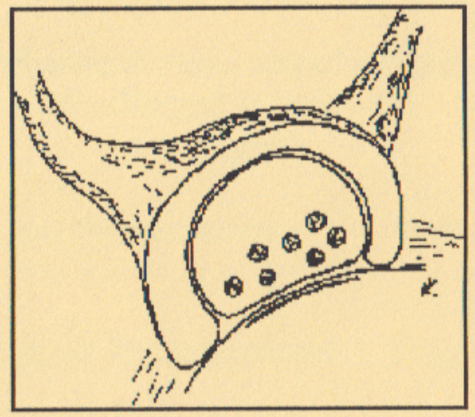
SATELLITE SYMPOSIUM
TO THE 17TH EUROPEAN NEUROSCIENCE ASSOCIATION
MEETING

THE ROLE OF GLIA IN SYNAPSE DEVELOPMENT, SYNAPSE LOSS, AND SYNAPTIC PLASTICITY



September 11 - 14, 1994

Castle Ringberg
Tegernsee
Bavaria
Germany

PROGRAM

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Sunday, September 11	16.00 - 18.30
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Duration of Talks 20 minutes plus
10 minutes discussion

Poster boards width: 150 cm
height: 125 cm

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Sunday, September 11

16.00 **Registration**

17.30 - 18.30 **Welcome and Introduction**
Chairperson: Harry G. Goshgarian

Georg W. Kreutzberg, Max-Planck-Institute for Psychiatry, Martinsried, Germany
Astrocytes and structural plasticity of synapses

18.30 **Departure for Dinner in the village Kreuth**

19.00 **Dinner**

Monday, September 12

8.00 - 9.00 **Breakfast**

9.00 - 10.30 **1. Session:**
Chairpersons: Marion Wienrich and Marco Celio

Glenn I. Hatton, University of California, Riverside, USA
Functional roles of astrocytes in synaptic and non-synaptic plasticity

Terry C. Pellmar, Physiology Department, AFRRI; Bethesda, USA
A role for glial cells in synaptic transmission

Fredrick J. Seil, Office of Regeneration Research Programs, Veterans Affairs Medical Center, Portland, USA
Glial regulation of synapse density in cerebellar cultures

10.30 - 11.00 **Coffee Break**

11.00 - 12.30

Rosemarie Grantyn, Max-Planck-Institute for Psychiatry, Martinsried, Germany
In vitro development of vertebrate central synapses

Helmut Kettenmann, Max-Delbrück-Center for Molecular Medicine, Berlin-Buch, Germany
Glial receptors in the grey matter

Christian Steinhäuser, Institute of Physiology, University of Jena, Germany
Expression of Ca^{2+}-permeable AMPA/kainate-receptors in hippocampal glial cells

12.30 - 13.30 **Lunch**

13.30 - 17.00 **Social Program**

17.00 - 18.30 **2. Session**
Chairpersons: Fredrick Seil and Rosemarie Grantyn

Vivian I. Teichberg, Department of Neurobiology, The Weizmann Institute of Science, Rehovot, Israel
Properties of the kainate binding protein from chick Bergmann glia

Amin Derouiche, Zentrum der Morphologie, Klinikum der J. W. Goethe-Universität Frankfurt, Germany
Roles for astrocytic glutamine synthetase in glutamatergic systems

J. Glowinski, Laboratoire de Neuropharmacologie, INSERM U 114, Collège de France, Paris, France
Role of astrocyto-neuronal interactions in the control of striatal glutamate

18.30 **Dinner**

Tuesday, September 13

8.00 - 9.00 **Breakfast**

9.00 - 10.30 **3. Session**
Chairpersons: Glenn I. Hatton and Jacques Glowinski

Brian MacVicar, Neuroscience Group, University of Calgary, Canada
GABA receptors and synaptic potentials in astrocytes

G. Moonen, Department of Human Physiology and Physiopathology, University of Liège, Belgium
Cultured astroglia release a negative allosteric modulator of the $GABA_A$ receptor

Joachim W. Deitmer, Abteilung für Allgemeine Zoologie, FB Biologie der Universität Kaiserslautern, Germany
Neurotransmitter-induced calcium and proton signals in glial cell

10.30 - 11.00 **Coffee Break**

11.00 - 12.30

Marco R. Celio, Institut d'Histologie et d'Embryologie générale, Université de Fribourg, Switzerland
Perineuronal nets of extracellular matrix in the brain

Hans Thoenen, Abteilung Neurochemie, Max-Planck-Institute für Psychiatrie, Martinsried, Germany
Activity-dependent synthesis and release of neurotrophins - implications for neural plasticity

G. M. Gilad, Faculty of Medicine, Technion-Israel Institute of Technology, Haifa, Israel
Chemotaxis and accumulation of nerve growth factor by microglia

12.30 - 14.00 **Lunch**

14.00 - 15.30 **4. Session**
Chairpersons: Gustave Moonen and Terry Pellmar

C. L. Poitry-Yamate, University of Geneva Medical School, Switzerland
An anaplerotic role of Müller glial cells in providing energy substrates for neurotransmitter glutamate in photoreceptor-neurones from a mammalian retina

Harry G. Goshgarian, Wayne State University School of Medicine, Detroit, USA
Combined effects of phrenicotomy and spinal cord hemisection on neuronal and glial plasticity in the phrenic nucleus

Christian M. Müller, Max-Planck-Institute for Developmental Biology, Tübingen, Germany
Role of glial cells in activity-dependent visual cortex development and plasticity

15.30 - 17.00 **Postersession and Coffee Break**

17.00 - 18.00 L. M. Garcia-Segura, Instituto Cajal, C.S.I.C., Madrid, Spain
Gonadal hormone regulation of neuro-glial interactions in the rat hypothalamus

Dionysia T. Theodosis, Neuroendocrinologie Morphofonctionelle, INSERM U 378, Université de Bordeaux, France
The magnocellular oxytocinergic system: a model for activity-dependent neuronal-glial and synaptic plasticity in the adult CNS

18.30 - 20.00 **Dinner**

20.00 **Round Table Discussion**

Wednesday, September 14

8.00 - 9.30 **Breakfast and Departure**

Marco R. Celio, Institut d'Histologie et d'Embryologie générale, Université de Fribourg, Switzerland
Perineuronal nets of extracellular matrix in the brain

Joachim W. Deitmer, Abteilung für Allgemeine Zoologie, FB Biologie der Universität Kaiserslautern, Germany
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Activity-dependent synthesis and release of neurotrophins - implications for neural plasticity

Claudia Eder, Institut für Neurophysiologie, Universität Köln, Germany
Voltage-gated currents in microglia treated with colony-stimulating factors

