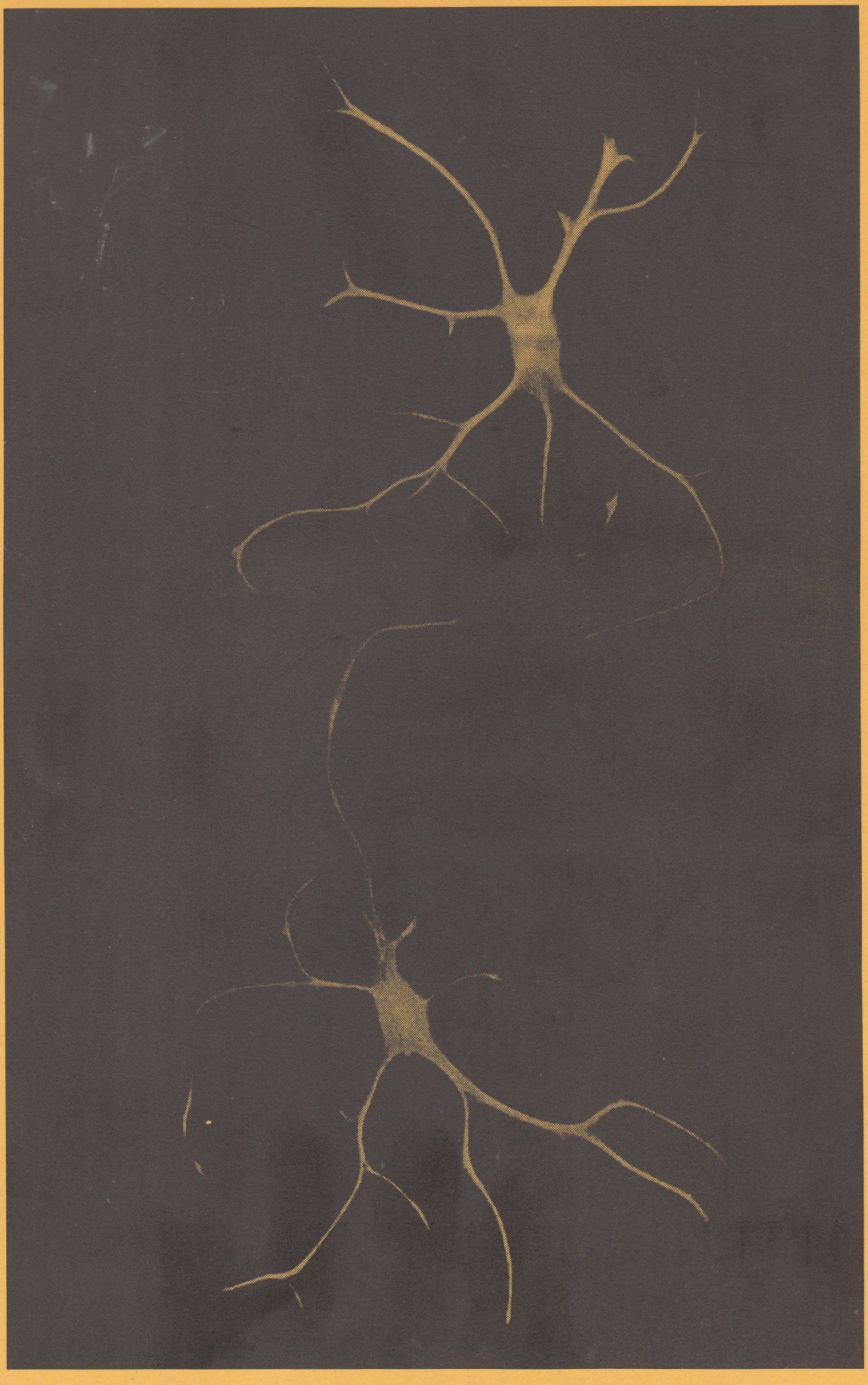
GLIAL RESEARCH IN GERMANY 1993



The figure shows cells of the clone SVK1 stained by indirect immunofluorescence with antibodies recognising glial fibrillary acid protein. This clone was derived from cerebellar glial cells from 6-day old mice, immortalised with a Murine Moloney Leukemia Virus-derived retroviral vector carrying a temperature-sensitive mutant of the SV 40 T oncogene. (Picture supplied by courtesy of Dr. M. Jung).

Preface

This brochure summarizes glial research activities in Germany. The "*Deutsche Forschungsgemeinschaft*" (German Research Council) has formalized these activities as a group grant, the "*Schwerpunkt*" on "*Functions of Glial Cells*". At present, 42 groups have joined on this common research program. The "*Schwerpunkt*" was established in 1991 and has the perspective to continue for at least four more years.

The introduction to this brochure is based on the proposal which was submitted to the "*Deutsche Forschungsgemeinschaft*" for establishing the present "*Schwerpunkt*". The planning committee consisted of Bernd Hamprecht, Helmut Kettenmann, Christian Müller and Jutta Schnitzer.

Most of the contributors to the brochure are members of this "*Schwerpunkt*". We, however, learned during the assembly of this brochure that even more groups are focussing on glial research which are not yet included. It will be the opportunity for a second addition to also incorporate these groups. We hope that this brochure will facilitate cooperations among groups within Germany, but also may be a nucleus for common projects between partners within Europe.

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Helmut Kettenmann
Department of Neurobiology
University of Heidelberg
Im Neuenheimer Feld 345
D-6900 Heidelberg
Phone: (49) 6221/56 3996
Fax: (49) 6221/56 3801

Max-Delbrück-Center
for Molecular Medicine
Robert-Rössle-Str. 10
D-1115 Berlin Buch
Phone: (49) 30 9406 3325
Fax: (49) 30 9406 3801

Introduction

World wide research programs in the last decade have shown that glial cells play an important role in the function of the nervous system. The functional capabilities of these cells go far beyond the original concept which regarded glial cells as supportive elements of the nervous system. New research has redefined the role of these cells and determined the following functions:

1. Glial cells play a pivotal role in the development of the central nervous system. They form guiding structures for migrating neurons and interact with these cells and each other via cell adhesion molecules. Moreover, they synthesize a number of important (neuro)trophic factors that appear to be critical for normal development.

2. Astrocytes and microglial cells - subpopulations of glial cells - play important roles in immune function within the central nervous system (CNS). For example, it has been recently demonstrated that astrocytes can act as antigen-presenting cells in vitro. Microglial cells, in particular during brain injury or in response to immune-mediated disease, phagocytose and remove cellular debris.

3. Glial cells, in contrast to the classical concept, are not electrically passive, but express a large repertoire of neurotransmitter receptors, transport molecules and ion channels. They are therefore equipped to influence the electrical acitvity of the CNS in a great variety of ways.

4. Glial cells play important roles in studies of the clinical neurosciences. The disease multiple sclerosis provides a clear example of this. The paralysis is due to degeneration of myelin in the CNS and myelin is, of course, produced by oligodendrocytes. The initiating steps for the destruction of myelin are at present unknown as are the factors which regulate the formation of new myelin. The majority of primary brain tumors, including the most lethal varieties, are formed by an uncontrolled growth of glial cells. Thus, an understanding of the factors which control the development of glial cells will be necessary to understand CNS specific diseases and may help to develop strategies for treatment.

It is thus important to carefully consider glial cells for understanding complex brain functions. New methological approaches with cell biological, immunocytochemical, molecular biological, and electrophysiological methods open the possibility to study identified glial cells under defined conditions. Due to these advancements a large number of laboratories have been attracted to glial research. The present

"Schwerpunkt" will coordinate German scientists working in this field and it is meant to stimulate further research on this topic. The specific aims of the Schwerpunkt are as follows:

1. Which factors control the development and proliferation of glial cells? How do glial cells influence other brain cells?
2. Are glial cells functionally involved in plasticity of the central nervous system and what role do they play during regeneration?
3. How are glial cells involved in the immune responses of the central nervous system?
4. Are there glial cell specific biochemical pathways in the central nervous system?
5. What is the role of glial cells in the regulation of the extracellular ion concentration of the brain and the volume of the extracellular space?.
6. Are glial cells involved in signal transduction in the central nervous system?

State of glial research

The brain is composed of three main classes of cells: neurons, endothelial cells and glial cells. Glial cells outnumber neurons ten to one in man and represent about half of the volume of the brain. In spite of this, CNS research has mainly focussed on neurons. Thus, glial cells have been largely ignored in most investigations of the brain functions. Entire areas of neurobiological research, such as the study of neuronal networks, disregarded glial cells altogether.

With the discovery of glial cells, Rudolf Virchow believed that they formed a kind of brain connective tissue and represented the passive matrix in which neurons were embedded. At present, we know that glial cells are metabolically highly active and fulfill a number of functions for the developing and adult central nervous system. We now know that glial cells participate in all of the brain's major functions although the details of this participation are, in most instances, unclear.

Three main classes of glial cells have been described in the CNS: astrocytes, oligodendrocytes and microglial cells. Astrocytes are thought to play an important role in the homeostasis of the extracellular environment with regard to ions, transmitters and metabolites. Oligodendrocytes in the CNS, and Schwann cells in the peripheral nervous system, insulate the axons of nerve cells by formation of myelin. Mircoglial cells have a functional importance for the immune system of the CNS. The subsequent summary of the present state of research on functions of glial cells will deal with each of these three cellular groups. It has, however, become evident that there are many interactions between these glial cell subtypes. This is particularly important for the

differentiation of glial cells, for the formation of myelin and in regard to immunological processes. Moreover, there are multiple important interactions between neurons and glial cells, and the number of these is most likely far beyond our present knowledge.

The present understanding of glial cell function makes it evident that the action and reaction spectrum of glial cells goes far beyond the original concept of a passive matrix in the central nervous system. Glial cells are involved in the development of the CNS and the normal functioning of the brain, including its interaction with the immune system. Furthermore, it is becoming evident, that these cells fulfill their tasks by interaction with each other and with other brain cells. Glial cells are therefore an important part of the brain and must be taken into consideration to explain brain function. In addition, disturbances of glial function may importantly be related to neurological diseases. Thus, the clarification of these mechanisms is an important step in understanding the brain.

Aims of the "*Schwerpunkt*" program

The aim of the "*Schwerpunkt*" program is to discover the importance of glial cells for the function of the CNS. In detail, we would like to answer the following specific questions:

- How are glial cells involved during the development of the nervous system?

These projects address the questions of how glial cells develop from precursor cells and how their properties change during these developmental stages. Glial cells can be identified at different developmental stages using cell type-specific and developmental- or stage-specific antibodies. In this context, the question will be studied whether glial cells are different in different areas of the brain. Moreover, the identification of new markers to identify stages in glial cell development should be possible. In this context, functionally important molecules may play a key role, as for instance, proteins which are involved in the formation of myelin. By incorporation of oncogenes it is possible to freeze glial cells in defined developmental stages. With proper transfection, differentiation can be controlled by external stimuli such as temperature shift. Of particular interest are the early developmental forms of glial cells which are not well characterized. These precursors may play key roles in regeneration, since they are still proliferative. A better knowledge may help in understanding regeneration.

- Which factors control the development and proliferation of glial cells?

We would like to determine which factors control proliferation and differentiation of glial cells. These factors could either be substances such as well-known growth factors or components of extracellular matrix and cell membranes. These factors need to be characterized with respect to their function and molecular structure. This directly leads to the question of which cells produce factors and what regulates their production. This implies that a number of regulating circuits may interact where a number of different cell types are involved. This basic research is also of clinical importance since most brain tumors of the CNS where the control of proliferation and differentiation is disturbed are of glial origin.

- Do glial cells influence the behavior of other cells?

As glial cells are influenced with respect to their own proliferation and differentiation by their environment, they in turn can also influence other cells. Most often the partner for such an interaction is a neuron and many previous studies have concentrated on neuron-glia-interactions. It is known that glial cells release soluble factors which control differentiation and survival of neurons. Moreover, glial cells participate in the production of the extracellular matrix which can influence the growth of neurons. A further focus of the interest are contact related interactions, such as those mediated by cell adhesion molecules which are not only important for cell recognition but can also cause changes in the physiological properties of the interacting cells. In addition to their interactions with neurons, astrocytes interact with endothelial cells. It is speculated that this interaction induces the tight junctions between the endothelial cells forming the blood-brain barrier. This structure controls the exchange of substances between blood and brain and guarantees the CNS cells in an controlled environment.

- Are glial cells involved in the regeneration of neuronal connections?

In a number of projects the role of glial cells during the regeneration of the nervous system will be analysed. The reduced ability of the CNS to regenerate after injury is important in the understanding of many CNS deseases such as spinal cord injury. Glial cells play a pivotal role in the regeneration of axonal tracts. After injury axonal tracts in the central nervous system fail to regenerate, in contrast to the peripheral nervous systems where axon tracts show much better regeneration after injury. While this is true for mammals, fishes and amphibia show enhanced CNS regeneration even in the adult. In the meantime, we have learned that the surface of oligodendrocytes in the mammalian CNS expresses non-permissive substances which may contribute to the lack of regeneration of neuronal processes. In addition, differentiated CNS glial cells of mammals may lack molecules which are expressed in the periphery by Schwann cells and encourage regeneration. Groups will study how oligodendrocytes differ

between mammals and fishes and why fish oligodendrocytes are growth permissive for neuronal regeneration. In addition, the permissive properties of the myelinating glial cells of the peripheral nervous system, the Schwann cells, will also be studied. A further aspect deals with the influence of the vasculature after damage. Questions on the nteraction of glial cells with endothelial cells and their role in controlling regenerative processes will be addressed.

- *Which factors control the formation of myelin?*

The myelination of vertebrate axons permits saltatory conduction and thus rapid signal transfer. Myelin is produced in the CNS by oligodendrocytes, and in the periphery by Schwann cells. Some of the groups address the question of which factors control the formation of myelin during development. These factors can be either cell-cell contacts or mediated by soluble substances. This research will be important in contributing to the understanding of multiple sclerosis.

- *Are glial cells involved in plasticity?*

Neuronal plasticity is linked to the formation of connections during development and to learning and memory in the adult brain. CNS plasticity is mainly explained on the basis of synaptic contacts. It is, however, known that glial cells closely interact with synapses. A number of new studies imply that glial cells and in particular astrocytes may be involved in CNS plasticity. The mechanisms involved are so far entirely speculative. Some of the groups will study such mechanisms in the hope of revealing the molecular interactions between neurons and glial cells that control plasticity.

- *What is the role of glial cells in the immune response of the CNS?*

In recent years we have learned that glial cells are involved in the immune functions of the central nervous system. It has become evident in the last few years that cells of the immune system such as activated lymphocytes can pass into the brain tissue and interact with glial cells in a complex manner. Two aspects of these interactions have become important:

1. One interaction is mediated by soluble substances. Lymphocytes can activate glial cells via interleukins. In return, glial cells release substances which can modulate the response of immune cells.
2. Presentation of antigen by glial cells.

After induction of MHC class II antigens (for instance by interferon gamma) astrocytes and microglial cells can present protein antigens on the surface in conjunction with the major histocompatibility complex; this can be recognized by T-cells. This research will be a basis for the understanding of CNS diseases with an autoimmune component.

- Are there glial cell-specific pathways in the CNS?

The recently postulated functions of glial cells are the support of neurons and the removal of components from the extracellular space such as neurotransmitters. To fulfill this task, glial cells must express a defined repertoire of enzymes and transport molecules. Some of the projects address the question whether glial cells express specific enzyme and transport molecules which are optimised for these tasks. Examples are

1. uptake carries for glutamate, formation of taurine as a potential osmoregulator, and storage of creatine as energy supply.
2. detailed investigation of carboanhydrase (for pH and volume regulation), particularly in oligodendrocytes
3. enzymes of metabolic pathways such as for the genesis of glucose. In return, one can also ask the question as to which enzymes are specifically expressed by glial cells. This, in return, may give answers to new functions of glial cells.

- How do glial cells control extracellular ion and volume homoestasis - importance for epilepsy and brain damage?

Kuffler and colleagues recognized that glial cells control the extracellular potassium concentration. During neuronal activity potassium rises in the extracellular space and glial cells modulate these peak increases. Disturbances in these glial functions could lead to excess potassium accumulation and may induce epilepsy or spreading depression. The transport systems of glial cells have not yet been studied in complex systems. Such an example would be hippocampal slices which is a very established model for epilepsy research. New research has indicated that neuronal activity not only affects the extracellular potassium concentration, but has also a profound impact on the intracellular pH, chloride concentration or the volume of the extracellular space. It has therefore become increasingly important to understand the transport systems for pH and chloride, and, in particular, the factors which regulate the activity of these systems. This knowledge will also be the basis for understanding the changes in volume which are induced by neuronal activity. A number of transport systems are involved, and some groups focus on the properties, control mechanisms and the expression of these transport systems in the various types of glial cells. Such volume regulation is of large clinical importance since brain damage leads to the swelling of astrocytes. This swelling is the cause for most casualities after brain damage. Therapeutical approaches can only be developed if the basic regulatory mechanisms for glial ion activities and volume are understood.

- Are glial cells involved in the signal transduction of the CNS?

Glial cells express a variety of receptors for neurotransmitters and neurohormones. This implies that the cells may sense neuronal synapse-mediated activity. From there, four lines of reseach have been developed:

1. Does the activation of glial receptors lead to a change in gene expression or proliferation of glial cells? In return, these modulated glial cells could again influence neurons.
2. Most of the glial receptors have been characterized in cultured glial cells. It is therefore an important question as to whether these receptors are expressed in the intact CNS and if there are differences among the glial cell types and between the brain regions.
3. Which second messenger pathways are coupled to glial receptors?
4. Activation of receptors can lead to waves of calcium which can spread in glial environments. This signal has been implied to complement the neuronal activity.

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Structure of the Group Grant ("Schwerpunkt") "Functions of Glial Cells"

The "Schwerpunkt" is under the auspices of

Deutsche Forschungsgemeinschaft (DFG)
Postfach 20 50 04
D-5300 Bonn
Germany

Phone: (49) 228 885-1 Fax: (49) 288 885 2221

DFG-Representative for the "Schwerpunkt

Dr. Hans-Joachim Bode

Phone: (49) 288 885-2297 Fax: (49) 228 885-1

Coordinator of the "Schwerpunkt"

Dr. Helmut Kettenmann

Institut für Neurobiologie
Universität Heidelberg
Im Neuenheimer Feld 345
D-6900 Heidelberg

Phone: (49) 6221 56 3996
Fax: (49) 6221 56 3801

Max-Delbrück-Centrum für
Molekulare Medizin
Robert Rössle Str. 10
D-1115 Berlin-Buch

Phone: (49) 30 9406 3325
Fax: (49) 30 9406 3819

Secretary of the "Schwerpunkt"

Meino Thomae
Institut für Neurobiologie
Unversität Heidelberg
Im Neuenheimer Feld 345
D-6900 Heidelberg

Phone: (49) 6221 56 3996 Fax: (49) 6221 56 3801

Advisory Board of the "Schwerpunkt"

Prof. Dr. Adriano Fontana
Universitätsspital, Innere Med.
Abt. für klinische Immunologie
Haldärieweg 4
CH-8044 Zürich
Schweiz

Prof. Dr. W. W. Franke
DKFZ
Inst. f. Zell- u. Tumorbiologie
Im Neuenheimer Feld 280
6900 Heidelberg

Prof. Dr. Michael Frotscher
Anatomisches Institut
Universität Freiburg
Alberstr. 17
7800 Freiburg

Prof. Dr. Christo Gorides
Centre d'Immunologie de
Marseille Luminy
Case 906
F-13288 Marseille
France

Prof. Dr. K. P. Hoffmann
Allg. Zoologie u. Neurobiologie
ND 7, Zi. 31
Universitätsstr. 150
4630 Bochum

Prof. Dr. Ferdinand Hucho
Institut für Biochemie
Freie Universität Berlin
Thielallee 63 - 67
1000 Berlin

Prof. Dr. Hans Lassmann
Universität Wien
Neurologisches Institut
Schwarzspaniergasse 17
A-1090 Wien

Prof. Dr. Oksche
Anatomisches Institut
Universtiät Gießen
Postfach
6300 Gießen

Prof. Dr. Werner Rathmayer
Fakultät für Biologie
Universität Konstanz
Postfach 5560
7750 Konstanz

Herrn Prof. Dr. K. Unsicker
Phillips Universität
Abt. Anatomie u. Zellbiologie
Robert Koch Str. 6
3550 Marburg

Prof. Dr. Heinz Wässle
MPI für Hirnforschung
Deutschordenstr. 46
6000 Frankfurt 71

Head of the Research Unit

Hans H. Althaus

Address:
MAX-PLANCK-INST. f. EXPERIMENTELLE MEDIZIN
H.-REINSTR. 3
34 GÖTTINGEN
GERMANY

Phone: 49 551 3899 327
Fax: 49 551 3899 388
Bitnet:

General Research Interests

Capabilities of Mature OL

Oligodendrocytes (OL) are the cells in the CNS which manufacture and maintain the myelin sheath. Two major line of research interests were followed after culturing and characterisation of OL were possible: a) to study the capabilities of mature OL and b) to investigate the differentiation steps within the glial lineage. Mature OL were previously considered as lazy cells, which have more or less fulfilled their tasks. Recent culture and in vivo studies, however, have shown that mature OL can undergo plasticity and are integrated in the neural orchestra as an active member. We have isolated and cultured OL and astrocytes from the adult pig brain. We have shown that OL can produce and release PGs, respond to neuroactive substances such as NGF, can undergo proliferation, and are capable to myelinate. OL express a number of Oct-proteins which may be important for regeneration and proliferation.

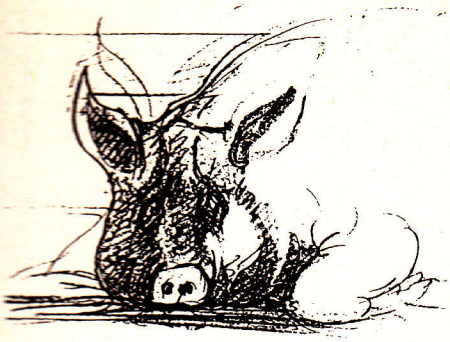
Present and/or Future Project in the DFG Schwerpunkt

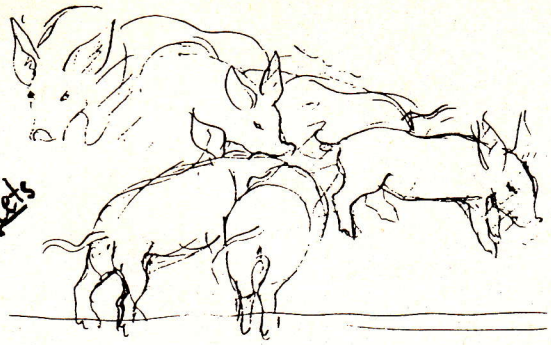
Signaltransduction in Oligodendrocytes

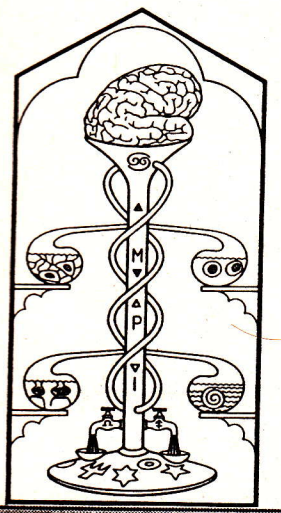
We have shown that protein kinase C (PKC) activating substances such as phorbol esters induce OL proliferation and process formation. On the search for replacing the phorbol esters surprisingly NGF was found to mimick the TPA effects. The kinetics, however, were different. Low and high affinity NGF receptors were identified. NGF induces the tyrosine phosphorylation of several proteins among which MAPK may be present. Studies on the NGF signal transduction pathway are initiated. They may help us to define the rails which are important for proliferation, process formation, and the production of myelin proteins.

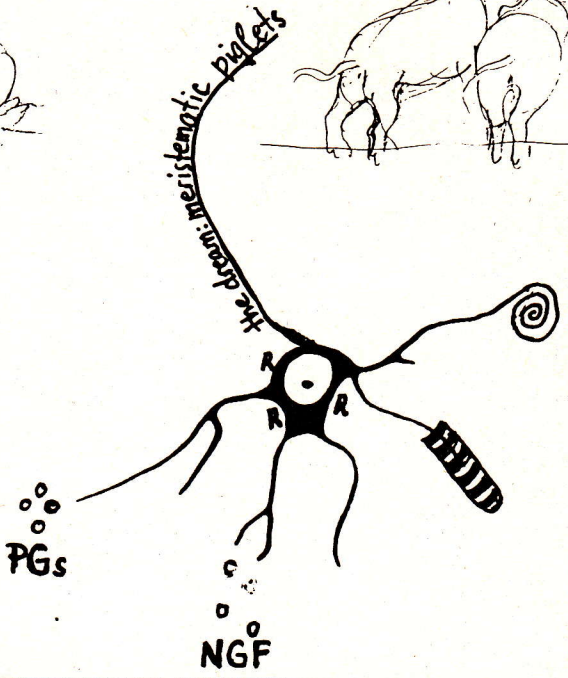
Methods Available

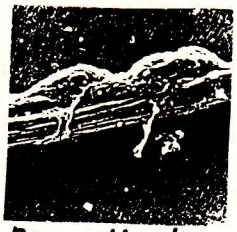
tissue culture techniques
immuno-methods
biochemical methods











Remyelination

Regeneration

Members of the Research Team

Dr. Tyede Schmidt-Schultz
Frank Werner

Collaboration with Other Teams

W. Blakemore,
R. Franklin/Cambridge
P. Gruss/Göttingen
R. Heumann/Bochum
H. Persson/Stockholm

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Head of the Research Unit

Mathias Bähr

Addresses: Max-Planck Institut für Entwicklungsbiologie,
Spemannstr. 35/I und Neurologische
Universitätsklinik, Hoppe-Seylerstr.3,
W-7400 Tübingen
F.R.G.
Phone: 49-7071-292051 and 7071-601327
Fax: 49 7071-601300
Bitnet:

General Research Interests

Functions of glial cells during development and regeneration

It has been generally accepted that the environment plays a major role in pathfinding, targeting and regeneration of axons during development and after lesions. There are major differences between the abilities of different glial cell populations to support neuronal survival and axonal growth. - To examine the abilities of different glial cell populations like Schwann cells, astrocytes or oligodendrocytes and their precursors, tissue culture techniques were established that allowed to analyse survival and axonal growth of embryonic and regenerating adult CNS neurons in a controlled environment. The aim of these studies is to determine the influences of different glial cells and their functional states on CNS neurons and to characterize putative cell surface or extracellularmatrix molecules which might be relevant for axon growth. We have recently described conditions which support the survival and allow regeneration of lesioned CNS neurons in vitro and in vivo.

