Regeneration and Plasticity of the Central Nervous System

S18-01

CELL ADHESION MOLECULES AND SYNAPTIC PLASTICITY IN MEMORY FORMATION

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Training chicks on a one-trial passive avoidance task results in a cascade of molecular and cellular processes culminating within 60-90 minutes in posttranslational glycosylation of synaptic membrane proteins and expresion of immediate early genes c-fos and c-jun. 4 hr downstream of training, there is again vulnerability of memory to the protein synthesis inhibitor anisomycin. By 5.5hr posttraining this window closes, to be replaced by a window of sensitivity to blockade of glycoprotein synthesis. The pre- and post-synaptic membrane glycoproteins involved at both first and second time windows include cell adhesion molecules, L1 (at both times) and NCAM (at the later). Molecular dissection of the external membrane domains of L1 distinguish between a requirement for the IgG domain at the early time, the fibronectin-like domain at the later. The second time window only occurs if the animal is trained on a stimulus strong enough to be remembered for a long period; weak memories do not persist beyond 6-8hr and the second wave of glycoprotein synthesis does not occur. Treatments which enhance the salience of the experience to be remembered, such as corticosterone injection, also activate the second wave even for weak memories and thus ensure their persistence.

S18-02

THE NEURAL CELL ADHESION MOLECULE L1 AND HIPPOCAMPAL LONG-TERM POTENTIATION A. Lüthi, J.-P. Laurent, H. Mohajeeri# and M. Schachner#. Preclinical Research, F.Hoffmann-La Roche Ltd, CH-4002 Basel, Switzerland: #Neurobiology, Swiss Federal Institute of Technology, CH-8093 Zürich, Switzerland.

Long-lasting changes in the efficacy of synaptic transmission likely involve modifications in synaptic morphology or even the formation of new synaptic contacts. As L1, a neural cell adhesion molecule of the immunoglobulin superfamily, contributes to the morphogenetic processes of development, we examined the role of L1 in hippocampal long-term potentiation (LTP), a model widely used to study the mechanisms of synaptic plasticity in the vertebrate brain.

Theta-burst stimulation (TBS)-and pairing-induced LTP in the CA1 area of rat hippocampal slices was markedly reduced by local application of L1-antibodies and recombinant L1-fragments. Similarly, transgenic mice overexpressing L1 in astrocytes exhibited a clear reduction in TBS- and pairing-induced LTP. In contrast, basal synaptic transmission, post-tetanic potentiation and paired-pulse facilitation were not modified. We suggest that overexpression of L1 and the application of L1-antibodies inhibit the expression of LTP by interfering with signal transduction or adhesion processes, which may also involve other adhesion molecules like NCAM or integrins. These results support the idea that modifications of adhesion molecule-dependent cell-cell interaction, leading eventually to changes in synaptic morphology, contribute to a stable expression of LTP.

S18-03

THE INVOLVEMENT OF BDNF IN HIPPOCAMPAL LONG-TERM POTENTIATION.

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BDNF is a member of the NGF gene family, which has been shown to influence the survival and differentiation of specific classes of neurons *in vitro* and *in vivo*. The possibility that neurotrophins are also involved in processes of neuronal plasticity has only recently begun to receive attention. To determine whether BDNF has a function in processes like long-term potentiation (LTP), we produced a strain of mice with a deletion in the coding sequence of the BDNF-gene. We then used hippocampal slices from these mice to investigate whether LTP is affected by this mutation. Mutant mice show significantly reduced LTP in the CA1 region. The magnitude of the potentiation as well as the percentage of cases in which LTP could be induced successfully was clearly affected. According to the criteria tested, important pharmacological and morphological parameters in the hippocampus of these animals appear to be normal. Adenoviral vectors were used to re-express BDNF in acute slices of BDNF-knock-out mice. In most cases LTP could be rescued with this approach These results suggest that BDNF might have a functional role in the expression of LTP in the hippocampus.

S18-04

ANALYSIS OF AXONAL REGENERATION IN TRANSGENIC MICE

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Lack of axonal regeneration in the adult CNS is, in part, attributed to the low abundance of permissive molecules and to the presence of inhibitory molecules. Mice were generated in which the neurite growth-promoting adhesion molecule L1 is expressed under regulatory sequences of the GFAP gene. Axonal elongation from neurons cultured on astrocyte monolayers or cryosections of heavily myelinated CNS tissue from transgenic mice was significantly improved when compared to the same substrates prepared from wild-type mice. Mice deficient for the myelin-associated glycoprotein (MAG) were analyzed to test the hypothesis that MAG is a potent inhibitor of axonal regeneration. Myelin prepared from the CNS of mutant and wild-type mice was a similarly poor substrate for different cell types *in vitro* and axonal regrowth in the optic nerve and corticospinal tract *in vivo* was not improved in mutants when compared to wild-type mice.

S18-05

ANTIBODIES AGAINST MYELIN-ASSOCIATED NEURITE GROWTH INHIBITORS ENHANCE PLASTICITY AND REGENERATION IN THE ADULT SPINAL CORD.

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The capacity of spinal cord fiber tracts to regenerate following axotomy or to undergo plastic changes e.g. in response to denervation of neighboring territories greatly decreases during the first postnatal week in rats. This is paralled by a decrease in GAP-43 levels and occurs simultaneously with myelin formation. Prevention of myelin formation in the caudal spinal cord led to high GAP-43 levels (comparable to newborns) at 1 month of age, to successful regeneration of a population of corticospinal tract (CST) fibers following bilateral CST lesions, and to a high potential for plastic growth of unlesioned dorsal root or CST fibers in response to partial sensory or CST deafferentation. Very similar results were obtained for long distance regeneration (following partial spinal cord transections) and for CST plasticity in normally myelinated 1 - 3 months old rats as a consequence of the application of the monoclonal antibody IN-1. mAB IN-1 or a recombinant Fab fragment neutralize the powerful neurite growth inhibitory activity associated with the CNS myelin proteins NI-35 and NI-250. In spinal cord lesioned rats NI-250 treatments also result in very significant functional improvements of locomotion and certain reflexes. These results as well as preliminary anatomical data indicate that fibers induced to grow (plasticity) or to regrow (regeneration) in the juvenile or adult spinal cord are able to find appropriate targets and integrate into functionally meaningful circuits.

S18-06

EXPRESSION OF EXTRACELLULAR MATRIX MOLECULES IN THE DEVELOPMENT OF THE DORSAL LATERAL GENICULATE NUCLEUS IN TUPAIA

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The goal of the present study was to see if the extracellular matrix (ECM) may play a role in the development of the cytoarchitectonic lamination of the dorsal lateral geniculate nucleus (dLGN), in Tupaia. We investigated the organization of ECM from birth (P0) to the end of the third week after birth (P23) using peanut agglutinin lectin (PNA) and antibodies to tenascin.

Preliminary results show that at P0 the staining with PNA yields a very faint pattern of lamination. At P2, which is before cytoarchitectonic laminae will appear, we can observe two interlaminar zones. At P3 all the interlaminar zones of the adult animal can be detected, the zones between lamina 1 and 2 and between lamina 4 and 5 being more pronounced than the others. Thus, the organization of ECM seems to precede the segregation of neurons in the dLGN.

S18-07

FUNCTIONAL ASPECTS OF CALLOSAL CONNECTIONS IN THE TREE SHREW

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The goal of the present study was to investigate the functional consequences of callosal connections on neurons of the primary visual cortex in the tree shrew (Tupaia belangeri).
Single-unit activity from cortical area 17 neurons in the area 17/18 border region was recorded while the callosal influence from the contralateral hemisphere was reversibly inactivated by using the method of cryoblocking. Of 20 neurons analyzed so far 75% showed a decreased activity, 10% an increased activity, and 15% did not respond to the cryoblocking of the contralateral primary visual area. In our conditions the spontaneous and the evoked activity of neurons ceased at a temperature of about 17.5°C. The temperature gradients measured ipsilaterally and contralaterally to the cooling device in the cortical tissue of the striate cortex were 1-2°C per mm.
In summary, the preliminary results show mostly excitatory but also inhibitory effects on neurons of the primary visual cortex mediated through the corpus callosum.