Orlando Guntinas-Lichius, Klinik für Hals-, Nasen-, Ohrenheilkunde, Universitätskrankenhaus Eppendorf, Hamburg, Germany
Reactive deafferation of inhibitory axosomatic synapses in the rat brainstem

G. Hager, Max-Planck-Institute for Psychiatry, München, Germany
Granule cell migration and Bergmann-Glia in the developing rat cerebellum

Frank Kirchhoff, Department of Neurobiology, University of Heidelberg, Germany
Expression of glycine receptor subunits in glial cells of the rat spinal cord

Andrea Pastor, Cellular Neurosciences, Max-Delbrück-Center for Molecular Medicine, Berlin-Buch, Germany
Glycine and GABA induced currents in glial cells of the rat spinal cord slice

Martin Reddington, Max-Planck-Institute for Psychiatry, Martinsried, Germany
Increased expression of tissue plasminogen activator and plasminogen activator inhibitor in neurons and glia following motoneuron injury

P. Verkade, Rudolf Magnus Institute, Utrecht, The Netherlands
In the regenerating rat sciatic nerve the increase of the B-50/GAP-43 level occurs predominantly in unmyelinated axons, and less in myelinated axons

PERINEURONAL NETS OF EXTRACELLULAR MATRIX OF THE ADULT RAT BRAIN M. R. Celio, E.S. Wahrenberger, F. Rathjen, M. Paulsson, Institute of Histology and general Embryology, University, CH-1700 Fribourg, Switzerland, Ctr. for Molec. Neurobiol. University, Hamburg, Maurice-Müller Institute, Bern.

"Perineuronal nets" are ill-known formations surrounding the cell bodies and proximal dendrites of certain neurons in the brain. They are labelled by lectins and monoclonal antibodies, and appear as a fine, honeycomb-like, reticular covering. It is as yet not clear if "perineuronal nets" represent a cytological entity or extracellular material. Since hylauronic acid and proteoglycans have been found in a "perineuronal localization" an extracellular component must be postulated. We therefore looked for the presence in "perineuronal nets" of known matrix proteins. Using immunohistochemical methods we have detected the presence of the large extracellular matrix-proteins restrictin/janusin and ostonectin in the "perineuronal nets" surrounding certain cortical interneurons. At the ultrastructural level the label is limited to the extracellular space interposed between glial processes and the surface of neurons. It is absent from synaptic clefts. By double labelling fluorescence we find that antibodies to these glycoproteins mark the circumference of parvalbumin-immunoreactive neurons preferentially. We conclude that these matrix protein are a major component of "perineuronal nets" and may play a role in the stabilization of previously formed contacts between glial cells and neurons.

NEUROTRANSMITTER-INDUCED CALCIUM AND PROTON SIGNALS IN GLIAL CELLS. J.W. Deitmer, Abteilung für Allgemeine Zoologie, FB Biologie der Universität, Postfach 3049, D-67653 Kaiserslautern, Germany

Neurones and glial cells communicate with each other via the extracellular spaces by means of chemical and ionic signals. Neurotransmitters released by neurones can elicit membrane responses not only in postsynaptic neurones, but also in neighbouring glial cells, and may hence constitute part of a signal transfer between these different cell types in nervous systems. We have investigated membrane and ion responses of giant leech glial cells and cultured rat astrocytes to neurotransmitters with ion-sensitive microelectrodes and fluorescent dyes to measure intracellular calcium and pH. Glutamate and the non-NMDA agonist kainate produce distinct intracellular calcium and pH signals in both types of glial cells. The responses to kainate are blocked by the receptor antagonist CNQX (20-100 uM) and by the divalent cation nickel. We have tried to identify the sources of the rises in calcium and protons, i.e. to differentiate between ion flux through agonist-gated membrane channels and intracellular release, and possible interaction between calcium and protons in these cells.

Supported by the Deutsche Forschungsgemeinschaft, SFB 246, TP C7.

ROLES FOR ASTROCYTIC GLUTAMINE SYNTHETASE IN GLUTAMATERGIC SYSTEMS.

Amin Derouiche* and Michael Frotscher Institute of Anatomy -University of Frankfurt/M, 60590 Frankfurt/M, and -University of Freiburg, 79104 Freiburg; Germany

Two immunocytochemical observations suggest that glutamine synthetase (GS) - a glutamate metabolizing enzyme contained exclusively in the cells of the astrocyte family - uses the neurotransmitter as its substrate: A) It is contained in the astrocytic processes around identified glutamatergic synapses, and B) its lamina-specific distribution in the rat brain corresponds to the relative density of glutamatergic terminal fields, which is particularly obvious e.g. in hippocampus and cortex. These observations promt the idea that glutamate in turn may be active in regulation of GS expression and/ or glial morphology (not only during development). This was investigated by deafferentation of the rat hippocampus, either acutely (entorhinal cortex lesion, ECL) or chronically (explantation of hippocampus for slice culture). ECL produced a rise in GS- immunoreactivity (ir) which was most pronounced in the terminal field of the perforant path, whereas there is no lamination in immunostained slice cultures. Here, astrocytes display a rarified process pattern and reduced GS-ir. These observations can be explained by an excess glutamate release from degenerating terminals, and the absence of the laminated, powerful glutamatergic input from the perforant path, respectively. Intrahippocampal injection of t-ACPD, a glutamate analogue activating the metabotropic glutamate receptor, also leads to an increase in GS-ir.

Although not excluding alternative explanations, these experiments support the hypothesis of a reciprocal, feedback regulation of GS and glutamate, which could constitute an element of neuronal self-organization.

VOLTAGE GATED CURRENTS IN MICROGLIA TREATED WITH COLONY-STIMULATING FACTORS.

C. Eder*, H.-G. Fischer and U. Heinemann. Institut für Neurophysiologie, R.-Koch-Str.39, 50931 Köln, FRG

Microglial cells were isolated from murine brain culture where they differentiated by supplementation of either the macrophage-colony stimulating factor (M-CSF) or the granulocyte/macrophage-colony stimulating factor (GM-CSF). We investigated voltage gated K^+ currents from both cell populations. Currents were recorded using the whole cell patch clamp technique. In most cells derived from M-CSF-driven culture only inward K^+ currents could be observed. In contrast, in GM-CSF-grown cells both inward and outward K^+ currents could be recorded.

Currents evoked by hyperpolarizing commands showed properties of inward rectifying currents. These currents activated negative to the K^+ equilibrium potential. Their inactivation proved to be abolished when external Na^+ was replaced by NMG. The currents could be blocked by extracellularly applied Ba^{2+} and Cs^+. Tetraethylammonium (TEA) partially reduced the inward rectifier while 4-aminopyridine (4-AP) did not show any effect.

