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# Abstracts of Poster Presentations—Session 2

## P42 IMMUNE-PRIVILEGED SERTOLI CELLS AS GENE DELIVERY VEHICLES FOR SPINAL CORD INJURY

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We have evaluated transplantation of immune-privileged Sertoli cells as a proprietary means for delivery of recombinant human neurotrophin-3 (hNT-3) into the injured spinal cord. Sertoli cells infected with recombinant adenovirus expressing hNT-3 were found to produce high amounts of biologically active hNT-3. We tested the bioactivity of the secreted hNT-3 from infected cells on embryonic neocortical cultures and found increased axonal outgrowth in cells cultured in the presence of conditioned medium from modified Sertoli cells. Adult Sprague Dawley rats were implanted with allogeneic Sertoli cells genetically modified to produce both hNT-3 and green fluorescent protein (eGFP) or the eGFP alone. Cells were implanted into either the uninjured spinal cord or immediately after contusion injury at the T8 vertebral level. Viable eGFP fluorescing cells, positive for hNT-3, were detected in intact spinal cord. Injured animals that were implanted with NT-3 secreting Sertoli cells were found to produce significantly large amounts of hNT-3 as compared to animals that were injured but did not receive any cell implants (untransplanted control). Animals that received transplantation of Sertoli cells, expressing either eGFP or eGFP and hNT-3, had significant improvement in functional recovery, at earlier time points after injury, as compared to those animals subjected to injury alone (untransplanted control) as assessed by the BBB scale. Moreover, the rate of functional recovery was improved in these animals. In addition, recovery of automatic reflex emptying of the bladder was significantly improved in animals that had been implanted with cells secreting hNT-3. We found that Sertoli cells significantly attenuated early macrophage infiltration based upon quantitative assessment of inflammatory cells in the acutely injured spinal cord. Together, these results suggest that hNT-3 delivery by allogeneic Sertoli cells is a promising strategy for the treatment of spinal cord injuries.

## P43 GENERATION OF MULTIPLE CELL TYPES WITH REGULATABLE TYROSINE HYDROXYLASE PRODUCTION: A POSSIBLE METHOD TO IMPROVE CELL BASED THERAPIES FOR PARKINSON'S DISEASE

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Effective treatment of neurological diseases such as Parkinson' disease, Huntington's disease, and epilepsy, remains problematic. The development of new therapies that capitalize on our growing understanding of molecular genetics and developmental cell biology provides some promise for clinical applications. The unfortunate disappointments of the recently reported double-blind trials of cell transplant therapy for Parkinson's disease have necessitated a return to the bench, and a rethinking of the approach to cell based therapies. We have genetically engineered three conditionally immortalized cell lines [one neuronal, and two glial (astrocytic and oligodendrocytic)] with the cDNA coding for the dopamine synthesizing enzyme tyrosine hydroxylase (TH). The cDNA was inserted downstream of a doxycycline responsive (tet-off) promoter in the LINX vector, using a PCR-based cloning strategy. The

LINX vector produces a transactivating protein (tTA) that drives the expression of the gene of interest. Doxycycline binds the tTA and halts transcription. The resulting construct was used to transfect multiple cell types that had been previously transfected with a temperature sensitive oncogene. Following transfection, the cells were found to produce immunologically detectable TH (western blots and immunocytochemistry). Importantly, with the addition of doxycycline to the medium, the ability of the tTA to drive gene expression was suppressed by more than 90%. Several cell lines of each type were generated using these means and they are currently being evaluated for regulatable catecholamine production. The ultimate goal of producing transplantable cell populations that have controllable expression of therapeutic genes will likely be achieved using the methods used here. Combining stem cell technology and molecular genetics may provide a way to overcome the recently reported side effects produced by cell replacement strategies for Parkinson's disease. These cells provide new tools for basic exploration of improved cell based therapies for neurodegenerative disorders.

### P44 HUMAN NEURONAL CELL LINES FOR THE TREATMENT OF PAIN AND SPASTICITY FOLLOWING SCI

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Chronic neuropathic pain and spasticity after spinal cord injury (SCI) are common clinical problems. SCI pain has often proven difficult to treat and no pharmacologic approach has been shown to be effective over time in a significant number of individuals. Spasticity is often treated with an implanted mechanical pump to supply the agent baclofen, but such technology needs improvement. The possibility of transplanting cultured cells, derived from expandable cell lines, that release biologic agents into the subarachnoid space offers a new approach to the treatment of chronic pain and spasticity in SCI. Our laboratory has pioneered the development of cell lines bioengineered to express specific antinociceptive and antispastic agents, such as the inhibitory neurotransmitter gamma-aminobutyric acid (GABA). GABA also occurs endogenously in the motor and pain-processing pathways of the spinal cord, but is not likely to be present in sufficient concentrations after SCI to function effectively in dampening the sensory imbalance that induces chronic neuropathic pain and spasticity. From the human NT2 cell line, we have derived two unique neuronal human cell lines: The hNT2.19 GABA and hNT2.19 serotonin (5HT) lines. Each has a characteristic phenotype, including the synthesis and secretion of their respective neurotransmitters after as little as a week of differentiation in vitro. Each cell line was transplanted into the subarachnoid space at two weeks after injury in two different models of SCI pain and spasticity. The SCI pain model uses spinal injection of quisqualic acid (QUIS) to induce tactile allodynia and thermal hyperalgesia in the rat hindlimbs. The S2 sacral spinal cord transection is used as the model for spasticity. Only the GABAergic hNT2.17 cell line improved spasticity behaviors; transplants of either cell line improved the behavioral sensitivity associated with neuropathic pain. Each of these cell lines is being developed for testing in clinical trials after SCI.

### P45 TRANSPLANTATION OF MULTIPOTENTIAL AND LINEAGE-RESTRICTED PRECURSOR CELLS DERIVED FROM FETALSPINAL CORD INTO THE MATURE CENTRAL NERVOUS SYSTEM (CNS)

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Transplantation of neural precursor cells may be an ideal therapeutic strategy for cellular replacement and repair because of advantages they provide in achieving a desired phenotypic

fate. Multiple classes of precursor cells have been isolated and characterized from the CNS including multipotential neural stem cells and lineage-restricted precursor cells. However, the optimal transplantation protocols for cell replacement and repair in the mature CNS are not known. We have previously reported that in developing spinal cord multipotential neuroepithelial (NEP) stem cells predominate at E10.5, whereas at E13.5, the caudal neural tube consists mostly of precursor cells—neuronal restricted precursors (NRPs) and glial restricted precursors (GRPs)—with lineage restrictions for neurons and glia, respectively. In the current study, we examined the fate of E10.5 multipotential NEP cells and a mixed population of E13.5 lineage-restricted cells after transplantation into the adult spinal cord, striatum, and hippocampus. To track transplanted cells reliably in vivo, cells were prepared from transgenic rats expressing the human placental alkaline phosphatase gene. Our results show that grafted NEP cells survive poorly at 3 days, and no cells were observed at later time points in all three CNS regions. These results indicate that most CNS regions do not support survival of multipotential cells derived from fetal CNS. In contrast, at 4 weeks post-graft, lineage-restricted precursor cells showed selective migration along white matter tracts and robust survival in all three CNS regions. These cells also expressed the mature neuronal markers NeuN and MAP2, as well as the oligodendrocyte marker RIP and astrocyte marker GFAP. We conclude that mixed populations of lineage-restricted CNS precursors are well suited to the adult CNS and provide a promising transplant for cellular replacement because of their good survival, robust differentiation and migration properties.

## P46 USE OF TRANSGENIC RAT CELLS TO TRACK TRANSPLANTED SCHWANN CELLS WITHIN THE INJURED AND INTACT ADULT RAT SPINAL CORD


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Transplantation of Schwann cells can promote regeneration and myelination of axons after spinal cord injury. However, the relative contribution of host and transplanted cells to this repair has been difficult to determine. Recently transgenic rats that ubiquitously express human placental alkaline phosphatase (PLAP) have been developed and their cells subsequently demonstrated to express the transgene in vitro and in vivo for up to six weeks following transplantation of precursor cells. PLAP cells provide a useful tool for evaluating transplanted cell survival and their interactions with host tissue. The PLAP protein is detectable via histochemistry and immunohistochemistry, and the gene construct via PCR. In the present experiment, Schwann cells were isolated from transgenic PLAP rats and transplanted into the uninjured, contused or transected spinal cord. Prior to transplantation PLAP Schwann cells maintained PLAP expression in vitro, continued to express Schwann cell antigenic markers (p75, S100, GFAP), and formed myelin when cocultured with DRG axons. When transplanted into the spinal cord, double labeled PLAP+/p75+ Schwann cells were initially observed at the injection site. Over time double labeled cells decreased. Few double labeled cells were observed two weeks after transplantation, however many cells expressed p75. Decreased PLAP staining corresponded with a decrease in PLAP DNA within the first 24 hours. Together this suggests that the decrease in PLAP staining is due to the death of the transplanted cells and that host Schwann cells enter the spinal cord following transplantation. These initial studies suggest that PLAP cells

may be a useful marker for identifying transplanted Schwann cells, and their interactions with host cells, as well as a useful tool for optimizing the conditions for cells survival following transplantation.

