

**EIGHTH INTERNATIONAL SYMPOSIUM
ON NEURAL REGENERATION**

The Asilomar Conference Center
Pacific Grove, California
December 8-12, 1999

INTRODUCTION

Eighth International Symposium on Neural Regeneration

The Eighth International Symposium on Neural Regeneration was held at the Asilomar Conference Center, Pacific Grove, California from December 8-12, 1999. The meeting was cosponsored by the Medical Research Service of the Department of Veterans Affairs, the Paralyzed Veterans of America, the National Institute of Neurological Disorders and Stroke, The Christopher Reeve Paralysis Foundation, and the Eastern Paralyzed Veterans Association. The purpose of these biennial symposia is to cover the field of neural regeneration research by varying the topics presented at the different symposia, while emphasizing areas in which some notable progress has been made. Six topics were selected for platform presentation by invited speakers, including: (1) Strategies for Spinal Cord Injury, chaired by Bradford Stokes of The Ohio State University; (2) Impact of Neuroprosthetic Applications on Functional Recovery, chaired by John Chapin of MCP Hahnemann University; (3) Neurotrophins and Activity-Dependent Plasticity, chaired by Fredrick Seil of the VA Office of Regeneration Research Programs in the absence of Hans Thoenen of the Max-Planck-Institut, Martinsreid; (4) Plasticity of the Injured Spinal Cord: Retraining Neural Circuits to Promote Motor Recovery, chaired by Bruce Dobkin, University of California, Los Angeles in the absence of Reggie Edgerton, also of the University of California, Los Angeles; (5) Candidate Cells for Transplantation into the Injured CNS, chaired by Itzhak Fischer of MCP Hahnemann University; and (6) New Directions in Regeneration Research, chaired by Jeffrey Kordower from Rush Medical School, Chicago. The following section on Abstracts of Oral Presentations contains the abstracts of the presentations of the session speakers.

Free communications were presented in the form of posters. The posters in many cases reflected the themes of the oral presentations, but were by no means restricted to these themes, and, in fact, ranged well beyond the boundaries of the platform sessions. The following section on Abstracts of Poster Presentations contains the poster abstracts. An attempt was made to group the posters by subject matter, which explains why there are following abstracts in some cases from the same institution or even the same laboratory. As always, the posters added a great deal of excitement to the symposium.

Not included among the abstracts, because these particular speakers were not asked to submit abstracts, were the very valuable introductory overviews presented by the session chairs at the beginning of each of the sessions, and the keynote and featured talks. Martin Raff of University College, London, was the keynote speaker, and his topic was "Control of Oligodendrocyte Numbers." Dr. Raff noted that varying the numbers of myelin-receptive axons increased

or decreased oligodendrocyte numbers, and that this was likely mediated by neuregulin. The featured speakers were John Sladek of The Chicago Medical School, who reviewed the history and progress of neural transplantation under the title of "Neural Repair Strategies: Factors, Grafts, Gene Therapy or All of the Above?" and Glenn Hatton of the University of California, Riverside, who talked about formation of double synapses on hypothalamic neurons relative to glial ensheathment in different functional states in his presentation entitled "Function Related Neuronal-Glial Plasticity in the Adult Mammalian Hypothalamus." These extra-length talks were critical pillars in a well balanced program.

The Ninth International Symposium on Neural Regeneration is scheduled for December, 2001 (specific dates to be determined) at the Asilomar Conference Center.

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**ABSTRACTS
OF
ORAL
PRESENTATIONS**

PHARMACOLOGIC THERAPY FOR ACUTE SPINAL CORD INJURY: A SYSTEMIC REVIEW OF THE EVIDENCE

M.B. Bracken, Yale University, New Haven, CT

Medline from 1966 to the present, and CINAHL 1982 to present, were searched using key words acute spinal cord injury, pharmacotherapy, corticosteroids and methylprednisolone for all published randomized or quasi-randomized human trials in any language. Data were abstracted from original trial reports and by direct communication with authors. Meta analysis of trials of the same therapy was conducted. High dose methylprednisolone therapy is the only pharmacologic therapy shown to have efficacy in a Phase III randomized controlled trial when administered within 8 hours of injury. A recent trial indicates additional benefit by extending the maintenance dose from 24 hours to 48 hours when start of treatment is delayed to between 3 and 8 hours after injury. There is an urgent need for more randomized trials of pharmacologic therapy for acute spinal cord injury.

DEMOGRAPHICS ANALYSIS AND PHARMACOLOGIC EFFECTS IN THE GM1 GANGLIOSIDE ACUTE SPINAL CORD INJURY (SCI) STUDY

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The objective was to determine the efficacy and safety of GM1 in acute SCI after standard methylprednisolone (MPSS) treatment. GM1, a cell membrane component abundant in the CNS, has shown acute neuroprotective and long-term regenerative effects in experimental ischemia and injury. The study was designed as a double-blind parallel investigation of placebo vs. GM1, stratified by injury level, age and baseline severity in 28 North American centers. Follow-ups were at 4, 8, 16, 26, and 52 weeks. Marked recovery was defined as a 2-grade improvement in the modified Benzel scale post-baseline vs. the baseline ASIA Impairment Scale (AIS). Of 797 patients enrolled, 760 were analyzable. Overall, 10.6%, 41.2% and 77.6% of patients in baseline severity strata A, B, and C+D respectively showed marked recovery at week 26. The percentages of patients with marked recovery at week 26 (principal endpoint) were similar for both groups (32.5% for GM1; 31.2% for placebo; $P=0.741$). In the time course of marked recovery, treatment differences always favored GM1, and were statistically significant at weeks 8 and 16 ($P=0.003$ and 0.043 respectively.) In the non-operated group, spinal cord contusions (62 GM1 and 60 placebo), there was a statistically significant difference in the proportion attaining marked recovery by week 26 in favor of GM1 (51.6% vs. 25%, $P=0.003$, Fisher's exact test). Initial safety analyses indicated no significant differences in frequencies or numbers of adverse events. Our conclusion was that the GM1 100mg group demonstrated earlier marked recovery than did the placebo group. In severity group A this effect was modest, and possibly constrained by the limited recovery attainable in these subjects. Greater effects were seen in severity groups B and C+D.

CELL DEATH AND TISSUE REPAIR AFTER EXPERIMENTAL SPINAL CORD INJURY

M.S. Beattie and J.C. Bresnahan, The Ohio State University, Columbus, OH

Experimental spinal cord injury can be produced using a variety of models ranging from complete transection to mild contusion injuries. Recently, several models of contusion or compression injury have been shown to provide reliable and clinically relevant means for testing neuroprotective agents and for testing strategies for neural repair. Our laboratory has concentrated on studying recovery of locomotion after contusion injuries and on the biology of this complicated lesion. The hope is that a thorough examination of the biological events that underlie secondary injury and repair will lead to novel treatment strategies that can themselves be tested in the models. Contusion lesions made using the NYU weight-drop device were examined for evidence of secondary injury and apoptosis. We found mostly necrosis early after injury at the lesion center. However, at time periods later than six hrs, substantial numbers of cells were found to be apoptotic. The presence of apoptosis spread circumferentially and rostrocaudally over days, and even weeks following injury. At later time

periods, most apoptosis was found within white matter tracts, and was associated with activated microglia and degenerating axons. Both microglia and oligodendrocytes (OLs), but not astrocytes, were seen undergoing programmed cell death. Severe lesions (25 mm weight drop) resulted in chronic loss of OLs in the dorsal columns, whereas moderate lesions (12.5 mm) did not, even though substantial numbers of apoptotic cells were present. This suggests that OLs may be replaced after injury in this model. We hypothesize that cytokines produced by microglia may contribute to OL cell death. However, other factors may be involved, including the p75 low affinity neurotrophin receptor. P75 is not normally expressed by spinal cord OLs, but after contusion injury or hemisection, p75 is expressed in OLs in regions where apoptosis occurs. Thus, it is possible that neurotrophins like NGF contribute to cell death after injury. In addition to cell death, there is evidence of substantial tissue repair and regeneration in this model of cord injury. Cells around the central canal proliferate and appear to form cellular trabeculae within the lesion cavity. Other cells within the cord parenchyma divide and express nestin. Many axons from the dorsal roots grow into the lesion cavity and some CNS axons can also grow into the lesion. Together, these results provide a picture of coordinated cell death and repair that may be amenable to manipulation to enhance the final functional outcome after cord injury. Support: NIH NS-38079, APA BB02 and PVA-SCRF 1734

GUIDANCE OF NEURONAL PROCESSES BY ASTROCYTE-DE-RIVED EXTRACELLULAR MATRIX MOLECULES

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The regrowth of severed axons in the mammalian central nervous system is hampered by the presence of the glial scar. While the glial scar may present a physical barrier, other data suggest that it is extracellular matrix (ECM) molecules within the glial scar that prevent regrowth. Using cell culture models of the glial scar, we have dissected the effects of individual ECM molecules on neurite growth. Astrocytes in culture respond to certain cytokines and chemokines with an increase in their production of ECM molecules, and this ECM can affect two different aspects of neurite growth: the extent of growth (outgrowth) as well as the direction of growth (guidance). We have also found that individual regions or fragments of the ECM molecules laminin and tenascin-C can alter outgrowth and guidance of neuronal processes in culture in ways that differ from that of the intact ECM molecule. We also show that the response of neurites to these proteins can be altered by treatment of cultures with agents that affect specific signal transduction pathways. Given the evidence for proteolysis of ECM following brain trauma, our results suggest ways in which to overcome the inhibitory ECM of the glial scar. Supported by NIH NS-25168 to HMG.

SPINAL CORD INJURY-INDUCED INFLAMMATION: A DUAL EDGED SWORD

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Traumatic spinal cord injury (SCI) causes the destruction of axon pathways that transmit information required for proper sensory and motor integration resulting in paralysis below the level of injury. One consequence of traumatic SCI is an aggressive inflammatory response that may contribute to the neuropathology initiated by traumatic injury. Interleukin-10 (IL-10) is a potent anti-inflammatory cytokine with demonstrated neuroprotective effects following traumatic spinal cord injury (SCI). IL-10 reduces contusion volume, attenuates SCI induced inflammation and improves functional recovery two months after traumatic SCI. Alternatively, several studies have demonstrated that inflammation if properly regulated, may facilitate wound healing and promote functional recovery. SCI-induced inflammation can be characterized, in part, by the coordinated infiltration of peripheral blood leukocytes, transcription factor activation and the secretion of reactive radicals, cytokines, chemokines and lipid metabolites that may participate in neurodestruction. We will discuss different components of an inflammatory response, such as how pro-inflammatory cytokines elicit cytotoxic responses, mechanisms by which anti-inflammatory cytokines may reduce these cytotoxic events and finally, how the timing of an inflammatory response may be a central regulator in determining if the response is

neurodestructive or neuroconstructive. Therefore, developing a better understanding of SCI-induced inflammation may lead to the development of more effective strategies for treating SCI and improving functional recovery.

TARGETED IMMUNE THERAPIES: STRATEGIES TO PROMOTE REPAIR OF THE INJURED SPINAL CORD

P.G. Popovich, D.M. McTigue, D.M. Basso, B.T. Stokes and C.C. Whitacre, The Ohio State University, Columbus, OH

Neuroinflammation is an inevitable consequence of traumatic spinal cord injury (SCI). Unfortunately, the biological significance of cross-talk between cells and proteins of the nervous and immune systems, especially in the context of CNS trauma, is poorly understood. Resident microglia and infiltrating leukocytes can cause neuronal cell death and demyelination. However, these same cells also can promote neuronal survival, axon regeneration, remyelination and revascularization. To clarify this dichotomy of neuroinflammation, we have completed studies that have systematically explored the role played by macrophages, microglia and lymphocytes in mediating secondary injury and regeneration of the injured spinal cord. Specific analyses have included characterization of cytokine/chemokine cascades (e.g., IL-6, TNF- α , TGF- β , MCP-1), cell-specific immunomodulation therapies (e.g., macrophage-depletion, antigen-specific T-cell activation) and analysis of endogenous immunosuppressive mechanisms. From these data we conclude that the manifestation of immune-mediated injury or repair is largely determined by local microenvironmental cues and systemic influences which regulate immune cell function (e.g., neuroendocrine mechanisms). As we continue to explore the duplicity of immune function in the injured spinal cord, a multilevel approach is needed. For example, compromised neuroendocrine pathways or concomitant trauma and inflammation in peripheral tissues could affect acute and delayed immune processes in the injured CNS. Differences in location and severity of injury also may determine whether inflammatory leukocytes provoke CNS regeneration or degeneration. Experimental oversight of these variables may adversely affect our ability to interpret and successfully apply immune-based therapies in SCI models. Supported by NS37846.

CORTICAL MOTOR AREAS AND THEIR PROPERTIES: IMPLICATIONS FOR NEUROPROSTHETICS

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Devices that use naturally occurring signals within the central nervous system to functionally activate paralyzed muscles or prosthetic limbs offer great potential for achieving a sophisticated level of motor control. Although many different brain regions might provide appropriate signals, motor areas of the cerebral cortex are most accessible and offer the best opportunity for long-term recording of neural signals through chronically implanted electrode arrays. The effective use of these devices raises a number of questions which will be the focus of this presentation: 1) What specific cortical areas might provide optimal signals for prosthetic control? 2) What is the topography of the output map for each motor area? 3) Are motor output maps fixed or do they show plasticity? 4) Can the activity of neurons within cortical motor areas be shaped through feedback? 5) What is the impact of spinal cord lesions on the long-term viability of cortical neurons and can these neurons be activated in appropriate ways following spinal cord lesions? The monkey represents a useful model for exploring many of these issues. In addition to primary motor cortex (M1), several secondary cortical motor areas have been identified in the monkey (Luppino et al., 1991; He et al., 1995; Preuss et al., 1996). All of these areas contain neurons whose activity is consistently related to movement, although the degree to which neuronal activity patterns resemble muscle patterns varies as does the tuning of neurons for specific features of movement. Recent studies have also revealed that many secondary cortical areas possess a substantial corticospinal projection (He et al. 1995). Our work demonstrates that maps of forelimb M1 in the macaque monkey show a consistent core representation of distal muscles surrounded, except at the 3a/4 border, by tissue representing

proximal muscles. The maps of individual muscles within the distal and proximal representations exhibit considerable overlap. The synaptic connections of individual neurons with different motoneuron pools is also of interest. Rather than having their terminations confined to a single motoneuron pool, the target muscles of individual corticospinal neurons form functional synergies involving multiple muscles. Such synergies often involve muscles at multiple joints. Understanding these and other properties of the corticospinal system is essential to the optimal design of neural prosthetics.

