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SYMPOSIUM

SIGNAL TRANSFER BETWEEN NEURONS AND GLIAL CELLS

September 16-20
1991

Castle Ringberg
Tegernsee
Bavaria
Germany

PROGRAM

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Office Hours:	Monday, Sept. 16:	15.00 - 21.00
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ORGANIZATION

Under the auspices of the Max Planck Society

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Monday, September 16, 1991

15.30 Registration

18.30 Dinner

20.00 Key note lecture

Chairperson: S. Waxman

G. W. Kreutzberg (MPI for Psychiatry, Martinsried):
Microglia: a cell for all seasons

21.00 Informal get-together

Tuesday, September 17, 1991

9.00 Session 1

Chairpersons: J. de Vellis and B. Hamprecht

C. Walsh (Harvard Medical School, Boston):
Cell lineage in the neocortex studied with a new retroviral library PCR rescue technique.

J. Price (MRC, London):
Cell lineage in the rat cerebral cortex

J. Trotter (University, Heidelberg):
The establishment and characterisation of immortalised lines of glial precursor cells

10.30 Coffee break

11.00 R. P. Skoff (Wayne State University, Detroit):
The differentiation of oligodendrocytes: from cell division to myelination

B. R. Ransom (Yale University, New Haven):
Glial -mediated changes in extracellular pH may influence neuronal excitability

I. Sommer (Southern General Hospital, Glasgow):
Interaction of viruses with rat glia in vitro

12.30 Lunch

14.00 Session 2

Chairpersons: B. R. Ransom and R. P. Skoff

B. Hamprecht (University, Tübingen):
Neuron - astroglia interaction in energy metabolism

B. A. Barres (University College, London):
Physiological properties of optic nerve glia

H. Kettenmann (University, Heidelberg):
Ligand- and voltage activated ion channels in glial cells in situ

15.30 Poster Session

16.30 G. Levi (Istituto Superiore di Sanita, Rome):
Release of endogenous amino acids induced by non-NMDA receptor agonists in cultured neuronal and glial cells

E. A. Newman (Eye Research Institute, Boston):
Electrogenic Na^+/HCO_3^--cotransport in retinal glial (Müller) cells

S. J. Smith (Stanford Medical School, Stanford):
Neuronal activity elicits astrocyte Ca^{++} waves and oscillations within hippocampal slices

K. D. McCarthy (University of North Carolina, Chapel Hill):
Calcium regulation in glia

18.30 Dinner

20.00 Session 3

Chairpersons: G. W. Kreutzberg and G. Lemke

B. A. MacVicar (University, Calgary):
Voltage-activated ion channels in acutely isolated hippocampal astrocytes

R. K. Orkand (Universidad de Puerto Rico, San Juan):
Neuron-glia interactions in the amphibian optic nerve

S. Murphy (University, Iowa):
Receptor-mediated generation of intra- and intercellular messengers from astrocytes

J. A. Coles (INSERM, Bordeaux)
Physiological interactions between glia and neurons in drone retina.

Wednesday, September 18, 1991

9.00 **Session 4**

Chairpersons: R. K. Orkand and H. Wekerle

G. P. Wilkin (Imperial College, London):
Substance P receptors on cells of the O-2A lineage

J. deVellis (UCLA, Los Angeles):
Neurotrophic interactions between glia and neurons

H.-W. Müller (University, Düsseldorf):
Astroglial factors supporting differentiation and long-term survival of brain neurons

10.30 **Coffee break**

V. Amberger (University, Zürich):
Oligodendrocyte-associated inhibitors of neurite growth and cell migration; the role of proteases for glioblastoma invasion

D. Monard (Friedrich Miescher Laboratory, Basel):
Interactions of glia-derived nexin with components of the extracellular matrix

A. L. Prochiantz (INSERM, Paris)
The control of neuronal shape

12.30 **Lunch**

14.00 **Excursion**

18.30 **Dinner**

20.00 **Open Air Jazz Concert**

Social Program

Wednesday, September 18, 1991
Time: 20.00

Open Air Jazz Concert

"Müller Braig Jazz Unit"

H. Müller, saxes **U. Frenzel, bass**
R. Braig, piano **H. Wachter, drums**

featuring: Freddy Wilkes, vocals

Thursday, September 19, 1991

9.00 Session 5

Chairpersons: D. Giulian and A. L. Prochiantz

A. Faissner (University, Heidelberg):
The tenascin glycoproteins - extracellular matrix molecules involved in neurohistogenesis

J. Taylor (ETH, Zürich):
The influence of the glial-derived J1 glycoproteins on growth cone behavior in vitro

C. M. Müller (MPI for Developmental Biology, Tübingen):
Astrocytic involvement in activity-dependent plasticity

10.30 Coffee break

R. K. Small (University College, London):
Functional properties of retinal Müller cells following transplantation

J. Schnitzer (MPI for Brain Research, Frankfurt/M.):
Responses of retinal glial cells following transection of the optic nerve of rabbits

M. Nieto-Sampedro (Cajal Institute, Madrid):
Glial scar effect: central neurite outgrowth over gliotic tissue

12.30 Lunch

14.00 Session 6

Chairpersons: G. Levi and G. P. Wilkin

S. David (General Hospital, Montreal):
Heterogeneity of reactive astrocytes in the lesioned adult rat optic nerve

D. Giulian (Baylor College, Houston):
Microglia and wound healing in the central nervous system

P. J. Gebicke-Haerter (University, Freiburg):
Responses of cultured microglia to inflammatory stimuli - implications for acute and chronic diseases of the CNS.

15.30 Poster Session

K. Frei (University, Zürich):
Microglial cell-mediated neurotoxity involves reactive oxygen intermediates and excitatory amino acid receptors but not cytokines

H. Wekerle (MPI for Psychiatry, Martinsried):
MHC gene expression in the central nervous system

W. Risau (MPI for Psychiatry, Martinsried):
Glial-endothelial cell interaction in blood-brain barrier differentiation

18.30 Dinner

20.00 Session 7

Chairpersons: S. David and K. D. McCarthy

S. G. Waxman (Yale University, New Haven):
Ion channel organization in myelinated axons and perinodal astrocytes

G. C. Owens (Washington University, St. Louis):
Modifying myelin protein gene expression

G. Lemke (Salk Institute, San Diego):
The transcription factor scip and glial differentiation

T. Amédéé, INSERM, Bordeaux
Electrophysiological and pharmacological characterization of K^+ and Ca^{2+} channels in mammalian Schwann cells. Interactions with dorsal root ganglion neurons

F. Aloisi, Istituto Superiore di Sanita, Rome
In vitro interactions between 0-2A lineage cells and immune cells

R.B. Banati, W. E. F. Klinkert, D. Hoppe, K. Gottmann, H. Kettenmann and G. W. Kreutzberg, MPI for Psychiatry, Martinsried
The ion channel pattern of microglia, dendritic cells and Langerhans cells

A. Bleuel, R. Meier, E. Reinhard and D. Monard, Friedrich-Miescher Institut, Basel
Expression of glia-derived nexin after in vitro lesion

J. Deitmer, University, Kaiserslautern
Electrogenic bicarbonate secretion by glial cells

B. Flott, W. Seifert, MPI for Biophysical Chemistry, Göttingen
Glutamate transporters in glial cells in primary cultures

C. A. Haas, M. Reddington and G. W. Kreutzberg, MPI for Psychiatry, Martinsried
Calcitonin gene-related peptide elicits differential expression of early respronse genes in cultured astrocytes

C. O. Hanemann, G. Kuhn, P. Spreyer, C. Gillen, H. Schaal, H. W. Müller, University, Düsseldorf
A Schwann cell transcript homologous to gas3 is differentially regulated by axonal growth during peripheral nerve regeneration

M. Jung, J. Crang, W. Blakemore, A. Aguzzi, and J. Trotter, University, Heidelberg
Immortalization of oligodendrocyte precursors by infection with a t-NEU containing retrovirus

F. Kirchhoff and H. Kettenmann, University, Heidelberg
GABA triggers a [Ca^{2+}] increase in murine precursor cells of the oligodendrocyte lineage

D. R. Marriott, G. W. Taylor and G. P. Wilkin, Imperial College of Science and Technology, London
Prostaglandin release from rat cerebral cortex and spinal cord astrocytes

D. Piani, K. Nohava, U. Malipiero, K. Frei and A. Fontana, University Hospital, Zürich
Production of cytokines by microglial cells

W. Reichelt, T. Müller, P.M. Orkand, J. Schnitzer, H. Kettenmann,University, Heidelberg and Leipzig
Membrane currents recorded in Müller (glial) cells of the isolated intact mouse retina

A. Rohlmann, J. Neuhaus, A. Gocht and H. Wolburg, University, Tübingen
Freeze-fracture analysis of astrocytic membranes in the optic nerve of the myelin-deficient rat during postnatal development

J. Scherer and J. Schnitzer, MPI for Brain Research, Frankfurt/M
Characterization of cultivated retinal glial cells and effect of growth factors

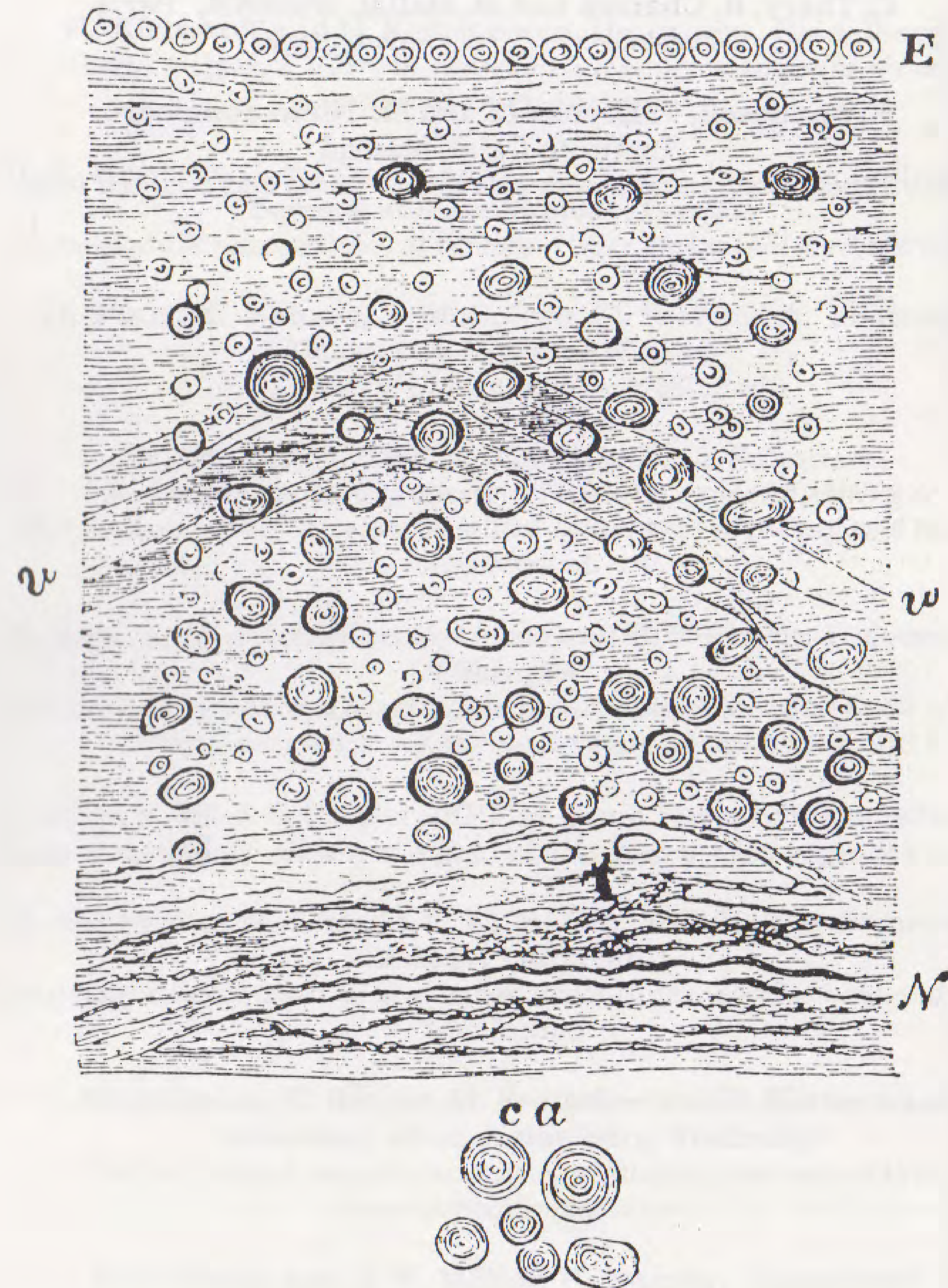
M. Schweizer, W. J. Streit, C. M. Müller, MPI for Developmental Biology, Tübingen
Perisynaptic lectin-binding sites in cat visual cortex: development and glial localization