### P47 ELEVATION OF CYCLIC AMP ENHANCES REGENERATION AND IMPROVES BEHAVIORAL RECOVERY IN SCHWANN CELL-GRAFTED ANIMALS AFTER SPINAL CORD INJURY

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The search continues for interventions that will enable axons to regenerate across the injury site and into the uninjured tissue beyond after damage to the spinal cord. It is now known that central neurons are able to regenerate if an appropriate environment is introduced. Inhibition of axonal growth is due, in part, to the abundance of myelin debris and a glial scar containing proteoglycans and semaphorins. Central neurons may also be unable to regenerate as a result of an imbalance between growth "stop" and "go" signals within the cell soma. A promising new strategy to stimulate neurons to overcome inhibitory signals is the activation of cAMP signaling pathways. We have employed this strategy after thoracic (T8) moderate contusion in adult rats, transplanted with Schwann cells (SCs) 1 wk post-injury. Elevation of cAMP in injured-only and SC grafted animals was achieved by (i) a one-time db-cAMP injection near the growth cones at the injury site (transient elevation); (ii) inhibition of cAMP hydrolysis globally by subcutaneous administration of the phosphodiesterase IV inhibitor, Rolipram, immediately after injury (acute) or at 1 wk (delayed) for 2 wk (to prolong the elevation of cAMP); or (iii) a combination of these treatments. We show that global inhibition of cAMP hydrolysis by rolipram can produce significant sparing of supraspinal and proprioceptive axons and promote myelination when combined with SC grafts. Furthermore, combination of rolipram with local increases in cAMP at the growth cone increased the amount of sparing and myelination, promoted regeneration of serotonergic fibers into and beyond SC grafts, and led to significant improvements in locomotor function. By using a combination strategy, we found that cAMP levels are important for protection and regeneration of injured central nervous system axons in vivo and suggest that this treatment could be useful for human spinal injuries.

Supported by the Christopher Reeve Paralysis Foundation, NINDS, Miami Project and Buoniconti Fund.

### P48 THE NICHE FOR NEURAL STEM CELLS AND PROGENITOR CELLS IN THE ADULT RAT BRAIN

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Fractones are newly revealed extracellular matrix structures associated with neural stem cells and progenitor cells, as well as astrocytes and macrophages, in the ventricle walls of the adult mammalian brain (Mercier et al., 2002; 2003). We speculate that fractones, which are labyrinthine protrusions of perivascular basal lamina within the subependymal layer, are the sites for growth factor/extracellular matrix interactions, and form the "niche" that govern stem cell and progenitor cell proliferation, differentiation, and migration. Here, we identified protein components of fractones, and established anatomical relationships between post-mitotic neural cells and fractones. Components of fractones were assessed by immunocytochemistry using Confocal Laser Scanning Microscopy on frozen sections of adult rat brains. Post-mitotic cells were identified by bromodeoxyuridine (BrDU) antibodies on frozen sections generated from rats treated with intracerebroventricular (ICV) injections of

BrDU 1-5 days prior animal sacrifice. In these experiments, the animals were either control animals or animals treated with ICV perfusion of brain derived neurotrophic factor (BDNF). In all sections examined, we found that fractones were uniformly immunoreactive for collagen-IV and laminin-gamma-1, two basic components expected in basal lamina structures. Fractones were also immunoreactive for both matrix metalloproteinase-2 (MMP-2) and MMP-9. Interestingly, some fractones were intensely immunoreactive for these MMPs. In addition, some fractones exhibited collagen-1 immunoreactivity. Because both collagen-1 and MMPs are involved in remodeling during both development and adulthood, we suggest that the presence of these molecules in fractones reflect remodeling activity associated with adult neurogenesis. Furthermore, we frequently observed post-mitotic BrDU-labeled cells in close proximity to- or directly in contact with-fractones. These associations were frequent when neurogenesis was stimulated via BDNF injections. Together, these results suggest that fractones may participate in morphogenic events, including proliferation of neural stem cells and progenitor cells throughout adulthood.

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## P49 FRACTONES AND THEIR COMPONENTS IN ADULT BRAIN AND SPINAL CORD

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Fractones are newly revealed extracellular matrix structures protruding from perivascular basal laminae, and directly associated with neural stem cells, progenitor cells, plus local macrophages, microglial cells, and astrocytes in the ventricle walls of the adult mammalian brain (Mercier et al., 2002; 2003). We previously found that laminin and collagen IV, two ubiquitous components of basal laminae are present in fractones. Here, we extended our investigation of fractone composition and extent in the central nervous system (CNS), using immunocytochemistry and Confocal Laser Scanning Microscopy. First, we confirmed that fractones are ubiquitous specialized basal laminae in adult mammals, characterizing these structures in the ventricle walls of adult humans, rats, and mice. The presence of fractones was investigated in mice in the entire CNS, using serial frozen sections, laminin and collagen-IV immunoreactivity. In addition to third and lateral ventricles, where they were initially characterized, fractones were identified in the walls of the olfactory ventricle, fourth ventricle, brain stem, and spinal cord. Series of fractones, as imaged by serial punctuation of laminin immunoreactivity, were also found along the rostral migratory stream (RMS), the migrating pathway for neuroblasts generated in the dorsal portion of the lateral ventricle, en route towards the olfactory bulb. Immunoreactivity for perlecan, a major heparan sulfate proteoglycan (HSPG) involved in the binding, activation, and presentation of growth factors involved in tissue remodeling, cell proliferation and differentiation such as FGF2, was specifically associated with fractones in all ventricle walls. Nidogen, a matrix molecule binding laminins to collagen-IV, was specifically associated with a subpopulation of fractones. These results suggest that fractones may form a functional extracellular interface between connective tissue cells (such as macrophages always associated with fractones), neural cells or their progenitors, contributing to the “niche” that regulates growth factor and cytokine activity, and governs neural cell proliferation, differentiation, migration, and function throughout adulthood.

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## P50 THE EFFECTS OF P75 AND RHO PATHWAY INHIBITION ON THE SPROUTING OF DESCENDING AMINERGIC FIBRES FOLLOWING DORSAL ROOT INJURY

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A dorsal rhizotomy and the subsequent degeneration of distal axons and terminals results in the deafferentation of the dorsal grey matter in the spinal cord. The increase in deafferented post-synaptic targets may promote the sprouting of descending supraspinal axons and possibly lead to changes in the sensorimotor function of injured animals. Previous work has shown that a variety of inhibitory molecules and environments may be overcome by treatment with pharmacological inhibitors of the Rho-GTPase pathway, leading to an increased propensity for axonal regeneration and/or sprouting. Recent evidence indicates that inhibitory myelin proteins signal through the p75 neurotrophin receptor (p75NTR) and Rho. Here, the Rho-kinase inhibitor Y-27632 was applied intrathecally in rats for seven days following septuple dorsal rhizotomy. As well, wild-type and p75NTR knockout mice were similarly treated with NGF or NT-3. The sprouting of serotonergic (5-HT) and noradrenergic fibres in the spinal cord dorsal horn was then assessed. The application of Y-27632, NGF, and NT-3 significantly increased the density of 5-HT- and tyrosine hydroxylase (TH)-immunolabled fibres in both the ipsi- and contralateral spinal cord. Increases in the density of both fibre types were also seen in p75NTR knockout mice compared to wildtype in the presence and absence neurotrophins. The data suggest that sprouting of aminergic fibres is influenced by neurotrophins and signaling through the p75/Rho pathway.

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## P51 THE ROLE OF P75 IN REGENERATION ACROSS THE DORSAL ROOT ENTRY ZONE

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The biological function of the p75 neurotrophin receptor (p75NTR) in axonal regeneration failure is poorly understood. Although this receptor has been implicated in the promotion of regeneration through neurotrophin binding, p75NTR has also been suggested to act as a transducer for myelin-derived inhibitory proteins, responsible for the activation of GTPase Rho, and the consequential collapse of growth cones. Following septuple dorsal rhizotomy in homozygotic p75NTR knockout mice, we have been able to directly examine the effect of p75NTR on the regenerative capacity of sensory neurons, in both the absence and presence of exogenous neurotrophic treatment in vivo. Immunohistochemical analysis of the of the transganglionically-transported tracers (B-fragment of cholera toxin and wheat germ agglutinin), injected intraneurally, several days prior to sacrifice, revealed a significant increase in p75-/- neural regeneration into the central nervous system through the dorsal root entry zone, compared with the wild-type strain. Interestingly, topical treatment of neurotrophin-3 (NT3) or nerve growth factor (NGF) in the p75NTR knockout strain only provided a small, limited advancement of this regenerative ability compared to a similar treatment in wild-type mice. In a similar model, poor regeneration of injured dorsal root sensory axons occurred in Wistar rats treated intrathecally with a specific Rho Kinase antagonist (Y-27632). These results suggest that p75NTR participates in the inhibition of regeneration following dorsal root injury in the absence of Rho Kinase activation. Based on these results, we propose that the absence of p75 in sensory neurons leads to an increased availability of Schwann-cell derived neurotrophins for trk binding in the regenerating dorsal root, and enhanced regeneration into the spinal cord.