Outward K^+ currents activated at depolarizing potentials positive to -30 mV. They were very sensitive to extracellularly applied 4-AP, charybdotoxin and noxiustoxin, but were reduced only to a small extent by TEA. Various divalent cations (Cd^{2+}, Co^{2+}, Ni^{2+}, Zn^{2+}, Ba^{2+}) shifted the steady-state activation curve to more positive values, but seemed to produce no blocking actions.

Upon long-lasting depolarizing voltage pulses microglial cells exhibited slowly activating proton currents and their amplitudes were increased by acidification of the internal solution or by alcalization of the extracellular milieu. These H^+ currents were reduced after extracellular application of anorganic polyvalent cations as well as by 4-AP and TEA.

Supported by DFG grant He 1128/6-2 and SFB 194

GONADAL HORMONE REGULATION OF NEURO-GLIAL INTERACTIONS IN THE RAT HYPOTHALAMUS. L.M. Garcia-Segura*, J.A. Chowen, S. Busiguina, A. Párducz and F. Naftolin. Instituto Cajal, C.S.I.C., 28002 Madrid, Spain, Institute of Biophysics, Biological Research Center, H-6701 Szeged, Hungary and Dept. OB/GYN, Yale Univ. School of Medicine, New Haven, CT, USA

The influence of gonadal steroids on astroglia and number of axo-somatic GABAergic synapses was assessed in the arcuate nucleus. Glial fibrillary acidic protein (GFAP) and its mRNA levels, the number of astroglial profiles in the arcuate neuropil and the proportion of neuronal perikaryal membrane covered by glia showed a testosterone-dependent sexually-dimorphic pattern that was linked to the sexual differentiation of synaptic connectivity. In females, astroglia cell shape, GFAP levels and the number of axo-somatic synapses showed fluctuations associated with the modifications of estradiol in plasma during the estrous cycle. These results suggest that coordinated changes in astroglia and synapses may be actively involved in the modulation of neuroendocrine events.

CHEMOTAXIS AND ACCUMULATION OF NERVE GROWTH FACTOR BY MICROGLIA. G.M. Gilad* and V.H. Gilad. Faculty of Medicine, Technion-Israel Institute of Technology, Haifa, Israel.

It has been long known that both microglia and astrocytes are activated by CNS injury. Microglia are the first to react and their reaction is transitory, while reactive astrocytes, once transformed, persist in their newly acquired state. It was recently proposed that astrocytes and microglia exert opposing actions on the survival of neurons by releasing neurotrophic and neurotoxic factors, respectively. However, evidence indicating that, like astrocytes, microglia can also be stimulated to produce nerve growth factor (NGF, a potent neurotrophic factor for several classes of neurons), was at odds with that proposal and prompted us to investigate the localization of NGF. The present study in primary CNS cultures, provides evidence not only for the acummulation of NGF selectively in microglia, rather than in astrocytes, but also for chemotaxis towards and sequestration of NGF by microglia. The findings suggest that a local increase in NGF release may serve to attract more microglia and perhaps peripheral macrophages. It is proposed that NGF may serve as a paracrine/autocrine mediator of astroglia-microglia-neuron interactions. By means of sequestration, microglia can regulate NGF availability in a site-specific manner at critical times during CNS development and aging, and after neurotrauma, when this neurotrophin is needed for nerve cell survival and axonal growth and regeneration.

ROLE OF ASTROCYTO-NEURONAL INTERACTIONS IN THE CONTROL OF STRIATAL GLUTAMATERGIC TRANSMISSION

J. Glowinski*, P. Marin, N. Stella, M. Tence, J. Prémont
Lab. Neuropharmacology, INSERM U.114, Collège de France, Paris 5, France

In cultured striatal astrocytes, the combined stimulation of A1 adenosine and a1-adrenergic receptors or of somatostatin and of a1-adrenergic receptors induced a prolonged influx of calcium, the activation of phospholipase A2 and the production of arachidonic acid (AA). AA in turn inhibits the reuptake of glutamate spontaneously released from cells. Glutamate extracellularly accumulated then enhanced the a1-adrenergic-evoked activation of phospholipase C by acting on metabotropic receptors coupled to this enzyme. In addition, through glutamate, conditioned media from treated astrocytes stimulated phospholipase C activity in cultured striatal neurons. Moreover, glutamate itself can stimulate phospholipase A2 in astrocytes through receptors pharmacologically distinct from those coupled to phospholipase C and the glutamate evoked formation of AA is potentiated by ATP.

COMBINED EFFECTS OF PHRENICOTOMY AND SPINAL CORD HEMISECTION ON NEURONAL AND GLIAL PLASTICITY IN THE PHRENIC NUCLEUS.

Harry G. Goshgarian* and Wayne W. Liou. Wayne State University, School of Medicine, Detroit, Michigan, USA.

This study was carried out to determine if chronic phrenicotomy has an influence on the functional and morphological plasticity that is normally demonstrated by phrenic motoneurons following spinal cord injury. Adult experimental rats were subjected to chronic phrenicotomy at 1,2,3, and 4 weeks prior to induction (by spinal cord hemisection and contralateral phrenicotomy) and physiological recording of the crossed phrenic phenomenon (CPP). Quantitative EM morphometric analysis of the phrenic nucleus was carried out on other animals treated identically. The physiological results showed that there is a transient, but statistically significant depression of crossed phrenic nerve activity at 2 weeks with a recovery to the normal activity level at 4 weeks post phrenicotomy (PP). Correlative quantitative morphometric analysis showed that the number of synapses contacting phrenic profiles is significantly decreased from control levels at 2 weeks PP, but this number returns to a level not significantly different from control values at 4 weeks. Quantitative analysis also showed that phrenic dendritic area and the number of dendrodendritic appositions were reduced at 2 weeks PP. A significant increase in the microglia area fraction was seen at 2 weeks PP whereas a significant increase in the astroglia area fraction was only seen at 4 weeks PP. These results suggest that peripheral axotomy imposes a transient effect on spinal cord injury-induced plasticity of phrenic motoneurons most obvious at 2 weeks post phrenicotomy. This effect involves a depression of phrenic nerve activity which may be caused by a reduction of synaptic input to phrenic motoneurons. Both the physiological and morphological effects of phrenicotomy are no longer present by 4 weeks PP.

In vitro Development of Vertebrate Central Synapses.