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S18-08

UPREGULATION OF BDNF EXPRESSION BY VIP AND PACAP IN CULTURED CORTICAL NEURONS.

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The regulation of BDNF expression by VIP was analyzed by Northern blot in primary cultures of neurons originating from the mouse cerebral cortex. In these cultures, VIP stimulates in a concentration-dependent manner BDNF expression. The neuropeptide PACAP, which shares a high degree of sequence identity with VIP, also stimulates BDNF expression. VIP and PACAP induction of BDNF expression is completely inhibited by the NMDA receptor antagonist MK-801, therefore indicating that the effect of VIP is indirect being mediated by glutamate acting on NMDA receptors. Taken together with previous observations (Martin et al., J. Neurochem. 65, 1-9, 1995) demonstrating that the induction of *c-fos* expression by VIP or PACAP is also mediated by glutamate acting on NMDA receptors, these results strongly suggest that both peptides can increase the "throughput" or "strength" of glutamate-containing circuits in the cerebral cortex.
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S18-09

MONOCLONAL ANTIBODIES AGAINST LACTATE DEHYDROGENASE LDH5 (A) AND LDH1 (B) SUBUNITS REVEAL CELL-SPECIFIC LABELING IN THE HUMAN BRAIN.

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Polyclonal antibodies against Lactate Dehydrogenase (LDH) subunits have shown that LDH5 and LDH1 subunits are selectively distributed in the human brain. Thus, in a previous study performed in the hippocampus and occipital areas 17 and 18, the LDH5 subunit was found to be exclusively immunolocalized in astrocytes, whereas the LDH1 subunit was localized in both astrocytes and neurons. We have recently produced and engaged in the further characterization of three monoclonal antibodies against LDH subunits that display a similar pattern. Monoclonal antibody (MAb) 1 reveals neuronal perikarya, while MAb 2 and 3 selectively react with different populations of astrocytes. In the occipital region, monoclonal antibody 2 reveals astrocytes in layer I and in the white matter. Monoclonal antibody 3 reacts with protoplasmic astrocytes in the gray matter.

S18-10

CALRETININ-IMMUNOREACTIVITY DURING THE POSTNATAL DEVELOPMENT OF THE RAT CORTEX: A QUANTITATIVE AND QUALITATIVE ANALYSIS

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During the postnatal development of the rat cortex many Ca^{2+}-dependent processes occur. An overload of nerve cells with calcium(Ca^{2+}) is known to cause neurodegeneration Thus, there is a need for neuro-protective mechanisms that help to control the intracellular Ca^{2+}-concentration. One could be the presence of calretinin (CR), an EF-hand Ca^{2+}-binding protein, which is thought to act as a Ca^{2+}-buffer suppressing neurotoxic Ca^{2+}-overload. We investigated the temporal and spatial distribution of CR in five different cortical areas of the rat cortex. Quantitative immunohistochemistry revealed only minor differences between the areas examined and a transient increase of CR-expression during the second postnatal week. The same dynamics for CR were observed using ELISA and immunoblots. Thereby, we coincidentally detected an additional band at 36-38kD, whose identity has to be determined. The temporal correlation with Ca^{2+}-dependent events such as synaptogenesis, neurite elongation and spontaneous Ca^{2+}-waves suggests that CR may play a general neuro-protective role during early postnatal development of the rat cortex.

S18-11

REGULATION BY VIP AND NORADRENALINE (NA) OF GLYCOGEN SYNTHASE mRNA EXPRESSION.

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VIP and NA have been shown to promote two opposed cAMP-dependent and temporally regulated actions on glycogen levels in primary cultures of cerebral cortical astrocytes: glycogenolysis within a few minutes (Sorg and Magistretti, Brain Res 563: 227-233, 1991), and a subsequent resynthesis of glycogen, resulting within 8-10 hours in a 6-10 fold increase in glycogen content (Sorg and Magistretti, J. Neurosci.12: 4923-4931, 1992). The long-term action of VIP and NA is sensitive to protein synthesis inhibitors, suggesting that the synthesis of one or more enzymes involved in glycogen metabolism is modulated by VIP and NA. Two potential candidates for this regulation are glycogen synthase(GlyS) and glycogen phosphorylase (GlyP). We have cloned and characterized the astrocyte GlyS isozyme. As revealed by Northern blot analysis, VIP and NA increased the level of GlyS mRNA within 8-10 hours, while GlyP mRNA levels were not affected. On the other hand, VIP and NA did not affect GlyS mRNA levels in cortical neurons. This study indicates that VIP and NA, by increasing cAMP levels, simultaneously trigger a short-term effect (glycogenolysis), as well as a delayed one that is transcriptionally regulated (glycogen resynthesis). This long-term effect ensures that sufficient substrate is available for the continued expression of the short-term action.

S18-12

DOWN-MODULATION OF VOLTAGE-DEPENDENT Na^+ CURRENTS IN SLOW AND FAST GATING MODE BY PROTEIN KINASE C.

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Protein kinase C has been shown to down-modulate V-dependent Na^+ currents in Xenopus oocytes or Chinese hamster ovary cells (CNaIIA-1) transfected with the α-IIA subunit. Down-modulation was achieved by a change in the activation process in X. oocytes whereas the inactivation process was affected in CNaIIA-1 cells. α-IIA-channels in X. oocytes existed in the slow gating mode whereas α-IIA channels in CNaIIA-1 cells showed fast current kinetics.
In order to investigate wether the effect of PKC stimulation is gating mode dependent we expressed the α-IIA subunit either alone or together with the β1-subunit in X. oocytes. Coexpression of the β1-subunit caused the Na^+ currents to shift from the slow to the fast gating mode. The two-electrode voltage clamp technique and agarose-cushion electrodes designed for fast clamp speed and stable long term recording were used. We observed significant positive shifts in the half-activation voltage, decreased steepness of the voltage dependence of activation and inactivation and decreased maximal conductance for both gating modes after addition of phorbol ester (5 nM) to the bath. The half-inactivation voltage, the time course of inactivation and the reversal potential did not change. α-phorbol (600 nM) had no effect which renders direct PMA effects (not involving PKC) unlikely. Our results suggest that PKC activation acts primarily on the activation process of Na^+ channels and independent of the gating mode.

S18-13

MATURATION OF THE CYTOSKELETON IN ORGANOTYPIC SLICES OF RAT CEREBRAL CORTEX.

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Variations in the composition and modification (such as phosphorylation) of the cytoskeletal proteins were studied in organotypic slices of rat cerebral cortex. Modifications were in many instances correlated with the degree of maturation of cortical neurons and their synaptic connections. The present study was designed to evaluate the contribution of the cortical neurons, in absence of their extrinsic connections, towards the maturation process of cytoskeletal elements. We have analyzed biochemically and immunocytochemically changes in the composition of the cytoskeleton in slice cultures which were prepared from neonatal rats and maintained up to three weeks in culture using MEM-medium supplemented with normal horse serum. Changes in expression or phosphorylation of neurofilament (NF-L, NF-M, NF-H), MAP2-isoforms and tau proteins followed in qualitative terms changes observed *in vivo*. However, some differences in the ratio of neuronal vs. glial proteins and in the temporal sequence of expression were observed between cultures and their "age-matched" *in vivo* samples. It is concluded firstly that, in isolation of their extrinsic connections, the cytoskeleton of cortical neurons still differentiates according to the pattern expressed *in vivo*, and secondly, that this *in vitro* system is a suitable tool to evaluate chemical factors which could influence the maturation of the cortical cytoskeleton. Supports: SNF 31-43137.95 to B.M.R. and 31-40852.94 to J.P.H.

S18-14

RETINAL FIBER ORGANIZATION ON THE ANTERIOR POLE OF THE OPTIC TECTUM IN THE CHICK: A ^{14}C-DEOXYGLUCOSE AND TRACER STUDY

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Retinal fibers are chronotopically organized in the optic tract, i.e. late generated axons are located superficially. Since peripheral temporal fibers are generated late and located superficially, the problem arises how they can get access to the most anterior part of the optic tectum and if they form a topographic map. This problem was studied using the ^{14}C-deoxyglucose method and injections with various tracers.