C. Steinhäuser, T. Berger, M. Frotscher and H. Kettenmann, University, Jena, Heidelberg, Freiburg
NMDA induced inward currents in identified glial cells of the mouse hippocampal slice

C.C. Stichel and H. W. Müller, University, Düsseldorf
Cortical neuron-astrocyte, -substrate and -medium interactions: transmitter-phenotype-dependent morphological effects

C. Thery, B. Chamak and M. Mallat, INSERM, Paris
Neurotoxicity of brain macrophages

F. Wandosell, P. Bovolenta and M. Nieto-Sampedro, Instituto Cajal, Madrid
Neurite outgrowth inhibitors in gliotic CNS tissue and glial cell lines



IN VITRO INTERACTIONS BETWEEN O-2A LINEAGE CELLS AND IMMUNE CELLS

Francesca Aloisi, Laboratory of Pathophysiology, Istituto Superiore di Sanità, Rome, Italy

Multiple sclerosis (MS) lesions are characterized by demyelination and by infiltration of activated T lymphocytes and macrophages. In order to better understand the immunopathogenic mechanisms involved in MS, we investigated whether cells belonging to the oligodendrocyte-type-2 astrocyte (O-2A) lineage, which play a central role in the formation and maintenance of myelin sheets and probably of nodes of Ranvier, may function as stimulators and/or targets of a T cell-mediated immune response. Purified populations of O-2A bipotential progenitors (mainly $LB1^+$, $O4^+$, $GFAP^-$, $GalC^-$ cells) were isolated from primary cultures of neonatal Lewis rat forebrain and cultured either in 10% FCS-containing medium or in 0.5% FCS-chemically defined medium. Maximal stimulation of MHC class II (Ia) antigens was detected on type-2 astrocytes and O-2A progenitors following the combined treatment with IFN-γ and TNF-α. When cytokine-treated O-2A cells were confronted with syngeneic, MHC class II-restricted, MBP-specific T-lymphocyte lines, they failed to induce antigen-specific proliferation of T-lymphocytes. However, morphological screening of the O-2A cell T-lymphocyte cocultures and ^{51}CR-release assays indicated that MBP-specific T cells caused lysis of cytokine-treated O-2A cells in the presence but not in the absence of MBP. This finding suggests that O-2A cells may be able to present antigen to T cells, but do not provide the signals necessary to induce T cell proliferation. On the basis of the present data it can be hypothesized that antigen-specific interactions between O-2A lineage cells and T-lymphocytes infiltrating the brain tissue may be responsible for O-2A cell damage and contribute to the demyelination process and/or to the failure of successful remyelination in MS.

OLIGODENDROCYTE-ASSOCIATED INHIBITORS OF NEURITE GROWTH AND CELL MIGRATION; THE ROLE OF PROTEASES FOR GLIOBLASTOMA INVASION.

V.Amberger; M.E.Schwab; P.A.Paganetti; Brain Research Institute, University of Zurich,August-Forel-Str.1,CH-8029 Zurich.

Human glioblastomas are known as highly invasive CNS tumors. The rat C6 glioblastoma line is a good model to study infiltrative properties of CNS tumor cells. We showed that the C6 cells are able to overcome the inhibitory substrate properties for cell migration expressed by oligodendrocytes and CNS white matter. A proteolytic activity enables the C6 cells to inactivate these inhibitors. Using various known protease blockers we identified it as a metalloproteolytic activity which is associated with the C6 plasma membranes (Paganetti et al.; J. Cell Biol. 107:2291, 1988). Its blocker profile is distinct from that of known metalloproteases. The enzyme is strongly membrane-bound, but can be solubilized by detergents, and has a pH-optimum between 5.5 and 6.5. Blockade of the metalloprotease by oligopeptides strongly depresses the migration of C6 cells through myelin and into optic nerve explants.

ELECTROPHYSIOLOGICAL AND PHARMACOLOGICAL CHARACTERIZATION OF K^+ AND Ca^{2+} CHANNELS IN MAMMALIAN SCHWANN CELLS. INTERACTIONS WITH DORSAL ROOT GANGLION NEURONS.

T. AMEDEE, Unité de Neurobiologie Intégrative, INSERM, U 176, 1 rue Camille Saint Saëns, 33077 BORDEAUX Cedex, FRANCE.

Schwann cells ensheath axons with myelin in the peripheral nervous system in order to increase conduction velocity. However, if some mammalian Schwann cells possess voltage-dependent K^+ and Na^+ channels which are similar in many respects to those found in neuronal cells (Chiu et al., 1984), these channels do not seem to participate to the insulation of the axons. In this study, using the patch clamp technique, we have investigated the electrophysiological properties of Schwann cells from organotypic culture of dorsal root ganglia (DRG) of the mouse.

In standard conditions, the estimated resting membrane potential was about -40 mV. From a holding potential of -70 mV, depolarizing steps activated two sets of K^+ currents: a fast transient current similar to the "A" current, far more sensitive to 4-AP (half-maximal dose: 1.3 mM) than to TEA (half-maximal dose: 100 mM), and the well known delayed rectifier K^+ current.

In K^+ free conditions, depolarizing steps from -70 mV evoked inward Ca^{2+} channel currents as they were unaffected by tetrodotoxin and inhibited by cobalt. Although the vast majority of the cells displayed T-type Ca^{2+} channel currents, few cells exhibited an additionnal L-type Ca^{2+} channel current. Interestingly, the expression of Ca^{2+} channel currents in the Schwann cells appeared to be dependent on the presence of DRG neurons in the culture as axonal degeneration following excision of the explants led to loss Ca^{2+} channel currents.

In summary, cultured mouse Schwann cells possess voltage-dependent K^+ and Ca^{2+} channels. The expression of Ca^{2+} channel currents depends on the presence of DRG neurons in culture which suggest that DRG neurons interacts with Schwann cells even at a distance. Similar requirement for the expression of Ca^{2+} channel currents has been reported recently for cultured rat astrocytes (Corvalan et al., 1990). Although the physiological relevance of these ionic channels remains to be clarified, their putative modulation by neurotransmitters and/or second messengers could interact with neuronal activity.

Chiu et al., (1984). *Nature* **311**, 156-157.
Corvalan et al., (1990). *Proc. Natl. Acad. Sci. USA* **87**, 4345-4348.

THE ION CHANNEL PATTERN OF MICROGLIA, DENDRITIC CELLS AND LANGERHANS CELLS

R. B. Banati[1], W. E. F. Klinkert[1], D. Hoppe[2], K. Gottmann[1], H. Kettenmann[2] and G. W. Kreutzberg[1]

[1] Max-Planck-Institut für Psychiatrie, Neuromorphology, Am Klopferspitz 18A, D-8033 Martinsried; [2] Dept. of Neurobiology, University of Heidelberg, INF 345, D-6900 Heidelberg

Microglia, the endogenous tissue macrophages of the brain, are characterized by a distinctly different pattern of voltage dependent K^+ channels in the cell membrane (Kettenmann et al., 1990) Unlike macrophages, that possess outward and inward K^+ currents and only show inwardly rectifiying K^+ currents, regardless of the isolation or cultivation method employed. Morphological, antigenic and functional similarities of microglia with a group of cells, termed *dendritic antigen presenting cells*, lead to the suggestion that microglia might in fact belong to the same cellular entity.

In our study, however, we found that in dendritic cell from thymus, lymph nodes and skin (Langerhans cells) only outward K^+ currents could be ativated. These dendritic cells are thus clearly distinguished from microglia as wellas from peritoneal macrophages. The fact that these cell types differ in the profile of the functionally important K^+ channels support the assumption that mononuclear phagocytes, lymphoid dendritic cells and microglia have diverged so considerably that, despite numerous similarities, they should be thought of as distinct cellular lineages.

PHYSIOLOGICAL PROPERTIES OF OPTIC NERVE GLIA

B. A. Barres, University College London

The electrophysiological properties of rat optic nerve glia were characterized using whole-cell and single-channel patch recording. Type-1 astrocytes (1As), the O2A glial progenitor cell, and its two descendant cell types, type-2 astrocytes (2As) and oligodendrocytes (OLs) were studied *in vitro* and after acute isolation. To ensure that the properties of the acutely-isolated cells represented those found *in vivo*, a simple enzymatic dissociation technique called "tissue printing" was developed that allowed isolation of viable cells still bearing processes.

Each cell was found to express a unique voltage-dependent ion channel phenotype *in vitro* and *in vivo*. *In vitro*, 1As express mainly an outward K^+ current, while 2As have a complex "neuronal" phenotype including Na^+, Ca^{++}, and K^+ currents. OLs have a large K^+ current and an occult Cl^- current. These properties suggest a new mechanism for K^+ homeostasis that involves neuron to glia signalling.

Direct comparison of the Na^+ channels in 1As and neurons showed that they were qualitatively similar but quantitatively distinct: the glial channel has slower kinetics and a more negative voltage dependence. Both forms of the channels were found in 2As.

While cells of the O2A lineage have similar ion channels *in vitro* and *in vivo*, the 1As do not. Early postnatal 1As in tissue prints resembled those in culture, but by P10 the channel phenotype of cells in prints had reached the adult pattern, more complex than in culture. The development and maintenance of the adult phenotype depended on neurons; it was prevented by optic nerve transection, and could be induced by co-culture with purified neurons. The presence of voltage-dependent currents in glia *in vivo* suggests that glia are not as distinct from neurons as has been thought, and may be involved in active signalling processes.

EXPRESSION OF GLIA-DERIVED NEXIN AFTER IN VITRO LESION

Bleuel A., Meier R., Reinhard E. and Monard D.
Friedrich Miescher Institut, P.O. Box 2543, CH-4002 Basel.

Glia-derived Nexin (GDN) is a serine protease inhibitor promoting neurite outgrowth . In the peripheral nervous system, the expression of GDN is down regulated, but can be dramatically induced after lesion (Meier et al., Nature 343, 548-550 [1989]. In cultured dorsal root ganglia explants, it is possible to mimic the lesion in vitro. GDN, which is down regulated, can be induced following lesion of the processes emerging of the explants. Dotblotting quantification shows a maximal increase of 4-5 fold after 7 days. Double staining with specific antibodies demonstrates that the induction of GDN synthesis occurs only in Schwann cells and not in fibroblasts. The GDN increase is observed only in the Schwann cells distal to the site of injury, where neuronal contact has been lost as consequence of nerve degeneration. These results show that the effects seen in vivo can be replicated in vitro. They suggest the existence of a neuronal component which represses the expression of GDN in Schwann cells.

GLIAL SCAR EFFECT: CENTRAL NEURITE OUTGROWTH OVER GLIOTIC TISSUE

Paola Bovolenta, Francisco Wandosell and Manuel Nieto-Sampedro. Instituto Cajal, Av. Doctor Arce, 37, Madrid.

