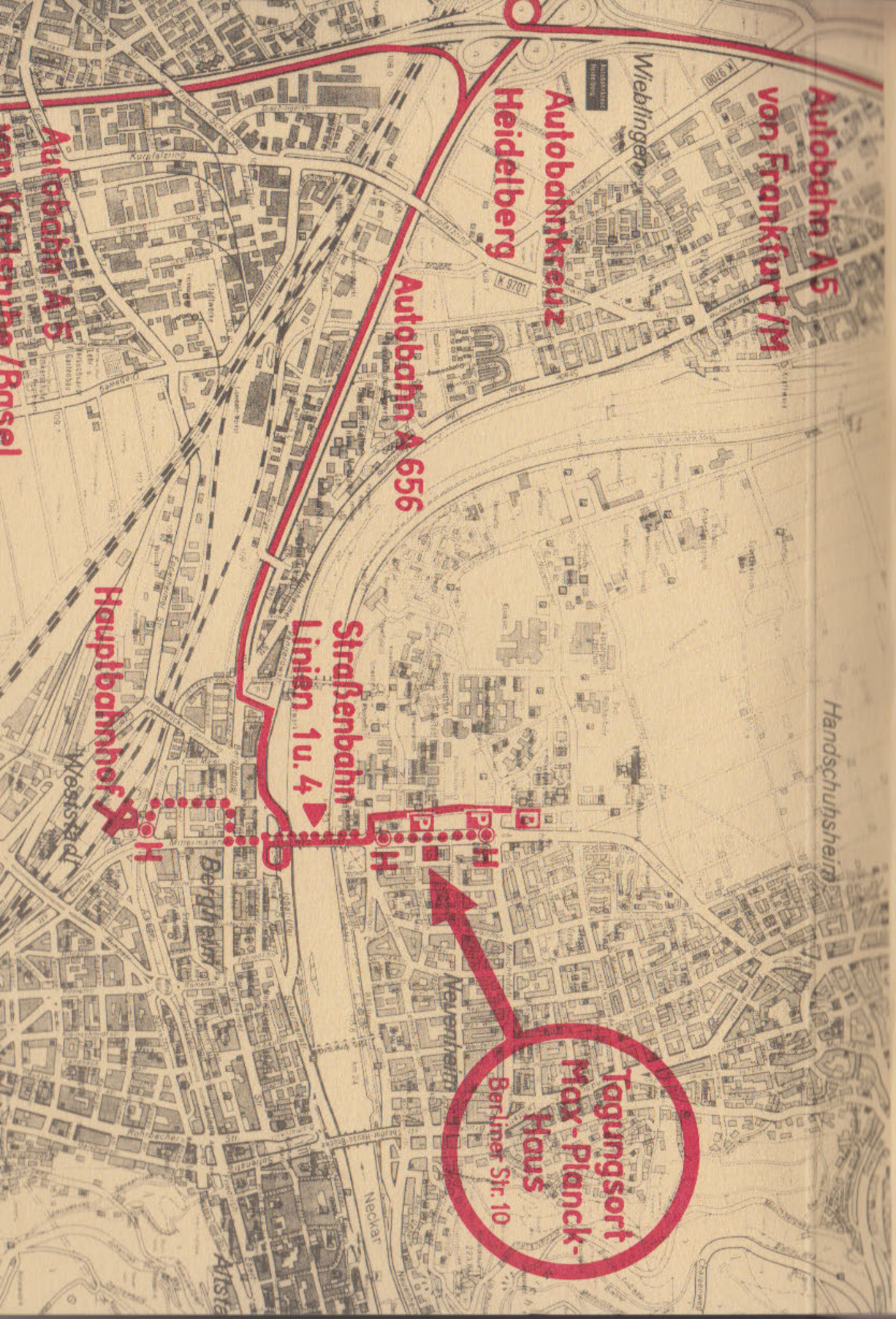

FIRST

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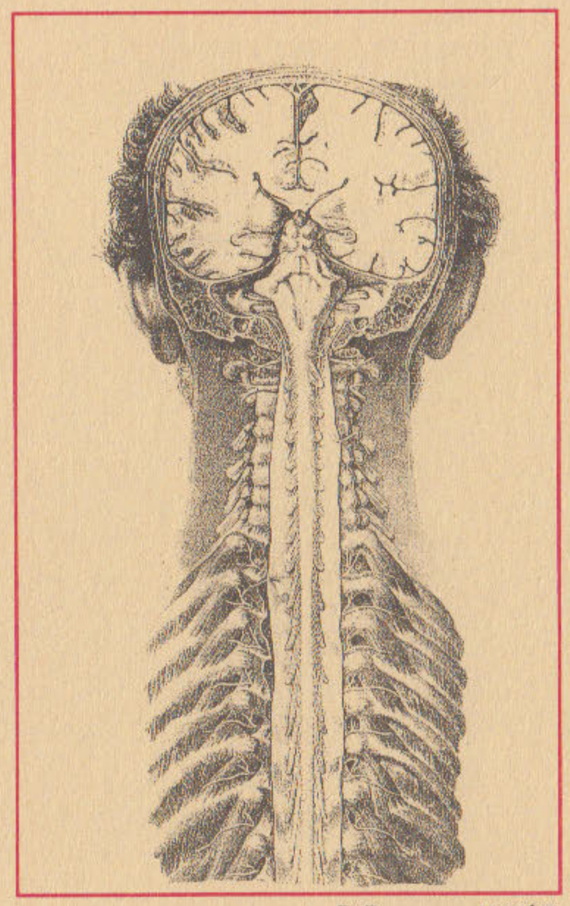
OF THE DFG-SCHWERPUNKT

FUNCTIONS OF GLIAL CELLS

TOPIC:

REGENERATION



B. Bourgery, 1866/67

HEIDELBERG, MAY 7 - 9, 1992

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General Information

Office Hours:	Thursday, May 7	16.00 - 20.00
	Friday, May 8	8.30 - 20.00
	Saturday, May 9	8.30 - 13.00

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Organizers

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Robert-Rössle-Str. 10
O-1115 Berlin-Buch
Phone: 0037 2 946 3278
Fax: 0037 2 949 7

Thursday, May 7 , 1992

16.00 - 18.30 **Registration**

18.30 - 19.30 **Key note feature**

Chairman : G. W. Kreutzberg

K. Beyreuther, Center for Molecular Biology, University of Heidelberg
The Alzheimer amyloid ßA4 and its precursor in health, disease and aging

19.30 **Informal Get-Together**

Friday, May 8, 1992

9.00 - 10.30 **Lectures and Short Communications**
Chairmen: B. Hamprecht & W.- R. Schlue

J. Zimmer, PharmaBiotec, Institute for Neurobiology, University of Aarhus
Axonal growth and regeneration studied in intracerebral neural transplants and organotypic slice cultures

S. Thanos, Department of Ophthalmology, University of Tübingen
Adult retinofugal axons regenerating through peripheral nerve grafts can restore the light-induced pupilloconstriction reflex

J. Scherer, Max Planck Institute for Brain Research, Frankfurt/Main
Postnatal transection of rabbit optic nerve does not lead to an altered astrocyte and microglial cell topography in the mature retina

M. Bähr, Max Planck Insitute for Developmental Biology, Tübingen
Support or inhibition? Influences of glia on survival and axon growth

10.30 - 11.00 **Coffee Break**

11.00 - 12.05 **Lectures and short communications**
Chairmen: A. Oksche & G. Brückner

G. Raivich, Max Planck Institute for Psychiatry, Martinsried
Expression of receptors for colony-stimulating factors in regenerating nervous system

A. Derouiche, Institute of Anatomy, University of Frankfurt/Main
Perisynaptic glutamine synthetase-containing astrocytic processes in organotypic cultures of rat hippocampus

K. Unsicker, Department of Anatomy and Cell Biology, University of Marburg
Functions of FGFs and TGF-ßs related to CNS lesions and regeneration

12.05 - 14.00 **Lunch**

14.00 - 16.00 **Lectures and Short Communications**
Chairmen: J. R. Wolff & A. Reichenbach

M. Reddington, Max Planck Institute for Psychiatry, Martinsried
Calcitonin gene-related peptide (GGRP) and neuron-glia interaction during neuronal regeneration

J. Haas, Department of Anatomy, University of Göttingen
QUIS, GLU, ASP, but not other glutamate agonists induce cell death of astrocytes in vitro. A GLU-receptor-independent type of cytotoxicity

W. Blakemore, Department of Clinical Veterinary Medicine, University of Cambridge
Transplantation of glial cells into areas of demyelination in adult animals

M. Jung, Department of Neurobiology, University of Heidelberg
Oligodendrocyte precursor immortalized by infection with a T-neu containing retrovirus

A. Privat, INSERM U 336, USTL Montpellier
Reinnervation of specific targets by trans-planted monoaminergic neurons in the de-afferented spinal cord of adult rats.

W. Nörenberg, Department of Pharmacology and Psychiatry, University of Freiburg
Expression of an outward current in rat microglia during activation

16.00 - 17.30 **Poster Session and Coffee Break**

17.30 - 18.50 **Lectures and Short Communications**
Chairmen: A. Maelicke & J. Deitmer

R. B. Banati, Max Planck Institute for Psychiatry, Martinsried
Characterization of respiratory burst activity in microglial cells as mediators of tissue destruction in the central nervous system

B. R. Finsen, PharmaBiotec, Institute for Neurology, University of Aarhus
Microglial reactions in the postischemic hippocampus

H.W. Müller, Department of Neurology, University of Düsseldorf
Differential gene expression in regenerating mammalian nerve

M. Sendtner, Max Planck Institute for Psychiatry, Martinsried
Ciliary neurotrophic factor (CNTF): regulation of expression after peripheral nerve lesion and action on lesioned and degenerating motoneurons

18.50 - 19.45 **Meeting of the Members of the DFG-Schwerpunkt**

19.45 - 21.15 **Dinner**

21.15 **Social Program**

Saturday, May 9, 1992

9.00 - 10.05 **Lectures and Short Communications**
Chairmen: P. Layer & R. Dermietzel

M. Schwab, Brain Research Institute, University of Zürich
Myelin-associated neurite growth inhibitors influence development of fiber tracts, stabilize the CNS, and impede regeneration

W. Walz, Institute of Neurobiology, University of Heidelberg
P_2-purinergic receptor induced currents in cultured microglial cells

C. Stürmer, Department of Biology, University of Konstanz
Success of axonal regeneration in the fish CNS

10.05 - 12.00 **Poster Session and Coffee Break**

12.00 - 12.40 **Lecture and Short Communication**
Chairmen : R. Heumann & H. Althaus

F. Miragall, Institute for Anatomy, University of Regensburg
Expression of connexins in a regenerating system: the olfactory organ

M. Schachner, ETH, Zürich
Glial recognition molecules and neuron-glia interactions

12.40 **Lunch**

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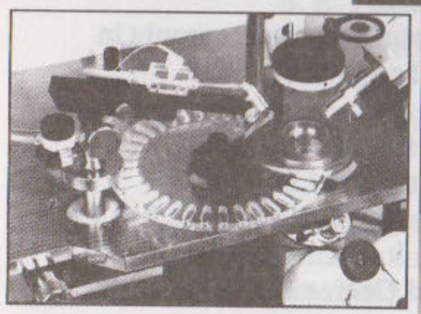

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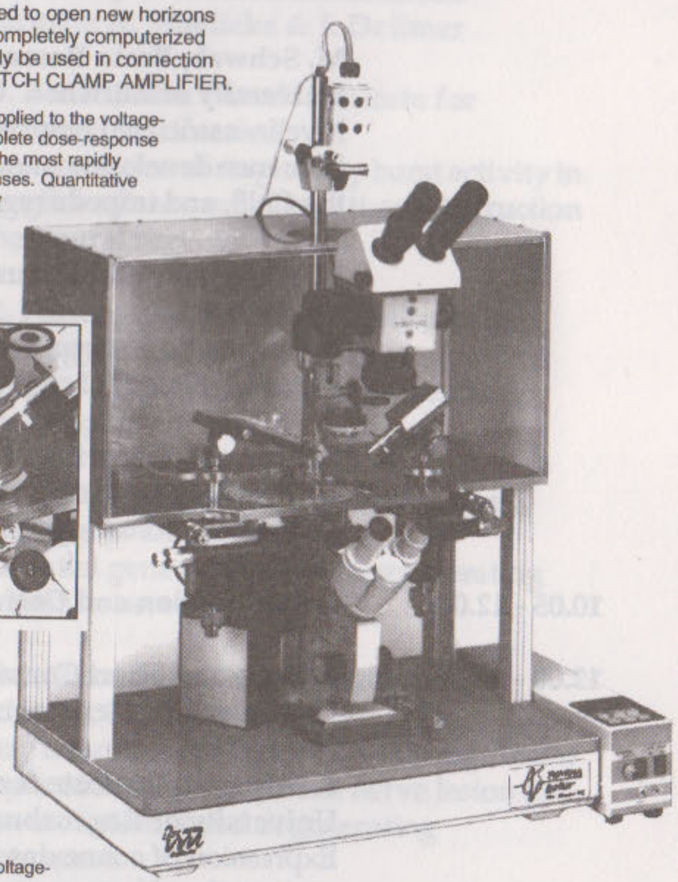

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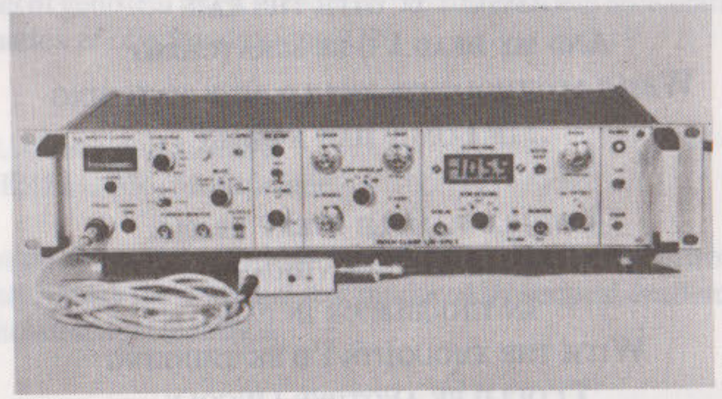

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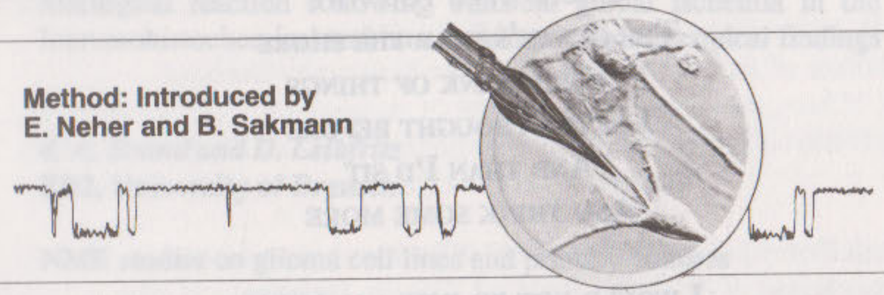

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I could while away the hours
Confirm it with the flowers
Consult it with the rain
And my head I'd be scratching
While my thoughts would busy hatching
...If I only had a brain

I would ramble every riddle
for any individual
In trouble or in pain
With the thoughts I'd be thinking
I could be another Lincoln
...If I only had a brain

Oh, I
Could tell you why
The ocean's near the shore
I could think of things
I never thought before
And than I'd sit
And think some more

I would not be just a nothing
with my head all full of stuffing
My heart all full of pain
I would dance and be merry
Like i'd be a ding-a-derry
...If I only had a brain

The Wizzard Of Oz — Song Of the Scarecrow

Poster Presentations

1. H. Althaus, S. Klöppner, P. Schwartz, and T. Schmidt-Schultz
Max Planck Institute for Experimental Medicine, Göttingen

Effect of genistein on NGF induced proliferation and accelerated process formation of oligodendrocytes (OL)

2. G. Alonso and A. Privat
INSERM U 336, Montpellier

Hypothalamic neuropeptide Y-producing neurons regenerate lesioned axons through the astroglial scar produced by surgical deafferentation of mediobasal hypothalamus

3. P. Bonnekoh, J. Gehrmann, Takahito Miyazawa, U. Oschlies, G. W. Kreutzberg, and K.-A. Hossmann
Max Planck Institute for Neurological Research, Cologne

Microglial reaction following transient global ischemia in the rat. Immunohistochemical and immunoelectron microscopical findings

4. A. Brand and D. Leibfritz
FB2, University of Bremen

NMR studies on glioma cell lines and primary cultures

5. G. Brückner, K. Brauer, W. Härtig, G. Seeger, B. Delpech, A. Derouiche, M. J. Rickmann, and J. R. Wolff
Paul-Flechsig-Institute for Brain Research, University of Leipzig

Polyanionic perineuronal nets: specialized glia-associated microenvironment of neurons

6. A. J. Crang, A. Wilkins and W. F. Blakemore
Department of Clinical Veterinary Medicine, University of Cambridge

Interactions between glial cell lines and normal glia in rotation mediated aggregating cell cultures

7. R. Dörner and W. - R. Schlue
Institute for Zoology, Neurobiology, University of Düsseldorf

pH-changes during glutamatergic stimulation in the leech central nervous system

8. R. Dringen and Bernd Hamprecht
Physiological-Chemical Institute, University of Tübingen

Investigation on the function of glycogen in astrocytes

9. U. Ebbinghaus-Kintscher and P. Hochstrate
Institute of Animal Physiology, Ruhr-University Bochum

Activation of the Na^+/K^+ pump in the pigmented glial cells of the fly retina

10. J. Engele, M. C. Bohn, and C. Pilgrim
Department of Anatomy and Cell Biology, University of Ulm

Survival-promoting effect of mesencephalic glial cell lines on cultured dopaminergic neurons

11. T. Fahrig
Institute for Neurobiology, Troponwerke Köln

Receptor subtype involved and mechanism of norepinephrine induced stimulation of glutamate uptake into primary cultures of rat brain astrocytes

12. A. Faissner, A. Lochter, A. Streit, and M. Schachner
Department of Neurobiology, University of Heidelberg

Purification of a CNS proteoglycan with neurite outgrowth promoting properties

13. F. Förster, W. Gerdes, B. Flott, and W. Seifert
Max Planck Institute for Biophysical Chemistry, Göttingen

Wound experiments in cortical cultures: a model for in-vitro regeneration studies

14. J. Gehrmann, R. Gold, C. Linington, Joseli-Lannes-Vieira, H. Wekerle, and Georg Kreutzberg
Max Planck Institute for Psychiatry, Martinsried

Spinal cord microglia in experimental allergic neurites: evidence for fast and remote activation

15. C. Gillen, C. O. Hanemann, P. Spreyer, M. Gleichmann, and H. W. Müller
Department of Neurology, University of Düsseldorf

Identification of transcriptionally regulated genes after sciatic nerve injury

16. M. Giménez-Ribotta, N. Rajaofetra, M. Mersel, and A. Privat
INSERM U 336, Montpellier

Experimental spinal cord injury in rats: effect of 7ß-hydroxycholesteryl oleate on glial scar

17. R. Gold, W. E. F. Klinkert, C. Linington, H. Wekerle
Max Planck Institute for Psychiatry, Martinsried

Regulation of I-A and ICAM expression on Schwann cells and correlation with T-cell activation

18. S. Graf, G. Lemke, and K. - A. Nave
Center for Molecular Biology (ZMBH), Heidelberg

Induction and transcriptional regulation of the myelin proteolipid protein (PLP) gene

19. C. A. Haas and G. W. Kreutzberg
Max Planck Institute for Psychiatry, Martinsried

Differential expression of early response genes after facial nerve lesion

20. W. Härtig, K. Brauer, and G. Brückner
Paul-Flechsig-Institut for Brain Research, University of Leipzig

Cytochemistry of lectin-binding components of perineuronal nets in relation to neuronal structures in rat brain

21. R. Hanitzsch and W. - U. Mättig,
Carl-Ludwig-Institute of Physiology, University of Leipzig

Normal and abnormal light induced potassium changes in the isolated rabbit retina

22. D. Hartmann and J. Sievers
Department of Anatomy, University of Kiel

Interface cultures of hyppocampal slice explants as a model for the experimental analysis of brain development

23. U. Heinemann, D. Albrecht, B. Nixdorf, and G. Weber-Luxemburger
Institute for Neurophysiology, University of Cologne

Glial cells and spatial K^+ buffering in rat hippocampus

24. A. Henke, W. Eberhardt, and A. Reichenbach
Carl Ludwig Institute for Physiology, University of Leipzig

Voltage-dependent K^+ channels of rabbit retinal Müller (glial) cells

25 . A. Höhne, C. Herkert, U. Wollscheid ,and B. Seeliger
Hematology/Oncology, University of Mainz

Isolation and characterization of a murine glia precursor cell line

26. K. Husmann, M. Schachner, and A. Faissner
Department of Neurobiology, University of Heidelberg

Tenascin promotes cerebellar granule cell migration and neurite outgrowth by different domains in the fibronectin type III homologous repeats

27. S. Ilschner, C. Ohlemeyer ,and H. Kettenmann
Department of Neurobiology, University of Heidelberg

ATP induces an increase of intracellular calcium in cultured mouse microglial cells

28. M. Jung, J. Crang, W. Blakemore, A. Aguzzi, and J. Trotter
Department of Neurobiology, University of Heidelberg

Glial cells immortalized by infection with an SV 40 T containing retrovirus retain in vitro and in vivo properties of primary cells

29. P. Kahle and C. Hertel
PharmaDivision Hoffmann-LaRoche, Basel

Internalization of nerve growth factor mediated by the low affinity NGF receptor $p75^{NGFR}$

30. U. Konietzko, F. - J. Binmöller, and C. M. Müller
Max Planck Institute for Developmental Biology, Tübingen

Dye-coupling amongst astrocytes in rat hippocampal formation

31. G. Kuhn, A. Lie, G. Zoidl, and H. W. Müller
Department of Neurology, University of Düsseldorf

Coexpression of a new 22 kD Schwann cell protein with other peripheral myelin genes during regeneration and development

32. G. Kurz, H. Wiesinger, and B. Hamprecht
Physiological-Chemical Institute, University of Tübingen

Immunocytochemical localization of malic enzyme in neural primary cultures

33. W. Lehmann, W. Naumann, W. Härtig , H. Garn, U. -K. Hanisch, and P. Debagge
Section Biosciences, University of Leipzig

Lectin interactions in the secretory product of the subcommissural organ (SCO) under functional aspects.