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## P52 VESICULAR GLUTAMATE TRANSPORTER 1 AS AN ANATOMICAL MARKER OF ADULT RAT DORSAL COLUMN AXON SYNAPSE FORMATION

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Several strategies have recently been reported that allow dorsal column axonal regeneration after spinal cord injury. For this regeneration to be meaningful, functional synapses must be restored with target neurons. Here we report that vesicular glutamate transporter 1 (vGluT1) may be useful as an anatomical marker of functional synapses made by adult rat dorsal column (DC) axons with secondary cuneate nucleus (CN) neurons. At low intensity (1.5 mA) forepaw stimulation, short latency somatosensory evoked potentials (SSEPs) were recorded from the contralateral somatosensory cortices of both normal and incomplete cervical dorsal spinal cord hemisection (CDH) rats but not from complete CDH rats at varying post-SCI times. The SSEPs recorded from incomplete CDH rats were of longer latency and smaller amplitude compared to normal SSEPs. After bilateral forepaw cholera toxin B subunit (CTB) injections, confocal images of transverse sections through the brainstem revealed vGlut1/CTB co-labeled terminals throughout the forepaw representation area of the normal CN. These vGluT1/CTB-labeled terminals made apparent synapses with neuronal nucleus/microtubule associated protein 2-labeled CN neurons. In contrast, fewer vGluT1/CTB co-labeled terminals were observed in incomplete CDH rats and none were observed in a complete CDH rat. Additionally, vGlut1-labeled terminals seen in a complete CDH rat were largely restricted to the areas of the CN corresponding to the locations of terminals from primary afferent axons originating above the injury. Little vGlut1 was observed at the end of CTB-labeled axons at the DC injury site. These results suggest that terminals of DC axons that have made functional synapses express vGlut1. Furthermore, SSEPs recorded from the somatosensory cortex reflect the number of vGlut1/CTB-labeled DC axon terminals observed in the CN.

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## P53 ATTENUATION OF RUBROPSPINAL ATROPHY BY LENTI-VIRAL VECTOR MEDIATED BDNF EXPRESSION IN THE RED NUCLEUS

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Rubrospinal neurons undergo a massive atrophy after axonal injury at the level of the cervical spinal cord. This atrophy occurs during the second week and reaches a plateau after 1–2 months. In previous studies we have shown that this atrophy can be fully prevented by the infusion of BDNF into the vicinity of the rubrospinal cell bodies (Kobayashi et al. 1997, J. Neurosci 17:9583). Moreover, even a delayed treatment with BDNF, as late as one year post lesion, reverses this atrophy and restores the regenerative capacity of these neurons (Kwon BK, Liu J et al. 2002, PNAS 99:3246). This suggests that regeneration after chronic spinal cord injury might be feasible eventually. Here we have asked whether alternative methods of BDNF delivery could replace the rather traumatic intraparenchymal infusion via osmotic minipumps. We injected a lentiviral vector expressing BDNF into the vicinity of the rubrospinal neurons at the time of spinal injury (dorsolateral funiculotomy, C4) and in another group of animals at one months after the lesion. The expression of the viral construct was assessed by GFP fluorescence in and around the red nucleus as well as by

immunohistochemical staining of the injection site for BDNF. Inflammatory changes at the injection site were minor as evidenced by some mononuclear cell infiltration. Cell profile sizes were found around 71% in animals treated with BDNF-virus versus 56% in GFP-virus controls (p=0.06; n=3) at one months after axotomy. In situ hybridization for Ta1 and GAP-43 are currently underway to test whether this viral BDNF delivery is sufficient to sustain the regenerative mode of these neurons. In addition, we will present data for rats that received the BDNF-virus treatment as late as 2 months to test whether it can reverse chronic atrophy of rubrospinal neurons.

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## P54 INTEGRIN-LIGAND BINDING MODULATES NEURONAL BDNF AND TRKB EXPRESSION

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Integrin class adhesion proteins influence signaling and surface expression of tyrosine kinase trophic factor receptors in various cell types. Moreover, integrin binding to extracellular matrix (ECM) ligands regulates gene expression in non-neural cells. Findings in our laboratory indicate that integrins also regulate trophic activities of adult central nervous system (CNS) neurons. Studies of mature hippocampal slices showed that treatment with the integrin ligand peptide gly-arg-gly-asp-ser-pro (GRGDSP) increases gene expression for the neurotrophins BDNF and NGF, and for the BDNF receptor trkB. The present study evaluated mechanisms through which integrins regulate neurotrophin expression and signaling. In situ hybridization and real time PCR analyses show that GRGDSP-induced increases in gene expression depend entirely on NMDA receptors and, in part, on L-type voltage sensitive calcium channels (VSCCs). Western blot analyses show that GRGDSP treatment increases tyrosine phosphorylation of NMDA receptor subunits NR2A and NR2B and of the a1 L-type VSCC subunit, and that co-treatment with the NMDA receptor antagonist APV blocks GRGDSP-induced increases in a1 phosphorylation. Together these findings indicate that integrin-ligand binding upregulates neurotrophin expression via sequential effects on NMDA receptor and L-type VSCC phosphorylation and function. Current studies indicate that integrin ligation also influences cell surface expression of codistributed receptors including the BDNF receptor trkB. Together these findings highlight the importance of matrix interactions in sustaining endogenous trophic factor expression and signaling, and the potential value of manipulating the ECM environment to facilitate neuronal growth and survival effects of endogenous neurotrophins following damage to the adult CNS.

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## P55 NEUROTROPHIC FACTORS EXPRESSED IN BOTH THE CORTEX AND THE SPINAL CORD INDUCE AXONAL PLASTICITY AFTER SPINAL CORD INJURY

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We recently reported that overexpression of neurotrophin-3 (NT-3) by motoneurons in the spinal cord of rats will induce sprouting of corticospinal tract (CST) axons (Zhou et al., 2003). We now report that overexpression of brain-derived neurotrophic factor (BDNF) or glial cell-derived neurotrophic factor (GDNF) in the rat sensorimotor cortex near the CST neuronal cell bodies together with overexpression of NT-3 in the lumbar spinal cord significantly increases axonal sprouting over that induced by NT-3 alone. Two weeks after unilaterally lesioning the CST at the level of the pyramids, we injected rats with saline or adenoviral vectors (Adv) carrying genes coding for BDNF (Adv.BDNF), GDNF (Adv.GDNF) or EGFP (Adv.EGFP) at six sites in the sensorimotor cortex, while delivering Adv.NT-3 to motoneurons in each of these four groups on the lesioned side of the spinal cord by retrograde transport from the sciatic nerve. Four days later,

biotinylated dextran amine (BDA) was injected into the sensorimotor cortex on the unlesioned side to mark CST axons in the spinal cord. Morphometric analysis of axonal sprouting 3 weeks after BDA injection showed that the numbers of CST axons crossing the midline in rats treated with Adv.BDNF or Adv.GDNF were 46% and 52% greater, respectively, than in rats treated with Adv.EGFP or PBS ($p<0.05$). These data demonstrate that sustained local expression of neurotrophic factors in the sensorimotor cortex and spinal cord will promote increased axonal sprouting after spinal cord injury, providing a basis for continued development of neurotrophic factor therapy for central nervous system damage.

**P56 DECORIN SUPPRESSES NEUROCAN, BREVICAN, PHOSPHACAN AND NG2 EXPRESSION AND PROMOTES AXON GROWTH ACROSS ADULT RAT SPINAL CORD INJURIES**

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The formation of misaligned scar tissue by a variety of cells types expressing multiple axon growth inhibitory proteoglycans presents a physical and molecular barrier to axon regeneration after adult spinal cord injuries (Tang et al., J. Neuro. Res. 71:427-444 [2003]). Decorin is a small, leucine rich proteoglycan that has previously been shown to reduce astrogliosis and basal lamina formation in acute cerebral cortex stab injuries (Logan et al., Exp. Neurol. 159:504-10 [1999]). We have therefore tested whether mini pump infusion of hr-decorin into acute stab injuries of the adult rat spinal cord can not only inhibit formation of an astroglial limitans but also deposition of the axon growth inhibitory proteoglycans neurocan, NG2, phosphacan and brevican. Combined immunohistochemical and quantitative Western blot analysis revealed major reductions in levels of core protein expression (>80 for 130kD neurocan, 145/80kD brevican, 300kD phosphacan) and immunoreactivity for all four CSPGs within decorin treated injuries compared to untreated controls. Astrogliosis within lesion margins and the accumulation of OX42+ macrophages/microglia within lesion centers were also significantly reduced. These decorin induced changes in scar formation combined to promote the striking ability of axons from microtransplanted adult sensory neurons to enter, grow within and exit decorin infused spinal cord injuries, in sharp contrast to the complete failure of GFP+ axons to cross untreated, CSPG rich lesions. The ability of decorin to promote axon growth across acute spinal cord injuries via a coordinated suppression of inflammation, CSPG expression and astroglial scar formation make decorin treatment a promising component of future spinal cord regeneration strategies.