Present and/or Future Project in the DFG Schwerpunkt

Adult glial cells in tissue culture

We have rently developed tissue culture techniques which allow studying the survival and axonal regrowth of adult CNS neurons under controlled conditions. We could show that Schwann cells from sciatic nerves of newborn rats were able to support survival and axonal growth of adult rat retinal ganglion cells (RGC). Immature astrocytes were permissive for axon regeneration but did not support survival of RGCs from adult rats. We have now adopted these tissue culture techniques to prepare glial cells from the adult rat and (in collaboration with Prof. Claudia Stürmer) from adult fish optic nerves. We are currently examining the reaction pattern of glial cells after optic nerve axotomy in adult rats. Furthermore, adult glial cells from rat and fish are used as substrates for regenerating axons from embryonic or adult rat and fish retinae. Our aim is to determine the species differences in neuron-glia interaction during regeneration and to examine the molecular background of this specific behaviour. Furthermore, signals that are present in the adult rat optic nerve after axotomy, which seem to lead to a cascade of dedifferentiation and proliferation of adult glial cells, are examined.

Methods Available

Optic nerve axotomy, schiatic nerve transplantation, labeling of retinal ganglion cells *in vivo* and *in vitro*, tissue cultures of glial cells from embryonic, neonatal and adult rats, cocultures of glial cells with embryonic or adult neurons, staining techniques, electron microscopy.

Members of the Research Team

Dr. Mathias Bähr, MD

Dipl.Biol. Christine Przyrembel

Collaboration with Other Teams

Prof. Claudia Stürmer, Faculty of Biology, Univ. Konstanz

Prof. Hartwig Wohlburg, Dept. Pathology, Univ. Tübingen

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Head of the Research Unit

Gert Brückner

Address

Paul Flechsig Institute for Brain Research
University of Leipzig
Jahnallee 59
O-7010 Leipzig
Germany
Phone: 49 341 7974 603
Fax: 49 341 2114492
Bitnet:

General Research Interests

Cytochemistry of Glia and Extracellular Matrix

Glial cells structurally adapt to morpho-functional features of neurons. Recent data indicate that the cytochemical properties of glial cell processes and the extracellular matrix at the neuron-glia interface may substantially determine the neuronal environment. Glycoconjugates such as proteoglycans seem to play an important role. They are concentrated as polyanionic macromolecules in perineuronal nets and at nodes of Ranvier. Many structural aspects and the exact chemical composition of the glia-matrix complexes are unknown and their functions are still obscure. Similar to the ensheathment of nodes of Ranvier perineuronal nets may provide a special ion buffering capacity required around various, perhaps highly active types of neurons. It is the aim of our studies to analyze the construction of those perineuronal specializations and to characterize the glial components and associated glycoconjugates with respect to the types of ensheathed neurons using cytochemical methods at the light and electron microscopical level. We hope to contribute new data on glia-neuron communication systems mediated by the extracellular matrix.

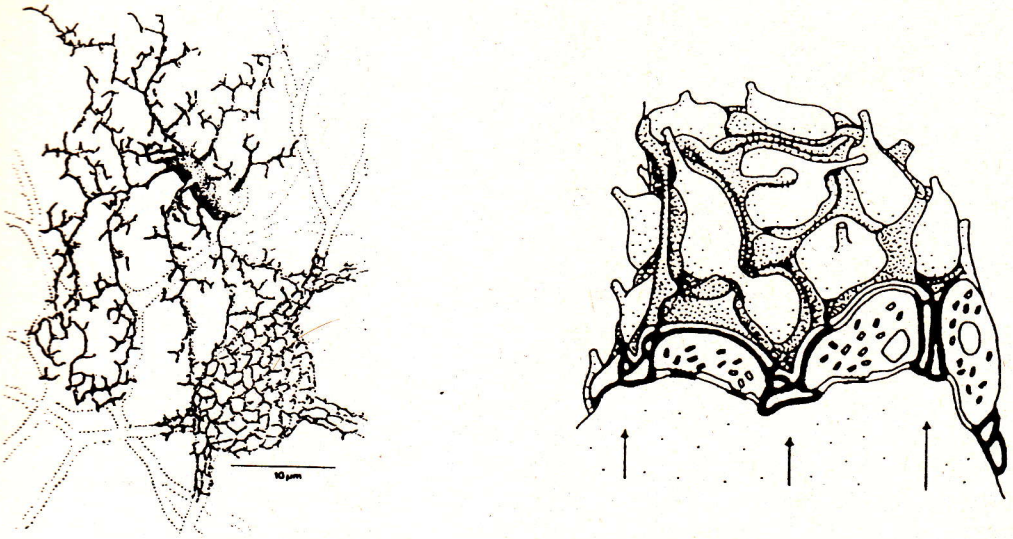
Present and/or Future Project in the DFG Schwerpunkt

Cytochemical and Structural Properties of Perineuronal Nets

The polyanionic character of perineuronal nets visualized in the rat brain with the Golgi technique (Brauer et al., J.Hirnforsch. 23: 701, 1982) was histochemically demonstrated in the present project using the colloidal iron hydroxide staining and the detection of hyaluronan by biotinylated hyaluronectin. Three lectins (Vicia villosa agglutinin, VVA, Wisteria floribunda agglutinin, WFA, soybean agglutinin, SBA) with affinity for N-acetylgalactosamine (GalNac) visualized perineuronal nets similar to those rich in anionic components. These structures ensheath somata, parts of dendrites and axon initial segments of various types of neurons. They were found in more than 100 brain regions. In the neocortex and hippocampus the nets were associated with non-pyramidal cells. Dual labelling demonstrated that GalNac-containing nets frequently surround neurons characterized by the calcium-binding protein parvalbumin. Electron microscopically, VVA-binding sites were scattered throughout perisynaptic profiles but accumulated at membranes and in the extracellular space except synaptic clefts. To investigate the spatial relationship between glial cell processes and glycoconjugates of perineuronal nets, GFAP, S100-protein and glutamine synthetase were detected in dual-label experiments. It is concluded that perineuronal nets are composed of perisynaptic astrocytic processes associated with polyanionic, GalNac-containing matrix material.
Our future project comprises studies based on similar cytochemical methods but it will be focused on developmental and phylogenetical aspects, and will also consider activity-dependent variations in the formation of perineuronal nets.

Methods Available

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



Left: Golgi impregnation of a glial cell forming perineuronal nets on non-pyramidal neurons in the rat cerebral cortex (drawing by Dr.K. Brauer). Right: Perineuronal net consisting of perisynaptic glial profiles associated with glycoconjugates (black) as demonstrated in electron microscopical lectin-binding studies.

Members of the Research Team

Dr. Kurt Brauer
Dr. Wolfgang Härtig
Gerlinde Köppe

Collaboration with Other Teams

Bertrand Delpech, Rouen, France
Amin Derouiche, Frankfurt, Germany
Wolfgang H. Oertel, Munich, Germany
Andreas Reichenbach, Leipzig, Germany
Michael J. Rickmann, Göttingen, Germany
Gudrun Seeger, Leipzig, Germany
Joachim R. Wolff, Göttingen, Germany

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Head of the Research Unit

Joachim W. Deitmer

Address: Abteilung für Allgemeine Zoologie
FB Biologie, Universität Kaiserslautern
Erwin-Schrödinger-Str. 13
D-6750 Kaiserslautern
Germany
Phone: 49 631 205 2887, 2497
Fax: 49 631 205 2998
Bitnet: Internet: deitmer@sun.rhrk.uni-kl.de

General Research Interests

Ion regulation by nerve and glial cells

Ion activities in nerve and glial cells, as well as in the extracellular, interstitial spaces, may undergo rapid and large changes, when ion fluxes are initiated across the membranes of neurones and glial cells. Such ion fluxes can be elicited by membrane depolarization, activating voltage-gated ion channels, receptor activation by transmitters, hormones and neuromodulators or ion carriers. We use ion-sensitive microelectrodes and fluorescent dyes to monitor intracellular and extracellular ion activities, in particular H^+ and Ca^{2+}, and electrophysiological techniques to study the mechanisms of ionic fluxes and the regulation of ions by nerve and glial cells.

Present and/or Future Project in the DFG Schwerpunkt

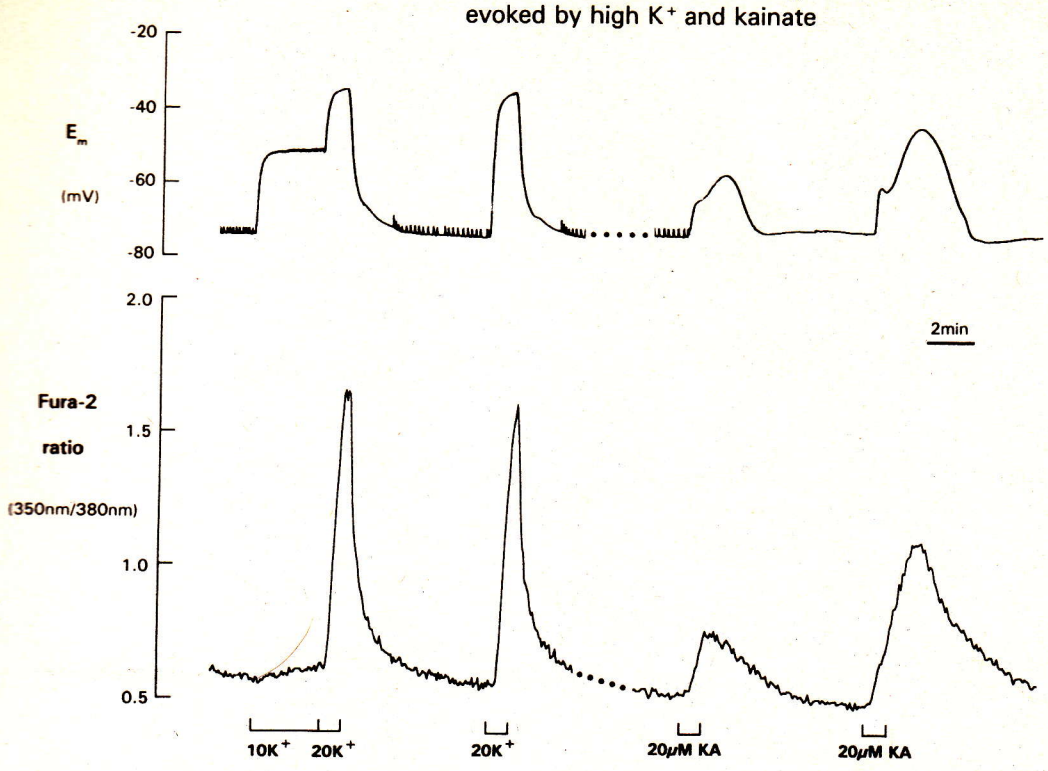
Ca^{2+} regulation by glial cells

Ca ions play an important role as charge carrier and second messenger, modulating intracellular enzymes and ion channels. We look at different pathways of Ca^{2+} into identified glial cells and aspects of Ca^{2+} regulation by glial cells. Glial cells display voltage- and transmitter activated membrane channels and different kinds of intracellular Ca^{2+} stores. We want to study mechanisms by which glial cells control their low intracellular Ca^{2+} and extrude Ca^{2+}, and whether they are involved in Ca^{2+} regulation in the extracellular spaces.

Methods Available

- Ion measurements using ion-sensitive microelectrodes and fluorescent dyes in cells and tissues
- Conventional electrophysiological techniques, current and voltage-clamp
- Patch-clamp technique of cultured and in situ cell

Ca^{2+}-transients in identified leech glial cells evoked by high K^+ and kainate



Members of the Research Team

Backus, Kurt Harald, Dr.
Brune, Thomas
Glowatzki, Elisabeth
Meis, Susanne
Munsch, Thomas, Dr.
Lönnendonker, Ulrich, Dr.
Rose, Christine

Collaboration with Other Teams

Altenberg, Guillermo, Galveston
Augustine, George, Duke, USA
Golowasch, Jorge, Harvard, USA
Martins da Silva, Antonio, Porto
Reuss, Luis, Galveston, USA
Szatkowski, Marek, London
Thomas, Roger, Bristol

5 References

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Rolf Dermietzel

Institute of Anatomy
University of Regensburg
Universitätsstraße 31
8400 Regensburg
Germany
49 941 943 2820
49 941 943 2868

General Research Interests

Structural and functional identity of glial gap junctions

Gap junctions allow the direct intercellular exchange of ions and low molecular weight metabolites, including second messengers, between adjacent cells. By this mode of communication signal molecules are directly transferred without leakage into the extracellular space. Glial cells, especially astrocytes, are furnished with an extensively elaborated complement of gap junctions. Thus the degree of coupling between this glial species is regarded to be high. Functional communication between glial cells in form of Ca^{2+} osszilations and IP3 movement has been shown to be mediated via gap junctions. Insofar gap junction provide the structural backbone by which glial cells are assembled in form of a functional syncytium.

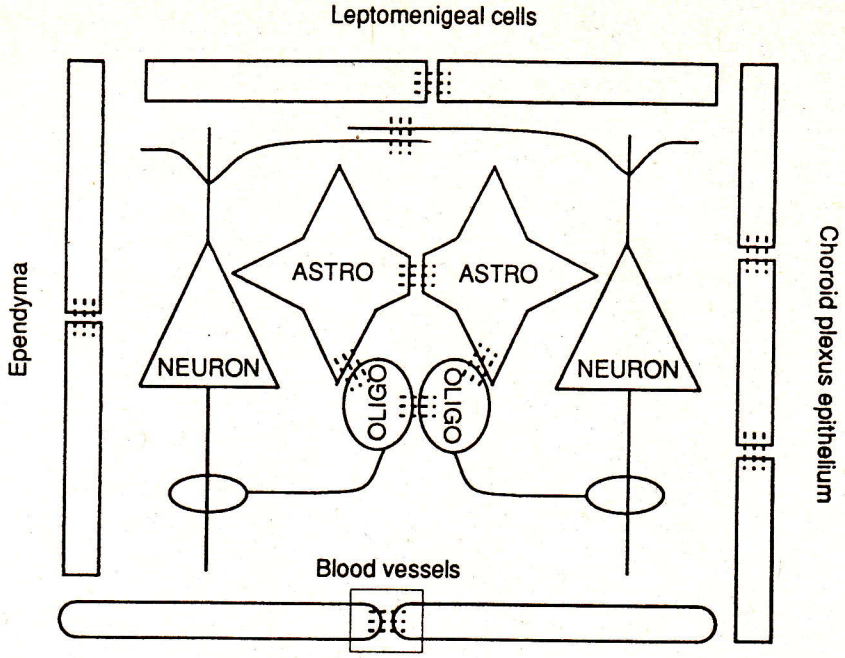
Present and/or Future Project in the DFG Schwerpunkt

Molecular identity and plasticity of interglial gap junction communication

Connexins are the channel-forming proteins of gap junctions. Diverse isoforms are expressed in different tissues. Astrocytes and oligodendrocytes display a diversity in connexin expression. While connexin43, originally cloned from heart tissue, is highly expressed in astrocytes, oligodendrocytes express connexin32. Both connexins, however, provide different functional properties, i.e. voltage dependence, pH sensitivity. One of our present projects is to elucidate whether heterologous coupling between both types of makroglial cells is feasible and to characterize the functional properties of such chimeric junctions. Accumulating evidences also indicate that the strength of coupling between glial cells shows a high degree of plasticity according to the actual functional requirements of the brain tissue. A further goal of our project is to define the cell biological aspects which prove responsible for the plasticity of interglial communication.

Methods Available

Isolation of gap junction proteins
Molecular biological techniques
Microinjection of dye tracers
Light and electron microscopical immunolabelling
Tissue culture techniques



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

Members of the Research Team

Michael Davids
Irmgard Hertting
Andreas Hofer
Marian Kremer
Jörg Kunz
Fernando Miragall
Dorothee Puchner

Collaboration with Other Teams

Michael Bennett, New York
Helmut Kettenmann, Heidelberg
Christian Müller, Tübingen
Harold Kimmelberg, Albany, New York
David Spray, New York
Otto Traub, Bonn
Klaus Willecke, Bonn
Reto Weiler, Oldenburg

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Head of the Research Unit

Juergen Engele

Address: Department of Anatomy and Cell Biology
University of Ulm
Albert-Einstein-Allee 11
7900 Ulm
Germany
Phone: 49 731 502 3223
Fax: 49 731 502 3217
Bitnet:

General Research Interests

Humoral glial-neuronal interactions

Neurotrophic factors play a crucial role in neuronal development, maintenance and regeneration. While it is generally assumed that neurotrophic factors are specifically provided by the target tissue of a given neuronal phenotype, our studies and studies of others have indicated that growth factors may also act on developing central nervous system neurons through glial-mediated processes. Specifically, we observed that a number of identified growth factors primarily affect glial cells which in return influence neuronal survival and differentiation by a yet unknown humoral mechanism. The aim of our present studies is to further characterize these humoral glial-neuronal interactions. These studies may not only provide new insights into the role of growth factors during brain development, but may also help to understand the mechanisms underlying certain neurodegenerative diseases e.g. Parkinson's disease

Present and/or Future Project in the DFG Schwerpunkt

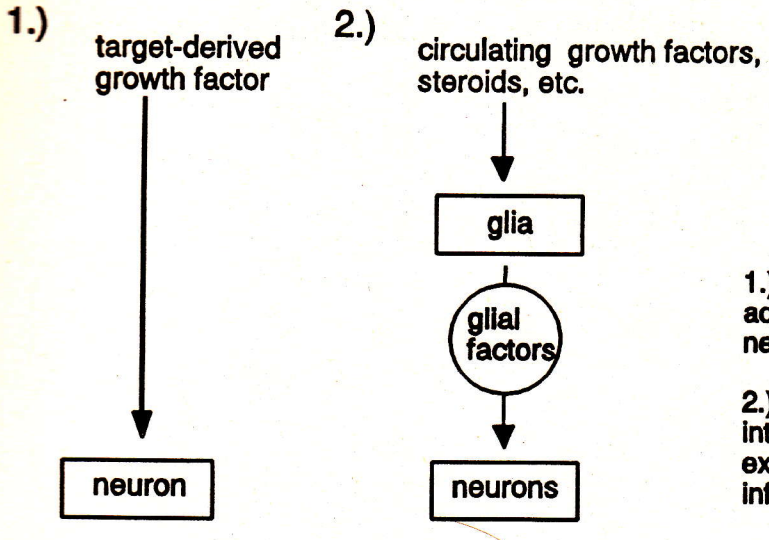
Identification of glial-derived neurotrophic factors for mesencephalic dopaminergic neurons

Our previous studies have identified mesencephalic glia as a putative source for a neurotrophic factor for mesencephalic dopaminergic neurons. To circumvente quantitative problems in the identification and isolation of the responsible neurotrophic activity, we have immortalized primary mesencephalic glia by oncogene transfection. Similar to primary mesencephalic glia, conditioned media obtained from the established glial cell lines increased the survival and differentiation of dopaminergic neurons in serum-free cultures of the dissociated embryonic mesencephalon. Interestingly, this neurotrophic effect was significantly decreased after pretreatment of the cell lines with glucocorticoids. Differential screening of conditioned medium obtained from steroid-pretreated and non-treated glial cell lines resulted in the identification of a steroid-regulated 40 kD protein with survival-promoting effects on dopaminergic neurons.

Our future research interest will be mainly concerned with the purification and sequence analysis of the identified 40kD protein. Moreover, the expression and distribution of the identified protein in the developing brain will be studied by the use of specific antibodies raised against the glial factor. With respect to a possible role of the glial factor in the etiology and/or therapy of Parkinson's diesease, we further intend to more fully characterize the physiological regulation of the identified glial factor by testing various growth factors, cytokines and steroids for effects on its synthesis and/or release.

Methods Available

Cell culture techniques for neurons and glia
Evaluation of growth factor effects on neurons and glia
Immortalization of primary neural cells
Biochemical isolation of growth factors



1.) Classical view of a direct action of growth factors on neurons.

2.) Glial cells as a integrative element of extracellular growth factor influences.

Members of the Research Team

Henry Rieck

Collaboration with Other Teams

Martha Bohn, Rochester, USA
Dave Schubert, LaJolla, USA

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Head of the Research Unit

Andreas Faissner

Address:

Department of Neurobiology
University of Heidelberg
Im Neuenheimer Feld 364
W-6900 Heidelberg
Germany

Phone: 49 6221 563 819
Fax: 49 6221 563 700
Bitnet:

General Research Interests

Glial Extracellular Matrix in Neural Pattern Formation and Regeneration

Astrocytes play an important role in forming neural tissues, for example by guiding migrating neurons and growth cones to their destination or by forming transient tissue boundaries designed to segregate neuronal assemblies. We are attempting to identify glial extracellular matrix (ECM) molecules which might mediate these morphogenetic functions. In this context, we study in more detail tenascin glycoproteins and the recently discovered chondroitin sulfate proteoglycan syneuran. Tenascin glycoproteins are transiently expressed by astrocytes during CNS development and delineate functional processing units in some areas, e.g. the somatosensory barrel field. The generation of a library of monoclonal antibodies and the design of in vitro bioassays served to uncover at least four functional properties of tenascin glycoproteins, namely neuron-binding, the control of neuron migration and of neurite outgrowth, and the repulsion of neurons and of their processes. Current investigations aim at the structural characterization of these functional domains and at the identification of complementary neural receptors. Particular emphasis will be devoted to the elucidation of the repulsive effects of the molecule. Interestingly, the glial proteoglycan syneuran influences neuronal differentiation and might also contribute to glial functions.

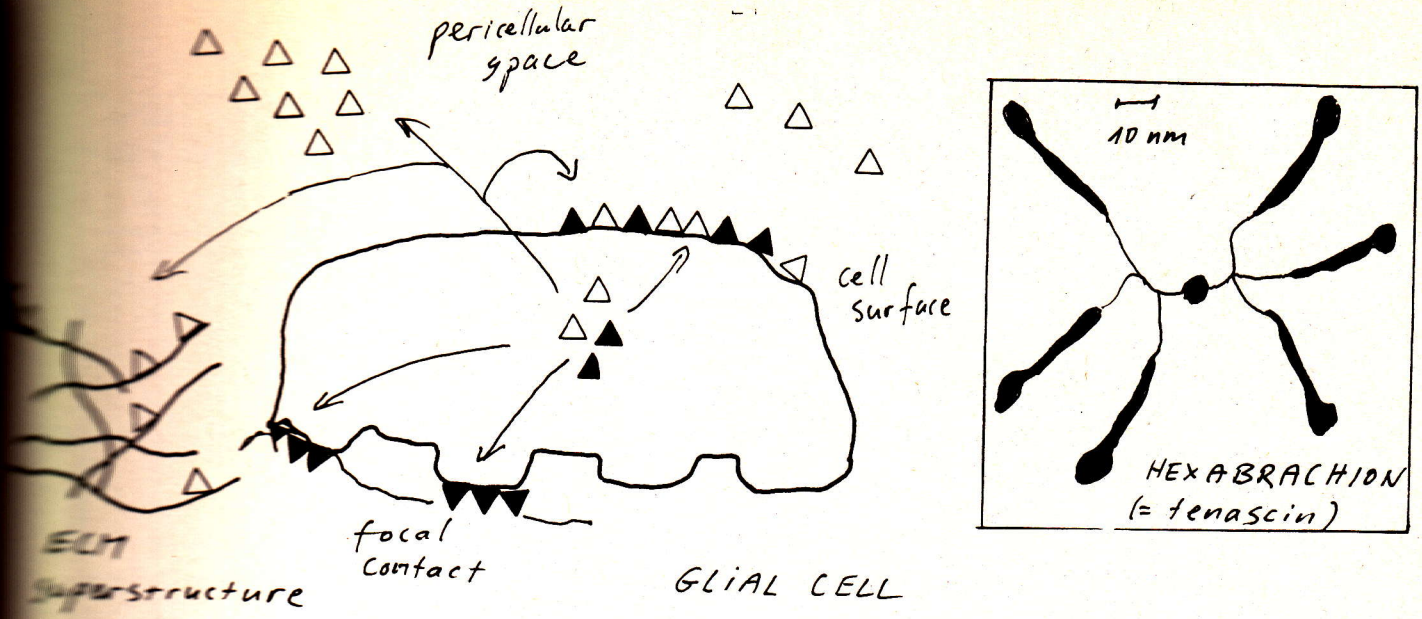
Present and/or Future Project in the DFG Schwerpunkt

Structural and Functional Characterization of Syneuran

Proteoglycans (PGs) consist of glycoprotein cores with at least one glycosaminoglycan (GAG) side chain covalently attached to serine or threonine residue(s). PGs are expressed in various tissues on cell surfaces and in the extracellular matrix. For example, at least 25 PG core proteins have been distinguished in rodent brain. The functions of individual PGs of the nervous system are, however, not well characterized, partly because specific reagents which would permit their isolation are missing. We used the monoclonal antibody (mab) DSD-1 which binds to the surface of a subclass of murine glial cells in vitro to purify the chondroitin sulfate proteoglycan (CSPG) syneuran from detergent-free postnatal mouse brain extracts by a combination of immunoaffinity with ion exchange chromatography. Syneuran displays an Mr of 800-1000 kD and possesses a major core glycoprotein of Mr 350 kD. Differential digestion of purified syneuran with chondroitinase ACI or ACII and subsequent ELISA demonstrated that DSD-1 recognizes a chondroitin sulfate/dermatan sulfate hybrid epitope. DSD-1 is the first reagent with this type of specificity. In order to investigate potential functional properties, embryonic day 18 (E18) hippocampal neurons were grown on syneuran adsorbed to poly-DL-ornithine. Under these conditions, syneuran promoted neurite outgrowth by 100% and neurite elongation by 65%, processes which could be blocked by mab DSD-1 and by enzymatic removal of the dermatan sulfate-containing GAG chain(s). Recent observations have established that syneuran is up-regulated in CNS lesions, implying a role of the CSPG not only during neurohistogenesis, but also in neural de- and regeneration processes. For these reasons, the structure-function analysis of syneuran will be advanced and refined by molecular cloning of the CSPG.

Methods Available

Primary culture of defined neural cell types
In vitro bioassays for determination of cell adhesion, cell repulsion and neurite outgrowth
Generation of monoclonal antibodies to neural ECM molecules
Purification and biochemical characterization of neural ECM components
Molecular cloning of neural ECM components, expression of fusion proteins, in situ hybridization



Glial cells are known to express various ECM molecules. These are distributed to distinct cellular compartments. For example, ECM components are expressed on cell surfaces or detected in focal contact points. In addition, they are also released into the pericellular space where they might be present as soluble components or assembled into ECM superstructures. Scheme on the right side shows tenascin, a growing family of ECM glycoproteins which are expressed by astrocytes and assemble to hexamers under native conditions.