34. R. Martini, Y. Xin, B. Schmitz, and M. Schachner
Department of Neurobiology, Swiss Federal Institute of Technology

The L2/HNK-1 carbohydrate is involved in preferential neurite outgrowth of motoneurons on ventral (motor) versus dorsal(sensory) nerve roots.

35. K. W. McDermott and M. B. L. Luskin
Department of Clinical Veterinary Medicine, University of Cambridge

Lineage relationships among retrovirally infected glial cells in the neonatal rat cerebral cortex: an ultrastructural analysis

36. J. Mey and S. Thanos
Ophthalmology, University of Tübingen

Neurotrophic factors support the survival of axotomized retinal ganglion cells in adult rats in vivo

37. M. Müller, W. Hanke, and W.- R. Schlue
Institute for Zoology, University of Düsseldorf

Ion channels in neuropile glial cells of the leech nervous system

38. T. Müller, T. Möller, T. Berger, J. Schnitzer, and H. Kettenmann
Department of Neurobiology, University of Heidelberg

Calcium entry through kainate receptors blocks resting potassium currents in cerebellar Bergmann glial cells

39. T. Munsch and J. W. Deitmer
Department of General Zoology, University of Kaiserslautern

Intracellular calcium of identified leech glial cells

40. C.Naujoks-Manteuffel
Institute for Brain Research, University of Bremen

Are there glial scars in salamanders?

41. T. Naumann, G. M. Peterson, and M. Frotscher
Institute of Anatomy, University of Freiburg

Long-term survival of septohippocampal neurons following fimbria-fornix transection: a fine-structural study of axotomized neurons and reactive glial cells

42. C. Pavlidis and S. Thanos
Ophthalmology, University of Tübingen

Growth of chick trigeminal neurites and interactions with corneal slices in embryonic organ co-culture

43. B. Pfeiffer and B. Hamprecht
Physiological-Chemical Institute, University of Tübingen

Immunohistochemical colocalization of glycogen phosphorylase brain isozyme with astroglial markers in rat brain sections

44. G. O. Pflugfelder, B. Poeck, and S. Ingendahl
Theodor-Bovery-Institute Würzburg

Glia cells in drosophila optic lobe development

45. V. Philippi, H. Korr, and G. W. Kreutzberg
Institute for Anatomy, Technical University Aachen

Axotomy of the mouse facial nerve leads to temporary increased unscheduled DNA synthesis (UDS) and mitochondrial DNA synthesis (MiDS) in the regenerating motor neurons

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Department of Pharmacology and Toxicology, Medical Academy, Magdeburg

Can dentate area transplants take the function of destructed hippocampal granular cells?

47. C. Przyrembel and M. Bähr
Max Planck Institute for Developmental Biology, Tübingen

The reaction of adult optic nerve glia to axotomy

48. A. Pühlhofer and K. - A. Nave
Center for Molecular Biology (ZMBH), Heidelberg

MS-1: A DNA binding protein in brain recognizing a sequence element common to myelin-specific genes

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Institute of Physiology, University of Leipzig

Light pulses evoke membrane currents in Müller cells of the guinea pig retina

50. M. K. Reinicke, E. Willbold, H. Wolburg, and P. G. Layer
Max Planck Institute for Developmental Biology, Tübingen

Müller glia endfeet and a retinal basal lamina together stabilize the laminar structure of chicken *retinospheriods* as derived from the pigmented eye periphery

51. S. Richthof, M. Mauch ,and H. Kettenmann
Department of Neurobiology, University of Heidelberg

Adrenaline reduces the membrane K^+ conductance of cultured oligodendrocytes from mouse brain

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Molecular Neurobiology Laboratory, Department of Neurology, University of Düsseldorf

Astroglial cells support longterm survival of central nervous system neurons in culture

53. T. Schmidt-Schulz and H. H. Althaus
Max Planck Institute for Experimental Medicine, Göttingen

Monogalactosyldiglyceride (MGDG), a potential modulator of protein kinease C (PKC) activity in mature oligodendrocytes (OL)

54. A. Schneider, P. Montague, L.R. Griffiths ,and K. A. Nave
Center for Molecular Biology (ZMBH), Heidelberg

An ile-to-THR mutation of the proteolipid protein (PLP) gene causing hypomyelination in *rumpshaker* mice

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Paul-Flechsig-Institute for Brain Research, University of Leipzig

Distribution of perineuronal nets in rat brain

56. J. Seeger and S. Thanos
Paul-Flechsig-Institute for Brain Research, Leipzig

Microglial cells during axotomy-induced neuronal degradation: combined function-dependent fluorescence staining and electronmicroscopy study

57. B. Seilheimer and W. D. Matthew
Department of Neurobiology, Harvard Medical School, Boston

The expression of basement membrane molecules on cultured Schwann cells and their role in neurite outgrowth

58. B. Seliger, A. Thews, and U. Wollscheid
University Hospital, Department of Hematology, Mainz

Establishment and characterization of a rat neuronal progenitor cell line

59. U. Sprick, C. Schmalenbach, and H. W. Müller
Department of Biopsychology, University of Bochum

Survival of fetal neurons implanted into the neurotoxically damaged hippocampus of rat is enhanced by co-grafted astroblasts but not by fibroblasts

60. U. Sprick, C. Schmalenbach, and H. W. Müller
Department of Biopsychology, University of Bochum

Facilitation of learning by co-grafts of astroblasts and fetal neurons into the neurotoxically damaged hippocampus of rat

61. C. Steinhäuser, R. Jabs, W. Haschke, and H. Kettenmann
Institute for Physiology, University of Jena

L-glutamate activates receptor-mediated ionic currents and simultaneously blocks potassium currents in identified glial cells of the mouse hippocampal slice

62. C. C. Stichel, R. Kruczewski, G. Wunderlich, W. Schroeder, and H. W. Müller
Department of Neurology, University of Düsseldorf

Glial response to transection of the rat postcommissural fornix

63. W. Stoffel, R. Janz ,and M. Körner
Institute for Biochemistry, University of Cologne

Regulation der Genexpression des Proteolipidproteins (PLP) und basischen Myelinproteins. Regulatorische Elemente und Transgene.

64. G. Struckhoff, A. Giese, and J. Sievers
Institute for Anatomy, University of Kiel

Astrocyte and meningeal cell conditioned media (ACM; MCM) contain astrocyte differentiation factors

65. S.Tix, E. Eule, A. Fröhlich, and K. -F. Fischbach
Institute Biology III, University of Freiburg

Characterization of glial cells in the optic lobe of Drosophila melanogaster

66. J. Trotter, J. Crang, M. Schachner, and W. Blakemore
Department of Neurobiology, University of Heidelberg

Transplantation of immortalised glial cell precursors into areas of demyelination

67. J. Urban, C. Sachs, J. Sohn, G. Nase, and G. M. Technau
Institute for Genetics, University of Mainz

An approach for the identification of glia determining genes in Drosophila melanogaster

68. C. Volk, C. Klawe, and O. Kempski
Institute for Neurosurgery and Pathophysiology, University of Mainz

An in vitro model for the investigation of intracellular pH regulation of glial cells

69. M. Wanner, C. Fabian, and C. A. O. Stürmer
University of Konstanz

Regeneration of transected spinal cord axons in goldfish

70. H. Weigel, C. Distler, and K. - P. Hoffmann
General Zoology and Neurobiology, Ruhr-University Bochum

Glial cells in the monkey retina: morphology, distribution and relationship to vasculature and neuronal structures

71. T. Wemmer, A. Klaes, and C. Klämbt
Institute for Developmental Physiology, University of Cologne

Molecular and genetic analysis of glial-neuronal interactions in the embryonic CNS of Drosophila.

72. A. Westmeyer, U. Junghans, and H. W. Müller
Department of Neurology, University of Düsseldorf

A protein:chondroitinsulfate-proteoglycan-complex with neurotrophic survival activity for central neurons

73. H. Wiesinger
Physiological-Chemical Institute, University of Tübingen

Arginine uptake in cultured glial cells

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Institute of Pathology, University of Tübingen

Induction of tight junctions in bovine brain endothelial cells (BBE) by various cocultured cells and their conditioned media

75. G. Wunderlich, C. C. Stichel, W. Schroeder, and H. W. Müller
Department of Neurology, University Düsseldorf

Immature astrocytes promote axonal regeneration in the adult rat brain

Support or inhibition ? Influences of glia on survival and axon growth in the mammalian CNS

Mathias Bähr, Neurologische Universitätsklinik and Max-Planck Institut für Entwicklungsbiologie, Tübingen

The ability of adult CNS neurons in mammals to survive axotomy and to regrow axons mainly depends on the glial environment. We have investigated the influences of different glia populations on retinal ganglion cells (RGCs) in vitro. Schwann cells (SCs) from peripheral nerves were cultured as purified populations in different functional states. Schwann cells which had been allowed to form a basal lamina and to ensheathe axons and were then 'activated' by Wallerian degeneration in vitro were able to support long term survival, ensheathment and myelination of regenerating RGCs. Astrocytes prepared from newborn rats as 'immature' populations produced a basal lamina which contained laminin and were growth supportive for RGC axons but did not support RGC survival. In vitro 'matured' astrocytes did not support RGC survival and growth rates of RGC axons were substantially lower than on immature astrocytes. Glia from adult rat retinae (astrocytes and Müller cells) were able to promote RGC axon growth. Adult rat optic nerve astrocytes (and oligodendrocytes), however, were not permissive for RGC axon growth from retinal explants. These findings suggest that besides growth-inhibiting oligodendrocytes different subpopulations of astrocytes exist in the adult retina and optic nerve which are either growth permissive or interfere with axonal growth.

CHARACTERIZATION OF RESPIRATORY BURST ACTIVITY IN MICROGLIAL CELLS AS MEDIATORS OF TISSUE DESTRUCTION IN THE CENTRAL NERVOUS SYSTEM

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A number of recent experimental and clinical studies document that ischemia-induced nerve cell death can be prevented by the xanthine derivate propentofylline (HWA 285). It is presently hypothesized that propentofylline acts via adenosine or itsself possesses adenosine-like properties. As adenosine is known to modulate the production of reactive oxygen intermediates, i.e. the respiratory burst, in cells of mononuclear-phagocyte lineage we studied the effect of propentofylline on peritoneal macrophages and microglial cells, the source of endogenous brain macrophages.

Using a new flow cytometric assay propentofylline in therapeutic dosis of 50 μM was shown to completely inhibit the Ca^{2+}-dependent conA-induced increase in the production of reactive oxygen intermediates in peritoneal macrophages. In isolated and cultured microglial cells, which showed a high spontaneous respiratory burst, the production of reactive oxygen intermediates was reduced by approximately 30%. A phorbol myristate acetate (PMA) induced rise in the respiratory burst activity could not be inhibited by propentofylline in either cell type.

An increased generation of reactive oxygen intermediates particularly after ischemia and in brain edema is thought to contribute to nerve cell death. We therefore suggest that the neuro-protective properties of propentofylline might be in part due to a decrease in the production of the potentially harmful reactive oxygen intermediates possibly by the suppression of respiratory burst activity in microglial cells.

THE ALZHEIMER AMYLOID ßA4 AND ITS PRECURSOR IN HEALTH, DISEASE AND AGING

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The deposition of amyloid ßA4 protein in the brain is characteristic of Alzheimer's disease (AD). This protein is derived from a larger precursor (APP) which is normally expressed in many tissues, including the brain. Because in an increasing number of families with AD a mutation at position 22, 42 and 46 of the ßA4 region has been linked with the disease, these mutations provide compelling evidence for the central role that APP and its pathologic breakdown product ßA4 must play in brain function and dysfunction. The prevalence of ßA4 deposition increases as a function of age in the normal population and in DS. Using immunocytochemistry, we have estimated that in normal caryotypes and in individuals with Down syndrome, a period of 30 years of preclinical ßA4 deposition precedes the onset of clinical symptoms for AD. The APP gene consists of 19 exons which code for a typical transmembrane protein. The ßA4-amyloid sequence is part of exons 16 and 17. Several APP isoforms can be generated by alternative splicing of exons 7, 8 and 15. Alternative splicing of exons 7 and 8 in APP mRNA's encoding domains with homologies to Kunitz-type protease inhibitors and the MRC OX-2 antigen, respectively, is changed in brain during aging. Splicing more proximal to the ßA4 region, leading to exclusion of exon 15 of the APP-gene (König et al. *J. Biol. Chem.* in press) generates transmembrane, ßA4-sequence containing APP variants, lacking 18 amino acid residues close to the amyloidogenic region. The resulting novel L-APP (leukocyte-specific) forms are expressed by T-lymphocytes, macrophages and microglial cells. The presence of APP (Mönning et al. *FEBS Lett.* **277**, 261-266 (1990)) and L-APP in lymphocytes and cells of the mononuclear phagocyte system might be necessary to allow the rapid transition of the dual functions of nonadherence and adherence in the immune system. This is in accordance with the proposed role of APP's in establishment, maintenance and regeneration of cell adhesion in the nervous system and in the periphery. Together with our observation of a preferential localization of APP at synaptic sites of human and rat brain (Schubert et al. *Brain Res.* **563**, 184-194 (1991)), and in the light of the proposed role of redox potential as one possible factor for amyloidogenesis, it is suggested that activation of microglial cells and failure to regenerate could be critical events leading to aberrant processing of neuronal and synaptic APP to ßA4.

TRANSPLANTATION OF GLIAL CELLS INTO AREAS OF DEMYELINATION IN ADULT ANIMALS

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Central to the interpretation of any glial cell transplantation experiment is the need to unequivocally distinguish transplanted glial cells or their effects from those of host-derived glial cells. When ethidium bromide solution (EB) is injected into spinal cord white matter which has been locally exposed to 40 Grays of x-irradiation, an area of glial-free demyelination is created which shows no evidence of remyelination by either oligodendrocytes or Schwann cells. The validation of the locally x-irradiated EB lesion as an experimental system in which to demonstrate with certainty the effects of transplanted glial cells is provided by the results of a further control experiment. Following transplantation of glial cell preoperative which alone have no inherent remyelinating potential, but which have been shown capable of recruiting host glial cells into lesions made in non-irradiated white matter, no evidence of remyelination can be observed. Thus the locally x-irradiated EB lesion provides an ideal *in vivo* situation in which to examine the differentiation potential of genetically engineered glial cells. This paper will report the results obtained following transplantation of two different engineered glial populations: firstly, the differentiation of glial cell progenitors expanded under the influence of a temperature-sensitive SV40 large T oncogene into oligodendrocytes and oligodendrocytes, and secondly, the differentiation of glial progenitors expanded by continuous exposure to PDGF and basic FGF.

GLUTAMINE SYNTHETASE CONTAINING ASTROGLIAL PROCESSES REACT TO DEAFFERENTATION

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Brain glutamine synthetase (GS), a glutamate degrading enzyme, is localized in very thin processes of astrocytes around glutamatergic synapses in the rat hippocampus. Here, possible factors determining this organization were studied by using cultured hippocampal explants as a deafferentation model.

Transverse slices of hippocampus from 2-4 day old rat pups were cultured for about 3 weeks by using the roller-tube method (B.H. Gähwiler, J.Neurosci.Meth. 4:329-342 (1981)). Immunocytochemistry was performed with anti-GS and enhanced by silver.

In organotypic slice cultures, the layer specific distribution of GS - immunoreactivity typical of the in situ hippocampus was not found. Instead, GS - immunoreactivity was distributed homogenously. Electron microscopic analysis showed that - as in situ - many asymmetric synapses were contacted by GS - immunoreactive astrocytic processes. It appears that in the hippocampus the layer specific intensity of GS - immunoreactivity mirrors the density distribution of glutamatergic terminals. This applies as well after processes of re-organization such as the shift of terminal fields caused by collateral sprouting of intrinsic fibres in the culture. A dramatical glial reaction to deafferentation is also evident in the hippocampus in situ two days after entorhinal lesion, which results in marked changes of GS - immunoreactivity within only the terminal fields concerned.

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MICROGLIAL REACTIONS IN THE POSTISCHEMIC HIPPOCAMPUS

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Microglial reactions were studied 1-21 days and 6 months after 10 min of global cerebral ischemia, causing degeneration of dentate hilar neurons and hippocampal CA1 pyramidal cells. Normal and reactive microglial cells were visualized with the histochemical NDPase stain and with OX42-immunohistochemistry for the C3 receptor (CR3). Reactive microglial cells were also shown with markers of MHC antigen class I (OX18) and II (OX3).

The **early, transient reactions** consist of coarsening of the microglial processes, increased NDPase/CR3 reactivity and class I induction. They are obvious in all subfields of hippocampus up to day 4. At day 7 the microglial cells in CA3 revert to resting microglial cells, while the ones in the dentate hilus and CA1 undergo further activation and take part in the **protracted, lesion-specific reactions**. Around degenerating hilus neurons there is satellitosis. In CA1 there is rod cell and ultimately macrophage-like transformation of microglial cells in str. radiatum and macrophage-transformation of microglial cells in str. pyramidale. In the dentate hilus and CA1 there is proliferation and persistent class I expression of microglial cells. Class II immunoreactivity, in contrary, is sparse, although it is present in a time-sequential pattern in the dentate hilus and CA1. Six months after ischemia the CA1 area is severely atrophic, and densily, but homogenously populated with resting microglial cells.

In conclusion, we find a **two-stage activation** of microglial cells in the hippocampus following cerebral ischemia, with evidence that class I is a sensitive marker for microglial activation.