R. Grantyn, Developmental Neurobiology -Group, Max-Planck-Institute for Psychiatry, D-82152 Martinsried

This contribution addresses the basic determinants os synaptic efficacy in central nervous circutits: location and number of release sites, release probability and single cell activated (unitary) conductance changes. We shall forward the hypothesis that development of glutamatergic and GABAergic synapses differs in major aspects of synaptogenesis. Disregarding, for the moment, that various test models and cells types could render diverging results, one may notice the following tendencies. Glutamatergic terminals display a preference for dendites, whereas GABAregic terminals select soma locations at initial stages of development. Glutamatergic synapses are characterized by receptor accumulation in the region of terminal apposition, while in GABAergic synapses receptor concentration is weak, if present at all. The expression of GluRs, but not $GABA_A$ receptors is under control of inter-neurons.

Developmental changes in synaptic transmission have not yet been assessed by quantal analysis. Now we present data showing that in predominantly GABAergic cultures from the rodent superior colliculus a considerable fraction of terminals remains in a low efficacy release state ($p<0.2$). A developmental increase in synaptic strength is reached by the appearance of singular highly effective release sites. Presynaptic maturation is to some degree under environmental control and can be mani-pulated by chronic drug treatment. Addition of GluR antagonists significantly increased amplitudes and decreased the coefficients of variations of evoked IPSCs.

Our results suggest that development of central inhibition depends on the activation status of heteronymous synaptic receptors. It is conceivable that the latter can be modified by astroglia.

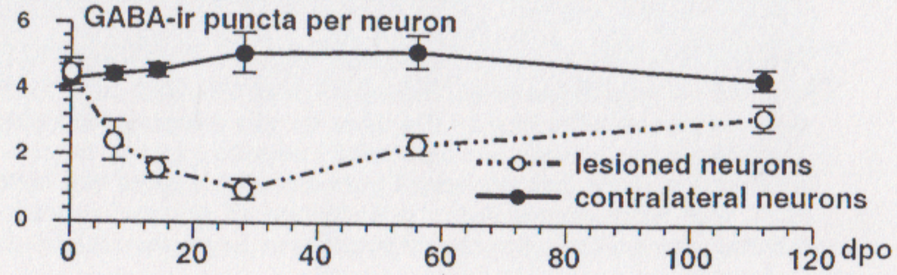
Reactive deafferation of inhibitory axosomatic synapses in the rat brainstem

O. Guntinas-Lichius[1], J. Lebek[2], R.W. Veh[3], E. Stennert[1] and W.F. Neiss[2]
[1]Klinik für Hals-, Nasen-, Ohrenheilkunde und [1]Institut I für Anatomie der Universität zu Köln; [3]Abtl. für molekulare Neurobiologie, Universitätskrankenhaus Eppendorf, Hamburg; FRG

There is controversy, whether the synaptic stripping after axotomy of motoneurons exclusively concerns excitatory boutons. Now we examined the number of axosomatic inhibitory GABA-ergic synapses attached to hypoglossal motoneurons after separation of these cells from their target muscles.

After resection of the hypoglossal nerve 30 rats were fixed by perfusion 7 to 112 days post operation (dpo), vibratome sections of the brainstem embedded in LR White and semithin sections incubated with anti-GABA. The number of GABA-ir puncta per neuronal cell profile was counted lightmicroscopically, resulting in these means of means (± S.D.) of GABA-ir axosomatic puncta per hypoglossal motoneuron (see figure).

The results show that gaba-ergic synapses are stripped from hypoglossal motoneurons after nerve resection reaching a minimum at 28 dpo. At 112 dpo the number of axosomatic inhibitory synapses has partially recovered. On the contralateral side the number of GABA-ir puncta increases transiently, this contralateral coreaction suggests a synaptic plasticity in the untreated hypoglossal nucleus.



NOTES

GRANULE CELL MIGRATION AND BERGMANN-GLIA IN THE DEVELOPING RAT CEREBELLUM. G. Hager, P. Liesi*, H.-U. Dodt and W. Zieglgänsberger Max-Planck-Institute of Psychiatry, Clinical Institute, Clinical Neuropharmacology, Kraepelinstr.2,80804 München; *Lab. of Molecular and Cellular Neurobiology, NAAA, NIH,12501 Washington Avenue, 20852 Rockville MD, USA

The established model of neuronal migration in the postnatal rat cerebellum relies on the glial guidance: The external granule cells are thought to initiate their migration by extending horizontal processes followed by an adaption of a T-shaped morphology and gliding in a third vertical leading process along the Bergmann glial fibres. Recent studies have shown, that cerebellar granule neurons migrate on a laminin substrate either along other neuronal fibres or by a process-extension-contact-formation type of nuclear movement inside the preformed neuronal processes. The contact to glia cells seems to be not essential for neuronal migration. Laminin can be demonstrated by antibody binding studies at Purkinje cells and Bergmann glia cells and as punctate deposits in between the cells in the external granule layer.

We used *in vitro* cerebellar slices in combination with infrared videomicroscopy and time lapse recording to study the migration of external granule cells (EGC). Slices of 0 - 14 day postnatal rat cerebellum were placed in a slice chamber with a coverslip bottom. The chamber was mounted on a inverted microscope and perfused with artificial CSF.

Initially (P0) vertical pathways of neuronal fibers can be observed. These pathways are probably established by early bipolar granule cells that attach to the external basal lamina via one process and send another process vertically towards the Purkinje cell layer. Beginning with P2 EGC´s undergo a horizontal transition, extend long parallel fibres, and migrate horizontally. At day 8 postnatally the horizontal orientated cells can be seen as a large separate layer. These horizontal migrating cells undergo a second transition in vertical direction and migrate in the internal granule cell layer either along the glial fibres or along the vertical pathways formed by other neuronal cell processes. The vertical migration pathways disappear by P14 when the glia processes have adopted a fully radial orientation. Both vertically and horizontally migrating granule neurons move by nuclear translocation inside their preformed processes with a speed that varies between 6-120 µm/h. Our result suggest that a new concept of neuronal migration via a process-extension-contact-formation should be added to the standard model of glial guidance.

FUNCTIONAL ROLES OF ASTROCYTES IN SYNAPTIC AND NON-SYNAPTIC PLASTICITY. G.I. HATTON. University of California, Riverside, Riverside, California, U.S.A.

The roles played by glia in nervous system functioning have been increasingly recognized over the past decade or so. It has become clear that glial cell types participate importantly in response to functional demands placed on the organism by changing physiological conditions.