Members of the Research Team

Bernhardt Goetz
Oliver Schnädelbach
Angela Scholze

Collaboration with Other Teams

March D. Ard, Jackson, USA
Alain Prochiantz, Paris, France
Stefan Riedl, Heidelberg, Germany
Melitta Schachner, Zürich, Switzerland
Dennis Steindler, Memphis TN, USA
Leslie Tolbert, Tucson AZ, USA
Anton Wernig, Bonn, Germany

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Head of the Research Unit

Michael Frotscher

Address:
Institute of Anatomy
University of Freiburg
Albertstrasse 17
7800 Freiburg
Germany

Phone: 49 761 203 2561
Fax: 49 761 203 2557
Telex:

General Research Interests

Development and Regeneration of Neuronal Projections

We use the septohippocampal projection in the rat as a model to study the factors underlying the formation of central pathways. Since this projection can easily be transected, it may also be used as a model to study degenerative and regenerative processes of central neurons. This includes studies on the role of trophic factors during development of the septohippocampal projection and after axotomy. Glial cells may play a crucial role in the development of the septohippocampal projection as well as in degenerative and regenerative processes following transection of this pathway in the adult animal. We recently succeeded in establishing the septohippocampal projection in vitro by using slice cultures of septum and hippocampus. Some of the factors underlying the normal development of this pathway, its degeneration and regeneration following axotomy may be better analyzed in this in vitro system.

Present and/or Future Project in the DFG Schwerpunkt

At present there is no project of this research group in the DFG Schwerpunkt. However, it can be seen from our general research interests that we are very interested in the role of glial cells during development and regeneration. Thus, we have plans to submit a proposal in the near future.

Methods Available

Histological techniques and tracing techniques for the study of interneuronal connections
Correlated light and electron microscopic immunocytochemistry
Slice cultures
Intracellular labeling of neurons and glial cells in fixed slices
In situ hybridization, PCR technique

Members of the Research Team

Dr. Giselind Adelmann
Roland Bender
Dr. Thomas Berger
Eckart Förster
Dr. Bernd Heimrich
Dr. Rüdiger Linke
Thomas Naumann
Dr. Martina Plaschke
PD Dr. Herbert Schwegler
Angela Straube

Collaboration with Other Teams

Amin Derouiche, Frankfurt, Germany
Helmut Kettenmann, Heidelberg, Germany
Csaba Leranth, Yale University, New Haven, USA
Robert Nitsch, Frankfurt, Germany
Uwe Otten, Basle, Switzerland
Gary M. Peterson, Greenville, USA
Eduardo Soriano, Barcelona, Spain
Christian Steinhäuser, Jena, Germany

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Head of the Research Unit

Peter J. Gebicke-Haerter

Address: Dept. of Psychiatry
University of Freiburg
Hauptstr. 5
7800 Freiburg i. Br.
Germany
Phone: 49761/270-6835/6592
Fax: 49761/270-6523
Bitnet:

General Research Interests

The Function of Microglia in Brain Injury and Disease

It has become increasingly evident that microglia are key elements in essentially any kind of brain injury or chronic diseases. Besides astrocytes, they appear to have developed the highest sensitivities towards cellular disfunctions or degeneration. Their responses entail proliferation, migration and differentiation at sites of injury. Inflammatory reactions in brain, however, seem to be less pronounced than in peripheral tissues. This may be due to a rather immature stage of microglial development or to efficient immunosuppressive molecules made in brain. The expression of immune mediators during microglial activation and the control of microglial immune functions by specific brain-derived factors, therefore, is a major focus of our research interest.

Present and/or Future Project in the DFG Schwerpunkt

The Molecular Biology of Microglia

The advent of suitable culture techniques for microglia has made it possible to study in more detail the expression and regulation of microglial genes both in isolation and in context with other cell types. Our laboratory has begun to investigate very early steps of microglial activation on the molecular level. Interleukin-3 expression appears to represent a good marker characterizing this activation, since mRNA $^{IL\text{-}3}$ is barely detectable in normal brain. Moreover, IL-3 adds another T-cell feature to microglia (L-APP : König et al., 1992; K^+-channel : Nörenberg et al., 1992) and gives rise to speculations as to their state of maturation within the monocyte/macrophage lineage as well as to potential compensatory functions due to a relatively low abundance of T-lymphocytes in brain. We have found that they not only express the microglial growth factor IL-3 but also the IL-3 receptor. Molecules that induce or increase IL-3 and regulate IL-3 receptor expression in microglia are presently under investigation. With the molecular tools at hand (PCR methodology, *in situ*-hybridization), expression of this cytokine and its receptor in brain cell aggregates and in the brain proper will be studied and extended to other cytokines and mediators of immune responses.

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Methods Available

Molecular biology methods including NORTHERN-, SOUTHERN-, and *in situ*-hybridization and sequencing of cloned genes or PCR-fragments,
tissue culture techniques and immunocytochemistry to characterize cell types.

Members of the Research Team

K. Appel
H. Frey

Collaboration with Other Teams

P. Illes, Pharmacology, Freiburg
J. Bauer, Psychiatry, Freiburg
H. Northoff, Transf.Mediz., Tübingen
D. Männel, Immunologie, Regensburg
B. Volk, Neuropathol., Freiburg

5 References

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Head of the Research Unit

Bernd Hamprecht

Address:
Physiologisch-chemisches Institut
der Universität Tübingen
Hoppe-Seyler-Straße 4
D-7400 Tübingen
Germany

Phone:
Fax:
Bitnet:

General Research Interests

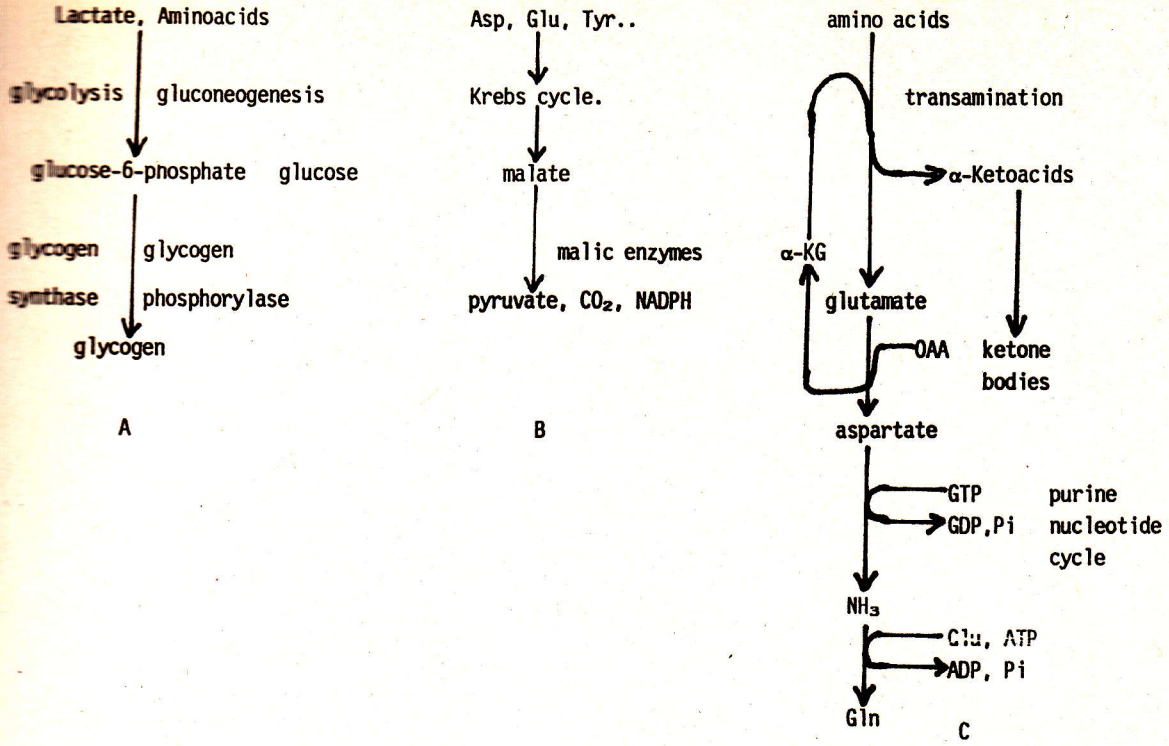
The strategic position of astrocytes between the blood capillaries and the neurons and oligodendrocytes supports the view that astrocytes play a key role in the energy metabolism of the brain. Thus, we are testing our hypothesis that one function of these cells is as processing plants for the generation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

Present and/or Future Project in the DFG Schwerpunkt

In the past we have shown that in brain the glycogen degrading enzyme glycogen phosphorylase is present in astrocytes, ependymal cells and sensory neurons. Of the 3 isozymes likely to occur in brain it is to be assessed whether or not each of them is associated with one cell type. The cellular location of glycogen synthase will be compared with that of phosphorylase. In astroglial cultures gluconeogenesis from various substrates and its regulation will be investigated. An effort will be made to immunocytochemically localize in brain the three key gluconeogenic enzymes. The generation of fuel material from amino acids will be studied by using the ketogenic amino acid leucine. Since for several amino acids the processing to fuel molecules will require the presence of the key enzymes malic enzyme and AMP deaminase, the cellular distribution in brain of the cytosolic and mitochondrial forms of malic enzyme and of the isozymes of AMP deaminase will be analyzed.

Methods Available

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



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

Members of the Research Team

Marija Cesar
Ralf Dringen
Brigitte Pfeiffer
Dieter Schmoll

Collaboration with Other Teams

R. Meyermann, Tübingen, Germany
E. Buse, Kiel, Germany

5 References

Pfeiffer, B., R. Meyermann and B. Hamprecht (1992) Immunohistochemical co-localization of glycogen phosphorylase with the astroglial markers glial fibrillary acidic protein and S-100 protein in rat brain sections, Histochemistry 97: 405-412.

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Dringen, R. and B. Hamprecht (1993) Inhibition by 2-deoxyglucose and 1,5-gluconolactone of glycogen mobilization in astroglia-rich primary cultures. J. Neurochem., in the press.

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Head of the Research Unit

Renate Hanitzsch

Address:
Carl-Ludwig-Institut of Physiology
University of Leipzig
Liebigstr. 27
O-7010 Leipzig
Germany

Phone: 0341 7167 248
Fax: 0341 7167 570
Bitnet:

General Research Interests

Glial Physiology of the Retina

Explanation of light evoked field potentials and the Electroretinogram in terms of glial Müller cell potentials by light induced extracellular potassium changes.

Present and/or Future Project in the DFG Schwerpunkt

Contribution of Müller cells to the Electroretinogram

We measured the extracellular potassium concentration in the dark adapted retina with Corning and valinomycin microelectrodes. There does not exist a potassium gradient in the retina.
The light induced potassium increases in the proximal retina are smaller than 0.5 mM in healthy preparations of rabbit, rat and frog retina; they increase in rabbit and rat retina in unhealthy preparations.
The slow potentials in the ERG (c-wave, slow PIII) are not only determined by the potassium decrease around receptors but also by long lasting potassium changes in the proximal retina of frog and rabbit.

Methods Available

Isolated superfused mammalian retina including RCS-rat-retina
Recording of field potentials
Measurement of extracellular potassium concentration

Members of the Research Team

W.-U. Mättig
Ch. Zeumer
U. Lang

Collaboration with Other Teams

5 References

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Head of the Research Unit

Rolf Heumann

Address:
Lehrstuhl für Molekulare Neurobiochemie
Ruhr-Universität Bochum
Universitätsstraße 150
4630 Bochum 1
Deutschland

Phone: 49 0234 700-4225/4230
Fax: 49 0234 7094-105
Bitnet:

General Research Interests

Intracellular Signal-Transduction-Mechanisms of Neurotrophins

Neurotrophins mediate neuronal survival and the differentiated functions of responsive cells. After release from target cells the neurotrophins nerve-growth-factor (NGF), brain derived growth factor (BDNF), neurotrophin 3 (NT3) and NT4/5 interact with their specific high-affinity receptors trk A, trk B, trk C and with the common NGF low-affinity receptor. Ligand activated receptor tyrosine kinase leads to a cascade of protein phosphorylations finally resulting in the multiple physiological responses. We have recently demonstrated that the small G-protein p21ras not only mimics the action of BDNF and NGF in responsive chicken sensory neurons, but in addition p21ras activity is essential for the neurotrophin mediated action. The putative proteins "upstream" and "downstream" of p21ras are under investigation. We are using antisense-oligonucleotides to identify proteins that are involved in the regulation of p21ras cycling between the active GTP-bound and the inactive GDP-bound conformation in neurons. The aim of these studies is to define the intracellular mechanisms underlying neural regeneration and synaptic plasticity.

Present and/or Future Project in the DFG Schwerpunkt

The Role of p21ras in Glial Cells

In the intact brain, specific populations of neurons constitute a major site of NGF synthesis. However, after lesion and probably during pathological situations also astrocytes and oligodendrocytes synthesize or respond to nerve-growth-factor: in cultured astrocytes activation of their platelet derived growth factor or epidermal growth factor receptors by the corresponding ligands leads to a relative increase of endogenous GTP-bound p21ras. Activated p21ras strongly induces the synthesis of interleukin-1 which in turn stimulates NGF synthesis in astrocytes. Interleukin-1 is able to upregulate its own synthesis, so that a "feed-forward" mechanism has to be postulated leading finally to an increase of NGF synthesis. NGF induces fiber outgrowth in cultured oligodendrocytes, stimulates the rate of cell division and increases the level of trkA mRNA coding for the NGF high-affinity receptor (collaboration with Dr. Althaus/Göttingen). An autocrine action of NGF has to be postulated, because a) there is evidence for the presence of NGF high-affinity and NGF low-affinity receptors, b) NGF synthesis has been demonstrated by using anti-NGF-antibodies, c) NGF developes a multiple action on these oligodendrocytes. The oligodendrocytes will serve as a model to dissect the mechanism of p21ras mediated action of NGF with respect to mitogenicity and induction of fiber outgrowth.

Methods Available

Methods of molecular genetics: PCR techniques, cloning techniques, quantative Northern blots etc.
Mikroinjection of single cells
Introduction of proteins into a whole cell population using the trituration method
Elektrophysiology: patch-clamp techniques on whole cells
Synapse formation in culture

Members of the Research Team

Irmgard Dietzel-Meyer
Marion Gries
Annette Markus
Lothar Kruska
Hartmut Berns
Nguyen Quang Vinh
Franz-Josef Klinz
Yildirim Algür
Kai Erdmann
Stefan Harjes
Jian Zhong
Volkmar Leßmann

Collaboration with Other Teams

Dr. H. Althaus / Göttingen/ Germany
Dr. M. Brown / Oxford/ England
Dr. H. Perry / Oxford / England
Dr. Wittinghofer / Heidelberg / Germany

5 References

R. Heumann, B. Hengerer, D. Lindholm, M. Brown and H. Perry: Mechanisms leading to increase in nerve-growth-factor synthesis after peripheral nerve lesion. In: Advances in neuronal regeneration research: Proceedings of the third international neural regeneration research symposium, pp125-145 (1990).

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Head of the Research Unit

Peter Illes and Wolfgang Nörenberg

Address: Department of Pharmacology
University of Freiburg
Hermann-Herder-Strasse 5
7800 Freiburg
Germany
Phone: 49 761 203 3465
Fax: 49 761 203 4235
Bitnet:

General Research Interests

Transmitter Receptors and Ion Channels of Microglial Cells

Although there is some controversy about their origin, microglia are generally believed to derive from monocytes/macrophages entering the brain during the early embryonic development. Peripheral immunocytes, including macrophages exhibit a variety of voltage-dependent conductances. Changes in the functional states of peripheral immunocytes seem to be closely related to alterations in ionic fluxes across the cell membrane. For example, voltage-dependent outward K^+ currents are more frequently expressed during the activation of human macrophages by lipopolysaccharide (LPS). Resting microglia in culture exhibits only inwardly rectifying K^+ channels.
Peripheral immunocytes and microglia posses also ATP-sensitive receptors. ATP opens nonselective cationic channels and leads, thereby, to an increase in the intracellular concentration of Ca^{2+}. In resting microglia the duration of the ATP-induced membrane depolarization is extremely long-lasting because of the absence of outwardly rectifying K^+ channels.

Present and/or Future Project in the DFG Schwerpunkt

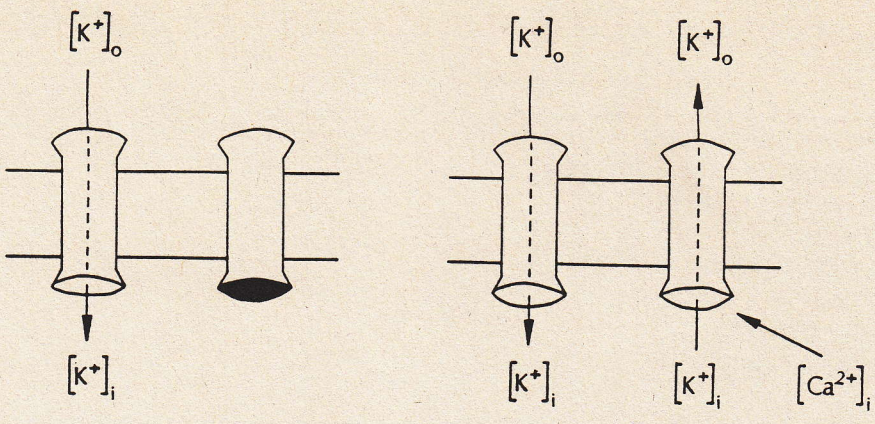
Voltage- and Ligand-Operated Channels of Activated Microglial Cells

In rat microglia, LPS and various other stimuli known to activate macrophages (interferon-γ, incubation in hydrophobic tephlon bags) lead to the expression of an outward K^+ current in addition to the inwardly rectifying K^+ channels. The two types of channels have a differential pharmacological sensitivity; Cs^+ and Ba^+ block the former, while Cd^{2+}, 4-aminopyridine, quinine and charybdotoxin block the latter conductance (see cartoon). An increase in intracellular Ca^{2+} inhibits the outward K^+ conductance, which thereby exhibits features similar to the K_n current of lymphocytes.

Our aim is to investigate voltage- and ligand-activated currents in cultured microglia. We intend to carry out experiments in rat and human microglia in order to answer the following questions. (1) Do these cells possess other types of ionic channels than those permeable to K^+? (2) Are ATP-sensitive receptors present? (3) Are neuropeptide- and excitatory amino acid-receptors present?

Methods Available

Study of whole cell and single channel currents with the patch-clamp technique
Measurement of intracellular Ca^{2+} concentrations with fluorescent dyes
Tissue culture techniques



K^+ channels and their pharmacological sensitivity in resting and activated microglia. There are no pre-formed outwardly rectifying K^+ channels in resting microglia; these channels are newly expressed during LPS-treatment.

Members of the Research Team

Jens Langosch
Oliver Gleichauf

Collaboration with Other Teams

Peter Gebicke-Haerter, Freiburg, Germany

5 References

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Head of the Research Unit

Gunnar Jeserich

Address:
Department of Animal Physiology
University of Osnabrück
Barbarastr. 11
4500 Osnabrück
Germany

Phone: 49 541 969 2880
Fax: 49 541 969 2870
Bitnet:

General Research Interests

Myelinogenesis in the CNS of trout

The myelin-forming cells in the CNS of fish are extraordinary in their biochemical properties whilst in terms of morphology they closely resemble mammalian oligodendrocytes. Instead of synthesizeing proteolipid protein, which is an established oligodendroglial marker they express two Po-like proteins which is a Schwann cell marker. Furthermore they synthesize a new myelin protein constituent of 36,000 dalton as a major myelin component for which now molecular equivalent exists in higher vertebrate myelin. During development fish oligodendrocytes originate from proliferative, A2B5+ progenitor cells and seem to require an adequate extrinsic stimulation for proper induction and maintenace of myelin gene expression. The major goal is to define the factors involved in the regulation of these processes at the cellular and molecular level.

Present and/or Future Project in the DFG Schwerpunkt

Proliferation and differentiation of oligodendroglial progenitor cells

An immunomagnetic cell separation method was developed for isolating oligodendroglial progenitor cells from the larval trout CNS, which yielded a highly enriched population of viable cells.
The factors potentially involved in the regulation of cell proliferation and differentiation of these cells are currently being analysed in cell culture. The functional differentiation of oligodendrocytes thereby is followed in parallel by the occurence of myelin-specific markers as well as the pattern of ion channel expression.
Furthermore work is in progress to clone the genes coding for the major myelin proteins of trout CNS to generate probes for in situ hybridization.

Methods Available

Protein biochemistry including electrophoresis and western blotting
Molecular cloning and sequencing of myelin genes
In situ hybridization and immunocytochemistry
Cell and tissue culture techniques
Single channel and whole cell recording with the patch-clamp technique

Members of the Research Team

Thomas Clasen
Günther Glassmeier
Silvia Janetzki
Dan Ngyuen
Astrid Stratmann
Jens Strelau

Collaboration with Other Teams

Sam Nona, Manchester, UK
Baruch Kanner, Jerusalem, Israel

5 References

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Oliver Kempski

Institute for Neurosurgical Pathophysiology
Johannes Gutenberg University Mainz
Langenbeckstr. 1
6500 Mainz
Germany
49 6131 172373
49 6131 176640

General Research Interests

Regulation of Glial Cell Volume and intracellular pH

Glial swelling has long been known to accompany pathophysiological events such as cerebral ischemia, trauma, or metabolic disorders, contributing to brain edema formation and detrimental rises of intracranial pressure. The mechanisms of glial volume homeostasis are not known in detail. So far we have identified various factors causing glial swelling in vitro: exposure to e.c. glutamate, K^+, arachidonic acid, and, best studied, acidosis. Swelling appears to be an active phenomenon, resulting from the activation of homeostatic mechanisms controlling the e.c. environment to facilitate neuronal function. Major elements of the homeostatic machinery include the Na^+/H^+-antiporter, Na^+/HCO_3^--cotransport, lactate transport, and, possibly, H^+-channels. Cell volume regulation is closely linked to intracellular (i.c.) pH-regulation, currently studied under controlled conditions in vitro. In earlier studies we could demonstrate that glial cells from primary culture and C_6 glioma cells (used as model cells, available in large quantities with standardized properties) swell considerably in e.c. acidosis. Swelling required the presence of bicarbonate and Na^+, was 50% inhibited by amiloride, and was believed to result from the activation of transport systems regulating i.c. pH.

Present and/or Future Project in the DFG Schwerpunkt

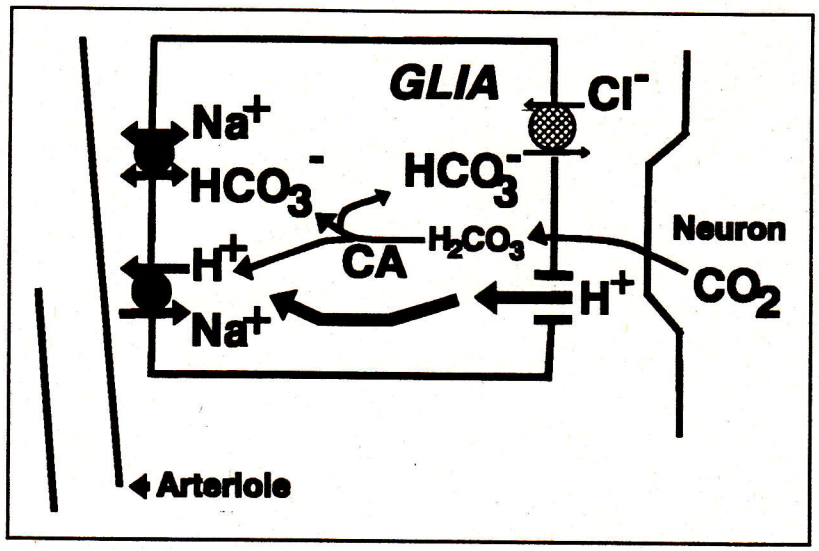
Mechanisms of pH Regulation in Glial Cells

Currently the i.c. pH changes accompanying e.c. acidosis are studied under close control of other e.c. factors. For comparison, the ability of glia to regulate i.c. pH after an i.c. acid load (NH_4 prepulse technique) is studied using inhibitors of ion channels and transporters. It turns out that glial cells acidify in e.c. acidosis, even in the absence of bicarbonate. Acidification is slower than realkalization upon e.c. pH normalization. An intracellular acid load (at normal e.c. pH) is rapidly compensated by glia, but in contrast to many other cell types, amiloride is not very effective to inhibit pH regulation. A complete block of pH normalization was achieved by $ZnCl_2$, which by itself causes i.c. acidosis, and, with longer incubation periods, glial necrosis. Zn^{++} blocks proton channels, which we hypothesize may in vivo turn out important to communicate local changes of e.c. pH, e.g. in the vicinity of neurons, via glial cells to the microcirculation. In vivo, a 'polar' spatial distribution of proton channels and ion transporters could serve to rapidly extrude acid equivalents to the microcirculation, or even to alkalize the neuronal environment during activity - in brief to cause local pH gradients in the brain. Future efforts will be devoted to verify this hypothesis using in vitro imaging techniques as well as in vivo approaches with an open cranial window technique.

Methods Available

Fluorescence measurements of cytosolic ion concentrations (dual wavelength excitation)
imaging techniques, video microscopy including image intensifier and averager, cell and tissue culture techniques, histological laboratory, lactate and LDH analysis, fully equipped operating room for small and large animal experiments: laser-Doppler flow, SEP, tissue pO_2, respiratory parameters, acid-base status etc.

Fig. 1:
Hypothetical polar distribution of ion channels and antiporters on certain glial cells supposedly acting as homeostatic elements between neurons and the microcirculation. The proposed polarity would enable the glial cell to communicate early and efficiently changes of neuronal activity to the vascular bed. In vitro the polarity is lost, thus allowing the investigator only to study the net effect of changed e.c. conditions.



Members of the Research Team

Christoph Volk
Christoph Klawe
Birgit Kloker

Collaboration with Other Teams

Alexander Baethmann, München
Frank Staub, München
Henry Weigt, Ulm
Gerd-Helge Schneider, Berlin

5 References

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Ch. Klawe, Ch. Volk, O. Kempski (in press) Effects of extracellular acidosis on glial cell intracellular pH: evidence for a glial spatial H^+-buffering mechanism? in: "Cerebral Ischemia and Basic Mechanisms" (Hartmann et al. eds.) Springer, Heidelberg

Head of the Research Unit

Helmut Kettenmann

Address: Department for Neurobiology
University of Heidelberg
Im Neuenheimer Feld 345
6900 Heidelberg
Germany
Phone: 49 6221 563 996
Fax: 49 6221 563 801
Bitnet:

General Research Interests

Transmitter Receptors and Ion Channels of Glial Cells

Glial cells were viewed as the electrically passive elements of the central nervous system. Recent advances in imaging and physiological techniques made it possible to demonstrate that glial cells in cell culture can express a variety of voltage gated ion channels and transmitter receptors. These studies implied that the glial cells have the structural repertoire to respond to rapid signals, but have not answered whether these cells indeed do so in an intact nervous system. To address this question, we have developed brain slice preparations and applied the modern imaging and physiological techniques to study individual glial cells. We can identify and analyze properties of microglial cells, oligodendrocytes and their precursors in the corpus callosum, Bergmann glial cells in the cerebellum, astrocytes in the spinal cord, cortex and hippocampus. Our recent studies demonstrate a diversity of physiologically distinct glial cells with receptor properties different to those found in neurons. The aim of these studies is to define the signals between neurons and glial cells and unravel the contribution of glial cells to the information processing in the central nervous system.