QUIS, GLU, ASP, BUT NOT OTHER GLUTAMATE AGONISTS INDUCE CELL DEATH OF ASTROCYTES IN VITRO. A GLU-RECEPTOR-INDEPENDENT TYPE OF CYTOTOXICITY ?

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Astrocytes were isolated from rat cortex (P2) and subcultures maintained in serum-free defined medium (G5). Cell death was quantified by measuring lactic dehydrogenase activity in the medium and by vital-dye-fluormetry. Cell death could be transiently (4-8 d.i.v.) induced by GLU, ASP, QUIS in a concentration-dependent manner (≥1mM), but not by NMDA, Kainate, Ibotenate, ACPD, AMPA, L-AP4. In contrast to excitoneurotoxicity, this gliotoxicity cannot be inhibited by antagonists of the iono- and metabotropic receptors, additionally, serum-supplemented medium supresses astrocytic but not neuronal vulnerability. Astrocytic cell death becomes quantitatively significant after more than 6 hours, while morphological reactions at the ultrastructural level are detectable earlier (≥2h). These results suggest that GLU-ASP-QUIS dependent cytotoxicity in astrocytes differs from neuronal excitotoxicity in several respects. Evidence will be presented to indicate the importance of GLU-uptake-mechanism for the induction of cell death in astrocytes.

OLIGODENDROCYTE PRECURSORS IMMORTALIZED BY INFECTION WITH A T-NEU CONTAINING RETROVIRUS

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We have used oncogene-carrying replication-defective retroviruses to immortalize murine oligodendrocyte precursors. Primary cultures of cerebellar cells originating from 6-day-old mice were freed from neurons by complement-dependent immunocytolysis and subsequently infected with a retrovirus based on the Murine Moloney Leukemia Virus containing the t-neu oncogene and a gene coding for resistance to the antibiotic G 418. Cells expressing the O4 antigen, expressed by oligodendrocytes and their precursor cells from primary cultures, could be continually passaged in vitro and O4$^+$ clones were isolated on the FACS. We obtained cells which showed immunological and morphological characteristics of oligodendrocyte precursors. Culture in the presence of 1mM dibutyryl cAMP for at least 10 days induced a shape change and a shift in antigen expression towards a more mature stage. The in vivo behaviour of the cells following transplantation into demyelinated lesions in adult rats was investigated. An uncloned population of an early passage of the cells specifically associated with demyelinated axons. Oligodendrocyte precursors can thus be immortalized with the t-neu oncogene and are capable of a degree of differentiation both in vitro and in vivo.

EXPRESSION OF CONNEXINS IN A REGENERATING SYSTEM: THE OLFACTORY ORGAN.

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The olfactory system represents a unique region in the CNS in which there is plasticity of neural connections and regenerative capacity in the adult. The cellular substrate of these exceptional properties appears to be two pools of precursor cells which persist in the adult olfactory system. The molecular mechanisms which underlie these unusual features, however, are still unclear despite that numerous studies have been made during the past few years.

Gap junctions are intercellular channels which mediate exchange of ions and small metabolites between cells. They have also been reported to play a regulative role in development. Biochemical analysis of gap junctions have led to the isolation and characterization of a protein family, the connexins (cxs). In the present study, the expression of cxs 43, 32 and 26 have been investigated in the developing and adult olfactory system of the mouse using cytochemical and biochemical methods. Cx 43 was detected from the beginning of the development at the stage of olfactory placode (E9). During early embryonic development the levels of cx 43 remained low. Expression of cx 43 became high during the postnatal phase including adulthood. Cx 43 was localized in non-neuronal cells. Cx 32 was not detected during embryonic and early postnatal stages. At later postnatal stages cx 32 immunolabeling was observed on cells tentatively identified as oligodendrocytes. Cx 26 was only clearly localized on leptomeninges of the olfactory bulb. Our results substantiate the cell specific expression of these three cxs. Furthermore they document the primacy of cx 43 in the olfactory tissues and confirm the importance of this gap junction protein in the nervous system.

DIFFERENTIAL GENE EXPRESSION IN REGENERATING MAMMALIAN NERVE

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Based on the hypothesis that the highly predictable sequence of cellular and molecular reactions following nerve lesions reflects the differential expression of specific genes, we have designed an experimental strategy that allows the cloning of those genes whose expression is either induced or repressed during nerve degeneration and/or repair (1,2). The differential hybridization screening of a cDNA-library of regenerating rat sciatic nerve led to the identification of a surprisingly high proportion of regulated clones representing novel sequences or known gene products that have not previously been detected in the nervous system. The clone encoding an axon-regulated Schwann cell gene (CD25/gas3) with potential growth regulatory functions will be characterized in more detail (3). This membrane protein may be critical for Schwann cell differentiation and myelin formation since evidence suggests that this gene is defect in the dysmyelinating neurological mouse mutant *trembler* and presumably in the human autosomal dominant Charcot-Marie-Tooth disease (CMTD). Supported by BMFT.

(1) Müller (1990) in: New Issues In Neurosciences, Vol 2, Wiley and Sons, pp 15-21.
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EXPRESSION OF AN OUTWARD CURRENT IN RAT MICROGLIA DURING ACTIVATION

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Rat microglial cells in culture displayed under whole-cell patch conditions only one voltage-dependent membrane conductance, i.e. a hyperpolarization-induced inwardly rectifying potassium current (see also Kettenmann et al., J Neurosci Res 26:278, 1990). However, when cells were exposed to bacterial lipopolysaccharide (LPS, 100 ng/ml), known to be a modulator of immunocyte function, an additional depolarization-induced outward current became detectable. The LPS action appeared 2 hours after application and vanished after an additional 3-5 days. The outward current was abolished by external 4-aminopyridine 1 mM, quinine 1 mM and charybdotoxin 100 nM, as well as by intracellular Cs^+ 150 mM; external tetraethylammonium 20 mM caused about 50% inhibition. This together with the fact that tail currents reversed near to the potassium equilibrium potential, characterized the conductance as K^+ specific. Increasing intracellular free Ca^{2+} from 0.01 to 1 μM depressed the current, while changes in extracellular Ca^{2+} (0, 2, 20 mM) had no effect. The current showed a time-dependent inactivation during voltage steps. Peak amplitudes were stable at 0.125 Hz, but not at 1 Hz stimulation with depolarizing pulses. Taken together these findings demonstrate a high similarity between the K_n-type potassium outward current found in B- and T-lymphocytes (Gallin, Physiol Rev 71:775, 1991) and the LPS-induced conductance in rat microglia. Since K_n-channels in lymphocytes are related to mitogenesis, the expression of the outward current in microglia might represent the electrophysiological correlate of the activation process (Streit et al., Glia 1:301, 1988) leading to proliferation.

REINNERVATION OF SPECIFIC TARGETS BY TRANSPLANTED MONOAMINERGIC NEURONS IN THE DEAFFERENTED SPINAL CORD OF ADULT RATS.

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After complete transection at lower thoracic level, the segment of the cord below the section is totally devoid of monoaminergic innervation. One week after the section, suspensions of serotonergic neurons prepared from the anlage of groups B1-B2 or B3 were injected in the dorsal fasciculi.
The animals were sacrificed after a survival of 2 to 3 month, and their spinal cord was processed for the immunocytochemical detection of serotonin.
Neurons from group B3 innervated specifically the dorsal horn, which is their target in the intact animal, and upon electron microscope examination, axonal varicosities appeared to contribute rarely to typical synapses. Conversely, neurons from groups B1-B2 innervated the ventral horn, and made numerous axo-dendritic synapses, as their counterpart in the intact animal. In addition, substance P was detected by dual immunocytochemistry in these synaptic boutons.
We conclude that immature serotonergic neurons transplanted in a denervated adult spinal cord are able to innervate their specific targets and to express a highly differentiated phenotype.

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EXPRESSION OF RECEPTORS FOR COLONY-STIMULATING FACTORS IN THE REGENERATING NERVOUS SYSTEM.

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Proliferation of microglial cells commonly occurs in the response of the central nervous system to injury, but little is known about how this process is regulated *in vivo*. Here we have studied the expression of receptors to macrophage colony stimulating factor (MCSF) and granulocyte-macrophage colony stimulating factor (GMCSF) in the normal and regenerating rat facial motor nucleus.

Under normal conditions, immunocytochemical staining with anti-MCSF receptor (MCSFR) antibody revealed a moderate but selective labelling of microglia-like cells of the facial motor nucleus. This immunostaining also colocalized with MUC102, a new monoclonal antibody raised against microglial cells in the rat central nervous system. Axotomy of the facial nerve led to a rapid increase in MCSFR-staining intensity 1 day after injury, became maximal at 7 days postoperatively and then decreased. A similar, but somewhat slower increase was also observed for the specific [^{125}I]-MCSF binding with a maximum at 7 days. Specific [^{125}I]-GMCSF binding also increased, peaking at 4 days postoperatively and then rapidly decreasing to normal levels at 21 days after axotomy.

In summary, axotomy of the facial nerve led to a rapid increase in receptors for MCSF and GMCSF which coincided with the pattern of microglial proliferation in the regenerating facial motor nucleus. This apparent upregulation of receptors for microglial growth factors may play an important role in preparing the microglia to participate in the cellular response to injury in the regenerating central nervous system.

CALCITONIN GENE-RELATED PEPTIDE (CGRP) AND NEURON-GLIA INTERACTION DURING NEURONAL REGENERATION.

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Recent evidence suggests that calcitonin gene-related peptide (CGRP) might play a role in peripheral nerve regeneration. After facial nerve transection, increases in CGRP immunoreactivity and CGRP mRNA are observed in facial motoneurons as early as 15 h after axotomy. Further analysis revealed a biphasic pattern of the CGRP increase with a first peak around day 3 and a second peak at day 21 after facial nerve transection. In contrast, sciatic nerve transection led to a decrease in CGRP levels and CGRP mRNA in the dorsal root ganglion. Thus CGRP synthesis is differentially regulated in motor and sensory neurons in response to injury.

The early and dramatic increase of CGRP in injured facial motoneurons indicates a possible role in the cellular reactions accompanying neuronal regeneration. In vitro studies demonstrated that CGRP has several effects on cultured astrocytes, including a change in morphology, a stimulation of cyclic AMP synthesis and a rapid and strong induction of the fos proto-oncogene. Taken together, these data suggest that the increased CGRP synthesised in response to facial nerve lesion might play a role as a regulator of astrocyte function.

GLIAL RECOGNITION MOLECULES AND NEURON-GLIA INTERACTIONS

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Neuron-glia interactions are mediated by diverse cellular and molecular mechanisms not only during morphogenesis, but also, in the adult, during modification of neural connectivity and regeneration. Glial recognition molecules, at the cell surface and in the extracellular matrix display adhesive and antiadhesive properties towards neuronal cell bodies and neurites. These recognition molecules form boundaries between units of functional neuronal assemblies during the establishment of topographic maps and display their dual functions of being adhesive and antiadhesive depending on the partner cell type. Glial cells produce neurite outgrowth promoting recognition molecules, the expression of which correlates with their potential to foster neuronal regeneration in the central and peripheral nervous systems of adult mammals. Glial cells also express carbohydrate structures that mediate the pathway selection of neuronal subpopulations in peripheral nerves. Finally, glial recognition molecules may influence the intra- and extracellular ionic milieu during development and in the adult. This broad repertoire of functional traits endows glia with hitherto unknown possibilities by which these cells exert their influences on neurons.

POSTNATAL TRANSECTION OF RABBIT OPTIC NERVE DOES NOT LEAD TO AN ALTERED ASTROCYTE AND MICROGLIAL CELL TOPOGRAPHY IN THE MATURE RETINA

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Following transection of the optic nerve (ON) of mammals, ganglion cells undergo retrograde degeneration. It has been shown that in the adult retina glial cells respond to the degeneration of ganglion cells (Schnitzer and Scherer, 1990; Scherer and Schnitzer, 1991). In this study the ON of rabbits were transected postnatally. The aim was to examine whether this experimentally induced degeneration process and the consequent absence of ganglion cell axons leads to an altered topography or morphology of glial cells in the mature retina.

Three months after postnatal transection of the ON, microglial cells, labeled by nucleoside diphosphatase histochemistry were observed in the nerve fiber layer (NFL), and in the inner plexiform layer (IPL). As in control retinae, microglial cells in the NFL were concentrated near the medullary rays. The preferential orientation of their processes along the course of ganglion cell axons, as observed in control retinae, were not evident in retinae with transected ON.

No morphological differences were observed between microglial cells in the IPL of retinae with transected or intact optic nerve. However, mature retinae, whose ON had been transected postnatally, showed a slight increase of microglial cell density in the IPL as compared to control retinae. Taking into account the reduced extension of these retinae, the total number of microglial cells in the IPL was calculated to be the same as in control retinae. This suggests that the density of microglial cells in the mature rabbit retina is not influenced by the degeneration process occurring postnatally.

No changes in the morphology and topography of astrocytes in mature retinae with postnatally transected ON were observed. The presence of astrocytes was still confined to the vascularized area around the optic nerve head. Thus it seems not to be the ganglion cell axons, which postnatally prevent a spread of astrocytes in further peripheral parts of the rabbit retina.

Antibodies against galactocerebroside and myelin basic protein labeled oligodendrocytes in control retinae, but not in 'transected' retinae indicating the absence of mature oligodendrocytes. This may largely be due to the fact, that after transection oligodendrocyte progenitors could no longer migrate from the ON into the retina. However, progenitors which already are present within the retina at that time, seem not to be able to develop a mature phenotype in the absence of ganglion cell axons.

In conclusion, the increased number of degenerating ganglion cells within the postnatal retina after optic nerve transection and the absence of ganglion cell axon in consequence do not lead to altered patterns of microglial cells and astrocytes in the mature retina. In contrast, after postnatal transection oligodendrocytes seem to be absent from the retina.

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MYELIN-ASSOCIATED NEURITE GROWTH INHIBITORS INFLUENCE DEVELOPMENT OF FIBER TRACTS, STABILIZE THE CNS, AND IMPEDE REGENERATION

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Oligodendrocytes and CNS myelin contain an activity which leads to arrest of neurite growth in culture. This activity was identified as two fractions of molecular weights 35 and 250 kD (NI-35/250. Partial amino acid sequencing resulted in novel sequences which are highly conserved accross the species. - Monoclonal antibodies (IN-1, IN-2) neutralizing the inhibitory activity of NI-35/250 have been generated and characterized in several in vitro systems. Application of the antibody IN-1 into the CSF of spinal cord lesioned rats led to successful regeneration and elongation of cortico-spinal tract (CST) fibers over 5 - 20 mm (in 2 - 4 weeks). In contrast, control antibodies only permitted local sprouting not exceeding 0.5 - 1 mm. Identical results were obtained in rats X-irradiated at birth, thus eliminating oligodendrocytes and myelin. A similar, highly significant regeneration response was recently obtained in the hippocampus (septohippocampal fibers) and in the optic nerve. - During development, oligodendrocyte differentiation occurs in a tract- and region-specific manner in the CNS. We studied the effect of oligodendrocyte elimination or of the antibody IN-1 on CST development. In both cases branching of CST fibers occurred into the sensory tracts, a phenomenon never observed under normal conditions. These results attribute a guidance function ("guard rails") to NI-35/250 for the developing CST. - In the developing hamster visual system oligodendrocytes appear in the stratum opticum of the superior colliculus (SC) soon after the optic fibers have grown in. If one SC is removed, the target-deprived optic fibers sprout to the other side, but grow only very superficially and never enter the stratum opticum. In presence of IN-1 this pattern is reversed to a normal neuroanatomical innervation. In this case NI-35/250 restrict the access of regenerating fibers to particular tectal layers. - Results in the oligodendrocyte-free rat optic nerve (P15) indicate that axons sprout along the nerve in the absence of oligoden-drocytes, indicating a stabilizing function of these inhibitory molecules in the adult CNS.

CILIARY NEUROTROPHIC FACTOR (CNTF): REGULATION OF EXPRESSION AFTER PERIPHERAL NERVE LESION AND ACTION ON LESIONED AND DEGENERATING MOTONEURONS

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CNTF is expressed in high quantities in Schwann cells of peripheral nerves, but not in target fields of responsive neurons during post-natal development of the rat. The absence of detectable CNTF expression during embryonic development suggests that CNTF might act on neuronal survival of responsive neurons in a manner different to that of nerve growth factor (NGF). In addition, the lack of a hydrophobic leader sequence and the immunohistochemical localization of CNTF within the cytoplasm of Schwann cells indicate that the factor might not be available to responsive neurons under physiological conditions. However, CNTF supports the survival of a variety of embryonic neurons, including spinal motoneurons in culture. Morevover, the exogenous application of CNTF protein to the lesioned facial nerve of the newborn rat rescues these motoneurons from cell death. This observation suggests that CNTF might act as a lesion factor under pathophysiological conditions in the adult peripheral nervous system. After adult rat sciatic nerve lesion, CNTF mRNA is down-regulated in Schwann cells after loss of axonal contact and re-expressed by the same cell type during ensuing regeneration. At the lesion site, where regeneration begins, significant amounts of CNTF immunoreactivity are found in the extracellular space. *Western* blot analysis and determination of ciliary neuron survival activity indicate that most of the CNTF protein detectable in these segments remains still biologically active. These observations suggest that endogenous CNTF might indeed play a functional role as a lesion factor in peripheral nerves.

SUCCESS OF AXONAL REGENERATION IN THE FISH CNS

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In contrast to mammals retinal ganglion cells (RGCs) in fish regenerate their axons upon injury and restore vision. Reasons for the success of fish RGC regeneration appear to lie in 1) the glial cell environment of the fish optic nerve and 2) the ability of the fish RGCs to reactivate the cellular maschinerie required for lengthy axonal regrowth.

1) In coculture experiments oligodendrocytes derived from the fish optic nerve did not inhibit but instead supported the regrowth of injured fish RGC axons. Moreover, even RGCs from retinal explants of adult rats regenerated axons in considerable density and length on the surface of the fish oligodendrocytes. Thus, fish oligodendrocyte obviously possess growth supportive cell surface molecules

2) During axonal regeneration fish RGCs re-express a set of "growth associated cell surface molecules" which are normally found on embryonic but not on mature axons in adults. The spatiotemporal expression pattern of such molecules on axons and oligodendrocytes and their significance for RGC regeneration will be discussed.

ADULT RETINOFUGAL AXONS REGENERATING THROUGH PERIPHERAL NERVE GRAFTS CAN RESTORE THE LIGHT-INDUCED PUPILLOCONSTRICTION REFLEX

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The optic nerve of adult rats was used as a paradigma to demonstrate that retinal ganglion cells can regrow their axons through peripheral nerve grafts, approach central target cells and form functional connections within central targets. In the experimental paradigma, severed ganglion cell axons were guided into the primary visual centers which subserve the pupillary constriction reflex in response to light. The ocular stump of the transected optic nerve of adult rats was connected by means of an autologous peripheral nerve graft with the pretectal region which contains the relay nucleus of pupillary constriction, the olivary pretectal nucleus (OPT). This nucleus is efferently connected with preganglionic neurons in the oculomotor nuclear complex which innervates parasympathetically the muscle constrictors of the iris. Six to sixteen weeks after optic nerve transection and peripheral nerve transplantation, brisk responses were observed in the pupils upon illumination of the transplanted eye. Recovery of the pupil responses indicated that retinal neurons used the peripheral nerve "bridge" to assess the pretectum, in which they established synaptic contacts in sufficient density and with appropriate specificity, in order to reconstitute the function of the traumatically interrupted neuronal circuitry. The experiments demostrate that adult neurons possess, in principle, the ability to reestablish functional synaptic contacts within the brain.

FUNCTIONS OF FGFs AND TGF-ßs RELATED TO CNS LESIONS AND REGENERATION

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Several members of the FGF (acidic and basic FGF, hst, FGF-5) and TGF-ß (TGF-ß 1, 2 and 3) gene families occur in the nervous system and serve important functions spanning from regulation of neuron death, neuritic growth and transmitter synthesis to glial cell proliferation and differentiation. My talk will present several of the most relevant examples and also discuss possible roles of FGF in animal models of Parkinson's disease and of FGF and TGF-ßs in astroglial scar formation.