Central neurite outgrowth over glial scar tissue was studied using an *in vitro* model. Neurites from explants of rat septum, hippocampus or dorsal root ganglion, grew profusely over polylysine coated with either laminin or plasma membranes from cultured astrocytes. However, growth was strongly inhibited when the substrate was either myelin or plasma membranes from deafferented (or kainic acid-treated) adult hippocampus. Membranes from either normal cortical tissue or cortical tissue adjacent to an open injury had intermediate properties.

The membranes from injured tissue from 3 to 20 days postlesion had inhibitory properties, but the underlying mechanisms of inhibition at various periods after injury were probably different. This was evidenced by solubilization of the inhibitory molecules with detergent and fractionation of the extract. Molecules capable of preventing explant attachment to the substrate were most abundant after 3 days postlesion and could be removed with 0.2 % CHAPS. Other growth inhibitors did not seem to interfere with explant attachment but were capable of preventing neurite initiation by laminin and repelled growing neurites. These molecules, solubilized by 0.5% CHAPS from 12 days postlesion tissue, were trypsin resistant, heparinase sensitive and fractionated as a heparan-sulfate. Work in progress suggests that.changes in the precise structure of the same type of proteoglycan (i.e. heparan sulfate or hyaluronic acid) determines whether the molecule is either neurite promoting or inhibiting.

PHYSIOLOGICAL INTERACTIONS BETWEEN GLIA AND NEURONS IN DRONE RETINA

J. A. Coles, INSERM, U176, F-33077 Bordeaux Cedex.

The drone retina is an essentially uniform population of neurons, the photoreceptors, set in a functional syncytium of glial cells; it has neither chemical synapses nor blood vessels. Recent studies of the behaviour[1] and photoreceptor physiology[2,3] of drones support the idea that the retina switches from a quiescent state in the dark hive to an active state when the drone flies out in bright sunlight. In superfused slices of retina glucose is taken up selectively into the glia[4]: most of it is incorporated into trehalose, and a little into glycogen[5]. Light stimulation increases O_2 consumption by the photoreceptors and, in the glia, both the incorporation of radioactive glycosyls into glycogen and the breakdown of glycogen and trehalose[4,5]. K^+ released by stimulated photoreceptors appears to be neither an adequate nor a necessary signal to the glia[5,6]. So far, no effects have been observed of addition to the superfusate of candidate substances for the signal to the glia or for the carbohydrate that is transferred to the photoreceptors. Brief light stimulation is followed by a transient alkalinization of extracellular fluid which is reversibly inhibited by the weak acid propionate at 20 mM and which suggests that coupled transmembrane transport is occurring at this time.

1. Vallet, A.M. & Coles, J.A. (1991) *Vision Res.* **31**, 1453-1455. 2. Coles, J.A. *et al.* (1991) *J. Physiol.* **435**, 104P. 3.Walz, B. *Verd. Dtsch. Zool. Ges.* (1990) **83**, 261. 4.Tsacopoulos, M. *et al.* (1988) *PNAS* **85**, 8727-8731. 5. Evêquoz-Mercier, V. & Tsacopoulos, M. (1991) *J. gen. Physiol.* (in press). 6. Coles, J.A. & Schneider-Picard, G. (1989) *Glia* **2**, 213-222.

NEURONAL ACTIVITY ELICITS ASTROCYTE Ca WAVES AND OSCILLATIONS WITHIN HIPPOCAMPAL SLICES.

John W. Dani, Alex Chernjavsky, JoAnn Buchanan, & Stephen J. Smith. *Department of Molecular and Cellular Physiology, Stanford University School of Medicine, Stanford, CA 94305.*

Using laser confocal microscopy and the Ca indicator dye fluo-3, we have imaged intracellular Ca ion concentrations in organotypic hippocampal slices following electrical stimulation of the dentate gyrus. Our experiments suggest that synaptic release of neurotransmitter glutamate triggers Ca signals that then propagate within newtorks of astrocytes. Specific cells within the slices were identified as astrocytes by retrospective GFAP immunocytochemistry and electron microscopy. Both neurons and astrocytes located within area CA3 exhibited elevated intracellular Ca levels with electrical stimulation. These responses were mediated through a TTX-sensitive nerve fiber pathway. Astrocyte Ca responses were delayed by 1-2 seconds when compared with the neuronal Ca response, which appeared within milliseconds following the onset of stimulation. The astrocytes displayed both propagating waves of intracellular Ca and Ca oscillations, whereas only tonic Ca rises were apparent in neurons. Kynurenic acid, a glutamate antagonist, completely blocked astrocytic responses to electrical stimulation, suggesting that neuronal glutamate release may mediate this aspect of the response. Astrocyte Ca waves and oscillations were observed with stimulus frequencies as low as 2 Hz. At room temperature, wave velocities ranged from 7 to 15 um/s, and the periods for oscillations averaged 20 seconds. If one supposes that such glial Ca signals in some way feed back to influence neuronal excitability or synaptic transmission, these findings suggest that astrocytes may be partners with neurons in the brain's information processing functions. Various possible means by which glial Ca signals actually may feed back to influence synaptic transmission will be discussed.

HETEROGENEITY OF REACTIVE ASTROCYTES IN THE LESIONED ADULT RAT OPTIC NERVE.

S. David, The MGH Res.Inst. McGill University, Canada.

We have been studying the injury-induced changes in astrocytes in the transected adult rat optic nerve in order to characterize some of the molecular and functional changes, and their implications for axon growth and regeneration. A few days after an optic nerve transection, the non-permissive nature of this CNS white matter tract is changed to a permissive state near the lesion site. The area of this change corresponds to the region of macrophage infiltration. These changes might be mediated via the action of cytokines on astrocytes, and by other mechanisms which inactivate inhibitory molecules associated with oligodendrocytes and CNS myelin. After astrocytes are activated by IL-1 and dBcAMP for 3 days in culture, they are able to support better neurite growth. Thus suggesting that reactive astrocytes might be able to promote axon growth, and may support the injury-induced axon sprouting in vivo. At the very site of transection, however, astrocytes which were within the core of the normal optic nerve, become associated with leptomeningeal cells. These reactive astrocytes form a new glia limitans, and express the ECM molecule tenascin, which has anti-adhesive properties. It is possible that tenascin which delineates the edge of the lesion might serve to limit the growth of axons across the lesion site. These results suggest a heterogeneity in the reactive astrocyte population.

ELECTROGENIC BICARBONATE SECRETION BY GLIAL CELLS

J.W. Deitmer, Abt. Allgemeine Zoologie,
Universität Kaiserslautern,
D-6750 Kaiserslautern, Germany

The production and shift of acid-base equivalents in the nervous system leads to pH transients in neurones, glial cells and in the extracellular spaces. In order to minimize these pH changes H^+ buffering and active pH regulaton are required. Addition of external bicarbonate produced a marked intracellular alkalinization in glial cells of the leech central nervous system, due to an electrogenic Na^+-bicarbonate cotransport into the cells (1). New experiments using double- and triple-barrelled ion-sensitive microelectrodes in identified leech glial cells suggest that this transport process can be reversed by reducing the external pH/bicarbonate concentration, or by hyperpolarizing the glial membrane. The outwardly directed cotransport can be inhibited by DIDS (0.3-0.5 mM), and can be transiently accelerated by reducing the external Na^+ or bicarbonate concentration. There are distinct changes of the intracellular pH and Na^+ as well as of the membrane potential, when the Na^+-bicarbonate cotransporter is activated in either direction. The bicarbonate uptake and secretion by glial cells may effectively counteract alkaline and acid shifts in the extracellular spaces, and may thus help to maintain H^+ homeostasis in the central nervous system.

(1) Deitmer, J.W. & Schlue, W.R. (1989) J. Physiol 411: 179-194

Supported by the Deutsche Forschungsgemeinschaft.

NEUROTROPHIC INTERACTIONS BETWEEN GLIA AND NEURONS. J. de Vellis, A. Espinosa de los Monteros, S. Kumar and S. Scully. UCLA School of Medicine, Los Angeles 90024-1759.

Previous studies have shown that transferrin (Tf), the iron transport protein, plays a developmentally regulated role in CNS neuronal and glial survival and that oligodendrocytes play a key role in transferrin synthesis and iron storage, hence iron homeostasis in the brain. Tf gene expression has been examined in vivo and in vitro by in situ hybridization, Northern analysis and immunocytochemically. Tf gene expression in the adult rat brain is restricted to oligodendrocytes and the choroid plexus, suggesting that Tf is an autocrine and paracrine factor. However, in vitro Tf expression also occur in astrocytes and some types of neurons under certain conditions. Its expression is regulated by several agents, cell-cell interactions and culture conditions. In addition to its survival effect Tf acts as a differentiation factor on brain cells. In conclusion, Tf is a neurotrophic agent produced by oligodendrocytes that regulate cell survival and gene expression in an autocrine and paracrine fashion. (Supported by NIH and DOE).

The tenascin glycoproteins - extracellular matrix molecules involved in neurohistogenesis. Andreas Faissner, Department of Neurobiology, University of Heidelberg, FRG.

Tenascin is transiently expressed in the developing central nervous system, for example on astrocytes delineating "barrels" in the rodent somatosensory cortex. Tenascin and related molecules consist of glycoproteins with apparent molecular weights of 190 to 260 kD. On the AA sequence level tenascin shows a modular organization with 13½ to 14½ EGF-type repeats on the aminoterminal followed by fibronectin (FN) type III repeats and homologies to fibrinogen ß and γ on the carboxy-terminal end. Different isoforms are generated from a single gene by differential splicing of variable numbers of FN III repeats. Under non-reducing conditions tenascin monomers are assembled to hexamers which appear as six-armed structures, so called hexabrachia, when examined by rotary shadowing and electron microscopy. In order to investigate functional properties of the molecule, a library of monoclonal antibodies - J1/tn1 to J1/tn5 which recognize different sites on the molecule - was generated and used for perturbation studies in bioassays in vitro. Tenascin adsorbed to polyornithine (PORN) reduced neuronal cell-substrate contact formation and induced neuron aggregation and neurite fasciculation. Further, neuronal cell bodies and fibers sorted to tenascin-free areas on patterned tenascin/PORN-substrates. Despite these repulsive properties, homogeneous tenascin/PORN-substrates stimulated neurite outgrowth by embryonic hippocampal and postnatal cerebellar granule neurons. In contrast, tenascin added as soluble component to the culture medium inhibited neurite outgrowth. The neurite outgrowth promoting property was blocked by J1/tn2 which binds to the 10th or 11th FNIII repeat of mouse tenascin. None of the antibodies interfered with the repulsive or inhibitory effects of the molecule, suggesting that its conducive and adverse effects are mediated by distinct domains. By virtue of the opposite functional influences on neuronal differentiation, tenascin may contribute to both segregation of neuronal assemblies and formation of ordered fiber tracts during the development of neural tissues. Supported by DFG (SFB 317).

Glutamate Transporters in Glial Cells in Primary Culture

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In the present study pyramidal neurons from rat hippocampus and astrocytes from rat cortex were chosen for autoradiographic and biochemical investigations concerning the participation of the respective cell types in glutamate uptake. Autoradiograpy indicates that hippocampal neurons in primary culture do not exhibit any uptake of glutamate even in "mature" cultures (i.e. 5 weeks in vitro). In contrast astrocytes show an intensive label after being incubated with tritiated glutamate (50 nM, 15 min).

As shown recently (1) glial glutamate uptake is dependent on either Na^{+} or Cl^{-} or Ca^{++}, exhibiting a low and a high affinity component respectively. The sodium- and the chloride-dependent uptake system represent two independent transport sites differing in their pharmacological properties. The sodium-dependent transporter accumulates aspartate as well as glutamate. The uptake is strongly inhibited by aspartate-ß-hydroxamate (AßH) and threo-ß-hydroxyaspartate (TßH). In contrast the chloride- and the calcium-dependent uptake systems do not handle aspartate as substrate. AßH and TßH are only poor inhibitors while quisqualate reduces glutamate uptake almost completely (1).