Present and/or Future Project in the DFG Schwerpunkt

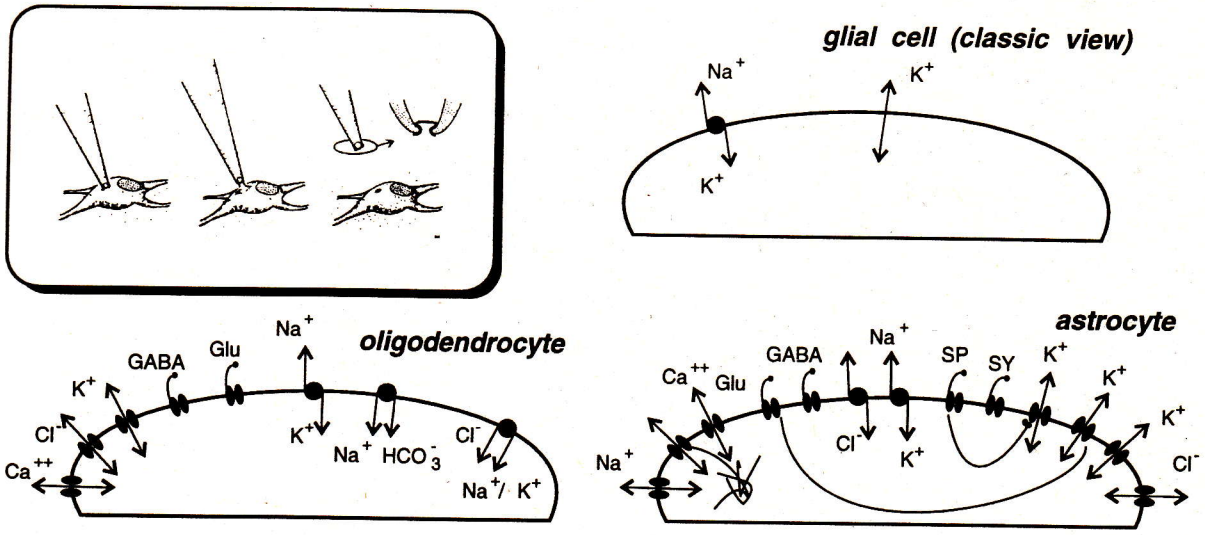
Physiological Properties of Microglial Cells

Microglial cells are considered as a resident macrophage population in the central nervous system. We have studied the physiological properties of microglial cells in culture; based on their membrane channel pattern, we could distinguish these cells, not only from macroglial cells and neurons, but also from non-brain derived macrophages. Such channel pattern was only found in a subpopulation of bone marrow cells, the stem cells of monocytes. Cultured microglial cells have the capacity to respond to neuronal signals; the transmitter ATP induced membrane and cytosolic Ca^{2+} responses in these cells. To overcome the restrictions of the cell culture model, we have recorded from comparable cells in brain slices, namely invading, ameboid microglial cells at early postnatal age. These cells show similar physiological properties as their cultured counterparts.

The ameboid microglial cells on the acute brain slice show a characteristic 'behavioural' repertoire such as phagocytosis and defined movements. One aim of the present project is to characterize these behaviours and determine its pharmacological properties. A second goal is to compare the physiological properties of the ameboid with the resting microglial cells. Since microglial cells are involved in most, if not all CNS diseases, the study of the influence of signal substances on their behaviour might be a prerequisite to determine their role in the normal and pathologic brain.

Methods Available

Study of single channel and whole cell membrane currents with the patch-clamp technique
Use of brain slices to analyze neuron-glia interactions
Fluorescence measurements of cytosolic ion concentrations combined with imaging techniques
Reconstruction of cells with confocal microscopy
Tissue culture techniques



A selection of channels, carriers and receptors described in astrocytes and oligodendrocytes (bottom) as compared to the classical view (top). On the top left, the patch-clamp technique is schematically illustrated.

Members of the Research Team

Karin Borges
Johannes Brockhaus
Jens Grosche
Dr. Susanne Ilschner
Dr. Frank Kirchhoff
Marianne Mauch
Thomas Müller
Carsten Ohlemeyer
Andrea Pastor

Collaboration with Other Teams

Richard Banati, Martinsried, Germany
Virginia Bocchini, Perugia, Italy
Michael Frotscher, Freiburg, Germany
Rosemarie Grantyn, Martinsried, Germany
Harold K. Kimelberg, Albany, USA
Hanns Möhler, Zürich, Switzerland
Paula M. Orkand, San Juan, Puerto Rico
Bruce R. Ransom, Yale University, New Haven, USA
Jutta Schnitzer, MDC, Berlin-Buch, Germany
Peter Seeburg, ZMBH, Heidelberg, Germany
Christian Steinhäuser, Jena, Germany
Alexandr Verkhratsky, Kiev, Ukraine
Wolfgang Walz, Saskatoon, Canada

5 References

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Head of the Research Unit

Christian Klämbt

Address: Institut für Entwicklungsbiologie
Universität zu Köln
Gyrhofstr. 17
5000 Köln 41
Germany
Phone: 49 221 470 3108
Fax: 49 221 470 5164
Bitnet:

General Research Interests

Glial neuronal interactions in the embryonic CNS of Drosophila

The formation of the CNS axon pattern depends on glial-neuronal interactions. We study these processes during *Drosophila* embryogenesis due to the manifold advantages this system offers (genetic analysis, detailed cell biology with many glial cell specific markers, germline transformation etc.). In our work we focus on questions on the determination of cell fate and cell function of specific midline glial cells during axonal patterning. Which genes are required for cell fate decisions? How do the midline glial cells mediate their instructive function during commissure formation?

Research Project on Glial Cell Structure and Function in Health and Disease

Analysis of the gene *argos*

We have identified the gene *argos*, which is specifically expressed in the midline glial cells in the embryonic CNS. Molecular and genetic analysis showed that it encodes a secreted, diffusible protein, which is involved in cell fate decisions during later stages of *Drosophila* development. To study the function of *argos* during embryogenesis we have isolated a set of X-ray and EMS induced alleles. They imply a function of the ARGOS protein during commissure development. To fully understand the CNS axon pattern defect caused by lack of *argos* function, it is important to determine the ARGOS protein distribution during development. Since *argos* encodes a secreted and diffusible protein this would reveal the target cells of argos function and in addition would indicate where ARGOS receptors would have to be expressed. To date we have isolated *argos* specific antibodies and identified some of the *argos* target cells in the embryonic CNS. To isolate the ARGOS receptor we are using a genetic approach, which so far has revealed one new locus which is closely interacting with *argos*.

Methods Available

- germ line transformation, genetics
- molecular biology techniques
- tissue culture techniques
- scanning and transmission electronmicroscopy

Members of the Research Team

Andreas Klaes
Thomas Wemmer
Thomas Menne
Henrike Scholz

Collaboration with Other Teams

Benny Shilo, Rehovot, Israel

5 References

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Klämbt, C. (1993) The *Drosophila* gene *pointed* encodes two *ets* like proteins, which are involved in the development of the midline glial cells. Development, in press.

Head of the Research Unit

Hubertus Köller and Mario Siebler

Address: Department of Neurology
Heinrich - Heine University
Moorenstraße 5
D - 4000 Düsseldorf 1
Germany
Phone: (49) 211 311 8979
Fax: (49) 211 311 8485
Bitnet:

General Research Interests

Electro - Immunological Coupling in Glial Cells

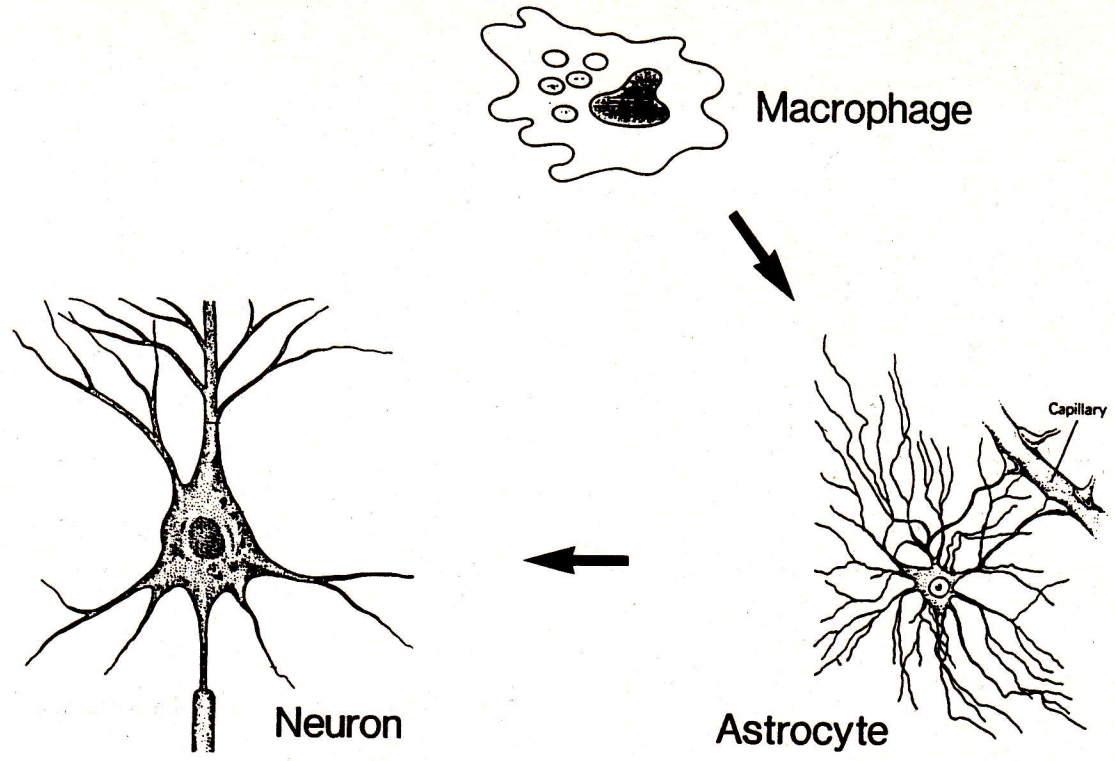
Glial cells, especially microglia and astrocytes are known to participate in immunological processes within the CNS and are able to produce cytokines and present MHC class II antigens. After immunological stimulation, e.g. by leukotriene B_4, we observed a modulation of ion channels, dependent on intracellular regulatory mechanisms and leading to a marked depolarization of cultured astrocytes. Our main interest is now to clarify the functional relevance of electrophysiological alterations during immunological activation, the underlying ional and intracellular mechanisms and their pharmacological regulation, and the coupling between different immunological stimuli and electrophysiological effects.

Research Project on Glial Cell Structure and Function in Health and Disease

Future projects include the investigation of different immunologically active substances released from macrophages or produced by immunologically competent cells within the CNS like astrocytes or microglia cells concerning their effects on electrophysiological properties of cultured astrocytes and neurons from new-born rats. In comparison with these results we plan to study putative effects of immunologically active substances on human astrocytoma cells.

Methods Available

Patch clamp technique, Fluorescence measurements of intracellular ion concentrations (in collaboration with Prof. H. Haas, Düsseldorf), primary cell cultures from CNS (in collaboration with Dr. H.W. Müller, Düsseldorf)



Members of the Research Team

Hubertus Köller
Mario Siebler
Sandra Wilms
Jochen Buchholz

Collaboration with Other Teams

Hans Werner Müller, Düsseldorf
Guido Stoll, Düsseldorf
Walter Däubener , Düsseldorf
Hans Peter Hartung, Würzburg
Helmut Haas, Düsseldorf
Winfried Reichelt, Leipzig

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Head of the Research Unit

Georg W. Kreutzberg

Address:

Max-Planck-Institute of Psychiatry
Dept. Neuromorphology
Am Klopferspitz 18a
8033 Martinsried near Munich
Germany

Phone: 49 88 8578 3650
Fax: 49 89 8578 3939
Bitnet:

General Research Interests

Resident, resting microglia are ubiquitously distributed in non-overlapping territories in the brain. While relatively little is known about the function of resting microglia, the activation of microglia has been intensively studied. Microglial activation becomes apparent by proliferation, recruitment to the site of injury, immunophenotypical changes (including an increased expression of the CR3 complement receptor, MHC class I and II antigens and the receptors for colony-stimulating factors) and finally their capacity to transform into intrinsic phagocytes. In this laboratory the microglial reaction is primarily studied in the facial nerve axotomy paradigm. This has the advantage of leaving the blood-brain barrier intact which allows the study of intrinsic microglia only. In addition, microglial reactions (with a similar repertoire of microglial activation changes) have been examined in several other models including ischemia, autoimmune diseases, traumatic and neurotoxic lesions. In vitro, microglia show a pronounced cytotoxicity as evidenced by their capacity to release reactive oxygen and nitrogen intermediates as well as proteinases. In summary, these results underline the fact that microglia form an intrinsic immune defense system of the CNS, and are primed at an early stage to participate in the CNS immune defense.

Present and/or Future Project in the DFG Schwerpunkt

The microglia may be the first cell type to become rapidly activated in response to a pathological stimulus. In recent years we have therefore examined their activation in several experimental neuropathologies as well as their electrophysiological and functional properties in vitro. Microglia in vitro have a characteristic potassium channel pattern which destinguishes them from peripheral macrophages. It has recently been shown that a subpopulation of bone marrow cells shares this potassium channel pattern. It is now planned to extend the electrophysiological characterization of the microglia, in particular in slice preparations (in collaboration with H. Kettenmann et al.). In addition, the in vivo characterization of microglial reactions will be continued and extended in several experimental neuropathologies. One feature of microglial activation is their rapid recruitment to the site of injury. Since microglia can migrate long distances in vitro, migration might be involved in the process of microglial activation. Using intracerebral transplants of dye-labelled microglia, the migration of microglia is now being studied in vivo. A new focus of research interest will be aging. Activated microglia newly synthesize the amyloid precursor protein (APP) and, moreover, a new alternative splicing form, the l-APP. In collaboration with K. Beyreuther et al. the contribution of activated microglia to APP formation and, possibly to amyloidogenesis in neurodegenerative disorders will therefore be further investigated. Furthermore, microglial morphological and immunophenotypical changes will be examined in aged rats as a model of "normal" aging.

Methods Available

Light and electronmicroscopy for standard histology
Immunocytochemistry for light and electron microscopy
Fluorescence microscopy
Cell culture techniques
molecular biology including in particular in situ hybridisation

Members of the Research Team

Georg W. Kreutzberg
Jochen Gehrmann
Richard B. Banati
Audrey N. Kalehua

Collaboration with Other Teams

Helmut Kettenmann, Heidelberg
Konrad Beyreuther, Heidelberg
Konstantin-A. Hossmann, Köln
Hartmut Wekerle, Martinsried
C. Linington, Martinsried
Johannes Noth, Aachen
Wilhelm Nacimiento, Aachen
Siegfried Schoen, Aachen
Rudolf Töpper, Aachen
Richard Meyermann, Tübingen
Günter Valet, Martinsried
Gregor Rothe, Regensburg
Jia Newcombe, London
Louise M. Cuzner, London
Ralph Myers, London

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Streit, W.J., M.B. Graeber and G.W. Kreutzberg (1988) functional plasticity of microglia: a review. Glia, 1: 301-307.

Head of the Research Unit

Dieter Leibfritz

Address:
Universität Bremen
Fachbereich Biologie/Chemie
NW2
2800 Bremen 33
Germany
Phone: 49 421 218 2818
Fax: 49 421 218 4264
Bitnet:

General Research Interests

Multinuclear in-vivo-NMR-Spectroscopy / NMR-Imaging

In-vivo-NMR spectroscopy of the brain, even if it is done volume selectively, inevitably records metabolites from different cell types because of the intrinsically large volume, which has to be recorded (approx. 1 ml in the case of human brain; several µl for rat brain). Therefore, isolated cultures are well suited to elucidate the origin of NMR detectable metabolites and to follow-up their metabolism by high resolution multinuclear NMR techniques. Depending upon the desired resolution the spectra are recorded from cell extracts or living cells (embeded in gel threads). For the living organism (rat) we have deveoped techniques for the fast volume selective spectroscopy (currently a voxel size of 4 µl, recording time 3.5 min) to record the transient metabolic stages of experimental brain infarcts and its penumbra. Complementary imaging techniques with diffusion weighted or magnetization transfer weighted images record the simultaneous swelling process.

Present and/or Future Project in the DFG Schwerpunkt

Multinuclear NMR Studies of Glial Cell Metabolism

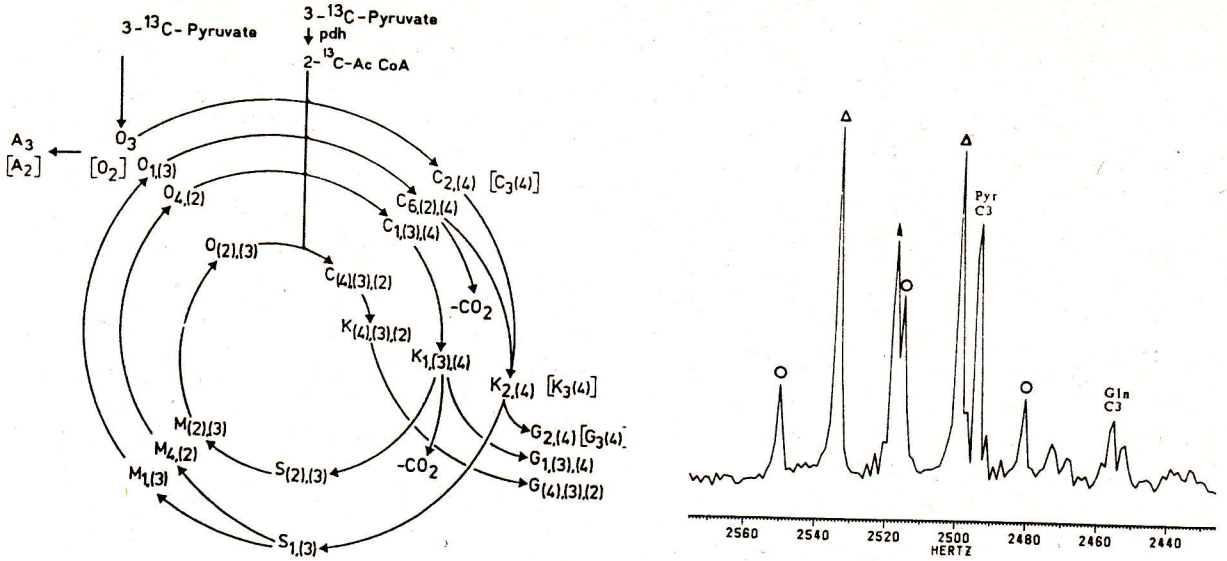
With C-13 labelled precursors as glucose, pyruvate etc. the fate of the label is followed in situ without metabolite isolation. The relative contribution of pyruvate dehydrogenase and pyruvate carboxylase to the TCA cycle are recorded simultaneously. Primary glial cell have much higher pc-activity (46 %) compared to neurons (28 %). Surprisingly the isotopomer pattern of glutamate and glutamine are different for primary glial cells. This is important with respect to similar experiments in living rats, where different isotopomer pattern have been interpreted as different TCA cycles for glia cells and neurons.

We have evidence that myo-inositol may be used as NMR marker for in-vivo proton NMR spectra. However, the turnover of myo-inositol seems to be rather unclear. As for various pathologies the observable myo-inositol concentration varies within a large range, this metabolite may become a diagnostic marker.

The NMR method is also well suited to characterize and quantify membran transport (c.f. pH regulation etc.) and metabolic events after drug treatment.

Methods Available

High resolution NMR spectroscopy (H-1; C-13; P-31; F-19; H-2; N-15 a.o.)
In-vivo-NMR cell techniques in gel threads
Volume selective NMR spectroscopy in intact organism (SI; CSI)
Fast Imaging techniques (diffusion-, MTC weighted images)



a) The fate of the 3-C* label of C-13 pyruvate during utilisation in the TCA-cycle. b) Extended part of the carbon spectrum of C-3 glutamate of C6 glioma cells with the mono-, double- and triple labelled isotopomer.

Members of the Research Team

Dr. Anette Brand
Dr. Wolfgang Dreher
Jörn Engelmann
Ulrich Flögel
Joachim Henke
Dr. Hansjörg Koch
Bernd Kühn
Thoralf Niendorf
Dr. David Norris
Natalie Perkowa
Wieland Willker

Collaboration with Other Teams

C. Richter-Landsberg, Bremen, Germany
K.A. Hossmann, Köln, Germany
F. Podo, Roma, Italy
G. Zimmer, Frankfurt, Germany

5 References

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Head of the Research Unit

Konrad Löffelholz

Address:
Department of Pharmacology
University of Mainz
Obere Zahlbacher Str. 67
6500 Mainz
Germany

Phone: 49 6131 173260
Fax: 49 6131 176611
Bitnet:

General Research Interests

Neuropharmacology of the cholinergic system in the CNS

Early studies of the cholinergic system of the heart showed a strong dependence of the synthesis of acetylcholine on choline availability and suggested the mobilization of choline from choline-containing phospholipids by muscarinic receptor activation via phospholipase D. In the CNS, the availability of choline is of particular importance in central cholinergic dysfunction. We demonstrated that the availability of choline in the CNS is subject to homeostatic mechanisms which keep the level of extracellular choline constant after exogenous choline administration. Both kinetic (rapid choline outward transport) and metabolic (rapid uptake and phosphorylation of choline by brain cells) mechanisms were identified as major contributors to choline homeostasis. The release of choline from choline-containing phospholipids, mainly phosphatidylcholine, in the CNS is mediated by phospholipases A2 and D which are both subject to regulation by neurotransmitter action. Glial cells, mainly astrocytes, may be deeply involved in the mechanisms of choline uptake and release and therefore influence the availability of choline for cholinergic neurons.

Present and/or Future Project in the DFG Schwerpunkt

Receptor-mediated activation of phospholipase D in astrocytes

Recent studies indicated that, in the hippocampal slice preparation, phospholipase D has a high basal activity (measured by formation of phosphatidylpropanol in the presence of propanol). The enzyme was further activated by aluminum fluoride, an activator of G-proteins, and by glutamate acting on metabotropic receptors. Preliminary experiments on astroglia-rich cultures, prepared from newborn rat cortex, indicate that astrocytes may be responsible for these effects. The activity of phospholipase D was increased by both glutamate (1mM) and aluminum fluoride (0,01 mM) by 30-100%. As phospholipase D, besides choline, forms phosphatidic acid and diacylglycerol, i.e. potent intracellular second messengers, we plan to characterize PLD in primary astrocyte cultures both with respect to activation by neurotransmitters (type of transmitter, subtypes of receptors involved, possible mediation by G-proteins) and with respect to intracellular signal transduction (activation of protein kinase C, mitogenic response). In addition, we will test the hypothesis that astrocytes contribute to the homeostasis of choline in the CNS. For this purpose, the choline uptake systems (high- or low-affinity uptake) of astrocytes will be characterized, and the possible receptor-mediated activation of the choline-releasing phospholipase A2 and PC-specific phospholipase C will be investigated.

Methods Available

Microdialysis in the conscious animal
Superfusion of brain slices from striatum, hippocampus, cortex
Cell culture: astroglia-rich culture from newborn rat cortex
Measurement of choline, acetylcholine and its metabolites by HPLC-ECD
Determination of phospholipase activities (PLA2, PC-PLC, PI-PLC, PLD)

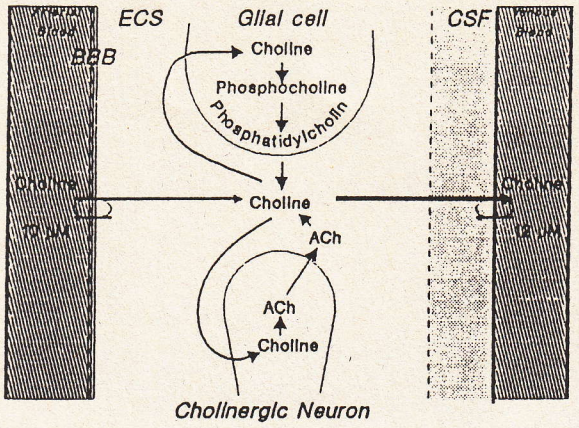

Fig. 1:
Pathways of choline
in the CNS

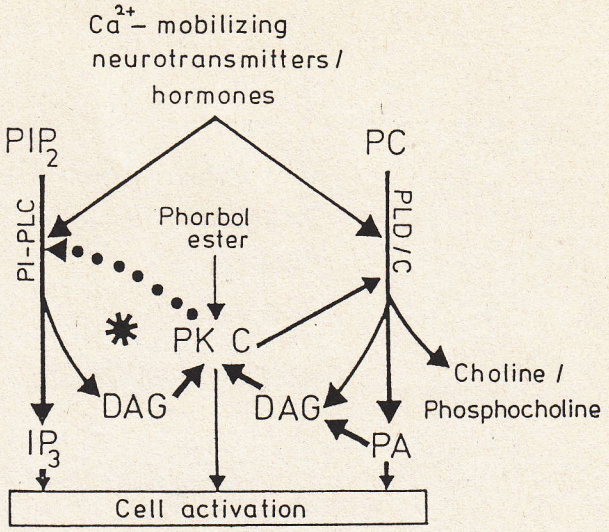

Fig. 2:
Regulation of protein kinase C
activity by receptor-mediated
phospholipid hydrolysis

Members of the Research Team

R. Gonzalez
T. Holler
Dr. J. Klein
A. Köppen
Dr. R. Lindmar
R. Lommel

Collaboration with Other Teams

V. Bigl, R. Schliebs, Paul-Flechsig-Institut für Hirnforschung, Leipzig, Germany
M. Liscovitch, Weizman Institute, Rehovot, Israel

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R. Lindmar und K. Löffelholz (1992) Phospholipases D in heart: basal activity and stimulation by phorbol esters and aluminium fluoride. Naunyn-Schmiedeberg's Arch. Pharmacol. 346, 607-613.

Head of the Research Unit

Christian M. Müller

Address:
Max-Planck-Institut für Entwicklungsbiologie
Spemannstr. 35/1
7400 Tübingen

Phone: 07071/601497
Fax: 07071/601300
Bitnet: Mueller@mpib-tuebingen.mpg.dbp.de

General Research Interests

Role of glial cells in central nervous plasticity

One of the unique features of the central nervous system is its ability to adapt to environmental influences. During development this process influences the self-organization of the brain circuitry, in the mature system it is the basis for memory and learning. Plasticity in the brain includes morphological changes in the circuitry, e.g. synapse formation and synapse elimination. Our aim is to understand the mechanisms underlying such processes. Our hypothesis is that glial cells play a dominant role in such mechanisms of activity-dependent plasticity.