P_2-PURINERGIC RECEPTOR INDUCED CURRENTS IN CULTURED MICROGLIAL CELLS

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Microglial cells in culture are distinct from neurons, macroglial cells and body macrophages by their characteristic inwardly rectifying current pattern. While these cells can rapidly respond to the degeneration of neurons, little is known on the specific membrane receptors which may mediate neuron-(micro)glia interactions. In this study, we studied purinergic receptors on purified microglial cells from rodent cortical cultures using the whole-cell patch clamp technique. 100µM ATP activated a partly transient inward current of 67 pA (range 7-360 pA, n=25). The purine receptor agonists ADP, AMP and adenosine resulted in no detectable inward current at the holding potential (-70 mV). There was a run-down of the ATP response depending on the electrode resistance from 20 min (3 MΩ) to >60 min (>10 MΩ). To analyze ATP dependent currents at different membrane potentials, the membrane was clamped to -35, 0, 35, -105 mV from a holding potential of -70 mV. The resulting current voltage curves at the peak of the ATP response indicated an increase of the membrane conductance as compared to a control (1.6nS, range 0.5 to 4.6 nS, n=7). The reversal potential of the ATP activated current was -21mV (range -60 to 8 mV, n=7). We conclude that extracellular ATP may act as a signal substance for microglial cells.

AXONAL GROWTH AND REGENERATION STUDIED IN INTRACEREBRAL NEURAL TRANSPLANTS AND ORGANOTYPIC SLICE CULTURES

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Intracerebral transplants of late fetal or neonatal rat hippocampal tissue were grafted into the hippocampus of - 1) normal newborn and adult rats, - 2) newborn and adult rats deprived of dentate granule cells by neonatal x-irradiation, - and 3) adult rats with excitotoxic and ischemic lesions of the CA3 and CA1 hippocampal subfields. Using histochemical, and immunocytochemical stains and neuroanatomical tracing techniques the growth of recipient brain fibers into the grafts were found to depend on - a) recipient age, - b) nerve type, - and c) lesion type. The results correspond with observations obtained in organotypic slice cultures of hippocampal tissue, co-cultures of septohippocampal tissue with formation of cholinergic connections and nigrostriatal co-cultures with formation of dopaminergic connections.

Based on these results it is proposed that a determining factor for the extent of axonal growth and regeneration of point-to-point connections is **axonal competition** in the target areas, while global, cholinergic and monoaminergic projections, with distinctly better regenerative abilities, depend on other factors.

EFFECT OF GENISTEIN ON NGF INDUCED PROLIFERATION AND ACCELERATED PROCESS FORMATION OF OLIGODENDROCYTES (OL)

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OL isolated from adult pig brain regenerated their processes in vitro. Treating of OL with NGF (10-100ng/ml) resulted in an enhanced process formation and induced proliferation in an OL subpopulation which concomitantly expressed c-fos. Furthermore, several proteins were tyrosin phosphorylated (5-60 min.). Genistein, an inhibitor of protein tyrosin kinases (PTK), blocked the ^{3}H-thymidine incorporation completely but reversibly at a concentration of 50 μM. No obvious inhibitory effect was observed for the enhanced process formation. The results indicate that the NGF signalling path consists of a PTK dependent and independent component.

HYPOTHALAMIC NEUROPEPTIDE Y-PRODUCING NEURONS REGENERATE LESIONED AXONS THROUGH THE ASTROGLIAL SCAR PRODUCED BY SURGICAL DEAFFERENTATION OF MEDIOBASAL HYPOTHALAMUS.

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Unilateral surgical deafferentation of the mediobasal hypothalamus was used to study the capacity of axonal regeneration of hypothalamic neurons in adult rats. The postlesional responses to axotomy were compared in two types of hypothalamic neurons including: 1) Vasopressin (VP)-neurons which project to the deafferented area and whose perikarya are outside this region, and 2) NPY-neurons located in the mediobasal hypothalamus, which project outside this region. The organization of axotomized neurons within the lesioned area was studied by confocal microscope observations of 40-50 m thick vibratome sections after double immunostaining for glial fibrillary acidic protein (GFAP) and for NPY or VP. In contrast to VP-neurons which degenerate after such a deafferentation, the surgical lesion induced a progressive increase in the number and immunostaining intensity of NPY-immunoreactive perikarya and fibers within the deafferented area. After 7 days, numerous highly immunoreactive NPY fibers were found to abut on the surgical cut. After 15 days and more frequently after 30 and 45 days, NPY-immunoreactive fibers were observed to cross the astroglial scar in the periventricular and retrochiasmatic portions of the cut. Electron microscope observations of adjacent ultrathin sections indicated that the numerous NPY-immunoreactive axonal profiles that were included in the scar matrix, were thightly apposed to the limiting membrane of astroglial processes containing dense bundles of microfilaments.
These data indicate that the lesional scar formed after surgical deafferentation of the mediobasal hypothalamus allows the regrowth of NPY-axons, whereas it constitutes a permanent obstacle to regeneration of VP-axons.

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MICROGLIAL REACTION FOLLOWING TRANSIENT GLOBAL ISCHEMIA IN THE RAT. IMMUNOHISTOCHEMICAL AND IMMUNOELECTRON MICROSCOPICAL FINDINGS

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Transient global ischemia of the brain may cause neuronal necrosis in selectively vulnerable areas. The role of microglial cells in the manifestation of neuronal cell death was investigated. Global ischemia of 30 min was induced in the rat using the four-vessel-occlusion model. After recirculation times of 8 h, 1, 3 and 7 d brains were prepared for immuno-cytochemistry or immunoelectron microscopy. Monoclonal antibodies against different microglial epitopes were used (e.g. MUC 101 and 102, Ox-6). An early (24 h) microglial reaction increasing with time was prominent in the striatum and the hippocampal sectors CA1 and CA4. A lesser involvement of other regions such as substantia nigra, thalamus and white matter areas was also observed. These findings were supplemented by immunoelectron microscopy (MUC 102 and Ox-6 against Ia antigen). The results show an early (24 h) phagocytic activity of MUC 102 positive microglial cells and expression of IA antigen on perivascular microglia. The transformation of microglia into phagocytic cells points at their role in removing the neuronal debris after neuronal death. However, microglial cells could also play a role in the mediation of ischemic injury by their cytotoxic potential.

NMR STUDIES ON GLIOMA CELL LINES AND PRIMARY CULTURES

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Since individual cell types are involved in different biochemical pathways it was the purpose of this work to follow the metabolic fate of nonradioactive labelled substrates (C-13 enriched glucose and pyruvate) in glioma cell lines (F98, C6 glioma) and primary cultures of glia cells.

The following main results are obtained from P-31, H-1 and C-13 NMR spectra of PCA cell extracts:

All glial cells contain a high amount of inositol, probably typical for glia cells and different to neurons. The inositol turnover is slow. No C-13 label appears after two hours.

All cells show creatine kinase activity. However, the concentrations of phosphocreatine and creatine are different for the individual cell types. The intracellular amounts of PCr and Cr also depend on the extracellular concentration of creatine.

The pyruvate flux into the TCA cycle via pyruvate carboxylase (pc) is substantial but more than three times smaller compared to pyruvate dehydrogenase (pdh) flux in all glial cells studied.

In contrast to primary glia the glioma cell lines have a higher glycolysis rate than oxidative metabolism (Warburg effect). Their lactate and alanine content is particularly high.

F98, C6 glioma and primary glia cells show marked differences in glutamine synthetase activity.

POLYANIONIC PERINEURONAL NETS: SPECIALIZED GLIA-ASSOCIATED MICROENVIRONMENT OF NEURONS

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The net-like system of polyanionic glycoconjugates surrounding subpopulations of non-pyramidal neurons in the cerebral cortex, and large neurons in deep cerebellar and vestibular nuclei was studied in the rat using light and electron microscopic methods. The aim was to characterize glial components involved in this cytochemically specialized microenvironment of neurons.

Astrocytes were stained by immunocytochemical detection of glial fibrillary acidic protein (GFAP), S100-protein, or glutamine synthetase (GS). Griffonia simplicifolia I B_4-isolectin (GSA) was used as a marker for microglia.

The perineuronal nets detected by the colloidal iron hydroxide staining or binding of Vicia villosa agglutinin (VVA) were shown to be spatially related to moderately S100-positive, putative astroglial, processes at the light microscopic level. The formation of perineuronal nets by astrocytic profiles was confirmed by electron microscopic demonstration of GS in corresponding structures of deep cerebellar nuclei, and S100 in the lateral vestibular nucleus. VVA binding in the cerebral cortex was associated mainly with membranes of astrocytic processes in close vicinity to neuronal perikarya and the presynaptic boutons attached to them.

The polyanionic perineuronal nets seem to be related to a special type of glial function supporting classes of highly active neurons.

INTERACTIONS BETWEEN GLIAL CELL LINES AND NORMAL GLIA IN ROTATION MEDIATED AGGREGATING CELL CULTURES

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Rotation mediated aggregating cell cultures (RMACCs) provide an opportunity to study 3-dimensional cellular interactions *in vitro*. We have examined both the ability of CNS glial cells to form aggregates and the morphology of these aggregates. Primary mixed glial cell cultures (MGCs) formed uniform RMACCs in which astrocytes and myelin membranes were found. By separating MGCs into a top-dwelling population, enriched in oligodendrocytes and glial progenitor cells, and a bottom-dwelling population, enriched in type-1 astrocytes, our findings suggest that **both** populations are required for aggregate formation. A cell line which exhibits some features of type-1 astrocytes, when co-cultured with top-dwelling glial cells, formed aggregates which differed morphologically from normal mixed glial RMACCs. Our observations suggest that both the ability of glial cells to form aggregates and the morphology of aggregates formed demonstrate the interaction of normal glia and glial cell lines, and may be used to test whether a particular cell line can substitute for a normal glial population.

PH-CHANGES DURING GLUTAMATERGIC STIMULATION IN THE LEECH CENTRAL NERVOUS SYSTEM

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Neuropile glial (NG) cells and Retzius (RZ) neurones in the central nervous system of the medicinal leech have glutamate-receptors of the non-NMDA-type. We have used pH-sensitive microelectrodes to investigate the effects of the glutamatergic agonists on the intra- and extracellular pH in isolated ganglia. In NG cells the application of 10^{-4} M kainate in HEPES-buffered solutions induced depolarizations of 23,1 ± 6,5 mV which were accompanied by transient acidifications of 0,26 ± 0,07 pH-units (mean ± SD; n = 12). The membrane depolarizations in RZ neurones during the application of kainate were 19,2 ± 6 mV and the concomitant acidifications were 0,22 ± 0,04 pH-units (n = 10). DNQX inhibited the membrane depolarizations and the acidifications in NG cells completely, but in RZ neurones only partially. In CO_2/HCO_3^--buffered solutions the amplitudes of the kainate-induced acidifications were 0,22 ± 0,07 pH-units in NG cells (n = 12) and 0,18 ± 0,03 pH-units in RZ neurones (n = 10). In the extracellular space the kainate-induced acidifications were up to 0,45 pH-units.In Ca^{2+}-free solutions the amplitudes of the kainate-induced acidifications in all three compartments were reduced considerably.

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INVESTIGATION ON THE FUNCTION OF GLYCOGEN IN ASTROCYTES

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The function of glycogen in brain astrocytes is unknown. To contribute to the elucidation of the function of glycogen in astrocytes, studies were carried out on astroglia-rich primary cultures derived from the brains of newborn rats. Although glucose deprivation rapidly depletes astrocytes of their glycogen, glucose cannot be detected in the medium surrounding the astroglial cells. This contrasts with the situation encountered with hepatocytes.

The specific activity of glucose-6-phosphate hydrolyzing enzymes is only 18 % of that of hepatocytes. The specific activity of hexokinase in cultured astroglia is 10 times that of phosphatases. In contrast to the apparent K_M value of the phosphatase activity in the mM range, the K_M value for glucose of hexokinase in the astroglia homogenates is 40 µM. Thus, in accord with the experimental finding, it appears unlikely for a glucose molecule generated from glycogen to be able to leave the cells before it is rephosphorylated by hexokinase.

Instead of glucose the astroglial cells release high amounts of lactate into the culture medium. Gluconolactone or 2-deoxyglucose in the culture medium prevent the glycogen breakdown in astroglial cells that normally follows glucose deprivation. Therefore these compounds allow to discriminate between lactate generated from glycogen and lactate from other sources. The amount of lactate found in the medium in the absence of gluconolactone (or 2-deoxyglucose) exceeds the amount found in the presence of the compound by the lactate equivalents calculated to be contained in the cellular glycogen. Consequently glycogen in astrocytes can be considered as a store not as much for glucose as for lactate.

ACTIVATION OF THE NA^+/K^+ PUMP IN THE PIGMENTED GLIAL CELLS OF THE FLY RETINA

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The pigment cells of insect eyes play an important role for the functioning of the retinal tissue, as mechanical stabilization, optical isolation and energy supply of the photoreceptors ('glial function'). In bees, the pigment cells have been shown to be involved in the homeostasis of the extracellular fluid (Coles, Glia 2: 1-9, 1989).
By using ion-selective electrodes we investigated the transport of K and Na across the membrane of the pigment cells in superfused cut eyes of blowflies. We found that [Na]i was high (≈80mM) in the absence of extracellular K whereas [K]i was low (≈20mM). Extracellular addition of small amounts of K (2-8mM) induced a considerable decrease in [Na]i within a few min and a corresponding increase in [K]i. The concentration changes were due to the activation of a Na/K pump:
1. In the presence of the K channel blocker Ba the cells hyperpolarized when [K]o was increased, and 2. ouabain had a strong inhibitory effect. This inhibition, however, was not complete suggesting a contribution of passive ion fluxes to the observed concentration changes. Passive Na-K exchange was particularly evident when extracellular Na was substantially replaced by K.
Under physiological conditions [K]o was found to be 4mM and increased by a factor of about ten after intense light stimulation. Our results show that part of the released K is taken up by the pigment cells and that at least 50% of the uptake is due to the Na/K pump.

SURVIVAL-PROMOTING EFFECTS OF MESENCEPHALIC GLIAL CELL LINES ON CULTURED DOPAMINERGIC NEURONS

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Our previous studies have demonstrated that conditioned medium obtained from mixed mesencephalic glia promotes survival and differentiation of embryonic dopaminergic neurons in vitro. To obtain sufficient material to identify this factor(s), primary mesencephalic glia were immortalized using an inducible oncogene system. Glial proliferation in E14.5 mesencephalic cultures was stimulated with aFGF and heparin and cells were cotransfected with two DNA-constructs: the SV40 T-antigen under control of the lac operator and a lac repressor/VP 16 herpes transactivator fusion protein under control of the actin promoter.

As expected, cell proliferation was reversibly inhibited in the presence of the galactose-derivate, IPTG. The established cell lines expressed a process-bearing morphology and were immunoreactive for A2B5. Moreover, cells expressed double-labeling for A2B5 and GFAP upon treatment with cAMP, suggesting that the immortalized cells represent cells of the O2A/astrocyte type-2 cell lineage. Inclusion of CM derived from the established cell lines to serum-free low density cultures of the dissociated embryonic day 14.5 rat mesencephalon induced a dose-dependent increase in the number of surviving tyrosine hydroxylase-immunoreactive neurons.

The present study demonstrates that immortalized mesencephalic glial cells provide neurotrophic factors for dopaminergic neurons and, thus, can be a convenient source for the isolation of glial factors affecting the survival and differentiation of this neuronal phenotype.

RECEPTOR SUBTYPE INVOLVED AND MECHANISM OF NOREPINEPHRINE INDUCED STIMULATION OF GLUTAMATE UPTAKE INTO PRIMARY CULTURES OF RAT BRAIN ASTROCYTES

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Glutamate uptake into rat brain astrocytes is potently stimulated by addition of norepinephrine (NE). This effect is mediated by α_1-adrenergic receptors expressed by these cells (Hansson and Rönnbäck, 1989, 1991). The present study was undertaken in order to identify the adrenergic receptor subtype involved, and to determine the sequence of events following receptor activation. NE increased glutamate uptake rates in a dose- and time-dependent manner (EC_{50}=6 mM). Both, the selective α_1-receptor antagonist prazosine (IC_{50}=2.5 µM) and the α_{1b}-adrenergic receptor subtype specific alkylating agent chlorethylclonidine (CEC, 100 µM) prevented NE (100 µM) evoked stimulation of glutamate uptake. Furthermore, omission of Ca^{2+} from the extracellular medium had no significant influence on NE-induced increase in glutamate uptake, indicating that the stimulatory effect is mediated by α_{1b}-adrenergic receptors. Treatment of cells with pertussis toxin (PTX) for 24h or with 12-O-tetradecanoylphorbol-13-acetate (TPA) for 30-45 min prior to NE addition abolished the NE mediated effect on glutamate uptake. Addition of TPA alone resulted in a rapid increase of glutamate uptake rates, which declined to control levels when TPA was applied 30 min prior to uptake initation by glutamate. The increase in glutamate uptake elicted by TPA and NE added at the same time showed no additivity of the stimulatory effect resulting from treatment with each agent alone. Treatment of cell cultures with 1mM Ba^{2+} influenced glutamate transport principially in the same manner as found for TPA and NE, respectively. Again no additivity of Ba^{2+} and NE effects on glutamate uptake was observed. Finally, NE, TPA and Ba^{2+} stimulated glutamate uptake exhibited nearly identical time-response curves. These results suggest that NE predominantly acts via α_{1b}-adrenergic receptors, resulting in an activation of protein kinase C, which in turn leads to a blockade of outwardly directed K^+-currents. This increases the intracellular K^+-concentration and thereby stimulates glutamate uptake.

PURIFICATION OF A CNS PROTEOGLYCAN WITH NEURITE OUTGROWTH PROMOTING PROPERTIES. Andreas Faissner[1)], Andre Lochter[1)], Andrea Streit[1)] und Melitta Schachner[2)].
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Proteoglycans (PGs) are expressed in various tissues on cell surfaces and in the extracellular matrix and display substantial heterogeneity of both protein and carbohydrate constituents. For example, at least 25 proteoglycan core proteins have been distinguished in rodent brain. The functions of individual proteoglycans of the nervous system are not well characterized, partly because specific reagents which would permit their isolation are missing. We report here that the monoclonal antibody DSD-1, which binds to the surface of a subclass of murine glial cells (formerly 473, A. Faissner, Soc. Neurosci. Abstr. **14**, 920 (1988)), reacts with a dermatan sulfate/chondroitin sulfate hybrid epitope on a chondroitin sulfate PG. The molecule was purified from postnatal mouse brain and displays an Mr between 800 kD and 1000 kD with a major core glycoprotein of 300 kD. The PG promotes neurite outgrowth by embryonic hippocampal neurons in vitro, a process which can be blocked by monoclonal antibody DSD-1 and enzymatic removal of the dermatan sulfate-containing glycosaminoglycan chain(s). These results show that dermatan sulfate-containing glycosaminoglycans promote the morphological differentiation of CNS neurons. We propose to name the proteoglycan syneuran.

WOUND EXPERIMENTS IN CORTICAL CULTURES : A MODEL FOR IN-VITRO REGENERATION STUDIES

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A "wound culture system" has been developed in our laboratory for in-vitro studies on regeneration. Dense cultures from rat cortex are "lesioned" by scratching a wound in the cell layer. Dynamic changes of glial cells and neurons after wounding are followed by phasecontrast microscopy, timelapse cinematography and flourescence microscopy. These changes concern cell migration into the open wound space, process formation and changes in cellular morphology (F. Förster and W.Seifert, in "Neural Dev. and Regeneration", 491-503, Springer 1988).
The different cell types found in the wound at various times were characterized (astrocytes, microglial cells, oligodendrocytes, neurons) by using several antisera for immunofluorescence microscopy. The effects of basic fibroblast growth factor (bFGF) on these dynamic processes were investigated. FGF stimulates outgrowth of mainly astrocytic processes into the wound.
We like to propose this wound culture system as a useful two-dimensional lesion model for in-vitro studies on regeneration in the central nervous system.