We are investigating if depolarization causes alterations in glutamate uptake efficacy both in glial and in neuronal cells. Different uptake systems might offer several possibilities for neural cells to regulate the extracellular concentration of the excitatory transmitter and therefore be related to phenomena of synaptic plasticity and glutamate neurotoxicity.

(1) B. Flott and W. Seifert: Characterization of Glutamate Uptake Systems in Astrocyte Primary Cultures From Rat Brain. *GLIA, Vol. 4, 1991.*

MICROGLIAL CELL-MEDIATED NEUROTOXICITY INVOLVES REACTIVE OXYGEN INTERMEDIATES AND EXCITATORY AMINO ACID RECEPTORS BUT NOT CYTOKINES

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The colocalization of activated microglial cells and destructed neurons as observed in degenerative brain diseases may point to microglia-induced neuronal cytotoxicity. Furthermore, destruction of neurons leading to neurological sequelae are also observed in bacterial meningitis, which is paralelled by activation of macrophages secreting TNF-α and IL-1β. In the present study, we report that microglial cells are highly cytotoxic for cerebellar neurons in vitro. Cytotoxicity follows two pathways, one being due to secretion of hydrogen peroxide, the effect of which is inhibited by the oxygen radical scavangers SOD and catalase. The other pathway involves microglia-derived neurotoxin(s), which interact with the neuronal N-methyl-D-aspartate (NMDA) receptor complex. This interaction is characterized by neutralization of the neurotoxic effects by NMDA receptor antagonists but not by drugs which inhibit non-NMDA receptor-mediated pathways or calcium influx.

The microglia-derived cytokines TNF-α, IL-1β, IFN-α/-β and IL-6 caused no significant neuronal damage when added to the cultures. These data indicate that in addition to the classical view that microglia phagocytose neuronal debris, microglia may also directly damage neurons.

RESPONSES OF CULTURED MICROGLIA TO INFLAMMATORY STIMULI. - IMPLICATIONS FOR ACUTE AND CHRONIC DISEASES OF THE CNS.

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The microglia culture system described here offers a variety of advantages to study responses to inflammatory stimuli as well as regulation of mediators of inflammation produced by the cells themselves (J.Neurosci. 9, 183-194; 1989). Microglia react to brain lesioning by proliferation, migration to the sites of injury and differentiation into macrophages. Proliferation of the isolated cells in culture was induced by recombinant IL-3, GM-CSF, by fetal calf sera or by as yet unidentified factors synthesized in astrocyte cultures. These factors apparently synergized with IL-1 and TNF in stimulation of microglial proliferation, whereas IL-1 and TNF by themselves tested on the isolated cells had no effect. Steroids, such as aldosterone, hydrocortisone and dexamethasone reversibly inhibited microglial proliferation. The progesterone receptor antagonist RU 38486 abolished glucocorticoid-mediated inhibition. LPS, the bacterial endotoxin, irreversibly inhibited microglial proliferation induced by serum, IL-3, GM-CSF, or astrocyte conditioned media, and induced secretion of IL-1, IL-6 and TNF. This induction was inhibited by dexamethasone. Inhibition of proliferation by LPS or glucocorticoids, therefore, involves basically different mechanisms. Glucocorticoids appear to "freeze" the cells in their actual stages of maturation, whereas LPS drives them further into differentiation. The present data encompass the beginning of an understanding of how a variety of blood- and brain-derived factors interact and bring about the glial reaction upon injury.

MICROGLIA AND WOUND HEALING IN THE CENTRAL NERVOUS SYSTEM

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Reactive microglia participate in nearly every type of CNS injury including trauma, stroke, neoplasm, infection, demyelination, and neurodegeneration. When "activated", these cells respond by migration to the sites of injury, phagocytosis of debris, and release of protein factors that promote astrogliosis and neovascularization. In addition, reactive microglia release small, heat-stable, protease-resistant molecules which are toxic to neurons in culture. These same neurotoxins are found in highest concentrations within tissues damaged by stroke or trauma. Suppression of microglia secretory function by chloroquine and colchicine reduces the production of neurotoxins in vivo. Control of inflammatory responses during acute CNS damage may, therefore, reduce neuronal loss and improve the recovery of neurological function.

CALCITONIN GENE-RELATED PEPTIDE ELICITS DIFFERENTIAL EXPRESSION OF EARLY RESPONSE GENES IN CULTURED ASTROCYTES

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The early and dramatic increase of calcitonin gene-related peptide (CGRP) in injured motoneurons indicates a possible role for this neuropeptide in the cellular reactions accompanying neuronal regeneration. Therefore possible effects of CGRP were investigated on astroglial cells in culture. Quantitative Northern blot analysis revealed the differential regulation of early response gene expression (c-fos, c-jun, jun B and PC4 (TIS7)) in cultured astrocytes challenged with CGRP. A strong and transient increase in c-fos and jun B mRNA was observed after treatment with CGRP; the levels of c-jun and PC4 mRNAs, however, were constitutively expressed in cultured astrocytes and not effected by the CGRP treatment. Induction of the c-fos gene by CGRP was concentration-dependent, half maximal stimulation of c-fos mRNA being obtained with 100 nM CGRP. The CGRP effect appeared to be mediated by a CGRP receptor, since calcitonin was found to mimic only weakly the action of CGRP on cultured astrocytes. Calcitonin transiently induced c-fos gene expression with a similar time course to CGRP, but its effect was much less pronounced. Agents affecting the intracellular cyclic AMP level, forskolin and Ro 20-1724, stimulated c-fos mRNA in a strong and transient fashion with a temporal sequence similar to the response to CGRP. Further, the phosphodiesterase inhibitor Ro 20-1724 potentiated the action of CGRP on c-fos mRNA induction, suggesting a role for cyclic AMP in mediating the action of CGRP. The present results strongly indicate that CGRP may play a physiological role as a regulator of astrocyte gene expression.

NEURON-ASTROGLIA INTERACTION IN ENERGY METABOLISM

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The location of astrocytes between blood capillaries on the one side and neurons and oligodendrocytes on the other side suggests that they may play a key role in the energy metabolism of the brain. In immunocytochemical studies with monoclonal antibodies against the brain isozyme of glycogen phosphorylase, in brain this enzyme was detected solely in astrocytes, including Bergmann glial cells and in ependymal cells. The enzyme was found only in astroglia-rich but not neuron-rich cultures. Compatible with this is that glycogen was found always in astroglia-rich primary cultures but not in neuron-rich primary cultures. In astroglia-rich primary cultures derived from the brains of rats or mice the level of glycogen was regulated by glucose and by neurohormones that are found in brain neurons. Thus, besides withdrawal of glucose, also elevation of the concentration of cyclic AMP decreases the glycogen content of the cultures. In contrast, exposure of the cultures to insulin-like growth factor I elevates the level of glycogen. Part of the lactate generated by degradation of glycogen is released from the astroglial cells. In the brain such lactate may function as a supply of energy to neurons and oligodendrocytes.

A SCHWANN CELL TRANSCRIPT HOMOLOGOUS TO GAS3 IS DIFFERENTIALLY REGULATED BY AXONAL GROWTH DURING PERIPHERAL NERVE REGENERATION.

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A cDNA-library from crushed rat sciatic nerve was screened for mRNAs which are differentially expressed in regenerating and intact nerve. One cDNA-clone (1.8kb), which shows significant homology to "growth arrest specific cDNA" (gas3) from mouse fibroblasts, is expressed in Schwann cells as revealed by *in situ* hybridization with a cRNA probe. The mRNA level was rapidly down-regulated within 2 days after lesion as measured by RNase protection assay. In the distal part of crush-lesioned nerves, where regeneration occurs, the mRNA levels were restored to control values within 4-6 weeks. In the distal part of transected and ligated nerves, which are prevented from regeneration, the mRNA levels stayed at 10% of control levels for at least 12 weeks post operation.

To study the possible correlation between axonal regeneration and changes in the mRNA levels, transected and ligated nerves were reanastomozed after 6 weeks. *In situ* hybridization of nerve segments distal to the lesion site and at different time intervalls after anastomosis clearly showed that recovery of the mRNA signals occured in a proximal to distal orientation. Correlation of these data with results from immunohistochemistry using monoclonal antibodies against neurofilament revealed that expression of this gene product depends on axonal regeneration from the proximal into the distal segment. In cultured Schwann cells which are devoid of axonal contact expression level was elevated following treatment with forskolin.

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IN VITRO EXPRESSION OF MYELINOGENIC PROPERTIES BY OLIGODENDROCYTES OF TROUT CNS

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The myelin forming cells of fish CNS are exceptionel in their biochemical properties since they produce a major myelin protein of about 36,000 dalton MW (36K) which is unique to the CNS of bony fish. Furthermore they do not express PLP which is an established molecular marker for mammalian oligodendrocytes but synthesize instead two Po-like glycoproteins termed IP1 and IP2 and hence appear antigenically related to mammalian Schwann cells.

In a cell culture approach the in vitro differentiation of oligodenrocytes from embryonic trout brain was analysed immunohistochemically. Evidence was obtained that trout oligodendrocytes develop from proliferative A2B5-positive progenitor cells which in the absence of axonal contacts can differentiate into immature oligodendrocytes expressing the IP2 glycoprotein. Further maturation of the cells as indicated by the appearance of the late differentiation markers 36K and IP1 does not occur under these conditions.

An immunomagnetic cell separation technique was worked out to isolate the oligodendrocyte precursors from embryonic trout brain allowing a more detailed characterization of these cells.

IMMORTALIZATION OF OLIGODENDROCYTE PRECURSORS 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. Infected cells were selected in G 418 after several in vitro passages and cells expressing the O4 antigen were cloned on the fluorescent activated cell sorter. This antigen is expressed by oligodendrocytes and their precursor cells. 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 "differentiated" maturation stage. The *in vivo* behaviour of the cells following transplantation into demyelinated lesions in adult rats was investigated. A bulk population of an early passage of the cells specifically recognized and ensheathed 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*.

Ligand- and voltage activated ion channels in glial cells *in situ*

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Glial cells have been classically characterized as electrically passive elements in the nervous system. Recent advances in techniques have demonstrated that glial cells in culture can express a variety of voltage- and ligand activated channels. With the application of the patch-clamp technique to brain slices it has become possible to unequivocally characterize such channels in situ. We used a mouse corpus callosum slice preparation to study the current patterns of glial cells during their development *in situ* (1). To correlate physiological data with the cell type, we identified glial cells ultrastructurally after recording. We found that the pattern of voltage-gated channels markedly changed when glial precursor cells developed into oligodendrocytes (1). Moreover, precursor cells and oligodendrocytes expressed GABA and glutamate receptors (2). This study demonstrates that the expression of voltage- and ligand-gated channels by glial cells is not an artefact of the tissue culture system which makes it likely that these channels may have functional importance.

[1] Berger, T, J Schnitzer and H Kettenmann (1991) Developmental changes in the membrane current pattern, K^+ buffer capacity and morphology of glial cells from the corpus callosum slice, J. Neurosci.,in press.
[2] Berger, ., W Walz, J Schnitzer and H Kettenmann (1991) GABA and glutamate activate currents in glial cells of the corpus callosum slice, submitted.

GABA triggers a $[Ca^{2+}]_i$ increase in murine precursor cells of the oligodendrocyte lineage.