The hypothalamo-neurohypophysial system (HNS) has become a model system for the study of function-related neuronal/glial plasticity in the mammalian central nervous system. Much has been learned about the dynamics and functional consequences of astrocyte mobility since the initial discovery, two decades ago, that physiologically increasing the demand for peptide synthesis and release by the HNS caused profound alterations in the morphology of this system. These changes in which the astrocytes are active elements, have subsequently been shown to be entirely reversible. Physiological stimulation has been found: (a) to result in formation of new, specialized synapses, (b) to permit or increase the occurrence of dendritic bundling and electrotonic coupling (electrical synapse formation) among the HNS neurons in the hypothalamus, (c) to permit the peptidergic terminals to increase their contact with the basal lamina surrounding the perivascular space in the neurohypophysis, and (d) to increase peptidergic terminal synaptoid contacts with neurohypophysial astrocytes (pituicytes). Recent work has identified some of the receptors that appear to mediate both the hypothalamic and neurohypophysial astrocytic responses to physiological stimulation. Also, the roles played by glia in controlling neuronal excitability via release or uptake of neuroactive substances have begun to be elucidated. Ways in which these factors might affect synaptic plasticity will be discussed.

TRANSMITTER RECEPTORS AND ION CHANNELS IN BERGMANN GLIA

H. Kettenmann, Max-Delbrück Center for Molecular Medicine, Berlin-Buch, Germany

Bergmann glial cells are closely associated with neurons: during development they provide guiding structures for migrating granule cells and in the adult cerebellum they display intimate interactions with Purkinje cells. While Bergmann glial cells in mice of postnatal day (P) 20 to P30 have thick processes with arborized, irregularly-shaped leaf-like appendages, the processes of cells from younger mice (P5-P7) are thinner and smoother. This morphological maturation is accompanied by a variation in voltage-gated currents. In cells from P5-P7 delayed outward and inward rectifying K^+ currents were recorded from, while older Bergmann glial cells were characterized by large, voltage- and time-independent K^+ currents. In addition, application of γ-aminobutyric acid (GABA) induces two effects, a rapid activation of a Cl^- conductance and a longer-lasting decrease in the (resting) K^+ conductance. Both effects were mediated by benzodiazepine-insensitive $GABA_A$ receptors. Responses in cells of P5-P7 mice were large as compared to the small or even undetectable responses in P20-P30 cells.

Moreover, these cells respond to kainate and NMDA agonists, selectively activating non-NMDA and NMDA receptors, respectively. The kainate/AMPA receptor in Bergmann glial cells not only triggers a cationic conductance but leads to a substantial increase in cytosolic Ca^{2+} which in turn modulates the resting K^+ and the gap junctional communication. These changes in the Bergmann glial cell membrane properties suggest a transition between functional states during development of the Bergmann glial cells and imply that these cells are involved in synaptic transmission.

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EXPRESSION OF GLYCINE RECEPTOR SUBUNITS IN GLIAL CELLS OF THE RAT SPINAL CORD.

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By electrophysiological methods it was shown that different glial cells, namely astrocytes, oligodendrocytes and precursor cells of the rat spinal cord respond to the inhibitory neurotransmitters glycine.

In this study the reverse transcription mediated polymerase chain reaction (RT-PCR) in combination with the patch-clamp technique was applied to individual glial cells to investigate subunit expression of the receptor. After electrophysiological recording in the whole-cell configuration the cytoplasm of those glial cells was harvested which displayed a response to glycine. After reverse transcription of cellular mRNA glycine receptor subunit specific DNA fragments were amplified by PCR and analyzed by agarose gel electrophoresis. The α1 subunit of the glycine receptor but not α2, 3 or 4 could be detected in all glial cell types investigated in rats of postnatal day 6 to 9. In addition, a truncated β subunit lacking the first transmembrane domain was found in a subpopulation of cells. The sequence of the amplified fragment was confirmed by subsequent sequencing. The physiological role of this truncated β subunit remains to be determined.

The detection of glycine receptor subunit mRNAs in glial cells confirms the electrophysiological studies showing glycine evoked inward currents and substantiates that glial cells themselves express the receptors. The physiological role of them may be controlling the microenvironment in the vicinity of neurons during synaptic transmission. The contribution of other neurotransmitter receptors known to be expressed in glial cells is conceivable.

ASTROCYTES AND STRUCTURAL PLASTICITY OF SYNAPSES

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The surface of CNS neurons is generally covered either by boutons or by astrocytic processes. The latter also provide the perisynaptic ensheathing of the axon terminals. The question arises as to what extent the territories are assigned to glial or axonal elements and to what extent this distribution can be disputed, e.g. in a competitive way.

A more or less complete covering by Schwann or glial cells is seen in sensory neurons of the PNS but also in the mesencephalic nucleus of the trigeminus. Neurons of this modality have a highly monopolized input. The wrapping by glial processes could represent a way to protect neurons from undesired input.

Prominent perineuronal glial lamellae have been described in various nuclei of the hypothalamus and will be discussed in other presentations of this symposium. The density of synaptic terminals on these neurons depends on hormones and varies greatly with changes of hormonal levels.

Post-traumatic changes and/or adaptations are also frequently accompanied by a change in the relation of synaptic and glial territories on the neuronal surface. Axotomy of brain stem and spinal motoneurons leads to synaptic stripping by microglial cells that are replaced by astrocytic processes leading to a permanent deafferentation of these neurons, even after complete axonal regeneration and peripheral target reinnervation. Morphologically, the appearance of astrocytic processes and glial lamellae is very similar in the post-traumatic and in the hormonally regulated situations. Goshgarian has reported another example for the competition between astrocytes and synapses in the rat phrenic nucleus following spinal cord lesion. Normally, the motoneurons and their dendrites are partially covered by glial processes. Within hours after hemisection of the spinal cord, the territories of dendro-dendritic appositions and, later, the number

of double or even triple and quadruple synapses increases at the expense of the astrocytic territories. An active retraction of glial processes from neuronal surfaces seems to occur and enables ineffective synapses to become functional.

In the development of the CNS, neuronal differentiation can also ínvolve a change in neuron/glial relationships. Purkinje cells lose the axosomatic climbing fiber terminals and become ensheathed by Bergman glia as they mature postnatally. At the same time the ectoenzyme 5'-nucleotidase changes from a synaptic to a glial form. In cerebellar explants, Purkinje cells become ensheathed by astrocytes to such a degree that a 60% reduction in the number of somatic synapses occurs. Astrocytic hypertrophic processes seem to be involved in the phagocytosis of supernumerary boutons in postnatal development and in synaptic remodelling of the cortex in aging.

These observations challenge the view of the structural stability of synapses and raise several questions to be discussed at this symposium:

(a) Is there a competition between glia and axon terminals or synaptic sites?

(b) What are the glial mechanisms for receiving and transducing signals from the neuron?

(c) What is the cell biology of retraction or extension of astrocytic processes or axon terminals? Is it a growth cone-like behaviour?

(d) What stabilizes axon?

(e) Finally, what can we learn from pathology and is this relevant for physiology?