**P57 ROLE OF SULFATED PROTEOGLYCANS IN THE GLIAL SCAR ASTROCYTE**

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It is our hypothesis that soluble mediators cause changes in astrocyte physiology which cause them to become reactive and inhibitory to neuronal regeneration, in large part to their synthesis of chondroitin sulfate proteoglycans (CSPGs). Here we present data using cell culture models that 1) transforming growth factor-beta1 (TGF-B1) treated astrocytes are a model of the glial scar and 2) proteoglycan glycosaminoglycans and sulfation are essential for the inhibitory activity of CSPGs. Treatment of monolayers of mouse astrocytes with TGF-B1 reduced the ability of these astrocytes to support neuronal growth and upregulated the synthesis of sulfated proteoglycans. Addition of inhibitors of glycosaminoglycan (GAG) chain synthesis or sulfation reduced this inhibitory activity to neuronal growth, suggesting that sulfation is an essential feature. RT-PCR data indicated that TGF-B1 also upregulated the transcriptional levels of several sulfotransferases that produce 4- and 6- sulfated proteoglycans. In order to

more directly test the role of GAG chain sulfation, we investigated the behavior of axons at a boundary between CS-A (a predominantly 4-sulfated sugar) and PLL. We found that axons from neurons on PLL did not cross onto CS-A, but the crossing percentage is significantly increased after treatment with 4- and/or 6-sulfatase. FACE sugar structure analysis confirmed that 4- or 6-sulfate is removed by these sulfatases. Furthermore, overexpression of chondroitin-4-sulfotransferase in astrocytes reduced the length of axons plated onto these astrocytes. In summary, our current observations demonstrate that TGF-B1 induces the upregulation of astrocyte-associated sulfated proteoglycans and sulfotransferase mRNA, and this matrix inhibits neuronal growth. Moreover, the sulfation composition and pattern of the sugar moiety is very critical to the inhibitory properties of CSPGs.

## P58 LIBERATION OF HEAT SHOCK PROTEINS (HSPS) FROM STEROID RECEPTORS IS A POTENTIAL MECHANISM OF STEROID-INDUCED NEUROPROTECTION IN MOTONEURONS *IN VITRO*

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Steroid hormone treatments such as testosterone and estrogen have been found to be neuroprotective in both *in vivo* and *in vitro* injury models. The protective effects of both testosterone and estrogen have been investigated in our laboratory *in vivo* following various peripheral nerve injuries. We have found that physiological levels of both steroid hormones accelerate regeneration and behavioral recovery following peripheral nerve injury in vivo. The mechanism of steroid-induced neuroprotection is unknown. The present study investigated the ability and the potential mechanism of steroid hormone treatments to rescue injured embryonic rat spinal motoneuron hybrid cells transfected with the human androgen receptor gene (a generous gift from Dr. K.H. Fischbeck). In addition to expressing the androgen receptor, these motoneurons hybrid cells may also express the estrogen receptor. Steroid hormone treatment was found to be protective in vitro following a 30-minute heat shock at 11° above normal incubation temperature. Future experiments have been established to investigate the mechanism for steroid-induced neuroprotection. It is hypothesized that the existence of protective HSPs complex with steroid hormone receptors may be the mechanism for steroid-induced neuroprotection. In vitro co-immunoprecipitation experiments are planned to investigate whether or not steroid treatment liberates reputedly protective HSPs from the steroid receptor thus providing a potentially protective pool of HSPs following steroid treatment and injury.

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## P59 CADHERIN LOCALIZATION AND INJURY-INDUCED PLASTICITY WITHIN THE ADULT RAT SPINAL CORD

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Different somatic sensations originating in peripheral tissues are conveyed via sensory axons that have highly precise, distinct central termination patterns within the dorsal horn of the spinal cord. Some large-diameter myelinated axons (light touch) terminate in lamina III-V, while small-diameter non-myelinated axons (pain and temperature) terminate in lamina I/II. The mechanism of such laminar-specific targeting is poorly understood. Various families of cell adhesion molecules have been implicated in this process during development, including the Ig superfamily, integrins, ephrins, and cadherins. Because of their involvement in neurite outgrowth, laminar targeting and synaptogenesis during development, we chose to investigate cadherin expression and localization with subpopulations of sensory afferent central terminations within the adult rat dorsal horn. Sciatic nerve injections of cholera subunit B (CTB) and wheat germ agglutinin (WGA) label specific central terminations of distinct

sensory afferent fibers. CTB labels terminations of large diameter myelinated fibers in lamina III and WGA labels terminals of small diameter non-myelinated fibers in lamina I/II. Confocal and immuno-gold EM analyses confirm that E-cadherin is present specifically at WGA-labeled terminals, while N-cadherin associates with CTB-labeled terminals. Therefore, cadherins may be involved in specifying synaptic targets of primary afferent fibers. In addition we are investigating the involvement of cadherins in injury-induced plasticity within the spinal dorsal horn. Peripheral nerve injury causes tactile allodynia, where a normally non-noxious stimulus is increasingly perceived as nociceptive. It has been postulated that structural reorganization of sensory afferents within the dorsal horn may be involved. Our results suggest a bidirectional change in E-cadherin and N-cadherin protein levels within the dorsal horn after sciatic nerve transection. E-cadherin protein levels are decreased specifically within the sciatic nerve termination zone of lamina II inner, while N-cadherin protein levels is increased within lamina I/II.

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### P60 THE THROMBIN PROTEASE ACTIVATED RECEPTOR (PAR-1) AFTER SPINAL CORD CONTUSION INJURY: UPREGULATION IN GFAP POSITIVE ASTROCYTES

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Spinal cord injury produces prominent disruption of the blood spinal cord barrier, an acute inflammatory reaction and an astrocytic response. One of the earliest mediators of these effects following tissue injury is the serine protease thrombin. It has previously shown that thrombin, acting through the Protease Activating Receptor (PAR-1) opens endothelial gap junctions, causes neutrophil infiltration, directly modulates the shape of astrocytes and induces neurite retraction. We used a spinal cord contusion model to examine PAR-1 receptor expression following injury. PAR-1 receptor expression was characterized by immunocytochemistry in both uninjured and 1,3,7,14,and 21 day post injury tissue. In uninjured mice, the PAR-1 receptor appears to be weakly expressed primarily throughout the spinal cord gray matter in cells with a motoneuron phenotype. Following injury, there appears to be a robust PAR-1 signal within GFAP positive astrocytes at the injury epicenter and rostral and caudal to the injury site. Our findings demonstrate that the thrombin PAR-1 receptor appears to be upregulated in GFAP positive astrocytes following contusion injury and may play an important role in subsequent gliosis.

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### P61 INVOLVEMENT OF N-END RULE PROTEIN DEGRADATION IN NEURITE OUTGROWTH AND AXONAL REGENERATION WITH PARTICULAR REFERENCE TO THE UBIQUITIN-CONJUGATING ENZYME E2-14KD

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We are investigating the role of the ubiquitin/proteasome system—the principal regulator of intracellular protein levels—in neurite outgrowth and axonal regeneration. Previous work demonstrated that NGF-mediated neuronal differentiation of pheochromocytoma (PC12) cells is accompanied by increased levels of high molecular weight ubiquitin-conjugates in PC12 nuclei and decreased levels of free ubiquitin in cytoplasmic and nuclear extracts. The search for enzymes responsible for increased utilization of ubiquitin revealed an up-regulation of E2-14kD mRNA in NGF-treated PC12 cells. E2-14kD mRNA is a component of the N-end rule pathway, which is implicated in the cleavage of proteins with destabilizing N-terminal residues that include bulky hydrophobic or

basic amino residues. We show that E2-14kD is present in peripheral neurons and localizes to the nucleus and plasma membrane. Down-regulation of E2-14kD mRNA by small interfering RNA or treatment of cells with dipeptide-inhibitors of the N-end rule pathway suppress NGF-induced neurite outgrowth and axonal elongation of peripheral neurons obtained from adult rats. The present data indicate that E2-14kD is up-regulated during neuronal differentiation and is required for axonal regeneration in peripheral neurons in vitro.