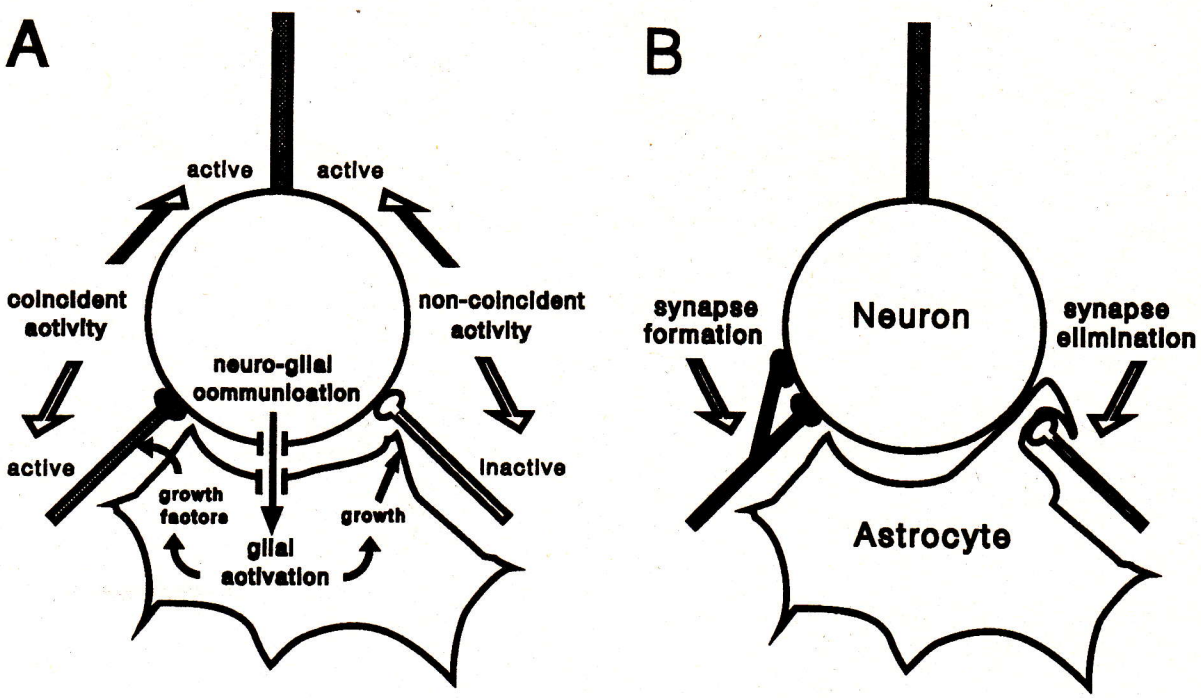
Present and/or Future Project in the DFG Schwerpunkt

Are astrocytes involved in hippocampal long-term potentiation?

Our reasearch in the DFG-Schwerpunkt focusses on a possible role of astroglial cells in hippocampal long-term potentiation. Such a role for glial cells may be based on an activity-dependent neuro-glial information transfer and a glia-neuronal interrelation which influences the efficacy of synaptic transmission or synaptogenesis. The presence of both features is well documented, albeit a direct relation to the induction or manifestation of long-term potentiation is as yet unproven. We investigate this issue by specific activation or inhibition of astrocytes and monitoring long-term potentiation in hippocampal slice preparations.

Methods Available

Extra- and intracellular recordings / immunocytochemistry / intracellular dye injection / organotypic cultures



Members of the Research Team

R. Brechtmann

S. Gardziella

Y.-M. Hong

U. Konietzko

G. Schöbel

M. Schweizer

Collaboration with Other Teams

T. Bonhoeffer, Frankfurt, Germany

N. Daw, Yale Univ., New Haven, USA

R. Dermietzel, Regensburg, Germany

A. Faissner, Heidelberg, Germany

H. Kettenmann, Heidelberg, Germany

M. Schwab, Zürich, Switzerland

5 References

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Head of the Research Unit

Hans Werner Müller

Addresse: Department of Neurology
Molecular Neurobiology Laboratory
University of Düsseldorf
Moorenstr. 5
4000 Düsseldorf 1
Germany
Phone: 49 211 311 8410
Fax: 49 211 311 8485
Bitnet:

General Research Interests

Glia-Neuron Molecular Interactions in Neurological Disease and Regeneration

Neural trauma induces a sequence of distinct molecular reactions that reflect the differential expression of a group of specific genes with putative functions in nerve degeneration and repair. We have recently cloned several of these genes by differential colony hybridization screening of a cDNA library of rat sciatic nerve. Currently the structure and regulation of these genes, their relation to neurological diseases and the biological function of the gene products are investigated using molecular and cell biological, biochemical and immunologcial methods. One of the identified genes, PMP22, that encodes a new peripheral myelin protein 22kD, could be linked to the hereditary demyelinating Charcot-Marie-Tooth 1A neuropathy.
We further investigate the molecular basis of long-term neuronal survival and axonal regeneration in the mammalian central nervous system. In this respect we focus on the role of astrocytes. Our recent studies demonstrate the expression of a new class of neurotrophic chondroitin-sulfate proteoglycans of astroglial origin. Finally, the capacity of the adult mammalian nervous system to regenerate after lesion is investigated using stereotactically applied cell suspension transplants of juvenile astroblasts.

Present and/or Future Project in the DFG Schwerpunkt

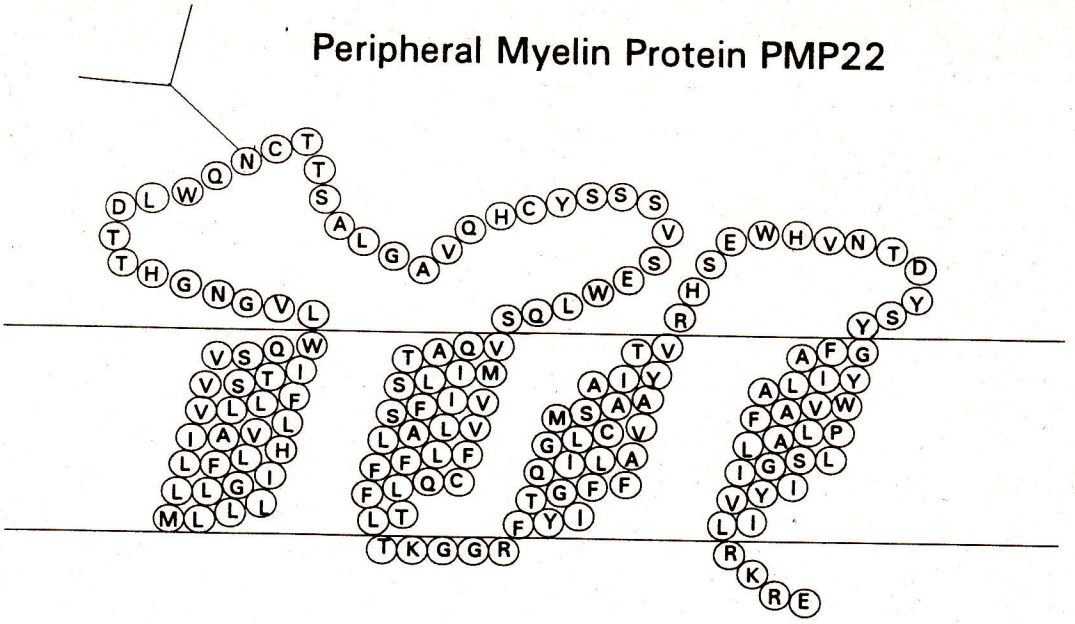
Astroglial Proteoglycans with Neurotrophic Activity

We have shown that extracellular matrix substrat adhesion glycoproteins, cell contact-mediated molecular interactions as well as diffusible neurotrophic factors of astroglial or meningeal origin synergistically interact to support differentiation and long-term survival of defined CNS neuronal cell cultures. A 300kD neurotrophic chondroitin sulfate proteoglycan that is biologcially active at nanomolar concentrations has been purified from serumfree conditioned medium by FPLC fractionation procedures under dissociating conditions. This molecule is not identical with one of the known neurotrophic factors suggesting a novel principle of neurotrophic action.

In the future project we aim at the functional characterization of this proteoglycan and its mechanism of action as well as at the biosynthesis and cellular regulation of expression and release by astrocytes. Specific antibodies should allow to investigate the spatio-temporal distribution of the neurotrophic proteoglycan in the central nervous system during development, maturation and aging.

Methods Available

Gene cloning (plasmid/phage/cosmid) and differential library screening; Quantitative PCR-methods;
Retroviral gene transfer; In situ hybridization; Immunocytochemistry;
Protein isolation and purification techniques; Biosynthetic protein labeling;
Primary cell cultures from CNS and PNS; Stereotactic brain lesions;
Cell transplantations into brain.



Hypothetical membrane topology of glycoprotein PMP22. Note the four putative transmembrane regions and the two hydrophilic loops (one of which is glycosylated) on the outer surface of the myelin membrane. The N- and C-termini of PMP22 are probably located at the cytoplasmic surface of the membrane.

Members of the Research Team

Frank Bosse
Dr. Donatella D'Urso
Wolfgang Fricke
Clemens Gillen
Marc Gleichmann
Dr. Oliver Hanemann
Dr. Ulrich Junghans
Dr. Georg Kuhn
Dr. Joachim Kappler
Katrin Lips
Dr. Corinne Schmalenbach
Dr. Christine Stichel-Gunkel
Sandra Wilms
Dr. Georg Zoidl

Collaboration with Other Teams

David Colman, New York
Christine van Broeckhoven, Antwerpen
Phil Chance, Salt Lake City
Joseph Huston, Düsseldorf
Rainer Kuhn, Basel
Greg Lemke, La Jolla
Helmut E. Meyer, Bochum
Kurt Naujoks, Penzberg
Guido Stoll, Düsseldorf

5 References

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Timmerman et al. (1992) Myelin gene PMP22/Gas3 is contained within the Charcot-Marie-Tooth disease type 1a duplication. Nature Genetics 1, 171-175.

Head of the Research Unit

Klaus-Armin Nave

Address: Zentrum für Molekulare Biologie (ZMBH)
Universität Heidelberg
Im Neuenheimer Feld 282
D-6900 Heidelberg
Germany
Phone: 49-6221-566898
Fax: 49-6221-565894
Bitnet:

General Research Interests

Molecular Genetics of Glial Cells and Myelin Formation

The differentiation of myelin forming glial cells provides a model system to study principles of neural development and cellular interactions in the nervous system. Myelin assembly depends critically on the coordinate and cell type-specific expression of a set of genes encoding the structural myelin proteins. The expression of these genes is endpoint of a genetic program that underlies the differentiation of oligodendrocytes (or Schwann cells) from pluripotent neural stem cells. We are using molecular and genetic techniques to study the function of myelin-specific genes in normal brain development and in neurological mutant mice which display genetic defects of myelination. Our major interest focusses on the role of the proteolipid protein (PLP) in oligodendrocyte differentiation. More recently, we have begun to investigate the molecular mechanism which underlie the coordinated expression of the major myelin-specific genes.

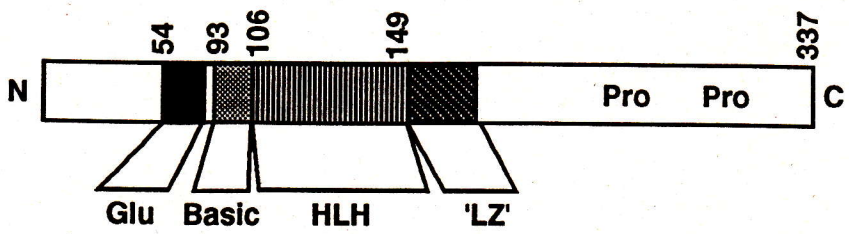
Present and/or Future Project in the DFG Schwerpunkt

Helix-loop-helix (HLH) proteins in the glial cell lineage

Following the commitment of neural stem cells to the glial cell lineage, the differentiation of oligodendrocytes is a default pathway *in vitro* and follows an intrinsic genetic program. The final stage of this program includes the high-level expression of myelin-specific structural genes. The molecular mechanism that determine glial cell fate and underlie the coordinated expression of the structural genes, however, are unknown. In Drosophila and in muscle cell development, comparable cell fate decisions are determined by a set of "master regulatory" DNA binding proteins which belong to the HLH class of transcription factors. Using highly degenerate oligonucleotide primers we have been able to clone NEX, a mammalian bHLH protein that is specifically expressed in pyramidal neurons of the adult central nervous system. With the goal to identify related "master regulatory" proteins of the glial cell lineage, we will redirect this search to well defined glial cell populations of the central and peripheral nervous system.

Methods Available

cDNA and genomic cloning techniques, generation and analysis of transgenic mice, tissue culture and gene transfer techniques, immunofluorescence, gene expression analysis.



Predicted domain structure of NEX, a novel helix-loop-helix (HLH) protein of the mammlian central nervous system (Glu, glutamic acid-rich; Basic, predicted DNA binding domain; Pro, prolin-rich domains).

Members of the Research Team

Angelika Bartholomä
Hartmut Krischke
Anja Pühlhofer
Moritz Rossner
Armin Schneider
Markus Schwab

Collaboration with Other Teams

Carol Readhead, Caltech, Pasadena, U.S.A.
Greg Lemke, Salk Institute, California, U.S.A.
Ian Griffiths, U. of Glasgow, U.K.

5 References

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Bartholomä, A. and Nave, K.-A. (1993) NEX: a mammalian neural helix-loop-helix protein and high-level expression in cortical pyramidal neurons, submitted.

Head of the Research Unit

Andreas Oksche

Address: Department of Anatomy and Cytobiology
University of Giessen
123 Aulweg
W-6300 Giessen

Phone: 49 641 702 3904
Fax: 49 641 702 3977
Bitnet:

General Research Interests

The Subcommissural Organ: An Ependymal Brain Gland

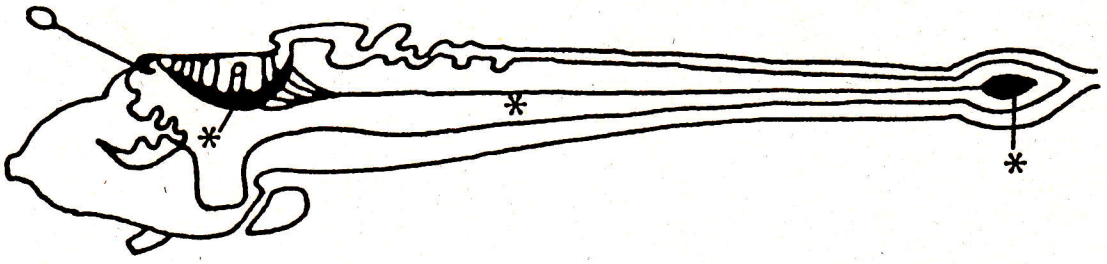
The subcommissural organ (SCO) is a complex of ependymal and ependyma-derived (non-neuronal) secretory cells covering the posterior commissure. In evolutionary terms, the SCO is a very ancient and persistent structure of the vertebrate brain. The cells of the SCO express several of the molecular markers characteristic of tanycytes. The bulk of the secretory products of the SCO, a complex containing different glycoproteins, is released into the ventricular cerebrospinal fluid. In addition, this material also gains access to the blood vessels. In contrast to a remarkable progress in the fine-structural and chemical analysis of the SCO and its secretion (including Reissner's fiber), the biological function of the SCO is still enigmatic.

Present and/or Future Project in the DFG Schwerpunkt

We have no project in the present DFG Schwerpunkt. Our investigations have been supported over 8 years by the Volkswagen Stiftung. We are open with respect to a future association.

Methods Available

Immunocytochemistry
Tracer techniques - Imaging techniques
Confocal laser microscopy - Electron microscopy (TEM, SEM)
Tissue culture techniques
Chemical analysis (E.M. Rodriguez and associates)



Schematic representation of the Subcommissural organ-Reissner's fiber complex (asterisks) in the vertebrate brain (for details see Oksche et al. 1992; Review article).

Members of the Research Team

Andreas Oksche
Frank Nürnberger
Graduate and post-graduate students

Collaboration with Other Teams

Esteban M. Rodríguez and associates, Valdivia, Chile
Pedro Fernández-Llebrez and associates, Malaga, Spain
Graduate and post-graduate students, Valdivia and Malaga

5 References

Nualart, F., S. Hein, E.M. Rodríguez and A. Oksche (1991) Identification and partial characterization of the bovine subcommissural organ-Reissner's fiber complex. Evidence for the existence of two precursor forms, Mol.Brain Res., 11: 227-239.

Rodríguez, E.M., A. Oksche, S. Hein and C.R. Yulis (1992) Cell Biology of the Subcommissural Organ, Int.Rev.Cytology, 135-121.

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Oksche, A., E.M.Rodríguez and P. Fernández-Llebrez, eds. (1993) The Subcommissural Organ. An Ependymal Brain Gland. ca 300 pp. Springer, Heidelberg Berlin New York.

Head of the Research Unit

Torsten Pietsch

Address:
Department of Neuropathology
University of Bonn
Sigmund-Freud-Straße 25
5300 Bonn
Germany

Phone: 49 228 280 3605
Fax: 49 228 280 4331
Bitnet:

General Research Interests

Production of Colony-stimulating Factors by Human Glial Cells

Glial cells are known to produce a battery of polypeptide cytokines that help them to interact with the other cell types of the central nervous system. In the last two years a lot of data have been raised to elucidate this glia-neuron and glia-endothelial cytokine network in physiological conditions and pathological alterations like tumors and inflammation in the central nervous system. A class of polypeptide cytokines that can be produced by glial cells are the colony-stimulating factors (CSFs). The target cells of these factors are inflammatory cells and endothelial cells, which are known to be activated to migrate and proliferate by CSFs.

We have established test systems to assay the production of colony-stimulating factors by glial cells on the mRNA level as well as on the protein level. G-CSF and GM-CSF is inducible in glial cells by inflammatory cytokines like TNF and IL-1. Our recent studies indicate that these cytokines are expressed in high amounts in activated glial cells in inflammatory brain diseases as well as in transformed glial cells.

Present and/or Future Project in the DFG Schwerpunkt

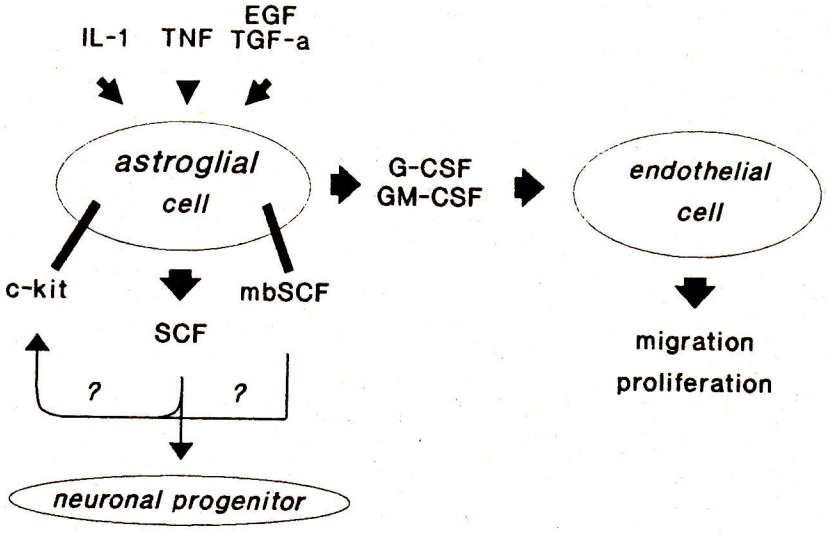
Expression of Stem Cell Factor by Astroglial Cells

One of these glial cell derived factors first identified as a stimulator of the growth of early hematopoitic progenitor cells termed **stem-cell factor** (SCF) was found to be expressed in the central nervous system by in-situ hybridization techniques. Our group was able to detect expression of SCF mRNA species encoding both for the soluble as well as the membrane bound SCF molecules in glial tumor cell lines as well as in normal cultured glial cells. Furthermore, the cell surface receptor of this factor has been identified to be the ***c-kit*** **proto-oncogene** product, a member of the tyrosine-kinase receptor family. This SCF-receptor is also widely expressed in certain glial cells as well as progenitor cells of the CNS, so that local iuxtracrine as well as autocrine mechanisms of the action of this factor seem likely.

The aim of our studies is to investigate the regulation of SCF expression in glial cells. We have recently identified specific regulators that down- and upregulate SCF expression in fibroblasts and endothelial cells. The soluble as well as membrane bound SCF proteins will be identified using antibodies generated in the last year in our laboratory. SCF protein producing cell types of the CNS as well as possible targets (SCF receptor expressing) cell types will be identified by immunohistochemistry using fresh frozen slices of human brain as well as on cultured glial cells. Finally we want to test the hypotheses that glial cells use SCF as an autocrine factor by the addition of saturating concentrations of rhSCF as well as blocking experiments using neutralizing antibodies and antisense oligonucleotides against SCF and its receptor.

Methods Available

Cell culture of normal and malignant glial cells
Cytokine assays (bioassays and immunological assays)
Cytokine receptor studies (Binding studies, chemical crosslinking)
Immunophenotyping of glial cells, immunohistology
RT-PCR and Northern blots for G-CSF, GM-CSF, SCF and its receptors



The production of colony-stimulating factors by glial cells is controlled by inflammatory and trophic factors. Target cells for G-CSF and GM-CSF are known to be brain endothelial cells which proliferate and migrate in response to these CSFs. Astroglia also expresses soluble and membrane bound (mb) stem cell factor (SCF).

Members of the Research Team

Selim Corbacioglu
Thorsten Scharmann
Martin Stanulla
Elif Yakisan
Dorothea Kajetanowitz

Collaboration with Other Teams

Karl Welte, Hannover, Germany
Kris Zsebo, Thousand Oaks, USA
Robert Thompson, Boulder, USA
Martin R. Hadam, Hannover, Germany

5 References

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Head of the Research Unit

Manfred F. Rajewsky

Address: Institute of Cell Biology (Cancer Research)
University of Essen Medical School
West German Cancer Center Essen
Hufeland-Strasse 55
D-4300 Essen 1
Germany
Phone: 49 - 201 - 723 - 2802
Fax: 49 - 201 - 723 - 5905
Bitnet:

General Research Interests

Molecular and Cellular Mechanisms in Carcinogen-Induced, Cell Lineage-Specific Malignant Transformation and Tumor Progression in the Rat Nervous System

Carcinogenesis is a multistep process interfering with the differentiation program of cells and varying mechanistically with both the type and developmental/differentiation stage of cells and the nature (molecular reactivity) of the carcinogenic agents involved. In the rat, the magnitude of the neuro-oncogenic effect and the relative proportions of different types of neural tumors induced, are thus strongly dependent on the developmental stage of the nervous system at the time of pulse-exposure to the DNA-reactive carcinogen ethylnitrosourea (EtNU). Malignant phenotypes ultimately resulting after the initial (mutagenic) interaction of the carcinogen with the target cell system appear to originate from proliferation-competent glial and Schwann precursor cells. Altered control mechanisms of proliferation and differentiation, and abnormal cell-cell and cell-matrix interactions of the "initiated" subset of cells play a crucial role in this process. We are thus focusing our investigations on genes and molecular control mechanisms critically involved in carcinogen-induced neuro-oncogenesis in a cell lineage-specific way.

Present and/or Future Project in the DFG Schwerpunkt

Structure and Function of Differentiation- and Transformation-Associated Genes in the Brain of the Rat

Our project aims at the characterization of genes whose expression, aberrant expression, or inactivation, are critically associated with both differentiation and the process of oncogenesis in the developing nervous system of the rat. Specifically, we intend to clone, and to analyze with respect to possible mutations and biological function, a gene coding for a cell surface differentiation antigen that has very recently been partially sequenced. This glykoprotein (gp130$^{RB13\text{-}6}$) is recognized by the monoclonal antibody RB-13-6 (Kindler-Röhrborn et al., Differentiation 30:53-60, 1985). On prenatal day 18 (the developmental window used for neural tumor induction by pulse-exposure to the DNA-reactive carcinogen ethylnitrosourea, EtNU) gp130$^{RB13\text{-}6}$ is transiently expressed by a small subpopulation of neural precursor cells which differentiate into a subtype of astrocytes, microglia, and ependymal cells *in vitro* (S. Blass-Kampmann, Ph.D. thesis, 1991). However, all of the EtNU-induced brain tumors thus far analyzed express the antigen, as do a collection of 16 malignant cell ines transformed in culture after exposure of fetal brain cells to EtNU *in vivo*; reminiscent of the persistent expression of the mutant *neu* (*erbB-2*) gene in malignant schwannomas induced by EtNU in the peripheral nervous system of the rat (investigated in a separate project presently supported by the Fritz Thyssen Foundation).

Methods Available

Primary cell cultures (CNS and PNS); *In situ* hybridization; Immunocytochemistry; Quantitative immunofluorescence; Electronic multiparameter analysis, cell sorting (FACS); Protein isolation and purification; PCR techniques; Gene cloning and sequencing; microinjection; hybridoma technology; transgenic animals.

Members of the Research Team

Dr. Sabine Blass-Kampmann
Helmut Deissler
Jian-Jian Jin
Dr. A.Yu. Nikitin
Jörg Papewalis

Collaboration with Other Teams

Kazymir M. Pozharisski,
St. Petersburg, Russia
Carmen Sapienza,
La Jolla, CA, USA

5 References

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Head of the Research Unit

Fritz G. Rathjen

Address:
Center for Molecular Neurobiology
University of Hamburg
Martinistr. 52
2000 Hamburg 20
Germany

Phone: 49 4717 4746
Fax: 49 4717 4839
Bitnet:

General Research Interests

Regulation of axonal growth and cell adhesion by glycoproteins with iummunoglobulin- and fibronectin type III-related domains

In the past years we have been interested in identifying macromolecules implicated in axon extension and cell adhesion during embryonic development. By using immunological techniques our work has led to the identification of several axon associated surface proteins and the ECM molecule restrictin in the chick nervous system. cDNA cloning revealed that neurofascin and F11 are composed of multiple immunoglobulin (Ig) C2- and fibronectin type III (FNiII)-like domains. Neurofascin which exists in multiple isoforms arose by alternative pre-mRNA splicing is a transmembrane protein whereas F11 interacts with the plasma membrane through a covalently linked glycolipid. Restrictin is not a member of the Ig superfamily but contains multiple FNIII- and epidermal growth factor-like repeats. Recent binding studies reveal that the Ig-like proteins and restrictin are interacting in a complex manner with each other to regulate axonal growth.