GABA RECEPTORS AND SYNAPTIC POTENTIALS IN ASTROCYTES

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GABA receptors are expressed in astrocytes both in cell culture and in tissue slices. Recent experiments on astrocytes acutely isolated from the hippocampus indicate that these cells have $GABA_A$-like receptors that have similar properties to those expressed in cell culture. GABA activates Cl^- specific channels that are modulated by barbiturates and benzodiazepines and the neuronal inverse agonist DMCM enhances the current in a subpopulation of astrocytes. GABA receptor activation depolarizes astrocytes sufficently to open voltage-gated Ca^{2+} channels. Visualization of GABA receptors on live astrocytes indicates that they are distributed in discrete clusters on the cell soma and a subset of processes. In stellate glial cells of the pituitary pars intermedia, GABA as well as dopamine released from synaptic terminals activates postsynaptic potentials directly. The physiological significance of astrocytic $GABA_A$ receptor activation remains unknown, however GABA-activated Cl^- fluxes may be involved in extracellular ion homeostasis, pH regulation and/or Ca^{2+} signalling.

CULTURED ASTROGLIA RELEASE A NEGATIVE ALLOSTERIC MODULATOR OF THE $GABA_A$ RECEPTOR

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We previously showed that the low molecular weight (<1000 Da) fraction of a chemically defined medium conditioned for 24 h. by cultured astrocytes derived from newborn rat cerebral cortex (ACM) has a neurotoxic activity. By several criteria it was shown that this toxic activity is not related to the excitotoxins. Neurotoxicity was prevented by GABA and benzodiazepines. This led us to look for an effect of ACM on GABA currents as measured using the whole cell patch clamp technique. ACM inhibits GABA induced inward currents recorded in cerebellar granule cells and hippocampal neurons in culture. This effect is specific for ACM, as medium conditioned by PC12 cells does not affect the GABA response of these cells. It is also specific for GABA-induced currents because glutamate-induced currents do not change either in amplitude or in shape in the presence of ACM. The inhibitory effect on the GABA response in cerebellar granule cells of ACM could be suppressed by flumazenil, a specific benzodiazepine (BZD) antagonist and could be mimicked by two BZD inverse agonists. These data thus demonstrate the presence of a BZD inverse agonist-like activity in ACM. This effect of ACM on different neuronal cell types was heterogenous since no detectable effect could be observed on the GABA-induced current in GABA-responsive dorsal root ganglion (DRG) neurons, presumably reflecting a functional heterogeneity of the $GABA_A$ receptors present in these different neuronal subsets. Further binding studies ($[^3H]$ flumazenil, $[^3H]$ muscimol and $[^{35}S]$ TBPS) did confirm an interaction between ACM and the $GABA_A$ receptor although this interaction does not seem to occur at the BZD site. By releasing a negative allosteric modulator of the $GABA_A$ receptor, astrocytes could modulate inhibitory neurotransmission.

ROLE OF GLIAL CELLS IN ACTIVITY-DEPENDENT VISUAL CORTEX DEVELOPMENT AND PLASTICITY

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During a restricted period in postnatal development the thalamo-cortical circuitry of the cat visual cortex undergoes an activity-dependent reorganization which includes synapse elimination and axonal sprouting. In cat, this period is limited to about the first three postnatal months.

We have analyzed possible interrelations of glial development and neuronal plasticity in the vertebrate visual cortex. Immunocytochemical studies have shown a coincidence of the maturation of glial cells and the time course of the critical period for cortical malleability. These data suggested that immature glial cells are essential for cortical plasticity. This hypothesis was underscored by the reinduction of adaptive changes in the mature cat visual cortex following transplantation of immature astrocytes. Using biochemical techniques we have shown a prominent expression and release of plasminogen activators in the developing visual cortex which may be involved in synapse elimination and/or axonal growth. These activators of proteases have been shown to be expressed by astrocytes.

With respect to the termination of the critical period for cortical malleability we used an in vitro assay where embryonic neurons are cultured on unfixed sections of cat visual cortex. It is shown that neuronal growth gradually declines with increasing developmental age of the substrate tissue. Immunocytochemical studies revealed a concomitant expression of myelin associated neurite growth inhibitors in oligodendroglial cells. Furthermore, there is a reduction of the astroglial derived matrix protein tenascin, which may promote neurite growth. It is suggested that glial cells control cortical malleability by influencing axonal growth. The contribution of the different types of glial cells to individual mechanisms will be discussed.

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GLYCINE AND GABA INDUCED CURRENTS IN GLIAL CELLS OF THE RAT SPINAL CORD SLICE

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In a recent study four types of glial cells could be distinguished in the rat spinal cord slice using electrophysiological and morphological methods. Astrocytes, oligodendrocytes and two types of precursor cells showed a distinct type of current pattern. In the present study, we demonstrate that these cells respond to the inhibitory neurotransmitters glycine and GABA, as revealed with the whole cell recording configuration of the patch clamp technique. All astrocytes and glial precursor cells and a subpopulation of oligodendrocytes responded to glycine. Similarly, large GABA currents could be elicited in astrocytes and precursor cells while oligodendrocytes showed only small responses. The GABA-activated current is due to the activation of $GABA_A$ receptors since the reversal potential was close to the Cl^- equilibrium potential. The response could be reversibly blocked by bicuculline and mimicked by muscimol. The involvement of glycine receptors is infered from the observation that the response was blocked by strychnine and that it reversed close to the Cl^- equilibrium potential. Besides of the activation of the Cl^- current, $GABA_A$ receptor activation also induced a block of the resting K^+ conductance, similar as observed in Bergmann glial cells. The presence of the glycine receptor seems to be unique for spinal cord glial cells since such responses have not yet been described in any other preparation. This further implies that such glial receptors may be involved in synaptic transmission since glycine is a dominant inhibitory neurotransmitter of the spinal cord.

A ROLE FOR GLIAL CELLS IN SYNAPTIC TRANSMISSION. T. C. Pellmar* and D. O. Keyser. Physiology Department, AFRRI, Bethesda, MD 20889-5603, USA.

Glia are an important functional component of the nervous system and are known to regulate the microenvironment in support of neuronal activity. We now find that glia play a dynamic role and are crucial to the maintenance of synaptic transmission.

Disruption of glial metabolism with fluoroacetate (FAC), a glial-specific metabolic blocker, causes rapid attenuation of evoked and spontaneous synaptic transmission in field CA1 of the guinea pig hippocampal slice. Both intracellular synaptic potentials (PSPs) and extracellular population PSPs are inhibited. PSPs in area dentata are significantly less sensitive to FAC (IC_{50} = 554 μM) than in field CA1 (IC_{50} = 65 μM). FAC does not decrease iontophoretic responses to glutamate (GLU), the excitatory neurotransmitter, eliminating postsynaptic receptors as a site of action. FAC reduces the ATP concentration in hippocampal slices (both CA1 and dentate) and in cultured glial cells (both C6 cells and primary astrocytes), but not in synaptosomes, a purely neuronal element. Isocitrate, a metabolic substrate selective for glia, prevents the FAC-induced decrease in synaptic transmission and in ATP. Glutamine (GLN) also prevents the decrease in PSPs by FAC, suggesting a compromised GLU/GLN cycle. However, GLN also could be protecting by providing a metabolic substrate for glia.

