysiologically characterized for their expression of K^+ currents by patch-clamp recording and, subsequently, immunostained with Kv1.1 to Kv1.6-specific antibodies. Oligodendrocytes were found to express Kv1.1 (10 %), Kv1.4 (40 %), Kv1.5 (80 %) and Kv1.6 (90 - 100 %) channel proteins, while no immunoreactivity for Kv1.2 or Kv1.3 proteins could be detected.

The single-cell RT-PCR technique was applied by use of degenerate oligonucleotide primers allowing the simultaneous amplification of transcripts derived from the six *shaker* K^+ channel genes. Subsequently, capillary electrophoresis of the amplified cDNA fragments was performed not only to identify individual K^+ channel transcripts, but also to quantify their relative expression levels in single cells. This analysis revealed a rather heterogeneous expression of K^+ channel mRNAs. The majority of oligodendrocytes (14 of 18 investigated cells) expressed several (2 and more) transcripts simultaneously, while 4 out of 18 expressed one type only. Combinations of transcripts encoding Kv1.2, Kv1.4; Kv1.5 and Kv1.6 were most commonly detected. So far, Kv1.1 transcripts were found in one cell only. In contrast, to the immunocytochemical investigation, Kv1.2 was found in the majority of oligodendrocytes (13 out of 18).

Young oligodendrocytes and its progenitors express a wide repertoire of different Kv1 channel combinations. To understand the physiological role of the channel heterogeneity, we have to search for techniques allowing subtype-specific channel modulation.

DSD-1-PROTEOGLYCAN MODULATES THE MIGRATORY BEHAVIOUR OF THE OLIGODENDROCYTE CELL LINE OLI-NEU

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DSD-1-Proteoglycan (DSD-1-PG) is a chondroitin sulfate proteoglycan which carries the DSD-1-epitope specifically recognized by monoclonal antibody (mAb) 473HD. Due to its strong expression on immature oligodendroglial cells at differentiation stages known to display migratory behaviour, a potential involvement of the DSD-1-PG in this context was investigated.
The expression of the DSD-1-epitope could be enhanced in vitro by members of the TGF-β family of growth factors, with TGF-β1 being able to induce a five-fold increase. This regulation was used to study potential influence on the motile behaviour of the immortalized oligodendrocyte precursor cell line Oli-neu. TGF-β1 treated Oli-neu cells displayed a reduced migration performance on laminin-1 by 34%, while migration on either merosin, fibronectin or poly-ornithine was unaffected. Coincubation with TGF-β1 and mono- or polyclonal antibodies directed against the proteoglycan neutralised the inhibition on laminin-1. The specific effect of the DSD-1-PG on motile behaviour of Oli-neu might be due to a reduced short-term adhesion to laminin-1 caused by the up-regulation of the proteoglycan. This may result in insufficient grip and, therefore, reduction in the length of processes extended by Oli-neu. From these data we conclude that the DSD-1-PG is involved in the control of motile behaviour of the cell line Oli-neu.

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Synergistic effects between brain capillary endothelial cells and astrocytes in antioxidative defense during hypoxia/ reoxygenation

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Astrocytes (AC) induce typical properties of the blood-brain barrier (BBB) in brain capillary endothelial cells. However, the influence of AC on the antioxidative potential of the BBB has not been investigated yet.

This study used a co-culture model of the BBB consisting of immortalised rat brain endothelial (RBE4) cells and primary rat AC. RBE4 cells were cultivated on a Falcon filter (0.45 µm pore size, 1.6 x 10^6 pores/cm^2). AC were growing on the bottom of the same well. The antioxidative potential of the cells was determined by measurement of enzyme activities and endogenous antioxidants involved in decomposition of reactive oxygen species: superoxide dismutase (SOD), catalase (Cat), glutathione peroxidase (GPx), and reduced glutathione (GSH). In monoculture, AC possessed a higher antioxidative potential in comparison to RBE4 cells (activity of enzymes: MnSOD 2.7-fold, Cat 1.5-fold, GPx 4-fold; content of GSH 1.4-fold). When RBE4 cells and AC had been co-cultivated, both cell types expressed significantly higher activities of antioxidative enzymes (especially MnSOD which was increased in AC 5.2-fold, in RBE4 cells 9-fold). As a model for changes in BBB under ischemia/reperfusion injury, the influence of short-term hypoxia followed by reoxygenation on antioxidative defense mechanisms was investigated. In monoculture a decrease in activity of CuZnSOD, Cat, GPx and in content of GSH was observed during hypoxia/reoxygenation whereas activity of MnSOD was increased after 2h of hypoxia. Under these conditions, there was a higher tolerance of AC towards hypoxia/reoxygenation as compared to RBE4 cells in monoculture (higher increase of MnSOD activity, lower decrease in activity of other enzymes and GSH in AC). In contrast, antioxidative defense potentials were not altered during hypoxia/reoxygenation, when RBE4 cells and AC had been co-cultivated.

In conclusion, AC can protect brain endothelial (RBE4) cells from oxidative stress due to their higher antioxidative potential. Co-cultures of brain endothelial (RBE4) cells with AC are more resistant to hypoxia/reoxygenation. These results give further evidence that AC support the formation of the BBB by brain endothelial cells.

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CALEB IS A NOVEL NEURAL MEMBER OF THE EGF FAMILY OF DIFFERENTIATION FACTORS

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The differentiation of the nervous system is the result of a complex series of cell communications mediated by molecules shown to participate in different aspects of development such as mitogenic signaling, axonal growth and pathfinding and the establishment of synaptic junctions. When screening for unknown cell surface proteins binding to known ones in an interaction trapping approach we were able to identify CALEB in the chicken nervous system due to its interaction with specific extracellular matrix molecules as a novel member of the EGF family of differentiation factors. cDNA cloning indicates CALEB (**c**hicken **a**cidic **l**eucine-rich **E**GF-like domain containing **b**rain protein) to be a multidomain protein consisting of a N-terminal glycosylation region, a leucine-proline-rich segment, an acidic box, a single EGF-like domain, a transmembrane and a short cytoplasmic stretch. The EGF-like domain of CALEB is most similar to the EGF-like domains of EGF, TGF-α and the neuregulins. In the developing nervous system CALEB is associated with glial and neuronal surfaces. CALEB binds to the extracellular matrix proteins tenascin-C and tenascin-R. In vitro antibody perturbation experiments reveal a participation of CALEB in neurite formation.

Reference: Schumacher et al. (1997) *J. Cell Biol.* 136:895-906

NO RELEASE FROM ASTROCYTES AND INTERVENTIONS DURING HYPOXIA/REOXYGENATION

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Among others, astrocytes (AC) are considered to induce specific properties in brain endothelial cells forming the blood-brain barrier. Under hypoxic conditions, this barrier can be damaged by reactive oxygen species (ROS) mainly formed during reoxygenation. The role of nitric oxide (NO) under hypoxic conditions is controversially discussed. NO plays a physiological role in signal transduction. On the other hand NO can readily react with superoxide radicals forming peroxynitrite which was shown to be cytotoxic. In this study the ability of rat AC to release NO and the NO-dependent signal transduction were examined. Furthermore, the effects of NO donors and of expression of inducible NO-synthase (iNOS) on hypoxic alterations were investigated.

Although AC did not possess a constitutive NO-synthase basal NO release was detected due to a weak iNOS expression. As shown by RT-PCR, AC have a soluble guanylate cyclase and a cGMP-dependent protein kinase (PKGI) enabling to NO-mediated signal transduction. Incubation with cytokines (IL-1β/IFNγ) lead to the induction of iNOS and pronounced formation of ROS and NO. As a result of cytokine incubation, cell viability was reduced to about 60%. During hypoxia/reoxygenation, NO seemed to protect AC from damage by ROS formed due to reoxygenation. Thus a reduced lipid peroxidation was shown in AC treated with NO donors or preincubated with cytokines.

In general AC are able to produce NO which can act protectively under hypoxic conditions; however, simultaneous formation of ROS is cytotoxic.

LONG-TERM ACTIVATION OF CAPACITATIVE CALCIUM ENTRY IN MOUSE MICROGLIAL CELLS

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Microglial cells react very rapidly to numerous pathological events in the brain by transforming from the resting state to the activated microglia. The nature of the signalling system which relays the changes in the brain microenvironment into the long-term changes of microglial gene expression and consequently the reorganisation of the cell phenotype remains largely unknown. The fluctuations of cytoplasmic calcium concentration ($[Ca^{2+}]_i$) might be regarded as a likely candidate underlying initiation of microglial activation.

We have measured $[Ca^{2+}]_i$ in cultured microglial cells with the Ca^{2+}-sensitive fluorescent dye fura-2 using a digital imaging system. Stimulation of P_2 purinergic receptors of microglial cells by ATP or UTP always evoked a $[Ca^{2+}]_i$ elevation. The Ca^{2+} response was comprised of both Ca^{2+} influx through ionotorpic receptors and Ca^{2+} release from intracellular pools. When intracellular Ca^{2+} release was stimulated in the absence of extracellular Ca^{2+}, the readmission of extracellular Ca^{2+} caused a large rebound $[Ca^{2+}]_i$ increase. Following this rebound, $[Ca^{2+}]_i$ did not return to the initial resting levels but remained for long periods of time (up to 20 min) at a new, higher steady-state level. Both the amplitude of the rebound Ca^{2+} transient and the new plateau level strongly correlated with the degree of intracellular Ca^{2+} depletion, indicating the activation of a store-operated Ca^{2+} entry pathway. The long-term activation of this Ca^{2+} entry pathway appears to be associated with a dramatic decrease in the capability of the intracellular Ca^{2+} stores to reuptake cytosolic Ca^{2+} once they have been maximally depleted. This mechanism could have important consequences for the understanding of the processes which lead to the activation of the microglial cells.

ACTIVATION OF CULTURED PRIMARY ASTROGLIAL CELLS BY GLIADIN PEPTIDES OF EVERYDAY HUMAN DIET.

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Celiac disease (CD), a condition in some individuals who are sensitive against wheat gluten proteins present in the everyday diet of a large human population consuming flour and bread, is associated by various neurological and/or psychiatric disorders. These disorders are characteristic for CD but may range from multifocal intracranial calcifications mimicking a Sturge-Weber syndrome to early progressive senile dementia (before 60) or schizophrenia-like symptoms. The appearance of all of the various neurological or psychiatric symptoms in CD can get prevented or ameliorated at their early stage of onset by a gluten-free diet. It is now well established that degradation products of gluten, the gliadin peptides may reach the central nervous system in CD. Our studies were undertaken to see whether can gliadin peptides affect glial cells. Cultured rat primary Type-I astroglial cells were derived on coverslips as it has been described (Latzkovits, L. et al., 1993, J. Am. Physiol. 264, C603-C608) and exposed to a gliadin product of SIGMA (G-3375) for various periods. Study of ^{3}H-thymidine incorporation and immunostaining of GFAP showed that gliadin peptides can activate the cultured astroglial cells.

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BERGMANN GLIAL CELLS *IN SITU* EXPRESS ENDOTHELIN$_B$ RECEPTORS LINKED TO CYTOPLASMIC CALCIUM SIGNALS

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The endothelin (ET) isoforms ET-1, ET-2 and ET-3 applied at 100 nM triggered a transient increase in $[Ca^{2+}]_i$ in Bergmann glial cells in cerebellar slices acutely isolated from 20 - 25 days' old mice. The intracellular calcium concentration ($[Ca^{2+}]_i$) was monitored using fura-2-based $[Ca^{2+}]_i$ microfluorimetry. The ET-triggered $[Ca^{2+}]_i$ transients were mimicked by ET_B receptor agonist BQ-3020 and were inhibited by ET_B receptor antagonist BQ-788. ET elevated $[Ca^{2+}]_i$ in Ca^{2+}-free extracellular solution and the ET-triggered $[Ca^{2+}]_i$ elevation was blocked by 500 nM thapsigargin indicating that the $[Ca^{2+}]_i$ was released from $InsP_3$-sensitive intracellular pools. The ET-triggered $[Ca^{2+}]_i$ increase in Ca^{2+}-free solution was shorter in duration. Restoration of normal extracellular $[Ca^{2+}]$ briefly after the ET application induced a second $[Ca^{2+}]_i$ increase indicating the presence of a secondary Ca^{2+} influx which prolongs the Ca^{2+} signal. Preapplication of 100 µM ATP or 10 µM noradrenaline blocked the ET response suggesting the involvement of a common Ca^{2+} depot. The expression of ET_B receptor mRNAs in Bergmann glial cells was revealed by single-cell RT-PCR. The mRNA was also found in Purkinje neurones, but no Ca^{2+} signalling was triggered by ET. We conclude that Bergmann glial cells are endowed with functional ET_B receptors which induce the generation of intracellular $[Ca^{2+}]_i$ signals by activation of Ca^{2+} release from $InsP_3$_sensitive intracellular stores followed by a secondary Ca^{2+} influx.

Ca^{2+} SIGNALS INDUCED BY ACTIVATION OF SERINE PROTEASE RECEPTORS IN CULTURED RAT ASTROCYTES

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Thrombin seems to be involved in physiological processes within the central nervous system such as synapse formation and stabilization[1]. The protease causes oscillations of $[Ca^{2+}]_i$ in rat C6 glioma cells[2]. Most cellular responses induced by thrombin are mediated by proteolytic activation of a G protein-coupled thrombin receptor. Recently, a second member of this protease activated receptor family (PAR-2) has been identified, which is soecific for trypsin. In the present study the effect of thrombin and trypsin on the $[Ca^{2+}]_i$ of astrocytes, obtained from total brain of newborn rats, was investigated.