In the ^{14}C-deoxyglucose study, a stationary pattern was projected onto the retina. The exposition of the 20 µm flatmount slices of the tectum to a film revealed the fully established retinotopical projection on the anterior pole of the tectum.

In the tracer study four different tracers were used: WGA-HRP, fluorogold and two dextran amines. In each animal all four tracer substances were injected vertically aligned into the superficial layers of the anterior tectum. The fields of retrogradely labeled ganglion cells in the peripheral temporal retina show a topographical correspondence to the tectal injection sites. Thus retinotopy is even present on the anterior pole of the tectum.

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S18-15

IMMUNOCHEMICAL ANALYSIS OF THE NMDA RECEPTOR NR 2D SUBUNIT DISTRIBUTION IN BRAIN AND RETINA

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The expression patterns of NMDA receptors containing the NR2D subunit in developing and adult rat brain were analyzed using a polyclonal NR2D-specific antiserum on histo-blots (Wenzel et al., Neuroreport, 7, No.1 1995 and J. Neurochem., in press). Throughout postnatal development, the NR2D subunit was detected in the globus pallidus, parts of the thalamus, subthalamic nuclei and superior colliculus. A developmental downregulation of the NR2D subunit was observed in the ventrobasal complex (thalamus), hippocampus, inferior colliculus and brainstem reticular formation indicating a functional relevance of NR2D-containing NMDA receptors only during early development of these areas. In the other regions the contribution of these receptors to excitatory neurotransmission is maintained up to adulthood.

Using confocal laser scanning microscopy, NR2D immunoreactivity (IR) was analyzed on the cellular level in the retina (rabbit and rat). In both species exclusively PKCα-positive rod-bipolar cells were stained. The NR2D-IR was detected mainly on dendritic arbors receiving synaptic input from rods, on the axonal processes spanning the inner plexiform layer (IPL) and, with the highest intensity, on axonal endfeets which are presynaptic to AII amacrine cells. The localization of the NR2D subunit to both post- and presynaptic terminals of retinal bipolar cells suggests a function of NMDA receptors in the transmission from the outer plexiform layer to the ganglion cell-layer as well as a contribution to lateral signal integration in the IPL.

S18-16

Size and asymmetry of the intra/infrapyramidal mossy fibre projection correlate with paw preference in the mouse

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Paw preference was assessed in 26 mice from two lines bred for strong (HI) or weak (LOW) preference, 38 mice of a F3-generation randomly bred from an 8-way cross (RAND), and 22 mice with variably sized corpus callosum (CC). We also compared the strain means in paw preference in nine inbred strains. The size of the intra/infrapyramidal mossy fibre projection (IIP-MF) was assessed by Timm's stain. HI mice had a 70% larger IIP-MF than LO and CC mice, with RAND mice falling in-between. The inbred strain with the largest IIP-MF had the highest degree of paw preference. In pooled HI, LO, RAND and CC mice, the individual degree of paw preference correlated positively with the IIP-MF size. In CC mice, the IIP-MF size correlated positively with test-retest reliability of paw use. The left-right *asymmetry* of the IIP-MF was positively correlated with the *direction* of paw preference in HI, LO, and RAND mice. We conclude that the size and asymmetry of the IIP-MF projection are two of many factors influencing the direction and strength of paw preference. These results provide another example of correlations between IIP-MF variations and non-spatial behaviour. Swiss NF NF 31-37497, Julius-Klaus Stiftung für Genetik, DFG (Schw 252/3), Fondation Fyssen (Paris), CNRS (URA 1294).

S18-17

Peripheral Myelin Protein PMP22 Overexpressing Mice as a Model for the Analysis of Schwann Cell Differentiation

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The peripheral myelin protein 22 (PMP22) accounts for 2-5% of PNS myelin protein. Mutations affecting the PMP22 gene have been linked to hereditary peripheral neuropathies in mice and human. A large intrachromosomal duplication containing the PMP22 gene and the resulting putative PMP22 overexpression have been suggested to be responsible for the most frequent form of demyelinating Charcot-Marie-Tooth disease. To prove that PMP22 overexpression alone is sufficient to cause myelin deficiencies in the PNS, we have generated transgenic mice that carry additional copies of the PMP22 gene. Sciatic nerves and femoral nerves of PMP22-overexpressing animals display severe hypomyelination as characterized by complete lack of myelin without signs of myelin degeneration. Continous proliferation leads to a increased number of Schwann cells which are not forming cellular onion bulbs but rather align in association with axons. Our results indicate that strong overexpression of PMP22 prevents Schwann cells from differentiation to the myelinating phenotype, leading to impaired nerve conduction and muscle atrophy comparable to severe cases of hereditary motor and sensory neuropathies.

S18-18

Native chick laminin-4 containing the s-laminin/β2-chain promotes rather than inhibits motor axon growth

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Following denervation of muscle, motor axons reinnervate original synaptic sites. A recombinant fragment of the synapse-specific protein s-laminin/β2-chain containing the sequence Leu-Arg-Glu (LRE) was shown to selectively inhibit motor axon growth. Consequently, the s-laminin/β2-chain was proposed to mediate specific reinnervation at the neuromuscular junction (Porter et al. Neuron 14, 549-559). We report here that native chick laminin-4, which contains the s-laminin/β2-chain and is present in the synaptic basement membrane does not inhibit but rather promotes motor axon growth. In native laminin, the LRE sequence of the s-laminin/β2-chain is found in a triple coiled-coil region that is formed by all three subunits. We show here that the effect of LRE depends on the structural context. Whereas monomeric LRE peptides indeed inhibited outgrowth by chick motor neurons, a small recombinant triple coiled-coil protein containing the LRE sequence did not. Moreover, the LRE sequence of the s-laminin/β2-chain is not conserved.

S18-19

LESION OF THE ZEBRAFISH OPTIC NERVE AFFECTS GENE TRANSCRIPTION IN RETINAL GANGLION CELLS.

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Each stage of neuronal differentiation is characterized by the expression of a specific set of genes. The neuronal growth-associated genes (GAPs), for example, are primarily expressed in neurons extending processes and are selectively silenced when synapses form. GAPs, such as GAP-43 and α-tubulin are re-expressed in regenerating zebrafish retinal ganglion cells (RGCs) after optic nerve lesion. Both genes are strongly induced 22-48 h after crush. However, whereas no GAP-43 expression is detected anymore 15 days after lesion, α-tubulin mRNA levels decline much slower and are still elevated in RGCs 4 weeks after crush. This indicates that distinct mechanisms silence the GAPs in differentiated neurons. To isolate additional genes that are regulated in response to nerve injury, we compared gene expression in regenerating and non-regenerating retinas by differential display. We have so far isolated three partial cDNAs encoding novel genes that are similarly regulated as GAP-43 after optic nerve lesion in that they are induced 24-48 h after crush and are downregulated to pre-crush levels after 13 days. We are in the process of isolating full-length cDNA clones to determine the regulation and function of these genes during nerve regeneration.

S18-20

THE α1 SUBUNIT OF THE GABAA RECEPTOR IN THE DEEP CEREBELLAR NUCLEI (DCN): A DEVELOPMENTAL STUDY IN THE MOUSE.

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We use the gabaergic projection of Purkinje cells unto the neurones of the DCN to study the formation of synaptic specializations(here receptor aggregates). In the confocal microscope, at P8, the label is uniformly weak for cytoplasm, soma membrane and dendrites; rare patches could be seen on soma membranes. At P10, cells with little and those with heavy cytoplasmic label showed both large patches on the membrane, and the neuropil exhibited densely labelled zones. In the adult, weak granular label of the cytoplasm is completed by a nearly continuous dense label of somatic and dendritic membranes. Synaptophysin surrounded somatic membranes in a near continuous manner, in good accordance with the continuous label of the receptor. The soma membrane appears to be gradually enriched in receptors during development; as a rule, the α1 subunit appears as aggregates, in large hot zones in the adult, in smaller spots in the young. Support: FNRS 364.017.93.