The role of the GLU/GLN cycle in the actions of FAC is currently under investigation. The pool of presynaptic GLU could be decreased by interference with GLU uptake or by reduced availability of the GLU precursor GLN. Fluorometric measurements of GLU in C6 cells and astrocytes show that FAC does not alter intracellular GLU levels or the uptake of extracellular GLU. Experiments to measure changes in GLN production are in progress.

These data demonstrate that metabolic integrity of glia is essential for maintenance of synaptic transmission. We hypothesize that glia can rapidly modulate synaptic transmission by regulating the neuronal supply of the GLU precursor, GLN, through the energy-dependent GLU/GLN cycle.

AN ANAPLEROTIC ROLE OF MÜLLER GLIAL CELLS IN PROVIDING ENERGY SUBSTRATES FOR NEUROTRANSMITTER GLUTAMATE IN PHOTORECEPTOR-NEURONES FROM A MAMMALIAN RETINA

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One important role of CNS glia may be the biosynthesis, release and transfer of glucose-derived carbohydrate intermediates (CI) and amino acids (AA) to neurones consequent to neurotransmitter release. In intact mammalian retina Müller cells intensively phosphorylate glucose, contain the essential stores of glycogen, and envelop synapses in the inner and outer retinal layers. Moreover, after acute isolation, guinea pig Müller cells retain their *in situ* metabolic activity and cell polarity. Biochemical quantification of ^{14}C-CI's and ^{14}C-AA's formed from ^{14}C(U)-glucose revealed the biosynthesis and predominant release of ^{14}C-lactate and of ^{14}C-glutamine from Müller cells; ^{14}C-metabolites contributing to carboxylic and/or amino moieties of glutamate were also released extracellularly. However, identical substrate incubations of acutely isolated Müller cells still attached to photoreceptors showed a light/dark modulated transfer of lactate, glutamine and glutamate between these cells. Glutamine formation in Müller cells was dependent on extracellular glutamate which remained below neurotoxic levels. Since only photoreceptors respond directly to illumination changes, these results provide evidence of a photoreceptor-dependent modulation of LDH and pyruvate carboxylase activity in Müller cells. The net release of ^{14}C-lactate produced by anaerobic glycolysis in Müller cells probably serves a dual function: to maintain their redox potential and thus the glycolytic flux; and to fuel oxidative metabolism in photoreceptors. Anaplerotic and anaerobic sources of energy substrates from glia appear important towards maintaining the pool of neurotransmitter glutamate in photoreceptors.

INCREASED EXPRESSION OF TISSUE PLASMINOGEN ACTIVATOR AND PLASMINOGEN ACTIVATOR INHIBITOR-1 IN NEURONS AND GLIA FOLLOWING MOTONEURON INJURY M. Reddington*, C.A. Haas and G. W. Kreutzberg. Department of Neuromorphology, Max Planck Institute of Psychiatry, Am Klopferspitz 18a, 82152 Martinsried, Germany

Plasminogen activators are proteases that have been implicated in tissue repair and remodelling processes. However, very little is known about the reaction of the plasminogen activator system following injury of the nervous system. Here we report immunocytochemical studies on the reaction of tissue plasminogen activator (tPA) and the plasminogen activator inhibitor-1 (PAI-1) in neurons and glia of the rat facial nucleus to axotomy of the facial nerve. An increase in motoneuronal tissue plasminogen activator (tPA) immunoreactivity and tPA-mRNA, detected by in situ hybridisation, occurred after axotomy. These reached a peak after 3-5 days, then declined to control levels by 14 days, at which time reinnervation of the target muscle begins. From 14 days to 8 weeks after axotomy, tPA immunoreactivity was observed in astrocytes surrounding injured motoneurons. This also occurred if regeneration was delayed by resection of the facial nerve, indicating that target-derived factors are not involved in the late astrocytic tPA response. In addition to changes in tPA expression, an increase in PAI-1 immunoreactivity was also observed 3 days after axotomy in perineuronal astrocytes. This increase was sustained at all times studied (up to 8 weeks). The results suggest a role for the plasminogen activator system in the cellular events accompanying transection and subsequent regeneration of motoneurons. Moreover, the fact that changes occur in tPA and PAI-1 in both the damaged neurons themselves and in astrocytes suggests an interplay between these cell types in mediating the cellular reorganisation of the facial nucleus following axotomy.

GLIAL REGULATION OF SYNAPSE DENSITY IN CEREBELLAR CULTURES. Fredrick J. Seil. Office of Regeneration Research Programs, Veterans Affairs Medical Center, and Departments of Neurology and Cell Biology & Anatomy, Oregon Health Sciences University, Portland, OR 97201, USA

Organotypic cerebellar cultures derived from neonatal mice were depleted of granule cells and functional glia by exposure to cytosine arabinoside for the first 5 days *in vitro* (DIV). In the absence of parallel fibers (granule cell axons), there was a remarkable sprouting of Purkinje cell recurrent axon collaterals, which hyperinnervated Purkinje cell somata unensheathed by astrocytic processes and formed heterotypical synapses with Purkinje cell dendritic spines. Such cultures were subsequently "transplanted" at 9 DIV with three different preparations, including: 1) granule cells and functional glia, 2) optic nerve (glia without granule cells) and 3) granule cells without functional glia. Transplantation with granule cells and glia resulted in reduction of the excess sprouted recurrent axon collaterals, myelination, astrocytic ensheathment of Purkinje cells, reduction of axosomatic Purkinje cell synapses to control levels, and formation of homotypical parallel fiber-Purkinje cell dendritic spine synapses, which outnumbered persistent heterotypical synapses by a 2.4:1 ratio. Transplantation with optic nerve did not result in neurite reduction or homotypical synapse formation, but did induce myelination, astrocytic ensheathment of Purkinje cells and reduction of axosomatic synapses, and a reduction of synapses in the cortical neuropil by 50%. Cultures transplanted with granule cells in the absence of functional glia remained unmyelinated, excess neurites were not reduced, Purkinje cells were not ensheathed by astrocytes and their somata remained hyperinnervated. Homotypical axospinous synapses formed in the cortical neuropil, but in a 1.4:1 ratio to heterotypical synapses, which persisted in almost twice the number found in cultures transplanted with functional glia. These collective studies suggest a role for astrocytes in the regulation of synaptic density. Supported by the U.S. Dept. of Veterans Affairs and NIH grant NS 17493.