Brief stimulation (60-90 s) with thrombin resulted in transient elevation of $[Ca^{2+}]_i$. The concentration-effect curve was best fitted by a double sigmoidal curve. Long-term superfusion with thrombin resulted in three different types of kinetics. i) A single transient $[Ca^{2+}]_i$ rise superimposed with fast fluctuations (48% of the cells tested), ii) a biphasic $[Ca^{2+}]_i$ elevation composed of an inital peak followed by a sustained plateau (43%), ii) thrombin-induced $[Ca^{2+}]_i$ oscillations (9%). The initial $[Ca^{2+}]_i$ transient could be elicited in the absence of extracellular Ca^{2+} and was abolished by prior emptying of intacellular stores by thapsigargin. Furthermore, the long-term Ca^{2+} signals depend on the presence of the agonist and extracellular Ca^{2+}, could be mimicked by the thrombin receptor activating peptide (TRAP) and were inhibited by the endogenous thrombin inhibitor protease nexin-1 (PN-1).

Besides thrombin, superfusion of astrocytes with trypsin induced similar changes of $[Ca^{2+}]_i$, suggesting that rat astrocytes express the PAR-2. In contrast to thrombin, the Ca^{2+} response induced by trypsin showed a sigmoidal concentration dependence. Omission of extracellular Ca^{2+} revealed that trypsin similarly to thrombin caused the release of Ca^{2+} from intracellular stores. Taken together these results indicate that receptors for thrombin and trypsin are present on astrocytes inducing long-term $[Ca^{2+}]_i$ changes, which are generated by an interplay of Ca^{2+} release from intracellular stores and Ca^{2+} entry across the plasma membrane.

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DO THE GLUTAMATE METABOTROPIC RECEPTORS INFLUENCE THE PRODUCTION OF ENDOGENOUS NEUROPROTECTANT KYNURENIC ACID?

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Kynurenic acid (KYNA) is the physiological brain constituent synthesized along kynurenine pathway, preferentially within astrocytes. Due to its antagonistic properties at ionotropic glutamate receptors, especially that of N-methyl-D-aspartate (NMDA) type, KYNA displays potent neuroprotective and anticonvulsive effects. Glutamate binding sites comprise the ligand-gated ion channels named ionotropic receptors and G-protein-coupled metabotropic receptors (mGLU). Both are largely represented in the mammalian nervous system on neuronal and glial cells. We have investigated the effect of ionotropic and mGLU receptors activation on KYNA synthesis in vitro.

KYNA production was examined in rat brain cortical slices according to the method of Turski et al., (J. Neurochem. 1989, 52:1629-1636). KYNA was subjected to HPLC and detected fluorimetrically. The mean control production of KYNA in the presence of 10 M L-kynurenine was 12.3 +/- 1.9 pmol/2 h/well.

None of the selective ionotropic EAA agonists such as N-methyl-D-aspartic acid (NMDA), kainate (KA) or (S)-a-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA), up to 0.5 mM, was able to diminish the production of KYNA in vitro. mGLU receptor agonist, (+/-)-1-aminocyclopentane-trans-1,3-dicarboxylic acid (t-ACPD) at 0.1 and 0.2 mM reduced KYNA synthesis to 71 and 61% of control levels, respectively. Similarly, L(+)-2-amino-4-phosphonobutyric acid (L-AP4), another mGLU receptor agonist, used at 0.1 and 0.2 mM, diminished KYNA production to 79 and 72% of control values, respectively. The agonist of all ionotropic and mGLU receptors, glutamate, used at 0.5 and 1.0 mM, also inhibited KYNA synthesis to 64 and 47% of controls, respectively.

Our results suggest that KYNA production within the brain can be modulated by the activity of mGLU receptors. Further investigations with use of recently available antagonists will be required to evaluate the involvement of specific mGLU receptors subtypes in the modulation of KYNA production.

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GLIAL AND NEURONAL GLUTAMATE TRANSPORTERS CONTAIN AN SH-BASED SITE RESPONSIBLE FOR REDOX MODULATION AND OXIDANT VULNERABILITY

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Glutamate (Glu) uptake controls the extracellular Glu level in the brain and prevents excitotoxicity. We have recently reported that Glu uptake is long-lastingly inhibited by a number of endogenous oxidants, including oxygen radicals, hydrogen peroxide (H_2O_2)[1] and peroxynitrite ($ONOO^-$)[2]. All of these agents affect uptake by direct interaction with the Glu transporters at unidentified "oxidant-vulnerable" site(s). We show here that 3 recombinant rat transporter subtypes, i.e. the two major glial transporters, GLT1 and GLAST, as well as the neuronal EAAC1, expressed in HeLa cells and reconstituted in liposomes, respond to thiol-specific oxidants (DTNB, 500 μM) and disulfide-reductants (DTT, 3 mM) with loss and gain of function respectively. The 3 transporters show almost identical sensitivity to SH-redox reagents (±20-30%). Moreover, whole-cell clamped rat cortical astrocytes respond to sequential applications of DTT and DTNB with dynamic up- (+80%) and down-changes (-40%) of the Glu uptake current. In the same paradigm, H_2O_2 (1 mM) and $ONOO^-$ (250 μM), like DTNB, antagonize DTT potentiation, bringing the current level below control; subsequently, DTT mostly reverses H_2O_2 but not $ONOO^-$ inhibition. Therefore: (a) reversible redox interchange between free SH groups and disulfides on glutamate transporters is a novel regulatory mechanism of glutamate uptake; (b) GLT1, GLAST and EAAC1 contain a similar SH-based redox site, probably formed by conserved cysteine residues; (c) this site is most likely targeted by biological oxidants leading to transporter oxidation and inhibition. In view of the predominant role for glial transporters in maintaining extracellular Glu within its physiological range[3], our findings may help to understand the link between glial Glu uptake alterations, excitotoxicity and oxidative stress in a number of neurodegenerative pathologies (e.g. Amyotrophic lateral sclerosis, Alzheimer's disease etc.).

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ALTERED EXPRESSION OF MACROPHAGE ANTIGENS BY PHAGOCYTIC MICROGLIA DOES NOT IMPROVE NEURONAL SURVIVAL AFTER SEVERE LESION OF THE FACIAL NERVE IN RATS

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In response to lethal injury of the facial motoneurons, the finely-branched resting microglia rapidly proliferate, migrate toward neuronal somata, and transform into neuronophages with upregulated synthesis of macrophage-specific antigens. Whereas these alterations in neuronophages have been extensively documented, their functional significance remains obscure. On one side, the expression of immune-related antigens is thought to represent an important step in initializing immune responses in the CNS. On the other side, it has been suggested that it even suppresses local immune responses. In this report, we tried to draw functional implications on the upregulated expression of immune-related antigens by their selective suppression. This was achieved through chronic oral administration of the dihydropyridine type calcium-entry blocker nimodipine.

A total of 132 adult female Wistar rats (strain HsdCpb:WU) were used. Following stable vital labeling of neuronophages in the facial nucleus (injection of 50 µl 4% Fluoro-Gold into the whisker pad prior to resection of 10 mm of the facial nerve), half of the animals were treated with 1000 ppm nimodipine in the food pellets, and half with placebo, i.e. standard laboratory chow. Thereafter we traced synchronously (1) the expression of immune-related antigens by FG-labeled neuronophages and (2) the changes in neuron number in the lesioned facial nucleus. The time course of changes in these two parameters were compared between nimodipine- and placebo-treated animals for 3-224 days post resection.

The postoperative treatment with nimodipine caused attenuated expression of CR3, MHC class I and class II antigens (detected by immunocytochemistry with mabs OX-42, OX-18, and OX-6), enhanced the expression of the macrophage specific antigen (mab ED1), and did not change the expression of rat macrophage differentiation antigen (mab ED2). These altered expressions, however, had no effect on the loss rate of motoneurons in the lesioned facial nucleus (counts after immunostaining for neuron-specific enolase).

We conclude that the degree of expression of immune-related antigens by neuronophages has no influence on the delayed neuronal cell death induced by permanent target deprivation.

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Localization and Regulation of Intercellular Adhesion Molecule 1 (ICAM-1) on Microglia in the Regenerating Mouse Facial Motor Nucleus

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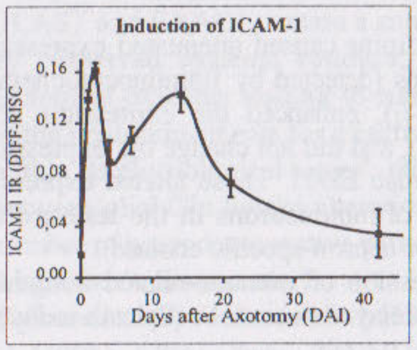
Intercellular adhesion molecule 1 (ICAM-1) is a widely expressed adhesion protein, which is a member of the immunglobulin superfamily. ICAM-1 is a ligand for the lymphocyte function-associated-1 antigen (LFA-1/$\alpha_L\beta_2$-integrin) and plays an important role in the extravasation of leukocytes during inflammation of neural and peripheral tissue. In the injured CNS, activated microglia also express high levels of $\alpha_M\beta_2$-integrin, the second leukocyte receptor for ICAM-1. In the current study we examined the localization and regulation of ICAM-1 after CNS injury in the axotomized mouse facial motor nucleus.

In the normal facial nucleus, ICAM-1 immunoreactivity is restricted to endothelial cells. Transection of the facial nerve led to a strong and selective upregulation of ICAM-1 on the activated microglia. Interestingly, quantitation of ICAM-1 immunoreactivity using confocal laser microscopy and double labelling for ICAM-1 and constitutively expressed mouse IgG-reactivity revealed a biphasic increase. The first increase was observed 1-2 days after injury, at the early stages of microglial activation and proliferation. The second increase occurred around day 14 during the late posttraumatic neuronal cell death. It is associated with microglial phagocytosis of neuronal debris, synthesis of proinflammatory cytokines and lymphocyte infiltration. Here, immunoelectron microscopy showed strong ICAM-1 immunoreactivity on the cell membrane of phagocytotic microglia and associated lymphocytes which aggregate around dying neurons and neuronal debris. No immunolabelling was observed on other cell types such as neurons, astrocytes and oligodendrocytes.



In summary, the current study shows the induction of ICAM-1 on activated microglia and its expression on lymphocytes invading the injured nervous tissue. The data suggest the involvement of ICAM-1 in lymphocyte infiltration and in mediating cell to cell contacts between endothelial cells, lymphocytes and microglia during the cellular response to injury and the ensuing repair of the central nervous system.

High-affinity glutamate transporter in the rat retina: A major role of the glial glutamate transporter in transmitter clearance

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Glutamate is the primary mediator of excitatory synaptic transmission and excitotoxic neuronal injury in mammalian retina and brain. High-affinity glutamate transporter proteins control the extracellular glutamate concentration and thus play a key role in regulating the balance between physiological signaling and pathological overactivation.

Using membrane vesicle preparations of total rat retina, we show that retinal glutamate uptake is a high-affinity, electrogenic and Na^+-dependent transport process which is driven by the transmembranous Na^+/K^+ ion gradient. RT-PCR revealed the presence of three different high-affinity glutamate transporters in the rat retina identified as GLAST-1, GLT-1 and EAAC-1. No message was found in retina for EAAT-4, a transporter recently cloned from human brain. Autoradiography of intact and dissociated rat retinae indicated that Müller glial cell glutamate uptake dominates the total retinal glutamate transport and that this uptake is strongly influenced by the activity of glutamine synthetase. RT-PCR, immunoblotting and immunohistochemistry revealed that Müller cells express only GLAST-1. In retinal tissue GLAST-1 was found to be the most abundant transporter followed by the neuronal subtypes EAAC-1 and GLT-1. Furthermore, the interaction between glutamate transport and glutamine synthetase activity in Müller cells was investigated, suggesting a common transcriptional regulation of GS gene expression and GLAST-1 gene expression.

By regulating the glutamate concentration within the synaptic cleft, the action of GLAST-1 in Müller cells may extent beyond protection of neurons from excitotoxicity and we suggest a mechanism by which Müller cell glutamate transport might play an active role in shaping the timecourse of excitatory transmission in the retina.

EMBRYONIC BRAIN TRACT FORMATION IN *DROSOPHILA*

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A general feature of brain development in all higher, bilaterally symmetrical organisms is the ordered interconnection of cephalic hemispheres by a series of horizontal and longitudinal brain tracts. During embryogenesis in insects the axonscaffold of the brain is build around the embryonic foregut which separates the anlagen of the brain hemispheres. Here we use immunocytochemical and reporter gene techniques to investigate this process in *Drosophila*. Three major axonal pathways are formed across the midline. These are the primary preoral commissure linking the two cephalic hemispheres, a pathway via frontal commissure and frontal connectives linking the two frontal ganglia with the tritocerebrum, and the postoral tritocerebral commissure located below the foregut. We show that the major longitudinal and horizontal tracts of the embryonic brain form superficially near the interface between the foregut and the embryonic brain hemispheres. We identify three types of cellular structures which might be involved in tract formation. These are rows of glial cells at the medial brain margin, cellular bridges composed of neuronal somata, and the epithelial surface of the foregut itself. Staining for the expression of the glial-specific transcription factor repo indicates that the interhemispheric glial rows are transient cellular structures. The close vicinity to the outgrowing axons suggest that the structures at the brain-foregut interface may play a role in the morphogenesis of embryonic brain tracts in *Drosophila*.