S18-21

FUNCTIONAL RECOVERY IN CO-CULTURES BETWEEN OLD AND YOUNG HIPPOCAMPAL SLICES.

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To investigate the factors that modulate the age-dependent capacity for lesion-induced sprouting and recovery expressed in hippocampal organotypic cultures (Stoppini et al., 1993), we carried out co-culture experiments using hippocampal slices maintained 1 week (young) or 3-4 weeks (old) in vitro. At these time points, slices were sectioned longitudinally into 2 pieces and placed in co-culture by associating younger with older tissue. The capacity for sprouting and synaptogenesis was tested one week after the lesion using biocytin labellings and field potential recordings across the section. The results indicate that young tissue grows quickly and effectively in young cultures and that this process is significantly slowed down when young tissue has to grow into old co-cultures. Conversely, old tissue regenerates slowly and poorly in old cultures, whereas this process is very much improved and fastened when the old tissue has to regenerate in young slices. Taken together, these results indicate a clear environmental influence on the capacity of functional recovery following a lesion in the CNS (work supported by FNRS).

S18-22

A HIGH MOLECULAR WEIGHT PROTEIN OF BOVINE CNS MYELIN INHIBITS NEURITE OUTGROWTH

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Inhibitors of neurite growth play a substancial role in the development and regeneration of the central nervous system (CNS). In rat, two of them (NI-35 and NI-250) cause collapse of neuronal growth cones and strongly inhibit neurite growth *in vitro*.

We report here that a high molecular weight protein of bovine CNS myelin is responsible for the inhibition of neurite outgrowth in PC-12 and in rat dorsal root ganglion neurons (P1). Using a purification protocol, which includes discontinous sucrose density centrifugation, strong anion exchange- and size exclusion chromatrography, a highly inhibitory protein fraction (1000x stronger biological activity) was obtained which could be neutralized by the specific monoclonal antibody IN-1 (raised against NI-250). A high molecular weight protein (250 kd) from this fraction, which was enriched in the SDS-PAGE analysis, was gel-eluted and microsequenced. The obtained amino acid sequences showed no homologies to known proteins (SwissProt and GenEMBL data bases).

S18-23

STAUROSPORINE BLOCKS LESION-INDUCED SPROUTING IN HIPPOCAMPAL ORGANOTYPIC CULTURES.

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Previous work (Stoppini et al., 1993) has shown that making a lesion using a razor blade in organotypic cultures is followed by a recovery process involving scar formation, sprouting of new fibers and formation of new functional synapses. Here we tested the effect of staurosporine, a protein kinase C antagonist, on this regeneration process. At a concentration of 1 μM, staurosporine prevented functional recovery assessed by measuring synaptic field potentials across the lesion. No alterations of synaptic transmission were found either outside the lesion nor on other control cultures. Immunostainings carried out using antibodies directed against neurofilament showed that there was a marked reduction in the number of regenerating fibers. However, in contrast to this, chelerythrine (50 μM), another PKC antagonist, did not prevent lesion-induced sprouting and functional recovery. We conclude that staurosporine blocks sprouting not by inhibition of a PKC-dependent phosphorylation, but probably through other interactions such as eventually receptors to growth factors (work supported by FNRS).

S18-24

DISRUPTION OF THE BENZODIAZEPINE BINDING SITE OF $GABA_A$ RECEPTORS IN MICE: ELECTROPHYSIOLOGICAL AND BEHAVIOURAL STUDIES.

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Using targeted mutagenesis, $GABA_A$ receptor γ_2 subunit deficient mice were generated to elucidate the physiological functions of the benzodiazepine (BZ) binding site. Patch clamp recordings on dorsal root ganglion cells revealed a decrease in the whole cell GABA current in homozygous mutant ($\gamma_2^{0/0}$) mice. The single channel conductance was reduced to a value consistent with that obtained from recombinant $GABA_A$ receptors composed of α and β subunits. Pharmacologically, the GABA response in $\gamma_2^{0/0}$ neurones was potentiated by pentobarbital but not by flunitrazepam. Acute administration of diazepam (10-30 mg/kg po), in contrast to ethanol (3 g/kg po), failed to produce sedation and loss of righting reflex in 14-day old $\gamma_2^{0/0}$ mice. Postnatally, the lack of BZ sites was associated with strong growth retardation, sensorimotor impairments and a drastically reduced life span (Günther *et al.*, 1995, PNAS 92, 7749-53). Thus, the γ_2 subunit is dispensable for the assembly of functional $GABA_A$ receptors but is required for normal channel conductance and the formation of BZ sites *in vivo*.

In heterozygous mice ($\gamma_2^{+/0}$), a reduction in the expression of the γ_2 subunit resulted in alteration of a small population of $GABA_A$ receptors (- 22%). Behaviourally, $\gamma_2^{+/0}$ mice were characterized by an increased reactivity to aversive stimuli and an enhanced sensitivity to agonists and inverse agonist of the BZ site, suggesting that these $\gamma_2^{+/0}$ mice may be useful as an animal model of emotional reactivity.

S18-25

MELANOCORTIN RECEPTORS AND ACTIONS IN DEVELOPING RAT BRAIN

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We previously observed region- and stage specific developmental patterns of melanocortin binding sites in rat central and peripheral nervous system (Lichtensteiger et al.,in press). In situ hybridization data for MC3 and MC4 receptor mRNA indicate that the majority of fetal locations with high density of melanocortin binding sites express MC4 receptor mRNA. These results suggest that the MC4 receptor may represent an important type of melanocortin receptor during the fetal period. Melanocortin actions on proliferation and various markers of growth and differentiation are being investigated in serum-free cultures of striatal cells taken at E18, i.e., shortly before the regional perinatal receptor peak. Preliminary results indicate an effect on neurofilament content.

S18-26

Function of the Prion Protein: Activation of Astrocytes after Peripheral Nerve Injury

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Infection with prions leads to a fatal disease characterized by various pathological changes such as gliosis, degeneration of neurons, spongiosis and activation of microglia. In cell cultures, degeneration of neurons as well as the activation of astrocytes seems to be caused by a direct action of the disease-specifc form of the prion protein (denoted PrP^{Sc}) or a peptide thereof. We have analyzed the reaction of astrocytes and microglial cells in the facial nucleus and the spinal cord in response to a lesion of the facial or the sciatic nerve, respectively. Using different genotypes ranging from mice lacking PrP ($PrP^{0/0}$) to PrP overexpressing mice (tgc35) we analyzed the influence of the level of the prion protein on the glial reaction. The astrocytic response was reduced in $PrP^{0/0}$ mice to about 20-30% of wild type mice while tgc35 mice did not differ significantly from controls. The microglial response was equal in all types of mice. These results suggest that PrP is involved in the activation of astrocytes after axotomy. We therefore suggest that PrP may serve as a signaling molecule between neurons and astrocytes. We are currently testing whether the activation of astrocytes *in vivo* is a direct effect of PrP or whether PrP serves to prepare astrocytes for activation.

S18-27

The zebrafish *zp-50* POU gene is a target for *sonic hedgehog* signalling in the rostral embryonic brain.

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We have characterized the expression pattern of the zebrafish *zp-50* gene containing a class III POU domain. The gene is expressed after gastrulation in a very dynamic and complex fashion in all major subdivisions of the CNS. The arrangement of different expression domains makes *zp-50* an excellent marker to study signals involved in dorsoventral patterning of the anterior brain. The gene is first activated in the prospective diencephalon at the beginning of somitogenesis. Correct *zp-50* expression in the ventral fore- and midbrain requires a functional *cyc* gene: *Zp-50* transcripts are absent from the ventral rostral brain of mutant $cyc^{-/-}$ embryos. In wildtype, but not in $cyc^{-/-}$ embryos, this region expresses *sonic hedgehog* (*shh*). *Shh* transcripts encoding an intercellular signalling molecule appear shortly before *zp-50* expression. Microinjection of synthetic *shh* mRNA into fertilized eggs causes ectopic *zp-50* expression at more dorsal positions of the embryonic brain. The close temporal coincidence of expression in the rostral brain, the similar response to the $cyc^{-/-}$ mutation, and the ectopic *zp-50* expression in the injection experiments all suggest that *zp-50* may be an early response gene to the Shh signal. A close link has been noticed in *Drosophila* between *hedgehog* signalling and the downregulation of intracellular protein kinase A activity. By blocking PKA activity in zebrafish embryos we observe similar *zp-50* misexpression as that caused by *shh*. Signalling by *shh* and PKA seems therefore to be tightly connected also in vertebrates.