STIMULATION, RECORDING AND MODULATION OF NERVE ACTIVITY USING IMPLANTED CUFFS

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Implanted nerve cuffs can be used to electrically activate axons, record the electrical signals that travel along axons, or modulate nerve functions with pharmacological agents. Nerve cuffs share three basic elements: an insulating wall made of biocompatible material, internal electrodes (or fluid ports) connected to insulated lead wires (or catheters), and a method for controlled cuff opening, installation and sealing. The mechanical properties of nerve cuffs and leads, the anatomical location and the installation procedure are critically important. Nerves bend and stretch considerably in the course of normal movements, and can be severely damaged by compressive forces or disruptions to their blood supply. However, when appropriate choices of materials, design and placement are made, nerve cuffs are the safest, most stable devices available for establishing permanent electrochemical interfaces with peripheral nerves, cranial nerves or spinal roots. This presentation will summarize practical issues and highlight results obtained with implanted nerve cuffs in the following applications:

- stimulation of peripheral motor axons to activate limb muscles paralyzed by central neurological injuries,
- stimulation of ventral root axons to control bladder function after central neurological injuries or disorders,
- selective stimulation of subpopulations of motor axons using multi-chambered nerve cuffs,
- recording of sensory nerve activity arising from cutaneous receptors,
- recording of sensory nerve activity arising from proprioceptors in muscles or joints,
- selective recordings from subpopulations of sensory axons using multi-chambered nerve cuffs,
- using paired stimulation and recording cuffs to monitor long-term stability of nerves and cuff electrodes
- using stimulation and recording cuffs to periodically monitor axonal recovery after nerve injury or repair,
- transiently blocking all activity in the nerve by anesthetic infusion into a cuff,
- transiently blocking activity in small-diameter (γ) motoneurons by anesthetic infusion into a cuff,
- treating axons by selective infusion of pharmacological agents.

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ROLE OF CORTICOFUGAL PROJECTIONS IN IMMEDIATE SOMATOSENSORY PLASTICITY

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Peripheral deafferentation of portions of the rat's mystacial whisker pad by subcutaneous injection of lidocaine results in an immediate reorganization of facial receptive fields throughout the trigeminal somatosensory system, including the VPM thalamus and SI cortex (Faggin et al., PNAS 94:9428-33, 1997). The present study was designed to examine the network level properties underlying this plasticity; specifically, the role of descending cortical projections in the reorganization seen in the VPM. Long Evans rats were implanted with 16 microelectrode wires in VPM and 8 wires in the barrel field of SI cortex. A cannula was also implanted in SI, 0.5 mm lateral to the microelectrode array. Seven days post surgery, rats were lightly anesthetized (pentobarbital) and single neuronal units were isolated from

each microelectrode. Each of the mystacial facial whiskers was stimulated. Muscimol (25-150 ng) was then infused into SI cortex and the whisker stimulation was repeated. While cortex remained inactive, lidocaine (10-40 μ l, 1%) was injected subcutaneously into the region of the upper gum and the whisker stimulation was repeated again. Muscimol completely abolished neuronal activity (recorded through the SI array) in SI for 6-8 hours. Cortical inactivation resulted in the reduction or elimination of long latency (>20 ms) responses to whisker stimulation. In some cells, however, long latency responses were unmasked. In a small subset of cells, short latency (~10 ms) responses were modified. Injection of lidocaine near the whisker pad resulted in receptive field reorganization within VPM. However, this immediate thalamic reorganization was significantly reduced following cortical inactivation. These results suggest that thalamic plasticity is dependent upon both corticofugal projections and ascending trigeminal pathways. Supported by NIH-DE 11121-02.

REORGANIZATION OF SOMATOSENSORY AND MOTOR CORTEX AFTER PERIPHERAL NERVE OR SPINAL CORD INJURY IN PRIMATES

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Somatosensory cortex contains orderly maps or representations of tactile and other inputs, while motor cortex systematically represents body movements. These orderly maps are greatly altered by the loss of afferents as a result of peripheral nerve or spinal cord injury, or the loss of muscle targets for motor control. In monkeys studied long after therapeutic amputation of the forelimb following injury, the portion of primary somatosensory cortex formerly devoted to tactile inputs from the hand represented the face and stump of the arm instead. Comparable reorganizations were observed after afferents were damaged in the dorsal columns of the spinal cord. After arm amputation, motor cortex formerly devoted to moving the digits represented movements of the shoulder and arm stump instead. The alterations in both the somatosensory and motor representations appear to depend in part on the growth of new connections. Surviving afferents grow to innervate deafferented neurons in the brainstem, and motor neurons in the spinal cord acquire new muscle targets.

BDNF AND ACTIVITY-DEPENDENT PLASTICITY OF HIPPOCAMPAL INTERNEURONS

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Inhibitory GABAergic interneurons play a crucial role in controlling excitatory transmission in the hippocampus. During the postnatal period, these interneurons mature their dendritic tree, and increase the expression of selective neuropeptides. Neuronal activity is a likely candidate to pace the development of interneurons, since it also exhibits a postnatal maturation. Furthermore, effects of neuronal activity on interneurons could be mediated by the neurotrophin BDNF, since this neurotrophin is synthesized and released in an activity-dependent manner and modulates the neurochemical characteristics of interneurons. The role of neuronal activity and BDNF in promoting the morphological and neurochemical development of hippocampal interneurons was evaluated using *in vitro* preparations. In neuronal cultures taken from E17 rat embryos, the GABA_A receptor agonist muscimol exerted a depolarizing effect during the first week *in vitro*, and increased the soma size and neuropeptide Y (NPY) immunoreactivity of interneurons. Opposite effects were observed at later times *in vitro*, when GABA_A receptor activation was hyperpolarizing. Early GABA_A receptor activation failed to increase NPY immunoreactivity in cultures from BDNF knockout embryos. Thus, neuronal activity modulates the morphology and neuropeptide content of developing interneurons, and BDNF mediates the activity-dependent regulation of NPY. To determine whether the same mechanism regulates different neuropeptides, the activity-dependent modulation of NPY was compared with that of somatostatin. In organotypic slice cultures taken from 7-day-old rat hippocampi, the GABA_A receptor blocker bicuculline induced a strong increase in the number of NPY-immunoreactive neurons. An opposite effect was observed following blockade of excitatory transmission. The expression of somatostatin was regulated by neuronal activity to the same extent as NPY. However, although BDNF induced a strong up-regulation of NPY, it did not affect somatostatin

immunoreactivity. Thus, neuronal activity could regulate the levels of different neuropeptides localized within the same interneurons via different mechanisms, i.e., the number of GAD65 and GABA immunoreactive puncta. These results suggest that increased excitatory activity increases the number of inhibitory synapses, which could represent a negative feedback aimed to limit the excitability of hippocampal networks.

REGULATION OF EXCITATORY AND INHIBITORY SYNAPTIC TRANSMISSION IN HIPPOCAMPAL CULTURES BY BRAIN-DERIVED NEUROTROPHIC FACTOR

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Brain-derived neurotrophic factor (BDNF) has been proposed to be a key signaling molecule in regulating synaptic function and plasticity. In this context, we have studied the effects of chronic BDNF treatment on excitatory and inhibitory synaptic transmission in conventional dissociated hippocampal cultures as well as in microcultures of individual hippocampal neurons in order to isolate cell-autonomous effects. We found that BDNF dramatically increases the frequency of spontaneously initiated action potentials in conventional hippocampal cultures. Although overall excitability in these cultures was increased, we found that BDNF in fact potentiates both excitatory and inhibitory synaptic transmission, but via different mechanisms. We further examined regulation of excitatory transmission by BDNF in individual CA1 hippocampal neurons maintained in microculture. These neurons underwent similar regulation of excitatory synaptic transmission as was observed in conventional cultures, indicating that BDNF regulation of excitatory transmission is likely to be direct. Regulation of excitatory transmission by BDNF was not dependent on activity *per se* as blocking action potentials with tetrodotoxin (TTX) for the entire duration of BDNF treatment had no effect on the magnitude of synaptic enhancement. Finally, we found that these effects of BDNF were quite selective as BDNF did not alter intrinsic membrane excitability, synapse number, or neuronal survival. This work was supported by grants from the NIH (NS32742) and the McKnight Endowment Fund for Neuroscience.

NEUROTROPHINS AND ACTIVITY-DEPENDENT INHIBITORY SYNAPTOGENESIS

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Development of the full complement of inhibitory synapses in organotypic cerebellar cultures derived from newborn mice requires the presence of neuronal activity (Seil, F.J. and Drake-Baumann, R., J. Comp. Neurol. 342:366-377, 1994). Neurotrophins BDNF, NT-3 and NT-4 were applied exogenously to cerebellar cultures at explantation, along with activity blocking agents, tetrodotoxin and elevated levels of Mg²⁺. After 2 weeks *in vitro*, Purkinje cell axosomatic synapses (inhibitory) were identified by electron microscopy and quantified, and extracellular recordings were obtained from the explants. Purkinje cells in untreated activity blocked cultures had approximately half the control numbers of axosomatic synapses. Purkinje cells in activity blocked cultures simultaneously exposed to the TrkB receptor ligands, BDNF and NT-4, developed axosomatic synapses quantitatively equivalent to control explants. By contrast, the TrkC receptor ligand, NT-3, did not prevent the reduced formation of Purkinje cell axosomatic synapses resulting from exposure to activity blocking agents. In a corollary experiment to assess the role of endogenous neurotrophins, exposure of cerebellar explants to a combination of antibodies to BDNF and NT-4 for 2 weeks *in vitro* resulted in a reduced development of Purkinje cell axosomatic synapses, similar to the effects of activity blockade. Electrophysiological results correlated with the morphological findings. After transfer to a physiological recording medium, all cultures exposed to activity blocking agents were silent for at least 10 minutes. After recovery from activity blockade, untreated cultures developed a state of sustained hyperactivity, the cortical spikes representing primarily Purkinje cell discharges. Activity blocked explants treated with BDNF and NT-4 exhibited spontaneous cortical discharge rates after recovery comparable to control cultures, while activity blocked cultures exposed to NT-3 became hyperactive.

Collectively, these results support the concept of a role for TrkB receptor ligands in activity-dependent inhibitory synaptogenesis. Supported by the Medical Research Service of the Department of Veterans Affairs.

MOLECULAR MECHANISM UNDERLYING BDNF MODULATION OF HIPPOCAMPAL LTP

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Recent studies have revealed that neurotrophins may play a critical role in learning and memory. Our laboratory has demonstrated that the brain-derived neurotrophic factor (BDNF) promotes hippocampal long-term potentiation (LTP), a cellular model for learning and memory. We showed that application of BDNF to developing hippocampus enhanced LTP at CA1 synapses, while inhibition of BDNF activity in the adult by the BDNF scavenger, TrkB-IgG, attenuated LTP. BDNF also potentiated synaptic responses to high-frequency stimulation (HFS) without affecting low frequency transmission. A series of physiological experiments demonstrated that BDNF attenuates synaptic fatigue through a presynaptic mechanism. Thus, BDNF may facilitate LTP at CA1 synapses by a presynaptic enhancement of high-frequency synaptic transmission. We have further investigated the cellular and molecular mechanisms of BDNF action using BDNF knockout mice. Compared with wild type (+/+), the homozygotes (-/-) and heterozygotes (+/-) exhibited more pronounced synaptic fatigue during HFS, leading to a severe impairment in LTP. The overall ultrastructural appearance of excitatory synapses on CA1 dendritic spines was similar in all genotypes. Quantitative analysis, however, revealed a striking reduction in the number of vesicles docked at presynaptic active zones in both +/- and -/- mutant mice. The number of reserve pool vesicles, active zone length, and presynaptic terminal area remained unchanged in the mutant mice. Hippocampal synaptosomes prepared from mutant mice exhibited a marked decrease in the levels of synaptophysin as well as synaptobrevin, a protein known to be involved in vesicle docking. Other synaptic proteins, including synaptotagmin, syntaxin-1 and SNAP-25, were unaffected. Treatment of the mutant slices with BDNF reversed the electrophysiological and biochemical deficits in the hippocampal synapses. Using a conditional knockout mouse with specific deletion of the BDNF receptor TrkB in the CA1 region, we showed that BDNF modulates LTP and HFS response in the CA1 synapses through mechanisms independent of postsynaptic CA1 pyramidal neurons. Taken together, these results suggest a novel role for BDNF in the mobilization and/or docking of synaptic vesicles to presynaptic active zones. Our studies may have general implications in understanding the mechanisms of learning and memory, and in treatment of learning disorders in both children and adults.

NEUROTROPHIN-EVOKED RAPID EXCITATION OF CENTRAL NEURONS

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The known rapid actions of neurotrophins (NTs) include the modification of the efficacy of transmission at several types of central synapses, as well as the transient elevation of the intracellular Ca^{2+} concentration. These processes are initiated within seconds up to several minutes following the application of NTs and involve mechanisms that were not entirely clarified. By using whole-cell patch clamp recordings in acute brain slice preparations, we found that NTs evoke within milliseconds action potential firing in various types of central neurons (Kafitz et al., 1999). Already at concentrations of 0.5-2.0 nM, brain-derived neurotrophic factor (BDNF) excited neurons in the hippocampus, cortex and cerebellum. Remarkably, BDNF and neurotrophin-4/5 (NT-4/5) depolarized neurons, at a more than thousand-fold lower concentration, just as effectively as the neurotransmitter glutamate. The onset of the BDNF-evoked response could not be distinguished from that of glutamate. A response delay of about 9 ms was observed under our experimental conditions. This includes the diffusion time and the build-up of the appropriate concentration at the receptor site. Neurotrophin-3 (NT-3) produced much smaller responses, while nerve growth factor

(NGF) was ineffective. The NT-induced depolarization resulted from the activation of a Na^{+} -conductance which was reversibly blocked by K-252a, a protein kinase blocker with preference for Trk-receptor tyrosine kinases. The extremely rapid onset of the response suggests a direct interaction of the transmembranous tyrosine-kinase TrkB receptor and an as yet unidentified Na^{+} -permeable channel. The rapidity of their action combined with the previous findings that they are transported to axon terminals, that they activate postsynaptic dendrites and that they are secreted in an activity-dependent manner, assigns to NTs in the low nanomolar range functional properties similar to those of conventional excitatory neurotransmitters.