DEVELOPMENT OF THE BLOOD-RETINA BARRIER IN THE CHICKEN

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The blood-retina barrier of the avian eye is composed of the retinal pigment epithelium (RPE) and the endothelial cells (ECs) of the pecten oculi. Recently, we have introduced the pecten oculi of the chicken as a new *in vivo* model for blood-brain barrier (BBB) investigations (1). Whereas the RPE cells loose their barrier properties during invasion into the pecten and become the pectinate pigmented cells, the ECs acquire them during invasion into the pecten primordium from the choroidal vascular bed. In analogy to astrocytes which are suggested to be inducers of BBB properties in ECs in the brain, the glial pigmented cells in the pecten are favourable candidates to do so in this organ. During pecten development, we investigated the freeze-fracture morphology of tight junctions (TJs), the lanthanum permeability and the immunoreactivities (IR) of the glucose transporter Glut-1, the HT7 antigen, the TJ-protein occludin and of N-cadherin, an important component of adherens junctions (AJs).

HT7 (2) and Glut1 (3) were almost uniformly distributed in retina and pecten on embryonic day (E) 7 and E10, and become more and more restricted to the RPE and the pecten ECs. Asking for the crosstalk between AJs and TJs, we found an early downregulation of N-cadherin prior to TJ formation in the RPE. In contrast, invading blood vessels of the pecten primordium revealed concomitantly increasing IRs for N-cadherin and occludin up to E16. In later stages, we observed a further increase in occludin-IR and a decrease of N-cadherin-IR to a punctuate appearance. The different expression patterns of N-cadherin and occludin in the RPE and the ECs of the pecten oculi speak in favor of a different developmental mode of epithelial (glial) and endothelial TJs.

Acknowledgements: With support by the DFG. We thank Dr. Redies for collaboration. We acknowledge the gift of the anti-occludin- and the HT7-antibody by Drs. Tsukita and Dr. Risau, respectively.

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PRESENCE OF OLIGODENDROCYTE PRECURSOR CELLS IN MULTIPLE SCLEROSIS LESIONS

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The central nervous system (CNS) of patients in a chronic stage of multiple sclerosis (MS) typically contains multiple non-repairing demyelinated lesions or *plaques*. Why loss of myelin and of myelin-forming cells, i.e., oligodendrocytes, is not adequately compensated for in MS, especially during later stages of the disease, is not known. The present study has examined whether any precursor cells to oligodendrocytes survive the demyelinating damage in MS and are present in chronic MS lesions. The presence of oligodendrocyte precursor cells was determined by immunolabelling 10 µm sections of postmortem MS tissue (obtained from the Netherlands Brain Bank) with the O4 antibody and antibodies to galactocerebroside (GalC) and glial fibrillary acidic protein (GFAP). In total, 17 demyelinated lesions from 11 patients with long-standing MS (9 - 49 years disease duration) were analysed. These immunolabellings revealed for the first time that MS lesions contain significant numbers of oligodendrocyte precursor cells, identified as process-bearing cells that bind the O4 antibody, but not antibodies to GalC and GFAP. Cell counts showed that the density of the oligodendrocyte precursor population varied considerably from lesion to lesion (range: 5 - 29 cells/mm^2). No correlation was found between the density of the oligodendrocyte precursor population in a particular lesion and the age of the MS patient, the relative age of the MS lesion or the length of the disease process. Preliminary data suggest that very few, if any, oligodendrocyte precursor cells in the MS lesions bind antibodies to the proliferation markers Ki67 or Proliferating Cell Nuclear Antigen (PCNA). Thus, the present study suggest that factors other than the disappearance of oligodendrocyte precursors from areas of demyelinating activity may play a role in the development of chronically demyelinated MS lesions. The identification of such factors may prove crucial in attempts to stimulate the oligodendrocyte precursor population in the lesions to proliferate, migrate and differentiate into remyelinating oligodendrocytes. This would represent an alternative to transplanting oligodendrocyte lineage cells into lesions site to promote myelin repair.

MODULATION OF PHAGOCYTOSIS, NO-PRODUCTION AND PROLIFERATION OF MICROGLIAL CELLS IN VITRO BY PRO- AND ANTI-INFLAMMATORY CYTOKINES

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Activation of microglial cells in neurological diseases involves proliferation, and the induction of phagocytic and cytotoxic properties. To get further insights into the signals modulating different aspects of microglial activity we studied the effects of four different cytokines/growth factors on microglial phagocytosis of latex-beads. using a quantitative assay based on flow-cytometry. Granulocyte/macrophage-colony stimulating factor (GM-CSF) and tumor necrosis factor-α (TNF-α) enhanced microglial phagocytic activity while a phagocytosis inhibiting effect could be observed after preincubation with transforming growth factor-β1 (TGF-β1) and interleukin 4 (IL-4). To further test if the phagocytosis inhibitory cytokines TGF-β1 and IL-4 negatively influenced other microglial activation parameters, we investigated their effect on microglial proliferation and production of nitric oxide (NO). While TGF-β1 slightly decreased microglial proliferation IL-4 significantly inhibited proliferation. Basal NO-production was not affected by IL-4 and TGF-β1. However, when NO-production was stimulated with LPS, this level was significantly increased after pretreatment with IL-4.

In conclusion, the deactivating cytokines in our in vitro assays differentially regulate distinct microglial activation parameters and might also *in vivo* play an important role in modulating microglial activation to keep the balance between the protective, defensive and destructive/chronic-inflammatory properties of microglia.

AGE-RELATED CHANGES IN THE PROLIFERATIVE RESPONSE OF MICROGLIA TO THE BRAIN INJURY.

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Microglia are frequently reported as the cell type most sensitive to pathological changes in the central nervous system. Their reactive transformations and proliferation are the earliest phenomena characteristic to the process of posttraumatic scar formation. However, age-dependent changes in the spatiotemporal pattern of injury-induced microglial proliferation are still a weakly explored research area. To examine the development of microglial reactivity, a mechanical injury was inflicted to the left cerebral hemisphere in rats of four age groups: newborns, 6, 14 and 30 day-old. Proliferating cells were labeled with [^{3}H]thymidine injected at different time intervals following the injury. Brain sections were processed for lectin BSI-B4 histochemistry and subjected to autoradiography. During microscopical observations autoradiographically- and lectin-labeled macrophages and three morphological types of microglial cells were distinguished. Numbers and and locations of the cell types within the injury area were recorded separately . Profiles of injury-induced changes in the total number of proliferating lectin-labeled cells as well as changes in their distribution in different age groups were analysed and compared with each other.

The results suggest that the microglial proliferative response to injury cannot be regarded as proportional to the developmental progress of the brain but it displays quantitative and qualitative features typical to different developmental stages.

Supported by the Foundation for Polish Science (grant 18/94), PECO Program (grant ERBCIPDCT930410) and BW15/95.

Identification of differentially expressed mRNAs and novel zinc-finger transcription factors in the developing peripheral nervous system.

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The major stages in the embryonic development of Schwann cells in rodent peripheral nerves have become more clearly defined recently. However, in contrast to our understanding of the Schwann cell lineage at the cellular level, only a very small number of genes that are implicated in embryonic Schwann cell development has been identified. It is therefore likely that many as yet undefined genes are associated with this process. They could be characterized by a rapid up- or down-regulation at the mRNA level within short periods of time when cells proceed through major transition stages. On the basis of this assumption we have developed two different strategies to identify genes that may be associated with Schwann cell development. Firstly, we have established a non-radioactive RNA fingerprinting approach that has enabled us to identify 20 differentially expressed genes when comparing early (embryonic day E12) and late (E13) Schwann cell precursor stage mRNAs. We will descibe this method and demonstrate a primary characterization of clones. Secondly, we have taken advantage of the modular structure of zinc-finger tranciption factors, built by single or tandem arrays of multiple copies of zinc-finger motifs to design degenerate primers against consensus sequences. Total RNA derived from the developing mouse sciatic nerve on E12 and the first postnatal day was compared by degenerate RT-PCR. Using this approach we were able to identify a number of previously unknown zinc-finger transcription factors in the PNS. We will describe the RT-PCR strategy, the cloning and characterization and the differential expression in vivo and in vitro of these zinc-finger genes.

Project Leader

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General Research Interest

After CNS lesions, e.g. optic nerve transection axonal regeneration is inhibited and the parental neurons (retinal ganglion cells - RGCs) die within 2-3 weeks. We have developed in vitro and in vivo models where RGCs are retrogradely labeled with fluorescent dyes to examine survival and axonal regrowth under controlled conditions and the interaction of retinal neurites with various types of glial cells. By applying single-cell patch-PCR in combination with subtractive cloning we have established cDNA libraries from de- and regenerating RGCs which are currently analysed.

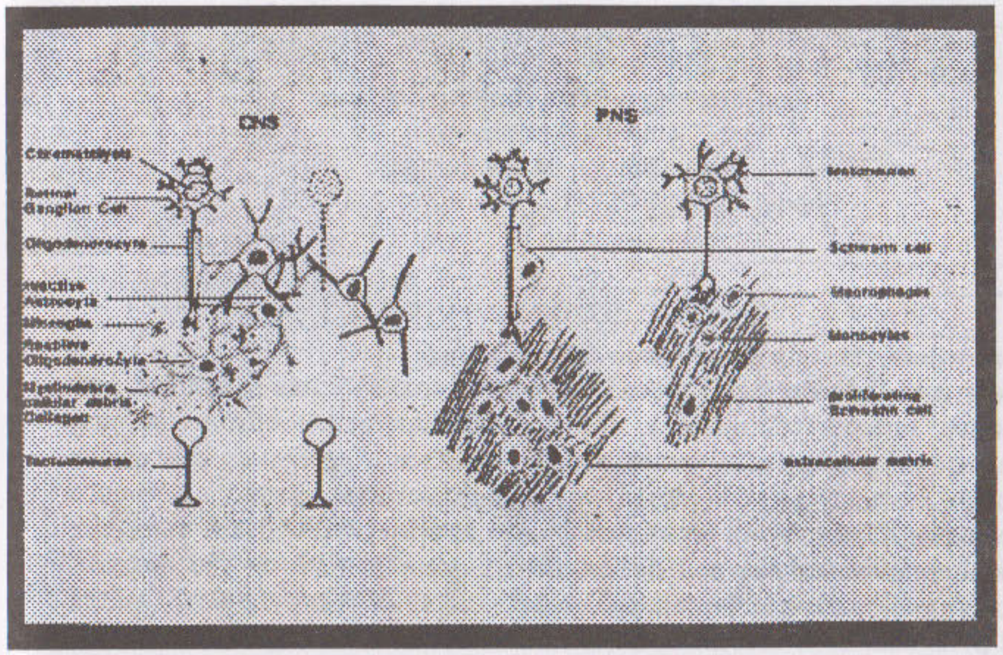
Project in the Schwerpunkt

Properties of reactive optic nerve glia
Several factors contribute to the reactive transformation of glia in the lesioned adult optic nerve. We have established astrocyte cultures from neonatal and adult rat optic nerves to describe the expression of cell surface and matrix molecules on reactive optic nerve glia. Futhermore, we have examined the influences of myelin-associated inhibitors for neurite growth on pathfinding and axonal guidance.

Methods Available

In vitro and in vivo regeneration models
Tracing with fluorescent dyes,Single-cell
patch PCR,Subtractive cloning, Differential display
Adenovirus vector mediated gene transfer.

Illustration



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4.T.Herdegen, P. Skene and M. Bähr (1996) **TINS**: in press
5.G.Struckhoff,C.Przyrembel, M. Bähr and A. Gocht (1996)
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General Research Interest

Extracellular Matrix and Neuron-Glia Interface

Recent data indicate that extracellular matrix proteoglycans associated with neuronal surfaces contribute to the neuronal microenvironment and may influence the properties of the neuron-glia interface. Aggregating chondroitin sulphate proteoglycans complexed with hyaluronan show distinct distribution patterns in different brain regions which can be correlated with the number of perineuronal nets (PNs) and diffuse matrix accumulations in the neuropil. Cortical areas can be differentiated by the number of non-pyramidal neurons and pyramidal cells associated with PNs. The functional significance of the heterogeneous matrix distribution and composition is still unknown.

Project in the Schwerpunkt

Properties of Perineuronal Nets in vivo and in vitro

Our previous lectin-histochemical and immunocytochemical studies showed characteristic distribution patterns of perineuronal nets (PNs) in different brain regions. the relative invariability of some of these patterns during evolution, as well as their postnatal development in the rat. One aim of the present project was to confirm the association of PNs with known types of neurons in vivo and in unfixed tissue slices. The in vivo labelling as well as experimental degradation of PNs will be used to study the consequences of these modifications in adult animals.

Methods Available

Lectin- and immunocytochemical detection of glial and neuronal markers including dual peroxidase and dual- or triple fluorescence techniques.
Electron microscopy

Illustration

Extracellular matrix and neuron-glia interface

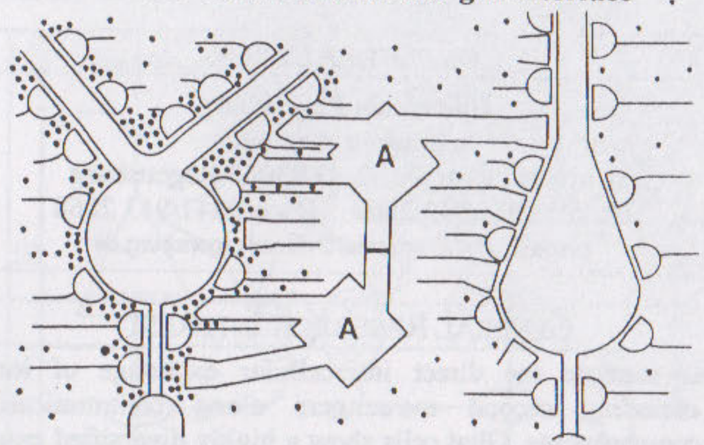

Fig. 1: Differential association of extracellular chondroitin sulphate proteoglycans (black dots) with non-pyramidal neurons and pyramidal cells in the neocortex. In perineuronal nets (left) the matrix components are interposed between neuronal surface and processes of astrocytes (A).