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The development of oligodendrocytes from their precursor cells can be studied *in vitro* by identifying cultured cells at different developmental stages. We used this culture system to compare the effect of the inhibitory neurotransmitter GABA on $[Ca^{2+}]_i$ using a fura-2 fluorescence measurement system. Application of GABA-evoked transient $[Ca^{2+}]_i$ increases in the precursor cell. In contrast, $[Ca^{2+}]_i$ levels were not affected in oligodendrocytes which were identified by their positive labelling for the antibody O1. The precursor cells, identified by a lack in the O1 staining, responded to GABA in the concentration range between 10^{-6} to 10^{-4} M. Since muscimol mimicked and bicuculline blocked the GABA response, we conclude that the response is mediated by activation of $GABA_A$ receptors. An involvement of Ca^{2+} channels is infered by the observation that the $[Ca^{2+}]_i$ changes could either be blocked by omitting Ca^{2+} from the bath solution or by adding the Ca^{2+} channel blocker Cd^{2+}. Both, $GABA_A$ receptors and Ca^{2+} channels have indeed been previously identified on these precursor cells with the aid of the patch-clamp technique. We thus propose the following mechanism: The Cl^- efflux via the GABA receptor depolarizes precursor cells. The depolarization leads to an activation of Ca^{2+} channels resulting in an influx of Ca^{2+} and the observed rise in cytosolic $[Ca^{2+}]$. The physiological importance of this event is yet speculative but could serve as a signal from GABAergic neurons to glial precursor cells.

MICROGLIA: A CELL FOR ALL SEASONS

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The review will be introduced by alluding to the history of the microglia concepts. It is now widely, though not unequivocally, accepted that microglia are a subpopulation of the monocyte/macrophage family and that the precursors enter the brain of vertebrates early during embryogenesis. The ameboid type seems to mature to a ramified type which settles with some regional differences throughout the CNS tissue. This resting microglial cell seems to have little known functions in the normal brain. It is, however, a most easily activated cell which responds non-specifically to any irritation of the nervous tissue. The rat facial nerve axotomy model allows the study of graded local glial reactions in a sterile remote lesion. The nerve was either cut or injected with toxins that undergo retrograde suicide transport. Astrocytes and microglia show reactions in situ. Non-phagocytic microglia undergo mitosis, participate in synaptic changes, and increase in and newly express a number of immuno-molecules following axotomy. In addition, local microglia can be shown to represent a major source of brain macrophages under conditions of retrograde neuronal degeneration. Microglia in an activated, but albeit non-phagocytic state show differences from macrophages in a couple of markers and in ther ion channel pattern. Development into full blown macrophages is possible and depends on local cell death, e.g., by the effect of toxic ricin. In vitro such cells are equipped with cytotoxic weapons such as respiratory burst or cathepsin activities. Various cytokines have been shown to play roles as signals in microglia activation.

THE TRANSCRIPTION FACTOR SCIP AND GLIAL DIFFERENTIATION

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We have investigated the structure and regulated expression of a new POU domain transcription factor expressed during the course of glial differentiation in the peripheral and central nervous systems. This protein - named SCIP - is largely restricted to the nervous system. It recognizes an AT-rich binding motif related to the "octamer" motif of immunoglobulin enhancers. Unlike previously described POU proteins, SCIP is not highly expressed in fully differentiated cells, but is instead maximally expressed in proliferative progenitors. In the PNS glial lineage, it is expressed by dividing Schwann cells immediately prior to their differentiation into myelin-forming cells. In the CNS O-2A lineage, expression is again maximal in dividing O-2A cells and very much reduced in differentiated oligodendrocytes and astrocytes. In dividing glial progenitors, SCIP appears to act as a repressor of myelin-specific gene expression, as assayed by transient co-transfection. This repressor activity is dependent upon the presence of both an intact DNA binding domain and an amino terminal hydrophobic domain. These observations suggest that SCIP functions in concert with additional DNA proteins, which we are currently attempting to identify.

RELEASE OF ENDOGENOUS AMINO ACIDS INDUCED BY NON-NMDA RECEPTOR AGONISTS IN CULTURED NEURONAL AND GLIAL CELLS

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Endogenous amino acid release was studied by HPLC using purified cultures of rat cerebellar granule cells or of rat cortical type-1 and type-2 astrocytes (AS). In neuronal cultures kainate (KA, 50 μM) enhanced the release of glu (3 fold) and, to a lesser extent, that of gln, gly, thr, tau and ala. Quisqualate (QA) and AMPA (50 μM) stimulated the release of glu>asp>tau, but not that of other amino acids. All the effects were CNQX sensitive. NMDA did not enhance amino acid release. Newly synthesized ^{3}H-glu and ^{3}H-asp were released similarly to endogenous glu and asp, whereas exogenous D-[^{3}H]asp was released twice as effectively. In contrast, high [K^+] released more effectively endogenous glu than exogenous D-[^{3}H]asp. It is concluded that non-NMDA receptor activation causes essentially a carrier-mediated efflux of glu from a pool that is preferentially labeled by exogenous D-[^{3}H]asp. On the other hand, D-[^{3}H]asp has a more limited access to the pool released exocytotically by depolarization. In type-1 AS cultures KA and QA were ineffective, even at high concentrations (300 μM), while both agonists (50 μM) enhanced the release of glu, asp, ser, gly, tau and ala from type-2 AS, in spite of the generally lower endogenous amino acid levels present in these cells. The evoked release was blocked by CNQX and was not due to cell swelling, since cell volume was not affected. In contrast, depolarization with high [K^+] caused cell swelling and release of tau>glu>asp in both type-1 and type-2 AS. In conclusion, non-NMDA receptor agonists stimulate the release of several endogenous amino acids (some of which are neuroactive) from type-2 AS. Extrapolating from results previously obtained on the release of preloaded [^{3}H]GABA, the release of endogenous amino acids induced by non-NMDA receptor agonists from type-2 AS is likely to be carrier-mediated.

PROSTAGLANDIN RELEASE FROM RAT CEREBRAL CORTEX AND SPINAL CORD ASTROCYTES

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It is now established that astrocytes possess receptors for a range of neuro-transmitters and neuropeptides. Recently, we have reported regional heterogeneity in the ability of substance P (SP) to elevate phosphoinositols, and release prostaglandins (PGs) from astrocytes in vitro.

In this present study we have used gas chromotography mass spectrometry (GCMS) to determine the profile of released PGs stimulated by various ligands from different CNS regions. Unlike conventional radioimmunoassay, CGMS is chemically specific, and allows the simultaneous measurement of several PGs from a single sample.

Astrocytes cultured from cerebral cortex (CX) and spinal cord (SC) exposed to luM SP, bradykinin (BK) or 10um ATP showed the following profiles of released PGs. (Mean {n=3} percent stimulation over basal ± SEM. * = $P<0.05$.).

	PGE_2		PGD_2		PGF_{2a}	
	CX	SC	CX	SC	CX	SC
SP	5±3	108±7*	2±3	88±8	6±3	64±6*
BK	121±9*	112±10*	94±7*	98±11*	84±9*	88±11*
ATP	146±14*	90±*6	98±12*	121±14*	102±16*	92±6*

These data show hitherto unrecognised complexity to the stimulated release of PGs from astrocytes. In particular, the effects of SP are strongly polarised in favour of SC astrocytes. Currently, we are working to assess the significance of astrocyte SP receptor expression in vivo.

VOLTAGE-ACTIVATED ION CHANNELS IN ACUTELY ISOLATED HIPPOCAMPAL ASTROCYTES

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The expression of voltage-activated ion channels in astrocytes from the CNS was analyzed using whole-cell voltage clamp techniques and microspectrofluorimetry. Astrocytes, which immunohistochemically stained for glial fibrillary acidic protein, were prepared by trituration of the CA1 region from hippocampal slices that had been treated with papain and hyaluronidase. Whole cell voltage clamp sutides indicated that these cells had 3 types of voltage-activated K channels: 1) a TEA-sensitive slowly inactivating current that was similar to the neuronal delayed rectifier; 2) a 4-AP sensitive transient current, similar to the neuronal A-type current; and 3) an inward rectifier activated by hyperpolarization. Analysis of fura-2 or indo-1 fluorescence indicated that depolarization of these cells increased intracellular Ca by activating verapamil-sensitive voltage activated Ca channels. Intracellular Ca was increased by levels of extracellular K greater than 20 mM. These results indicate that voltage-activated ion channels are expressed in astrocytes in the mature hippocampus. These channels may be important in regulating extracellular homeostasis. **Supported by MRC Canada.**

CALCIUM REGULATION IN GLIA

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Recent experiments from this and other laboratories indicate that glia exhibit a variety of neuroligand receptors linked to the regulation of intracellular calcium levels. We have used the calcium indicator dye fura-2 and a video-based imaging system to monitor simultaneously the response of many individual glial cells to different neuroligands. The results of these studies indicate that 1) type 1 and type 2 astroglia as well as oligodendroglia exhibit a variety of neuroligand receptors linked to calcium regulation, 2) there is marked heterogeneity among these cells with respect to their ability to respond to different neuroligands, 3) heterogeneity develops within clones of astroglia with time in culture and 4) type 1 astroglia are able to proprogate calcium signals between cells.

Among receptor types linked to calcium regulation, P_{2Y} purinergic receptors appear to be the most common receptor system found on type 1 astroglia. Other receptor agonists found to regulate calcium levels include alpha-adrenergic agonists, serotonin, glutamate, histamine and muscarinic cholinergic agonists. Each of these appear to increase calcium levels in a subpopulation of type 1 astroglia. Type 2 astroglia isolated from several different brain regions appear to exhibit a similar array of receptors linked to calcium regulation as type 1 astroglia. The relative percentage of astroglia responding to the different neuroligands varies among brain regions and with time in culture. The number of oligodendroglial receptor systems linked to calcium regulation appears to be markedly less than that expressed by astroglia. (Supported by NS 31677)

INTERACTIONS OF GLIA-DERIVED NEXIN WITH COMPONENTS OF THE EXTRACELLULAR MATRIX

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Glia-derived nexin (GDN), originally purified as a neurite-promoting factor, turned out to be a potent serine protease inhibitor interacting with thrombin, plasminogen activator, urokinase or trypsin. Sequence analysis, subsequent to cDNA cloning, confirmed human and rat GDN as members of the serpin superfamily. The primary sequence of protease nexin 1, a thrombin inhibitor detected in the medium conditioned by human fibroblasts is identical to that of human GDN. *In vivo*, GDN is induced following lesion of the rat sciatic nerve and is upregulated following the death of pyramidal neurons caused by transient ischemia in the CA1 region of the gerbil hippocampus. It is considered that GDN modulate neurite outgrowth through its regulation of the extracellular proteolysis. A role of an interaction between the SDS-resistant complex GDN-target protease and components of the extracellular matrix is however not yet excluded. For example, the complex GDN-thrombin binds to vitronectin and laminin with a higher affinity than GDN. The interaction of GDN with heparin or heparan sulfates strongly potentiates the affinity of the inhibitor for thrombin. Site-directed mutagenesis, followed by expression in COS cells and insect cells, allows a better definition of the GDN domains involved in the interactions with components of the extracellular matrix.

ASTROCYTIC INVOLVEMENT IN ACTIVITY-DEPENDENT PLASTICITY

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Activity-dependent central nervous plasticity is the very basis for the self-organization of the developing brain and memory formation in the mature animal. This kind of plasticity is governed by neuronal activity and is to a major extent manifested in changes of the circuitry. Glial cells have been shown to be able to monitor presynaptic, as well as postsynaptic neuronal activity and are, thus, capable to convey information about coincidence of synaptic activity. Coincidence of pre- and postsynaptic activity is thought to underly plastic changes in the brain. In addition, glial cells can influence synapse formation and synapse elimination, the prerequisite for long-term manifestation of plasticity. Evidence from the developing visual system of higher vertebrates will be presented which supports the notion that immature astrocytes may be a causal link in activity-dependent plasticity.

ASTROGLIAL FACTORS SUPPORTING DIFFERENTIATION AND LONG-TERM SURVIVAL OF BRAIN NEURONS

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Under the trophic influence of cerebral astrocytes serum-free neuronal cultures from different brain regions (neocortex, hippocampus, septum) of embryonic rat survive for more than 30 days; differentiate into tight neuritic networks; express ion channels, neurotransmitters and their receptors; generate network-driven spontaneous bioelectrical activity and eventually develop distinct spike patterns.