SPINAL CORD MICROGLIA IN EXPERIMENTAL ALLERGIC NEURITIS: EVIDENCE FOR FAST AND REMOTE ACTIVATION

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Activation of microglial cells has been demonstrated in the spinal cord after transection of peripheral nerves (Gehrmann et al. (1991) Restor. Neurol. Neurosci. 2: 181-198). To assess the role of microglial cells during neuroimmune processes occurring outside the CNS, we have examined the response of CNS microglial cells to an autoimmune inflammation of the peripheral nervous system, i.e. acute and chronic relapsing experimental allergic neuritis (EAN). Activation of microglial cells became apparent through changes in their immunophenotype and morphology within 48 hours of T cell transfer thus preceding the onset of clinical disease. The microglial reaction occurs at a site remote from the inflammatory lesions in the peripheral nerve. Similar changes, however, were also observed in the terminal projection fields of the primary, afferent fibres, such as the nucleus gracilis. Subsequently, after seven days, perineuronal microglial cells were found to detach afferent synaptic terminals from the neuronal surface, a process generally refered to as "synaptic stripping". This early activation of microglial cells in EAN suggests that a rapid and remote signaling might be operating in the microglial response during T cell-mediated autoimmune diseases.

IDENTIFICATION OF TRANSCRIPTIONALLY REGULATED GENES AFTER SCIATIC NERVE INJURY

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Axotomy of peripheral nerves in mammals leads to a complex but stereotypic pattern of histopathological reactions known as Wallerian degeneration. We want to get access to the genes involved in the degenerative and the subsequent regenerative responses. Therefore we constructed a cDNA library from the distal part of crushed sciatic nerves one week after injury. Differential screening of 5000 cDNA clones with ^{32}P-cDNA of crushed versus intact nerve delivered 14 cDNA-clones which are upregulated and 9 cDNA-clones which are downregulated after sciatic nerve injury. Sequence analysis and computer-assisted sequence comparison leads to the identification of 15 known and 8 novel sequences. The regulated sequences can be divided into three groups:

1. Eight novel sequences which will be further characterized.
2. Gene products, although known from other tissues, but which have never been detected in the PNS like Apo D (1), CD25/gas3 (2), Decorin, SPARC, SGP-1/SAP-1 and X16-mRNA.
3. Known sequences e.g. the myelin protein P_0, y-Actin, Vimentin, α-Tubulin, Chargerin and the Cytochrom C-Oxidase Subunit I

We will present the screening strategy and show the tissue specificity, the pattern of expression after nerve lesion and during development for some selected cDNA-sequences. Supported by BMFT.

(1)Spreyer et al. (1990), EMBO Vol. 9, p.2479-2484
(2)Spreyer et al. (1991), EMBO Vol.10, p.3661-3668

EXPERIMENTAL SPINAL CORD INJURY IN RATS: EFFECT OF 7ß-HYDROXYCHOLESTERYL OLEATE ON GLIAL SCAR.

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A classical problem in the adult CNS fiber regeneration is the astroglial response to injury.

In the present study, we have used an ester of cholesterol (EC) namely 7ß-hydroxycholesteryl oleate to prevent the formation of a glial scar. Adult rats underwent an hemisection of the spinal cord at low thoracic level and, two days later were injected intradurally through a thin catheter, with a suspension of liposomes (10 µl) containing 150 µg of EC. The animals were sacrificed after survival times of 9 days and 3 weeks. Immunocytochemistry was performed with polyclonal anti-GFAP and anti-serotonin antisera. Sections treated with the anti-GFAP antiserum were digitalized with an image analysis system (Samba 2005, Alcatel) in order to evaluate by densitometry the intensity of glial reaction.

Untreated control animals 9 days postlesion showed statistically significant increase in the immunodensity level (74.53%, $p<0.001$) on the lesioned as compared to the intact side. In contrast, in the treated animals, the immunodensity on the lesioned side was only slightly increased (27.60%) with statistically non significant difference versus the intact side. In addition, 3 weeks postlesion, we have observed in the treated animals, regrowing serotonergic fibers on the lesioned side originating from the intact side. These results show that EC decreases the proliferation of reactive astrocytes after a surgical lesion thus allowing axonal regrowth in the denervated territory.

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REGULATION OF I-A AND ICAM EXPRESSION ON SCHWANN CELLS AND CORRELATION WITH T-CELL ACTIVATION

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We studied the regulation of I-A and ICAM expression on rat Schwann cells (SC) which served as antigen-presenters (APC) in an in-vitro model for experimental allergic neuritis. SC cultures were of greater than 95 % purity after Thy 1.1 mediated complement kill.

In contrast to astrocytes, µ-IFN alone only weakly induced I-A on SC. TNF-α, however, potentiated the effect of µ-IFN and upregulated I-A expression in a dose-dependent manner. ICAM was readily upregulated by µ-IFN alone within 24 hours, with only a minimal additive effect of TNF-α. I-A and ICAM expression could be further enhanced by repetitive cytokine treatment. In contrast to astrocytes, elevation of intracellular cAMP during the induction period upregulated I-A on SC, whereas protein kinase C activation was downregulating as in astrocytes. Proliferation studies with BrDU disclosed a correlation between I-A expression and S-phase entry. Adhesion to a matrix and chronic mitogen treatment downregulated I-A inducibility of SC.

The ability of SC to activate T cells reached a maximum within 24 hours , one day earlier than professional APC's. Enrichment of $I\text{-}A^+$ SC by cell sorting and treatment with graded doses of cytokines did not lead to a further enhancement of T-cell activation and thus points towards the lack of accessory molecules on SC .

INDUCTION AND TRANSCRIPTIONAL REGULATION OF THE MYELIN PROTEOLIPID PROTEIN (PLP) GENE

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The terminal differentiation of postmitotic oligodendrocytes is marked by the transcription of a set of genes encoding structural proteins of myelin and enzymes of myelin lipid metabolism. Because glial precursor cells can not be isolated in sufficient quantities required for the biochemical analysis of gene regulation, little has been learned about the induction and regulated expression of these myelin-specific genes. We have investigated the ability of glial cell lines to "differentiate" in culture and transcribe the gene for proteolipid protein (PLP), the major integral membrane protein of CNS myelin. The glioblastoma line C6 differentiates in culture and expresses a high levels of PLP and CNP mRNA. Following growth arrest at high cell density or following serum removal, the endogenous PLP gene was induced. In exponentially growing C6 cells cultured with frequent changes of media containing fetal calf serum, PLP mRNAs were virtually absent. Using the chloramphenicol acetyl transferase (CAT) gene as a reporter in fusion constructs, the activity of the cloned rat PLP promotor (CAT-1038) was analysed in transiently and stably transfected C6 cells and various heterologous cell lines. Promotor constructs with increasingly truncated 5' ends revealed multiple positive and negative regulatory elements within 1 kb upstream of the transcription start sites. Most of these elements were matched by the location of protein binding sites as visualized by DNaseI footprints. Since transient expression of PLP was also observed in some non-glial cell lines (NIH-3T3 cells, B78 melanoma), we suggest that DNA methylation and/or chromatin packing are involved in the regulation of the endogenous PLP gene.

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DIFFERENTIAL EXPRESSION OF EARLY RESPONSE GENES AFTER FACIAL NERVE LESION

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Facial motoneurons respond with a number of molecular changes to peripheral transection of the facial nerve. In order to obtain insight into the transcriptional mechanisms underlying the changes induced by axotomy the expression of a number of early response genes (IEGs) was investigated after facial nerve lesion. Some IEGs (such as c-fos, c-jun or jun B) are known to encode transcription factors that bind to DNA at sites known to regulate gene expression and they could therefore contribute to long-term changes in motoneurons. Northern blot analysis of RNA extracted from the facial nucleus from post-operative intervals covering hours, days and weeks revealed that injured motoneurons show a unique pattern of IEG induction. c-Jun and jun B RNAs, also present in low amounts in the unoperated nucleus, are strongly induced after injury. The increase of jun B and c-jun mRNA levels starts at 7 hours and these levels are maintained up to eight days after axotomy. c-fos, however, known to act in concert with c-jun in other systems, was not expressed at basal levels in the unoperated nucleus, nor was c-fos mRNA induced by axotomy at all time points studied. Two members of the TIS family of early response genes, TIS 7 (PC 4) and TIS 11 RNA, however, were detectable at low levels in normal facial nucleus. TIS 7 mRNA levels were unaffected by lesion, whereas TIS 11 mRNA levels were increased in a similar fashion as c-jun and jun B with an early rise at 10 h that lasted until day eight. So far, c-jun mRNA could be clearly localized in motoneurons of the facial nucleus by in situ hybridization histochemistry indicating that c-jun induction happens in the course of the retrograde reaction of the motoneurons and not in the glial cells which also respond to injury of the facial nerve.

CYTOCHEMISTRY OF LECTIN-BINDING COMPONENTS OF PERINEURONAL NETS IN RELATION TO NEURONAL STRUCTURES IN RAT BRAIN

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Perineuronal nets contain components which can be recognized by the N-acetylgalactosamine (GalNac)-binding plant lectin Vicia villosa agglutinin (VVA). Two main reasons led to the assumption that a heterogeneous group of glycoconjugates is cytochemically visualized. Tollefsen and Kornfeld (1983, J.Biol.Chem. 258: 5172) found that several glycopeptides with O-glycosidically linked oligosaccharide units have a much higher affinity to VVA than monosaccharides such as GalNac. Furthermore, Naegele and Katz (1990, J.Neurosci. 10: 540) demonstrated some VVA-binding polypeptides after electrophoresis and blotting of cat cortical membrane fractions. We present modifications for the sensitive cytochemical demonstration of VVA-binding sites. It is shown that the lectins from the plant Trichosanthes kinlowii and the snail Helix aspersa reveal perineuronal nets in a manner which is different from the distribution of the VVA-label in the cortex. On the other hand, the localization of binding sites of the lectin from Wisteria floribunda and VVA seems to be similar.

To characterize cortical structures surrounded by perineuronal nets a double fluorescence labelling of VVA and markers for GABAergic neurons (glutamate decarboxylase and parvalbumin) was performed. It was confirmed that the majority of VVA-positive strutures is in close vicinity to GABAergic neurons. However, the cytochemical properties of structures enwrapped in perineuronal nets are further to be elucidated in other brain regions.

NORMAL AND ABNORMAL LIGHT INDUCED POTASSIUM CHANGES IN THE ISOLATED RABBIT RETINA

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Light induced extracellular potassium changes were measured with Corning- and valinomycin-electrodes in the isolated rabbit retina superfused by a plasma-saline mixture. There was no potassium gradient over the retina which needed to be buffered by Müller cells in the dark, when the measurements were done with potassium-specific valinomycin electrodes, but there was a gradient when Corning electrodes were used. This seems to be an experimental artefact. There was, however, no difference in the light-induced potassium changes when these two different kinds of electrodes were used. In apparently healthy preparations the increases of potassium at the vitreal surface and in the proximal retina are small: mostly less than 0.5 mM. This changed dramatically when the upper vitreal surface of the retina accidentally lay beneath a still solution which probably lacked oxygen. Potassium increases of more than 2 mM then occured at the vitreal surface. Under this condition the ERG was mostly cornea-negative. This marked effect of inadequate superfusion upon the light-induced potassium changes in the proximal retina may serve as an experimental model of pathological conditions under which there is a greater than normal need for the buffering capacity of Müller cells.

INTERFACE CULTURES OF HIPPOCAMPAL SLICE EXPLANTS AS A MODEL FOR THE EXPERIMENTAL ANALYSIS OF BRAIN DEVELOPMENT

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In vivo analysis of brain development during the past century has accentuated the important role of radial glial cells for the migration and laminar deposition of cortical neurons. The mode of and external influences on the ontogenesis of this guiding scaffold itself are mostly unknown. With previous experiments we have shown in vivo, that interactions with the surrounding mesenchyme are one prerequisite for radial cell differentiation and orientation. To analyze mesenchymal influences on radial glial cell development in more detail we have developed a modified type of organotypical cultures of neural tissue. Slice preparations of the hamster hippocampal formation explanted during the first two postnatal weeks are transferred to a filter substratum (cellulose nitrate or polycarbonate, pore size 0,4 μm) held at the medium surface. This 'interface assemblies' can be maintained in a medium consisting of a mixture of DMEM and Ham F12 supplemented with 20% horse serum for up to at least five weeks. By contrast to conventional slice culture preparations (in roller tubes or Maximov chambers) slice cultures in interface assemblies are directly exposed to an inert substratum and the culture medium without being surrounded by an ill-defined plasma clot or other adhesives. Thus, the effects of defined substratum coatings and soluble factors supplemented to the medium can directly be analyzed. Additionally, the complete separation of the culture well into two subcompartments connected only by filter pores of a defined size allows for various coculture assays.

Glial cells and spatial K^+ buffering in rat hippocampus

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Stimulus and seizure related rises in $[K^+]_o$ are limited to a ceiling level of about 12 mM in adult nervous tissue, while in young neocortex and hippocampus abnormal rises in $[K^+]_o$ may occur. The ceiling of rises in $[K^+]_o$ involves active reuptake into nerve cells and K^+ uptake into glia via the Na-K-ATPase and KCl cotransport. Glial cell contribute likely also to K^+ regulation by spatial redistribution. A factor in generation of rises in $[K^+]_o$ is also the myelinisation of fibres and the neuronal excitability. Studies on the expression of transient A currents and delayed rectifier K currents in hippocampal pyramidal cells studied in culture or after acute isolation suggest that these currents are about mature when abnormal K elevations appear. A measure of the pump activity is the relationship between rises in $[K^+]_o$ and subsequent undershoots. Findings from our lab suggest that similar sized rises in $[K^+]_o$ are followed by larger undershoots in weeks 1, 2 and 3 after birth. Ontogenetic studies on the appearance of astrocytes in hippocampal area CA1 show that the number of astrocytes is only normalized at about 3 weeks in all hippocampal layers. However, their spatial organisation seems to still differ at weeks 2 and 3 after birth. To test whether a delayed maturation of glial cells can cause abnormal $[K^+]_o$ rises we blocked glial K^+ conductances by Ba^{2+}. This resulted in abnormal rises in $[K^+]_o$ and slowed decay kinetics. The findings are in line with the hypothesis that glial cells play an important role in K^+ regulation.

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VOLTAGE-DEPENDENT K^+ CHANNELS OF RABBIT RETINAL MÜLLER (GLIAL) CELLS

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Enzymatically isolated Müller cells of adult rabbit retinae were used to study the expression of voltage-dependent channels, by means of the whole-cell configuration of the patch-clamp technique. In K^+free solutions, almost no current was evoked by applied voltages. When current responses to voltage ramps were recorded in solutions containing various $[K^+]_e$, a shift of the reversal potential occurred which was according to that expected from the NERNSTian equation for K^+ ions. The current response of the membrane of all cells to voltage ramps showed distinct inward rectification which was eliminated by 5 mM Cs^+ in the bath solution. The inwardly rectifying current could be isolated by using a voltage protocol with 0 mV holding potential, and showed inactivation at stronger hyper-polarizations. In addition to the inwardly rectifying current $I_{K(IR)}$ we found two different outward currents viz. a delayed rectifier $I_{K(DR)}$ and a rapidly inactivating current $I_{K(A)}$. The latter was activated by depolarizing voltage steps of more than -60 mV, and was fully inactivated at +20 mV. $I_{K(DR)}$ was activated by depolarizing voltage steps of more than -30 mV, showed almost no inactivation, and was specifically and reversibly blocked by 0.5 mM 4-AP. $I_{K(IR)}$ may provide for spatial buffering of excess K^+ ions during retinal activity, whereas $I_{K(A)}$ and $I_{K(DR)}$ may be involved in control of cell proliferation.

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Isolation and characterisation of a murine glia precursor cell line

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The establishment of primary brain cells, that exhibit early neuronal and glia stem cell properties and resemble freshly isolated neuroepithelial cells turned out to be quite difficult. Such cells must be stably immortalized without changing morphological and growth properties as well as gene expression patterns.

After electroporation of a retroviral vector carrying the myc oncogene we isolated a specific glia precursor cell line from murine brain cultures E10. The cells expressing the myc oncogene were subcloned and submitted to FACS immunofluorescence analysis using glia and neuronal specific monoclonal antibodies. We could show a disinct expression pattern of glia and oligodendrocyte markers in the various clones. This was accompagnied by differences their morphology. The modulated expression of brain specific markers and the different phenotype of the clones suggest, that we established a panel of glia progenitors which could be used as tools to study the role of oncogenes, growth factors and growth factor receptors during glia differentiation.

TENASCIN PROMOTE CEREBELLAR GRANULE CELL MIGRATION AND NEURITE OUTGROWTH BY DIFFERENT DOMAINS IN THE FIBRONECTIN TYPE III HOMOLOGOUS REPEATS.

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The extracellular matrix molecule tenascin is a hexameric glycoprotein which is involved in neuron-glia recognition. To study its role in astrocyte-mediated neuronal migration, five mAbs were used in an *in vitro* assay (modified from Lindner et al., Nature, 305: 427, 1983) to analyse the migration of cerebellar granule cells. Rotary shadowing electron microscopy was used to localize the antibody binding sites on tenascin. The mAbs J1/tn1, -4, and -5 bound within the third to fifth fibronectin type III repeats, while J1/tn2 reacted with the eleventh repeat and J1/tn3 within the third to fifth EGF-like repeats (for J1/tn1 and -2, see Lochter et al., J. Cell Biol., 113:1159, 1991). Only mAbs J1/tn1, -4, and -5 inhibited cerebellar granule cell migration. To study which of the domains are involved in neurite extension of granule cells, the influence of tenascin adsorped to polyornithine on neurite extension was measured in the absence or presence of the mAbs. Only mAb J1/tn2 inhibited the stimulatory effect of tenascin as described for hippocampal neurons (Lochter et al., ibid.). We conclude that tenascin influences neurite outgrowth and neuronal migration by different domains in the fibronectin type III repeats.

ATP INDUCES AN INCREASE OF INTRACELLULAR CALCIUM IN CULTURED MOUSE MICROGLIAL CELLS

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Microglial cells are not considered as elements of a fast signal transfer in the nervous system and thus as cells expressing neurotransmitter receptors. In this study, however, we found that the cotransmitter ATP (10^{-7}M to 10^{-4}M) induced a transient, rapid rise in intracellular [Ca^{++}] in cultured microglial cells from mouse cortex. 30s application of ATP induced a rise in intracellular calcium with a peak after 20s as measured with a fura-2 based Ca^{++} imaging system. Subsequently resting Ca^{++} levels were restored within several minutes. ATP was more potent than ADP, while AMP and adenosine were uneffective indicating the involvement of P_2 receptors. The lack of an ATP-induced Ca^{++} response in Ca^{++}-free bathing solution points to an Ca^{++} influx; this influx is not mediated by voltage-gated Ca^{++} channels, since depolarization (by 50mM K^+) did not trigger a Ca^{++} elevation. Simultaneous current recordings with the perforated patch clamp technique indicates that the rise in Ca^{++} is accompanied by an inward current. The ATP-induced response could be a means for microglia to sense neuronal activity. Moreover, it could serve as a mediator for detecting injured neurons which may leak ATP; the rise in Ca^{++} could induce a cellular response in microglial cells, e.g. such as secretion, migration or phagocytosis.

GLIAL CELLS IMMORTALIZED BY INFECTION WITH AN SV 40 T CONTAINING RETROVIRUS RETAIN IN VITRO AND IN VIVO PROPERTIES OF PRIMARY CELLS.