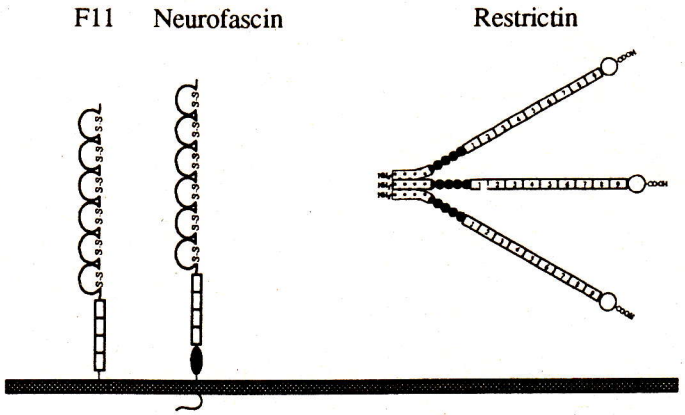
Present and/or Future Project in the DFG Schwerpunkt

Extracellular matrix and cell surface components expressed by glial cells

During extension, navigating growth cones are confronted with the surface of glial cells. *In vitro* experiments have shown that the surface and the ECM of astroglial cells support whereas those of oligodendrocytes of some animal species inhibit axonal growth. In contrast to the axonal surface the composition of the glial plasma membrane has not been studied in great detail. Although it is known that glial cells synthesize several extracellular matrix proteins including tenascin and laminin it has not been analyzed whether they also express restrictin and the Ig-like proteins F11 and neurofascin. Furthermore, receptors of the axonal Ig-like proteins including F11, neurofascin and NgCAM have not been characterized so far on glial surfaces. The major aim of our future research in the DFG Schwerpunkt is therefore to identify proteins on glial surfaces implicated in axon extension and which interact with the axonal Ig-like proteins and restrictin.

Methods Available

Protein chemical methods
Tissue culture techniques
Immunological methods including hybridoma technology
DNA recombinant technology



Basic domain organization of the axonal cell surface proteins F11, neurofascin and the ECM glycoprotein restrictin. Ig-like domains are shown as loops and FNIII-related repeats are represented as rectangles. Restrictin is composed of three identical polypeptides which are linked at the NH_2-terminus by disulfide bridges.

Members of the Research Team

Burkhardt Hassel
Michael Hubert
Roland Leuschner
Dr. Ursel Nörenberg
Dr. Hansjürgen Volkmer

Collaboration with Other Teams

Thomas Brümmendorf, Tübingen, Germany
Rainer Frank, Heidelberg, Germany
Peter Sonderegger, Zürich, Switzerland

5 References

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Head of the Research Unit

Andreas Reichenbach

Address: Carl Ludwig Institute of Physiology
Leipzig University
Liebigstrasse 27
D (O)-7010 Leipzig
Germany
Phone: 49 0341 7167 265
Fax: 49 0341 7167 570
Bitnet: ReiA@server3.medizin.uni-leipzig.de

General Research Interests

Development and Electrophysiology of (Retinal) Glial Cells

The vertebrate retina has been established as a suitable model of the central nervous system. This tissue contains one dominant form of (radial) glia *viz.* the Müller cells. Müller cells are, in the postnatal period, morphologically and immunocytochemically strikingly similar to retinal stem cells, and undergo considerable morphological changes during the period of neuronal retinal network maturation. It has been suggested that the mature phenotype of Müller cells develops by optimal adaptation to neuron-glia interactions. We studied the qualitative and quantitative morphological development of rabbit Müller cells, and the pattern of ion channels and membrane conductivities expressed by adult mammalian Müller cells. Our data demonstrate that postnatal development leads to a Müller cell phenotype that allows for great support of neuronal information processing in the retina. The aim of these studies is to define the signals mediating the development of these "symbiotic" neuron-glia interactions.

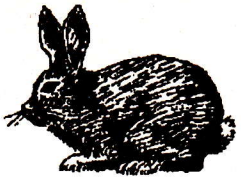
Present and/or Future Project in the DFG Schwerpunkt

Types and Topography of Ion Channels in Glial (Müller) Cells - Single-Channel Patch-Clamp Studies

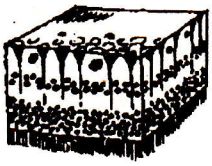
Own whole-cell and (preliminar) single-channel patch-clamp studies on mammalian Müller cells have shown that these cells express mainly K^+ channels. In adult rabbit Müller cells, we found at least four types, *viz.* inward-rectifiers, A current channels, delayed rectifiers, and symmatrical channels; probably, ATP-dependent K^+ channels are expressed as well. Furthermore, we got preliminary evidence that the different types of K^+ channels are not randomly distributed across the membrane, but rather form a distribution pattern that allows for optimal K^+ clearance. One aim of the intended project is to reveal a "map" of ion channel expression across the adult and developing Müller cell membrane, in order to detect spatio-temporal rules of channel insertion into the Müller cell membrane. Possible neuron-to-glial signals controlling this program will be tested in cell culture systems.
Another goal is to evaluate the genetic identity of the expressed K^+ channels. Recently, several families of genes have been identified that encode different types of voltage-sensitive K^+ channels, mainly for A-type and delayed rectifying currents. Presently, the first inward-rectifier channels are cloned. Thus, it will become possible to reveal the molecular identity of channels expressed by Müller cells, and to learn more on the mechanisms controlling both development and mature function of these glial cells.

Methods Available

Study of whole cell membrane currents with the patch-clamp technique (in isolated living cells)
Use of retinal wholemounts to analyze neuron-glia interactions
Measurement of glial cell surface complexity with fractal analysis
Retroviral vector labeling of retinal cell clones
Cell and organ culture techniques

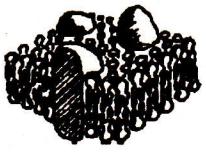


The rabbit is choosen as experimental animal since we collected ample data on structure and development of its retina and Müller (glial) cells.



Isolated retinal wholemount preparations are used for recording of currents over the Müller cell membrane during light stimulation.

Isolated living Müller cells are used for whole-cell patch-clamp studies of voltage- and ligand-activated ion currents.



Ion currents through channel proteins in clamped membrane patches will be studied in both cell-attached and inside-out configuration.

Members of the Research Team

Ivo Chao
Wolfgang Eberhardt
André Friedrich
Catrin Frömter
Katharina Kühnel
Thomas Pannicke
Susanne Pritz-Hohmeier
Winfried Reichelt
Jens-Uwe Stolzenburg

Collaboration with Other Teams

Gert Brückner, Leipzig, Germany
Dietrich Dettmer, Leipzig, Germany
Alfred Maelicke, Mainz, Germany
Dietrich L. Meyer, Göttingen, Germany
Neville N. Osborne, Oxford, U. K.
Jack Price, London, U.K.
Stephen R. Robinson, Brisbane, Australia
Jutta Schnitzer, MDC, Berlin, Germany
Thomas G. Smith, NIH, Bethesda, U. S. A.
Ernst Winkelmann, Leipzig, Germany
Hartwig Wolburg, Tübingen, Germany
Joachim R. Wolff, Göttingen, Germany

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Head of the Research Unit

	Regina Reszka	Wolfgang Walther	Friedrich Weber
Address	Max-Delbrück-Centrum for Molecular Medicine (MDC) Research Group "Drug Targeting" Robert-Rössle-Str. 10 O-1115 Berlin Germany	MDC Molecular Tumor Therapy	Neurosurgery Hospital University Steglitz Hindenburgdamm 30 W-1000 Berlin 45 Germany
Phone:	(030) 9406 2497	(030) 9406 2687	(030) 798 3516
Fax:	(030) 949 4161		(030) 798 2798
Bitnet:			

General Research Interests

Liposomes and Immunoliposomes as Carriers for Cytostatic Drugs, Magnetic Resonance Contrast Agents and Genetic Material

Liposomes are spherical lipid vesicles with one or more aqueous compartments. Depending on the liposome preparation method and the physico-chemical properties of hydrophilic or hydrophobic or even macromolecules e.g. DNA can be incorporated into the aqueous or lipid phase. For cell specific targeting of liposomes antibodies can be covalently attached to the liposome surface.
The investigations are focused on the use of liposomes as carriers in chemotherapy and gene therapy of tumor cells. This approach will be made by the i)encapsulation of cytostatic agents (carboplatin, 5-fluorouracil, mitoxantron), ii) encapsulation of lipophilic Fe^{3+}-MR contrast agents, the physico-chemical characterization of the encapsulated substances and the in vitro and in vivo testing and the iii) encapsulation of cytokine gene carrying retroviral vectors into anti-CEA (carcino-embryonic-antigen) harbouring immunoliposomes and their in vitro/in vivo testing in appropriate human tumor cells, such as glial tumors.

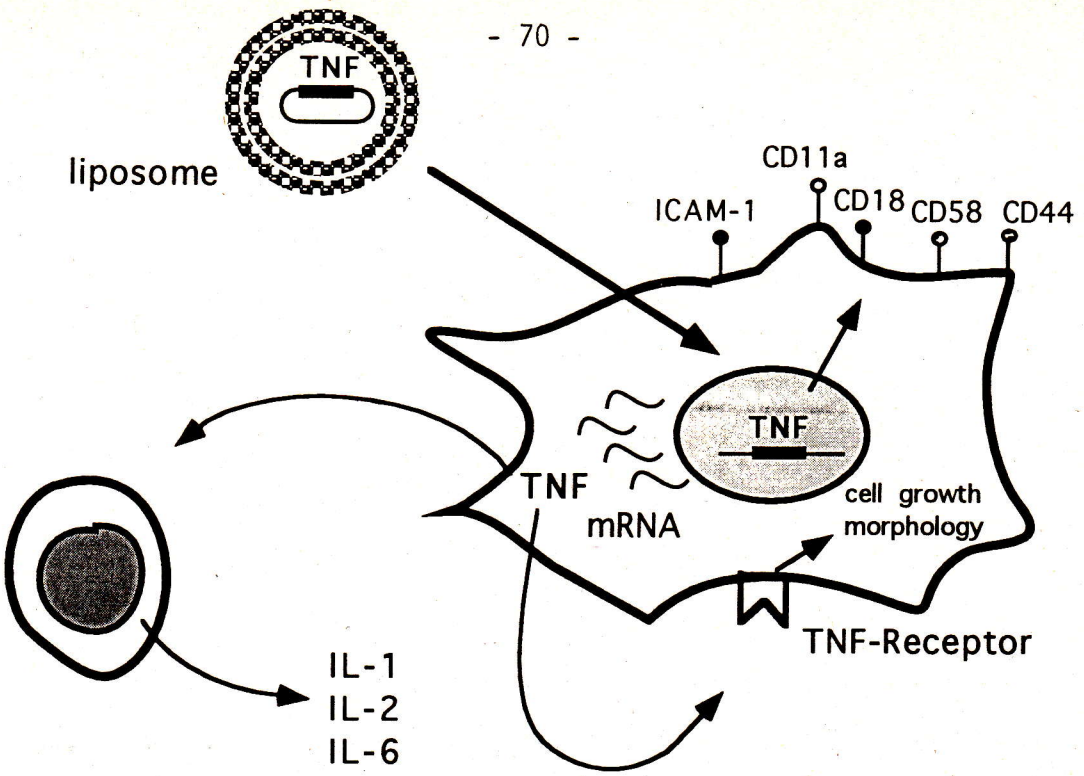
Present and/or Future Project in the DFG Schwerpunkt

Liposomal mediated gene transfer to glioblastoma cells in vitro and in vivo and studies on changes of cell to cell interactions after transfer

The aim of the study will be the preparation of liposomes containing cytokine gene carrying retroviral vectors and the cell specific gene transfer of these vectors into rat and human glioblastoma in vitro and in vivo. The pM3 neo based retroviral vector carrying the human tumor necrosis factor (TNF) cDNA will be transferred into glioblastoma cells. The gene transfer efficiancy will be assessed, the integration of the vector DNA and the expression of the cytokine in tumor cells will be analyzed on DNA, RNA and protein level respectively. We will investigate the influence of the expressed TNF on cell morphology, surface antigens and potential alterations of these antigens, the expression of adhesion molecules (ICAM-1 etc.) or the expression/induction of other cytokines (IL-1, IL-2, IL-6) in vivo.

Methods Available

Liposome preparation and characterization methods
Retroviral vector construction methods, DNA, RNA and protein detection
Surface antigen and adhesion molecule determination
Tissue culture techniques
Surgery techniques establishment of in vivo rat glioblastoma model



Liposomal gene transfer of human TNF into glial tumor cells and subsequent changes of surface antigens and induction of cytokine expression in the tumor environment.

Members of the Research Team

Angelika Gärtner
Renate Staak
Bärbel Pohl
Dr. Jörg List
Dr. Thomas Höll
Dr. Stefan Patt
Prof. Jian-Hong Zhu
Marianne Schumacher

Collaboration with Other Teams

Dr. Helmut Kettenmann, MDC, Berlin, Germany
Dr. Karin Jacobi
Dr. Michael Rudolph
Dr. Ulrike Stein
Dr. Gisela Sparmann

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Christiane Richter-Landsberg

Department of Biology
University of Bremen, NW II
2800 Bremen 33
Germany

Phone: 49 421 218 4578
Fax: 49 421 218 4042
Bitnet:

General Research Interests

Development and Differentiation of Neuronal and Glial Cells in vitro

Environmental factors, such as cell-substratum-adhesion, soluble cytoplasmic factors and cell-cell-adhesion molecules, are involved in the process of brain cell differentiation. Also, trophic interactions between developing nerve and glia cells are regulated by cell surface components, and cell surface glycoproteins may provide the specificity which is required to regulate cell-cell-recognition processes. We have applied primary cultures of embryonic rat forebrain and clonal cell lines (PC12, Neuroblastoma N115) to investigate the molecular events underlying neuronal differentiation in vitro. In particular the biochemical regulation of glycoproteins and their functional role and the involvement of extracellular factors (NGF, ACTH-fragments, cAMP-analogues) in nerve cell maturation was investigated.

Present and/or Future Project in the DFG Schwerpunkt

Neurotoxic Influences on Growth and Differentiation of Glial Cells in vitro

In response to injury a sequence of pathological changes occurs in the central nervous system, termed reactive gliosis. During this process astrocytes become hypertrophied, increase their mitotic activity and accumulate glial fibrillary acidic protein (GFAP). The molecular events that characterize this process are far from being understood, and also the role and reactions of oligodendrocytes in this process remain elusive.

The aim of our project is to characterize the growth and differentiation of rat brain macroglia (astrocytes and oligodendrocytes) in vitro on the cellular and molecular level. Glia cell cultures will be analysed (1) under normal growth conditions and in the presence of cAMP-derivatives, and (2) in the presence of neurotoxic agents (organotin derivatives). In particular, the molecular organization of cytoskeletal elements and the involvement of second messenger systems during the process of polymerization/depolymerization, and the formation of myelin-membranes will be investigated.

Methods Available

Cell culture techniques
Immunocytochemical methods
Protein analytics (1- and 2-dimensional gel-electrophoresis, Western blotting)
RNA, DNA analytics (Northern, Southern blotting), HPLC-analysis

Members of the Research Team

Angela Dippel
Michael Heinrich
Susanne Reuver

Collaboration with Other Teams

Bernd Jastorff, Bremen, Germany
Dieter Leibfritz, Bremen, Germany
Ludger Rensing, Bremen, Germany
Ephraim Yavin, Rehovot, Israel

5 References

A. Brand, C. Richter-Landsberg, D. Leibfritz (1993) Multinuclear studies on the metabolism of neuronal and glial tumor cell lines and primary cells. Physicy Medica Acta, in press.

C. Richter-Landsberg, E. Maronde, A. Besser (1993) Ecto-5'-nucleotidase in PC12 cells is synergistically modulated by nerve growth factor and 8-Bromo-cAMP. Neuroscience Res. Comm. 12, 51-56.

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M. Zorn, B. Jastorff, C. Richter-Landsberg (1993) N^6-substituted cyclic AMP analogues differentially affect cell growth and differentiation of C6 glioma cells in vitro. Eur. J. Cell. Biol., in press.

Head of the Research Unit

Wolf-Rüdiger Schlue

Address: Institut für Zoologie 1, Lehrstuhl für Neurobiologie
Heinrich-Heine-Universität Düsseldorf
Universitätsstr. 1
4000 Düsseldorf 1
Germany

Phone: 49 211 311 3414
Fax: 49 211 311 3085
Bitnet:

General Research Interests

Interaction between Neurons and Glial Cells

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

Present and/or Future Project in the DFG Schwerpunkt

Functional Role of Neurotransmitters in Intercellular Communication

In recent years we have found several receptors for neurotransmitters in neurons and glial cells. The receptors characterized so far are associated with cation channels. Receptor stimulation evokes marked changes in the intra- and extracellular concentrations of Na^+, K^+, H^+, Ca^{2+} and Cl^-. The different receptors are not homogeneously distributed in the nervous system: Neuropile glial cells possess receptors for serotonin, acetylcholine and glutamate, whereas Retzius neurons are lacking acetylcholine receptors. Furthermore, our unpublished results suggest that other neurons have neither acetylcholine nor glutamate receptors. By using electrophysiological methods and fluorescent-dye techniques we investigate the mechanisms by which neurotransmitters induce the ion activity changes. We want to map the distribution of neurotransmitter receptors in the leech central nervous system, and from that we shall get more insight into their role in neuron-glial interaction.

Methods Available

* Conventional electrophysiological methods
* Ion-sensitive microelectrode techniques
* Simultaneous monitoring of two different ions using a microscope-based fluorometric system
* Patch-clamp technique for single channel and whole-cell recording
* Histochemistry and autoradiography

Members of the Research Team

Gudrun Frey
Dorothee Günzel
Peter Hochstrate
Jörg Klusemann
Michael Müller
Michael Tesche

Collaboration with Other Teams

Walter F. Boron, New Haven, USA
Wolfgang Hanke, Stuttgart, FRG
Terry E. Machen, Berkeley, USA
Dieter Scheller, Neuss, FRG

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Schlue, W.-R. & Dörner, R. (1992) The regulation of pH in the central nervous system. *Canadian Journal of Physiology and Pharmacology* **70** Suppl., 278-285

Frey, G. & Schlue, W.-R. (1993) The pH recovery from intracellular alkalosis in Retzius neurones of the leech central nervous system. *Journal of Physiology* **462**, 627-643

Head of the Research Unit

Jutta Schnitzer

Address: Max-Delbrück-Center for Molecular Medicine
Robert-Rössle-Strasse 10
O-1115 Berlin-Buch
Germany

Phone: 49 30 9406 2490
Fax: 49 30 949 7008
Bitnet:

General Research Interests

Properties of glial cells in the developing vertebrate retina

During ontogenesis, the vertebrate retina develops as an extension of the diencephalic part of the neural tube. Cells generated at the ventricular zone of the retina give rise to all classes of retinal neurons and to Müller cells. We have been interested in the question why some mammalian retinae contain in addition astrocytes in the nerve fiber layer and, in the case of the rabbit retina, also oligodendrocytes. We could show that astrocytes and oligodendrocytes are immigrating from the optic nerve into the retina. The time course of their time of ontogenesis, development, and differentiation is independent from that of retinal neurons and Müller cells. In the course of these studies we became interested to further evaluate the questions how the development and differentiation of glial cells but also their response to injury is determined by surrounding neuronal and other glial elements and how this signal transfer takes place.

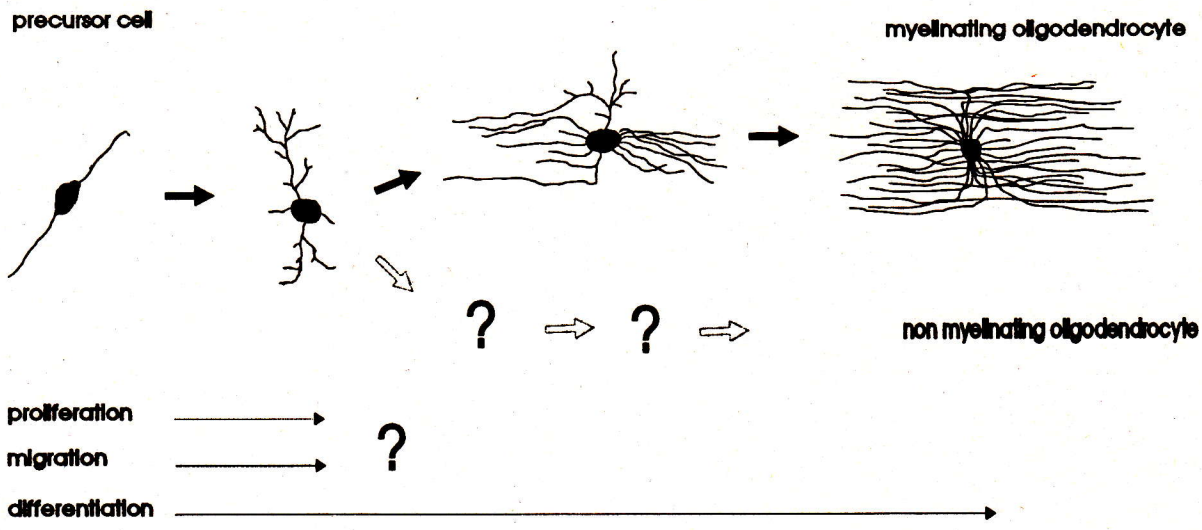
Present and/or Future Project in the DFG Schwerpunkt

Differentiation of oligodendrocytes from their precursors

The myelin-producing glial cells of the central nervous system develop during ontogenesis from highly motil precursor cells which in some instances have to migrate over large distances before differentiating. In the past we have asked the question at what stage of differentiation oligodendrocyte precursor cells are still capable to migrate. The factors influencing the migration of precursor cells are largely obscure. It is unknown why and how oligodendrocyte precursor cells are immobilized at their final destination. One of the aims of our present study is to address the question how migration of oligodendrocyte precursor cells is initiated and terminated, which interactions occur between precursors and their neighboring neuronal elements, glial cells and/or extracellular components. We plan to address these question by analyzing the role of growth factors in chemotactic assays, and by studying the role of transmitter receptors and intracellular signal pathways. We plan to quantify these effects by time lapse video microscopy. This project will be important in understanding why some parts of the central nervous system lack myelination. It might help to understand which cellular processes are necessary to perform successful regeneration.

Methods Available

Tissue culture techniques
Immunocytochemical staining techniques
Ultrastructural evaluation of lucifer yellow filled photoconverted cells



Members of the Research Team

Jürgen Scherer
Gerd Friedrich

Collaboration with Other Teams

Andreas Reichenbach, Leipzig, Germany
Helmut Kettenmann, Heidelberg, Germany
Paula M. Orkand, San Juan, Puerto Rico

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Head of the Research Unit

Wilfried Seifert

Address: Laboratory of Molecular Neurobiology
Max-Planck-Institut für biophysik. Chemie
Am Faßberg, Postfach 2841
3400 Göttingen,
Germany
Phone: 49 - 551 - 201 678
Fax: 49 - 551 - 201 779
Bitnet:

General Research Interests

Neurotrophic Factors in Neuron-Astroglial-Interaction of the Mammalian CNS

Several neurotrophic factors - such as NGF, BDNF and CNTF - have been characterized and were found to be essential for the survival, neurite outgrowth and maintenance of certain neuronal cell population. Basic FGF (Fibroblast Growth Factor) and acidic FGF are neurotrophic for the major cell population of the mammalian CNS, the pyramidal neurons of the cortex and hippocampus. Seven members of this FGF gene family (FGF 1 to FGF 7) are presently known and four different FGF receptors FGF-R1 to FGF-R4. The particular roles of these FGFs and their respective receptors in the developing and adult nervous system remain to be established.
We are investigating in our laboratory the gene expression of FGFs, FGF receptors and several immediate early genes both in astrocytes and in hippocampal neurons under normal and under neurotoxic conditions. Neurotoxicity can be induced by free radical production from hydrogene peroxide and by toxic concentrations of the neurotransmitter glutamate. The fact that glutamate can induce both FGF and NGF in astrocytes points to a new avenue of neuron-astroglial interactions and an interaction between the activity-dependent glutamate receptor activation and the gene expression of neurotrophic factors.

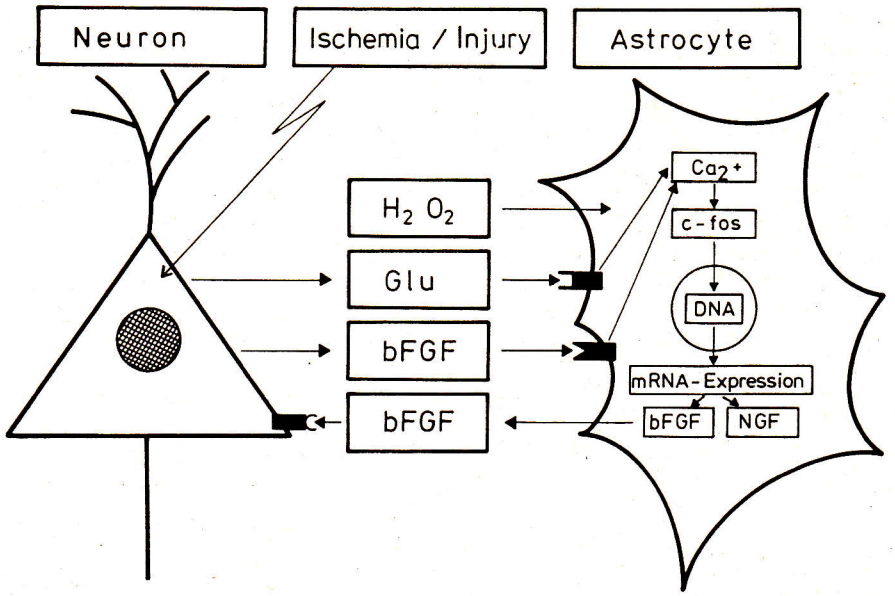
Present and/or Future Project in the DFG Schwerpunkt

We will further investigate the induction of FGFs and FGF receptors by small molecules which eventually could lead to clinical applications in neurodegenerative diseases and in processes of neuronal regeneration. Our basic research interest is the interaction between the glutamate neurotransmitter system and its receptors on one hand and the neurotrophic factors and their receptors (especially of the FGF gene family) on the other hand. Both types of receptors are found on neurons and on astrocytes and are probably regulated by complex interactions including the second messenger pathways and the regulatory immediate early genes. Understanding these interactions at the molecular level will be the foundation for understanding neuron-astroglial interaction in health and in disease.

Thus we will continue to explore the role of the different ionotropic and metabotropic glutamate receptors on astrocytes in the induction of gene expression for individual immediate early genes (oncogenes) and for neurotrophic factors (FGFs and NGF). The protective effect of FGF under toxic conditions induced by glutamate and/ or free radicals will be further inverstigated - both in-vitro in hippocampal cell cultures and in-vivo in the ischemic rat. Finally gene-transfected astrocytes will be used for cell transplantation in neuropathological conditions.

Methods Available

Primary cell cultures of astrocytes and neurons from rat cortex and hippocampus.
Fluorescence assays for cell survival and for calcium measurement.
Immunohistochemistry and in-situ hybridization.
Methods of molecular biology for gene expression and antisense strategy.
Biochemical methods for glutamate receptor binding, glutamate uptake and protein analysis.