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## P62 NEURON-INTRINSIC LIMITATIONS TO AXON REGENERATION IN THE DEVELOPING SPINAL CORD

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Unlike adults, embryonic birds and mammals show a remarkable ability to regenerate axons in the injured spinal cord. This regenerative ability is lost during a discrete developmental transition. Two main factors likely cause this transition: The emergence of inhibitory molecules in the spinal cord environment and a decline in the intrinsic ability of neurons to regenerate injured axons. To quantify the contributions of environmental versus neuron-intrinsic factors we are using as a model the embryonic chicken, which loses the ability to regenerate spinal cord axons on embryonic day 13 (E13). We have developed a culture system in which explants of brainstem neurons are placed adjacent to spinal cord tissue, and axon regeneration is quantified as the ages of brainstem neurons and spinal cord environment are varied independently. We find that as the spinal cord ages from E9 to E17, it supports 50% less axon regeneration. Thus changes in the spinal cord alone reduce but do not abolish axon regeneration. However, as brainstem neurons age from E9 to E15 they show a 90% reduction in axon regeneration, even in the permissive environment of the younger spinal cord. A similar reduction in axon growth from older brainstem neurons is observed on substrates of laminin. TUNEL staining reveals no difference in cell death in cultured brainstem of different ages, suggesting that the reduction in axon regeneration is not secondary to increased cell death. In addition, video analysis of living axons shows that older brainstem neurons elongate axons 50% more slowly than younger neurons. Combined, these data suggest that neuron-intrinsic changes contribute significantly to the failure of axon regeneration in the developing spinal cord.

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## P63 VERTEBRAL COLUMN FIXATION ALLOWS BETTER STUMP SEALING AFTER COMPLETE SPINAL CORD LESION

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A treatment for spinal cord injuries is still a major challenge. In the past, a variety of approaches in rodents elicited regeneration of spinal axons and partial functional recovery. Complete spinal cord transection, after multiple laminectomy, seems the most proper model to study axonal regeneration in experimental animals. However, after these extensive lesions any vertebral column movement may increase the gap between the cord stumps, change their dorsoventral orientation or cause spinal cord bending and, consequently, additional damage. To provide spinal cord stability during the process of healing, we have designed in rats a method to fix the vertebral column by using a bridge made of inlay pattern resin (Duralay). This bridge was placed covering the gap created by laminectomy, immediately after lesion, and both ends were attached to the spinous processes and laminae of the adjacent intact vertebrae. Sealing effectiveness of the stumps was analyzed fifteen days, one and four months post-surgery. To evaluate possible side effects of the bridge, we performed laminectomies, without spinal cord lesion, in sham operated animals. In all paraplegic rats, dense connective tissue was formed beneath the bridge and

above the spinal cord. Both stumps were attached with no gap between them suggesting that in addition to stabilization, the bridge may protect the spinal cord lesion site. Our bridges prevent the development of scoliosis, allowing correct walking and limb position. Moreover, placing a bridge seems essential to properly maintain the cord stumps for cell transplantation time after injury (chronic stage). In summary, we have developed a reliable and simple method for vertebral column fixation in rats with spinal cord injury, that may be advantageously used in combination with different repair strategies to reduce the variability between individuals and between different regeneration studies.

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### P64 COURSE OF MOTOR RECOVERY FOLLOWING VENTROLATERAL SPINAL CORD INJURY IN THE RAT

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The purpose of this study was to determine the importance of the pathways running in the ventrolateral spinal funiculus for overground locomotion in adult, unrestrained rats. Left-sided ventrolateral cervical spinal cord injury was performed in adult female Long-Evans rats. The behavioural abilities of these animals were analyzed at 2 days, and at 1.5, 2.5, 3.5, 4.5 and 5.5 weeks following spinal cord injury. Behavioural testing consisted of von Frey filament testing, ladder walking, a paw usage task, and the assessment of ground reaction forces during trotting. Animals with injury to the left ventrolateral cervical (C3) spinal cord did not develop enhanced sensitivity to pedal mechanical stimulation at 5.5 weeks following injury. At 2 days following injury, animals had impaired skilled locomotion as indicated by increased number of footslips during ladder walking. At 2 days, these animals also used both limbs together more often for support while rearing and the forelimb ipsilateral to the injury less than before spinal cord injury. Ground reaction force determination revealed that animals tended to bear less weight on the forelimb ipsilateral to the spinal cord injury and have substantially different footfall patterns during locomotion 2 days after injury. All animals returned to normal or near normal sensorimotor, including locomotor, abilities by 5.5 weeks following spinal cord injury. Only very subtle alterations in ground reaction forces were detectable by 5.5 weeks following spinal cord injury. These results support the current dogma that there is substantial functional redundancy of pathways traveling in the ventral spinal cord of the adult rat. Presumably, therapies need not be directed at regenerating pathways in the ventral spinal cord in their entirety.

### P65 BIODEGRADABLE POLYMER IMPLANTS AS A PLATFORM FOR OPTIMIZING SPINAL CORD INJURY REPAIR STRATEGIES

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Spinal cord axons have the potential to regenerate into a supportive microenvironment. To gain functional recovery, three questions must be answered: 1) What is the optimal microenvironment for axonal regeneration? 2) How can axons be guided to grow through residual cord towards their cellular targets? 3) What is required to re-establish interneuronal functional connections? Biodegradable polymer scaffolds may be used to gain answers to the first question and may contribute information to the second two. Using polymer chemistry and microtissue engineering techniques, we have used biodegradable polymer implants to begin to optimize the structural, molecular and cellular micro-environment to promote regeneration. Poly DL-lactic-co-glycolic acid (PLGA) and

polycaprolactone fumarate (PCLF) scaffolds containing multiple parallel aligned channels have been used to bridge a 2 mm gap in the rat spinal cord (T8-10). Channels of different diameter have been constructed (450 and 660 µm). Scaffolds have been pre-loaded with Schwann cells (SC), a SC precursor line (SpL201), or fibroblasts in matrigel. The bridge has also been implanted with or without concurrent systemic treatment with prednisolone and/or minocycline or tetracycline. In parallel experiments we have studied the release kinetics of chondroitinase-ABC and a synthetic analog of pigment epithelium derived growth factor (PEDF44). Microscopic magnetic resonance imaging, morphometry and three dimensional tissue reconstruction have been used to determine the ability of different micro-environments to support regeneration. Morphometric analysis of neurofilament stained axons demonstrated that axons and capillaries grew through the length of the channels. Wild-type SC provide the best cellular support. Biologically active PEDF44 and chondroitinase-ABC were released from PLGA microspheres demonstrating that encapsulation does not degrade the peptides. Data will be presented to address the role of channel diameter and potential neuroprotective strategies (minocycline and prednisolone). In the future, this model will provide a valuable platform to rapidly assess the value of different protective or supportive strategies to repair function after spinal cord injury.

### P66 AXONAL REGENERATION THROUGH NERVE COAPTATIONS IS ENHANCED BY DEGRADATION OF CHONDROITIN SULFATE PROTEOGLYCAN

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Injury to peripheral nerve initiates a degenerative process that converts the denervated nerve from a suppressive environment to one that promotes axonal regeneration. We investigated the role of matrix metalloproteinases (MMPs) in this degenerative process and whether effective predegenerated nerve grafts could be produced in vitro. Rat peripheral nerve explants were cultured for 1-7 days in various media and their neurite-promoting activity was assessed by cryoculture assay, in which neurons are grown directly on nerve sections. The neurite-promoting activity of cultured nerves increased rapidly and, compared to uncultured nerve, a maximum increase of 72% resulted by 2-day culture in the presence of serum. Remarkably, the neurite-promoting activity of short-term cultured nerves was also significantly better than nerves degenerated in vivo. We examined if in vitro degeneration is MMP-dependent and found that the MMP inhibitor GM6001 largely blocked the degenerative increase in neurite-promoting activity. In the absence of hematogenic macrophages, MMP-9 was trivial whereas elevated MMP-2 expression and activation paralleled the increase in neurite-promoting activity. MMP-2 immunoreactivity localized to Schwann cells and the endoneurium and colocalized with gelatinolytic activity demonstrated by in situ zymography. Lastly, in vitro predegenerated nerves were tested as acellular grafts and, compared to normal acellular nerve grafts, axonal ingress in vivo was approximately doubled and preliminary findings indicate improved functional recover with these grafts as well. We conclude that Schwann cell expression of MMP-2 plays a principal role in the degenerative process that enhances the regeneration-promoting properties of denervated nerve. Combined with their low immunogenicity, acellular nerve grafts activated by in vitro predegeneration may be a significant advancement for clinical nerve allografting.