USE OF FUNCTIONAL MAGNETIC RESONANCE IMAGING (fMRI) TO ASSESS PLASTICITY IN LOCOMOTOR NETWORKS

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fMRI studies of recovery of finger movement in a hemiparetic hand after stroke show considerable reorganization over time within the cortical networks that represent the hand. Locomotor retraining after incomplete spinal cord injury using treadmill stepping with partial weight support may also expand the effectiveness of uninjured descending and segmental afferent activity on the regulation of both spinal and cortical facilitation and recruitment. We are comparing active and passive alternating dorsiflexion and plantar flexion movement of the toes and ankle from control subjects to the same movements made by patients with stroke and spinal cord injury (SCI). Imaging artifacts can arise from head motion and always arise when more proximal leg movements are attempted. In controls, passive toe and ankle movements consistently activate the high medial primary sensorimotor and supplementary motor cortices. Voluntary movements produce larger activations. Compared to a group analysis of control subjects, some patients with chronic incomplete SCI who can walk show enlarged cortical representations and higher activations during passive movement. In SCI subjects who had clinically complete lesions, no activation was found. In SCI subjects who had no motor control and only vague awareness of passive foot motion, the cortex was activated, often over a wide representation. Serial fMRI studies during retraining of locomotion and after biological interventions to restore circuitry may reflect changes in network activity and offer insights into the efficacy of treatments over the course of gains in motor control.

ADAPTIVE PLASTICITY IN THE WALKING SYSTEM OF THE CAT

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An important issue in assessing the functional recovery of movement following damage to the nervous system is the extent to which recovery depends on use-dependent modification of intrinsic neural circuits in the spinal cord. My colleagues and I have recently addressed this issue in the cat by examining use-dependent changes in the motor pattern for walking following weakening of ankle extensor muscles. Weakening the ankle extensor muscles by either cutting the nerves to some of the muscles, or injecting botulinum toxin into the muscles, caused a marked increase in ankle flexion (yield) during the early part of the stance phase. There was also a reduction in ankle extension at the end of stance. The exaggerated yielding at the ankle and the reduction in ankle extension progressively decreased in the days following muscle weakening, and movements returned close to normal within two weeks. Associated with this functional recovery was an increase in the magnitude of electromyographic (EMG) activity in the innervated and/or non-injected muscles. The recovery of normal movements and the concomitant increase in EMG activity are use-dependent, since the onset of these adaptive changes was delayed if the leg was immobilized for a few days following muscle weakening. Data will be presented that indicate that the modification of the EMG activity depends on two processes: 1) a rapid increase in gain of spinal reflex pathways contributing to the generation of EMG activity during mid to late stance, and 2) a slower increase of the centrally-generated, feed-forward motor command generating the initial component of EMG activity in the ankle extensor muscles. The latter increases ankle stiffness and is primarily responsible for reducing the yield during early stance.

NEURAL PLASTICITY AS REVEALED BY THE NATURAL PROGRESSION OF MOVEMENT EXPRESSION - BOTH VOLUNTARY AND INVOLUNTARY - IN HUMANS AFTER SPINAL CORD INJURY

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We are examining the natural pattern and time course of movement expression (voluntary, reflex, or spontaneous) following acute spinal cord injury (SCI) in man. These studies may shed light on mechanisms of neurologic recovery, and may assist in subject selection and evaluation for clinical trials of therapeutic agents. More than 500 subjects with SCI have been examined to date, including roughly 200 with acute injury (typically <1 week). Measures include voluntary contractions, tendon taps, nerve stimulations, and evoked responses to transcranial magnetic stimulation (TMS). Recent studies of autonomic dysfunction include measures of arterial blood pressure, heart rate, and skin resistance following a variety of sensory inputs. Widespread alterations in spinal cord input/output properties are likely due, at least in part, to intrinsic alterations in the strength and/or distribution of synaptic contacts. A number of examples will be reported, including: 1) marked differences in spinal cord reflex excitability in persons with acute SCI having some retained (or recovered) movement in one or more lower limb muscles (i.e. motor-incomplete) compared to persons with motor-complete injury; 2) marked differences in excitability of some reflex pathways for acute versus chronic injury; 3) development in hand and forearm muscles of short-latency muscle contractions evoked by sensory stimulation of lower limb afferents, routinely seen in persons with motor-complete cervical SCI and occasionally seen in persons with motor-incomplete cervical SCI. These evoked responses suggest 'regenerative sprouting' of surviving lower limb afferents onto partially-denervated motoneurons of the cervical enlargement as the mechanism; 4) changes in autonomic nervous system properties leading to autonomic dysreflexia, which may be due to a similar mechanism as in '3' above; 5) expression of involuntary rhythmic leg contractions which can be similar to stepping movements, suggesting a spinal 'pattern generator' for such movements; and 6) clinical improvements in strength without change in the routinely-delayed TMS-evoked muscle contraction, suggesting that remyelination of central motor axons is not crucial for improved motor function after human SCI.

LOCOMOTOR (LAUF BAND) THERAPY IN SCI PERSONS

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Locomotor training on the treadmill (Laufband therapy) focuses on intensive walking in upright position facilitated by body weight support via a harness, the moving band of the treadmill and initial limb setting by two therapists, if necessary. These principles are derived from recent observations in spinal animals on activity-related "learning" of the isolated spinal cord and on the "rules of spinal locomotion" in lower vertebrates. First, results of a 5-year study are reported in which 89 incompletely paralyzed (44 chronic and 45 acute) patients exercised on the Laufband are compared with a total of 64 patients treated conventionally for comparable periods of time (median 10.5 weeks). Laufband therapy achieved significantly better results in all comparisons (Wernig et al., *Europ.J.Neurosci.* 7,823-829, 1995). A number of chronically wheelchair-bound patients (not capable of rising from the wheelchair or walking without help from other persons) became independent and walked with help of a rollator or two canes for distances of at least 100 meters. Most chronic patients not capable of staircase walking learned to do so either by themselves or with help from another person following Laufband therapy. Also acute patients treated on the Laufband achieved better results than conventionally treated patients. The results of a follow-up evaluation are reported, in which walking capability of Laufband treated patients, immediately following therapy, is compared with that after 1-6 years in domestic surrounding. All but one originally chronic patients and all originally acute patients maintained or even improved their walking capability (Wernig et al., *Spinal Cord*, 36, 744-749, 1998). Supported in part by Deutsche Stiftung Querschnittlähmung.

AUTOIMMUNE T-CELLS ENHANCE RECOVERY FROM SEVERE SPINAL CORD INJURY: IMMUNE NEUROPROTECTION M. Schwartz, The Weizmann Institute of Science, Rehovot, Israel

The mammalian central nervous system (CNS) has long been viewed as off-limits to the immune system, a concept expressed in terms of 'immune privilege'. Accordingly, the notion has been that all forms of CNS inflammation are detrimental, and hence the less immune intervention, the better. We showed that the innate immune system, in the form of activated macrophages, facilitates processes of regeneration in transected spinal cord (Rapalino et al., *Nat.Med.* 4, 814-821, 1998). More recently, we found also that autoimmune T-cells specific for a component of myelin can significantly protect the optic nerve from secondary degeneration following traumatic injury (Moalem et al., *Nat.Med.* 5, 49-55, 1999). In the present work, we show that such autoimmune T-cells can also promote recovery after contusive injury of the rat spinal cord. Lewis rats were injured by spinal contusion at the level of T7-T8 or T8-T9, and were then treated by a single intraperitoneal injection of 10^7 T-cells specific to the CNS self-antigen myelin basic protein. Injured control rats were similarly injected with T-cells specific to the foreign antigen ovalbumin or with phosphate-buffered saline. Treatment with the autoimmune T-cells dramatically enhanced recovery after spinal cord contusion, as expressed in significantly heightened locomotor activity, a 5-fold increase in the number of intact axons descending from the red nucleus, and a significant increase in the mass of spared neural cord tissue shown by diffusion MRI and by confocal microscopy. The results support our suggestion that the CNS, like other tissues, can benefit from immune system protection, and that autoimmune T-cells are not necessarily detrimental, but may play a beneficial role in tissue protection. Beneficial autoimmunity, normally suppressed in the CNS because of immune privilege, may be therapeutically boosted without inducing an autoimmune disease.

OLFACTORY ENSHEATHING GLIA TRANSPLANTS AS TOOLS TO RESTORE FUNCTION AND REPAIR INJURED SPINAL CORDS OF ADULT MAMMALS

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The lack of axonal regeneration in the adult mammalian central nervous system (CNS) causes permanent disabilities in patients with spinal cord injuries. We have used adult rat olfactory bulb ensheathing glia (OEG) transplants as a repair strategy to promote functional and structural recovery after spinal cord transection. Spinal cords of adult rats were sectioned at the thoracic level (T8) and suspensions of pure Hoeschst-labeled OEG were injected into the midline of both cord stumps. From three to seven months post-surgery all OEG-transplanted paraplegic animals recovered specific locomotor and sensory functions. Biotin dextran amine (BDA) was used to trace corticospinal axons and immunohistochemistry to label regenerating raphespinal, coeruleospinal, and sensory fibers, and also to delimit the glial scar. The extent of axonal regeneration in the damaged cords of paraplegic animals was analyzed eight months after surgery. In OEG-transplanted rats, injured axons crossed the gliotic tissue formed at the transection site and OEG occupied the entire injury region and intermingled with reactive glia. Corticospinal, raphespinal, and coeruleospinal axons regenerated for long distances (3 cm, L6) within the distal spinal cord stumps, and sensory axons elongated rostrally and crossed the transection site. OEG migrated from the injection sites and were found in the same locations as regenerating axons. By comparison, neither functional nor long-distance axonal regeneration was observed in any of the injured non-transplanted rats. Our results demonstrate that OEG transplantation provides a useful repair strategy in adult mammals with spinal cord traumatic injuries. OEG can be obtained from adult donors offering the possibility of autologous transplantation. Therefore, these cells open new perspectives in the search for a therapeutic procedure to treat spinal cord injuries and CNS trauma in humans.

LINEAGE RESTRICTED PRECURSORS FOR TRANSPLANTATION - CELL TYPES, SOURCES AND METHODS OF ISOLATION

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Acquisition of cell-type specific properties in the nervous system is a process of sequential restriction in developmental potential. At least two classes of pluripotent stem cells, neuroepithelial (NEP) stem cells, and EGF-dependent neurosphere stem cells, have been identified and shown to be present in distinct spatial and temporal domains. Pluripotent stem cells likely generate central nervous system (CNS) and peripheral nervous system (PNS) derivatives via the generation of intermediate lineage-restricted precursors that differ from each other and from multipotent stem cells. Neuronal precursors termed NRP's (Neuronal Restricted Precursors), multiple classes of glial precursors including GRP's (Glial Restricted Precursors), and PNS precursors termed NCSC's (Neural Crest Stem Cells), have been identified. Antibodies to cell surface epitopes that distinguish between these cell types have been identified and these antibodies have been used to select purified populations of cells. Multipotent stem cells and restricted precursor cells similar to those characterized from embryonic tissue can also be isolated from ES (Embryonic Stem) cell cultures, providing a non-fetal source of such cells. Analysis in multiple species illustrates similarities between rat, mouse and human cell differentiation, raising the possibility that similar factors and markers may be used to isolate precursor cells from human tissue or ES cells. Lineage restricted precursors have been transplanted into neonatal and adult rat brains and their ability to integrate and differentiate into appropriate phenotypes has been analyzed. Results from these transplantation studies will be compared to transplants of multipotent stem cells and the relevance of these results for potential therapeutic uses will be discussed.

STROMAL CELLS FROM BONE MARROW FOR CELL AND GENE THERAPY OF DISEASES OF THE CENTRAL NERVOUS SYSTEM

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In addition to precursors for hematopoietic cells, bone marrow contains stem-like cells for a variety of non-hematopoietic tissues. The cells are variously referred to as mesenchymal stem cells or marrow stromal cells (MSCs). Beginning over 20 years ago, Friedenstein and many other investigators demonstrated that MSCs in culture can differentiate into osteoblasts, adipocytes, chondrocytes, fibroblasts, and myoblasts (for review, see Prockop, *Science* 276:71-74, 1997). After systemic infusion, progenitors of MSCs appear in a variety of tissues and appear to differentiate into the specific phenotypes of the tissues (see Pereira et al. *PNAS* 92:4857-4861, 1995, and 95:1142-1147, 1998). Based on these observations, a clinical trial has been initiated in which allogeneic bone marrow transplantation is being tested for the therapy of osteogenesis imperfecta (Horwitz et al. *Nature Med.* 5:309-313, 1999). We initially observed that after infusion into the basal ganglia of rats, both rat and human MSCs integrate and migrate in a manner similar to astrocytes (Azizi et al. *PNAS* 95:3908-3913, 1998). Subsequently, we found that the cells can be gene-engineered so that they secrete L-DOPA after implantation into the basal ganglia of rats (Schwarz et al. *Human Gene Therapy*, Oct. 10, 1999). More recently, we have found that when murine MSCs are infused into the lateral ventricles of neonatal mice, the cells migrated both to the forebrain and to the cerebellum without disruption of the host brain architecture (Kopen et al. *PNAS* 96:10711-10716, 1999). Some MSCs within the striatum and the molecular layer of the hippocampus expressed glial fibrillary acidic protein and, therefore, differentiated into mature astrocytes. MSCs also populated neuron rich regions including the Islands of Calleja, the olfactory bulb, and the internal granular layer of the cerebellum. In addition, neurofilament positive donor cells were found within the reticular formation of the brain stem, suggesting that MSCs also may have differentiated into neurons. The results support previous indications that MSCs are potentially useful as vectors for treating a variety of disorders of the central nervous system.