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General Research Interest

Gap Junctions mediate the direct intercellular exchange of ions and small metabolites including second messengers along communicative traits in multicellular compartments. Glial cells show a highly diversified expression of gap junctions forming proteins with distinct local and temporal expression patterns. The goals of our studies are: i) to identify the diverse gap junction proteins (connexins) expressed in glia cells, ii) to evaluate the functional meaning of the diversification of connexin expression and iii) to understand the interrelationship of the communicative properties of the glial network with respect to neuronal control mechanisms. Diverse techniques including immunocytochemical and molecular biological methods are applied to approach these goals.

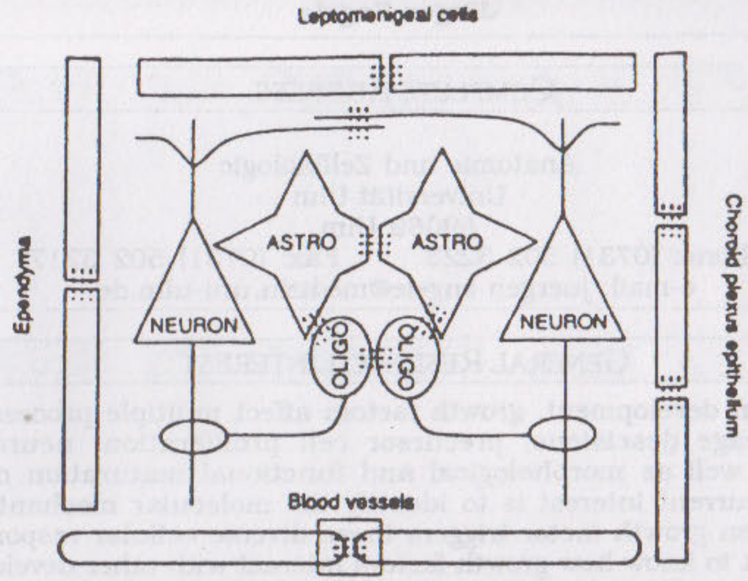
Project in the Schwerpunkt

1. Trafficking of the connexin43 in astrocytes and a concept of gap junction formation.
2. Phosphorylation of connexin43 and its consequence for the formation of communication competent gap junctions.
3. Pheripheral nerve regeneration and the expression of connexin32 and connexin46.
4. Identification of an additional connexin (connexin43) in oligodendrocytes and evidence of autocellular junctional territonies in white matter of the spinal cord.

Methods Available

Light/electron microscopical immunocytochemistry
Tissue cultures
Confocal laser microscopy
Molecular biology (RT-PCR, cloning)
Antibody production (monoclonal, polyclonal)

Illustration



Gap junctions between communicating cells in the central nervous system. Within most cell populations, gap junctions form the primary route of signal transmission. For neurons, gap junctions coexist with chemical synaptic transmission. Each compartment as drawn represents the aggregate of subcompartments with variable requirements for direct intercellular communication.

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Klaus Unsicker, Heidelberg
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General Research Interest

During brain development, growth factors affect multiple processes such as cell lineage descisions, precursor cell proliferation, neuronal cell survival as well as morphological and functional maturation of neural cells. Our current interest is to identify the molecular mechanism(s) by which a given growth factor triggers these diverse cellular responses. We further want to know how growth factors interact with other developmental signals such as neurotransmitters and extracellular matrix. These studies will not only open further insights into the physiological role of growth factors during brain development, but might also help to more clearly define the molecular events underlying regeneration and plastic processes in the brain.

Project in the Schwerpunkt

Growth factors for midbrain dopaminergic neurons
To optimize growth factor-based strategies in the therapy of Parkinson's disease, we have defined the role of growth factors in the development of midbrain dopaminergic neurons. We observed that during early embryonic development, dopaminergic cell survival and differentiation crucially depend on growth factors provided by mesencephalic glia as well as glia of the striatal/cortical target areas. We further obtained evidence that with ongoing maturation, individual dopaminergic neurons are affected by distinct sets of autocrine/paracrine growth factors. Interestingly, these differences in growth factor influences have no consequences for neuronal survival *per se*, but rather seem to play a role in the establishment of functional differences inherent to adult dopaminergic neurons.

Methods Available

Cell and tissue culture techniques
Immunohistochemistry
PCR techniques and in situ hybridization
Biochemical methods including gel electrophoresis and Western blotting

Illustration

Members of the Project Team

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Engele, J., and B. Franke (1996) Cell Tissue Res. 286: 235-240.

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General Research Interest

Molecular and Cellular bases of neuron-glia interactions
Astrocytes play an important role in forming neural tissues, for example by guiding migrating neurons and growth cones to their destination or by designing transient tissue boundaries designed to segregate neuronal assemblies. Glial boundaries might also inhibit regeneration in the form of neural scars which constitute an obstacle to axonal regrowth. In order to investigate the molecular bases of these interactions, we have focused on glial-derived extracellular matrix components and study in more detail tenascin-C glycoproteins, the chondroitin sulfate proteoglycan DSD-1-PG and INA, a proteoglycan-associated activity inhibitory to axonal growth which is derived from a culture model of reactive astrocytes.

Project in the Schwerpunkt

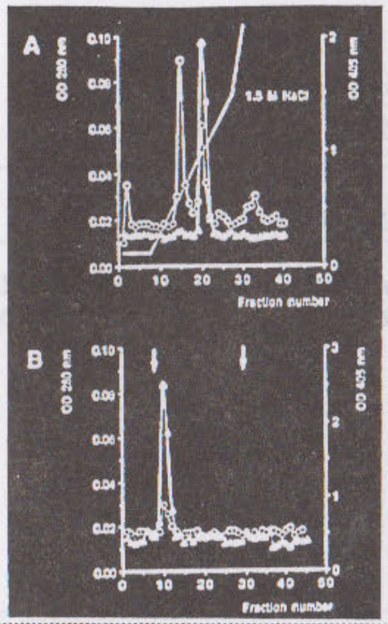
Structural and functional characterisation of DSD-1-PG
The neural chondroitin sulfate proteoglycan DSD-1-PG is transiently expressed on the surface of immature glia during development and at elevated levels after CNS lesion. DSD-1-PG is upregulated by TGF-β's and modulates the motility of an oligodendrocyte progenitor cell line, Oli-neu. The monoclonal antibody 473HD was used to purify DSD-1-PG from postnatal mouse CNS. DSD-1-PG promotes neurite outgrowth from embryonic day 18 hippocampal neurons and this effect involves the epitope of monoclonal antibody 473HD, a specialised glycosaminoglycan. The structural and functional characterisation of the DSD-1-epitope is currentyl being pursued. The proteoglycan core proteins are analysed on the molecular level by an integrated approach involving peptide sequencing and the screening of cDNA expression libraries.

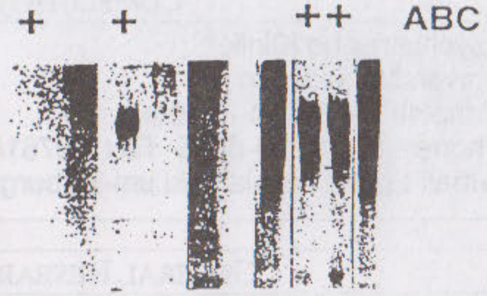
Methods Available

Primary culture of defined neural cell types; in vitro bioassays for determination of cell adhesion, cell repulsion and neurite outgrowth; generation of monoclonal antibodies to neural ECM molecules; purification and biochemical characterisation of neural ECM components and complementary receptors; molecular cloning of neural ECM components, expression of fusion proteins, in situ hybridisation

Illustration

BIOCHEMICAL PROPERTIES OF DSD-1-PG





1000 kD APPARENT Mr

CORE GLYCOPROTEIN
OF 400 kD

DSD-1-EPITOPE IDENTIFIED
BY 473HD

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General Research Interest

mRNA expression and regulation of cytokines / chemokines and their receptors in cultured microglia and in rat brain - as markers of microglial activation stages.

Project in the Schwerpunkt

IL-3 and IL-3 receptor mRNA expression in microglia

Methods Available

Molecular biological and protein chemical methods,
cell cultures, immunocytochemistry,
animal surgery

Illustration

Members of the Project Team

Collaboration with Other Teams

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B. Volk, Freiburg
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General Research Interest

We assess spatiotemporal maturation of brain focused on telencephalic motor centers of song birds in relation to behavioral development. Distinct anatomical features and the fact that one can manipulate its structure render the song control system a very attractive system for analysing functional relation between neurone and glial maturation and its consequences on the organizational rules and on the individual learning. Steroid therapy accelerates and the treatment with steroid receptor blockers inhibits the time schedule for the ontogenetic development.

Project in the Schwerpunkt

The ontogenetic expression of growth promoting and inhibitory molecules in the brain of two song bird species

In the developing CNS of mammals the topography of the expression of cell recognition molecules correlates with key elements (e.g. neuronal migration, process outgrowth and segregation of neuronal assemblies). We study side and time restricted expression of Tenascin, Proteoglycan and the neurite growth inhibitor Ni35/250 during the development in the zebra finch and in the canary. In the adult canaries we study whether neuroneogenesis and glioneogenesis are limited to the vocal control system and if the described changes in neuronal chracteristics might also be paralleled by seasonal alterations in glial gap-junctional coupling.

Methods Available

Immunocytochemistry
Tissue culture, cryoassay
Behavioural development

Illustration

Members of the Project Team

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Collaboration with Other Teams

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General Research Interest

The strategic position of astrocytes between the blood capillaries and the neruons and oligodendrocytes supports the view that astrocytes play a key role in the energy metabolism of the brain. Thus, we were testing our hypothesis that one function of these cells is as processing plants for the generation from various compounds such as amino acids, proteins and fatty acids nitrogen-freee molecules that can be used by other brain cells (neurons, oligodendrocytes) as combustible fuel material. To that end methods of enzymology, immunochemistry, cell culture and histochemistry have been used to test for the presence of such functions in astroglial cultures and the brain.

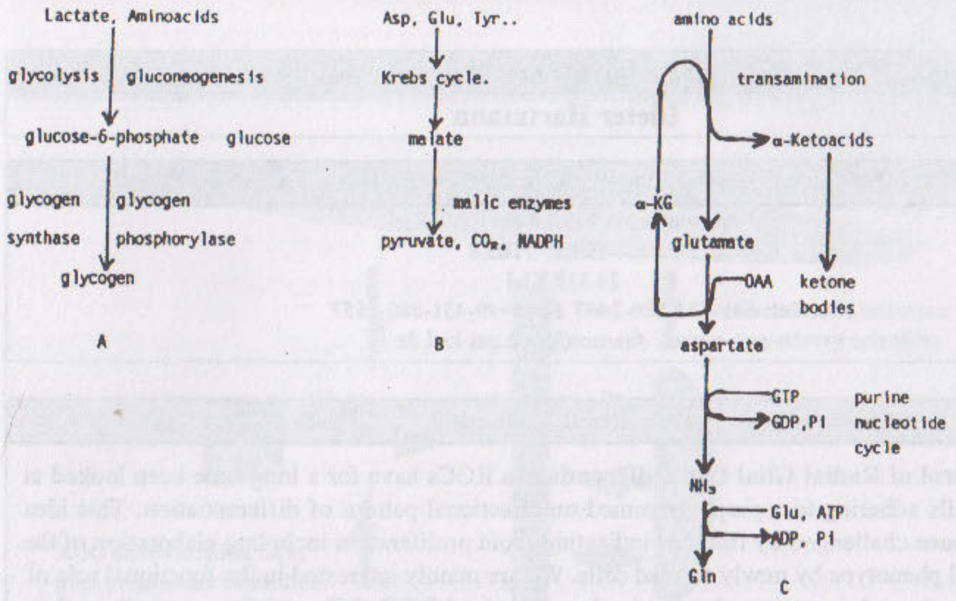
Project in the Schwerpunkt

We have shown that in brain the glycogen degrading enzyme glycogen phosphorylase is present in astrocytes, ependymal cells and sensory neurons. In astroglial cultures gluconeogenesis from various substrates has been demonstrated. Two of the 3 key gluconeogenic enzymes have been immunocytochemically localized in brain astrocytes only. The generation of fuel material from amino acids has been studied by immunocytochemically localizing the cytosolic and the mitochondrial isoforms of malic enzyme in glial cells and neurons, respectively. In addition it has been shown that astroglial cells can degrade the ketogenic amino acid leucine to the ketone bodies acetoacetate and 3-hydroxybutyrate and release them, most likely for being used as fuel molecules by other cell types.

Methods Available

Isolation and characterization of proteins - Enzymological techniques - Generation and characterization of mono- and polyclonal antibodies - Immunocyto - and histochemistry - Cell culture techniques (primary cultures, cell lines) - Studies of transport and metabolism in cultured cells

Illustration



Metabolic pathways in the fields of glycogen metabolism and gluconeogenesis (A), amino acid metabolism via Krebs cycle intermediates for the generation of i) pyruvate via malic enzyme (B) and ii) ketone bodies and ammonia (disposed of as Gln) (C).