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We have used oncogene-carrying replication-defective retroviruses to immortalize murine glia. Primary cultures of cerebellar cells originating from 6-day-old mice were freed from neurons by complement-dependent immunocytolysis and subsequently infected with a retrovirus based on Murine Moloney Leukemia Virus containing a temperature-sensitive mutant of the SV 40 T oncogene and a gene coding for resistance to the antibiotic G 418. After several in vitro passages cells expressing the 04 antigen were cloned on the fluorescent activated cell sorter. This antigen is expressed by precursors of oligodendrocytes and type-2-astrocytes, oligodendrocytes and Schwann cells. We obtained two different types of clones: 1) cells which gradually lost an oligodendrocyte-like morphology, lost expression of oligodendrocyte-specific markers and processes and became flat and 2) cells which showed several characteristics of Schwann cells. Culture of the latter cells in the presence of 1 mM dibutyryl cAMP for a period of at least 10 days induced a shape change and a shift in antigen expression towards a more mature stage. An uncloned bulk population of an early passage of the SV 40 T immortalized cells was transplanted into demyelinated lesions in adult rats. The transplanted cells ensheathed axons in a manner typical of Schwann cells and were capable of limited myelin formation. Cells immortalized with a temperature-sensitive mutant of the SV 40 T oncogene are thus capable of a degree of differentiation both in vitro and in vivo.

INTERNALIZATION OF NERVE GROWTH FACTOR MEDIATED BY THE LOW AFFINITY NGF RECEPTOR $p75^{NGFR}$

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Two glial cell lines, peripheral Schwannoma D6P2T and central 33B glioma cells were used to investigate the properties of the low affinity receptor for NGF ($p75^{NGFR}$). Binding of NGF was characterized by a Kd in the low nanomolar range, rapid dissociation kinetics and trypsin sensitivity at 4°C. On SDS-PAGE a heterogeneously glycosylated protein with a molecular weight of 60-80 kDa and its high molecular weight aggregates were identified as the NGF binding protein. All protein bands cross-linked to radiolabeled NGF were immunoprecipitated with anti-$p75^{NGFR}$ antibody, but not with anti-$p140^{prototrk}$ antiserum. In these cells, which were shown to express exclusively $p75^{NGFR}$, the time- and temperature-dependent appearance of a nondisplaceable, trypsin-resistant fraction of NGF was observed. The observed sequestration resembled receptor-mediated NGF internalization. Whether this internalization may indicate a functional role of $p75^{NGFR}$ in glial cells remains to be shown.

DYE-COUPLING AMONGST ASTROCYTES IN RAT HIPPOCAMPAL FORMATION

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There is increasing evidence that electrical coupling between astrocytes subserves important functions of these cells. For example, potassium can be redistributed from sites of neuronal activation to remote locations via a buffering mechanism utilizing gap junctions. In addition, recent data indicate that gap junctions allow astrocytes to exchange second messengers and thereby propagating intracellular calcium waves over considerable distances amongst coupled cells.
We investigated the presence and topography of junctional coupling in slices of rat hippocampus. Astrocytes were recorded intracellularly with glass micropipettes and subsequently filled by intracellular injection of the fluorescent dye Lucifer Yellow and biotin. The latter was visualized by horseradish-peroxidase-conjugated avidin. It is shown that hippocampal astrocytes of adult rat reveal extensive dye-coupling, while no coupling was detected within about the first two postnatal weeks. In adult rat injection of a single cell with low-molecular weight dyes often results in dyespread to more than 100 neighbouring cells covering an area of several hundred microns in diameter.
The data will be discussed with respect to a possible function of astrocytic communication in hippocampal physiology.

COEXPRESSION OF A NEW 22 kD SCHWANN CELL PROTEIN WITH OTHER PERIPHERAL MYELIN GENES DURING REGENERATION AND DEVELOPMENT

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Peripheral nerve lesion leads to axonal degeneration and repression of the myelin genes. Regeneration of axons is the prerequesite to reinduce myelin gene expression in Schwann cells.
Recently we described the molecular cloning of a novel Schwann cell gene, CD25, with possible growth-regulatory properties (1). The CD25-transcript is expressed at high levels in Schwann cells of adult intact sciatic nerve. However, within 2 days after peripheral nerve lesion CD25-expression is downregulated in the distal nerve stump.
Our present data suggest that CD25 is a novel peripheral myelin gene: (a) The CD25-transcript is induced by regenerating axons after reanastomosis of a transected sciatic nerve. (b) Expression of CD25 correlates well with the expression of the myelin protein P0 and the myelin basic protein (MBP) during regeneration and postnatal development. (c) In vitro translation and immuno-precipitation experiments show that the CD25 gene product is a 22 kD glycosylated membrane protein with structural similarities to the CNS myelin proteolipid protein (PLP). The recent chromosomal localization of the gas3 gene (2), the mouse homologue of rat CD25, makes it very likely that this gene is defect in the peripheral myelin deficient mouse mutant *trembler*.

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(1) Spreyer et al., 1991, EMBO J, 10:3661-3668.
(2) Colombo et al., 1992, Mammalian Genome (in press).

IMMUNOCYTOCHEMICAL LOCALIZATION OF MALIC ENZYME IN NEURAL PRIMARY CULTURES

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Malic enzyme (EC 1.1.1.40) catalyzes the formation of pyruvate, NADPH and CO_2 from malate and $NADP^+$. The cytosolic isoform (cME) has well known anaplerotic functions and is important for fatty acid synthesis. The existence of ME in glial primary cultures may be responsible for the fact that the glial cells can use glutamate as only energy source, at least for a limited period of time.
cME was purified from bovine brain 6,400-fold to apparent homogeneity. The homotetrameric enzyme had a specific activity of 50 U per mg of protein and a subunit molecular weight of 58 kDa.
Mouse monoclonal antibodies raised against cME were used to investigate the localization of this enzyme in rat astroglia-rich and neuron-rich primary cultures. cME was colocalized with the astrocyte marker glial fibrillary acidic protein and with the oligodendrocyte marker myelin basic protein. The intensity of staining of astrocytes was heterogeneous. Strong staining was found in nearly all oligodendroglial cells. Neurons did not show positive staining. The findings suggest that cME is predominantly a glial enzyme.

Lectin interactions in the secretory product of the subcommissural organ (SCO) under functional aspects.

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The glial SCO forms the Reissner's fibre (RF) extending caudally through the brain ventricles of almost all vertebrates. The function of the SCO is unknown.
We studied lectin properties of the SCO secretory material playing possibly a fundamental role in the RF forming process, and looking forward, they might be major candidates for influencing cell-cell communication in the embryonic nervous system.
Additionally, we characterized a putative ligand-receptor system in the secretion of the SCO, the interacting molecules of which might be similar either to Phaseolus vulgaris agglutinin-L (PHAL) or to the urinary Tamm-Horsfall glycoprotein (THGP).

The L2/HNK-1 carbohydrate is involved in preferential neurite outgrowth of motoneurons on ventral (motor) versus dorsal (sensory) nerve roots

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The carbohydrate epitope L2/HNK-1 is expressed on the majority of Schwann cell tubes of ventral spinal roots and motor nerves, but only on few Schwann cell tubes of dorsal roots and sensory nerves of adult mice. Based on these observations the possibility was investigated that the L2/HNK-1 carbohydrate may be involved in preferential growth of regenerating motoneuron axons. Using sections of fresh-frozen ventral roots or motor nerves and dorsal roots or sensory nerves of adult rats and mice as substrates for outgrowing neurons, motor neurons plated on ventral roots or motor nerves were found to grow 35% longer than on dorsal roots. In contrast, neurons from dorsal root ganglia (DRG) showed no significant difference in neurite length on dorsal versus ventral roots. The preferential growth of motor neurons on ventral roots could be inhibited by FAB-fragments to the L2/HNK-1 epitope. Outgrowth of DRG neurons on both roots was not affected by the antibodies. When glass coverslips were coated with both the outgrowth promoting extracellular matrix constituent laminin and a glycolipid containing the L2/HNK-1 carbohydrate, neurite outgrowth from motor neurons could be blocked by L2/HNK-1 antibodies by 50%, while neurite outgrowth from motor neurons on GM1 and sulfatide was not affected by the antibodies. - These experiments show that the L2/HNK-1 carbohydrate epitope promotes neurite outgrowth from motoneurons and is, therefore, a candidate to guide regenerating motoraxons into L2/HNK-1-positive motor nerves after a lesion of mixed nerves.

LINEAGE RELATIONSHIPS AMONG RETROVIRALLY INFECTED GLIAL CELLS IN THE NEONATAL RAT CEREBRAL CORTEX: AN ULTRASTRUCTURAL ANALYSIS.

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Previous studies have revealed that there are separate lineages for neurons, astrocytes and oligodendrocytes in the developing rat cerebral cortex as early as the onset of cortical neurogenesis. However it is unclear whether some glial progenitors in the neonatal cerebral cortex are bipotential giving rise to both astrocytes and oligodendrocytes. An ultrastructural analysis of clones generated by retroviral infection of neonatal progenitor cells has been undertaken to investigate this possibility. The Bag retrovirus containing the reporter gene *E.coli*. β-galactosidase (lac z) was injected into the cerebral ventricles of one day old rats which were perfused two weeks later and their brains serially sectioned. X-gal histochemistry revealed discrete groups of Lac-Z positive cells (clones) and these sections were subsequently processed for electron microscopy. All cells within such groups were identified according to ultrastructural criteria. In no case did a clone contain both astrocytes and oligodendrocytes. Most clones contained only astrocytes and were found in grey and white matter. Some clones contained only oligodendrocytes whereas others in the subependymal region were composed entirely of immature glia of unknown lineage. Although most clones were composed either solely of astrocytes or oligodendrocytes, both mature and less differentiated cells occasionally occurred within the same clone.

NEUROTROPHIC FACTORS SUPPORT THE SURVIVAL OF AXOTOMIZED RETINAL GANGLION CELLS IN ADULT RATS *IN VIVO*

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After transection of the optic nerve in adult rats, retinal ganglion cells (RGC) suffer from progressive degeneration until after two months a residual population of only about 5% of these cells survives.
In this study, we investigate the effect of BDNF, CNTF and regeneration associated factors from sciatic nerve on the survival of adult rat RGC after intraorbital optic nerve transection. Neurotrophic factors were injected into the vitreous: BDNF or CNTF, about 50ng/100g body weight, alternatively sciatic nerve exsudate (5ul/100g, prepared as described by Thanos *et al.*, *Europ.J.Neurosci.* 1(1989):19-26). Rats were allowed to survive 3, 5, or 7 weeks. The remaining viable RGC were then labelled by retrograde staining with the carbocyanine dye 4Di-10Asp, which was applied onto the proximal nerve stump in vivo. The animals were sacrificed 3 days later and RGC counted in retinal whole mounts.
After 3 weeks a 3-fold increase in the number of surviving RGC could be achieved using either of the neurotrophic factors (compared to controls, where HBSS was injected). The rescue effect was less pronounced after 5 and 7 weeks, and no additive effect could be seen when combining BDNF and CNTF treatment.

ION CHANNELS IN NEUROPILE GLIAL CELLS OF THE LEECH NERVOUS SYSTEM

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It is now established that glial cells surrounding the neurones are involved in the maintenance of ionic homeostasis in the central nervous system (CNS). We have used patch-clamp techniques to investigate single-channel properties of neuropile glial (NG) cells in the CNS of the medicinal leech. After opening the outer ganglion capsule the ganglia were enzyme treated with collagenase/dispase and the neuronal cell bodies were removed to expose the NG cells.
Ionic currents from single K^+-channels in the NG cell membrane were recorded in the cell-attached mode. The conductance of these K^+-channels was 50 pS, and they were regulated by the membrane potential. The application of 5-HT decreased their open state probability. The measured reversal potential of +10 mV indicates that the K^+-channels may also be permeable to other cations.
Intracellular marking of the NG cells was accomplished electrophoretically by injection, through the patch-pipette in the whole-cell mode, of a solution containing the fluorescent dye Lucifer Yellow.
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CALCIUM ENTRY THROUGH KAINATE RECEPTORS BLOCKS RESTING POTASSIUM CURRENTS IN CEREBELLAR BERGMANN GLIAL CELLS

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In this study, we characterized the glutamate receptor of Bergmann glial cells in a cerebellar slice preparation. The patch-clamp technique revealed a kainate-type glutamate receptor with an unusual sigmoid current to voltage relationship. Using a fura-2 based Ca^{++} imaging system, we observed a kainate induced increase in $[Ca^{++}]_i$. This Ca^{++} elevation was blocked by CNQX and by low external Ca^{++}. It was not mediated by the activation of Ca^{++} channels indicating an influx of Ca^{++} through the kainate receptor itself.

The entry of Ca^{++} led to a marked reduction in the resting (passive) K^+ conductance of the cell. This $[Ca^{++}]$ increase and the concommitant blockade of K^+ channels was either triggered by activation of the kainate receptor or by application of the Ca^{++}-ionophore ionomycin. Moreover, clear reversal potentials for the kainate-induced currents were only observed after the K^+ conductance was blocked. Since Purkinje cells with their glutamatergic synapses and Bergmann glial cells are closely associated, a functional interaction becomes likely.

INTRACELLULAR CALCIUM OF IDENTIFIED LEECH GLIAL CELLS

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The intracellular Ca^{2+} (Ca^{2+}_i) of identified glial cells in the neuropile of the central nervous system of *Hirudo medicinalis* was measured *in situ*, using the emission ratio of fura-2 at 350 nm and 380 nm excitation. The neuropile glial cells were exposed by mechanically removing the overlying ganglion capsule and neuronal cell bodies. This allows the impalement of microelectrodes under visual control to inject fura-2 iontophoreticaly. The resting Ca^{2+}_i was between 5 and 79 nM, on average 32 $\pm$ 23 nM ($\pm$ S.D., n=7). Adding 50 μM 5-hydroxytryptamine (5-HT), a common neurohormone in the leech nervous system, or raising the external K^+ concentration from 4 to 20 mM produced a rapid rise in Ca^{2+}_i, which remained high during prolonged exposure. Upon removing 5-HT or restoring normal external K^+, Ca^{2+}_i returned to its resting value within 1-2 min. Both Ca^{2+}_i responses were greatly reduced in nominally Ca^{2+}-free saline, suggesting influx of Ca^{2+} through ligand- and voltage-gated Ca^{2+} channels. The Ca^{2+}_i rise elicited by 5-HT was accompanied by a decrease of the input resistance by 44% and a twofold increase of the relative K^+-conductance, which indicates the existence of a Ca^{2+}-dependent K^+conductance in the glial membrane activated by 5-HT.

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ARE THERE GLIAL SCARS IN SALAMANDERS?

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Salamanders are the only vertebrates that are able to regenerate central tracts after lesions as adults. The occurrence and distribution of astroglial cells before and after cutting the di-mesencephalic junction was investigated with GFAP-immunocytochemistry. In plethodontid salamanders, no astroglial scars could be found even after survival times of 10 weeks. However, in the salamandrid salamander Pleurodeles, a glial scar could be induced. In addition, astrocytes proliferated at distant places in the telencephalon. It is discussed whether meningeal cells influence the formation of the astroglial scar and whether the late occurring proliferative acitivity of astroglial cells might influence the regenerative capacity of salamanders.

LONG-TERM SURVIVAL OF SEPTOHIPPOCAMPAL NEURONS FOLLOWING FIMBRIA-FORNIX TRANSECTION: A FINE-STRUCTURAL STUDY OF AXOTOMIZED NEURONS AND REACTIVE GLIAL CELLS

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Transection of the fimbria-fornix has widely been used as a model to study retrograde neuronal degeneration since immunolabeling for choline acetyltransferase disappears almost completely in the septal region. This is accompanied by a dramatic loss of large neurons in Nissl stain. It was concluded that fimbria transection interrupts the supply with target-derived trophic factor resulting in the degeneration of the projecting neurons.

Here we report on the fine-structural preservation of identified septohippocampal neurons following axotomy as well as on reactive changes in glial cells. Septohippocampal neurons were prelabeled by injection of the fluorescent tracer Fluoro-Gold into the hippocampus. Next, bilateral fimbria-fornix transections were performed. Retrogradely labeled and axotomized septohippocampal neurons were then intracellularly injected with Lucifer Yellow and photoconverted for an electron microscopic analysis. At all survival times studied (up to 10 weeks post lesion) surprisingly many intact septohippocampal neurons were observed. Mainly 3 weeks post lesion, a number of degenerating septohippocampal neurons and many microglial cells were found that had taken up Fluoro-Gold from sequestered neurons. In a few cases we observed glial lamellae covering vacant postsynaptic sites of axotomized, identified projection neurons.

Our data indicate that many more septohippocampal neurons survive axotomy than would be expected from the dramatic neuronal loss in immunolabeling. Degenerated neurons are phagocytosed by microglial cells which are identified by digested Fluoro-Gold particles.

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GROWTH OF CHICK TRIGEMINAL NEURITES AND INTERACTIONS WITH CORNEAL SLICES IN EMBRYONIC ORGAN CO-CULTURES.

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The present study was undertaken to examine the interactions between neurites derived from explanted embryonic chick trigeminal ganglia or control tissues and slices prepared from embryonic cornea which were co-explanted with the ganglia. Outgrowth of trigeminal neurites and ingrowth of these neurites into the corneal tissue were monitored over several days in culture. It appeared that trigeminal neurons started to extend growth-cone bearing neurites 2 to 5 hours after explantation. The growth of trigeminal neurites but not of neurites derived from control tissues such as trigeminal mesencephalic nucleus or ciliary ganglion was preferentially directed towards the co-cultured corneal slices, whereas ingrowth of trigeminal axons into the cornea was followed by formation of elaborate axonal terminal branches. Individual dissociated trigeminal neurons of either pseudounipolar or bipolar types developed their typical morphologies in culture. In co-cultures with corneal slices, they exhibited morphological plasticity and they frequently retracted some branches and formed or elongated other ones which were directed towards the target tissue. The data indicate that the pattern of trigeminal axonal growth in culture is influenced by the presence of corneal target tissue, which may produce secretable substances acting on the neurites.

IMMUNOHISTOCHEMICAL COLOCALIZATION OF GLYCOGEN PHOSPHORYLASE BRAIN ISOZYME WITH ASTROGLIAL MARKERS IN RAT BRAIN SECTIONS.

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The brain glycogen stores and the brain isozyme of glycogen phosphorylase (GP) are supposed to serve as parts of a system for energy supply during acute changes in regional metabolism. We set on (i) to elaborate our earlier findings that brain GP is exclusively found in astrocytes and ependymal cells of the ventricles, and (ii) answer the question whether all astrocytes in the adult rat brain contain GP. Immunofluorescence and immunoenzyme double-labeling methods were applied to formaldehyde-fixed, paraffin-embedded rat brain sections using a monoclonal antibody against bovine brain GP in combination with antibodies against the astrocyte markers glial fibrillary acidic protein (GFAP) and S-100 protein. In the cerebellum, the hippocampus and cortical areas, all astrocytes expressing GFAP and/or S-100 protein also contain GP. Ependymal cells show immunoreactivity for GP and S-100 protein, but not for GFAP. This emphasizes the fundamental role of astrocytes in brain energy metabolism. In conclusion, the brain isozyme of GP can be regarded as an astrocyte marker.

GLIA CELLS IN DROSOPHILA OPTIC LOBE DEVELOPMENT

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The optic lobes are the main processing sites for visual information in the adult fly brain. Viable combinations of mutations in the gene *optomotor-blind* (*omb*) are characterized by the lack of a set of giant neurons in the adult optic lobes. *omb* most likely codes for a transcription factor (Pflugfelder et al., 1992, PNAS, in the press). Null mutations of *omb* affect optic lobe development more drastically, in the extreme case eliminating nearly the entire optic lobe neuropil. An enhancer trap line with apparently glia-specific expression of the reporter gene *lacZ* (*rc56*, C. Klämbt, Köln) was crossed to *omb* mutants to analyze the effect of *omb* mutations on glial development. Lethal *omb* mutations eliminate a subset of the lacZ expression pattern in the developing optic lobes and in the eye imaginal disc without affecting the expression in other parts of the central nervous system. The relevance of these glia cells for optic lobe development is discussed.