Neuron-Astroglial Interaction mediated by bFGF and Glutamate (Glu)

Members of the Research Team

Dr. Wilhelm Gerdes

Dr. Peter Pechan

Dr. Miroslav Gottlieb

Katharina Koy

Manual Maler

Lore Dentzer

Collaboration with Other Teams

Fred Gage, Professor, San Diego, Ca, USA

Dr. Timothy Lee, National University, Singapore

Dr. Vera Valouskova, Prag,Tschech.Republik

Dr.Maria Grekova, Washington, USA

Dr.Wolfgang Brysch/ Dr. K.H.Schlingensiepen, Göttingen, Germany

Dr. Michael Hollmann, La Jolla, Ca, USA

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Head of the Research Unit

Jobst Sievers

Address: Anatomisches Institut
Universität Kiel
Olshausenstr. 40
2300 Kiel
FRG

Phone: 49 431/880 2459
Fax: 49 431/880 1557
Bitnet:

General Research Interests

Influences of meningeal cells on the development of the nervous system

Destruction of meningeal cells early in ontogeny induces severe defects in the development of the dentate gyrus and the cerebellum. Both the integrity of the secondary proliferative zones and the differentiation of the radial glia are disturbed, and this, in turn, results in defective migration and lamination of the postmitotic neurons. In vitro meningeal cells release a chemotactic activity that attracts neuronal cells from the external granular layer (EGL) of cerebellar slices. As a substrate meningeal monolayers induce the formation of radial glial fascicles from the (EGL) which are populated with migrating neurons. The study of meningeal cells and brain slices seems to be well suited for the assessment of the differentiation of radial glia and neuronal migration.

Interactions between astrocytes and mononuclear phagocytes

Microglia are thought to be resident macrophages of the brain derived from the myelomonocytic lineage which have several distinctive morphological and physiological properties. We have proposed that the specific characteristics of the microglia are induced in monocytic precursors by interactions with astrocytes, and shown that at least the ramified shape and the specific pattern of membrane currents develop only, when microglia are in contact with astrocytes, and also develop, when monocytes and macrophages are cultured on astrocytes.

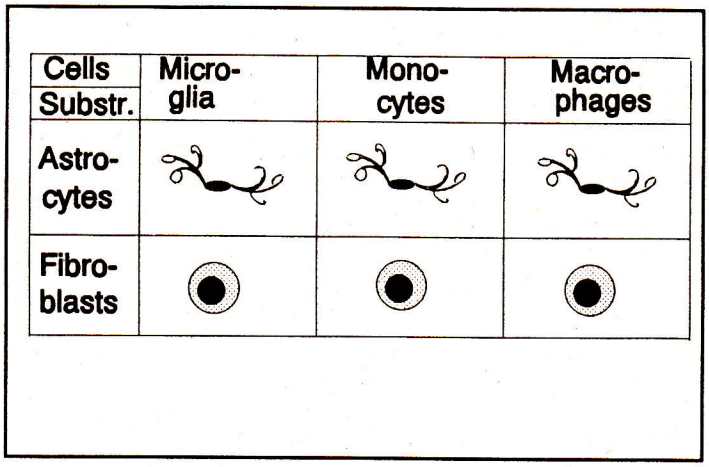
Present and/or Future Project in the DFG Schwerpunkt

Astroglial influences on the differentation of microglia, monocytes and macrophages

Microglial cells are involved in most pathological states of the nervous system, including aids and Alzheimer's disease. They differ from resident macrophages in other organs by several properties which confer a unique functional versatility to these immuneffector cells. We propose that the distinctive properties of these cells are induced in myelomonocytic precursors by interaction with astroglia, and have so far shown that at least two of the distinct properties of the microglia, i.e. their ramified morphology and membrane currents, are not intrinsic to this subpopulation of macrophages, but are acquired subsequent to their interaction with astroglial cells, since (i) they do not develop, when microglia are cultured on fibroblasts, and (ii) identical properties can be induced in both blood monocytes and spleen macrophages, when these are cultured on astrocytes. These findings demonstrate that distinct properties of microglia are induced by astroglia. We are now interested in studying other aspects of astroglial-microglial interactions, like e.g. proliferation, and additionally are starting to investigate the mechanisms of the interactions between these cells.

Methods Available

Cell culture of astroglia, microglia, oligodendrocytes, neurons, macrophages from different sources, monocytes.
Tissue culture of brain slices of different ontogenetic stages, retinal explants.
Immunohistochemistry, protein purification, in situ-hybridization, autoradiography.
Time lapse videomicroscopy.
Study of single channel and whole cell membrane currents with the patch-clamp technique.



Schematic drawing of the results of cross coculture experiments to test the hypothesis that astroglial cells induce morphological properties specific to microglial cells not only in these cells themselves, but also in monocytes and macrophages. Additionally, physiological properties like the specific pattern of membrane channels are also induced in all three groups of cells by astrocytes.

Members of the Research Team

Dr. Dieter Hartmann
Dr. Ralf Lucius
Dr. Gernot Struckhoff
Axel Wollmer

Collaboration with Other Teams

Prof. Martin Berry, London, UK
Prof. Reza Parwaresch, Kiel, FRG
Prof. Johann Schmidtmayer, Kiel, FRG
Prof. Jens Schröder, Kiel, FRG

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Head of the Research Unit

Jobst Sievers

Address:	Dr. Gernot Struckhoff Anatomisches Institut Universität Kiel 2300 Kiel
Phone:	49 431/880 2456
Fax:	49 431/880 1557
Bitnet:	

General Research Interests

Differentiation of Astroglial Cells

Different criterias are used to classify astrocytes. (i) in situ according morphological criterias (fibroblastic - protoplasmatic), (ii) in cell cultures according immunocytochemical criterias, (iii) biochemically according regional peculiarities (i.e. expression of receptors). It seems that different cells are described by every description. This discrepancy is the background of our investigations. We are seeking for the in vivo correlat of the immunocytochemically characterized type II astrocyte using as model system the nervus opticus of the myelin deficient rat. Another subject of our investigations are interactions of astrocytes with other cell types in cell culture.

Present and/or Future Project in the DFG Schwerpunkt

Interactions between Meningeal and Astrocytic Cells

Cultivated astrocytes are epithelioid cells. Conditioned media of meningeal cells induce in these cells the growth of cellular processes the astrocyte thus becoming a stellate cell. In cocultures of astrocytes and meningeal cells the astrocytic dendrites additionally develop terminal buttonlike extensions on adjacent meningeal cells and material of a basement membrane is found in the interface of both cell types. These observations lead to the working hypothesis that (i) meningeal cells in situ secrete a factor inducing an astrocytic dendritic growth in direction of the limiting membrane and (ii) that meningeal cells produce a second non diffusible factor, which is bound to the cell membrane and/or extracellular matrix produced by astrocytes and/or meninges, leading to the formation of the glia limitans superficiales.

Our goal is to characterize the processes involved and leading to the formation of the glial limiting membrane.

Methods Available

Tissue culture techniques
Protein chemical methods
Immunocytochemistry
Electronmicroscopy

Members of the Research Team

I am working in the Research Team of Prof. Dr. Sievers, Kiel

Collaboration with Other Teams

Dr. Andreas Gocht, Hamburg

5 References

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Head of the Research Unit

Jobst Sievers / Dieter Hartmann

Addresses: Anatomisches Institut der Universität Kiel
Olshausenstraße 40
D-2300 Kiel 1
FRG

Phone: 0049 - 0431 - 880 - 2467
Fax: 0049 - 0431 - 880 - 1557
Bitnet:

General Research Interests

Differentiation and function of radial glial cells during cortical development

In vivo experiments with late - developing radial glial cell populations in the cerebellar cortex and dentate gyrus and the corresponding neuronal strata have demonstrated a crucial role of the overlying mesenchyme, the meninges, for both the establishment of a radial phenotype in glial cells and the migration of undifferentiated neurons. Meningeal cells as well as other fibroblasts release a species -specific activity, that stimulates neuronal emigration from the cerebellar external granular layer (EGL). By a contact - mediated interaction, fibroblasts induce the differentiation of fasciculating radial glial cells guiding neuronal migration, an effect, that in part can be mimicked by the application of laminin substrata, but not other components of the basement membrane.

Functional role of cell - surface glycosyl transferases as adhesion molecules for neurons and glia

The ß(1,4) - galactosyltransferase (EC 2.4.1.38) mediate and regulate cell contacts to laminin substrata and possibly other cells in a number of in vitro cell systems. The specific blockade of this enzyme is also effective in inhibiting glial cell contacts to laminin and is discussed as a possible pathomechanism for the development of the reeler malformation of cortical development.

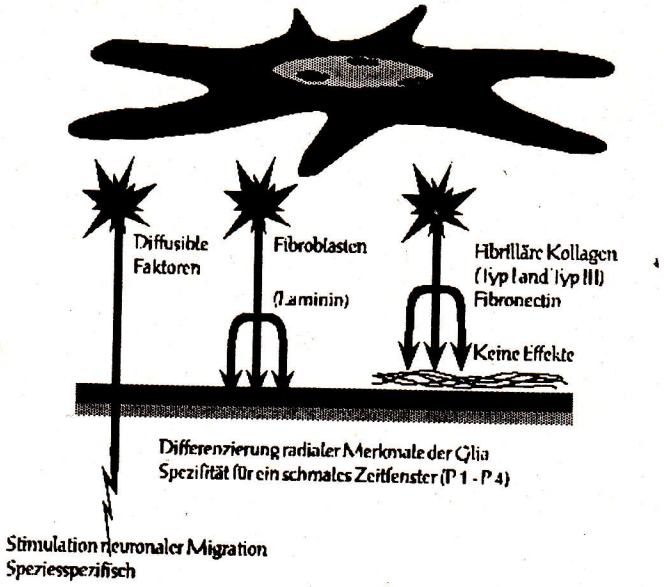
Present and/or Future Project in the DFG Schwerpunkt

Analysis of an in vitro model of cortical development

We have developed a two - chamber slice culture model to investigate the underlying mechanisms of cellular interactions not only between migrating neurons and radial glia, but also the mechanism of induction of the radial phenotype and neuronal migration due to mesenchymal influences. We are currently analyzing meningeal cell cocultures with slice explants as (i) a model for the surface - associated neuronal migration within the cerebellar EGL and (ii) their function of inductors of radial glial cells. Concerning the underlying molecular mechanisms, we are investigating a cell - surface associated galactosyltransferase as a cell contact mechanism. Thereby, we have set out to establish a culture model of the reeler malformation of cortical development to analyze enzyme distribution by incubation with labelled specific ligands and antibodies and function by applying blocking substances during migration in vitro in order to compare the effects observed with wild -type and reeler cultures.

Methods Available

Cell culture of astrocytes, meningeal cells and fibroblasts derived from other tissues
Tissue culture of brain slices of different ontogenetic stages
Two - chamber coculture systems to analyze contact- and diffusion-mediated interactions
Immunohistochemistry, in situ - hybridization
Time - lapse videomicroscopy



Members of the Research Team

Prof. Dr. Jobst Sievers
Dr. Dieter Hartmann

Collaboration with Other Teams

Dr. Susanne Fehr; Hamburg

5 References

Sievers, J.; Hartmann, D.; Pehlemann, F.W.; Berry, M. (1992) Development of astroglial cells in the proliferative matrices, the granule cell layer and the hippocampal fissure of the hamster dentate gyrus. J. Comp. Neurol. 320 : 1 - 32

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Hartmann, D.; Sievers, J. (1993) Meningeal cells stimulate neuronal migration and induce the formation of radial glial fascicles from the cerebellar external granular layer. Manuscript in preparation

Head of the Research Unit

Christian Steinhäuser

Address:
Institute of Physiology
University of Jena
Teichgraben 8
O-6900 Jena
Germany

Phone: 49 3641 8224954
Fax: 49 3641 8224286
Bitnet:

General Research Interests

Voltage- and ligand-operated ion channels of central glial cells

Recent studies have indicated that glial cells in cell culture can express a variety of voltage- and ligand-activated ion channels. To test for the presence of membrane channels and receptors on glial cells *in situ* as well as to access neuron-glia interactions we apply the patch-clamp technique to brain slices of the postnatal hippocampus. For cell identification, cells under study are filled with fluorescent dyes during the electrophysiological recordings. In addition, to quantify channel properties in more detail cells were acutely isolated from the same brain areas prior to current analysis. Based on the membrane current pattern, we distinguished at least four different glial cell types in the postnatal hippocampus. These cells express voltage-gated Na^+-, K^+- and Ca^{2+}-channels and receptors for the most abundant neurotransmitters in the brain, GABA and glutamate. Our studies demonstrate differences in the properties of glial transmitter receptors as compared with their neuronal counterparts.

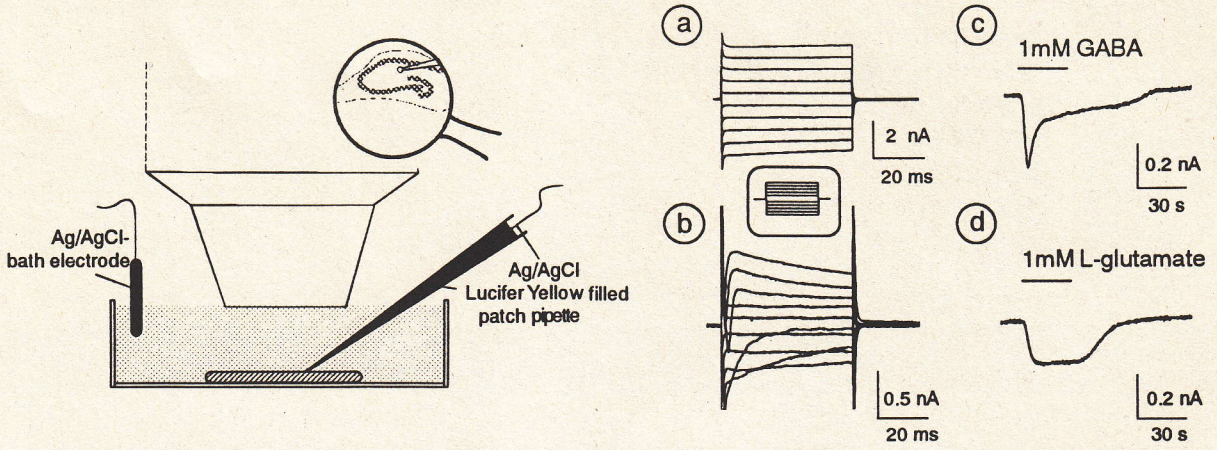
Present and/or Future Project in the DFG Schwerpunkt

Neuron-glia interactions

Glial cells lack the ability to generate fast action potentials but they are excitable cells. It seems very likely that they are activated during normal physiological processes. Judging from the *in situ* expression of various glial membrane receptors and the very close association between glial processes and synaptic regions we suppose that glial cells directly influence the information transfer between neurons. To analyze interactions between glial cells and neurons we stimulate neuronal tracts in the hippocampus and simultaneously record corresponding responses in identified neurons and nearby glial cells. We intend to induce long lasting changes in the synaptic efficacy such as LTP or LTD to look whether this change of neuronal activity feeds back onto glial cells.
It is well known from cell culture and a few *in situ* studies that glial ion channels are up- and down regulated during development. Therefore, in our analysis of hippocampal neuron-glia interactions we have to address the important issue of channel expression in glial cells during maturation.

Methods Available

Membrane current analysis with the patch-clamp technique in situ and in acutely isolated cells
Concentration clamp technique to record transmitter responses with high time resolution
Use of brain slices to analyze neuron-glia interactions
Fluorescence measurements for cell identification



On the left side, the application of the pach-clamp technique to hippocampal brain slices is schematically drawn. Glial cells are clamped at a negative holding potential (e.g. -70 mV) and voltage- (a,b) or transmitter-activated membrane currents (c,d) are analyzed.

Members of the Research Team

Marco Weber
Ronald Jabs
Klaus Kressin
Elena Kuprijanowa

Collaboration with Other Teams

Michael Frotscher, Freiburg, Germany
Helmut Kettenmann, Heidelberg, Germany

5 References

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Head of the Research Unit

Wilhelm Stoffel

Address: Institut für Biochemie
Medizinische Fakultät der Universität zu Köln
Joseph-Stelzmann-Straße 52
5000 Köln 41
Germany
Phone: +(49)-221-478-6980
Fax: +(49)-221-478-6979
Bitnet:

General Research Interests

Among the glial cellular elements oligodendrocytes play a pivotal role in myelogenesis. Our structural studies on myelin proteins (e. g. proteolipid protein) and the transition to the molecular biology approach has led to PLP gene structure, chromosomal location and studies of the temporal and spatial expression of the main myelin proteins. In addition, the molecular events of the X-chromosomal dysmyelinosis of the rat (md-rat) and man (Pelizaeus-Merzbacher disease) have been elucidated.
Besides the myelin proteins the enzymes responsible for the explosive synthesis of the complex oligodendrocyte-specific sphingolipids are the target of our contribution towards an understanding of the "maturation process" of the brain.
The incidental discovery of the neurotransmitter glutamate transporter of CNS has opened up an additional exciting field.

Present and/or Future Project in the DFG Schwerpunkt

Oligodendrocyte development

At present we are establishing the transgenic mouse model by homologous recombination in order to learn about the functions of PLP and its isoprotein DM20 each solely expressed during the differentiation of oligodendrocytes and also to establish disease models in myelination in the rat.
Of particular interest is the multiple fatty acylation of proteolipid protein and the biological meaning of the dynamics of this acylation process.

Methods Available

All recombinant DNA techniques – in vitro expression, stable expression cell lines (oocyte expression), in situ hybridization techniques, immunocytochemistry, protein purification and analysis, enzymology, complex lipid analysis, tissue culture techniques, embryonic stem cell and homologous recombination, transgenic mouse.

Members of the Research Team

Thomas Weimbs
Dr. Freimut Schließ
Detlev Boison
Birgit Fath
Michael Körner
Roger Janz

Collaboration with Other Teams

5 References

1) T. Weimbs and W. Stoffel (1992) Proteolipid Protein (PLP) of CNS Myelin: Positions of Free, Disulfide Bonded, and Fatty Acid Thioester-Linked Cysteine Residues. Implications for the Membrane Topology of PLP. *Biochemistry* **31,** 12289-12296.

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Head of the Research Unit

Guido Stoll

Address:

Department of Neurology
Heinrich-Heine-Universität
Moorenstr. 5
4000 Düsseldorf 1
Germany
49 211 3118415
49 211 3118485

General Research Interests

The cellular mechanisms that lead to immune-mediated demyelination in the PNS and CNS are not well understood. In culture systems not only T-cells and macrophages but also glial cells can be stimulated to synthesize cytokines and express immune activation markers such as MHC class I and II antigens and intercellular adhesion molecules. The roles of glial cells in concert with infiltrating leukocytes in the pathogenesis of autoimmune diseases of the nervous system are not well defined. Our investigations focus on the localisation of immune factors (cytokines, MHC class I and II antigens, immune adhesion molecules) in autoimmune demyelination of the peripheral and central nervous system in comparison to nonimmune nerve damage such as Wallerian degeneration.

Present and/or Future Project in the DFG Schwerpunkt

We have no project in the present DFG Schwerpunkt. However, we would be very interested in an association in the future.

Methods Available

Immunocytochemistry on 1um cryosections
in-situ-hybridisation

Members of the Research Team

Sebastian Jander
Guido Stoll

Collaboration with Other Teams

Hans-Georg Fischer, Düsseldorf
Hans-Peter Hartung, Würzburg
Hans Werner Müller, Düsseldorf

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Head of the Research Unit

Claudia A.O. Stuermer

Address:
Faculty of Biology
University of Konstanz
Am Gießberg 10
W-7750 Konstanz
Germany

Phone: Tel. ++49-7531 88-22 36
Fax: FAX ++49 7531 88-38 94
Bitnet:

General Research Interests

Glial cells in lower vertebrates and axonal regeneration

In contrast to mammals and birds neurons in the CNS of fish are capable of regenerating their axons after injury. One reason for the success of axonal regeneration in the fish optic nerve lies in the properties of the oligodendrocytes. Fish oligodendrocytes and CNS myelin lack the axon-growth inhibiting proteins that impair the regrowth of injured axons in mammals. Moreover, fish oligodendrocytes - at least in culture - possess axon-growth-supportive molecules on their surface. Thus, on a carpet of fish oligodendrocytes not only fish but even retinal explants from adult rat extend axons to considerable density and length. One of the growth supporting molecules on fish oligodendrocyte is the E 587 antigen, an L1-like cell adhesion molecule, that is also expressed by regenerating retinal axons. Ongoing studies are devoted to further characterize the properties of the fish oligodendrocytes in vivo, by determining their state of differentiation and expression of characteristic molecules when axons regenerate and after axonal regeneration is completed.

Present and/or Future Project in the DFG Schwerpunkt

Evolution of oligodendrocyte specific properties and axonal regeneration

Since oligodendrocytes and myelin in the fish visual system are axon-growth-permissive and probably contribute to the success of retinal axonal regeneration, we asked whether similar rules would apply to the fish spinal cord. We first determined five populations of neurons in the fish mid- and hindbrain that send their axons into the spinal cord. Upon spinal cord transection neurons of all five groups can regenerate their axons. The oligodendrocytes isolated from the regenerating spinal cord resemble in their morphology and by immunocytochemical criteria closely to those in the optic nerve. Spinal cord derived oligodendrocytes lack axon-growth inhibitors, express in vitro growth promoting molecules and thus are likely to support (as optic nerve derived oligodendrocytes) axonal regeneration in vivo.
A striking difference between oligodendrocytes in the forebrain and spinal cord has been detected in the CNS of the amphibian species Xenopus where axonal regeneration occurs - as in fish - in the optic nerve but not in the spinal cord. Current and futufre studies are devoted to compare further the properties of the fish and amphibian glial cells. Specifically we aim at detecting which species has "invented" axon-growth inhibiting molecules and whether this - as recent data in frog spinal cord suggest - goes in parallel with the lack of CNS axonal regeneration.

Methods Available

- Tissue culture of neurons and glial cells of lower vertebrates and mammals, cross-species, neuron glial co-cultures
- Neuronal tracing techniques in vivo
- Time lapse videomicroscopy for studies in vitro and in vivo
- Production of mono- and polyclonal antibodies, immunopurification of proteins

cDNA-cloning techniques

Members of the Research Team

Dr. Martin Bastmeyer
Dr. Suzanne Giordano
Rolf Käthner
Ute Laessing
Dirk Lang
Dr. Herbert Schaden
Thomas Schulte

Collaboration with Other Teams

Gunnar Jeserich, Osnabrück, Germany
Friedrich Lottspeich, München, Germany
Melitta Schachner, Zürich, Switzerland
Martin Schwab, Zürich, Switzerland
Pate Skene, Durham, USA
Monte Westerfield, Eugene, USA
Nicole Le Douarin, Paris, France
Dr. Brigitte Stecher
Ursula Topel
Marianne Wiechers

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Head of the Research Unit

Gerhard M. Technau

Address:
Institute for Genetics
University of Mainz
Saarstr. 21
6500 Mainz
Germany

Phone: 49 6131 395341
Fax: 49 6131 395845
Bitnet:

General Research Interests

The origin of cell diversity in the CNS

The mechanisms that underlie the transformation of the two-dimensional sheet of neural ectoderm into the highly specified three-dimensional nervous system belong to the major unsolved problems in developmental biology. The embryonic development of the CNS involves three principal steps, namely the specification of the neurogenic region of the ectoderm, of the metameric units (neuromeres) and their identities and of the precursor cell identities within these units (cell lineages). The determination of these regional and cellular qualities is mediated by positional information and cell-communication. We analyze on the cellular as well as molecular level aspects of these processes during early CNS-development of Drosophila.
To investigate the spatial and temporal dynamics as well as the contribution of intrinsic versus extrinsic factors during cell-fate determination we trace the development of individual cells in situ, in vitro and upon heterotopic, heterochronic and heterogenetic transplantations. To identify and characterize genes and their products involved in specific determinative events we use the tools of mutant analysis and molecular genetics.

Present and/or Future Project in the DFG Schwerpunkt

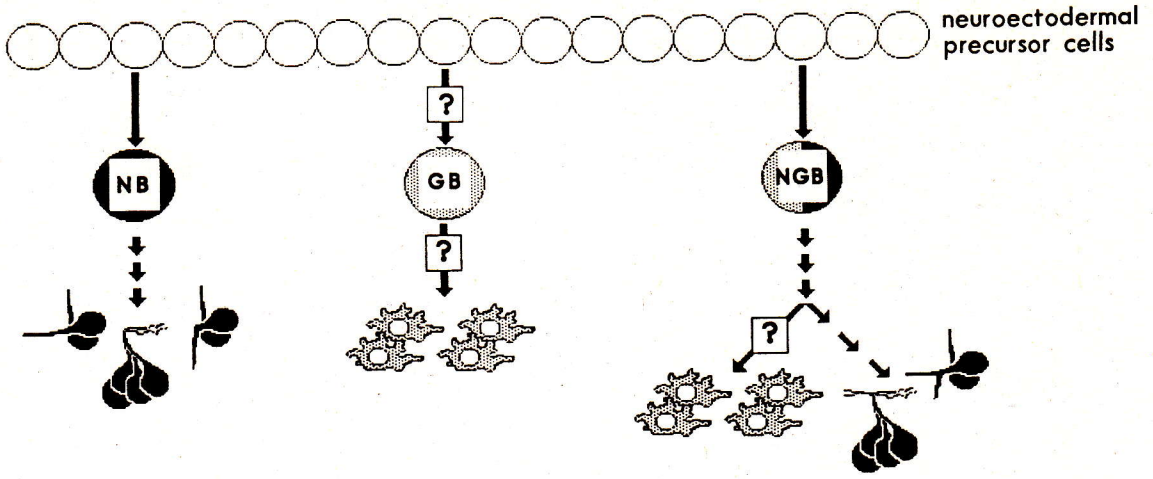
Molecular mechanisms underlying glia-determination in the CNS of Drosophila

In order to identify and characterize genes involved in the determination of glial cell types, and to produce cell-specific markers we performed a large scale enhancer-trap screen. We applied a modification of the method using the yeast transcription factor GAL4 as a reporter gene. Enhancer traps generated with this construct allow one to express any cloned gene linked to the GAL4 binding site (UAS) in the cells in which a particular GAL4 enhancer trap is active. The respective GAL4 expression patterns (usually reflecting the activity of a nearby Drosophila-gene) are demonstrated by crossing with a transformant strain carrying a secondary reporter construct (e.g. ß-gal-kinesin) linked to UAS. For a number of lines expressing the reporter in embryonic glial cells we analyzed the spatial and temporal patterns of expression in detail. One of these lines carries the p-element insertion in the gene eagle. To generate mutant alleles we remobilized the p-element. We began to clone this region.

Further studies are aimed at a detailed molecular characterization of the eagle gene to clarify the function of this gene with respect to glial development. Using our enhancer-trap lines we will investigate additional genes which are presumably involved in glia determination and differentiation. Taking advantage of the GAL4 system we also plan to ablate various glial cell types by the expression of cell autonomous toxins to look for possible defects in postembryonic development.

Methods Available

Labelling of single cells in vivo with lineage tracers (e.g. HRP; DiI); cell transplantations; primary culture of individual cells; immunohistochemistry; time-lapse videomicroscopy and image processing; 3D-reconstructions; genetics; mutagenesis; enhancer-trap techniques; germ line transformation; mosaic analysis; in situ hybridisation; molecular genetics



Three types of precursors segregate as single cells from the insect neurogenic ectoderm: neuroblasts (NB) with pure neuronal progenies, glioblasts (GB) giving rise only to glial cells and neuroglioblasts (NGB) which produce both, neurons and glia. The mechanisms leading to the decision towards glial cell fate are currently unknown and are the subject of our investigations.