S18-28

CONSEQUENCES OF MULTIPLE MUTATIONS IN GENES INVOLVED IN HEREDITARY MOTOR AND SENSORY NEUROPATHIES STUDIED IN MOUSE MUTANTS

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Genetic studies have demonstrated that mutations affecting PMP22, Protein zero (P0) and Connexin-32 (Cx32) are leading to similar diseases of the peripheral nervous system. While PMP22 has been suggested to play a role in cellular growth control and in cell recognition, P0 mediates the self adhesion of the Schwann cell plasma membrane. Cx32 is involved in the formation of gap junctions. Mice individually defective in each of these proteins show myelin degeneration in peripheral nerves characteristic for inherited human neuropathies. We have generated combinations of these mutations to examine the potential interactions between the previously mentioned myelin-associated proteins. It is anticipated that the approach will yield valuable insights into basic biological mechanisms underlying axon-glia interactions and myelination.

S18-29

CHANGES IN CALRETININ EXPRESSION BY NEURONS IN RAT DORSAL ROOT GANGLIA

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The expression of calcium-binding protein calretinin (CR) was studied in dorsal root ganglia during development. The immunocytochemical reaction revealed that CR appeared in 85% of cells in cervical, thoracic and lumbar DRG at E12. Afterwords, at later stages of embryonic development, the percentage of CR positive cells decrease rapidly. In fact at first two days after birth 14% of neurons displayed CR immunoreactivity again. And a continual decrease was observed in the number of CR immunoreactive neurons postnatally, in adult rat CR immunoreactivity was detected only in 5% of neurons.

In adult rat DRG, CR immunoreactivity was confined to both large and small neurons, but it was never detected in satellite or Schwann cells. The changes in CR expression during development was confirmed by western blot analysis.

These data show that calretinin is expressed very early in development and may thus have an important role during morphogenesis of dorsal root ganglion cells (SNF N° 3367-92).

S18-30

REGULATION OF NMDA RECEPTOR EXPRESSION IN CULTURED RAT CEREBELLAR GRANULE CELLS

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The developmental regulation of NMDA receptor expression was studied in rat cerebellar granule cell cultures maintained for 2-25 days *in vitro* (DIV). As monitored immuno-histochemically with polyclonal antisera recognising the subunits NR-1, NR-2A, and NR-2B, changes in protein expression were detected, which paralleled the variations in the subunit mRNA levels. Thus, a gradual increase in the NR1 and NR2A subunit staining was observed, while the NR-2B protein decreased progressively starting at 6-8 DIV. Further, we observed a partial colocalization of the synaptic protein synaptophysin with the NR-1 and NR-2A subunits, but not with the NR-2B protein. This suggests that granule cells *in vitro* express two distinct NMDA receptor populations. Finally, receptor down-regulation induced by CaM kinase inhibitors (Resink et al., J. Neurochem., in press) was accompanied by a redistribution of NMDA receptor subunits, as evidenced by a decrease in staining in neurites. This result provides an explanation for the neuroprotective effects of CaM kinase inhibitors against glutamate excito-toxicity.

S18-31

rMAL, A PROTEIN WITH FOUR HYDROPHOBIC DOMAINS IN CENTRAL AND PERIPHERAL MYELIN

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The rMAL (rat Myelin And Lymphocyte) mRNA was identified by a differential screening approach. It is the rat homolog of a human gene, previously cloned from T-cell lines. The predicted rMAL protein is highly hydrophobic (MW 16.8 kD) with four putative transmembrane domains. It thus shows structural similarities to other myelin proteins (e.g. PLP, PMP22) and to the connexins. Northern blot and In situ hybridzation show that rMAL mRNA is predominantly expressed by oligodendrocytes and Schwann cells in the nervous system (NS). Here, expression follows the time course of myelogenesis and is downregulated in the adult; outside the NS only low levels of mRNA are found in spleen and kidney. Immunocytochemistry localized the rMAL protein in all myelinated areas of the central and peripheral NS. Transfected cos-cells express rMAL in the Golgi network, the ER and on the plasma membrane and give evidence that the structural prediction for the protein is correct. Abundance and distribution of rMAL protein suggest an important role in myelin structure and function.

S18-32

TYROSINASE EXPRESSION IN MOUSE NEURAL TUBE

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Tyrosinase is the key enzyme in melanin synthesis and is expressed in pigment cells derived from both neural crest and neuroectoderm. This study was performed to detect tyrosinase promoter activity and tyrosinase gene expression during murine brain development. 6.1kb of mouse tyrosinase 5' region was used to direct lacZ expression in transgenic mice. During embryogenesis, the transgene reproduced tyrosinase expression in pigment cells but was also observed in embryonic neuroectoderm and migrating neural crest cells. Both tyrosinase and lacZ were detected in cell populations often organized in columnar arrangements and found throughout the entire neural tube. In the developing brain, the highest density of positive cells was localized to ventricular and subventricular zones and to evaginations of the neural tube. Tyrosinase promoter activity and tyrosinase expression are therefore not restricted to differentiated pigment cells, and we suggest that tyrosinase is a new marker for cell populations in the neural tube.

S18-33

STRATEGY FOR ANALYSING SINGLE UNIT PROPERTIES SIMULTANEOUSLY RECORDED FROM DIFFERENT AUDITORY CORTICAL FIELDS

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We recorded simultaneously 8 spikes trains from 8 microelectrodes placed in the cortical auditory fields on both sides of an anaesthetised cat. In order to understand the coding mechanism of complex sound we analysed single unit responses on peristimulus time histograms. An automatic feature detection algorithm developed by M. Abeles, was used in complementation to visual inspection.
We developed a appropriate database on 4th D in order to store all the relevant parameters for each single units. Data files from the feature detection program can be directly imported into the database. Graphical representation of these data are used as a classification tool of different response characteristics. E.g. for the coding of frequency-modulated (FM) sound we can directly represent the peak of the neuronal response as a function of the FM rate and attribute the cell to one of the following class: high pass, low pass, band pass, all pass and unresponsive. We can extract the data concerning specific populations of cells and treat them with appropriate statistical programs.

S18-34

CORTICOSTERONE LEVELS, TH- AND PNMT-ACTIVITY, AND MORRIS WATER MAZE PERFORMANCE IN PRENATALLY STRESSED WISTAR RATS

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Stress during fetal life has profound long-term effects on behavior in animals and human. Using rat as a model, we investigated the effects of prenatal stress on cognitive performance of the offspring in their later life. After mating, female Wistar rats were either left undisturbed (mothers of control animals; CON), or were stressed by immobilization during days 15 to 19 of pregnancy (mothers of prenatally stressed animals; PS). Animals were investigated in a spatial task (Morris Water Maze) at the age of 12 months. Briefly, in a circular swimming pool animals had to locate a submerged, and therefore invisible, escape platform by using statial learning strategies. One animal per litter and sex was tested at a water temperature of 9°-11°C, another one at a temperature of 19°-21°C. After 9 training days (acquisition) the escape platform was removed and the time spent in the platform quadrant searching for the platform was registered (transfer test). All animals reduced their escape latencies during acquisition irrespective of sex or prenatal treatment. However, in comparison to younger animals (3 months old) acquisition as well as transfer test performance were strongly impaired. A relation between swimming-induced corticosterone levels, escape times during acquisition, and water temperature was found for PS-males. The activity of the catecholamine synthesizing enzyms (TH and PNMT) shows only minor changes.