### P67 EMBRYONIC SPINAL CORD MOTOR NEURONS EXHIBIT PREFERENTIAL OUTGROWTH ON PRELESIONED PERIPHERAL NERVE TISSUE SECTIONS IN VITRO

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Previous studies have shown that motor neurons from embryonic rat spinal cord explants

exhibit preferential outgrowth on stripes of muscle membranes in vitro, according to their appropriate rostrocaudal position (Wang et al., J Neurosci. 19: 4984-93,1999; Chadaram et. al., J Neurobiol. 2003 Sep 15; 56(4): 347-59). In the present set of experiments, we explored the outgrowth of E-15 ventral spinal cord (motor neurons) on peripheral nerve tissue sections from normal or lesioned (7 days post-denervation) sciatic nerves of adult rats. Cryostat sections of alternating normal and lesioned nerves were plated onto a neucleopore filter. Explants attached and extended neurite bundles on both substrates, however more extensive outgrowth (measured both in terms of length and number of neurite bundles) was evident on sections from prelesioned nerve. Of the 121 individual cultures that were examined, 77 explants extended neurites on both substrates while 31 grew only on prelesioned nerve sections, the remaining 13 grew only on normal nerve sections. Comparing just the explants that grew on both types of substrate, the overall average length of the neurite bundles was longer on the prelesioned substrate (359 vs. 241 microns; $p < 0.005$), as was the average number of neurite bundles per explant (15.5 on prelesioned vs. 9.3 on normal; $p < 0.05$), Individually, 72 of the 77 explants that grew on both substrates had longer and more neurite bundles on the prelesioned nerve section. These results show both preferential outgrowth and more extensive outgrowth on the prelesioned tissue substrate. These findings are in agreement with our previous study looking at outgrowth on stripes of nerve membranes (Soc. Neurosci. 245.6, 2003), and reports from other laboratories using embryonic sensory neurons plated onto tissue-section substrates of adult sciatic nerve. The current report is the first to show that E-15 ventral spinal cord explants also preferentially extend neurites, most likely from primary motor neurons, onto sections made from prelesioned adult nerve.

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## P68 REDUCTION OF POSTOPERATIVE NEURONAL LOSS AND IMPROVED RECOVERY OF VIBRISSAE MOTOR PERFORMANCE BY COPOLYMER-1 AFTER FACIAL NERVE REPAIR IN MICE*


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Although the etiology of motoneuron degenerative diseases varies, they all share similar pathways which culminate in motoneuron death. Based on a recent discovery that the immune system protects from nerve insult, we show that vaccination with Copaxone (Cop-1, glatiramer acetate) protects mice motoneurons against acute degeneration. Animals were distributed in 3 groups (A, B, C). The mice of group A ($n = 8$) underwent transection and suture of the facial nerve (facial-facial anastomosis, FFA). Before FFA all animals of group B ($n = 10$) received a subcutaneous injection of Cop-1 plus Complete Freund's Adjuvant (CFA) and those of Group C—phosphate buffer saline (PBS) and CFA ($n = 9$). Two months after surgery, alterations in the vibrissal motor performance (2D/Manual Advanced Video System PEAK Motus 2000) and the degree of motoneuron loss (counts after retrograde labeling with 1% Fluoro-Gold injected into the whisker pad musculature) were estimated. All animals that underwent FFA after Cop-1 sensitization showed a significantly better recovery than those without treatment. This was best demonstrated by the amplitude of whisking, which measured 38.9 ± 10.6° (group B), 22.1 ± 9.9° (group C) and 11.0 ± 6.0° (group A). The mean value before surgery was 40.0 ± 14.0°. Cop-1 vaccination was effective in inhibiting motoneuron death. Eight weeks after FFA, the number of motoneurons which survived and reinnervated the whisker pad was 1172 ± 152 in group B, but 670 ± 178 in the axotomized non-vaccinated (group A) and 766 ± 104 in the axotomized vaccinated with CFA only. In line with the experimentally based concept of protective autoimmunity, the

present findings suggest that Cop-1 vaccination boosts local immune responses needed to combat destructive endogenous compounds associated with motoneuron death.

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**P69 EXPRESSION OF SEMAPHORIN RECEPTORS: THE PLEXINS, AFTER PERIPHERAL VS. CENTRAL NERVOUS SYSTEM INJURY**

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Semaphorins form a large family of molecules implicated in neuronal guidance events in development, as well as in adulthood. The mostly inhibitory nature of Semaphorins has led to the speculation that they may be involved in the inability of injured central nervous system (CNS) neurons to regenerate. The Semaphorin receptor complex is comprised of Neuropilins: The ligand binding subunit, and Plexins, the signal transducing subunit, which has been shown to link to intracellular pathways converging onto the cell cytoskeleton. In the present study, we show in injured, non-regenerating rubrospinal neurons CNS, Plexin-A2 mRNA expression increased, while Plexin-A1 mRNA did not change. In contrast, in injured facial neurons (peripheral nervous system: PNS), which are able to regenerate, Plexin-A2 mRNA expression decreased while Plexin-A1 mRNA did not change. We are presently expanding our study by analyzing the mRNA expression of other members of the Plexin receptor family: Plexins-A3, A4 and B1, in the injured rubrospinal CNS and facial (PNS) neurons. By understanding how Semaphorins and their receptors respond to injury, we hope this may lead to potential target molecules usable in a therapeutic approach to promoting regeneration after injury in the CNS.

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**P70 REGENERATION OF SUPERNUMERARY AXONS IN THE ADULT RAT SPINAL CORD IN A CAUDA EQUINA INJURY AND REPAIR MODEL**

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We have previously shown that lumbosacral ventral root avulsions lead to autonomic and motor neuron death in an adult rat model of cauda equina injury (Hoang et al., 2003, JCN, in press). However, implantation of the avulsed ventral root into the spinal cord can protect these neurons against cell death and promote reinnervation of the implanted avulsed ventral root. Here, we studied some of the long-term effects (6 weeks to 5 months) of this surgical neural repair strategy. We performed a unilateral avulsion of the L5-S2 ventral roots with an acute implantation of the avulsed L6 ventral root into the spinal cord. Using immunohistochemistry for choline acetyltransferase (ChAT), we demonstrated in the neuropil on the ipsilateral side of the cord several unusual and aberrant ChAT positive structures with an axonal phenotype. These structures exhibited a uniform diameter, an irregular course, occasional branching, and large terminal bouton-like swellings. Many supernumerary axons demonstrated growth towards the implanted avulsed ventral root. The atypical arbors were reminiscent of regenerating supernumerary axons. Double immunohistochemical labeling for ChAT and p75, a low affinity neurotrophic factor receptor, demonstrated in confocal microscopy colocalization of ChAT and p75 immunofluorescence in these atypical axon-like structures. The studies suggest that axotomized cholinergic neurons may generate ChAT positive supernumerary axons, many of which extend towards the implanted ventral root graft. Our studies also support recent suggestions for p75 to play a role in axonal elongation.

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## P71 INCREASED NEURONAL NITRIC OXIDE SYNTHASE EXPRESSION PRECEDES PROGRESSIVE DEATH OF AUTONOMIC NEURONS IN A CAUDA EQUINA INJURY MODEL

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We have previously demonstrated progressive death of autonomic preganglionic parasympathetic neurons (PPNs) and motoneurons in a unilateral L5-S2 ventral root avulsion injury model in the adult male rat. Here we report a transient increase in both the frequency and concentration of neuronal nitric oxide synthase (nNOS) expression in these populations that precedes neural death. PPNs and motoneurons were identified using retrogradely-transported FluoroGold, and nNOS was detected using immunohistochemistry. Stereological counts were used to determine the number of nNOS-positive neurons in uninjured control lumbosacral spinal cords, along with counts performed at 3-day, and 1, 2, 4, and 6-week post-injury survival times. These counts indicate a marked increase in the frequency of nNOS-positivity in PPNs, most notably at 3-days post-injury, as compared to the non-injured side. Densitometry and size measurements suggest increased density of nNOS immunoreactivity within PPN cell bodies at all post-injury time points. Qualitatively, PPNs on the injured side show much darker nNOS staining extending further into their dendritic projections. We believe an increased nNOS concentration may mediate the progressive death of PPNs and motoneurons following ventral root avulsion, and may provide an avenue for therapeutic intervention following traumatic cauda equina injury.

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## P72 FILUM TERMINALE IN THE RAT, NEW MODEL FOR STUDIES OF AXONAL REGENERATION IN THE ADULT CENTRAL NERVOUS SYSTEM (CNS)


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CNS myelin is inhibitory to regeneration of injured axons. Dysmyelination in the adult CNS, such as in the Long Evans Shaker (LES) rat coincides with spontaneous axonal plasticity observed as abundant sprouting and with regeneration of transected axons in the spinal cord. Massive injury of the spinal cord in the adult rat however, results in severe motor deficits and loss of bladder function requiring intensive and expensive animal care. We determined the anatomy and function of the filum terminale (FT), a slender extension of the terminal spinal cord—the conus medullaris (CM)—towards the tail. FT is defined by a pia limitans, contains the central canal surrounded by a narrow rim of axons interspersed by oligodendrocytes and astrocytes but not neurons. FT is >3 cm long and narrows from 170 microns at the CM to 50 microns at 3 cm distal to CM. In a study addressing axonal regeneration in the adult dysmyelinated CNS, FT was crushed 2 mm caudal to the CM and rats allowed survival for 2 weeks. Neurological deficits typical in the spinal cord injury such as paraplegia and urinary bladder dysfunction were absent. In cross sections of FT taken from the site of crush to 3 cm caudal, there was abundant axonal regeneration observed in close association with ependymal cells up to 3 cm distal to the crush. In a tracing experiment, a dextrane tracer microinjected into CM as detected in the FT >2 cm caudal to the crush site. This new model of adult CNS injury allows for detailed studying of axonal regeneration without the necessity of inflicting a crippling injury on the model animal.