DIFFERENTIAL EFFECTS OF SPINAL CORD GRAY AND WHITE MATTER ON PROCESS OUTGROWTH FROM GRAFTED HUMAN NTERA2 NEURONS (NT2N, hNT)

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To investigate host effects on grafts of pure, postmitotic human neurons, we assessed the morphological and molecular phenotype of purified NTera2N (NT2N, hNT) neurons implanted into the spinal cord of athymic nude mice. NT2N neurons were implanted into both spinal cord gray and white matter of neonatal, adolescent and adult mice and evaluated at post-implantation times up to 15 months. NT2N neurons remained at the implantation site, showed process integration into all host areas, and each graft exhibited similar phenotypic features regardless of location or host age at implantation. Evidence of host oligodendrocyte ensheathment of NT2N neuronal processes was seen and grafted NT2N neurons acquired and maintained the morphological and molecular phenotype of mature neurons. The microenvironments of host gray and white matter appear to exert differential effects on implanted neuronal processes as consistent differences were noted in the morphologies of graft processes extending into white matter versus gray matter. NT2N processes extended for long distances (over 2 cm) within white matter, while NT2N processes located within gray matter had short trajectories, typical of local host patterning. This suggests that NT2N neurons integrate into both gray and white matter sites in location-appropriate manners, extending processes that differentially respond to gray and white matter cues. Further studies of grafted human NT2N neurons may lead to the identification of host molecular signals that support and direct the successful integration of grafted human neurons and the outgrowth of their processes in the nervous system.

SMALL MOLECULE COMPOUNDS MIMICKING NEUROTROPHINS

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Neurotrophins have highly potent biological effects preventing neuronal death and promoting neurite outgrowth; however, like most proteins they do not have optimal pharmacological properties. Factors limiting their clinical application include stability, nervous system penetration and their wide array of local and systemic biological activities. An important approach for addressing these limitations is the development of synthetic small molecule neurotrophin mimetics with optimal profiles of stability, tissue penetration and targeted biological actions. Neurotrophin mimetic strategies include the following: development of agents that induce endogenous expression of neurotrophins; agents that act directly at neurotrophin receptors as agonists, partial agonists or antagonists; and agents that augment neurotrophin induced signal transduction. Compounds targeted to specific neurotrophin receptors have the potential to mimic the entire range of functions or a subset of functions of a given neurotrophin. For example, prevention of neuronal death in the absence of stimulating neurite outgrowth might constitute a desired activity profile in certain applications. The identification of specific neurotrophin protein domains likely to modulate receptor interactions has guided synthesis of neurotrophin peptidomimetics corresponding to individual domains and functioning via selected receptors to trigger neurotrophin-like signal transduction. Synthesis of domain-specific mimetics has also provided a key proof-of-principle that it may be possible to design neurotrophin antagonists that would inhibit neurotrophin actions in the contexts of neurotrophin-induced cell death, aberrant sprouting, etc. Current neurotrophin small molecule studies provide a basis and proof-of-principle to guide programs of rational drug design and large scale screening for compounds with medicinal properties and targeted neurotrophin activities

CLINICAL STUDIES OF K⁺ CHANNEL BLOCKADE IN CHRONIC SPINAL CORD INJURY

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The potassium channel blocker 4-aminopyridine (4-AP) has been explored as a symptomatic treatment for chronic spinal cord injury (SCI) in animal studies and human clinical trials. The history of this effort, spanning more than a decade, illuminates some of the general challenges facing therapeutic development in this field. The underlying basic science is relatively straightforward, and there is now good evidence of beneficial physiological effects of 4-AP on the injured nervous system. These effects are consistent with a direct action through the blockade of "fast" potassium channels in demyelinated or poorly myelinated nerve fibers. However, there have been no well-established structures for taking a promising approach from initial evidence of efficacy in chronic SCI to regulatory approval for human use. Building such structures raises some difficult questions. What would a successful therapeutic outcome (short of a miracle "cure") look like in a condition as diverse as chronic spinal cord injury? What is the minimal benefit that would be accepted as clinically significant, by patients, clinicians, and regulatory agencies, and how can such benefits be measured? Just how stable are the deficits in this condition, and to what extent are they susceptible to placebo effects? The current stage of development involves answering these questions. There are encouraging signs that the recipients of treatment themselves hold the key to understanding what an effective treatment looks like, and it is the task of the scientists and clinicians to find ways to quantify their experiences. In addition, the optimal dosage of 4-AP is under active investigation in both SCI and multiple sclerosis (MS). Current data appear to be consistent with concentrations of 4-aminopyridine that have been shown to be effective in improving conduction of impulses in injured nerve fibers *in vitro*.

TISSUE ENGINEERING STRATEGIES FOR REPAIRING THE NERVOUS SYSTEM

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Tissue engineering seeks to develop novel therapeutic approaches by combining elements of biological and engineering sciences. The approach typically involves implanting a construct that either contains living cells or is subsequently colonized by host cells to augment, repair or reconstitute specific tissue function. Two general approaches are being developed to repair the nervous system, namely, the use of encapsulated cell-containing implants as focal, sustained delivery systems to supply neuroactive substances or growth factors, and the use of guidance systems that bridge or reconnect severed or damaged nerve tracts. Recent studies indicate that cell-containing capsules show potential as a treatment for a wide range of indications including age-related degeneration, Alzheimer's disease, amyotrophic lateral sclerosis, chronic pain, neuroprotection, Huntington's disease, improving survival of fetal transplants, and Parkinson's disease. In addition, the approach has been used as a basic science tool to study the mechanism of functional recovery of transplanted embryonic tissue. Although the results are promising, fundamental technical issues regarding the extent of solute diffusion and the time frame over which the devices operate remain less well understood. The other major area has focused on creating substrates that direct or prescribe the course of axon trajectories in the central and peripheral nervous system. Research includes the development of materials with surfaces that are specifically engineered to promote and direct neurite outgrowth through the combined interactions of surface cues of such topographical features and the presence of biological ligands. Research ranges from fundamental 2-D cell-materials interactions studies to the development of 3-D matrices that promote regeneration of histiotypic architecture. Hollow fiber-based devices have been used with internal growth-promoting matrices to promote long tract regrowth. Fundamental issues regarding host response inhibition to all of the aforementioned approaches remain a major challenge to clinical implementation. However, recent studies employing biohybrid constructs containing glial elements as well as recombinant neurotrophin-secreting cells suggest novel methods of repairing target-directed long axonal pathways.

IN VIVO NEUROPROTECTION OF INJURED CNS NEURONS BY A SINGLE INJECTION OF A DNA PLASMID

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Spinal cord injury is a devastating event for which there is presently no effective treatment. Both descending and ascending axons are severed or otherwise damaged. Many of the axotomized neurons atrophy and/or die by an apoptotic process. Those neurons that survive are unable to regenerate their axons. Additional cells die at and around the injury site as the lesion expands during the days and weeks following injury (bystander death). The challenge is to design therapeutic approaches that prevent atrophy and death of central nervous system (CNS) neurons and promote regeneration of their processes. Since nervous tissue degeneration begins quickly after lesion, neuroprotective interventions need to be applied soon after the injury and should therefore be relatively non-invasive. We have developed a rapid method to introduce genetic information into CNS cells that protects injured neurons. The method consists of an injection of a DNA plasmid that codes for the antiapoptotic gene B-cell lymphoma 2 (Bcl-2). Our results indicate that a single intraspinal injection of the Bcl-2 plasmid just proximal to a total or subtotal hemisection lesion prevents death of axotomized Clarke's nucleus and red nucleus neurons in adult rats. Interestingly, the injection of the Bcl-2 plasmid also prevents atrophy of these axotomized neurons. We are presently evaluating the effect of a Bcl-2 plasmid injection on neurons of the horizontal diagonal band of Broca injured by an immunotoxin to determine whether these neurons can also be protected by this method. The mechanism by which the Bcl-2 injection protects cells probably includes both retrograde transport to the nucleus and local neuroprotective effects that ameliorate the hostile environment at or near the site of injury. The plasmid injection technique can be used to deliver other potential therapeutic genes, such as neurotrophins, into injured CNS neurons. Supported by NIH, EPVA, NS24707, NMSS and VA.

**ABSTRACTS
OF
POSTER
PRESENTATIONS**

DISTRIBUTION OF DESCENDING SUPRASPINAL AXONS AROUND ACUTE AND CHRONIC LESION CAVITIES AFTER SPINAL CORD CONTUSION INJURY IN THE ADULT RAT

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In an earlier study we were surprised by the presence of labeled central axons in a chronic contusion cavity within the cavity matrix in both the cortical and lateral paragigantocellularis (LPGi) injection cases six months after injury. This observation suggests that central axons penetrate into the lesion cavity, in addition to the known penetration of peripheral axons, as part of the endogenous repair response following SCI. There is some question as to whether the axons in the cavity at long survival times are spared or regenerating fibers. In the present study we examined various time points (1, 3, 8, 21 days and 14 weeks) to determine whether fibers present within the chronic lesion cavity are spared or regenerating fibers. Bilateral injections into the cortex or the LPGi were used to label axons. The distribution of anterogradely labeled axons was assessed after a 12.5 mm MASCIS contusion injury at T9-10. Corticospinal tract (CST) fibers anterogradely labeled prior to contusion injury were used to assess the distribution of labeled fibers over the first 3 weeks after injury. No labeled fibers were found within the lesion cavity at any of the time points (1, 3, 8 and 21 days). Instead labeled fibers were observed to stop abruptly rostral and caudal to the impact site. Rostral to the lesion cavity, labeled CST axons remain confined to the white matter tract and did not appear to have collateralized at the rostral edge of the lesion cavity as they did in the six month cases. Caudal to the lesion cavity, distal segments of labeled CST fibers persisted for up to three weeks, but were undergoing fragmentation. At 3 months proximal to the lesion cavity, the CST exhibited similar but not as extensive collateralization as was observed at six months. However, no fibers were observed to be growing into the lesion cavity. Similarly no fibers arising from the LPGi were observed to enter the cavity. These data suggest that the collateralization of the CST into the gray matter proximal to the lesion occurs between 3 weeks and 3 months, whereas the penetration of CST and brainstem axons into the lesion matrix occurs over an even longer time course. This is in contrast to primary afferent axons that can be observed to enter the lesion cavity as early as two weeks after injury, being escorted in by proliferating Schwann cells. Supported by PVA SCRF-1734 and NS-31193.

LOCAL HYPOTHERMIA LIMITS SECONDARY INJURY, NEURONAL DEATH AND PROGRESSIVE NECROSIS AND CAVITATION FOLLOWING SPINAL CORD INJURY

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This study evaluates the therapeutic effects of local hypothermia on secondary degeneration following spinal cord injury in rats. Crush injuries were produced at the T8 level using an extradural approach. Local hypothermia was then induced by irrigating the exposed surface of the spinal cord with artificial CSF under varying conditions (perfusion fluid temperature, perfusion velocities and durations). The optimal perfusion conditions were established in an initial experiment by evaluating lesion appearance 24 hours post-injury in sections stained with H&E. Then, in a second experiment, animals treated with the optimal condition were allowed to survive for 24 hrs and 1, 3, 8 weeks post-injury, and spinal cords were prepared for histological evaluation using H&E and silver staining for general histopathology. Quantitative assessments of lesion size and cavity area revealed a significant reduction (45%) in lesion volume at 24 hours post-injury in animals treated with optimal hypothermic perfusion (perfusion fluid temperature at 21°C, perfusion velocities at 50 ml/h, duration for 4 hours, started immediately after the crush). Lesion size and cavitation area remained smaller in treated animals throughout the post-injury survival interval. These results suggest that the induction of hypothermia immediately after spinal cord injury significantly reduces the secondary degeneration that would otherwise occur. The mechanism by which hypothermic perfusion exerts its beneficial effect is unknown, but may involve dialysis of noxious substances from the injured cord and/or hypothermic inhibition of inflammation.

CYTOKINE PROFILES AFTER ACUTE AND CHRONIC SPINAL CORD INJURY

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We have previously demonstrated that delayed transplantation and neurotrophin administration enhance axonal regeneration after spinal cord injury (SCI) in adult rats. Although it is likely that the molecular environment within the injured spinal cord influences the survival of transplants and the nature and extent of axonal regrowth, little is known about the local environmental changes after chronic SCI. We sought to determine whether a difference exists in cytokine profile between acutely and chronically injured spinal cord. Adult Sprague-Dawley rats received a midthoracic hemisection. Some rats were sacrificed at 3,6,12,24,48hr,4,7,14 days after hemisection (acute group). The others underwent removal of glial scar tissue 2 weeks after hemisection, and then were sacrificed at 3,6,12,24,48hr, 4 days after the second surgery (chronic group). In the control group, the rats received only a laminectomy. Cytokine profiles in spinal cord were analyzed quantitatively by RNase protection assay. In the acute group, pro-inflammatory cytokine-mRNAs such as IL-1 β , IL-6 and TNF α increased sharply, and reached their peaks between 6 and 12hr after hemisection. Their expressions decreased sharply at 24hr and declined to the control level by 4 days. The expression of TGF β 1, an anti-inflammatory cytokine, increased gradually after hemisection with the peak around 4 days. In the chronic group, pro-inflammatory cytokine mRNAs increased after the second surgery, and reached their peaks earlier. The expression of pro-inflammatory cytokine mRNAs, however, was significantly attenuated compared to the acute group. In contrast, TGF β 1 expression increased earlier and higher than the acute group. These findings suggest that over-production of pro-inflammatory cytokine mRNAs might contribute to the detrimental events such as secondary cell loss and glial scar formation after SCI. The attenuation of pro-inflammatory cytokine expression by TGF β 1 in chronically injured spinal cord might help to create a more favorable milieu for transplants as compared to acutely injured spinal cord. Supported by NIH grants NS27045 and DA04358.