S18-35

Cytoskeletal proteins during marsupial brain development

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In metatherians their extreme immaturity at birth and their extended maturation period in the pouch allows developmental studies without the necessity of caesarean surgery. We have investigated by immunoblotting and immunocytochemistry the development of the neuronal cytoskeleton in pouch young of a marsupial, the tammar wallaby *(Macropus eugenii)*. Intermediate filament components changed in the second postnatal month, vimentin decreased while glial fibrillary acidic protein (GFAP) concentration increased. Neurofilament subunits NF-M and -L were present already at birth, while NF-H appeared around postnatal day 75, a time that coincides with the completion of the six cortical layers. In contrast, microtubule proteins changed later, during the fifth postnatal month. Juvenile isoforms of tau proteins, MAP1b- and MAP2 disappeared, while the myelin basic protein MBP was expressed. These events coincide with an increase of thyroid hormone levels. An immunocytochemical analysis at P25 and P144 confirmed the cell-type and subcellular specific location of individual elements of the cytoskeleton, and antibodies against cytoskeletal proteins proved to be useful markers to study the development of radial glia, axons and dendrites. This work was supported by a Swiss NSF grant 31-33447.92 and by the Dr. Joachim De Giaccomi Foundation.

S18-36

EFFECTS OF GDNF ON MOTONEURON SURVIVAL IN VIVO

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Effects of glial cell-derived neurotrophic factor (GDNF) were studied in two animal models of motoneuron degeneration. First, GDNF was shown to be a potent motoneuron survival factor at 1 week following application onto the cut sciatic nerve of neonatal rats; however, the rescue effects were not long-term. In contrast, a slow-release delivery system with brain-derived neurotrophic factor (BDNF) combined with GDNF application onto the nerve caused long-term rescue. Secondly, we treated mutant mice showing motoneuron degeneration(*pmn*)with encapsulated GDNF-secreting cells. GDNF reduced the loss of motoneurons but it had no effect on either life span or nerve degeneration. Combinations of GDNF and BDNF are now being tested.

S18-37

HYPOMYELINATION IN THE PARALYTIC TREMOR (pt) RABBIT IS THE RESULT OF MISFOLDING AND ABNORMAL TRANSPORT OF THE PROTEOLIPID PROTEIN (PLP)

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Paralytic tremor is a point mutation in the gene encoding proteolipid protein (PLP) and its isoform DM-20, which results in a substitution of His^{36} by Gln. PLP is the major structural protein of central nervous system myelin and this mutation produces severe dysmyelination in the mutant animals. We have studied the transport of PLP by transfecting Cos-7 cells with corresponding cDNAs. Using intracellular markers for rough endoplasmic reticulum (RER) and lysosomes, we showed that normal rabbit PLP follows the usual pathway of membrane proteins, while PLP carrying the pt mutation accumulates in the RER and does not reach the membrane. Thus, hypomyelination in the pt mutant is the result of misfolding and abnormal transport of PLP.

S18-38

CYTOCHROME OXIDASE PATTERN IN HUMAN AUDITORY CORTEX

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Two normal human brains were studied for cytochrome oxidase (CO) pattern in cortex involved in audition (inf. bank of sylvian fissure, sup. temporal gyrus, insula). They were fixed within 12 hours after death and the 4 hemispheres were cut in serial coronal sections; 80 µm thick sections were stained for CO (histochem.), 40 µm thick sections for cell bodies or myelin. CO was revealed in neuropil and in individual neurons. The strength of staining varied radially and tangentially within the cortex. A dark band with gradual upper and lower limits was found in layer IV and a less dark band in layer VI of some regions. Four regions stained more darkly: i) gyrus of Heschl (area TC and part of TD); ii) posterior part of planum polare (TC/TG transition); iii) part of superior temporal gyrus (TA/TB); and iv) posterior and superior parts of insula (IB). Region (i) corresponds to AI, whereas the other 3 dark and the light CO regions may correspond to other functional areas.

S18-39

Localization of the putative secretion motif of the neuronal cell adhesion molecule axonin-1

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Axon-associated cell adhesion molecules (AxCAMs) are concentrated primarily on axons and growth cones. They are known to play an important role in pathfinding decisions. Axonin-1, a member of the immunoglobulin/fibronectin-type III family of cell adhesion molecules occurs as a glycosylphosphatidylinositol(GPI)-anchored membrane-bound and as a secreted form. The predominance of the secreted form is a unique and distinctive feature of axonin-1 compared to other AxCAMs. Soluble axonin-1 is known to perturb neurite fasciculation and pathfinding in the developing chick embryo. With domain-swaping experiments between axonin-1 and its non-released relative F11, deletion mutants and monoclonal antibodies we could show that the fourth FNIII-like domain of axonin-1 is necessary for release.

S18-40

NOVEL ISOFORMS OF THE MYELIN-ASSOCIATED/ OLIGODENDROCYTE BASIC PROTEINS (MOBPS)

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The Myelin-associated/Oligodendrocyte Basic Proteins (MOBPs) are the most recent identified myelin constituents specifically expressed by oligodendrocytes in the central nervous system (CNS). The MOBP proteins are small and highly basic and are abundantly present in CNS myelin as determined by Western blotting and immunohistochemistry. The identification of novel MOBP isoforms by cDNA cloning is reported. Interestingly, the MOBP-81 isoform, which is the most abundant one during myelinogenesis, has a significant clustering of positively charged amino acids at its unique carboxy-terminus, a feature that is also found in the myelin proteins MBP and P_0, both of which are key molecules for myelin sheath compaction at the major dense line. We propose that the main function of MOBP is related to myelin sheath compaction. This idea is further supported by the developmental expression of MOBP which tightly coincides with the appearance of compact myelin. We also demonstrate that certain MOBP mRNAs are transported into oligodendrocyte processes while others stay in the perinuclear area.

S18-41

NEUROSERPIN IS AN AXONALLY SECRETED SERINE PROTEASE INHIBITOR WHICH IS EXPRESSED LATE DURING NEUROGENESIS AND IN THE ADULT

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We have identified an axonally secreted glycoprotein of CNS and PNS neurons in a compartmented cell culture system. Based on its characteristics in two-dimensional-SDS-PAGE (IEP 4.8, M, 55 kD), we purified the protein by a three-step chromatographic procedure from ocular vitreous fluid of chicken embryos. Several peptides of the purified protein were microsequenced. Based on these sequences, a fragment of the corresponding cDNA was amplified from chicken embryonic brain mRNA by reverse transcription and PCR and used as a probe to isolate a full length cDNA from a chicken brain cDNA library. Because the deduced amino acid sequence qualified the protein as a novel member of the serpin family of serine protease inhibitors, we called it neuroserpin. Analysis of the primary structural features further characterized neuroserpin as a heparin-independent, functional inhibitor of a trypsin-like serine protease. *In situ* hybridization revealed a predominantly neuronal expression of the neuroserpin mRNA during the late stages of neurogenesis and in the adult brain in regions which exhibit synaptic plasticity. Thus, neuroserpin might function as an axonally secreted regulator of the local extracellular proteolysis involved *in the reorganization* of the synaptic connections during development and synapse plasticity in the adult.

S18-42

HUMAN ANTERIOR COMMISSURE CONTAINS AXONS ORIGINATING IN THE INFERIOR PART OF THE TEMPORAL LOBE

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Human anterior commissure (AC) is believed to contain fibres from orbitofrontal and polar temporal structures, as indicated by white-matter dissections done in man and by comparison with non-human primates. The contribution of inferior part of the temporal lobe to AC was studied using Nauta method for anterogradely degenerating axons in a case with unilateral right lesion. Inferior temporal, fusiform and parahippocampal gyri and hippocampal formation were damaged at levels posterior to AC. Amygdala, anterior perforated substance, olfactory tubercle, diagonal band of Broca and prepiriform cortex were spared. Degenerating axon segments were found in the posterior, but not the anterior, bundle of the CA on both sides. Thus, the inferior part of the temporal lobe, known to be involved in memory and visuocognitive functions, sends axons to the anterior commissure.