## P73 SEROTONIN 1A RECEPTOR ACTIVATION OF A LATENT MOTOR PATHWAY AFTER SPINAL HEMISECTION

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Cervical spinal cord injury (SCI) (C2 hemisection) results in paralysis of the ipsilateral hemidiaphragm, and subsequent alteration of the breathing pattern; breathing frequency increases and tidal volume decreases. A latent neural pathway, which runs down the cord, contralateral to hemisection and crosses distal to the site of injury, can be activated under conditions of elevated respiratory drive, thus, restoring activity to the paralyzed hemidiaphragm. Since, activation of serotonin 1A receptors increases respiration and can reverse the altered breathing pattern induced by SCI, we hypothesized that activation of serotonin 1A receptors would activate the latent motor pathway (crossed phrenic response) in paralyzed rats. C2 hemisection was performed under sterile conditions 24 hours prior to experimentation. Phrenic nerve activity (respiration) was recorded in anesthetized, vagotomized, paralyzed, ventilated Sprague Dawley rats, and the serotonin agonist, 8-OH-DPAT, was administered (IV). We found that SCI alone did not increase respiratory frequency in SCI rats (n=10) compared to controls (n=7). Since central drive was maintained constant and peripheral feedback was removed, the changes in breathing pattern observed in vivo by others must occur through modification of peripheral feedback mechanisms, but not at the central respiratory rhythm generator. 8-OH-DPAT increased frequency and peak height of phrenic activity in controls, whereas frequency increased only in 50% of SCI rats. While the peak height of phrenic activity on the contralateral side increased more in SCI rats than controls, the latent phrenic pathway was only activated in SCI rats that also showed an increase in frequency. This suggests that increasing the frequency component of neural impulses rather than total neural activity may preferentially drive activation of this latent pathway.

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## P74 SPONTANEOUS FUNCTIONAL RECOVERY IN A PARALYZED HEMIDIAPHRAGM FOLLOWING CERVICAL SPINAL CORD INJURY AND CAROTID BODY DENERVATION IN ADULT RATS

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Previous studies have demonstrated that in an animal model of spinal cord injury, a latent respiratory motor pathway can be activated to restore function to a hemidiaphragm paralyzed by upper cervical (C2) spinal cord hemisection. It has also been demonstrated that functional recovery of respiratory activity can occur spontaneously in C2 hemisected animals following prolonged post hemisection periods (2–4 months) without any therapeutic intervention. It is presently unknown whether peripheral respiratory chemoreceptors located in the carotid body are involved in spontaneous functional recovery after hemisection. The objective in the current investigation was to assess the effects of carotid body denervation on the onset of spontaneous functional recovery in C2 hemisected animals. Briefly, animals were anesthetized with ketamine, 70mg/kg and xylazine, 7mg/kg and were then subjected to bilateral carotid body denervation and allowed to recover for 5–6 days. All animals were monitored closely post surgery. Following recovery from carotid body denervation, all animals were reanesthetized and subjected to a cervical (C2) hemisection (HCBD group). Control animals were not subjected to carotid body denervation. Electrophysiological assessment of respiratory function was conducted 2 weeks after hemisection, i.e. approximately 3 weeks after carotid body denervation. In experiments thus far (n=8), recovery of respiratory activity in the hemidiaphragm paralyzed by C2 hemisection occurred in the majority (n=6) of cases at only 2 weeks post hemisection. Two animals did not survive the carotid body denervation and hemisection. Our findings thus far contrast markedly from the delayed onset of spontaneous recovery in C2 hemisected animals (with the carotid body

intact) noted in our previous study. Our results suggest that carotid body denervation accelerates mechanisms that underlie spontaneous recovery of the paralyzed hemidiaphragm following C2 hemisection.

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## P75 INJURY-INDUCED SPINAL CORD PLASTICITY IN THE MOUSE—THE CROSSED PHRENIC PHENOMENON

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The crossed phrenic phenomenon (CPP) describes respiratory functional plasticity that arises following spinal cord injury. Cervical spinal cord hemisection rostral to the phrenic nucleus paralyzes the ipsilateral hemidiaphragm by interrupting the descending flow of respiratory impulses from the medulla to phrenic motoneurons in the spinal cord. This loss of activity converts some synapses on phrenic motoneurons from a "functionally ineffective" state pre-hemisection to a "functionally latent" state post-hemisection. If the animal is subjected to respiratory stress by transecting the contralateral phrenic nerve, this latent respiratory pathway is activated and function is restored to the paralyzed hemidiaphragm. The mechanisms underlying this synaptic plasticity are not well-defined. Our ultimate aim is to understand the underlying molecular mechanisms of this functional recovery using a mouse model amenable to a molecular genetic approach. Although the phenomenon has been shown in dogs, cats, rabbits, guinea pigs and rats, this is the first report of CPP in mice. We have shown the CPP qualitatively in mice using electromyographic (EMG) recordings from the diaphragm. In particular, we examined the interoperative delay time between the hemisection and contralateral phrenicotomy required for a response. For each animal, a spinal cord hemisection was performed on the left side at C2. A contralateral phrenicotomy was performed the next day (overnight animals), 6–8 hours, 4–5 hours, or 1–2 hours post-hemisection. An abdominal laparoscopy was performed to expose the diaphragm and bipolar electrodes were placed on the left and right hemidiaphragms. EMG recordings were amplified and filtered to reduce spurious signals and electrocardiographic activity. While recording, the animals were observed to ensure that the EMG signal was respiratory-related inspiratory activity. As the interoperative delay was reduced, the proportion of mice displaying the CPP decreased from 100% for overnight animals, 94% in 6–8h, 85% in 4–5h, to 74% for 1–2h mice, and <30% for animals receiving a phrenicotomy under 1 hour.

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## P76 MK-801 ACCELERATES RECOVERY OF THE IPSILATERAL HEMIDIAPHRAGM FOLLOWING A LEFT C2 HEMISECTION IN THE RAT

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A left cervical (C2) hemisection disrupts the bulbospinal projections from the rostral ventral respiratory group to the phrenic motor nucleus and results in paralysis of the ipsilateral hemidiaphragm. Spontaneous recovery of the hemidiaphragm does occur, however, starting at six weeks post injury. Studies have demonstrated that increased mRNA levels for the 2A subunit of the N-methyl-D-aspartate (NMDA) receptor following a contusion injury at the thoracic level (T8) of the spinal cord correlate with improved hindlimb function in chronically injured rats. MK-801, a non-competitive NMDA receptor antagonist, has demonstrated the pharmacological ability to preferentially upregulate the mRNA levels of the 2A subunit

in neonatal rats. In the present study, we hypothesized that pharmacological upregulation of the 2A subunit would facilitate recovery of diaphragmatic function after C2 hemisection. Female Sprague-Dawley rats underwent a left C2 hemisection and were assessed for complete paralysis of the ipsilateral hemidiaphragm. One week post lesion, MK-801 was administered at a dosage of 0.50 mg/kg i.p. once per day for two days to upregulate the 2A subunit. Two days following the last administration of MK-801 (9 days post injury) the diaphragm was evaluated by electromyogram (EMG) recordings. Diaphragmatic EMG activity was observed in the initially paralyzed hemidiaphragm and was similar to spontaneous recovery observed at much later post hemisection periods in our earlier studies. The results suggest that administration of MK-801 accelerates the spontaneous recovery of diaphragmatic function following a left C2 hemisection through upregulation of the NMDA receptor 2A subunit.

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## P77 ROLE OF ATP/P2 RECEPTORS IN THE ACTIVATION OF ASTROCYTIC PROTEIN KINASE B/AKT BY TRAUMATIC INJURY

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Traumatic brain injury (TBI) activates protein kinase B (Jenkins et al., 2002), also known as Akt, a key signaling molecule that promotes cell survival and inhibits apoptosis. Akt phosphorylation has been implicated in neuronal survival after TBI (Noshita et al., 2002), but little is known about injury-induced Akt activation in astrocytes, cells that exhibit hypertrophic and hyperplastic responses to central nervous system injury. Here we have investigated the effect of mechanical strain on Akt activation in primary cultures of rat cortical astrocytes with the in vitro TBI model developed by Ellis et al. (1995). We have also examined the mechanisms that lead to trauma-induced Akt activation. Akt activation was determined by probing immunoblots with an antibody that recognizes Akt phosphorylated at serine 473; total Akt was measured as a loading control. We found that when astrocytes were subjected to mechanical strain (50 msec; 7.5 mm displacement), Akt phosphorylation was increased at 3 min postinjury, was maximal from 5 to 10 min and declined gradually thereafter. Akt activation was dependent on the severity of the injury. Stretch-induced Akt phosphorylation was attenuated by blocking phosphoinositide 3-kinase (PI3K), an upstream activator of Akt, with wortmannin. Because ATP is released after strain-induced injury (Ahmed et al., 2000), we examined the role of extracellular ATP in the activation of Akt. We observed that P2 receptor antagonists such as iso-pyridoxal-5’ phosphate-6-azophenyl-2’5’-isulfonate (PPADS) attenuated trauma-induced Akt activation. In uninjured astrocytes, we found that extracellular ATP stimulated Akt phosphorylation via P2 receptors (Broch et al., 2003). We conclude that mechanical strain causes activation of Akt in astrocytes via stimulation of P2 receptors and PI3K signaling. This suggests that the P2 receptor/Akt pathway promotes astrocyte survival and may play a role in the generation of reactive gliosis after TBI.