GLUTAMINE METABOLISM FOLLOWING SPINAL CORD INJURY

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Glutamine is a branch point substrate for several metabolic pathways in the central nervous system including the glutamine cycle, purine biosynthesis, protein production, and the urea cycle. In the central nervous system, the major synthetic enzyme for glutamine, glutamine synthetase (GS), is localized to glial cells. Since astrocytes undergo gliosis following injury to the nervous system, we investigated if glutamine metabolism is altered following spinal cord injury. Sprague-Dawley rats were anesthetized with sodium pentobarbital (40mg/kg), a laminectomy was performed and the thoracic spinal cord was transected at the T4-5 segments. Animals survived 12 hours to 7 days depending on the assay for glutamine metabolism. Several aspects of glutamine metabolism were examined following injury. Segments on either side of the transection and the cervical and lumbosacral enlargements were examined. Glutamine synthetase mRNA levels were investigated with in situ hybridization and RT-PCR. Within hours after spinal injury, mRNA levels for GS were elevated primarily in astrocytes in the white matter. GS and glutamine immunoreactivities in astrocytes were elevated within hours and remain elevated for days. At 7 days, GS enzyme activity and glutamine concentrations were examined in lyophilized, microdissected cryostat sections with HPLC. White and gray matter regions contained elevated GS activity and glutamine concentrations. These results indicate that glutamine metabolism is elevated early and is sustained following spinal cord injury. Because of its importance to cellular activity, glutamine metabolism may be a key component to allow astrocytes to enter into reactive astrocytosis. Supported by NIH27213 - KEM.

EXPRESSION OF MATRIX METALLOPROTEINASES IN THE TRANSECTED SPINAL CORD

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While the deposition of extracellular matrix (ECM) molecules (e.g., proteoglycans) and the influence of such molecules on neurodegeneration

and regeneration have been extensively investigated in various central nervous system (CNS) pathologies, many factors that might influence such deposition have not yet been examined. Matrix metalloproteinases (MMPs) are calcium-requiring zinc-containing endopeptidases that degrade ECM molecules. In spinal cord injury (SCI), expression or non-expression of MMPs may play a role in the development, progression and resolution (or fixation) of the lesion. This hypothesis stems from the observation that lesion formation in SCI involves tissue remodeling which, in turn, can be influenced by the activities of enzymes that degrade ECM components. Though lesion formation is degeneration per se, many lessons, pertinent to neural regeneration, can be learned from its scrutiny. For example, since growing neurites normally do not penetrate the boundaries of an SCI lesion, molecules found within the lesion (and whatever influences their formation) can be utilized for directed nerve regeneration (by repulsion). At the same time, tissue remodeling influenced by ECM degrading enzymes may have a direct effect on nerve regeneration; this is the case in the developing CNS where growth cones can proceed only if ECM molecules on their path are cleared by proteases. The expression of MMPs-2, 3 and 9 were examined up to 2 weeks after spinal cord injury. Preliminary data show that this two week period covers the acute, recovery and plateau points of locomotor behavior after dorsal overhemisection at T9 (mouse). MMP-9 is upregulated at the injury site during the acute period (maximum: 24 hours). At 1 week and 2 weeks, little MMP-9 activity is observed. MMP-2 is normally expressed in the spinal cord; it is upregulated 1 week after injury and normalized by 2 weeks. We could not detect MMP-3 with zymographic analysis.

EXPRESSION OF NEUROFILINS AND SEMAPHORINS IN RUBROSPINAL NEURONS AND THE INJURED SPINAL CORD FOLLOWING AXOTOMY

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The semaphorin family of axonal guidance molecules plays an important role in neural development by virtue of their chemorepulsive effects on most axonal growth cones. While little is known regarding the expression of semaphorins and their cognate receptors the neuropilins in the adult nervous system, many developmentally regulated genes are re-expressed following spinal injury. Therefore, we hypothesize that the re-expression of semaphorins, with their axonal growth inhibiting properties, may contribute to the failure of CNS regeneration in higher vertebrates. Here we have studied the expression of semaphorins and neuropilins in rubrospinal neurons, as well as the spinal cord lesion site, following cervical spinal cord injury of adult rodents. Using RT-PCR and in situ hybridization, we have found that the expression of semaphorin 3A mRNA was readily detectable in the micro-dissected spinal cord injury site, as well as within the uninjured spinal cord. In contrast, semaphorin D/III expression was not detected in either the normal or axotomized micro-dissected red nucleus. However, the expression of neuropilin-1 and neuropilin-2 was found in both the axotomized and contralateral red nucleus. Taken together, these data indicate a possible role of semaphorin 3A and the neuropilins in the axonal regeneration failure of rubrospinal neurons. Supported by the Medical Research Council of Canada.

IMMUNOHISTOCHEMICAL DETECTION OF THE NUMBER AND DISTRIBUTION OF GAP-43 POSITIVE NEURONS IN THE BRAIN OF THE SPINAL-TRANSECTED EUROPEAN EEL, *ANGUILLA ANGUILLA*

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The growth associated protein GAP-43 is known to be specifically involved in axon growth during development and regeneration. The European eel, *Anguilla anguilla*, is capable of central nervous system regeneration following spinal transection, making it a suitable animal model to study patterns of GAP-43 expression during regeneration. Under anaesthesia, the spinal cord of each eel was transected four segments caudal to the anus using fine microscissors. Animals were returned to the aquarium (24°C) for various times (2 to 56 days) before being reanaesthetised and perfused with fixative. Following perfusion, the brain was dissected out and sectioned for immunohistochemical investigation. Uninjured, control animals were treated in the same manner. The number and distribution of GAP-43 positive neurons in

the eel brain was determined by labelling with a monoclonal antibody for human GAP-43. In control animals a few reticulospinal cells reacted immunopositively for GAP-43 and an increase in this number was observed as early as two days post-transection. The number of GAP-43 reactive neurons peaked at approximately 20 days post-transection and then gradually declined until the number had almost returned to control levels by 56 days post-transection. This increase and decline in the number of positively reacting cells is inversely correlated with coordinated swimming performance. A comparison with a previous study (Bosch and Roberts, 1994, Brain Behav. Evol., 44: 50-60) using HRP-labelling of descending spinal neurons allowed identification of those cells in the brain that were GAP-43 immunoreactive and possessed projections to the spinal cord. All observed GAP-43 positive neurons were located in the brainstem and the distribution pattern was similar to the distribution of neurons with projections to the spinal cord, as determined previously. In addition, glial staining was seen around the ventricles in all the animals examined.

GENOMIC ANALYSIS OF ACUTE SPINAL CORD INJURY

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Early responses to acute spinal cord injury include inflammation, calcium influx, eicosanoid synthesis and production of reactive oxygen species. These lead to a secondary tissue damage, which can be a major component of long-term tissue damage in spinal cord patients. A glucocorticoid derivative, methylprednisolone (MP), has been found to reduce secondary tissue damage when administered within 8 hrs of injury in humans. It is argued that MP has its effects by blocking lipid peroxidation, but since MP also binds glucocorticoid receptors, a broad array of intracellular mechanisms may be affected. In order to characterize the constellation of gene expression responses to acute injury, and to determine which events are regulated by MP treatment, we analyzed the changes in expression of a large number of characterized genes using gene arrays. Groups of rats (n=3) were contused using the NYU impactor with a 10.0 g weight falling 25.0 mm onto exposed T9-10 spinal cord. One group of rats had their spinal cords surgically exposed but not contused (Sham). Injured animals were held under anaesthesia for 2 hrs, then killed to recover a 4 mm segment of cord from the site of injury. One group received infusion of 30 mg/kg MP (2 hr MP) and another group received an infusion of vehicle (2 hr saline). RNA was prepared from the pooled tissue, reverse transcribed into a radioactive cDNA probe, and used to hybridize a commercial array of 588 or 1,176 known rat genes (Clontech Atlas arrays). Hybridized radioactivity was quantified by phosphorimaging and analyzed using AtlasImage 1.0 software (Clontech). In preliminary studies, a majority of genes are not regulated, but several were strongly induced by injury. These included: plasminogen activator inhibitor (PAI-1), the heat shock proteins hsp70, hsp27 and hsp90, CuZn-SOD, IκB, complement lysis inhibitor, peripheral myelin protein-22, Na-K ATPase, urokinase receptor, LIF, and macrophage inflammatory proteins 1α and 2. Virtually all of these induced genes were inhibited by MP treatment. We are currently testing several of these gene responses for their role in mediating secondary tissue damage. Understanding the molecular mechanisms following spinal cord injury should allow a better management of the response to primary injury and a better preparation for regenerative therapies. Supported by a grant from the Christopher Reeve Paralysis Foundation.

ANALYSIS OF SHAM VS CONTUSED RAT SPINAL CORD GENES USING cDNA EXPRESSION ARRAYS

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The Atlas Rat cDNA expression arrays (Clontech, Palo Alto, CA) were used to study changes in gene expression in control and contusion injured rat spinal cord at 1-day post injury. A diverse temporal profile of gene expression was observed among the 588 genes on the Atlas rat cDNA expression array. Heat shock protein genes (27 and 70), genes involved in interleukin-6-induced STAT3 signaling, proteases and their inhibitors (proteasomes, tripeptidylpeptidase II, aminopeptidase B, tissue carboxypeptidase inhibitor and cathepsin H (F7i), tissue inhibitors of metalloproteinase 1 and 3; tissue plasminogen activator and its inhibitor were all observed to be induced. On the other hand, as expected, myelin PLP and cerebroside synthase gene

expression were downregulated. Studies utilizing the microarrays will be useful to observe coordinated regulation of groups of genes whose products act at different steps in a common process. Based on the changes in gene expression observed (increase or decrease) and what is known about the function of the gene, alternate strategies for the treatment of spinal cord injury can be determined. We also studied the effect of intramuscular methylprednisolone (MP) administration on the expression of certain inflammatory mediators and growth factors 6h, 1d and 3d following rat spinal cord contusion injury using the RT-PCR assay. Mechanical injury to rat spinal cord was produced at the T8 vertebra level with a weight drop device. 3 animals per time point received either MP treatment (60mg/kg immediately after injury and 30mg/kg 4 hours later) or saline injections. Following administration of MP, MCP-1, MIP-1 beta, GRO alpha, IL 1 beta, MMP9 and COX2 levels were decreased at 6h and/or 1d post injury. MIP 1 alpha levels were reduced by 55% at 3d while levels of MCP-3, TNF alpha and MMP2 were not altered. IP-10 levels showed a slight (20-35%) increase in gene expression in the presence of MP at both time points after injury. aFGF levels were not altered in the presence of MP. Our studies suggest that although MP therapy diminishes the post-traumatic inflammatory cascades, MP may hinder the endogenous repair mechanism by depressing the production of growth factors. Supported by the Department of Veterans Affairs Medical Research Service.

PERSISTING LABELING OF LUMBAR α -MOTONEURONS AND RUBROSPINAL AND CORTICOSPINAL TERMINALS IN RATS AFTER ISCHEMIA-INDUCED PARAPLEGIA

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Traumatic spinal injury can lead to long tract degeneration resulting in an upper motor neuron neurological profile. In the present study, using anterograde (biotinylated dextran [BD]; MW 10,000) and retrograde (Fluorogold; 0.004%) labeling methods, we sought to characterize the survival of spinal α -motoneurons and rubrospinal/corticospinal neurons and their spinal terminals in rat after a spinal ischemia-induced spastic paraplegia. To induce spinal ischemia, a 2F Fogarty balloon catheter was placed into descending thoracic aorta and inflated with 0.05 cc of saline for 10 min. After ischemia, the majority (>80%) of animals displayed spastic paraplegia. At 7, 14 or 28 days after ischemia, animals were anesthetized with 1.5% halothane. To label rubrospinal and corticospinal neurons, the L3-L5 spinal segments were exposed and 1 mm³ of Gelfoam infiltrated with 2% of Fluorogold solution was placed subdurally onto the lateral and dorsal aspect of the spinal cord. To label α -motoneurons, the sciatic nerve was cut just above its bifurcation and exposed to the 2% Fluorogold solution for 2 hrs. For anterograde tracing, 10% BD (1 μ l) solution was injected bilaterally into nucleus ruber and motor cortex. After application of tracers, animals survived for 7-14 days and were perfusion-fixed with 4% paraformaldehyde. Coronal brain and parasagittal spinal cord sections were cut and evaluated with fluorescence microscopy. In spastic animals, a clear fluorescence of rubrospinal and pyramidal neurons was seen at all time points. Numerically, no significant differences between the number of labeled neurons were detected, if compared with control non-ischemic animals. Similarly, a number of labeled α -motoneurons were detected in animals with spastic paraplegia and 1-4 weeks of survival. Labeling of rubrospinal and corticospinal terminals in lumbar spinal cords revealed numerous BD labeled terminals which showed "gigantoboutonal" type of degeneration resulting from the loss of target interneurons. These data show that in animals with spinal ischemia-induced spastic paraplegia there is a persistent labeling of spinal α -motoneurons and rubrospinal neurons which continues for a minimum of 5 weeks after spinal ischemia, and this corresponds with a long-term survival of the respective neuronal components. Supported by NIH NS32794, M.M.

LACK OF NEURONAL PROLIFERATION AFTER INJURIOUS OR NON-INJURIOUS INTERVALS OF SPINAL CORD ISCHEMIA IN RAT

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Transient brain ischemia leads to proliferation of neuronal progenitors in hippocampal dentate subgranular zone followed by terminal neuronal differentiation. In the present study, using rat balloon aortic occlusion model, we sought to characterize the proliferation potential of spinal neuronal progenitors after a transient spinal ischemia titrated to induce i) spastic paraplegia, ii) transient motor dysfunction, or iii) immediate and complete recovery. To induce spinal ischemia, a 2F Fogarty balloon catheter was placed into descending thoracic aorta and inflated with 0.05cc of saline for 8-10 min. After ischemia, animals displayed spastic paraplegia (n=4), transient motor weakness (n=3) or showed complete recovery (n=4). To label proliferating cells, animals were injected with BrdU (bromodeoxyuridine; thymidine analogue; 100mg/kg/i.p.) at 4, 8, 12 and 16 days after ischemia. After the last injection, animals survived for an additional 2 months and were then perfused with 4% paraformaldehyde. Frozen, transverse spinal cord sections (30 μ m) were then prepared and double stained for the presence of BrdU labeled nuclei and their colocalization with neuronal marker NeuN or astrocytic marker GFAP. At 3 months after spinal ischemia, BrdU labeled nuclei were detected with a predominant localization between laminae V-VII in lumbosacral segments. More labeled nuclei were seen in animals with developed spastic paraplegia as compared with animals which showed complete recovery of function (188 \pm 35 vs 76 \pm 29). In contrast to the brain ischemia study previously described, no colocalization of neuronal specific marker (NeuN) with BrdU labeled nuclei was detected in any case. Instead, a number of GFAP positive cells showed colocalization with BrdU labeled nuclei indicating that the majority of newly proliferated cells are astrocytes. At present, it is not clear why new neurogenesis does not occur in the spinal cord after gradual (injurious or non-injurious) ischemic insult. We hypothesize that it may result from i) limited proliferating capacity of spinal neuronal progenitors, or ii) from release and/or activity of a variety of cytotoxic/inflammatory factors known to be upregulated after ischemia. Supported by NIH NS32794, M.M.