AXOTOMY OF THE MOUSE FACIAL NERVE LEADS TO TEMPORARY INCREASED UNSCHEDULED DNA SYNTHESIS (UDS) AND MITOCHONDRIAL DNA SYNTHESIS (MiDS) IN THE REGENERATING MOTOR NEURONS

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The right facial nerve of 20 6-month-old mice was cut at the stylomastoid foramen. 4, 6, 8, 11, or 14 d after surgery 3-H-thymidine was injected, and the mice were killed 1 h later. Autoradiographs were prepared from deparaffinized and Feulgen prestained sections (ILFORD K2, 250 d exposure). Mean grain counts per nucleus (background corrected and normalized to the size of the nuclei) or grain densities of perikaryal cytoplasm were estimated for facial motor neurons, non-proliferating glial, endothelial cells (operated and non-operated side) and Purkinje cells, and led to the following results: The extent of UDS differed among the cell types being highest in the motor neurons. Interestingly, UDS of axotomized motor neurons 4 d after surgery significantly exceeded that of unoperated controls as well as 6-14 d after axotomy. The same result was obtained for perikaryal cytoplasm indicating increased MiDS, i.e. multiplication of mitochondria. Both results correlate to an increased protein synthesis of the regenerating cells. Furthermore, the increased UDS is in line with the fact that an increased protein synthesis leads to an increased production of free radicals and with it to enhanced DNA damages.

CAN DENTATE AREA TRANSPLANTS TAKE THE FUNCTION OF DESTRUCTED HIPPOCAMPAL GRANULAR CELLS ?

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A topical microinjection of colchicine can selectively destroy dentate area granular cells . Rats treated this way were unable to learn an active avoidance reaction by using a perforant path stimulation as a conditioned stimulus . It was also impossible to evoke potentials by stimulation of the perforant path .
Ten weeks after grafting neonatal dentate area into the damaged region potentials could be evoked again by stimulation of the perforant path , and the rats were again able to learn by using this stimulation as a conditioned signal . Some of the grafts showed the phenomenon of the post-tetanic potentiation . Histological and histochemical investigations showed , that the granular cells of the grafts exhibited a normal morphology , they innervated the CA3 sector of the recipient hippocampus by sprouting mossy fibers and were innervated by afferent fibers comming from the recipient brain .

The reaction of adult optic nerve glia to axotomy

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We investigated the response of adult rat optic nerve glial cells to axotomy in explant cultures.
Axotomized nerves (2-7 days after axotomy) were cultured for 6,10 or 16 days. Normal nerves were cultured 2-7 days longer to include the time interval of axotomy.
Glia migration from normal nerves was slower and so called typeII astrocytes (GFAP+/A2B5+) or mature oligodendrocytes (GalC+) were never observed. In cultures from axotomized nerves different precursor cells, so called adult (A2B5+/Vim-) and neonatal (A2B5+/Vim+) type 0-IIA progenitor cells as well as multipolar (O4+/Vim+) Oligodendrocyte precursors proliferated. High numbers of type II astrocytes and lower numbers of mature oligodendrocytes were found. After few days in vitro a dramatic decrease in oligodendrocytes and type II astrocyte numbers occured. These cell types, however, reappeared at later stages. Astrocyte proliferation in cultures from axotomized nerves was increased up to 4.5 fold as compared to normal nerves.
These findings suggest that axotomy leads to proliferation of astrocytes and different types of precursor cells, so called adult and neonatal O-IIA progenitors as well as Vim+/GFAP-/GalC-/O4-A2B5- cells. The latter ones seem to be able to aquire GFAP or O4 thus differentiating into astrocytes or oligodendrocytes.

MS-1: A DNA BINDING PROTEIN IN BRAIN RECOGNIZING A SEQUENCE ELEMENT COMMON TO MYELIN-SPECIFIC GENES

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The formation of myelin by glial cells requires the induction and transcription of a set of myelin-specific genes. These encode structural components of the myelin membrane, such as proteolipid protein (PLP), myelin basic protein (MBP), myelin-associated glycoprotein (MAG), and protein zero (Po). However, the molecular mechanisms underlying the cell type-specific and highly coordinate expression of these genes are largely unknown. We have cloned the 5' regulatory region of the rat PLP gene and have analysed its nucleotide sequence for elements it might share with other myelin-specific genes. One such element (MS-box: AGGGCT) was found 330 bp upstream of the first transcription start site in the PLP gene, immediately 5' to a consensus E-box motif (CACATG). The MS-box was found in the promotor regions of the mouse MBP gene, the rat MAG gene, and the rat Po gene at a similar position. Using a panel of labeled oligonucleotides in gel mobility shift assays (GEMSA) and competition experiments, we have identified a DNA binding protein in brain nuclear extracts (MS-1) that binds sequence-specifically to the MS-box. MS-1 is highly enriched in the adult rodent brain when compared to non-neural tissues and cells. However, some cell lines express related proteins with a slightly higher mobility in GEMSA. Interestingly, the DNA binding activity is present in both brain nuclear extracts and cytoplasmic fractions. MS-1 also binds single-stranded target DNA and DNA-protein complexes are sensitive to 1,10-phenanthroline, a property of zinc finger proteins. Following size fractionation and elution from a SDS polyacrylamide gel, MS-1 can be functionally renatured and has a molecular weight of approximately 45K. Transfection studies using glial cell lines are now used to determine whether MS-1 is a direct transcriptional activator of myelin-specific genes.

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LIGHT PULSES EVOKE MEMBRANE CURRENTS IN MÜLLER CELLS OF THE GUINEA PIG RETINA

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Müller cells are believed to act as controlling elements for the extracellular K^+ homeostasis in the retina. $[K^+]_o$ increases predominantly in the central part of the retina during neuronal activity, normally elicited by light stimulation. Indirect evidence indicated that Müller cells take up excess K^+ in the central part of the retina (in the somatic region) and release it from their endfeet at the vitreous surface. These passive currents, termed spatial buffer currents, are driven by the K^+ gradient throughout the retina and the concomitant depolarization of the Müller cell. To assess this glial property in an intact preparation, we used the patch-clamp technique to record membrane currents from Müller cells of a guinea pig retina. Wholemeounts were used to record currents from the endfeet, transverse slice to record currents from the soma. A light stimulus (4 Lux) for 2 sec elicited outward currents recorded in both, soma and endfoot of the Müller cell when the membrane potential was clamped at the resting potential. In the current clamp mode, both the membrane potential of the soma and the endfoot hyperpolarized. Longer light stimuli led to a decay of the outward current followed by a transient inward current after the offset of the light stimulus. These experiments are not compatible with spatial buffer currents and the results must arise from a so far unknown mechanism.

MÜLLER GLIA ENDFEET AND A RETINAL BASAL LAMINA TOGETHER STABILIZE THE LAMINAR STRUCTURE OF CHICKEN RETINOSPHEROIDS AS DERIVED FROM THE PIGMENTED EYE PERIPHERY.

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Dissociated embryonic chicken retinal cells regenerate in rotary culture into histotypic cellular spheres (*retinospheroids*). If the cells are derived from the central part of the retina, subareas of the spheres express all three nuclear layers with an inverted sequence (*rosetted spheroids*). However, cells from the eye margin including pigmented cells induce a correct sequence of layers (*stratospheroids*). Light and electron microscopic investigations show that an outer limiting membrane and radial Müller glia processes are expressed in both systems. However, an inner limiting membrane (ILM) including laminin expression is found only in *stratospheroids*. Here, juvenile Müller glia cells are properly fixed via their endfeet to a basal lamina. Since an ILM is absent in *rosetted spheroids*, Müller glia processes end freely in the IPL.
In order to determine the histogenetic roles of the RPE, of laminin and of the Müller glia, we performed two sets of experiments: 1) we raised *stratospheroids* in presence of laminin antibodies. Here, a significant number of *stratospheroids* shows a mediocre histotypic organization; in particular, an overproduction of Müller glia endfeet is indicated. 2) we raised *retinospheroids* in presence of the glia-specific toxin α-aminoadipic acid. This toxin leads to a destruction of cell laminae, to a disturbed columnar cell organisation and to a progressive decay of the *spheroids*. These experiments indicate crucial retinogenetic roles of both the retinal pigmented epithelium and of Müller glia processes.

ADRENALINE REDUCES THE MEMBRANE K^+ CONDUCTANCE OF CULTURED OLIGODENDROCYTES FROM MOUSE BRAIN

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The adrenergic ligand noradrenaline is extrasynaptically released from brainstem neurons which project throughout the central nervous system. In the forebrain 75-95 % of the (ß-)adrenergic receptors are localized on glial cells (Stone et al., 1990, Brain Res. 530:295-300). Oligodendrocytes and astrocytes, in vitro, express ß-adrenergic receptors; in the former the expression is correlated with the modulation of myelin-associated proteins suggesting a role of adrenergic receptors in the induction of myelination. In this study we investigated the effects of the ß-adrenergic receptor ligand adrenaline on the membrane properties of mature oligodendrocytes in culture. Cells were identified with monoclonal antibodies specific to developmental stages and by morphological criteria. Using the whole cell patch clamp technique the membrane conductance of the mature oligodendrocytes but not of the precursor cells was reduced by adrenaline (10^{-5} M). The adrenaline-induced effects were abolished in the presence of the K^+ channel blocker Ba^{++} (2 mM) suggesting that adrenaline acts as an inhibitor of potassium channels. The effects were insensitive to the application of the α_1-adrenergic antagonist prazosin (10^{-5} M), whereas they could be inhibited by the ß-antagonist propranolol (10^{-5} M) indicating the involvement of ß-receptors. These data suggest that the activation of ß-adrenergic receptors expressed in mature oligodendrocytes leads to a change in the physiological state of the cell which may have functional importance in the induction of myelination.

ASTROGLIAL CELLS SUPPORT LONGTERM SURVIVAL OF CENTRAL NERVOUS SYSTEM NEURONS IN CULTURE

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Serum-free medium conditioned by primary astroglial cells (ACM) isolated from the cerebrum of newborn rats promotes neurite extension and long-term survival of neuronal cultures from different embryonic brain regions (neocortex, hippocampus, septum). Under the trophic influence of ACM neuronal cells develop into tight and biolectrically active networks. These cultures survive for more than one month. The survival promoting activity of ACM acts in a dose dependent manner and is permanently required. Potassium induced depolarization or blockade of sodium channels by tetrodotoxin (TTX) has no further effect on ACM supported hippocampal survival.
Another critical parameter influencing hippocampal long-term survival is the cell contact density: The survival kinetics of highly enriched hippocampal cultures strictly depend on the number of seeded cells and achieve optimal values at a density of 10^5 neuronal cells per cm^2. In contrast, hippocampal cultures, seeded at low plating density survive only for more than one week in ACM when cocultivated in direct contact with astroglial cells, isolated from postnatal cerebral cortex.
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MONOGALACTOSYLDIGLYCERIDE (MGDG), A POTENTIAL MODULATOR OF PROTEIN KINASE C (PKC) ACTIVITY IN MATURE OLIGODENDROCYTES (OL)

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OL incorporated ^{3}H-galactose into various glyceroglycolipids. MGDG, the level of which increases during myelination and which is enriched in OL and myelin, exhibited the highest percentage among the radioactiv labeled glycerogalactolipids after 3 weeks in culture. The incorporation rate was enhanced by phorbol esters such as TPA. Addition of MGDG (prepared from white matter of pig brains) to the culture medium at a concentration of 500 μM enhanced the OL process formation significantly but less than TPA. MGDG activated PKC similarly to diacylglycerol (DAG), however, the Ca^{2+} dependence was different. The results indicate that DAG and MGDG activate different isoforms of PKC and that MGDG may act as a modulator of OL PKC activity.

AN ILE-TO-THR MUTATION OF THE PROTEOLIPID PROTEIN (PLP) GENE CAUSING HYPOMYELINATION IN *RUMPSHAKER* MICE

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Proteolipid protein (PLP) is the major structural protein of myelin in the central nervous system, but its cellular function remains obscure. Two isoforms with a relative molecular mass of 30K and 26K (DM-20) are produced by alternative RNA splicing, and their primary structure is 100% conserved between mouse and man. This suggests that PLP function involves critical protein-protein interactions. Mutations of the X-chromosome linked PLP gene are the primary defect of hypomyelination in the mouse mutant *jimpy*, in human patients with *Pelizaeus-Merzbacher Disease*, and in other animal models. In *jimpy* mice, hypomyelination is associated with unexplained early pathological changes of oligodendrocytes, including delayed differentiation, a cell cycle defect, and premature cell death. We have identified a novel mutation of the mouse PLP gene, termed *rumpshaker*. This mutation causes a less severe hypomyelination with remarkable phenotypic differences from all previously described PLP mutants, most notably without degenerative changes, the lack of oligodendrocyte cell death, and the normal life span of the affected animals. The expression of PLP mRNA in these mutants is only slightly reduced. We provide evidence that *rumpshaker* is a point mutation in exon 4, changing Ile(186) to Thr in the putative membrane embedded domain M4. Thus, minor changes of PLP cause directly hypomyelination that can be genetically separated from the degenerative changes in oligodendrocytes. We suggest that PLP is involved in both the architecture of compact myelin and in a distinct yet undefined "premyelin" function in glia which may be also relevant to the clinical spectrum of *Pelizaeus-Merzbacher Disease*.

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DISTRIBUTION OF PERINEURONAL NETS IN RAT BRAIN

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Net-like structures surrounding neuronal somata and their proximal dendrites can be visualized by using histological, histochemical, and immunocytochemical methods. Origin and function of these perineuronal nets have been controversely discussed.

Our investigations on their distribution pattern and the occurrence of regional specialization of these nets using the colloidal iron hydroxide staining for detection of polyanionic groups and the binding of Vicia villosa agglutinin (VVA) might provide some data which could help to elucidate their function. Perineuronal nets were found in brain regions and nuclei as neocortex, hippocampus, piriform cortex, basal forebrain complex, dorsal lateral septal nucleus, lateral hypothalamic area, reticular thalamic nucleus, zona incerta, deep parts of superior and inferior colliculus, nucleus ruber, substantia nigra, some tegmental nuclei, dorsal raphe and cuneiform nucleus, central grey, trigeminal nuclei, trochlear nucleus, pontine and medullar reticular nuclei, inferior olivary nucleus, and vestibular nuclei. The types of neurons, surrounded by these nets, were tried to be identified. Nets were seen on neurons which are known to differ in transmitter content and in morphological details, and which are involved in functionally different brain structures.

An association of perineuronal nets with neurons representing the fast firing type, is discussed.

MICROGLIAL CELLS DURING AXOTOMY-INDUCED NEURONAL DEGRADATION: COMBINED FUNCTION-DEPENDENT FLUORESCENCE STAINING AND ELECTRONMICROSCOPY STUDY.

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Retinal microglial cells can be selectively stained during the process of neuronal degeneration and subsequent ingestion and phagocytosis of the produced membrane material. To assess this, the membranophilic fluorescent styryl dye 4Di-10ASP was deposited either into the *superior colliculus* or into the optic nerve in order to retrogradely outline the ganglion cells destined to dye because of transection of the axons. Progressive degradation of the fluorescent ganglion cells resulted in uptake of the fluorescent debris by microglial cells, and since the lipid 4Di-10ASP can be not degraded, it accumulated within the phagocytosing cells and gave rise to their intense labelling. Interneurons of the retina and other neuroglia (astrocytes and Müller glia) are not directly labelled by incorporation of fluorescent debris, indicating that they may be not involved in degradation of the injured neurons. Fixation of tissue and photoconversion of the fluorescent microglial cells in presence of the chromophore DAB and examination with light- and electronmicroscopy revealed that only microglial cells contained stained phagosomes.

THE EXPRESSION OF BASEMENT MEMBRANE MOLECULES ON CULTURED SCHWANN CELLS AND THEIR ROLE IN NEURITE OUTGROWTH.

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We have analyzed the expression of Schwann cell (SC) basement membrane (BM) antigens and have investigated the ability of several purified monoclonal antibodies (mabs) to perturb neurite outgrowth (NO) onto cultured SCs. Dissociated dorsal root ganglia (DRG) from P1 rats were Percoll-fractionated in order to obtain pure populations of SCs and neurons. These studies analyzed the expression of antigens in three different cultures: (1) pure SCs; (2) pure neurons; and (3) pure N/SC co-cultures. The antibodies tested resulted from tolerizing mice to adult spinal cord and then immunizing with sciatic nerve.

Immunohistochemistry revealed the distinct pattern typical of SCBM staining in vivo and in vitro. Purified SCs also expressed the antigens on their surface but at low levels. Pure neuronal cultures were negative. Hence, contact with neurites promotes the accumulation of these antigens on Schwann cell surfaces—a characteristic trait of BM proteins. To test antibodies for their ability to perturb neurite growth onto established SC monolayers, the mabs were added after the neurons had attached (3-4 hrs). After 48 hours the cultures were processed for neurofilament staining to assess NO. One mab, PC1A6, was found to reduce NO dramatically. Furthermore, neuronal cell bodies and SCs tended to aggregate, indicating that this antibody interfered with substrate attachment. This data supports the fact that BM molecules are important in axon growth and, in addition, may play a role in SC adhesion and/or migration.

Establishment and characterisation of a rat neuronal progenitor cell line

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Lineage studies in several vertebrate species have shown that the immediate precursors of neurons are derived from a multipotential stem cell. Recently, we established a spontaneously immortalized cell line from embryonic (E18) rat brain. These cells express early neural stem cell properties, e.g. the intermediate filament protein nestin, without changing morphology and growth capacity as well as gene expression patterns. Treatment of these cells with 10-100uM cAMP causes differentiation which was obvious by morphological changes as neurite outgrowth and down-regulation of their proliferation rate. The differentiatiation was also accumulated by modulation of the expression of neuronal-specific markers: The expression of nestin decreased during cAMP treatment whereas the mRNA levels of various neurofilament subtypes NF 68, NF 145 and NF 200 and some neurotransmitters were induced. Taken together these results demonstrate the isolation of an early neuronal progentitor with the capacity to differentiate in a neuronal phenotype in the presence of cAMP.

FACILITATION OF LEARNING BY CO-GRAFTS OF ASTROBLASTS AND FETAL NEURONS INTO THE NEUROTOXICALLY DAMAGED HIPPOCAMPUS OF RAT

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Successful experiments on transplantation depend on achieving an adequate survival of fetal neurons. Co-grafts of fetal neurons and astroblasts producing neurotrophic survival factors as well as promoting neurite outgrowth in vivo[1] might provide a permanent source of growth factors thus enhancing graft survival as well as possible adaptive behavioral effects. One week after bilateral injections of kainic acid into their hippocampi adult rats received co-grafts of 1.5 ul fetal hippocampal tissue (approximately 100.000 cells) and 0.5 ul suspension of cerebral astroblasts from neonatal rats (approximately 30.000 cells). The astroblasts were derived from primary cultures in serum-free medium. A second group received co-grafts of fetal hippocampal neurons (100.000 cells) and cultured meningeal fibroblasts (30.000 cells) while a third group only received grafts of 100.000 neuronal cells. Controls received sham grafts after sham lesions or neurotoxin injections. Behavioral testing was done using the Morris-water maze for spatial memory. During a postoperative period of 12 weeks the animals were tested once a week. There was a beneficial behavioral effect of co-grafted astroblasts during a period of 7 weeks after grafting. Then the beneficial effect ceased and 3 months after grafting the co-grafted astocytes showed a maladaptive effect on spatial learning. Co-grafted fibroblasts did not show a substantial facilitation of learning.

Literature: 1) Smith et al., J. Comp. Neurol., 251:23-43, (1986).