Members of the Project Team

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Collaboration with Other Teams

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Schmoll, D., Führmann, E., Gebhardt, R. & Hamprecht, B. (1995) Eur. J. Biochem., 227: 308-315.
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General Research Interest

Control of Radial Glial Cell Differentiation RGCs have for a long time been looked at as cells adhering to a preprogrammed unidirectional pattern of differentiation. This idea has been challenged by findings indicating rapid proliferation including elaboration of the radial phenotype by newly formed cells. We are mainly interested in the functional role of mesenchymal / meningeal cells in the control of RGC differentiation as well as their influence on neuronal migration. **Glia cells in Neuropathology** Analysis of Arylsulfatase A knock-out mice as a model for human Metachromatic Leukodystrophy. **Ontogenesis of Microglia** Influence of epithelial cells on the development of phagocyte phenotypes

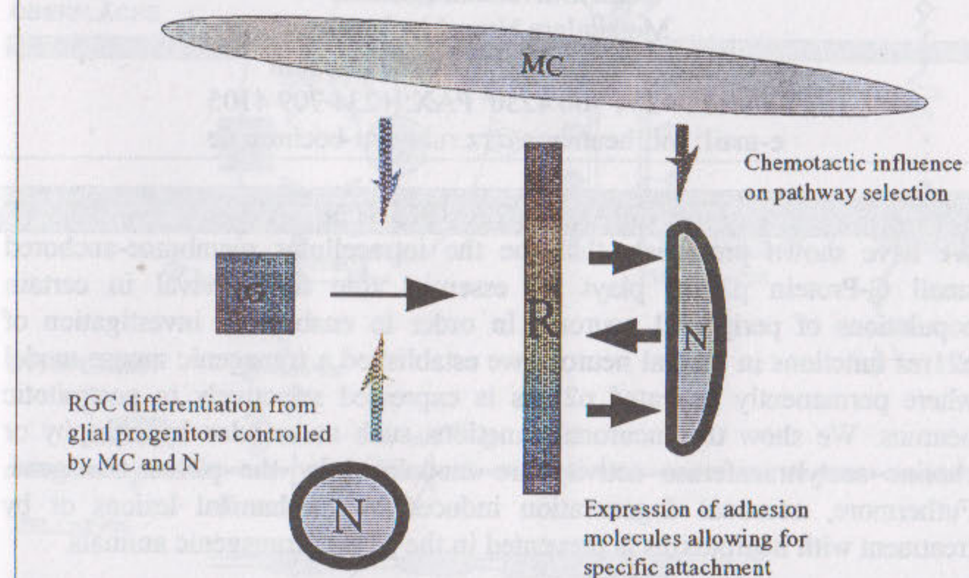
Project in the Schwerpunkt

Cell Adhesion and Differentiation of Radial Glia Radial glia cell differentiation is controlled by both immature neurons as well as meningeal fibroblasts. Specifically, fibroblasts induce expression of RGC-specific antigens such as RC 2 by diffusible factor(s) acting via protein kinase C and also stimulate radial differentiation in Bergmann glia progenitors during a critical ontogenetic period. In *reeler* mutant mice, the absence of an ECM protein secreted by early developing neurons not only impairs morphological differentiation of RGCs , but also seems to alter the expression of specific cell adhesion systems resulting in a pathologically increased neuron-glia adhesion. Here, we are currently investigating the function of an ectoenzyme, ß1,4-galactosyltransferase, in the establishment of transient 'migratory' cell contacts.

Methods Available

Cell and tissue culture
In vitro time lapse videography under incubator conditions
including real-time image processing
Image analysis
Immunohistochemistry

Illustration



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Hess, B; Saftig, P; Hartmann, D; Coenen, R; Lüllmann-Rauch, R; Goebel, H; Evers, M; von Figura, K; d'Hooge, R; Nagels, G; DeDeyn, P; Peters, C; Gieselmann, V (1996) PNAS 93 : 14821 - 14826
Wilms, H; Hartmann, D; Sievers, J (1997) Cell Tiss Res. 287 : 447 - 458

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General Research Interest

We have shown previously that the the intracellular membrane-anchored small G-Protein p21ras plays an essential role for survival in certain populations of peripheral neurons. In order to enable the investigation of p21ras functions in central neurons we established a transgenic mouse model where permanently activated p21ras is expressed selectively in postmitotic neurons. We show that neuronal functions such as cellular hypertrophy or choline acetyltransferase activity are modulated by the p21ras-transgene. Futhermore, neuronal degeneration induced by mechanical lesions or by treatment with neurotoxins is prevented in the p21ras-transgenic animals.

Project in the Schwerpunkt

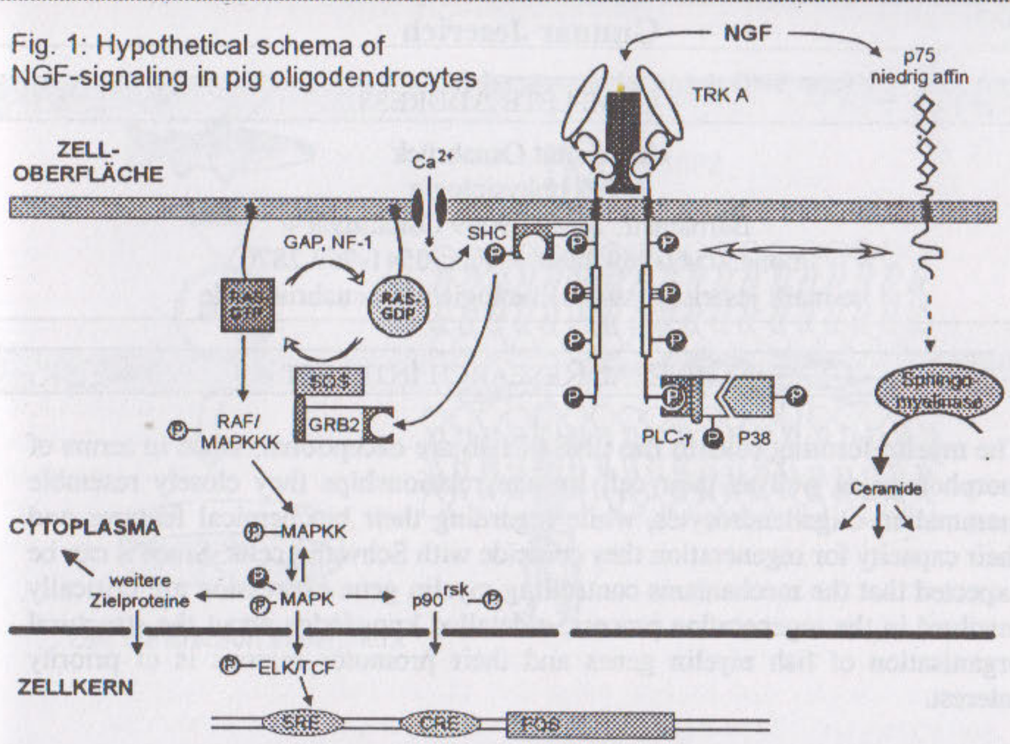
Cultured galactocerebroside positive oligodendrocytes derived from the adult pig carry both trkA tyrosine kinase receptors and neurotrophin low affinity receptors (p75NTR). Althaus et al. have demonstrated previously that these oligodendrocytes respond to nerve growth factor by enhancement of fiber outgrowth. We show that trkA mRNA levels are 2.5-fold increased in cultured oligodendrocytes by treatment with 50 ng/ml NGF for 6 days while levels of p75NTR mRNA are decreased, slightely. The intracellular signaling cascade evoked by nerve growth factor may involve activation of p21ras and mitogen activated kinase suggesting a mechanism similar to that described for central neurons.

Methods Available

Primary cell cultures of glia and neurons
Methods for intracellular application of proteins and recombinant DNA
Molecular biology methods: Two hybrid system. Phage display.
Transgenic animals. Autapses in single cell cultures of hippocampal neurons.

Illustration

Fig. 1: Hypothetical schema of NGF-signaling in pig oligodendrocytes



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General Research Interest

The myelin-forming cells in the CNS of fish are exceptional, since in terms of morphology as well as their cell lineage relationships they closely resemble mammalian oligodendrocytes, while regarding their biochemical features and their capacity for regeneration they coincide with Schwann cells. Since it can be expected that the mechanisms controlling myelin gene expression are critically involved in the regeneration process, a detailed knowledge about the structural organisation of fish myelin genes and their promotor regions is of priority interest.

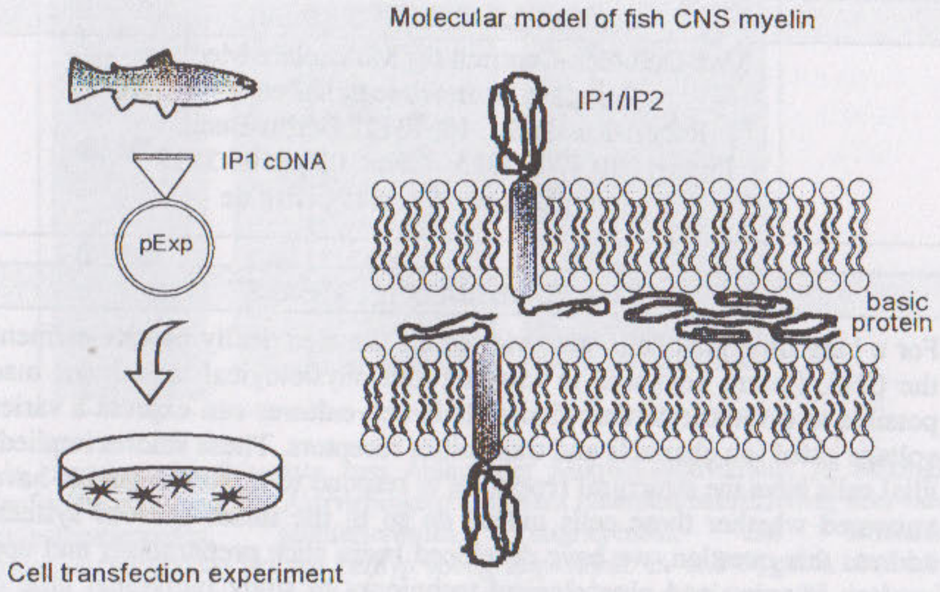
Project in the Schwerpunkt

Molecular structure of genes coding for fish myelin proteins
The cDNAs encoding the major structural proteins of trout CNS myelin (IP1/IP2 and 36K) were cloned and sequenced. Thereby a striking similarity between the two IP-isoforms and the major mammalian PNS myelin glycoprotein Po was revealed, while in the case of 36K no significant homology with any of the known mammalian myelin proteins was detected. To investigate the functional properties of these fish myelin proteins cell transfection experiments are currently being performed. Furthermore the 5'-flanking regions of the corresponding genes are comparatively analysed to identify *cis*-acting elements potentially involved in gene regulation.

Methods Available

Molecular cloning and sequencing
In situ hybridization, immunocytochemistry
Cell and tissue culture techniques
Cell transfection, single cell PCR
Patch-clamp analysis on cells and brain slices

Illustration



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5 References

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General Research Interest

For a long time glial cells were viewed als the electrically passive elements of the CNS. Recent advances in imaging and physiological techniques made it possible to demonstrate that glial cells in cell cultures can express a variety of voltage gated ion channels and transmitter receptors. These studies implied that glial cells have the structural repertoire to respond to rapid signals but have not answered whether these cells indeed do so in the intact nervous system. To address this question, we have developed brain slice preparations and applied modern imaging and physiological techniques to study individual glial cells. We can identify and analyse properties of microglial cells, oligodendrocytes and astrocytes. The receptors properties are distinct of those of neurons.

Project in the Schwerpunkt

Physiological Properties of Microglial Cells

Microglial cells are considered as a resident macrophage population in the CNS. We have studied the physiological properties of microglial cells in culture; based on their membrane channel pattern, we could distinguish these cells not only from macroglial cells and neurons, but also from non-brain derived macrophages.

The ameboid microglial cells on the acute brain slice show a characteristic 'behavioural' repertoire such as phagocytosis and defined movements. One aim of the present project was to characterize these behaviours and determine its pharmacological profile.

Methods Available

Path clamp technique
Tissue cultures and brain slices
Fluorescence measurement combined with imaging techniques
Reconstruction of cells with confocal microscopy
Video time lapse recording
Optical tweezer

Illustration

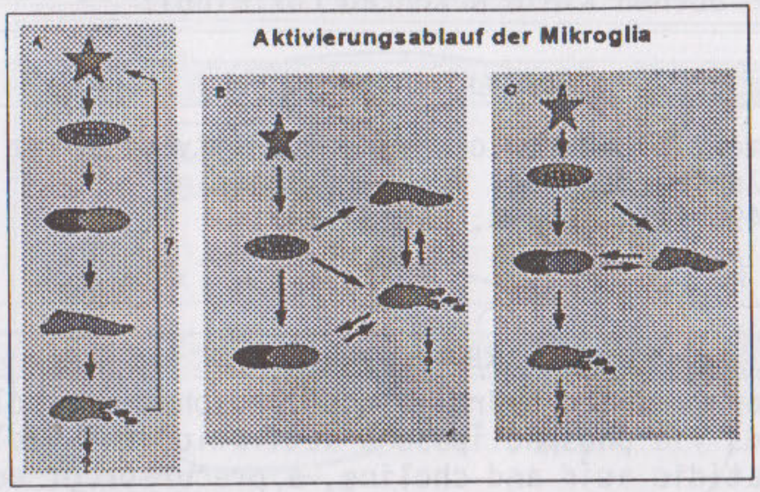

Abb. 1: Hypothetische Schemata zum Ablauf der Mikrogliaaktivierung; A. Mikroglia durchlaufen die verschiedenen Aktivierungsschritte vom ruhenden/ramifizierten, über einen Antigen-präsentierenden, proliferierenden, migrierenden und schließlich phagozytotisch/zytotoxisch aktiven Zelltyp streng sequentiell. B. und C. geben alternative Abläufe wieder, in denen parallele Aktivierungszustände existieren, die ineinander übergehen können.