The predominant glia-derived neurite growth-promoting activity isolated from conditioned medium (CM) is associated with a substrate-adhesion complex containing laminin B-chains and a heparan sulfate proteoglycan. Long-term maintenance of neuronal viability was supported by diffusible neurotrophic activity present in glia conditioned medium. This neurotrophic activity consists of several distinct proteins with molecular weights >10kD. By comparison of the biochemical properties of established peptide growth factors with neurotrophic functions we could exclude e.g. S100ß, FGF, IGF II, NGF, BDNF, NT-3 and neuroleukin as potential candidates for the long-term neurotrophic activity in CM. For the prolonged stabilization of differentiated brain neurons cell contact-mediated interactions are required. Supportive cell-cell interactions can be provided by cerebral astrocytes but not ,e.g., by meningeal fibroblasts.

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RECEPTOR-MEDIATED GENERATION OF INTRA- AND INTER-CELLULAR MESSENGERS FROM ASTROCYTES

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Astrocytes express an array of receptors for signal molecules but little is known of their functional role. Activation of a number of these receptors leads to a rise in intracellular calcium and the generation of diacyglycerol. To ascertain the functional consequences of such changes, we have focussed on the regulation of prostanoid synthesis, and the production of a guanylyl cyclase-activating factor (GAF). Only P_{2Y}-purinergic receptor activation leads to prostanoid synthesis. The specificity of this response appears to be due to direct coupling of the receptor to phospholipase A_2. Neither mobilization of intracellular calcium, nor diacylglycerol activation of protein kinase C are sufficient stimuli to promote prostanoid production. Activation of a number of receptors evokes the release of a non-prostanoid, nitrosyl compound termed astrocyte-derived relaxing factor (ADRF). This highly labile factor derives from arginine, and its vasodilatory and GAF activity can be blocked with oxyhemoglobin and methylene blue. Release of such potent factors from astrocytes in vivo will have profound paracrine and autocrine effects.

ELECTROGENIC Na^+/HCO_3^- COTRANSPORT IN RETINAL GLIAL (MÜLLER) CELLS.

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An electrogenic Na^+/HCO_3^- cotransport system was studied in freshly isolated salamander Müller cells using the whole-cell voltage-clamp technique. Outward currents were evoked when extracellular HCO_3^- was increased by pressure-ejecting a HCO_3^--buffered solution onto the cell surface. The HCO_3^--evoked current was almost completely blocked (96% reduction) by 0.5 mM DIDS and partially blocked by 2 mM DNDS (71% reduction) and 2 mM harmaline (72% reduction). The HCO_3^- response was Na^+-dependent (99% reduction when choline replaced Na^+) and Cl^--independent. These results indicate that the HCO_3^--evoked outward current was generated by a Na^+/HCO_3^- cotransport system with a $HCO_3^-:Na^+$ stoichiometry of > 1.

Na^+/HCO_3^- cotransporter sites were localized preferentially to the Müller cell endfoot. HCO_3^- ejection onto the endfoot evoked currents 10 times larger than were currents evoked by ejection onto the opposite (distal) end of the cell.

The reversal potential of the cotransporter varied as a function of the transmembrane Na^+ gradient (with the HCO_3^- gradient held constant at 1:1). The reversal potential equaled -0.1 mV for a Na^+ gradient of 1:1 and -25.2 mV for a gradient of 7.4:1 (outside:inside). These results indicate that the cotransporter has a stoichiometry of ~2.8 HCO_3^-:1 Na^+, close to the value of 3:1 reported in other systems.

Possible functions of the Na^+/HCO_3^- cotransporter in glial cells include the regulation of CO_2 and the control of cerebral blood flow in response to changes in neuronal activity. Supported by NIH grant EY04077.

NEURON-GLIA INTERACTIONS IN AMPHIBIAN OPTIC NERVE

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There is increasing evidence that neuroglia have a functional role in the homeostasis of the neuronal micro-environment with regard to ions and small molecules. The glial membranes are closely apposed to those of the neurons and constitute an immense surface area for exchange and interaction. In Necturus, studies with intracellular voltage and pH sensitive electrodes in glial cells demonstrate an electrogenic $NaHCO_3$ cotransporter and an electroneutral Na/H exchanger (Astion et al. Neurosci. Lett. 1989 107:167) which help not only to maintain the relative alkaline pH_i of glial cells but also the constancy of pH in the brain extracellular fluid.

In the frog, recording from the glia limitans of the optic nerve with the loose patch-clamp technique indicates that glial cells exhibit voltage gated Na channels which appear to be facilitated by impulses in the underlying axons (Marrero et al. Nature 1989, 339: 378). The results suggest that this facilitation, which decreases in raised $[Ca]_o$, results not simply from a transient increase in $[K]_o$ but from a longer lasting modification of the extracellular fluid produced by the nerve impulses. After all the axons degenerate, glial Na channels remain but have altered kinetics and TTX sensitivity. (Supported by the National Institutes of Health and the National Science Foundation)

MODIFYING MYELIN PROTEIN GENE EXPRESSION

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Myelin-associated glycoprotein (MAG) and Protein zero (P_o) are two identified cell adhesion molecules in peripheral myelin. At the early stage of myelination when Schwann cells segregate and selectively envelop large axons, forming 1:1 associations with them, MAG is highly expressed whereas P_o is not. Later P_o is expressed at high levels in compacted membranes and MAG is restricted to non-compacted membrane regions. To investigate the roles of MAG and P_o in myelination, retrovirus mediated gene transfer was used to alter the expression of these two proteins in Schwann cells, and the capacity of the genetically modified Schwann cells to differentiate was tested by coculturing them with sensory neurons. Overexpression of MAG in Schwann cells promoted the envelopment of axons by infected Schwann cells. Conversely inhibiting MAG by expressing MAG antisense RNA in Schwann cells prevented the cells from forming 1:1 associations with large axons. By contrast Schwann cells expressing P_o antisense RNA reached the 1:1 stage of Schwann cell axon interaction and either failed to differentiate further or elaborated thin myelin sheaths containing a variable number of compacted and non-compacted lamellae. These observations strongly suggest that a myelinating Schwann cell requires MAG to selectively envelop an axon to be myelinated, and requires P_o for further progress of the spiral of Schwann cell membrane and its subsequent compaction.

PRODUCTION OF CYTOKINES BY MICROGLIAL CELLS

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Infections of the central nervous system (CNS) give rise to intrathecal production of several cytokines; whereas TNF-α, IL-1, IL-6, IL8, GM-, M- and G-CSF are produced in the CNS in bacterial meningitis, IFN-γ, IL-1, IL-6, IL-8, GM- and M-CSF are synthesized in encephalitis or meningitis of viral origin. Cytokines produced in the CNS by activated astrocytes and/or microglial cells may provide chemotactic signals in order to recruit blood mononuclear cells. Furthermore, cytokines present in the brain tissue or cerebrospinal fluid may regulate the state of activation and differentiation of monocytes, lymphocytes and granulocytes invading the CNS compartment. Thus, cytokines produced intrathecally contribute to the clearance of neurotropic viruses, for instance by (1) guiding the response of B cells to produce antiviral antibodies and by (2) controlling the extent of expression of MHC class I and II antigenes required for the action of cytotoxic T cells on viral infected cells or for the activation of T helper cells by antigen presenting cells. The enhancement of the expression of NGF by IL-1 and IL-6 and the initiation of gliosis by TNF-α reflects the involvement of cytokines in tissue repair. In this regard it is interesting that in vitro microglial cells produce TNF-α, IL-1, and IL-6. Activation of microglia is achieved by astrocyte-derived signals such as GM-CSF and M-CSF.

CELL LINEAGE IN THE RAT CEREBRAL CORTEX

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In this laboratory, we have been studying cell lineage in the cerebral cortex of the rat using the retroviral labelling technique. This method allows the germinal cells of the cortical ventricular zone to be labelled, such that their fate and that of their progeny can be followed.

One of the questions this technique has allowed us to address is, how many different types of precursor cells are there in the ventricular zone, and what is their developmental potential? Experimentally, this issue is addressed by asking how many different types of clone can we detect and how stable are these different precursor cells phenotypes to manipulation, for instance by placing them in tissue culture?

We have discovered that there are at least four different types of precursor cells. One generates neurones, a second generates astrocytes of the grey matter, and a third generates white matter glia. The fourth type of clone contains both neurones and the white matter glial cells. We presume that the cell giving rise to this type of clone is the precursor of those cells that give rise to either neurones or white matter glia alone.

In this talk I will present the data that demonstrate this diversity of precursor cell types, and show that their different phenotypes are relatively stable in as much as they are still in evidence in dissociated tissue culture. Finally, I will discuss the different patterns of cell lineage suggested by these data and indicate how they differ from the patterns found in other regions of the CNS.

THE CONTROL OF NEURONAL SHAPE

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Astrocyte-derived matrix molecules modulate neurite growth and neuronal polarity. In particular, we have studied the role of laminin, fibronectin, proteoglycans and glycosaminoglycans and demonstrated that the initiation and elongation of axons and dendrites can be regulated separately by these molecules (Chamak and Prochiantz, 1989; Rousselet *et al.*, 1990; Lafont *et al.*, submitted). Neuronal morphogenesis has also been studied from the point of view of decision making and executing by the nerve cells. The possible influence of small G proteins of the rab family in the elaboration of axons and dendrites was examined and we demonstrated that "overexpression" of rab2 leads to an enhanced dendritic phenotype (Ayala *et al.*, 1990). Finally, we have some evidence that homeotic genes are involved in the definition of neuronal shape and that polysialilated NCAM might play a role in the control of neural differentiation by homeogenes (Joliot *et al.*, 1991; Joliot *et al.*, submitted).

Chamak and Prochiantz. (1989) Development, 106, 483-491.
Ayala *et al.* (1990) Neuron, 4, 797-805.
Rousselet *et al.* (1990) Dev. Biol., 137, 33-45.
Joliot *et al.* (1991) Proc. Natl. Acad. Sci. USA, 88, 1864-1868.

Glial-mediated changes in pH_o may influence neuronal excitability. Bruce R. Ransom. Yale Medical School, Dept. of Neurology, New Haven, Connecticut.

Extracellular ion concentrations in the brain can influence neuronal behavior as shown in the case of extracellular $[K^+]$ ($[K^+]_o$). Experiments will be discussed that suggest the hypotheses that neuronal excitability is influenced by extracellular pH (pH_o) and that activity-dependent extracellular acidification, mediated in part by glial cells, can act as a negative feedback mechanism on neuronal exctiability. Excitability may be influenced by direct membrane actions of pH_o and by pH_o modulation of the action of excitatory neurotransmitters at the N-methyl D-Aspartate (NMDA) receptor; specifically, acid shifts decrease excitability and block NMDA currents. In cortical slices large and long-lasting changes in pH_o occur with intense neural activity. Activity-dependent pH_o shifts in the acid direction appear to be mediated, at least in part, by glial cells; in the enucleated rat optic nerve, containing only glial cells, increases in $[K^+]_o$, as might occur with neural activity, cause an acid shift. Using microfluorometry and the pH-sensitive dye BCECF, isolated astrocytes depolarized by increasing $[K^+]_o$, exhibit an intracellular alkalinization that could cause extracellular acidification. These studies indicate that neural activity-dependent pH_o fluctuations, mediated in part by glial cells, might serve as an important mechanism of glial-neuronal interaction.

MEMBRANE CURRENTS RECORDED IN MÜLLER (GLIAL) CELLS OF THE ISOLATED INTACT MOUSE RETINA

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We studied membrane currents in mouse Müller cells of the intact retina *in situ* using the patch-clamp technique in the whole cell configuration. Cells were identified based on immunocytochemical and ultrastructural features after the physiologically characterized cells were dye-labelled. This combined approach revealed that the area under voltage-clamp control was most likely restricted to the endfoot region. This membrane area as determined from capacitance measurements ranged between 30 to 100 nm^2. The main current was a large almost voltage independent K^+-current (5-25 nS). In addition, we observed Ca^{++} currents and found that glutamate induced an inward current.