SURVIVAL OF FETAL NEURONS IMPLANTED INTO THE NEUROTOXICALLY DAMAGED HIPPOCAMPUS OF RAT IS ENHANCED BY CO-GRAFTED ASTROBLASTS BUT NOT BY FIBROBLASTS

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In order to increase the survival rate of fetal brain grafts in long-term experiments by application of neurotrophic factors, co-implantation of astrocytes seemed to be a promissing alternative. One week after bilateral kainic acid injections into their hippocampi adult rats (group A) received co-grafts of 1.5 ul fetal hippocampal tissue suspension (approximately 100.000 cells) and 0.5 ul suspension of cerebral astrocytes from neonatal rats (approximately 30.000 cells). The astrocytes were derived from primary cultures in serum-free medium. Another group (F) received co-grafts of fetal hippocampal neurons (100.000 cells) and cultured meningeal fibroblasts (30.000 cells) from neonatal rats. Two and 6 months after grafting the histological examination of the transplants revealed a rise of neuronal graft survival in those brains which had received co-grafts of neurons and astrocytes. Evaluation of the data showed an increase of graft survival of 18 % after 2 months and an increase of 26 % after 6 months in group A which had received co-grafts of neurons and astrocytes as compared to group F which received co-grafts of neurons and fibrocytes. The mean diameter of the giant pyramidal cells also increased in group A while pyramidal cells of grafts in group F did not show any morphological differences as compared to those of the normal hippocampus. In accordance to this general trophic effect the mean volume of grafts in group A was considerably larger than the volume in group F. Furthermore some organotypic cytoarchitectoral characteristics developed to the same degree in grafts of both groups indicating that there was no obvious difference between implanted astroglia or fibroblasts on the cytoarchitectoral development of the grafts.

ASTROCYTE AND MENINGEAL CELL CONDITIONED MEDIA (ACM; MCM) CONTAIN ASTROCYTE DIFFERENTIATION FACTORS

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Media conditioned by either astrocytes or meningeal cells contain biological activities which induce cultivated epithelioid type I astrocytes to develop into stellate cells. Parallel to the morphological transformation the astrocytes also increase their content of intracellular protein and the activity of the enzyme dipeptidylpeptidase II. Since this activity can be enriched from conditioned media, it seems likely that it is secreted by astrocytes and meningeal cells. Preliminary biochemical characterization of the biological activity indicates that it is a protein which can be concentrated by ultrafiltration over membranes with an exclusion molecular weight of 100 kD. The biological activity in both ACM and MCM shows similar biochemical characteristics during gel chromatography (Sephacryl S-300) and FPLC over an ionexchanger (Mono Q). In gel chromatography the activity is eluted as protein with an apparent molecular weight of 120-130 kD. In SDS gel electrophoresis a band appears at 60 kD. Comparisons of the biological activity in ACM and MCM with other factors known to induce a similar differentiation of astrocytes in vitro, like FGF, EGF, Nexin, cAMP show that (i) it has different biochemical properties, and (ii) that it has different biological effects on the differentiation of astrocytes, like induction of stellate cells even in serum containing media or the time in which the transformation occurs.

L-GLUTAMATE ACTIVATES RECEPTOR-MEDIATED IONIC CURRENTS AND SIMULTANEOUSLY BLOCKS POTASSIUM CURRENTS IN IDENTIFIED GLIAL CELLS OF THE MOUSE HIPPOCAMPAL SLICE

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In recent years, it became evident that glial cells, acutely isolated or in tissue culture, express a variety of different voltage- and ligand-activated ion channels which were previously thought to be exclusive to neurons. Among those are glutamate receptors (Sontheimer et al., 1988). The studies in cell culture advanced the knowledge on the biophysical and pharmacological properties of glial channels, but could not resolve the questions on their functional importance.

To overcome these restrictions we have applied the patch-clamp technique to hippocampal brain slices to study macroglial cells in the stratum radiatum with the cellular relationship left intact. To unequivocally identify glial cells ,we combined electrophysiology with immunocytochemical and ultrastructural characterization (Steinhäuser et al., 1992). In 90 % of the cells, L-glutamate (L-glu, 1 mM) induced inward currents ranging from 19 to 575 pA (N = 76). To identify whether the currents were receptor mediated or resulted from transmitter uptake, we applied L-glu agonists which are known to be no substrates for transport systemes, the uptake blocker dihydrokainate and analyzed the reversal potential of L-glu induced currents.

These experiments demonstrate that in glial cells of the hippocampus the L-glu response consists of at least two components: a) a receptor mediated current with a current voltage relationship which reverses at about 0 mV and b) a block of voltage-dependent potassium currents which in most cells covered the receptor-mediated currents.

Sontheimer, H., H. Kettenmann, K. Backus and M. Schachner (1988): Glia 1:328-336
Steinhäuser, C., T. Berger, M. Frotscher and H. Kettenmann (1992): Eur J Neurosci, in press

Glial response to transection of the rat postcommissural fornix

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Glial scars are thought to be important for the limited axonal regeneration after lesions to the mature CNS. They develop an extensive basal lamina and may act as mechanical barrier to growing neurites. In view of the little attention paid to glial changes after CNS lesions, we established a stereotactic lesion model for the study of spatio-temporal changes of astrocytes and microglial cells after unilateral transection of the postcommissural fornix tract. For that purpose we applied immunocytochemical methods using antibodies against glial fibrillary acidic protein (GFAP), vimentin (VIM) and CR3-receptor (OX42).

The unilateral transection of the fornix tract was accompanied by ipsilateral, strong and long-lasting (up to 6 month postlesion) upregulations in reactive astrocytes and microglia. However, the temporal and spatial patterns of these scar components differed. While microglial cells showed a very early reaction (2h after the lesion) the astrocytic response started at about 1-3d postinjury. Concerning the spatial distribution, we observed that both, astrocytic and microglial reactions always included the complete distal stump. However, the GFAP(+)- astrocytes and the microglial cells showed a more widespread response than the VIM(+)-astrocytes along the proximal stump.

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REGULATION DER GENEXPRESSION DES PROTEOLIPIDPROTEINS (PLP) UND BASISCHEN MYELINPROTEINS. REGULATORISCHE ELEMENTE UND TRANSGENE

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DNAse I-Footprint-Analysen und Gel-Retardierungsanalysen führten zur Definition einer Reihe von allgemeinen und hirnspezifischen cis-Elementen in der 5' nichttranslatierten Region der MBP- und PLP-Gene. Als Reporter-Gen für die regulatorischen Elemente diente das CAT-Gen, mit dem TK-Promotor versehen. Durch Deletion von Mutationen wurde eine 14 bp große Sequenz für einen gehirnspezifischen Transkriptionsfaktor für die Expression des MBP definiert. Der Beweis für die Oligodendrocytenspezifität wurde durch seine Expression als Transgen in der Maus geliefert, in der durch in situ Hybridisierungen und Northern Blots diese Zellspezifität nachgewiesen wurde.
Für das PLP-Gen wurden gleichermaßen die cis-DNA-Domänen durch Footprints und Gelshifts ermittelt.
Es wird über den Fortgang der Reinigung der trans-Faktoren (Enhancer-Proteine) für die Expression des PLPs und MBPs berichtet.

CHARACTERIZATION OF GLIAL CELLS IN THE OPTIC LOBE OF *DROSOPHILA MELANOGASTER*

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The enhancer trap technique allows to search for molecular markers of different cell types in *Drosophila*. We have characterized a number of enhancer trap lines with expression patterns in different populations of glial cells in the optic lobe. These patterns are used to catalogue glial cell types, as markers in developmental studies, and to improve the description of the phenotype of neurological mutants. Finally we use these patterns to begin a molecular characterization of *Drosophila* genes that are preferentially expressed in glial cells of the optic lobe.

At present our research is centered on the giant glial cells of the inner and outer optic chiasms, which are displaced in the *irregular chiasm C (irreC)* mutant. We suspect these giant glial cells may be involved in axonal guidance. Several enhancer trap lines show expression of the lacZ reporter gene in those cells. The reporter gene's expression pattern is thought to mimick that of near-by *Drosophila* genes. We are planning to produce loss-of-function mutant phenotypes by remobilization and imprecise excision of the P-vectors in order to learn something about the function of these genes.

TRANSPLANTATION OF IMMORTALISED GLIAL CELL PRECURSORS INTO AREAS OF DEMYELINATION.

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Retroviral vectors carrying a temperature sensitive mutant of the *SV 40 T* oncogene have been used to establish immortalised lines of oligodendroglial precursor cells from murine brain. Cells expressing the 04 antigen characteristic of immature oligodendrocytes have been immortalised. The established cell lines retain many properties of their partner cells from primary cultures, for example they express differentiation-stage specific adhesion molecules and ion channels. When transplanted into demyelinated lesions where host cell remyelination has been prevented by irradiation, early passages of lines are able to remyelinate demyelinated axons. Late passages of the lines and 04-positive cell clones do not form myelin but interact with the demyelinated axons, implying the retention of adhesion molecules specific for this interaction. Such cell lines and clones are providing useful tools for the study of oligodendroglial precursor cell-neuron interactions.

AN APPROACH FOR THE IDENTIFICATION OF GLIA DETERMINING GENES IN D. MELANOGASTER

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To identify genes expressed in neuronal and/or glial stem cells we are using the "enhancer trap" techinque (O'Kane and Gehring, PNAS 84, 9123, 1987) with a new modified vector where an enhancerless Gal4 gene was subcloned into the vector plwB (Brand et al., Crete abstracts, 1990). Gal4 is a yeast transcriptional activator that has no endogenous target sites in the *Drosophila* genome. By performing a cross between a strain carrying the Gal4 vector and a strain bearing the defective Δ2-3 element that expresses constitutively a large amount of transposase the Gal4 vector can be mobilized in the germline of the progeny. As a result the Gal4 gene is randomly integrated at new genomic sites, thereby coming under the control of different regulatory elements. Cell and tissue specific expression patterns of Gal4 can be visualized by mating the transformants to a strain carrying the lacZ gene under the control of UAS_G. The Gal4 protein binds to UAS_G sites and activates the transcription of the linked gene. We have screened about 1600 new insertion lines for gliaspecific expression patterns in 0-18 hr embryos and in the CNS of the third instar larvae and we will show some of our results.

AN IN VITRO MODEL FOR THE INVESTIGATION OF INTRACELLULAR pH REGULATION OF GLIAL CELLS.

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Acidosis in the brain, e.g. following cerebral ischemia leads to glial cell swelling. It had been shown that glial cells take up water from the extracellular (e.c.) compartment in acidosis. This was proposed as a mechanism to maintain a constant intracellular (i.c.) pH. To investigate this in detail we developed an in vitro model that allows i.c. pH measurements. The pH-sensitive dye BCECF is used in C6 glioma and rat primary astrocytes. The cells are illuminated at 442nm and 492nm, respectively, and wavelengths are interchanged at 50 Hz. Fluorescence is measured by a photomultiplier, and the pH can be calculated from the ratio of the two fluorescence intensities which is independent from cell density and dye concentration. Nigericin is used for pH calibration, causing i.c. pH to adjust to e.c. pH when e.c. K^+-concentrations are set to the i.c. level.

Exposure to acidosis down to pH 6.2 is followed by rapid i.c. acidification nearly down to e.c. levels, no matter whether HEPES, MOPS or bicarbonate are used as buffers. The i.c. pH drop is fully reversible after normalization of e.c. pH. Currently the mechanisms involved in i.c. acidification are studied in detail. It is hypothesized that glial cells serve as a sink for e.c. acid equivalents in order to protect nerve cells from acidosis.

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REGENERATION OF TRANSECTED SPINAL CORD AXONS IN GOLDFISH.

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Fish in contrast to warmblooded vertebrates regain their normal mobility after a complete transection of the spinal cord (Bernstein, 1964). This is, at least to some extent, based on regeneration of the transected spinal cord axons (Bunt and Fill-Moebs, 1984). To identify neurons in the fish brain 1. that project their axons into the spinal cord and 2. that regenerate their axons, we transected the axons on the level of the posterior end of the dorsal fin (anal region) and applied fluorescent tracers. Retrogradely labeled cells were found in the mesencephalic tegmentum (Nucleus of the median longitudinal fasciculus (NMLF) and Nucleus ruber), in the reticular formation, in the octaval area and in the facial lobe of the rhombencephalon.

To investigate the capacity of the identified cells to regenerate their axons a double labeling experiment using FITC coupled and Texas Red coupled dextran amins was performed: a first transection was made at the level of about the 18th vertebrate and the first dye was applied. 1-3 months later a second transection was carried out 2-4 segments caudal to the first and the second dye was applied. Only regenerated neurons should carry both dyes. After one month, 4-5% (n=4) and after 10 weeks 65-75% (n=4) of the neurons were double labeled. We never found double labeled cells in the facial lobe. These data imply that neurons in the CNS of fish have special properties allowing them to regenerate injured axons or that the environment of the injured axons encourages axon regrowth, or both.

GLIAL CELLS IN THE MONKEY RETINA: MORPHOLOGY, DISTRIBUTION AND RELATIONSHIP TO VASCULATURE AND NEURONAL STRUCTURES

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Astrocytes were studied in the retinas of adult rhesus monkeys using immunohistochemistry. Glial fibrillary acidic protein (GFAP) labelled cells were analyzed using a fluorescent microscope as well as a confocal microscope. In the periphery, astrocytes appear star-shaped, with 2-8 radial processes emerging from the mostly oval-shaped soma. Towards the center of the retina there is a tendency for the processes to change direction in a decreasing distance from the soma so that in the center but outside the fovea astrocytes have an almost bipolar appearance. We also found astrocytes close to the fovea. They are characterized by a high number of irregular processes and are arranged radially around the fovea. Astrocytes are unevenly distributed in the primate retina with a high in the center outside the fovea and a low in the periphery. Close to the fovea, astrocyte density is particularly low. Results of double label studies suggest that astrocyte morphology might at least in part be determined by their relation to neurites and vasculature.

Microglia was stained using the nucleoside-diphosphatase (NDPase) method. We distinguished three morphological types based on dendrite morphology and location in different retinal layers. In all layers, contacts of microglial cells and blood vessels could be demonstrated.

These results might contribute to the understanding of the role of glial cells in the primate retina.

MOLECULAR AND GENETIC ANALYSIS OF GLIAL-NEURONAL INTERACTIONS IN THE EMBRYONIC CNS OF *DROSOPHILA*

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In vertebrates as well as in insects glial cells are important cellular cues needed for the correct formation of the intricate axonal network. We are using the ventral midline of the embryonic Drosophila CNS as a model system to study glial-neuronal cell-cell interactions at a molecular and genetic level. Midline neurons as well as the midline glial cells play an important and instructive role during the establishment of commissural connections across the midline. During this process the midline glial cells specifically recognize the midline neurons and migrate along their cell processes posterior to finally separate the two segmental commissures.

To analyze this neuronal glial interaction we are studying mutations that affect the correct migration of the midline glial cells and therefore result in an abnormal axon pattern. The gene pointed is involved in the recognition of the midline neurons by the midline glial cells. We have cloned *pointed* and showed that it is expressed in the glial target cells at the midline. Sequence analysis revealed high homology to the vertebrate ets2 oncogene. To further investigate the function of the midline glial cells we are interested in genes that are specifically expressed in these cells. To identify such genes we are using the enhancer trap method. A genetic and molecular analysis of these genes will be presented.

A PROTEIN:CHONDROITINSULFATE-PROTEOGLYCAN-COMPLEX WITH NEUROTROPHIC SURVIVAL ACTIVITY FOR CENTRAL NEURONS

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In culture astrocytes and meningeal fibroblasts synthesize and release protein factors, which promote neurite outgrowth and support the survival of neuronal cells. The present study provides evidence that the survival activity for E 15 rat neocortical neurons of meningeal cell conditioned medium (MCM) correlates with chondroitinsulfate-PG (CSPG) immunoreactivity. The PG was isolated from MCM by anion exchange FPLC-chromatography. We yielded fractions with free laminin, laminin complexed to PG and fractions containing PG but virtually no laminin. These fractions were tested in a bioassay for their survival activity. Predominantly those fractions containing PG but only small amounts of laminin supported neuronal survival.The active PG could be identified as a CSPG due to immunoreactivity with anti-CS and because the immunoreactivity disappeared after treatment with chondroitinase ABC. Treatment of active CSPG containing fractions with 4 M guanidinium-chloride led to dissociation of the CSPG and an active protein of neutral or basic isoelectric point. We conclude that meningeal cells synthesize and release a diffusible negatively charged high molecular weight factor with neurotrophic survival activity for central neurons that consists of a CSPG complexed with an active protein component that appears to be different from laminin and fibronectin. Supported by DFG (Mu630/3)

ARGININE UPTAKE IN CULTURED GLIAL CELLS

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As a first step in elucidating the relationship between the production of nitric oxide,NO, and the supply of the NO-precursor L-arginine in glial cells, uptake of L-arginine has been studied in rat astroglia-rich primary cultures and glioma cells C6-BU-1 with the radioactive tracer method.

In both culture types, total uptake consisted of a diffusion-mediated process dominant at high concentrations of extracellular arginine and a saturable component with a K_M of about 30 µM and a V_{max} of 1.2 nmol per min and mg of protein. Uptake of arginine (25 µM) was strongly inhibited by lysine or ornithine (5 mM each), but not by alanine or serine (5 mM each), nor by cysteic acid and N-methyl-aminoisobutyric acid (0.5 mM each). Rate of uptake of arginine was not dependent on the concentrations of Na^+ or Cl^- in the incubation medium.
In conclusion, L-arginine at physiological concentrations is taken up by the glial cells with the help of the transport system "y" for basic amino acids.

INDUCTION OF TIGHT JUNCTIONS IN BOVINE BRAIN ENDOTHELIAL CELLS (BBE) BY VARIOUS COCULTURED CELLS AND THEIR CONDITIONED MEDIA.

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The maturation of the blood-brain barrier (BBB) includes the formation of complex tight junctions (TJs) between endothelial cells (ECs). BBB-characteristics are induced by brain-specific environment (e.g. astrocytes). In an in vitro model we cultured BBEs on one side of a cellagen filter and astrocytes on the other for up to 11 days. In separate experiments, astrocytes were replaced by fibroblasts or their conditioned media. Permeability to [^{3}H]-inulin and TJ complexity were determined. In cocultures of astrocytes, meningeal and 3T3 fibroblasts, the TJ complexity in ECs was increased. Conditioned media of these cells increased TJ complexity as well, but to a lesser extent. Forskolin treatment increased whereas phorbolesters decreased the complexity of TJs. These results suggest that second messenger-mediated mechanisms may be involved in the modulation of BBB-characteristics in cultured ECs and possibly also in vivo. Supported by the DFG.

IMMATURE ASTROCYTES PROMOTE AXONAL REGENERATION IN THE ADULT RAT BRAIN

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As astrocytes have been shown to support neuronal survival and fiber outgrowth in vitro, we have established a stereotactic brain lesion and transplantation model in the adult mammalian CNS to study the influence of transplanted astrocytes on de- and regeneration of axons in the unilaterally transected postcommissural fornix. Our preliminary WGA-HRP tracing experiments provided strong evidence for enhanced axonal regeneration in presence of an astrocytic transplant at the lesion site. To quantify this beneficial effect, we compared the number of axons in transected fornix tracts with and without a transplant. In view of the higher capacity of immature astrocytes in contrast to mature glial cells to stimulate neurite outgrowth and suppress glial scar formation, we used 4-6 postnatal day (pd) old astrocytes for transplantation.

The postcommissural fornix consists of about 29,000 myelinated axons throughout its entire extension. Unilateral fornix transection caused retrograde degeneration of 70-80% of the myelinated axons over 800-1,000µm proximal to the lesion. At the level of 1,200µm the number of axons was not decreased compared with the unlesioned tract. No further degeneration could be detected after 5d post lesion (pl). As the number of axons at 12w pl did not differ from that after 5-12d pl in the transection group, no spontaneous regeneration of myelinated fibres seemed to occur. Despite the lack of influence on the extent of degeneration up to 12d pl, transplanted immature astrocytes led to an almost twofold increase of myelinated axons 400µm proximal to the lesion within 12w pl. Supported by the DFG (SFB 194/B5)

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