EXPRESSION OF CA^{2+}-PERMEABLE AMPA/KAINATE-RECEPTORS IN HIPPOCAMPAL GLIAL CELLS. G. Seifert* and C. Steinhäuser. Institute of Physiology, University of Jena, Teichgraben 8, D-07740 Jena, Germany

Recently, it was demonstrated that glial cells in mouse hippocampal slices express glutamate receptor channels of the AMPA/kainate subtype (Pflügers Arch 426, 310, 1994). In the present study we further characterized this glial receptor. Since voltage clamp control is imperfect and diffusion barriers hinder a quantitative analysis of receptor currents in situ, the patch clamp technique was applied to glial cells acutely isolated from the CA1 stratum radiatum. A concentration clamp technique was used which enabled a very fast exchange of the extracellular solutions. Thus, it was possible to characterize fast transient receptor currents with high time resolution.

Application of glutamate and AMPA induced rapidly activating and fast desensitizing receptor currents in the suspended glial cells. The current decay was best fit by a single exponential. In contrast, kainate-induced currents were non-desensitizing. The corresponding dose-response curve revealed a half maximum of current activation at 350 µM. In some cases, however an additional, fast decaying component was observed. The current to voltage relationship of kainate evoked currents was linear with a reversal potential at about 10 mV. Replacing Na^+ by 50 mM Ca^{2+} in the extracellular solution the reversal potential was shifted to -33 mV. These results confirm earlier in situ data demonstrating a significant Ca^{2+}-permeability of the glial glutamate receptor in the hippocampus.

We conclude that glial cells of the juvenile hippocampus mainly express heteromeric high affinity AMPA receptors. Possibly, the receptor channel includes the edited GluR-2 subunit.

PROPERTIES OF THE KAINATE BINDING PROTEIN FROM CHICK BERGMANN GLIA.

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The avian Bergmann glia (BG) harbour very large amounts of a 49 kDa kainate (KA) binding protein (KBP) which displays the pharmacological properties of low affinity KA receptors, amino acid sequence homologies with the C-termini of KA/AMPA receptors and the hallmarks of ligand-gated channels. Vet, KBP cDNA or the KBP gene do not express KA-gated ion channels in Xenopus oocytes nor does KBP RNA contribute to the KA-induced currents in oocytes injected with total chick cerebellar mRNAs. The latter KA responses are likely to be due to AMPA receptor complexes which include the GluR2 subunit since linear I-V curves are observed. Thus, although KBP is apparently silent as a glutamate gated ion channel, its glutamate binding properties and its very high density within BG membranes that enwrap all molecular layer synapses, suggest that KBP ought to play a role in shaping the glutamate mediated synaptic responses. We are presently performing electrophysiological recordings from chick cerebellar slices to test this suggested role of KBP. The results of these studies will be presented.

THE MAGNOCELLULAR OXYTOCINERGIC SYSTEM: A MODEL FOR ACTIVITY-DEPENDENT NEURONAL-GLIAL AND SYNAPTIC PLASTICITY IN THE ADULT CNS

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Activation of the hypothalamic oxytocinergic system (parturition, lactation, osmotic stimulation) induces changes in the conformation of its neurons and glial cells such that glial coverage of the neurons diminishes and their surfaces become extensively juxtaposed. Concurrently, there is an increase in the number of GABAergic and glutamergic terminals making single and multiple synaptic contact onto the neurons. These changes are reversible with cessation of stimulation. We here discuss the possible role played by astrocytes of this system to allow such synaptic remodelling. We also show that these neurons and glial cells maintain certain embryological characteristics that may allow such plasticity. These include expression of adhesion molecules such as PSA-N-CAM, F3/F11 glycoprotein and tenascin, molecules normally found in large quantities during development and thought to intervene in synaptogenesis and establishment of tissue architecture. Such molecular features may allow these cells to manifest their capacity for morphological plasticity in adulthood, in response to factors such as central oxytocin and steroids, that we have shown to be essential in the induction of neuronal plasticity in this system.

IN THE REGENERATING RAT SCIATIC NERVE THE INCREASE OF THE B-50 / GAP-43 LEVEL OCCURS PREDOMINANTLY IN UNMYELINATED AXONS, AND LESS IN MYELINATED AXONS.

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We investigated whether myelination affects B-50 levels in the rat PNS by comparing the B-50 distribution in unmyelinated and myelinated axons of the sciatic nerve in a quantitative electron microscopic study. Rats received an unilateral sciatic nerve crush and were sacrificed eight days later. Ultrathin sections from Lowicryl HM20-embedded nerve pieces, proximal from the crush site, were labeled by specific B-50 antibodies and immunogold labeled second antibodies. The density of the B-50 immunoreactivity (BIR) was quantitated. In the intact nerve, more B-50 was associated with the plasma membrane of unmyelinated axons than with the plasma membrane of myelinated axons. The density of axoplasmic B-50, however, was similar in intact unmyelinated and myelinated axons. Comparison of BIR in the ipsilateral regenerating nerve versus BIR in the contralateral intact nerve revealed that there was a significant increase of B-50 density at the plasma membrane of unmyelinated axons, but no increase in myelinated axons. A similar comparison showed that the B-50 density in the axoplasm was increased in unmyelinated and myelinated axons, suggesting the occurrence of enhanced axonal transport in both. BIR was absent from Schwann cells surrounding the proximal nerve pieces.
We conclude that myelination affects the B-50 levels and distribution in axon shafts in the intact and regenerating peripheral nerve. On the basis of the role of B-50 in membrane signal transduction and neuroplasticity, we suggest that the differential association of B-50 with the axolemma is determined by the different properties of unmyelinated and myelinated axons.

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Departure Munich	**Arrival Tegernsee**
07.30 a.m.	08.34 a.m.
09.30 a.m	10.34 a.m.
11.30 a.m	00.34 p.m.
01.30 p.m	02.34 p.m.
03.30 p.m	04.34 p.m
05.30 p.m	06.34 p.m
07.30 p.m	08.34 p.m
10.35 p.m (bus)	00.10 a.m

And here are the connections back from Tegernsee to Munich on Wednesday September 14, 1994:

Departure Tegernsee	**Arrival Munich**
06.06 a.m (bus)	07.16 a.m
06.47 a.m	07.56 a.m
07.19 a.m	08.27 a.m
09.10 a.m	10.16 a.m
11.10 a.m.	00.16 p.m
01.13 p.m	02.20 p.m
03.10 p.m	04.16 p.m
05.46 p.m	06.56 p.m