INTRATHECAL INFUSION OF PEGYLATED BDNF RESULTS IN ACUTE FUNCTIONAL AND CHRONIC MORPHOLOGICAL CHANGES FOLLOWING TRAUMATIC SPINAL CORD INJURY (SCI) IN RATS

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Brain-derived neurotrophic factor (BDNF), a member of the neurotrophin family, has been shown to be efficacious at neuroprotection, facilitation of synaptic function, and stimulation of regeneration/sprouting of axons in a variety of CNS regions. Recent work in our laboratory has demonstrated that intrathecal infusion of a pegylated form of the BDNF protein (peg-BDNF) allows improved penetration into the spinal cord parenchyma with retention of some biological activity in vivo. To assess the effects of such activity following SCI, adult rats were subjected to contusion injury at the T8 level, and infused via an indwelling intrathecal spinal catheter with either: 1) 25 μ g/day peg-BDNF for 14 days beginning at 1 day post-injury (DPI) followed by vehicle for 14 days, 2) vehicle for 15 days and 25 μ g/day peg-BDNF on DPI 15-28, or 3) vehicle for 28 days. Animals were evaluated on a weekly basis using the BBB locomotor rating scale and examined for the presence of hindlimb airstepping on DPI 14, 21 and 28. Nine weeks post-injury, animals were sacrificed and processed for immunohistochemistry. Intrathecal administration of peg-BDNF beginning at 1 day, but not 15 DPI, resulted in an accelerated recovery of hindlimb function in the open field. peg-BDNF also acutely stimulated hindlimb CPG activity, as evidenced by the presence of rapid, alternating hindlimb airstepping during the period of factor administration. Finally, peg-BDNF infusion beginning on either 1 or 15 DPI resulted in increases of both peripherally originating myelin (visualized using the P0 antibody) and ChAT immunoreactive fibers at the injury epicenter. Together, these results demonstrate that peg-BDNF exerts both acute and long-term effects in the injured rat spinal cord, and that the timing of trophic factor administration is an important consideration in ameliorative therapies. Supported by NS37321. peg-BDNF generously provided by AMGEN, Inc. ALZET pumps supplied by arrangement from DURECT Corp.

BRADYKININ ANTAGONIST B9430 DECREASES THE PERMEABILITY OF THE BBB WITHOUT ALTERATION OF TNF α TRANSPORT AFTER SPINAL CORD INJURY

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Spinal cord injury (SCI) may stimulate kinin release. The injury as well as the kinins may further stimulate the production of other mediators of inflammation, including tumor necrosis factor α (TNF α), interleukins and prostaglandins. A bradykinin antagonist might block the cascade and ameliorate the deficit caused by SCI. For instance, the bradykinin antagonist B9430 (D-Arg-Arg-Pro-Hyp-Gly-Igl-Ser-D-Oic-Arg) reduces vascular permeability in general, and decreases the secretion of proinflammatory cytokines such as TNF α . Having found that SCI is related to partial disruption of the blood-brain barrier (BBB) and upregulation of the saturable transport system for TNF α on the BBB, we set out to determine whether pretreatment of mice with B9430 before SCI would affect the accumulation of albumin and TNF α in the CNS. SCI was generated by compression of the lumbar spinal cord of mice under general anesthesia. After the SCI, a significant increase in the entry of both ^{99m}Tc-albumin and ¹²⁵I-TNF α was found in the lumbar spinal cord immediately after SCI and again 72 h afterwards. Pretreatment of uninjured control mice with B9430 5 mg/kg ip did not change the permeability of the BBB measured 2 h later. By contrast, B9430 pretreatment 2 h before SCI abolished the immediate increase in the entry of albumin or TNF α . However, B9430 pretreatment followed by daily ip injection did not inhibit the entry of either albumin or TNF α at 72 h after SCI. Our results suggest that the immediate phase of BBB disruption is related to vasoactive substances, whereas the second phase is related to upregulation of TNF α transport. Thus, suppression of the action of bradykinin might reduce edema and lessen further damage to the spinal cord.

SPINAL CORD INJURY WITHOUT GLIAL SCARRING INHIBITS NEURITE GROWTH IN VITRO

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White matter of the central nervous system (CNS) has been reported to contain potent inhibitors of neurite growth that may contribute to abortive axonal regeneration. Recent studies of transplanted adult neurons directly into CNS fiber tracts have demonstrated extensive parallel axonal growth within the myelinated tracts, casting doubt on a physiological role for myelin-associated inhibitors in abortive axonal regeneration. Instead, these studies have provided strong evidence for the dominant role of glial scarring in preventing axonal regeneration through the injury site. We have found that neurons cultured using Neurobasal medium on unfixed cryostat sections of brain and spinal cord attach to white matter with extensive neurite outgrowth that is parallel with the longitudinal axis of the fiber tract. These same fiber tracts are inhibitory to neurites growing on adjacent gray matter indicating that the inhibitory properties of white matter are still detectable but that the geometry of the fiber tract may determine whether growth can occur. We sought to determine whether disruption of geometry is sufficient to inhibit neurite growth in the absence of glial scarring. Embryonic chick sympathetic neurons were seeded onto unfixed horizontal sections of mature rat spinal cord that had been crushed with forceps *ex vivo* then immediately frozen to prevent glial scarring. The neurons were subsequently labeled with a vital dye and neurite outgrowth was assessed using NIH Image software. Neurite growth on uninjured portions of the lateral funiculus was extensive and highly parallel with the longitudinal axis of the tract. Neurite growth on gray matter regions was mixed in orientation and inhibited from growing onto the same white matter tracts that supported parallel growth. At sites of crush injury, the lateral funiculus was interrupted by a region of tissue debris. Neurites could be observed growing on the lateral funiculus up to the border of the injury site but did not cross it. These observations suggest that glial scarring associated with spinal cord injury is not necessary to block axonal growth *in vitro*. It is possible that disruption of the parallel organization of myelin and its associated inhibitors, as occurs in the case of injury *in vivo*, may be sufficient to pose a significant barrier to regenerating axons. Supported by the Mayfield Clinic (MERF award).

THE CLASSICAL PATHWAY, BUT NOT THE ALTERNATIVE PATHWAY, OF THE COMPLEMENT PROTEIN CASCADE IS REQUIRED FOR THE IMMUNOLOGICAL DEMYELINATION OF THE ADULT RAT SPINAL CORD

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We have previously described a transient, myelin-specific, antibody-mediated, complement-dependent protocol that suppresses the onset of myelination in embryonic birds, and focally demyelinate the CNS of adult birds and rodents after intraspinal infusion. After a spinal injury, this procedure facilitates anatomical regeneration and functional recovery. In order to identify the relative necessity of the different complement proteins for this procedure, we assessed the effects of serum infusions deficient in a particular complement protein (e.g. C3, C4, CB and Factor B). We evaluated spinal treated tissue ultrastructurally using a transmission electron microscope and phenotypically by indirect immunofluorescence. Upon removal of the C3 protein, common to both the classical and alternative pathways, or the C4 protein, a classical pathway protein, normal myelin was observed. Using serum deficient in Factor B, an alternative pathway protein, demyelination was observed; in addition, large numbers of macrophages containing myelin debris were present in these regions. These results suggest that the classical serum complement pathway has a fundamental role in this immunological protocol. Upon removal of C6, a Membrane Attack Complex protein, a less extensive patchy distribution of naked axons was observed, once again accompanied by many macrophages, suggesting that some of the demyelination may be occurring through macrophage-mediated events involving complement-derived anaphylatoxins and membrane receptors. Supported by the Rick Hansen Neurotrauma Initiative of British Columbia (JAB, JKD) and the Medical Research Council of Canada (Operating Grant to JDS).

MYELIN REMOVAL DURING IMMUNOLOGICAL MYELIN SUPPRESSION IS MEDIATED BY BLOOD-DERIVED MACROPHAGES

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Complement mediated, myelin-specific antibody directed immunological suppression of rat myelin has been shown to be an effective method of promoting the axonal regeneration of traumatically injured descending, locomotor-associated brainstem spinal neurons. This has been demonstrated to be due to the removal of myelin, thus overcoming CNS myelin-associated inhibitors of axonal regeneration. Previous data indicates that the removal of myelin from the immunologically challenged spinal cord is macrophage mediated. This is induced by the production of complement protein C3 derivatives, which activate macrophages. The removal of C3 from serum complement results in a failure of macrophage activation for phagocytosis, and a subsequent maintenance of the myelin sheath. We now present data that further supports the role for macrophages in the removal of myelin. Utilizing dichloromethylene diphosphate (Cl₂MDP) encapsulated by liposomes to deplete systemic monocytes, we inhibited macrophage build up in the injured CNS. We observed (1) absence of O_x42⁺ macrophages in the CNS and liver, (2) preservation of the myelin (ultrastructure and MAG immunoreactivity), (3) a decrease in GFAP-like immunoreactivity around the infusion site. This suggests that macrophages are the primary mediators of myelin phagocytosis in the immunologically myelin-suppressed cord, and microglia/astrocytes, though capable of phagocytosis, do not compensate for the absence of macrophages in removing myelin. Also macrophages may play a role in the progression of astrogliosis and subsequent injury cavitation. Cl₂MDP (or clodronate) was a gift of Boehringer Mannheim GmbH, Mannheim, Germany. This work was funded by an MRC of Canada grant and by the BC Neurotrauma Initiative (Rick Hansen Institute).

THE EFFECTS OF BDNF AND cAMP ON BRAINSTEM-SPINAL NEURITES GROWN ON INHIBITORY MYELIN SUBSTRATES

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Regeneration of injured spinal cord projection neurons typically fails in higher vertebrates. One reason may be the presence of inhibitory molecules

in the central nervous system that restrict axonal growth. Recently, neurotrophic factors and the modulation of adenosine cyclic monophosphate (cAMP) have been shown to increase the growth of neurites on inhibitory substrates. To study this problem, we have developed an in vitro model that permits us to examine the effects of growth cone inhibitors on the neurite outgrowth of descending locomotor neurons germane to spinal cord injury. To selectively investigate these neurons, which include the reticular formations, raphe- and vestibulo-spinal nuclei, a crystal of carbocyanine dye (DiI) was inserted in ovo into the embryonic day (E) 5 cervical spinal cord which retrogradely labeled brainstem-spinal projection neurons as their axons grew towards spinal targets. Three days later (E8), labeled regions of the brainstem were dissected to produce explant cultures grown in serum-free medium on inhibitory substrates, e.g. myelin membrane fractions. Thus, the redistribution of DiI into regenerating processes permitted the measurement of neurite outgrowth from distinct neuronal populations that originally projected to the spinal cord. In preliminary experiments, we have observed that combinations of brain-derived neurotrophic factor (BDNF) with or without forskolin, a drug which increases intracellular cAMP levels, results in significantly increased neurite outgrowth from vestibulospinal explants grown on substrates composed of crude myelin fractions. A further characterization of the neurite outgrowth promoting effects of BDNF and cAMP in this model will be presented. Supported by the Rick Hansen Institute BC Neurotrauma Fund and the Natural Sciences and Engineering Research Council of Canada.

RECOVERY OF FUNCTION AND HISTOLOGICAL OUTCOME FOLLOWING CONTROLLED CONTUSION INJURY IN MICE

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A computer controlled electromagnetic injury device has been adapted to develop a mouse model of contusion injury in the spinal cord. In the present study, we completed the characterization of this model with behavioral and histopathological outcome assessment. Mice of the C57BL/6 strain received a laminectomy (LA) of the T9 vertebral process and were then assigned to groups receiving displacement injury (0.3mm, 0.5mm and 0.8mm), complete transection (TX) or LA only (n=4-8). Functional recovery was examined weekly for 9 weeks using the BBB open field locomotion scale. Grid walking and footprint analyses were also performed on the mice that had consistent stepping. Histological evaluation of cross-sectional lesion area and spared white matter at the impact site was tested for correlation with the biomechanical injury parameters and final behavioral outcome. Distinct patterns of locomotor recovery and differences in the percentage (%) of spared white matter at the lesion site were found across the injury groups. Mice with an 0.3mm injury showed locomotor deficits in BBB scores when compared to LA control at 1, 3 and 7 days post injury. After 14 days, BBB scores were not different from LA (21 ± 0 [SEM]), but the number of hindlimb misses on grid walking was greater in the 0.3mm group. BBB scores (12.4 ± 1.1), foot misses and toe drags from footprint analyses for the 0.5mm group were different from all other groups. Mice in the 0.8mm injury group had limited hindlimb movements; their BBB scores (3.8 ± 1.0) overlapped those with TX (2.3 ± 0.4). The % of spared white matter was correlated with final BBB scores ($r=0.935$), and inversely with the impact force ($r=-0.756$). These results suggest that graded controlled contusion injury can be produced reliably in mice by selecting the different displacement amplitude using OSU impact device. These behavioral outcome measures will be useful in future studies of recovery of function after molecular manipulation or therapeutic interventions. Supported by NS33696, the PVA-SCRF and the International Spinal Research Trust.