Members of the Research Team

Torsten Bossing
Rainer Dittrich
Dr. Kei Ito
Andreas Prokop
Urs Schmidt-Ott
Jürgen Sohn
Gerald Udolph
Dr. Joachim Urban
Olaf Vef

Collaboration with Other Teams

Rolf Bodmer, Ann Arbor, USA
Karl-Friedrich Fischbach, Freiburg, Germany

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Technau GM: Experimental approaches for studying the development of the central nervous system in Drosophila. Verh. Dtsch. Zool. Ges. 85.2, 111-131 (1992)

Head of the Research Unit

Jacqueline Trotter

Address: Department of Neurobiology
University of Heidelberg
Im Neuenheimer Feld 364
W-6900 Heidelberg
Germany
Phone: 49 6221 563 819
Fax: 49 6221 563 700
Bitnet:

General Research Interests

Development and function of myelin-forming Glia

The main focus of the group is the regulation of myelination by oligodendroglial cells and the cellular and molecular mechanisms regulating remyelination of demyelinating lesions. Prior to forming myelin lamellae, the oligodendroglial precursor cells migrate, proliferate and interact with the axonal surface upon which the myelin sheath is to be laid down. In order to generate sufficient quantity of material to facilitate biochemical analysis of the surface receptors involved in such interactions, we have generated a range of lines of immortalised glial cells using oncogene-carrying replication-defective retroviruses. These cell lines interact with axons *in vitro* and *in vivo* after transplantation into demyelinated lesions. They thus express the spectrum of cell surface receptors required for these specific interactions.

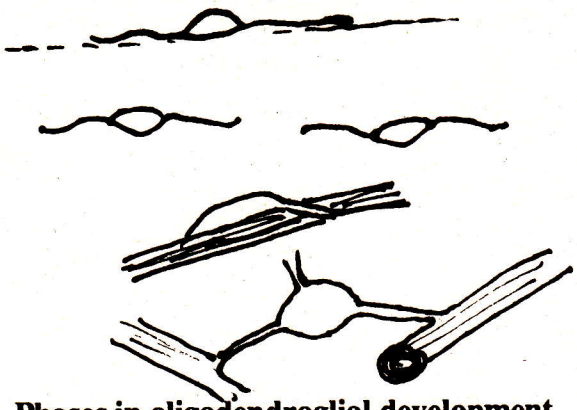
Present and/or Future Project in the DFG Schwerpunkt

Identification of new cell-surface receptors on oligodendrocytes

Since the cell lines described above mimic closely properties of their normal counterparts, we are using them as immunogens for the production of monoclonal antibodies against oligodendroglial cell surfaces. We hope to define new surface molecules which are not only useful as markers but also recognise molecules of functional importance such as those involved in signal transduction. Various strategies are being used: we have obtained some antibodies which further define subpopulations of oligodendrocytes and their precursors. We are using lectins to further delineate groups of glycoproteins on the cell surface against which antibodies are being generated. The new antibodies are being screened for their ability to influence cell behaviour, such as precursor cell proliferation or oligodendrocyte-neuron interaction. The cell lines we have generated are readily infected by a range of demyelinating viruses and can thus be used as a model to study these processes (in collaboration with I. Sommer, University of Glasgow) .

Methods Available

Tissue culture of glial cells and neurons.
Use of retroviruses.
Standard biochemical techniques.
Generation of monoclonal antibodies.



Phases in oligodendroglial development

Members of the Research Team

Dr. Marion Jung

Thomas Koch

Antje Niehaus

Collaboration with Other Teams

W. Blakemore, Cambridge, England.

H. Kettenmann, Heidelberg, Germany.

M. Schachner, Zürich, Switzerland.

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Head of the Research Unit

Reiner Westermann

Address: Department of Anatomy & Cell Biology
University of Marburg
Robert-Koch-Str. 6
W-3550 Marburg
Germany
Phone: -6421-284023
Fax: -6421-287066
Bitnet:

General Research Interests

Growth factors (GFs) in the nervous system (NS)

Nerve growth factor (NGF) has long been proposed to be the one and only GF with relevance to the NS. But, during the last years, around 50 GFs have been shown to be expressed by, or, to act on cells of the NS. It became obvious that several different GFs may play key roles during development, maintenance and regeneration in the NS. Analysis of GF function in the NS was complicated by the discoveries that GFs may directly regulate their own expression, the synthesis of other GFs and their receptors, and, that the effect of a (physiological) mixture of GFs may be quite different from that of each of the single factors.

We have chosen two model systems, C6 glioma cells (glia model) and adrenal chromaffin cells (neuron model), for the initial identification and characterization of the different GFs being expressed by and/or acting on glial cells and neurons. Our current interest is focused on three families of established or putative GFs and their receptors: FGFs, TGFbetas and granins, and we started to analyze regulation of synthesis, molecular properties, secretion, effects, and physiological as well as pathological functions of these GFs in neurons and glial cells.

Present and/or Future Project in the DFG Schwerpunkt

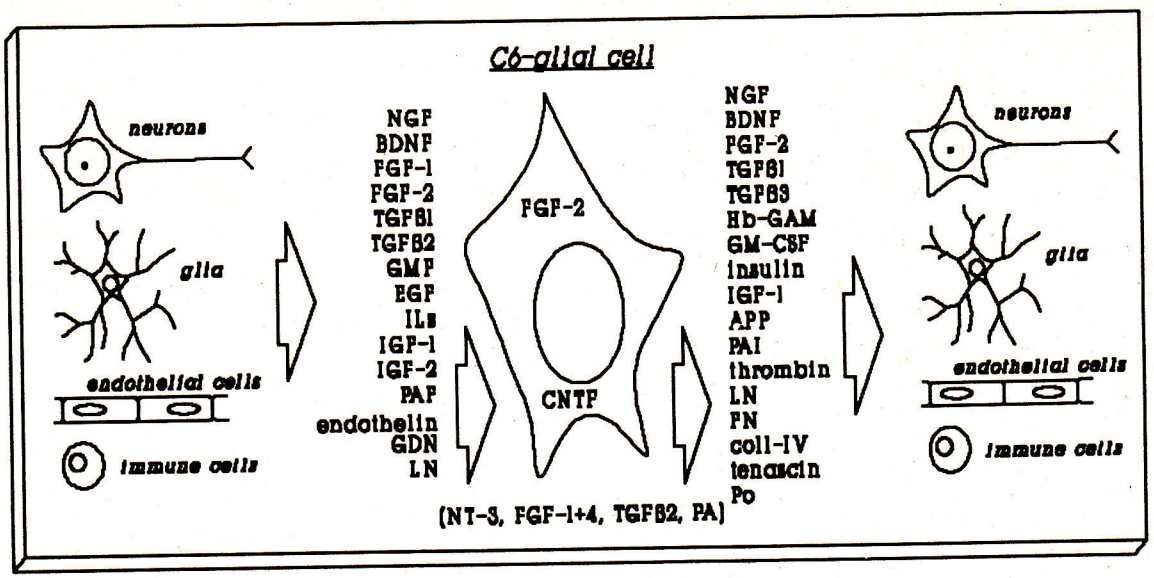
Growth factors in C6 glioma cells

In our previous work we have shown that C6 cells synthesize and release at least 15 different GFs and GF-related molecules, five of which need to be identified and characterized. Five established GFs were obviously not synthesized by C6 cells. For 17 GFs effects or functional receptors have been detected on the cells. Nine additional GFs have been identified in C6 cells by other groups. From the figure, which shortly summarizes these data, it may become obvious that, concerning GFs, C6 cells may be the best characterized glial cell type.

We are currently investigating the modulation of GF-synthesis in C6 cells by other GFs present in the glial environment (starting with TGFs, FGFs and their receptors). These alterations in GF-output may directly influence glia-glia, glia-neuron and glia-endothelial interactions. Experiments (in vitro & in vivo) with C6 cells with modified GF-expression (molecular or immunological knock out of GFs, etc.), and use of three-dimensional cultures (spheroids), should help to elucidate the function of these glia-derived GFs in the NS. Furthermore, we are interested in the possible therapeutic potential of C6-cells as GF-source in neurodegenerative diseases (e.g. Parkinsons disease).

Methods Available

Cell (tissue) culture: glial & glioma cells, neurons, spheroids
Biochemical methods: protein purification and characterization
Immunological methods: antibody production, IHC, Westerns, ELISA, RIA
Specific GF-assays: e.g. cell survival-, proliferation-, differentiation-assays
Histological methods: general



Summary of GFs acting on (left side), or being expressed (middle) and released (right side) by C6 glioma cells (GF in [ ] were not detectable in C6 cells).

Members of the Research Team

Christel Hanstein
Petra Hartmann

Collaboration with Other Teams

Gerd Aumueller, Marburg, Germany
Dieter Blottner, Berlin, Germany
Claudia Grothe, Freiburg, Germany
Thierry Janet, Heidelberg, Germany
Georgia Lahr, Ulm, Germany
Arthur Mayerhofer, Ulm, Germany
Satoshi Omura, Tokyo, Japan
Arndt Steuernagel, Goettingen, Germany
Klaus Unsicker, Heidelberg, Germany

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Head of the Research Unit

Heinrich Wiesinger

Address:
Physiologisch-chemisches Institut
der Universität
Hoppe-Seyler-Str. 4
7400 Tübingen
Germany

Phone: 49 7071 293338
Fax: 49 7071 295360
Bitnet:

General Research Interests

Transport Processes in Glial Cells

Transport of non-electrolytes as well as charged compounds across the plasma membrane is a fundamental process related to a variety of glial functions. We use cultured glial cells to investigate transport processes as a) part of substrate utilization for energy production (glucose, mannose, sorbitol) and the generation of second messengers (*myo*-inositol, arginine), b) part of volume and pH regulatory functions (*myo*- inositol, lactate), c) part of substrate channeling from the glial cells to neurons (glucose, lactate). The goal of our studies is to describe the mechanism of each of these transport processes and correlate them to the contribution of glial cells to the proper functioning of the brain.

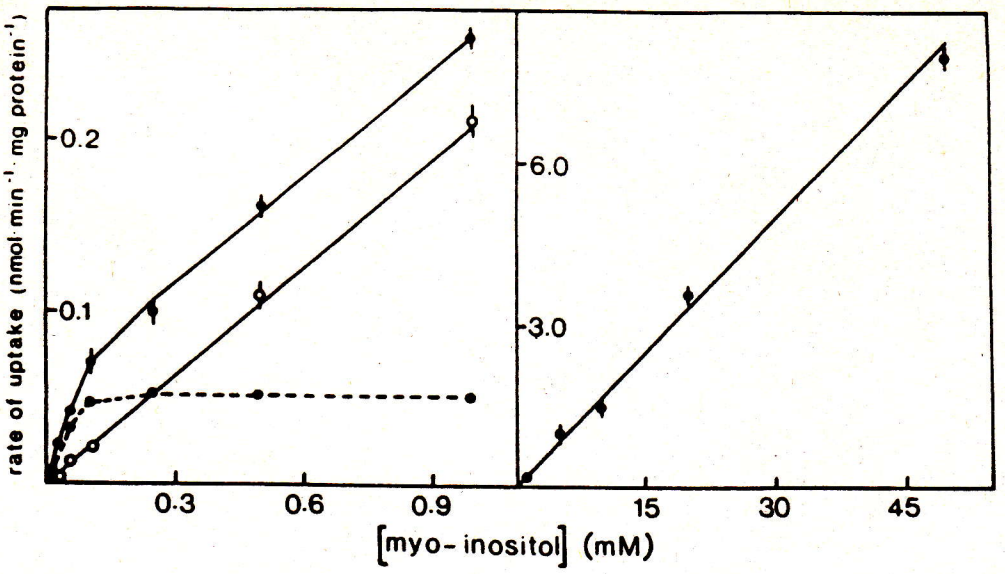
Present and/or Future Project in the DFG Schwerpunkt

Polyol Transport in Cultured Glial Cells

We have shown recently that astroglial cells can be cultured in a glucose-free medium as long as sorbitol is present. Sorbitol is taken up by the cells by diffusion through a proteinaceous pore in the plasma membrane. Since mouse oligodendroglial and microglial cells cannot survive in glucose-free medium in the presence of sorbitol we postulated that these cell types have not the unique membrane properties that cultured astrocytes exhibit. This hypothesis has to be investigated with pure cultures of oligodendrocytes and microglial cells. We also intend to characterize further the biochemical nature of the pore with protein modification methods. In contrast, transport of *myo*-inositol in astroglial cells consists of two components, a sodium-dependent, carrier-mediated process with a K_M-value of about 50 µM, and, at high concentrations, a sodium-independent diffusional process with characteristics similar to sorbitol transport. With respect to the potential role of *myo*-inositol as an osmolyte in the brain, transport measurements under varied osmotic conditions and after direct mechanical stretching will be performed together with volume measurements in a Coulter counter. In all these cases uptake of sorbitol will be investigated as a model process of diffusional *myo*-inositol transport.

Methods Available

Protein purification
Production of antibodies
Cell culture techniques
Immunocytochemistry
Radioactive tracer techniques
Coulter counter (volume determination)



Rates of myo-inositol uptake in a 17-day-old astroglia-rich mouse primary culture as a function of the concentration of extracellular *myo*-inositol in the presence (●) and absence (○) of 150 mM NaCl. In the latter experiment, Na^+ was isoosmotically replaced by choline. Dotted line represents saturable component of uptake alone. From: H. Wiesinger (1991) J. Neurochem. 56: 1698-1704.

Members of the Research Team

Hajo Peters
Andreas Schmidlin

Collaboration with Other Teams

5 References

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Head of the Research Unit

Hartwig Wolburg

Address:
Institute of Pathology
University of Tübingen
Liebermeisterstraße 8
D-7400 Tübingen
Germany

Phone: 49 7071 29 6890
Fax: 49 7071 29 2258
Bitnet:

General Research Interests

Induction of the blood-brain barrier

Brain microvessels are invested mainly by astroglial cell processes. The subendothelial as well as the subpial membranes of astrocytes are occupied by orthogonal arrays of particles (OAPs) which are visualized exclusively by means of of freeze-fracturing and hypothetized to carry ionic curents. Their expression seems to be dependent on both the presence of a basal lamina and neuronal activity. The distribution of OAPs across the surface of the glial cells is highly polarized.
Astrocytes are believed to be responsible for the induction of blood-brain barrier (BBB) properties such as tight junctions and glucose transporter in the endothelial cells (ECs). In an in vitro model, we cocultured astrocytes together with ECs and observed a modulation of the EC tight junctions by astroglial factors. In mammals, an intact BBB seems to be coupled with astrocytes highly polarized in terms of OAP distribution. Our aim is to define the conditions necessary for the complete induction of BBB properties and to understand the signal cascade between neurons, glial cells and the vascular system.

Present and/or Future Project in the DFG Schwerpunkt

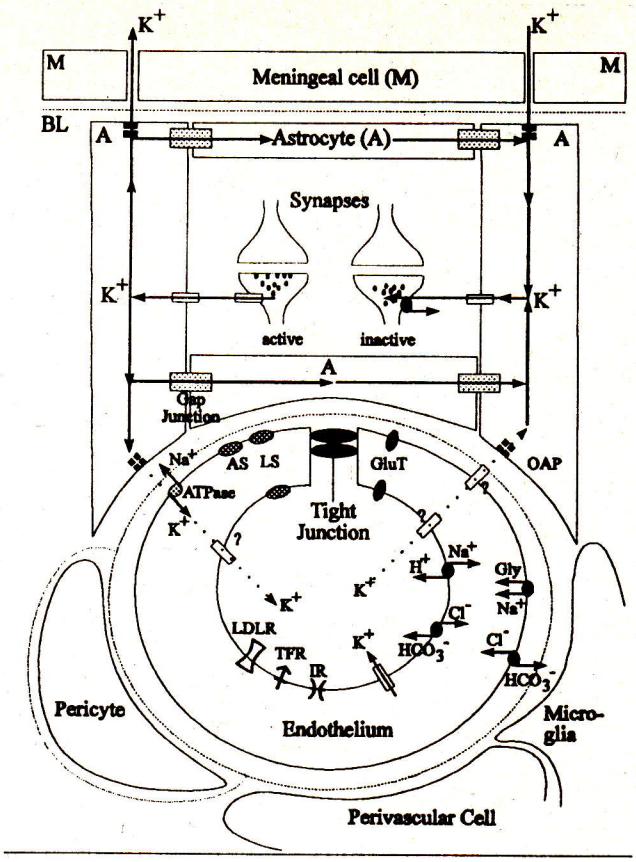
Membrane properties of the glio-vascular complex

Astrocytes are considered to be involved in the induction and maintenance of the blood-brain barrier (BBB). This barrier consists in interendothelial tight junctions (TJs). In vivo, the TJs build a densely meshed network of strands which are associated with the protoplasmic half (P-face) of the endothelial membrane as is known from epithelial TJs. However, if endothelial cells are cultured their TJs decrease their complexity and loose their association with the P-face switching to the extracellular half of the membrane (E-face). Recent data suggest that the mode of TJ-association within the membrane determines the transepi- or transendothelial electrical resistance and permeability. We have shown that astroglial factors are able to increase the complexity of endothelial TJs, but not to maintain the P-face-association. Obviously, the culture system suffers from insufficiency in maintaining all BBB properties in endothelial cells. This holds true also for the glucose transporter, a reliable marker of BBB endothelium. Its expression is not maintained in cultured endothelial cells. Regarding the observations that in unmyelinated sites of the CNS astrocytes are altered in terms of GFAP content, orientation and OAP polarity, and the BBB is leaky, our goal in the future project is to determine whether astro-oligodendroglio-vascular interactions are required to induce the expression of BBB parameters such as glucose transporter and truly tight "tight junctions".

Methods Available

Electron microscopy
Freeze-fracturing
Morphometry
Immunocytochemistry (light- and electron microscopy)

Schematic Illustration of glio-vascular Interactions involved in Blood-Brain Barrier Formation



Author Teams

Sylvia Bolz
Marcus Frank
Uwe Kniesel
Ria Schmid

Collaboration with Other Teams

Andreas Gocht, Hamburg, Germany
Paul Layer, Darmstadt, Germany
Andreas Reichenbach, Leipzig, Germany
Werner Risau, Bad Nauheim, Germany

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Joachim R. Wolff

University of Göttingen
Kreuzbergring 36
D-3400 Göttingen
Department of Anatomy, Developmental Neurobiology Unit

Phone: 49 551 39-7051,-7052,-7071
Fax: 49 551 39-7995
Bitnet:

General Research Interests

Roles of Astrocytes in Neuroplasticity

In neurons, "millisecond business" and neuroplasticity are partially interrelated functions. The role of glial cells in activity-dependent neuroplastic reactions is still enigmatic. Especially astrocytes appear suited for monitoring alterations of synaptic function in the neuropil: (1) We have demonstrated that astrocytic processes show differential attraction to various types of synapses with a maximum at synaptic clefts. As recently detected, their membranes carry receptors for various transmitters. (2) Astrocytes show a small but significant turnover in adult mammalian brains with different cells entering and leaving the growth fraction (Korr). We have recently shown that under certain condtions Glutamate can induce cell death of astrocytes. (3) Astrocytes also play some role in all types of synapse elimination known so far: Synaptic dissociation or "stripping" (retrograde reaction to axotomy of neurons), autophagy and lysosomal degradation (e.g. anterograde reaction to partial deafferentation), phagocytosis of isolated degenerating synaptic elements (consequence of cell death or lesions). The aim is to unravel the relation between these functions of astrocytes and their structural organisation.

Present and/or Future Project in the DFG Schwerpunkt

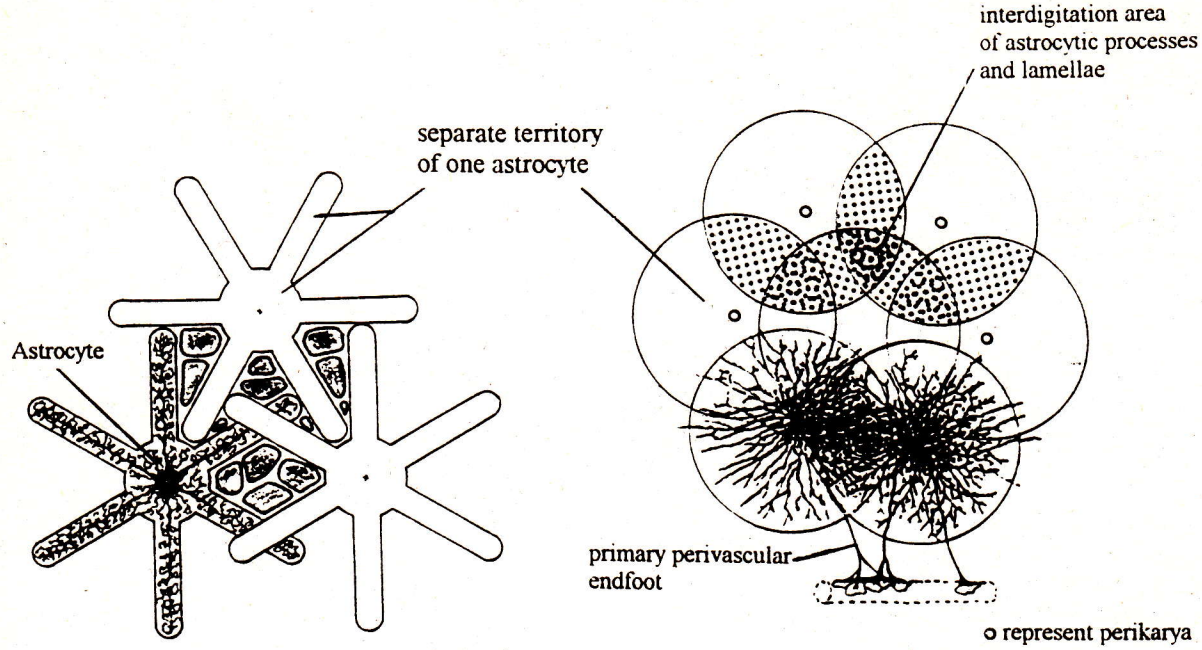
Structure and Dynamics of Astrocytes Serving Neuropil Monitoring

Astrocytes apparently form a network which is connected by gap junctions. In this project we want to define quantitatively the structural basis of this network and study its structural dynamics as function of astrocytes monitoring the extracellular milieu of adjacent neurons. We will focuss on the following features: (1) The rather even distribution of cell bodies ("contact spacing") provides a separate territory for each astrocyte. Its average size will be determined on astrocytes identified by intracellular filling and immunocytochemical markers. (2) In vitro, astrocytic cell bodies show movements within cell processes during phagocytosis. It remains to be studied whether movements also occur in situ, and which function they have. (3) Neighboring cells regulate interastrocytic contact formation by interdigitation of radial processes. This overlap will be quantitavely estimated. (4) The surface of perikarya and radial processes is extremely enlarged by lamellar expansions of astrocytic membranes. In vitro, formation of such lamellae with characteristic length has been induced in gel-like substrates rich in hyaluronic acid even in the absence of cell adhesion. Thus, position rather than size of astrocytic lamellae may be determined by surrounding neuropil components. We will have to estimate the average size of the lamellar control space by stereological measurements and find factors influencing its dynamics (e.g. Tenascin, with SCHACHNER) (5) Interastrocytic coupling is mediated by gap junctions. Using antibodies and oligoprobes for connexin 43 (with DERMIETZEL) we want to estimate the average number, size and turnover of gap junctions per statistical astrocyte and its dynamics depending on activity changes in neighboring neurons.

Methods Available

Organoid tissue culture and cell culture methods,
combined immunohistochemistry for several antigens on light- and electonmicroscopical level,
experimental models for inducing various types of transneuronal neuroplasticity,
3-D reconstruction techniques, stereology, morphometry, TV-image analysis

Interdigitation models of Astrocytes



Members of the Research Team

Dr. Siegfried Eins
Dr. Markus Missler
Dr. Michael Rickmann
Dipl. biol. Astrid Rohlmann
Thomas Rollmann

Collaboration with Other Teams

G. Brückner (Leipzig)
R. Dermietzel (Regensburg)
M. Frotscher (Freiburg)
F. Joo (Seged)
R. Laskawi (HNO Göttingen)
A. Reichenbach (Leipzig)
M. Schachner (Zürich)
H. Wolburg (Tübingen)

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Head of the Research Unit

Astrid Zimmermann

Address:

Institute of Zoology
Technical University
Schnittspahnstr.3
61 Darmstadt
Phone: 49 6151 163303
Fax: 49 6151 164808
Bitnet: DF6N@ DDATHD21

General Research Interests

Neuron-Glia Interactions

Glial functions in nervous system development are often seen primarily as late events , the final, functional establishment of the neural structure. In fact, one important glial activity with morphogenetic consequences- myelinization - occurs once phenotypic differentiation of the neuron and synapse formation has been completed. On the other hand, there is a lot of evidence that glial cells play an early and fundamental role in the structuring of the nervous system by providing essential cues for neuronal migration and axonal growth. Our goal is to pick up some of the molecules involved in the specific cross-talk between neuron and glia during nervous system development using suitable *in vitro* systems. The approach has been to study cellular behaviour of pure glial cultures as well as neuron-glia interaction cultures. Of particular interest are growth relevant components of the glial cell surface which we have defined by monoclonal antibodies (mabs) and diffusible signals produced and secreted by glial cells such as the neurotrophins, to which the neurons react in terms of survival or modified growth behaviour.

Present and/or Future Project in the DFG Schwerpunkt

Glial signalling for neuronal growth

In trying to analyze the molecular cross-talk between neurons and glial cells we have focused on a relatively simple part of the nervous system, the peripheral sensory ganglia. *In vivo* studies have shown that the axonal tips of sensory neurons grow out into the periphery in close association with glial cells. Part of the axons become myelinated in later development. We have developed mabs which define glial cell surface epitopes relevant to neuronal growth, the biochemical characterization of which shall be pursued. It will further be our goal to analyze the secretory potential of glial populations particularly in regard to neurotrophic factors.. The concept is being tested that glial cells may amplify or suppress diffusible factor signals provided by the tissue surrounding the growing axons or the target, thus serving as regulators of axonal growth and sprouting.

Methods Available

Single cell and explant cultures.
Hybridoma /mab production.
Immunological identification and separation of neural cell classes..
Radioreceptor binding analyses.
Receptor autoradiography.

Members of the Research Team

n.n.

Collaboration with Other Teams

5 References

Zimmermann, A. and Sutter, A. (1992) Mab E1 (SGIII-1) delineates neuronal cell classes in neuron-glial cell interaction and affects neurite outgrowth. Abstr. Soc. for Neuroscience, 399.3.
Zimmermann, A., Sutter, A. and Stephani, U. (1993) Autocrine regulation of neurotrophin secretion in Schwann cells. J. Cell. Biochem., Suppl. 17D, PZ334.