Members of the Project Team

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Andreas Reichenbach, Leipzig
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General Research Interest

Receptor-mediated hydrolysis of phosphatidylcholine proceeds via phospholipase D activation and yields phosphatidic acid and choline, a precursor of acetylcholine synthesis. Phosphatidic acid is a lipid second messenger in its own right and can be hydrolyzed to diacylglycerol; both lipids can activate protein kinase C isoforms. Our laboratory has been involved in the regulation and function of PLD activity in neural tissue, using brain slices, astrocytes, and synaptosomes.

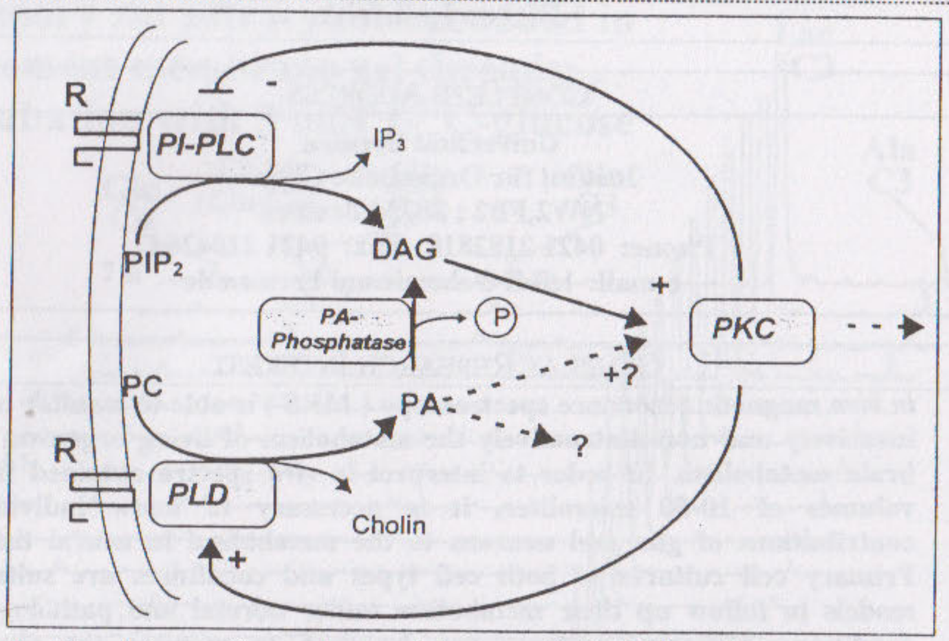
Project in the Schwerpunkt

Receptor-mediated activation of phospholipase D in primary astrocytes The project concentrates on three signals which activate PLD: noradrenaline, phorbol esters, and serum. The adrenergic PLD activation is characterized with respect to receptor subtype (alpha1) and its possible relationship to mitogenic signalling. In addition, we use bacterial toxins to probe the involvement of small G proteins in adrenergic PLD activation.

Methods Available

Primary astrocyte culture, brain slices, synaptosomes
Measurement of phospholipase activities and choline-containing metabolites
Microdialysis and HPLC determination of acetylcholine

Illustration



Activation of protein kinase C (PKC) subtypes by lipid second messengers formed from phosphoinositides via PI-PLC and from phosphatidylcholine via PLD.

Members of the Project Team

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Collaboration with Other Teams

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Ronald Gonzalez, San Jose
Moti Liscovitch, Rehovot

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Gonzalez R., Löffelholz K. and Klein J. (1996) Neurosci. Lett. 219, 53-56.

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General Research Interest

in vivo **magnetic resonance spectroscopy (MRS) is able to monitor non-invasively and non-destructively the metabolism of living organism i.e. brain metabolism. In order to interpret *in vivo* spectra obtained from volumes of 10-50 microliter, it is necessary to know individual contributions of glia and neurons to the metabolism in neural tissue. Primary cell cultures of both cell types and cocultures are suitable models to follow up their metabolism under normal and pathological conditions. Different isotopes can be used to monitor the energy metabolism, fluxes through various enzyme complexes (glycolysis, TCA cycle etc.), metabolic pathways, cell volume, intracellular calcium and others.**

Project in the Schwerpunkt

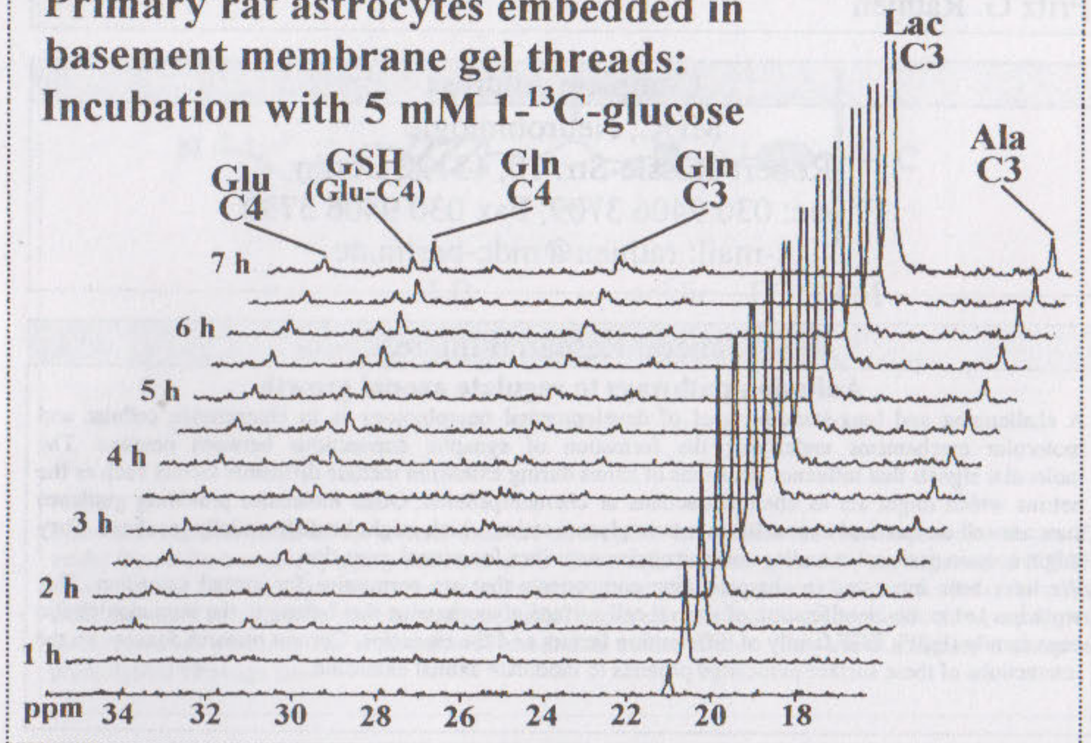
Metabolism of primary astrocytes
Astroglia cells are an essential part of the metabolic trafficking between astrocytes and neurons. They also maintain the homeostasis of various metabolite concentrations in the intercellular space.
Cytotoxic edemas are a major complication in focal brain ischemia. In this context the mechanism of volume regulation in glia cells is important to overcome such episodes.
NMR spectroscopy on cell extracts and living cells is used to monitor these processes.

Methods Available

Magnetic resonance imaging with various contrasts
in vivo magnetic resonance spectroscopy
cell cultures
mass spectroscopy
measurement of cell volumes by diffusion nmr

Illustration

Primary rat astrocytes embedded in basement membrane gel threads: Incubation with 5 mM 1-^{13}C-glucose



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5 References

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General Research Interest

Adhesion pathways to regulate axonal growth

A challenging and long-standing goal of developmental neurobiology is to characterize cellular and molecular mechanisms underlying the formation of synaptic connections between neurons. The molecular signals that influence decisions of axons during extension include diffusible factors such as the netrins which might act as chemoattractants or chemorepellents. Other molecules providing guidance cues are cell surface and extracellular matrix glycoproteins which might be differentially localized. They might contain permissive and/or non-permissive activities for axonal extension.

We have been interested in characterizing components that are permissive for axonal extension. This work has led to the identification of several cell surface glycoproteins that belong to the immunoglobulin superfamily (IgSF), EGF family of differention factors and the tenascins. Current research focuses on the interactions of these surface associated proteins to modulate axonal extension.

Project in the Schwerpunkt

Characterization of tenascin-R and CALEB

Tenascin-R is a homotrimeric extracellular matrix glycoprotein synthesized by glial cells and neurons in the developing nervous system. The interaction of tenascin-R with the IgSF member F11 in in vitro cultures enhanced F11 mediated neurite outgrowth and results in an enlargement of the surface area of growth cones. These results suggest that tenascin-R might modulate F11 mediated axon extension.

In order to identify proteins interacting with tenascin-R we have combined the sensitivity of binding detection in ELISAs with immunological screens. The application of these steps resulted in the identification of a novel member of the EGF family of differention factors which we termed CALEB. CALEB is associated with glial and neuronal surfaces.

Methods available

Molecular biology, Cell culture techniques
Protein biochemistry
Neurite outgrowth assay

Illustration

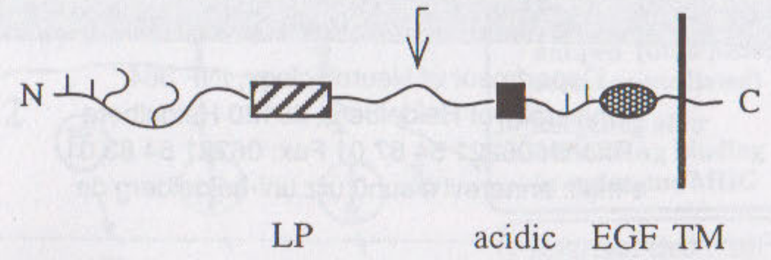

Fig. 1
Schematic representation of the overall structure of CALEB. LP denotes the leucine/proline rich motif, acidic the acidic box that is highly enriched in the amino acid residues glutamate and aspartate and EGF stands for the EGF-like domain. A transmembrane segment (TM) separates these extracellular domains from the short cytoplasmic stretch. Vertical dashes represent potential N-glycosylation sites, squiggles mark potential positions for attachment of chodroitin sulfate chains. The arrow indicates a putative proteolytical cleavage site.

Members of the Project Team

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5 References

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General Research Interest

Immune reactions develop in the brain following either internal or external stimuli. Astrocytes and microglia possess functional features of antigen presenting cells: regulated surface expression of MHC class II molecules, stimulation of antigen-specific T lymphocytes, secretion of cytokines. However the precise role of each of these cells in the initiation and/or maintenance of the brain immune response is not yet understood. We address this question by investigating the membrane traffic that underlies the immune competence of these cells and the modulatory effects of immune activation on this traffic.

Project in the Schwerpunkt

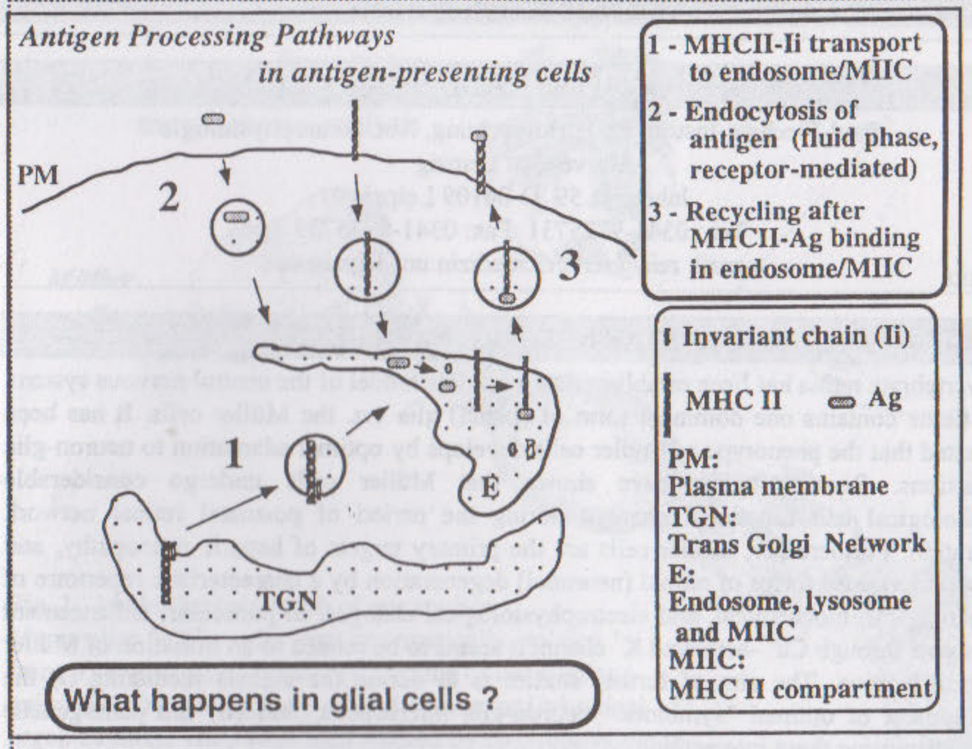
Comparative analysis of the antigen processing and presenting capacities of astrocytes and microglia

Using an established antigenic model for T cell stimulation, we are currently evaluating the capacities of immune(cytokines)-activated astrocytes and microglia to process and present the model antigen to specific T lymphocytes, i.e. the capacities of these cells to build an "antigenic repertoire" from a given antigen. In parallel, we want to analyse the intracellular compartments through which this specific antigen and the antigen-MHC class II molecule complex travel.