THE NATURAL TEMPORARY REPAIR OF THE LESION IN SEVERED ADULT RAT SPINAL CORD AS SEEN IN VIVO BY MRI

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An intrinsic repertoire of repair mechanisms exists in the adult spinal cord and it is activated in response to injury. As in other tissues it starts to reconstruct the cord tissue including the regrowth of severed axons and blood vessels. However, by the end of the 3rd week postinjury the natural repair is aborted and decay processes take over, yielding a permanent wound gap and paralysis beyond the lesion site. This tissue decay is the primary

cause for the devastating consequences of spinal cord injury: the severed nerve fibers fail to cross the wound gap, thereby the cord beyond the injury site is permanently disconnected from the brain and the related muscles remain paralyzed. Reactive astrocytes play a major role in aborting the intrinsic repair [Proc. Natl. Acad. Sci. USA (PNAS), 1990, 87:10058]. Using localized radiation therapy in transected adult rat spinal cord, we found that the destructive outcome of injury can be averted and the natural repair of structure and motor function can be attained provided that at the right time (3rd wk) after injury reactive glia at the damage site are destroyed (PNAS, 1996, 93:11179 & 93:11185). Here we obtained by MRI a dynamic in vivo view of the repair and the destructive events that take place at the lesion site of severed adult rat spinal cord. High resolution T1- and T2-weighted sagittal images of the lesion site in completely transected spinal cords were acquired using a spin echo sequence, starting on day 4 postinjury. The MRI data correlate well with the histological analysis obtained previously. A normal course of wound healing events seems to proceed through the 3rd week postinjury. At the first few days postinjury the cord tissue around the lesion site is abnormal (swollen/edema) and the cut can be discerned as a narrow dark (low signal) gap within the cord. During the 2nd and 3rd week postinjury the cord at the lesion site seems to be almost normal with very little evidence of the initial cut. The destructive processes (localized high signal/inflammation) seem to take over at the end of the 3rd week; these are augmented and become more pervasive with time after injury, leading eventually to a permanent wound gap. The MRI data further support the existence of a window of opportunity during which the destructive processes could be stopped/prevented, allowing thereby the natural wound healing to be accomplished.

SPONTANEOUS CORTICOSPINAL AXONAL PLASTICITY AND SPONTANEOUS RECOVERY AFTER ADULT CNS LESIONS

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It is commonly believed that little spontaneous structural and functional plasticity occurs after injury to the adult mammalian central nervous system. Nonetheless, various degrees of spontaneous functional recovery are not uncommon after acute human brain or spinal cord injury. To investigate potential naturally-occurring compensatory mechanisms for CNS injury, lesions of defined components of the corticospinal motor pathway were made in adult rats in the rostral cervical spinal cord or caudal medulla. Following lesions of more than 95% of the corticospinal motor pathway, functional recovery occurred that was paralleled by a spontaneous and significant increase in the number of corticospinal axon terminals on medial motoneuron pools in the cervical spinal cord. Motor performance highly correlated with the formation of new connections. These findings demonstrate the presence of significant structural and functional plasticity after injury to the adult central nervous system. Spontaneous plasticity mechanisms may be useful targets for enhancing recovery after adult CNS injury. Supported by Canadian Spinal Research Organization, Swiss Institute International de Recherche en Paraplegie and the NIH.

INVOLVEMENT OF ADENOSINE A1 RECEPTORS IN RESPIRATORY RECOVERY FOLLOWING CERVICAL SPINAL CORD INJURY IN ADULT RATS

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Upper cervical spinal cord injury can result in interruption of the major descending respiratory pathways. Invariably, this leads to respiratory paresis and respiratory distress. In animal models of spinal cord injury, however, a latent respiratory pathway can be activated to restore function to a hemidiaphragm paralyzed by cervical (C2) hemisection during a reflex known as the "crossed phrenic phenomenon" (CPP). In this model, C2 hemisection interrupts the major descending respiratory pathways and paralyzes the ipsilateral hemidiaphragm. Transecting the contralateral phrenic nerve and making the animal asphyxial has traditionally activated the latent respiratory pathway by enhancing central respiratory drive. Recently, we have demonstrated that the latent pathway can be activated pharmacologically with theophylline, a methylxanthine with equal affinity for the adenosine A1 and A2 receptor subtypes. In this pharmacological approach, the contralateral phrenic nerve is not transected, i.e., the animal is not made

asphyxic. The present study was undertaken to assess whether or not A1 receptors are involved in the activation of the latent respiratory pathway which leads to functional recovery in hemisectioned animals. Out of 10 hemisectioned animals that were tested intravenously with varying doses (0.05-2 mg/kg) of the adenosine A1 antagonist, 8-Cyclopentyl-1, 3-dipropylxanthine (DPCPX), 7 demonstrated marked respiratory functional recovery in a dose dependent manner. Furthermore, the A1 agonist, N⁶-Cyclohexyladenosine (CHA) at 0.05 mg/kg reversed the DPCPX-induced functional recovery, suggesting strongly that A1 receptors mediate respiratory recovery after cervical spinal cord injury. Our data also suggest that A1 selective compounds may be clinically beneficial in spinal cord injured patients with respiratory deficits. Supported by NIH Grant HD 35766.

SEROTONIN-INDUCED RESPIRATORY RECOVERY IN THE PHRENIC NERVE AFTER CERVICAL SPINAL CORD HEMISECTION

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The present study investigates the role of serotonin in respiratory recovery following cervical spinal cord injury during normocapnia. Experiments were conducted on C2 spinal cord hemisectioned, anesthetized, vagotomized, paralyzed and artificially ventilated rats in which end-tidal CO₂ was monitored and maintained in the range of normocapnia. During normocapnia, the phrenic nerve ipsilateral to hemisection has no respiratory-related activity due to the disruption of the descending bulbospinal respiratory pathways by spinal cord hemisection. 5-hydroxytryptophan (5-HTP), a serotonin precursor, was administered intravenously. The drug induced dose-dependent increases in respiratory-related activity in the phrenic nerve ipsilateral to hemisection without increasing central respiratory drive. Since experiments were conducted on animals subjected to C2 spinal cord hemisection, the recovery was most likely mediated by the activation of a latent respiratory pathway descending into the spinal cord contralateral to the lesion and then crossing the midline before terminating on phrenic motoneurons ipsilateral and caudal to hemisection. 5-HTP-induced effects were reversed by a serotonin receptor antagonist, methysergide. The present study indicates that serotonin is an important modulator in the unmasking of the latent crossed phrenic pathway following spinal cord injury. Since 5-HTP-induced respiratory recovery was achieved without increasing central respiratory drive, it is possible that the recovery may be mediated by the activation of serotonin receptors on phrenic motoneurons in the spinal cord. Thus, respiratory recovery conceivably could be achieved therapeutically without risk of fatigue or stress. This could potentially lead to pharmacological treatments that may improve respiratory function in cervical spinal cord injury patients. Supported by NIH grant HD31550 (H.G.G.).

ADDITIVE EFFECTS OF IGF-I AND GDNF TO RESCUE POSTNATAL SPINAL MOTOR NEURONS FROM SLOW GLUTAMATE INJURY

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Amyotrophic lateral sclerosis (ALS) is a chronic, progressive neurological disorder characterized by selective degeneration of motor neurons. Slow glutamate toxicity, resulting from abnormal synaptic clearance and metabolism of glutamate, is one of the prevailing hypotheses implicated in the pathogenesis of ALS. In this regard, neurotrophic factors could be beneficial for the treatment of ALS by exerting trophic actions on surviving neurons, or by decreasing neuronal sensitivity to glutamate neurotoxicity. The growth and survival of motor neurons may depend on multiple neurotrophic factors, acting additively or synergistically. Individually, glial cell line-derived neurotrophic factor (GDNF) and insulin-like growth factor I (IGF-I) are potent neurotrophic/survival factors for postnatal motor neurons. We used a spinal cord culture model of ALS to test whether the combination of IGF-I and GDNF can increase the number of surviving motor neurons from chronic glutamate injury in the spinal cord. Organotypic cultures were prepared from lumbar spinal cord of postnatal-day-8 rat pups. For a neuroprotective paradigm, cultures were treated either with an inhibitor of glutamate/aspartate transport (D,L-threo-β-hydroxy aspartic acid[THA]) alone, which elevates glutamate in the extracellular space and injures large motor neurons in the ventral horn with a morphology typical of slow

excitotoxic degeneration, or with THA in the presence of IGF-I, GDNF or the combination. Neuroprotection was assessed by choline transferase (ChAT) activity using [³H]-acetyl coenzyme A, and by counting the number of surviving motor neurons, as previous experience has shown that ChAT activity correlates strongly with the number of large pyramidal neurons in the ventral horn (>25 μm). Our data demonstrate that the combination of IGF-I and GDNF at active doses completely rescues motor neurons from chronic glutamate-mediated toxicity, and additively upregulates ChAT activity in otherwise untreated cultures. The results predict that IGF-I combined with GDNF may provide a better therapy for the treatment of motor neuron disorders such as ALS and spinal muscular atrophy, and may have implications for other spinal cord diseases.

EFFECTS OF LESION SITE BDNF APPLICATION ON CELL BODY RESPONSES OF SPINAL CORD INJURED RUBROSPINAL NEURONS IN ADULT MICE

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The increasing availability of transgenic mouse models for neural regeneration research prompted us to investigate the effects of spinal cord injury and lesion site BDNF treatment on the injury responses of rubrospinal (RS) neurons. The somatotopic arrangement of the mouse red nucleus was determined by double retrograde labeling from the cervical and thoracic spinal cord levels, and showed little overlap between the cervical (dorsomedial, DM) and lumbar (ventrolateral, VL) projecting RS neurons. Seven days after cervical (C3) spinal cord hemisection, the lumbar-projecting RS neurons displayed elevated levels of regeneration-associated genes (GAP-43 and α-tubulin). However, 21 days after C3 injury, the axotomized RS neurons were highly atrophic and levels of GAP-43 and α-tubulin mRNAs had decreased to baseline. Interestingly, many axotomized rubrospinal neurons failed to be retrogradely labeled with Fast Blue from the injury site. To examine how these injury responses might be modified by lesion site BDNF application, gelfoam soaked in Fast Blue ± BDNF was inserted into the lesion site at time of injury. Two weeks later, BDNF treatment has (i) reduced the RS cell body atrophy, (ii) increased their expression of regeneration-associated genes, and (iii) greatly enhanced their Fast Blue retrograde labeling. Currently, we are studying the responses of mouse RS neurons to thoracic injury and BDNF treatment, and are investigating the transcriptional basis of axotomy-induced changes in neuronal gene expression using Tα1-lacZ transgenic mice. Supported by the Rick Hansen Institute, NSERC, MRC of Canada, and the SCRF

THE COMBINED EFFECTS OF BRAIN-DERIVED NEUROTROPHIC FACTOR (BDNF) AND GLIAL DERIVED NEUROTROPHIC FACTOR (GDNF) ON MOTOR AXONAL REGENERATION AFTER CHRONIC AXOTOMY

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The time-dependent decline in the ability of motoneurons to regenerate their axons after axotomy is one of the principal contributing factors to poor functional recovery after peripheral nerve injury. A decline in neurotrophic support may be partially responsible for this effect. BDNF belongs to the neurotrophin family of neurotrophic factors, and mediates its effects by binding specifically to high affinity trkB and low affinity p75 receptors, whereas GDNF belongs to the TGF-β superfamily of growth factors and mediates its effects by binding to GDNFRα which dimerizes with its signal transduction component RET. The upregulation of BDNF and GDNF after injury, in Schwann cells of the distal nerve stump and in axotomized motoneurons, has led to the speculation that it is important for motor axonal regeneration. However, few experiments directly measure the effects of exogenous BDNF or GDNF on motor axonal regeneration. In adult female Sprague-Dawley rats, we have counted the number of chronically axotomized tibial motoneurons that have regenerated their axons a distance of 20 mm 1 month after surgical suture to a freshly denervated common peroneal distal nerve stump. Motor axonal regeneration was evaluated by applying fluorescent retrograde neurotracers to the common peroneal nerve 20 mm distal to the injury site, and counting the number of fluorescently labeled motoneurons in 50μm longitudinal sections of the T₁₁-L₁ region of the spinal cord. We found that, although low doses of 0.1 μg/day BDNF increased regeneration by 184% compared to vehicle control, the higher doses of 20 μg/day reduced

motor axonal regeneration to less than 10% of the vehicle control. Recent reports of increased motor axonal regeneration and neuronal sprouting in animals which do not express p75 suggest that the presence of the low affinity receptor is in fact inhibitory for growth. For example, our high doses of BDNF (20 µg/day) may be saturating the trkB receptors, leading to increased binding to p75 receptors, thus inhibiting motor axonal regeneration. Should this be true, then exogenous application of GDNF, which does not bind p75, should elicit only facilitatory effects on motor axonal regeneration. This work is supported by the Canadian Neurotrauma Research Foundation and the Alberta Paraplegic Foundation.

BASIC FIBROBLAST GROWTH FACTOR (bFGF) SIGNIFICANTLY ENHANCES HINDLIMB LOCOMOTOR RECOVERY FOLLOWING MODERATE AND SEVERE SPINAL CORD INJURY IN THE RAT

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To investigate whether bFGF enhances the process of self-repair following spinal cord injury (SCI), we intrathecally administered a continuous infusion of exogenous bFGF for 1 week after producing a moderate (12.5mm) or severe (25mm) contusion SCI in adult rats using the NYU impactor weight drop device. Separate Alzet minipumps connected to a catheter were immediately implanted into the lateral ventricle of the brain and lumbar thecal sac in order to deliver bFGF at either 3µg or 6µg per day versus control vehicle. Animals were behaviorally assessed weekly using the Basso, Beattie, Bresnahan (BBB) locomotor rating scale before being euthanized after 6 weeks for histological assessment of tissue sparing at the lesion sites. Behavioral and histological data from the bFGF groups were collapsed based on no significant differences between dosages in either study. After moderate SCI, there were significant ($P<0.05$) consistent coordinated hindlimb movements in bFGF-treated rats after 6 weeks compared to vehicle-treated rats that showed only modest coordination. Measurements of spared tissue through the injured segments and at the lesion epicenters revealed significant increases ($P<0.01$) with bFGF treatment versus controls. In comparison, all rats receiving a severe SCI demonstrated more prolonged and sustained functional deficits, but from 2-6 weeks, bFGF-treated rats demonstrated significant ($P<0.05$) consistent weight-supported hindlimb movements compared to controls with only limited hindlimb weight bearing. Unexpectedly, however, there was no significant effect of bFGF treatment for corresponding tissue sparing through the T10 segments, nor the lesion epicenters. In conclusion, we demonstrate that intrathecal infusion of exogenous bFGF following both moderate and severe contusion SCI in rats significantly enhances functional hindlimb recovery, but a positive correlation between significant behavioral improvements and significant tissue sparing at the injury site was found only after the moderate level of injury. Collectively, this speaks to the undefined cellular and molecular mechanism(s) that underlie the recovery of hindlimb function in rats, with or without bFGF treatment. Future studies will evaluate the effects of increasing the dosage of bFGF as well as the duration of delivery. Supported by KSCHIRT #7-18.