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## P78 DIETARY FACTORS CAN MODULATE THE CAPACITY OF THE BRAIN TO COMPENSATE FOR TRAUMATIC BRAIN INJURY (TBI)

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We have previously shown that a diet high in saturated fat (HF) decreases levels of brain-derived neurotrophic factor (BDNF), to the extent that compromises cognitive performance and aggravates the outcome of TBI. Here we present evidence that antioxidant therapy with vitamin E compensates for damage incurred by this diet. These results show that oxidative stress can interact with the BDNF system to modulate synaptic plasticity

and cognitive function. These studies suggest a mechanism by which events classically related to the maintenance of energy balance of the cell, such as oxidative stress, can interact with molecular events that modulate neuronal and behavioral plasticity. In addition, here we show that good diets such as a diet supplemented with omega-3 fatty acids (e.g. DHA) can help to compensate for TBI. DHA play a crucial role in signal transduction, regulation of gene expression, and neuronal protection from neuronal apoptotic death. We have examined the possibility that omega-3 fatty acids supplements may produce beneficial effects on the outcome of traumatic brain injured animals. Rats were fed a regular diet or experimental diet containing 8% fish oil, for 4 weeks before a mild fluid percussion injury (FPI) was performed. FPI resulted in impairment of learning ability in the Morris water maze and reduction in BDNF and its downstream effectors on synaptic plasticity such as synapsin I and CREB. The DHA diet compensated for the reduced learning capacity, and for the decrease in BDNF, synapsin I, and CREB, resulting from FPI.

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## P79 CHANGES IN EXPRESSION OF NOGO, NOGO-66 RECEPTOR AND SMALL PROLINE-RICH REPEAT PROTEIN 1A FOLLOWING EXPERIMENTAL TRAUMATIC BRAIN INJURY IN RATS

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Axonal injury is a common finding following experimental and clinical traumatic brain injury (TBI). The post-traumatic expression of inhibitors of axonal growth such as Nogo, acting on the Nogo-66 receptor (NgR), may potently prevent axonal outgrowth and contribute to the poor functional recovery seen following TBI. The small proline-rich repeat protein 1A (SPRR1A) was shown to be an important promoter of axonal outgrowth following peripheral nerve injury, but has not previously been detected in brain. Nogo, NgR and SPRR1A expression was evaluated 1, 3, and 7 days following fluid percussion (FP) brain injury of moderate severity in rats. The rats were perfused with 4% paraformaldehyde, and primary antibodies against Nogo (1:8000), NgR (1:3000) and SPRR1A (1:1000; all antibodies a generous gift from Dr. Strittmatter, Dept. of Neurology, Yale) were used on 40 µm brain sections. Additional sections were double labeled with the neuronal marker microtubule-associated protein (MAP)-2. In all injured rats (n=8), there was an increased expression of Nogo in ipsilateral cortex, hippocampal subfield CA3 and the reticular thalamus as compared to sham injured animals, and the increased expression was predominately seen in MAP-2 positive cells. In addition, increased expression of Nogo was seen in ipsilateral external capsule and fimbriae. Increased SPRR1A expression was seen in MAP-2 positive cells in ipsilateral cortex and CA3 from one day post-injury, with the most marked increase at 7 days post-injury. Changes in NgR were less prominent, although a modest increase was seen in ipsilateral cortex in occasional animals. Our results show that FPI causes a changed expression of proteins promoting (SPRR1A) and inhibiting (Nogo/NgR) axonal outgrowth. These changes may be important contributors to the cognitive and neurological motor sequelae of TBI.

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## P80 REDUCED CENTRAL NERVOUS SYSTEM (CNS) SCAR FORMATION AND LESS NEUROMOTOR DEFICIT WITH ENRICHED ENVIRONMENT COMBINED WITH MULTIMODAL STIMULATION AFTER TRAUMATIC BRAIN INJURY IN RATS*

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The present study was designed to investigate possible effects of enriched environment (EE) combined with multimodal early onset stimulation (MEOS) versus standard housing (SH) without stimulation on neuromotor function and lesion volume after experimental traumatic brain injury (TBI). Sprague-Dawley rats, randomized to the following groups: (i) injured/EE+MEOS; (ii) sham/EE+MEOS; (iii) injured/SH; (iv) sham/SH, underwent moderate fluid-percussion or sham injury. Thereafter EE+MEOS groups were placed into modified cages containing various types of bedding and stimulating objects. Along with EE animals underwent motor, olfactory, auditory and visual stimulation (MEOS). In contrast, injured and sham SH groups were housed individually without stimulation. Neuromotor function was assessed using a composite NeuroScan (NS) test battery at 24h, 7, and 15 days post injury (DPI). On 15 DPI animals were transcardially perfused and brains were harvested for single coronal section immunocytochemistry for NSE, Caspase 3 active, and GFAP. Nissl counter-staining allowed demarcation of zones containing reactive astrocytes enabling a quantification of the projection area occupied by GFAP-positive astrocytes in each immunoreacted section. Values for total area of all sections and the section thickness served for calculation of the lesion volume in each rat's brain. Neuromotor function was markedly reduced in both injured groups at 24h post-injury being non-significant. However, injured/EE+MEOS animals performed significantly better when tested for neuromotor function compared to injured/SH animals on DPI 7 ($p < 0.05$) and DPI 15 ($p < 0.5$). Better neuromotor function in EE+MEOS animals at 15 DPI was associated with a significantly smaller lesion volume compared to SH animals (14.28 +/- 6.83 mm3 vs. 23.46 +/- 4.31 mm3). This first report on combined EE+MEOS post-traumatic treatment indicates that exposure to EE+MEOS may reverse neurological deficits and reduce CNS scar formation after TBI in rats.

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## P81 DIFFERENTIAL RESPONSES OF AXONS TO TRANSECTION: INFLUENCE OF NG2

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On the basis of in vitro experiments, the NG2 chondroitin sulfate proteoglycan has been proposed as an inhibitor of central nerve regeneration. To test this possibility in vivo, we have used wildtype and NG2 null mice to examine the response of three types of axons to spinal cord transection. Corticospinal tract axons descending through the ventral region of dorsal white matter do not regenerate in both wildtype and NG2 knockout animals. In contrast, CGRP-positive sensory afferents are observed with equal frequency within the transection scars of wildtype and NG2 null animals. Limited sprouting of descending serotonergic axons is also seen within the scar tissue of both groups of animals, but is more common in the presence of NG2. These studies therefore do not support the concept of NG2 as a general inhibitor of axon regeneration in the central nervous system. In some cases NG2 may even be stimulatory to axon re-growth. Our results suggest that nerve regeneration is a heterogeneous process that depends on the specific responses of different classes of axons to molecular cues present in the injured nervous system.

## P82 CHEMOKINE EXPRESSION FOLLOWING SPINAL CORD INJURY IN DIFFERENT STRAINS OF MICE

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Spinal cord injury in mice triggers a different set of cellular responses than is seen in rats and other species. The present study was undertaken to asses expression of chemoattractant cytokines in different strains of mice. C57BL/6, C57BL/10, FVB/n, CD-1 and BALB/c mice received complete crush injuries at the T8 level, and mRNA was isolated from a 8mm section surrounding the lesion. Expression of Rantes, Eotaxin, MIP1alpha, MIP1beta, MIP2, IP10, and MCP1 were assessed using ribonucleotide protection assays at 0, 6, 12, 24, 48, 72 hours, 7 days and 10 days after injury. There was a dramatic induction of chemokine expression in all strains, with peak expression occurring at 6-12hrs post-injury. BALB/c mice exhibited lower levels of induction of lymphocyte attractant MIP1alpha, MIP1beta, and Rantes than other strains. C57BL/6 and C57BL/10 strains exhibited higher levels of IP10 at 12hrs post-injury than all other strains ($p < 0.0001$), whereas there were no strain differences in IP10 expression by 24 hr post-injury. C57BL/6, C57BL/10 and FVB/n strains exhibited a second phase of expression of MIP1alpha, MIP1beta, and Rantes that began at 3 days post-injury and continued to increase up to 10 days. This second peak was not seen in BALB/c mice; instead, cytokine expression remained low after the initial peak. CD-1 mice exhibited continuously elevated levels of MIP1alpha and MIP1beta up to 10 days after injury. The higher initial peak levels, and continued expression of Rantes, MIP1beta, MIP1alpha, and IP10 expression suggest that there may be a persistent signal for T-cell recruitment in C57BL/6, B57BL10, and FVB/n strains that is not present in BALB/c. The consequences of these differences in chemokine induction on inflammatory cell recruitment remain to be defined.

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