Methods Available

Primary cultures of murine astrocytes and microglia
Antigen specific T cell activation
Cell labelling and subcellular fractionation of glial cells
Immunoprecipitation and Immunoblotting
Immunofluorescence and electron microscopy analyses

Illustration



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General Research Interest

The vertebrate retina has been established as a suitable model of the central nervous system. This tissue contains one dominant form of (radial) glia viz. the Müller cells. It has been suggested that the phenotype of Müller cells develops by optimal adaptation to neuron-glia interactions. Recent studies have shown that Müller cells undergo considerable morphological and functional changes during the period of postnatal retinal network maturation. Furthermore, Müller cells are the primary targets of hepatic retinopathy, and respond to various forms of retinal (neuronal) degeneration by a characteristic repertoire of morphological, biochemical, and electrophysiological changes. In particular, enhancement of currents through Ca^{2+}-activated K^+ channels seems to be related to an initiation of Müller cell proliferation. The aim of further studies is to define the signals mediating (i) the development of optimal "symbiotic" neuron-glia interactions, and (ii) the pathogenetic events disrupting these interactions.

Project in the Schwerpunkt

Types and Modulation of Ion Channels, Transmitter Receptors, and Amino Acid Transporters in Glial (Müller) Cells

We have studied the physiological properties of Müller cells from various mammalian species including primates and man. Our studies have shown that Müller cells express at least four different types of voltage-dependent K^+ channels (inward-rectifiers, transient A type channels, delayed rectifiers, and large conductance, Ca^{2+}-activated channels), neuron-type Na^+ channels, electrogenic GABA- and glutamate uptake mechanisms, and GABA A receptors. We have observed that the pattern of channel expression changes dramatically during postnatal develop-ment, as well as in cases of retinal pathology. The aim of the present project was to characterize these patterns of normal and altered channel expression, and to begin with the search for candidate signals mediating their development and pathophysiology.

Methods Available

Single-channel and whole-cell patch-clamp studies on isolated cells
Cell and organ culture techniques
Retroviral vector-mediated clonal analysis
Fluorescence measurement combined with imaging techniques
Use of retinal wholemounts to analyze neuron-glia interactions
Immunocytochemistry

Illustration

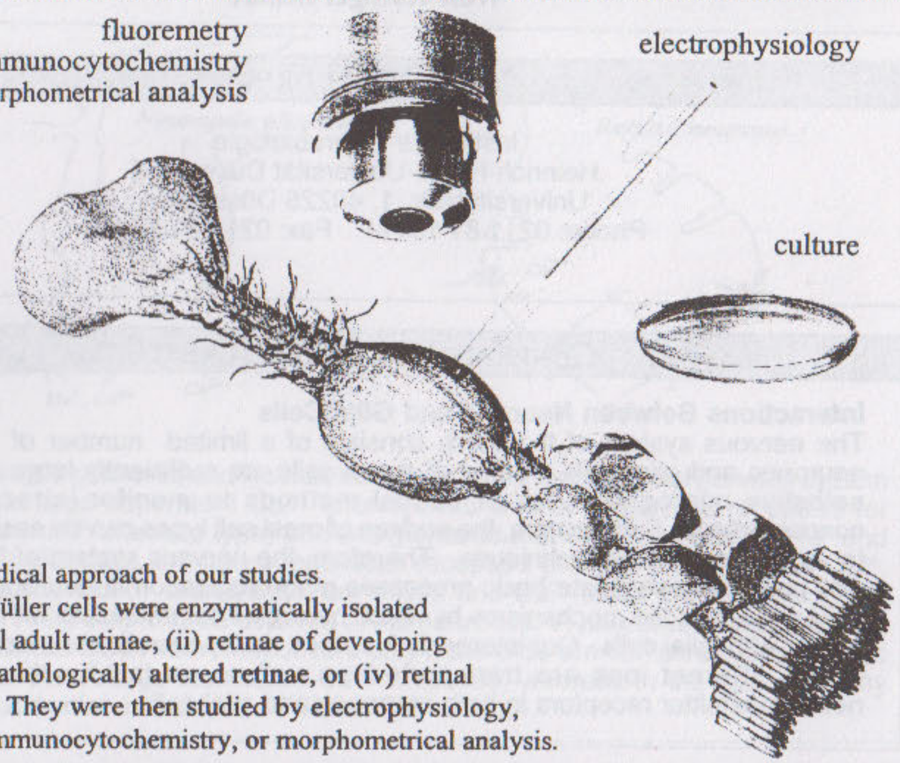

Fig. 1: Methodical approach of our studies. Mammalian Müller cells were enzymatically isolated from (i) normal adult retinae, (ii) retinae of developing animals, (iii) pathologically altered retinae, or (iv) retinal organ cultures. They were then studied by electrophysiology, fluorometry, immunocytochemistry, or morphometrical analysis.

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General Research Interest

Interactions Between Neurons and Glial Cells
The nervous system of the leech consists of a limited number of identifiable neurones and glial cells. Some of these cells are sufficiently large to use ion-selective microelectrodes or optical methods to monitor intracellular ion concentrations. Furthermore, the surface of most cell types can be easily exposed to apply patch-clamp techniques. Therefore, the nervous system of the leech is well suited to investigate basic processes of ion regulation in neurones and glial cells as well as the mechanisms by which neurones communicate with each other and/or with glial cells. Our interest is focussed on the membrane mechanisms by which different ions are transported as well as on the functional role of neurotransmitter receptors in both neurones and glial cells.

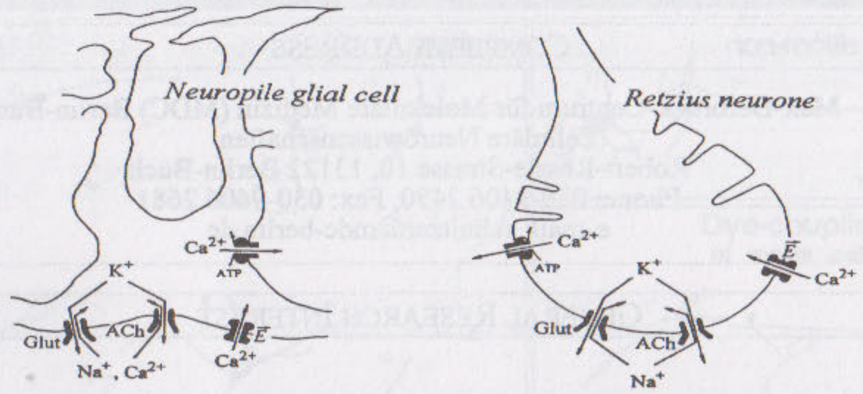
Project in the Schwerpunkt

Functional Role of Neurotransmitters in Intercellular Communication
In recent years we have characterized receptors for neurotransmitters such as serotonin, acetylcholine, and glutamate in leech neurones and glial cells. These receptors are associated with ion channels and their stimulation evokes marked changes in the intra- and extracelluar concentrations of various ion species. The neurotransmitter receptors are not homogeneously distributed: neuropile glial cells as well as motoneurones and several other neurones possess receptors for glutamate, while such receptors are lacking in mechanosensory neurones. By using electrophysiological and microfluorimetric techniques we investigate the mechanisms by which neurotransmitters change intracellular ion concentrations. We are mapping the distribution of neurotransmitter receptors in the leech nervous system to get more insight into their role in neuron-glial interaction.

Methods Available

Conventional electrophysiology
Ion-selective microelectrodes
Patch-clamp technique
Simultaneous microfluorimetric monitoring of two different ion species
Tissue cultures

Illustration



Both neuropile glial cells and Retzius neurones in the leech central nervous system possess voltage-dependent Ca^{2+} channels as well as ionotropic receptors for glutamate (AMPA/kainate type) and acetylcholine that are permeable to Na^+ and K^+. In the glial cells, the neurotransmitter receptors are also permeable to Ca^{2+}. Therefore, the loci of neurotransmitter action and Ca^{2+} influx are identical in the glial cells, while they may be different in the neurones. A neurotransmitter-induced Ca^{2+} influx into leech neurones occurs only if the cells are sufficiently depolarized to activate the voltage-dependent Ca^{2+} channels, whereas in the glial cells any receptor activation is associated with a Ca^{2+} influx.

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General Research Interest

During ontogenesis of the nervous system oligodendrocytes develop from precursor cells that originate from the subventricular zone. These precursor cells migrate over long distances to their final target regions where they differentiate into myelin-forming cells. Whereas neuronal migration has been studied extensively, the migration of oligodendrocyte precursor cells is still poorly understood. The aim of our project was to analyse and understand the properties of migrating precursor cells *in vitro* and in brain slices.

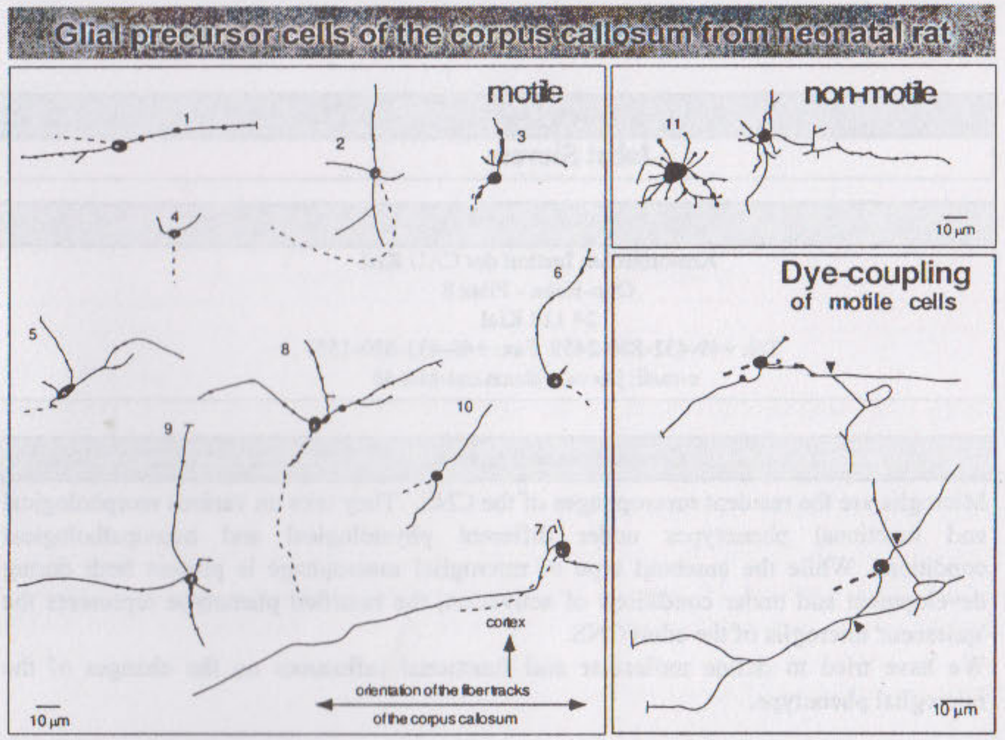
Project in the Schwerpunkt

Analysis of motile oligodendrocyte precursor cells *in vitro* and in brain slices
We characterized motile oligodendrocyte precursor cells in single cell cultures and in brain slices by video time lapse microscopy. Motility analysis was combined with electrophysiological, immunological, and morphological characterization of cells. *In situ* glial progenitor cells did not migrate along axon fascicles of the corpus callosum but rather traversed the fibers with an overall direction towards the cortex. Some motile progenitor cells *in situ* were dye-coupled to yet unidentified cells as revealed after Lucifer Yellow filling. The majority of motile cells *in vitro* expressed the A2B5 epitope. They showed saltatory movements as in brain slices. Substrate-coated laminin enhanced their motility. Strongly O4-positive cells were no longer motile. Our model systems are potent tools to investigate different aspects of oligodendrocyte precursor cell motility.

Methods Available

single cell and co-cultures
brain slices
video time lapse microscopy
Ca-imaging
immunocytochemistry
patch clamp technique

Illustration



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General Research Interest

Microglia are the resident macrophages of the CNS. They take on various morphological and functional phenotypes under different physiological and neuropathological conditions. While the ameboid type of microglial macrophage is present both during development and under conditions of activation, the ramified phenotype represents the 'quiescent' microglia of the adult CNS.
We have tried to define molecular and functional influences on the changes of the microglial phenotype.

Project in the Schwerpunkt

Influences of astrocytes and other epithelial cells on the morphology and membrane channels of microglia cells in vitro

When grown in coculture with astrocytes, ameboid microglia take on the ramified phenotype and a characteristic pattern of membrane currents, the most conspicuous of which is a Na^+-current. When monocytes and macrophages are grown under similar conditions, they take on the same morphological and electrophysiological characteristics. When microglia and other myelomonocytic cells are grown on monolayers of hepatoma, kidney epithelial or keratocyte cell lines, they again take on the ramified phenotype and develop the microglia-specific pattern of membrane currents. Molecular factors directing the induction of this transformation reside in the epithelial extracellular matrix.

Methods Available

Cell and tissue culture
In vitro time lapse videography under incubator conditions
including real-time image processing
Image analysis
Immunohistochemistry
Patch-clamp analysis

Illustration

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General Research Interest

There is a marked difference in the response to injury in the CNS of cold- and warmblooded vertebrates. We are interested in the cellular and molecular mechanisms underlying the success of axonal regeneration and repair of lesioned fiber tracts in the CNS of fish and frogs.