S18-43

Expression and activity studies of platelet activating factor acetylhydrolase (PAF-AH) suggest a link between platelet activating factor (PAF) metabolism and Miller-Dieker syndrome (MDS)

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MDS is a developmental brain malformation characterized by widespread agyria of the brain and neuronal migration defects. The LIS1 gene is hemizygously deleted in MDS patients and encodes α^{LIS1} protein, a subunit of PAF-AH. PAF-AH is a trimeric enzyme composed of α^{LIS1}, β and γ subunits. It deacylates and thereby inactivates PAF (1-O-alkyl-2-acetyl-*sn*-glycero-3-phosphocholine). To begin to understand the relationship between PAF-AH and neuronal migration in the brain we have used Northern analysis and *in situ* hybridization to determine the spacial and temporal expression of α^{LIS1}, β and γ subunits in mouse. In the prenatal brain the three genes were coexpressed. In the postnatal cerebellum, γ mRNA levels were high during granule cell migration and cerebellar extracts deacylated PAF efficiently. As migration ceased, γ gene expression declined along with a decreased ability of cerebellar extracts to deacylate PAF. However, expression of α^{LIS1} and β remained unchanged. A decrease of γ mRNA levels but not of α^{LIS1} and β was also observed in the adult cerebral cortex. We propose that PAF-AH is important for neuronal migration. Abnormal levels of PAF as a result from a partial loss of PAF-AH might be the cause of the neuronal migration defect observed in MDS.

S18-44

Chemotaxis che-14 gene required in development of sensory organs and associated glial cells in *Caenorhabditis elegans*

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In nematod *C. elegans*, sensory nervous system consists in 302 neurons and 56 glial-like cells. Worm responds to chemicals using 26 chemosensory neurons associated with 4 glia cells. The following results are based on DiO membrane lipophilic green-labeling 12 on 16 amphidial chemosensory neurones (at 20°C: 11.7 neurons n=60 in Wild-Type, 0.7 n=50 in che-14(*e1960*), and 6.0 n=50 in lin-26(*n156*)).

Che-14(*e1960*)$_{LGI}$ was localized at ~0.48 $_{MAP\ UNIT}$, and mapped using markers: 0.026$_{MU}$ with unc-40(*e271*) n=68, 0.42$_{MU}$ with bli-4(*e937*) n=68, 0.48$_{MU}$ with dpy-5 (*e61*) n=47, 0.86$_{MU}$ with unc-87(*e1216*) n=47, 1.68$_{MU}$ with unc-13(*e51*) n=12.

Testing lethal genes as potential alleles of che-14, *e1960* did not show any complementation with let-377(*h110*) n=334, let-376(*h130*) n=218, let-379(*h127*) n=222, let-393(*h225*) n=204. The situation was the same for lin-26(*n156*)$_{LGII}$ n=109

Transferring duplications, hDp 12, 16, 17, 18, 19, 31, 52, 61, 67, 72 covered and rescued che-14(*e1960*) (n=82, 114, 122, 142, 57, 309, 11, 207, 36, 144 respectively), and hDp 44, 68, 70 did not (n=133, 232, 94 respectively) (notice hDp72 covered unc-40(*e271*) n=185).

With an EMS-20µl/4ml-4h mutagenesis on ♂ unc-29(*e1072*)$_{LGI}$;him-5(*e1490*)$_{LGV}$ crossed with ♀ dpy-5(*e61*)che-14(*e1960*)$_{LGI}$, I have performed a non-complementation screen on 10 694 F1s and isolated only 1 ♀ with a new allele che-14(*mc16*)$_{LGI}$.

Cosmid injections C04F1 or M01E4 rescued *e1960* and *mc16* (with C24H1 cosmid rescuing dpy-5(*e61*) as positive marker, instead of plasmid markers pRF4 conferring Rol-6(*su1006*)$_{LGII}$ and pBx rescuing Pha-1(*e2123*), both had inefficient expression in che-14 animals).

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S18-45

INSERTION OF NEWLY SYNTHESIZED MEMBRANE-ANCHORED AXONIN-1 AT AXONAL GROWTH CONES ((U. Ziegler, L. Vogt, R.J. Giger, B. Kunz, and P. Sonderegger) Institute of Biochemistry, University of Zurich, Zurich Switzerland

Growth cones are the leading tips of neurites with the capability to probe constantly their environment for local growth stimulating or inhibiting cues during neurite outgrowth. Pathfinding decisions of growth cones are thought to be regulated by interaction of cell adhesion molecules on the surface of growth cones with surface molecules on neighboring cells or components of the extracellular matrix. Using an adenovirus based vector we expressed the glycosyl-phosphatidyl-anchored neural cell adhesion molecule axonin-1 of the chicken in rat dorsal root ganglia neurons. Kinetic studies demonstrated that heterologously expressed axonin-1 reached the neuronal surface at the growth cone and provided evidence that axonal axonin-1 was derived from growth cone axonin-1 by retrograde diffusion.

S18-46

THE HOMOPHILIC BINDING OF AXONIN-1 MOLECULES IS MEDIATED BY TWO HETEROTYPIC CONTACTS

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The axon-associated cell adhesion molecule axonin-1 engages in a homophilic binding (Rader et al., Eur. J. Biochem. 215:133-141, 1993) and forms heterophilic contacts with NgCAM (Kuhn et al., J. Cell Biol. 115:1113-1126, 1991) and NrCAM (Suter et al. J. Cell Biol. 131:1067-1081, 1995). To localize the homophilic binding site of axonin-1 we have tested Fab fragments of 7 preselected monoclonal antibodies known to interfere with the homophilic ligand binding function of axonin-1. The epitope of four antibodies was mapped to the 3rd and 4th fibronectin-type-III domains and three epitopes were on the 1st immunoglobulin domain. These results indicate that the homophilic interaction involves two binding regions, one on the fibronectin-type-III domains near the membrane where the molecule is GPI-anchored and one on the 1st aminoterminal Ig domain. We propose that the homophilic binding of axonin-1 molecules is achieved by a heterotypic binding pattern in which the N-terminal binding region of one molecule binds to the C-terminal region of the other and vice versa. We speculate that the same binding sites may be engaged in an intramolecular interaction within the same molecule, stabilizing the horseshoe-like structure of axonin-1 molecules that are not involved in a homophilic interaction.

S18-47

***IN VITRO* CHARACTERIZATION OF MACROGLIAL CELLS FROM NORMAL AND REGENERATING *XENOPUS* OPTIC NERVE**

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Glial cells have been identified in cultures of explanted optic nerve (ON) segments. Among the immunocytochemical markers for mammalian glial cells *in vitro*, monoclonals to epitope A2B5 (recognizing precursor cells of the astrocyte-oligodendrocyte lineage) and O4 (early oligodendrocyte differentiation) are the only well known antibodies cross-reacting with amphibian tissue. Staining for A2B5 is positive in 6day-old cultures. O4 is expressed later, at 2 weeks, and remains detectable in 7 week-old cultures. A2B5-bearing cells but not O4-positive cells, are twice as frequent in explants of regenerating ON than those from control nerve. Cytokeratin expression, an indicator of astrocytic differentiation *in situ*, is downregulated with culture duration. This contrasts with the stable expression of GFAP in ON cultures of mammals. We have assessed macroglial proliferation by BrdU incorporation and immunolabeling *in vitro* with respect to the *in situ* position of the ON explant. In cultures from normal ON, proliferative frequency is highest in cultures of the mid-nerve segment. In explants from freshly lesioned ON, maximal frequency is found in material from the region just distal to the lesion. Mitotic activation appears to propagate distally, since, in material explanted later after lesioning, maximal proliferation is obtained from more distal segments. This observation *in vitro* perfectly correlates with the proliferative wave along the distal nerve observed also *in situ*.