GLIAL-ENDOTHELIAL CELL INTERACTION IN BLOOD-BRAIN BARRIER DIFFERENTIATION

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Endothelial cells invade the neuroectoderm and differentiate to blood brain-barrier endothelium during embryonic and postnatal development. The interaction with astrocytes seems to be crucial for the induction and maintenance of blood-brain barrier endothelial cell characteristics (complex tight junctions, low number of vesicles, specialized transport systems). To study the underlying molecular mechanisms we have characterized and cloned a novel transmembrane glycoprotein which is specifically expressed in blood-brain barrier endothelium and is a new member of the immunoglobulin superfamily. This protein may be involved in cell adhesion or transport at the blood-brain barrier. By using pure cultures of brain endothelial cells we found that cells in primary culture rapidly loose some cell surface proteins (e.g. glucose transporter), and show a decrease in tight junction complexity and permeability compared to astrocyte-endothelial cocultures. Since these effects in cocultures were mediated through a filter that prevented direct cell-cell contact we conclude that soluble factors derived from astrocytes are responsible for the induction and maintenance of blood-brain barrier characteristics in cultured endothelial cells.

FREEZE-FRACTURE ANALYSIS OF ASTROCYTIC MEMBRANES IN THE MYELIN-DEFICIENT RAT DURING POSTNATAL DEVELOPMENT.

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Orthogonal arrays of particles (OAP) are typical elements of astrocytes. Reactive astrocytes were shown to increase the OAP density in parenchymal membranes. We investigated the distribution of OAP in the CNS of the myelin-deficient (md) rat in which reactive astrocytes occur. Endfoot and parenchymal membranes were compared during postnatal development of the optic nerve of md rats and normal littermates (myel). The md-endfoot OAP density remained constant during observation time. The myel-endfoot OAP density increased up to adult values. The md-parenchymal OAP density increased during observation time, whereas the myel-parenchymal OAP density remained constant during the first 40 days and thereafter increased rapidly. It is suggested that the disturbed myelinogenesis in the md mutant may be a signal for parenchymal membranes of reactive astrocytes to insert or assemble OAP subunits to complete arrays. - With support by the DFG.

CHARACTERIZATION OF CULTIVATED RETINAL GLIAL CELLS AND EFFECT OF GROWTH FACTORS

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The distinct topographic distribution of glial cell types, i.e. Müller cells, astrocytes, oligodendrocytes, and microglial cells, in the rabbit retina was used to prepare cultures containing Müller cells and microglial cells, but no astrocytes (P-cultures), and cultures containing in addition astrocytes and O4+ O2A-precursors (MR-cultures). The effect of various growth factors and fetal calf serum (FCS) on cell proliferation in these cultures was determined by ^{3}H-thymidine incorporation and by double labeling for cell type specific markers and incorporated bromodeoxyuridine.
We will show that Müller cell proliferation was stimulated by FCS, and EGF, but not by aFGF, bFGF, and PDGF. In MR-cultures an increase in ^{3}H-thymidine incorporation was found after addition of FCS, EGF, aFGF, and bFGF. Since FGF showed no stimulatory effect in P-cultures and O4+ cells are few in number, we suggest, that aFGF and bFGF are probably mitogens for retinal astrocytes. Microglial cell proliferation was stimulated only by FCS, but not by EGF, aFGF, bFGF, or PDGF. Proliferation of retinal O2A-precursor cells was stimulated by PDGF, aFGF, and bFGF, but not by EGF or FCS.
This culture system of peripheral and central rabbit retina seems suitable to identify factors which stimulate the proliferation of astrocytes but not Müller cells and may thus be helpful in understanding the distinct distribution of these glial cells *in vivo*.

RESPONSES OF RETINAL GLIAL CELLS FOLLOWING TRANSECTION OF THE OPTIC NERVE OF RABBITS.

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Following transection of the optic nerve of adult rabbits microglial cells residing in the retina begin to proliferate in the nerve fiber layer and in the inner plexiform layer, and phagocytose the degenerating ganglion cell axons and dendrites, respectively. By five months posttransection the number of microglial cells has returned to control level and ganglion cell degeneration has ceased. However, a substantial fraction of the ganglion cells (in the range of 10%) survive the complete nerve cut even for two years. The axons of these surviving ganglion cells are myelinated in the medullary ray (MR) region, as they are in the normal retina. Some oligodendrocyte cell bodies in the 'lesioned' retina were covered with myelin. Thus, after degeneration of about 90% of the axons, oligodendrocytes myelinate 'aberrant' cellular elements. The proportion of myelinated vs. unmyelinated axons in the MR was higher in the 'lesioned' that in the control retina. Whether the enhanced proportion of myelinated axons is caused by 'aberrant' myelination, or whether myelinated axons survive to a higher extent, is still open. It remains to be shown why some ganglion cells are capable of surviving and whether they could regenerate their axon in a 'suitable' environment.

PERISYNAPTC LECTIN-BINDING SITES IN CAT VISUAL CORTEX:

Development and Glial Localization

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Lectins with affinity for terminal n-acetylgalctosamine, such as Vicia villosa (VVA) or Glycine max (SBA), label somata and proximal dendrites of a subpopulation of cortical GABAergic neurons in a netlike fashion. In the cat visual cortex this labeling first appears at four weeks postnatally and reveals full expression not before several months of age. The lectin-binding epitope is shown to be located at distal astrocytic processes which surround asymmetric and symmetric synaptic boutons terminating on nonpyramidal cells. As the expression of the epitope parallels the maturation of the cortex, e.g glial development and the period of cortical malleability, we suggest that the shown molecular specialization in distal astrocytic processes may be involved in stabilizing mature synapses.

THE DIFFERENTIATION OF OLIGODENDROCYTES: FROM CELL DIVISION TO MYELINATION.

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We used ^{3}H-thymidine autoradiography to mark individual glial cells at a particular stage in their differentiation. Thymidine autoradiography, when combined with immunocytochemistry, permits one to determine the relative time of expression of the different oligodendroglial/myelin markers in relation to cell division. By sacrificing animals at different time points after an injection of thymidine, the approximate length of time from cell division until the expression of a given marker can be determined. The technique is also used to determine whether any of the oligodendroglial/myelin markers are expressed by dividing glial cells. Our work shows that both *in vivo* and *in vitro* cells which are synthesizing DNA already express certain myelin glycolipids. In contrast, myelin proteins do not appear to be made until cell division has been completed. While CNPase is detectable within 24hrs after an injection of thymidine, PLP does not appear until 72hrs. Antibodies which crossreact with sulfatide and galactocerebroside are detectable in cells which are synthesizing DNA. Proliferating glial cells which are also immunostained are not rare findings; in certains areas of the brain, the percentage of thymdine labeled cells which are immunostained for sulfatide approaches 100%. This finding coupled with the finding that many other dividing cells are immunostained for GFAP indicates that many dividing glial cells express astrocytic or oligodendrocytic specific markers. These dividing cells are appropriately called astroblasts and oligodendroblasts. They are not totally undifferentiated but are already in the process of differentiation in the astrocytic or oligodendrocytic lineage.

FUNCTIONAL PROPERTIES OF RETINAL MÜLLER CELLS FOLLOWING TRANSPLANTATION

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Two types of glial cells occur in the retina, Müller cells and astrocytes, and both cell types extend processes onto retinal blood vessels. Retinal vessels express features that constitute an impermeable barrier between the blood and retina similar to the blood-brain barrier (BBB) of the CNS. Astrocytes have been implicated in BBB induction, but the contribution of Müller cells to the blood-retinal barrier is not known. We have purified Müller cells from the guinea pig retina, which lacks astrocytes. Cells were prelabeled with diI and then injected into the anterior chamber of the eye. Two wks - 3 mos after injection, Müller cells were observed primarily within the ciliary body where they occurred as dense aggregates bordering blood vessels. Vessels of the ciliary body are freely permeable to circulating HRP and they remained permeable even when surrounded by Müller cells. These results indicate that cultured Müller cells express preferential orientation to vascular elements when transplanted to peripheral sites, a behavior that resembles the *in vivo* extension of processes onto retinal vessels. The interaction of Müller cells and endothelial cells appears to serve functions other than BBB formation, thus highlighting the functional distinctness of different glial cell types of the retina.

INTERACTION OF VIRUSES WITH RAT GLIA IN VITRO

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We are studying the interaction of glial cells with viruses, especially those known to cause CNS diseases in man. Cultures of rat optic nerve were used as a source of well-studied glia and cell types were identified with established immunological markers. Cells were infected with herpes simplex virus (HSV), measles virus and bunyamvera virus because of their known association with CNS encephalitits. Three fluorochrome-fluorescence was then used to identify the cell types in which virus specific antigens were present.
HSV was found to infect in preferential order fibroblasts, oligodendrocytes, O-2A progenitor cells, type 2 and 1 astrocytes. HSV infection also seemed to slow down the differentiation of progenitor cells into oligodendrocytes. Bunyamvera virus infected all cell types found in the optic nerve whereas measles virus infected mainly oligodendrocytes, type 1 astrocytes and the occasional 0-2A progenitor. Interestingly, measles virus did not spread from infected oligodendrocytes into neighbouring cells and therefore did not cause plaques of fused cells characteristic for measles in permissive cell lines. This may indicate an abortive or persistent infection similar to that found in cultured astrocytes (Schneider-Schaulies et al. Virology, 177, 802-806, 1990).

NMDA INDUCED INWARD CURRENTS IN IDENTIFIED GLIAL CELLS OF THE MOUSE HIPPOCAMPAL SLICE

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Glial cells, acutely isolated or in tissue culture, express kainate/quisqualate type of glutamate receptors while NMDA have so far not been detected. In this study, we have applied the patch-clamp technique to brain slices to investigate the glutamate sensitivity of glial cells in the 9-12 day old mouse hippocampus. To unequivocally identify glial cells we combined electrophysiological with ultrastructural characterizations. Therefore, cells were injected with the fluorescent dye Lucifer Yellow, the stain was converted to an electro-dense material and subsequently slices were inspected in the electron microscope. The glial cells obtained from the stratum radiatum were identified by the characteristic chromatin distribution, glial fibrils, and the lack of synaptic membrane specializations. In 78% of the cells, glutamate induced inward currents ranging from 50 to 800 pA (N=28). In contrast to previous studies, NMDA also activated inward currents (N=6) in 40% of glutamate responsive glial cells. This preliminary result contradicts the view that the expression of NMDA receptors is an exclusive property of neurons.

CORTICAL NEURON-ASTROCYTE, -SUBSTRATE AND -MEDIUM INTERACTIONS: TRANSMITTER-PHENOTYPE-DEPENDENT MORPHOLOGICAL EFFECTS.

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The influence of homotypic astrocytic monolayers (CAL) on the adhesion, survival and morphological differentiation of rat cortical neurons were studied in vitro and compared to those observed with heat-killed astrocytes or laminin. We also investigated the effects of chemically defined culture medium (N2) and an additional supply with diffusible astrocyte-derived factors (conditioned medium, CN) or foetal calf serum on the different substrate-neuron interactions.

Both, the strength of adhesion and the survival were significantly enhanced on viable astrocytes compared to any other substrate. While the former was independent of culture media, the survival rate on CAL was improved in the presence of CN. Neurons seeded on the other substrates only survived when cultured in CN. Replacement of astrocytic monolayers or the culture medium CN was mostly accompanied by (1) spreading of somata, (2) a drastic decrease in neuron area and (3) an increase in number of primary neurites. However, the latter change correlated with transmitter-phenotype and was never observed for GABAergic neurons.

THE INFLUENCE OF THE GLIA-DERIVED J1 GLYCOPROTEINS ON GROWTH CONE BEHAVIOUR IN VITRO.