Axon regrowth in the fish visual system is accompanied by upregulation in all axotomized neurons of sets of growth associated proteins which support axon growth. This demonstrates the competence of these neurons to reactivate their growth programms. Tests with mammalian CNS neurons indicate that only some produce a subset of the relevant proteins found in fish.

Project in the Schwerpunkt

Properties of oligodendrocytes. Adaptive plasticity: Oligodendrocytes in the fish and Xenopus visual system are free of neurite growth inhibitors (NI) typical of mammals. Upon lesion they dedifferentiate, produce growth supportive molecules (in parallel with axonal regeneration), but finally synthesize advanced myelin markers to remyelinate the regenerated axons.
In the Xenopus spinal cord, however, where axon regeneration fails to occur, oligodendrocytes do possess NI and fail to undergo these changes.
Exposure of mammalian oligodendrocytes to PDGF over 5-7 days caused a dramatic change in their growth inhibitory properties (and NI): Growth cones crossed them freely. Removal of PDGF, however, lead to the re-appearance of inhbitors eliciting growth cone collapse.

Methods Available

Glia cell and neuronal cultures
Time lapse videomicroscopy
Immuno EM
Surgery and in vivo regeneration
In situ-hybridization and molecular clonin

Illustration

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General Research Interest

Oligodendrocytes develop from as yet poorly defined precursor cells in the subventricular zone of the brain and spinal cord. These precursors are proliferative and migrate to the neuron tracts to be myelinated. The process of myelination can be divided into several distinct phases (see Figure). Although the main myelin proteins are well characterised, the surface antigens expressed by oligodendrocyte precursor cells and the molecules regulating their migration to and interaction with myelination-competent neurones are still largely unknown. Our aim is to define these molecules and to characterise and clarify surface molecules regulating the early stages of commitment to the oligodendrocyte lineage.

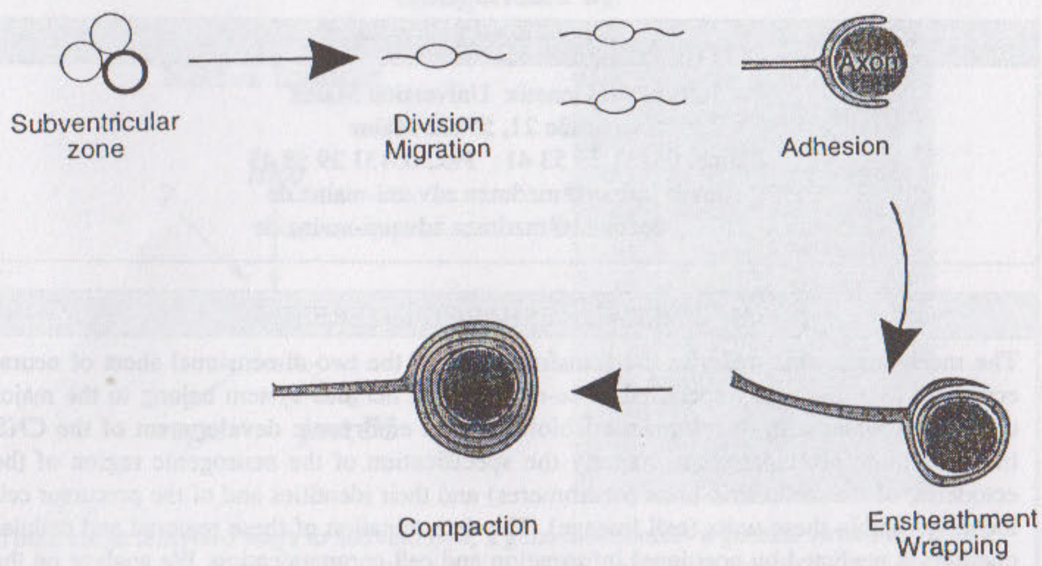
Project in the Schwerpunkt

Several monoclonal antibodies have been generated against lectin-binding glycoprotein fractions isolated from an immortalised line of oligodendrocyte progenitor cells. Two antigens have been studied in more detail. Both bind peanut lectin. One antibody defines a novel surface glycoprotein expressed by oligodendrocyte progenitor cells that is down regulated as the cells differentiate and express myelin proteins (Niehaus et al., submitted). It is also expressed by nestin-positive progenitor cells at embryonic stages. The differentiation potential of these cells (restricted or multipotential?) is under study. Subpopulations of Schwann cells also express the antigen in the developing and adult PNS. The second antigen is expressed by subpopulations of more mature oligodendrocytes.

Methods Available

Tissue culture of primary neural cells
Establishment of immortalised cell lines
Standard biochemical techniques
Screening of cDNA libraries
In vitro assays (e.g. adhesion, migration)

Illustration



Scheme showing the different phases of myelination

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General Research Interest

The mechanisms that underlie the transformation of the two-dimensional sheet of neural ectoderm into the highly specified three-dimensional nervous system belong to the major unsolved problems in developmental biology. The embryonic development of the CNS involves three principal steps, namely the specification of the neurogenic region of the ectoderm, of the metameric units (neuromeres) and their identities and of the precursor cell identities within these units (cell lineage). The determination of these regional and cellular qualities is mediated by positional information and cell-communication. We analyze on the cellular as well as molecular level aspects of these processes during early CNS development of Drosophila.

Project in the Schwerpunkt

Characterization of CNS glial cells and their origin
As a basis for the investigation of glial development we described the whole population of glial cells using own generated enhancer trap lines in conjunction with other markers. Using DiI as a lineage tracer we were able to link most of the glial cells to specific precursors.

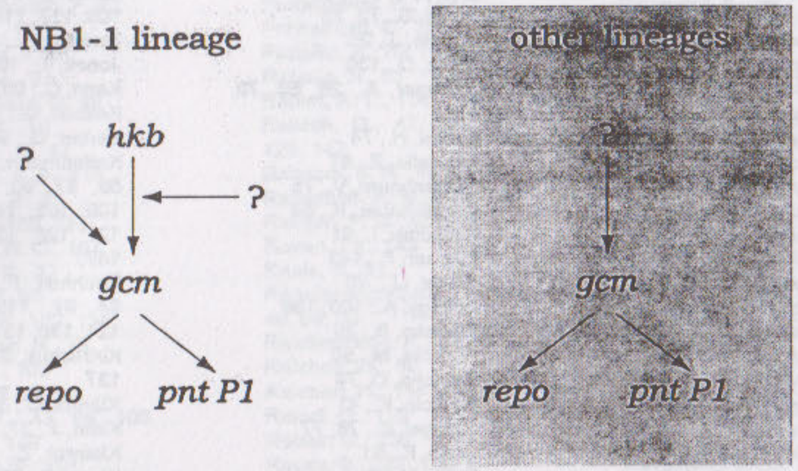
Cloning and characterization of *repo*, a gliaspecific homeoboxgene
Starting from a P-element insertion we identified this gene as an important factor for glial differentiation. It seems to be a direct traget of *gcm*, a recently identified gene conferring a molecular switch towards glial cell fate. In addition we investigated mechanisms leading to the actibvation of *gcm*. (see figure).

Methods Available

Molecular genetics, Genetics, Mutagenesis, Enhancer Trap techique, Germ line transformation, In vivo lineage tracing using DiI, Cell transplantation, Primary culture of single cells, time lapse videomicroscopy and image processing, 3D reconstructions, Immunocytochemistry.

Illustration

Gliogenesis in



There are at least two ways to activate *gcm*, a gene that confers a general switch towards a glial cell fate. One is dependent on the activitiy of the zink finger protein Huckebein (Hkb) and is used only in the lineage of NB 1-1. Judged on genetic data as well as on the expression pattern of Hkb we postulate additional factors which act either in parallel or in conjunction with Hkb leading to *gcm* activation in particular NB 1-1 progeny. Gcm in all lineages subsequently regulates the expression of gliaspecific downstream genes like *repo* and *pnt P1*.

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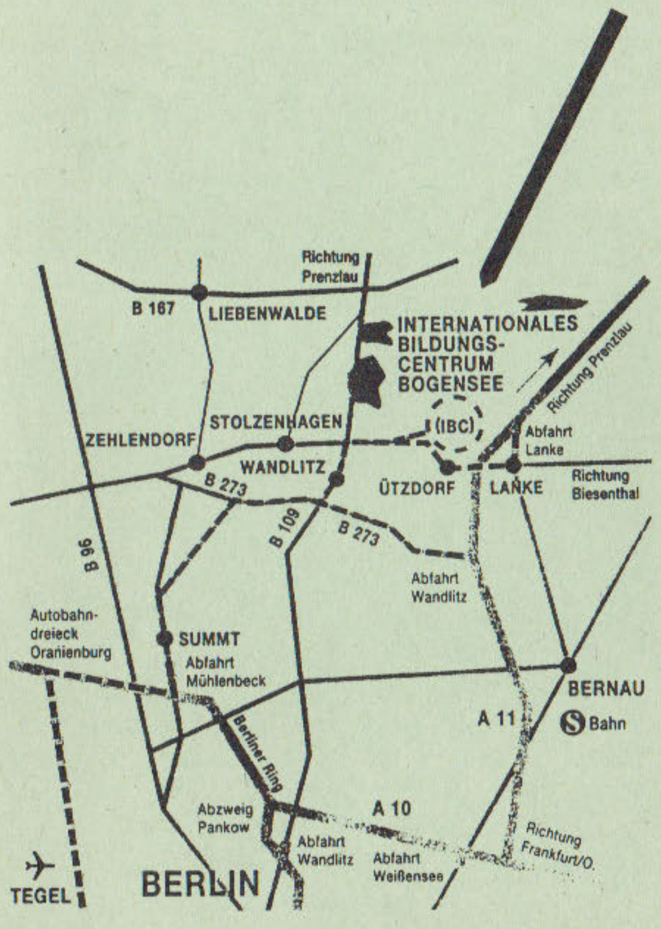
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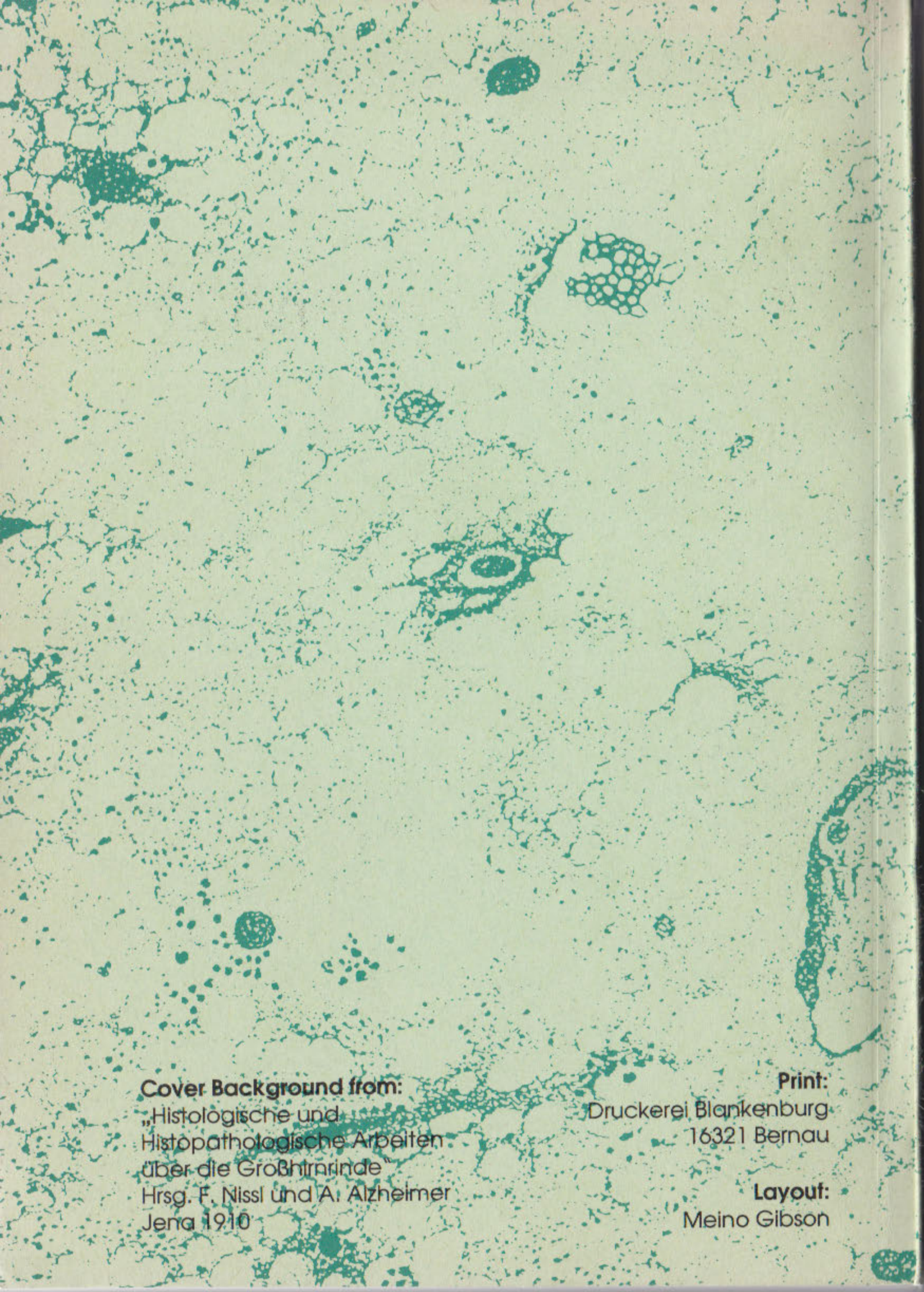

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