S18-48

THE NEURAL CELL ADHESION MOLECULES NGCAM AND AXONIN-1 INTERACT IN THE PLANE OF THE SAME MEMBRANE

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The axonal suface glycoproteins NgCAM and axonin-1 promote neurite outgrowth and fasciculation and regulate growth cone guidance. A direct binding between NgCAM and axonin-1 has been demonstrated using isolated molecules conjugated to the surface of fluorescent microspheres. During neurite outgrowth, axonin-1 functions as in vitro substratum for growth cones expressing NgCAM. To make NgCAM available for detailed topological studies on the NgCAM/axonin-1 interaction, we cloned the NgCAM cDNA by PCR and found a primary structure that deviates considerably from the published sequence. By expressing NgCAM and axonin-1 in myeloma and COS cells and performing cell binding studies we found that NgCAM and axonin-1 can not bind when present on the surface of different cells. In contrast, an association of NgCAM and axonin-1 in the plane of the same membrane was revealed by antibody-induced capping/cocapping experiments. Crosslinking studies in DRG neurons show that axonin-1 and NgCAM tend to form discrete heterodimeric complexes on the surface of non fasciculated neurons.

S18-49

THE PROTEOGLYCAN DSD-1-PG OCCURS IN "PERINEURONAL NETS" AROUND PARVALBUMIN-IMMUNOREACTIVE INTER-NEURONS OF THE RAT CEREBRAL CORTEX E.S. Wintergerst[+], A. Faissner[1], M. R. Celio; Institute of Histology and general Embryology, University of Fribourg, CH-1705 Fribourg and [1]Department of Neurobiology, University of Heidelberg, D-69120 Heidelberg

Proteoglycans involved in the shaping of the developing brain are often preserved in the adult brain in more restricted locations. We have studied the fate of DSD-1-PG, a chondroitin sulfate proteoglycan containing the hybrid epitope DSD-1. DSD-1-PG exerts neurite outgrowth promoting activity and has been shown to occur in the developing brain during late brain development and into adulthood. In the adult rat brain mono- and polyclonal antibodies against DSD-1-PG labelled only the circumference of a selected subpopulation of neurons. These nerve cells invariably expressed the calcium-binding protein parvalbumin. The label occupied the extracellular space in close vicinity to the cell body, surrounding axon terminals and glial end feet, but was absent from synaptic clefts. DSD-1-PG is thus shown to be an additional representative of the growing list of substances found in perineuronal location in the adult mammalian brain.

S18-50

EXCITATORY AMINOACIDS STIMULATE GLYCOLYSIS IN ASTROCYTES VIA ACTIVATION OF THE Na^+/K^+ ATPase

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Several lines of evidence indicate that astrocytes might play a pivotal role in the control of cerebral energy metabolism upon neural activation.We have observed that excitatory aminoacids (EAAs) like L-glutamate and L-aspartate increase 2-deoxyglucose (2-DG) uptake and phosphorylation by mouse cerebral cortex astrocytes in culture. The inhibitory aminoacid GABA, as well as other non-transmitter aminoacids were without effect. The stimulatory effect of EAAs, which is prevented by removal of Na^+ from the extracellular medium, is mediated by a Na^+-dependent glutamate transporter. Since the effect on 2DG uptake can be prevented by ouabain, an inhibitor of the Na^+/K^+ ATPase, we have engaged in a more direct demonstration of the involvement of the pump. Using ^{86}Rb uptake as an index of the activity of the Na^+/K^+ ATPase, we have found that glutamate increases Rb uptake into astrocytes. Like 2-DG uptake, this effect is also prevented by 100 µM ouabain, further supporting a functional link between these two processes. Since EAAs also increase lactate release by astrocytes, these observations also strongly support the view that EAAs stimulate glycolysis in astrocytes via activation of the Na^+/K^+ ATPase. When viewed in their physiological context, these data suggests that upon cortical activation, EAAs released by activated neurons provide a direct signal for astrocytes to take up glucose and metabolize it to lactate, which can be used by neurons as an energy source.

S18-51

CLONING AND CHARACTERIZATION OF G42, AN EPH-TYPE TYROSINE KINASE, EXPRESSED IN THE NEURAL CREST OF *XENOPUS LAEVIS*

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Receptor tyrosine kinases (RTKs) have been identified as key players controlling various stages of vertebrate embryonic development. In a search for RTKs expressed during early *Xenopus* develoment, we have recently identified an novel Eph-type tyrosine kinase called G42 [Brändli & Kirschner (1995) Dev. Dyn. 203: 119-140]. G42 shows an interesting expression pattern during neurulation as transcriptions is confined to migrating cranial neural crest of the second (hyoid) arch. We report here the cloning and sequencing of a full-length cDNA for G42, and of a partial cDNA, derived from second gene of G42, called G50. Computer-assisted sequence analysis suggests that G42 and G50 are most closely related to the mouse Eck tyrosine kinase gene. We are currently generating dominant negative mutants in order to dissect the function of the G42 in neural crest formation.

S18-52

EXPRESSION OF P-GLYCOPROTEIN IN BRAIN CAPILLARY ENDOTHELIAL CELLS

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P-glycoprotein is an ATP-dependent transport system with a broad substrate specificity which extrudes chemicals from the inside of the cell to its outside. P-glycoprotein is a product of the *mdr* gene which confers the multidrug resistance phenotype to tumor cells. P-glycoprotein is also expressed in various normal tissues such as the blood-brain barrier. Since brain capillary endothelial cells are the main constituents of the blood-brain barrier, expression of P-glycoprotein in primary cultures of these cells was studied. Using the monoclonal antibody C219, P-glycoprotein was localized on cultured cells by immunostaining. To exclude any cross-reactivity of the antibody, this finding was verified by the Western-blot technique using parental and multidrug resistant P388 tumor cells as a control. Uptake experiments revealed the functional intactness of P-glycoprotein: Uptake of daunomycin was enhanced in presence of verapamil or in ATP-depleted cells. Using a side-by-side diffusion chamber system, correct expression of P-glycoprotein at the apical side of the cell monolyer will be verified.

S18-53

G-PROTEIN-MEDIATED DESENSITIZATION OF A CATIONIC CURRENT ACTIVATED BY METABOTROPIC GLUTAMATERGIC AND MUSCARINIC RECEPTORS IN CA3 NEURONS *IN VITRO*

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A cationic conductance activated by bath-application of 1S,3R-ACPD (50 µM) or MCh (5 µM) undergoes rapid desensitization (tau of ~20 sec) upon prolonged agonist application. Experiments involving the consecutive transient application of agonists indicated that 3 minutes was required for 50% recovery from desensitization. Cross-desensitization between metabotropic glutamatergic and muscarinic receptors was observed, suggesting that the underlying mechanism occurs downstream to the membrane receptors. The desensitization process appears to be related to activation of a G-protein. Intracellular dialysis with GDPβS (1 mM, non-hydrolyzable analogue of GDP) inhibits desensitization such that the cationic current amplitude is greatly enhanced (~200 %) and in most cells becomes irreversible. This G-protein is pertussis toxin-insensitive. We are now attempting to determine whether the G-protein is directly linked to the cationic channel or is coupled to subtypes of mGluRs and muscarinic receptors different from those responsible for the cationic current.

S18-54

EVIDENCE INDICATING TROPHIC IMPORTANCE OF THYROID HORMONES IN REGENERATING RAT SCIATIC NERVE

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Attempts to enhance peripheral nerve regeneration by intraperitoneal injection of thyroid horomones lead to conflicting results. In the present study we provide evidence that a local treatment with triiodothyronine (T_3) promotes the regeneration of rat transected sciatic nerve. Rat sciatic nerve regeneration was examined in 10-mm impermeable silicone chambers filled either with T_3 or with sterile solvent as a control. Two or four weeks after surgery the regenerated nerves from within the silicone chambers were dissected and fixed for histological studies. Longitudinal sections of regenerated nerve immunostained with neurofilaments showed that the distance covered by regenerating axons was longer in T_3 treated animals than in controls. A great number of axons arriving at the distal end in experimental animals, while axons were less frequently seen at the distal end in controls. The semi-thin cross sections from T3 treated rats/controls showed a more mature-appearing regenerated nerve based on the size of nerve, the epinerium and vascular structures, and the percentage of myelinated or unmyelinated axons.

In conclusion, pharmacologic administration of exogenous thyroid hormones within a silicone chamber enhances regeneration of axons across the sectioned rat sciatic nerve (S.N.F., No: 31-43 254-95)