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The glia-derived J1 extracellular matrix glycoproteins exist as four major molecular species with apparent molecular weights of 160, 180, 200 and 220 KD (Kruse et al., 1985, Nature, 316,146). In vitro studies have shown both janusin (160 and 180 KD; Pesheva et al. , 1989, J. Cell Biol., 109, 1765) and tenascin (200 and 220 KD; Faissner and Kruse, 1990, Neuron, 5, 627) to be repulsive substrates for central nervous system neurons.

In order to study the effects of these molecules on growth cone advance and guidance we have observed the behaviour of neuronal growth cones when confronted with J1 glycoproteins, using video time lapse microscopy. Both tenascin and janusin repulse growth cones advancing on laminin when offered as a sharp border. Growth cones growing on mixed J1 and laminin substrates, however, advance at a faster rate than on laminin alone. We are currently investigating the signal transduction mechanisms underlying these repulsive and growth promoting properties of janusin and tenascin.

Title: NEUROTOXICITY OF BRAIN MACROPHAGES

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Brain macrophages (BM) are transiently present in different regions of the central nervous system when neuronal death or the elimination of axon collaterals occur. To investigate the influence of BM on neuronal growth, we have performed cocultures of BM and neurons derived from the cerebral cortex of 17 day-old rat embryos, in a serum free medium. The presence of BM led to a marked reduction of the number of neurons stained with an anti-MAP2 antibody, when compared with control neuronal primary cultures. This toxicity of BM was further evidenced by time-lapse video recording of the cocultures showing neurite retractions or neuronal degenerescences and phagocytosis. At the molecular level, we found that these neuronal involutions were due to a production of reactive oxygen intermediates (ROI) by BM. In fact, the addition of catalase (a scavenger of H_2O_2) into the culture medium efficiently reduced the extent of neuronal death in the presence of BM. Furthermore, cytochemical detection of ROI production revealed that the BM respiratory burst was stimulated by the contact with neurons. Studies are in progress to characterize the molecular signals involved in this stimulation. Altogether, these results suggest that BM might promote neuroregressive events during ontogenesis.

MHC GENE EXPRESSION IN THE CENTRAL NERVOUS SYSTEM.

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In striking contrast to most other tissues, parenchymal cells of the central nervous system (CNS) do not express determinants of the major histocompatibility complex (MHC). Several observations suggest that this deficit is due to downregulation, rather than to deletion of genes: MHC expression can be induced a) by inflammatory responses; b) by lesions of local neurons; c) in tissue culture.

To examine mechanisms possibly involved in modulation of CNS MHC, we developed a coculture system allowing controlled contacts between cortical neurons from newborn rats and syngeneic astroglia cells. Preliminary results suggest that viable, but not fixed neurons strongly downregulate constitutive MHC class I expression, and class I and class II inducibility. This regulation requires viable neurons; fixed neurons or membrane preparations are inert. It furthermore depends on cell-to-cell contacts, or acts via short range mediators, as supernates from neuron cultures do not affect MHC expression.

THE ESTABLISHMENT AND CHARACTERISATION OF IMMORTALISED LINES OF GLIAL PRECURSOR CELLS.

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To facilitate studies of glial cell development the availability of homogeneous populations of cells of defined differentiation stages would be advantageous. Immortalised lines of oligodendroglial precursor cells from murine brain have been established by infection of dividing cells in primary cultures witrh oncogene-carrying retroviruses. Retroviral vectors carrying a temperature sensitive mutant of the *SV 40 T* oncogene, the rat transforming *neu* oncogene and the *v-myc* oncogene have been used. In all cases 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. They express differentiation-stage specific adhesion molecules and ion channels which can in some lines be modulated by culture in the presence of dibutyrl cyclic AMP towards an expression pattern characteristic of a more mature differentiation stage. In vivo, early passages of lines established with the *SV 40 T* oncogene are able to remyelinate demyelinated axons. Late passages of these lines and lines established with other oncogenes specifically ensheath but do not remyelinate these axons implying the retention of adhesion molecules specific for this interaction. Such clonal cell lines will provide useful tools for the study of oligodendroglial precursor cell-neuron interactions.

CELL LINEAGE IN THE NEOCORTEX STUDIED WITH A NEW RETROVIRAL LIBRARY/PCR RESCUE TECHNIQUE.

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Whereas cell lineage compartments correspond to patterned homeobox gene expression in the developing vertebrate hindbrain, developmental compartments in the forebrain have been elusive. We have addressed this question using a new technique which allows the definition of clonal relationships regardless of the migration patterns of sibling cells.

A library of 95 retroviral vectors was constructed, all carrying the histochemical marker gene, lacZ. Each member of the library contained a unique, 30-600 bp fragment of genomic DNA, as a genetic "tag". This viral mixture was injected into the forebrain of embryonic rats, to label cortical progenitor cells and their progeny. The brains of adult animals were then sectioned, reacted for ß-galactosidase histochemistry, and the labeled cells plotted on drawings. Labeled cells were then removed from the tissue sections and processed using the polymerase chain reaction (PCR) to analyze the genetic tags. Cells of a clone share the same tag, while cells in different clones essentially always contain distinct tags.

Clonally related cortical neurons varied widely in their degree of separation from one another. About 60% of multicellular clones formed clusters of 2-5 cells from 50-1200 μm across. These clusters, similar to the pattern previously described (Walsh and Cepko, 1988, Science 241:1342), cross functional subdivisions of the cortex such as "barrels" of the somatosensory cortex. Surprisingly, the other 40% of multicellular clones spread for 1 millimeter or more, with several clones crossing major cytoarchitectonic subdivisions of the neocortex. The most widespread clones covered more than half of the antero-posterior or medial-lateral dimensions of the neocortex. Thus, the neocortex shows no simple compartmental or segmental development. Furthermore, the control of some aspects of cell number and phenotype may be independent of later specification of the neocortex into cytoarchitectonic zones. Supported by the Dana Foundation, the Howard Hughes Medical Institute, and the NIH.

NEURITE OUTGROWTH INHIBITORS IN GLIOTIC CNS TISSUE AND GLIAL CELL LINES.

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Damage to mammalian CNS leads to the formation of a "glial scar", predominantly made of reactive astrocytes and microglial cells. We have recently shown that glial scar tissue contains molecules that inhibit central neurite outgrowth, but did not characterize the cells of origin. In an attempt to establish the cellular source of the inhibitory components, we have compared central neurite outgrowth over damaged tissue membranes, over primary glial cultures (astrocytes and microglia from postnatal rat cortex), over glial cell lines (C6, RN22 and U937) and over the membranes prepared from these glial cells and glial cell lines.

Astrocytes and RN22 cells and their membranes were very neuritogenic, whereas microglial cells, the lines C6, U937 and their respective membranes, failed to induce any neurite outgrowth. As in the case of membranes from gliotic tissue, the growth inhibitory molecules were solubilized by low concentrations of mild detergent. The inhibitory components present in the various cell types were different proteglycans, as shown by fractionation of the solubilized activities on Dowex 1 column. Work is in progress to determine the precise nature of the inhibitory activities and their relation to the growth regulators responsible for central neurite inhibition by gliotic tissue.

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ION CHANNEL ORGANIZATION IN MYELINATED AXONS AND PERINODAL ASTROCYTES.

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Myelinated axons exhibit a non-uniform distribution of sodium channels, which are clustered in high density in the axon membrane at the node of Ranvier, but are present in only low densities in the internodal axon membrane under the myelin sheath. Recently, it has become clear that perinodal astrocytes constitute important components of the node of Ranvier in the CNS. Moreover, immunocytochemical studies demonstrate that, *in vitro* and *in situ*, astrocytes express sodium channels. Electrophysiological studies of optic nerve astrocytes *in vitro* indicate that type 2 astrocytes express sodium channels with physiological properties similar to those of neurons, while type 1 astrocytes exhibit sodium channels with different properties (slower time constant, more hyperpolarized study-state inactivation curve). In at least some types of optic nerve astrocytes, sodium channel expression varies with culture conditions. Preliminary results suggest that sodium channel expression in some astrocytes may be modulated by neuronal factors.

SUBSTANCE P RECEPTORS ON CELLS OF THE O-2A LINEAGE

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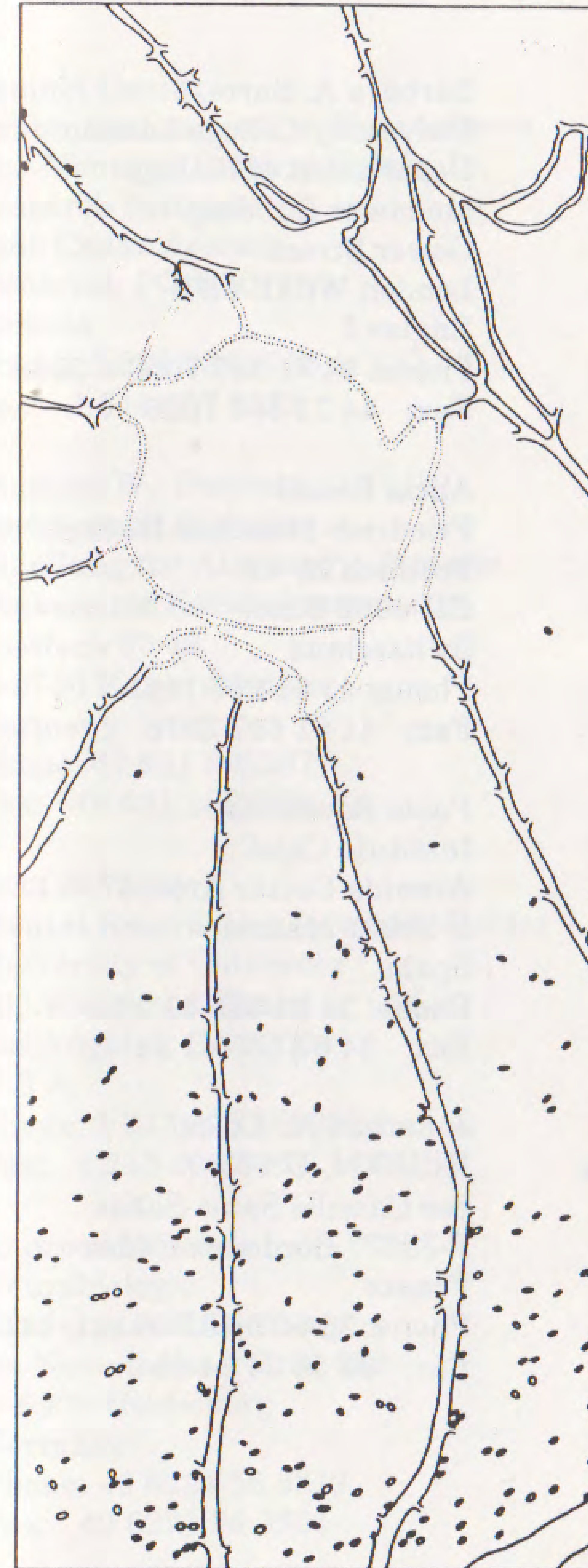
Comparative studies of rat astrocyte cultures from cortex, cerebellum and spinal cord revealed that only the latter possessed receptors for substance P (SP) (Cholewinski et al. Neurochem. Res. 13(4) 1988). More recent studies have shown that astrocytes derived from optic nerve and corpus collosum also possess SP receptors, and thus these receptors may be unique to white matter cells. In confirmation of this proposal both purified O-2A precursors and type 2 astrocytes respond to SP with increased phosphatidyl inositol turnover. The consequence of SP stimulation is the release of prostaglandins (Marriott et al. J. Neurochem 56(1) 1991). These molecules have a proposed role in the inflammatory process, which would be consistent with the recent studies of Mantyh et al. (Proc. Natl. Acad.Sci. 86, 1989) showing the upregulation of SP receptors on reactive astrocytes in damaged optic nerve. Together these data are therefore consistent with an upregulation of SP receptors on cells of the O-2A lineage following white matter damage.



CAMERA LUCIDA DRAWING OF THE FOVEAL REGION OF A WHOLEMOUNTED MONKEY RETINA

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