ACTIVITIES AND RECEPTORS OF NOVEL NEUROTROPHIC FACTORS THAT PROMOTE SURVIVAL OF SENSORY AND MOTOR NEURONS

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We have identified a fourth member of the GDNF (glial cell-derived neurotrophic factor) family of ligands which we have named Artemin (ARTN) and its GPI-linked receptor (GFR α 3). This family of factors also includes GDNF, Neurturin (NRTN) and persephin (PSPN) and its members share sequence similarity to the TGF- β superfamily of ligands. The receptor complexes for the GDNF family of ligands (GFLs) are comprised of the RET tyrosine kinase, which can be detected at all known targets of GFLs, and a GPI-linked co-receptor (GFR α 1-GFR α 4), which bind ligand directly, provide specificity and localize RET to lipid microdomains in order to enhance the efficiency of RET-mediated signal transduction. As with the neurotrophins, biochemical and genetic studies suggest that physiological pairing between the different GFR α s and the four ligands probably occurs in vivo, but some cross-talk interactions do occur. Expression patterns for

GFR α 3 and ARTN in the mouse embryo during development suggest that this newest family member may play an important role in the development of certain neuronal populations in the PNS, but not the central nervous system (CNS). Previous members of the GFLs have been shown to have potent activities on a broad spectrum of neuronal populations in the CNS, including midbrain dopaminergic neurons and motor neurons in culture and in vivo after sciatic nerve axotomy. In addition, GDNF, NRTN and ARTN support the survival of many peripheral neurons in culture, including sympathetic, parasympathetic, sensory and enteric neurons. Therefore, there is considerable interest in the potential use of GFLs in regeneration research and as therapeutic agents in the treatment of neurodegenerative diseases.

A MEAL MEANS MORE TO A STARVING PERSON -- INCREASE OF SENSITIVITY OF ASSESSMENT OF NEUROTROPHIC FACTORS BY "STARVING" THE NEURONS

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It is common practice to assess the effect of neurotrophic factors on neurons in vitro, particularly when novel substances are looked for. Due to general scantiness of these factors present in tissue, the effects may not be easily detectable. We have developed a method to deal with this issue by inducing controlled injury to the neurons, on which the effects of neurotrophic substances can be more markedly displayed. Effect of GDNF (20 µg/ml) on neonatal rat DRG neurons was used as a model. Types of injury: 1. Free radical: 100 µM FeSO $_4$ + 50 µM H $_2$ O $_2$; 2. Low glucose: 1 mg/ml; 3. Kainic acid: 10 µM; 4. Hemoglobin: 5 µg/ml. Assays for neuron viability: 1. MTT; 2. Cell count, Trypan Blue staining; 3. Total protein. For the normal neurons there was no statistically significant difference in MTT value and cell counts between groups with or without GDNF. However, in the cases of injured neurons, addition of GDNF could significantly or very significantly increase MTT value and cell numbers. The total protein assay was less sensitive and the value inconsistent. An example of its application: Adrenalectomy has been found to induce marked increase in GAP-43 expression and axonal sprouting of the nerve fibers innervating the anterior pituitary. No neurotrophic effect could be demonstrated by co-culturing the adrenalectomized anterior pituitary and the hypothalamic neurons. However, after inducing injury to the neurons before co-culturing, it became evident that the primed gland did have a trophic effect on hypothalamic neurons, and IL-6 might be the major factor that was responsible for this trophic effect.

PREVENTION OF MOTONEURON DEGENERATION IN ADULT RATS BY RE-IMPLANTATION OF VENTRAL ROOT FOLLOWING SPINAL ROOT AVULSION

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We have previously reported that motoneuron degeneration due to spinal root avulsion in adult rats can be prevented by a PN graft implantation. The aim of the present study is to test whether re-implantation of the avulsed ventral root, instead of PN graft implantation, could prevent the degeneration of motoneurons after root avulsion. Following the seventh cervical (C7) spinal root avulsion, animals were divided into two groups. Animals in the control group remained untreated. Animals in the experimental group received a re-implantation of ventral root immediately after avulsion. Animals were allowed to survive for three or six weeks with 6-8 animals in each survival time point. Regenerating motoneurons were labeled with fluorescent dye. In the control animals about 65% of injured motoneurons survived three weeks after the avulsion injury. More than 80% of them expressed nitric oxide synthase (NOS). By 6 weeks following root avulsion, only 30% of injured motoneurons survived and many of them were NOS positive. In contrast, re-implantation of ventral root significantly enhanced the survival of motoneurons. Near 80% or 70% of motoneurons survived by 3 or 6 weeks post-injury respectively if the ventral root was re-implanted immediately after root avulsion. Expression of NOS due to root avulsion was significantly inhibited in these experimental animals. Among the surviving motoneurons only 5-7% of motoneurons were NOS positive. More interestingly, over 90% of the surviving motoneurons were found to regenerate their axons into the re-implanted ventral root (labeled by FG), and all of these

regenerating motoneurons were NOS negative. Results of the present study show that re-implantation of ventral root after the root is avulsed can greatly enhance motoneuron survival and the surviving motoneurons can regrow their axons into the original ventral root. Compared with the PN graft implantation, re-implantation of ventral root has greater potential for clinical application. This study was supported by a grant from the Research Grant Council of Hong Kong.

TRANSFER OF THE ULNAR NERVE TO THE HIP (TO MAKE PARAPLEGICS WALK)

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It's generally known that spinal cord is non-permissive for axons to regrow. Experimental peripheral nerve grafts put from the upper to the lower stumps of the cord in rats showed ingrowth of the axons into the grafts. Axons however stopped when in contact again with the cord. To overcome this situation since 1982 I have done, first in rats and then in monkeys, direct connection of the upper cord to peripheral nerves by means of peripheral nerve grafts obtaining reinnervation of the graft, the peripheral nerves and the muscles (as witnessed by restored motor end plates) (Figures supplied). EMG showed good muscular responses. Clinical examination showed recovery of the lower limb. Research is still in progress. While waiting for final results and for transferring this operation to human beings I have done transfer of the ulnar nerve to the 3 main muscles of the hip: quadriceps, gluteus maximus and gluteus medius (in 4 human beings). Contemporary reconstructive surgery is done for interossei and adductor pollicis. The first man operated on is able to stand up and walk with the help of a light walker that he can fold and put in his car. Walking is rudimentary and the patient is able to walk 50 to 60 steps at a time. Then he must stop for a while before starting again. He is completely autonomous. He "feels" the movements he is doing with the lower limb. Late fMRI showed that by contracting the muscles of the hip, both the areas of the arm and leg were activated. Two more patients are still recovering and under reeducation.

DIAPHRAGMATIC MOTOR RECOVERY IN HEMISECTED RATS FOLLOWING SPINAL CORD REPAIR USING PERIPHERAL NERVE TRANSPLANTS AND AN ACIDIC FIBROBLASTIC GROWTH FACTOR (aFGF) SUSPENSION

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Hemisection at C2 level causes paralysis of the ipsilateral hemidiaphragm in the rat. The hemidiaphragmatic functional recovery is minimal. In order to enhance hemidiaphragmatic motor recovery, five small fragments of an intercostal nerve taken from the same animal were used to gap the descending respiratory pathway. The proximal stump of the graft was placed in contact with the anterolateral funiculus while the distal stump was placed in contact with the surface of the anterior horn. A matrix of fibrin glue mixed with an aFGF solution was used to encase the graft and keep it in place. All animals were subjected to hemisection. Eight adult rats were used as controls. Four rats received the hemisection alone, four different rats received the placement of the graft and fibrin glue without the aFGF solution immediately following hemisection. Four rats received the grafts plus the fibrin glue with the aFGF solution. EMG recordings were performed in all animals immediately following hemisection to demonstrate complete paralysis of the ipsilateral hemidiaphragm. EMG and phrenic nerve recordings were repeated 30 days after the initial surgery in all animals. No activity was demonstrated in any of the control animals without grafts. Minimal EMG activity was present in one animal of the four animals that received the graft alone. Three out of four animals that received the grafts embedded in aFGF glue showed EMG and phrenic nerve activity in the previously paralyzed hemidiaphragm. The nerve activity was moderate in two animals and robust in one. The results suggest that placement of peripheral nerve grafts embedded in an aFGF solution mixed with fibrin glue may induce functional regeneration of the descending respiratory pathway.

COMBINATORIAL TREATMENT STRATEGIES IMPROVE RUBROSPINAL MOTONEURON (RSMN) REGENERATION AFTER PARTIAL SPINAL CORD INJURY (SCI)

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Testosterone (T) is a neurotrophic factor that is able to enhance peripheral motoneuron regeneration by upregulating regeneration-associated genes (RAGs), including β II-tubulin. RSMN represents a population of spinal-projecting central neurons that express androgen receptor. T increases β II-tubulin mRNA levels in injured RSMN. Adding a peripheral nerve graft (PNG) at the site of a SCI stimulates migration of processes from injured RSMN through the graft. Using β II-tubulin as a marker of regeneration, this study examined the effect of a PNG on the intrinsic injury response of RSMN, and determined whether T could augment that response. Adult gonadectomized male hamsters were subjected to right peroneal nerve crush, and then subjected to a C7 dorsolateral aspiration lesion of the spinal cord a week later. Immediately following SCI, a segment of the peroneal nerve was autografted into the spinal aspiration cavity, with one end of the nerve segment sutured flush against the proximal end of the injury site. Half the animals received T implants at the same time of injury. Postoperative times included 2, 7 and 14 days. In situ hybridization was performed using a [32 P]-labeled cDNA probe specific to β II-tubulin. Analysis revealed that T treatment and PNG individually increased the levels of β II-tubulin mRNA in injured RSMN. However, RSMN in those animals that had received a PNG plus T treatment exhibited an increase in β II-tubulin mRNA levels that were higher than those levels following either treatment alone. These data suggest that the introduction of an environment that is permissive to neuronal growth in combination with a known neurotrophic factor can result in the heightened upregulation of genes that are critical in sustaining a regenerative effort by injured spinal-projecting motoneurons. The additive effect observed from an axonally transported factor originating from the graft, coupled with an exogenously applied gene-targeting hormone, suggests the existence of multiple pathways for the potential regulation of RAGs following SCI. Supported by VA grant V05-940A (KJJ).

AXONAL REGROWTH IN CHRONIC AND COMPLETE SPINAL CORD INJURY IN ADULT CATS TREATED WITH VITAMIN E, NEUROTROPHIC FACTORS AND PREDEGENERATED PERIPHERAL NERVE

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Our objective was to demonstrate axonal regeneration and functional recovery in cats with chronic and complete spinal cord injury (SCI) using vitamin E, neurotrophic factors and predegenerated peripheral nerve grafted in the injured zone. Six female adult cats, 3 in the experimental and 3 in the control group, were anesthetized with a mixture of xylazine hydrochloride (12.5 mg/kg) and ketamine clorhydrate (75 mg/kg) before each surgical procedure and for sacrifice. A T10 laminectomy was done and the spinal cord (SC) was subjected to complete section with microsurgical scissors. Ten days before the second surgery and under local anesthetized procedure, both sural nerves were transected distal to popliteal area. Five days before and after the second surgery, 280 mg of vitamin E was administered daily. Four weeks after the SCI, the surgical zone was reopened, the spinal cord scar was identified and removed along with 2 mm proximal and distal of preserved spinal cord. Then, in the experimental group, 8 μ l and 2 μ l of BDNF, CNTF and NT-3 mixture were injected into the anterolateral proximal and medial distal stumps respectively. At the same time, 3 cm long of each sural nerve were identified and removed. Six pieces of 8 mm length were grafted into the SC gap among the proximal anterolateral white and the distal medial gray substances. In the control group the gap was filled with Surgicel. The cats were followed during 9 months to observe functional recovery and finally were subjected to electrophysiological evoked potential studies and sacrificed for morphological analysis. A great number of regrowth axons were observed crossing the peripheral nerve grafts reaching to the gray distal substance. The peripheral nerve adjacent zones of white substance showed a great number of axons compared with the no adjacent. Nevertheless, during the follow-up there were no differences among experimental and control groups in clinical and electrophysiological evaluations. In conclusion, the association of neuroprotectors, trophic factors and grafts of predegenerated peripheral nerve allow axonal regrowth in chronic SCI adult animals. However, functional and electrophysiological recovery was not observed.

ORGANIZATION OF A CLINICAL TRIAL OF BODY WEIGHT-SUPPORTED TREADMILL TRAINING (BWSTT) FOR REHABILITATION AFTER ACUTE SPINAL CORD INJURY

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The first large single-blinded, multicenter, randomized clinical trial (RCT) of BWSTT after recent SCI will test the efficacy of a task-oriented intervention that optimizes locomotor-related sensory inputs. Based on a power analysis of past outcomes from the 5 clinical sites, 235 subjects classified as ASIA B, C and D who cannot ambulate without at least moderate assistance will be randomized within 8 weeks of onset of a traumatic SCI to 12 weeks of conventional rehabilitation plus 1 hour of mobility retraining vs substituting an hour of the latter with BWSTT and overground walking according to a specified strategy. The upper motor neuron group (100) will have lesions from C4 to T11. The lower motor neuron group (100) will have lesions from T12 to L3. Outcome measures for this intention-to-treat design are collected by a blinded observer. Primary outcomes are locomotor independence graded by the Functional Independence Measure of ASIA B and C subjects and overground walking speed with a reciprocal gait for ASIA D subjects. Secondary measures include rate of gains, strength, Berg Balance Score, Ashworth Score of tone, the MOS SF-54 for health-related quality of life, the walking Index for SCI (a walking impairment scale), community reintegration and immobility-related medical complications. Some sites will perform gait analyses, functional neuroimaging, or examine muscle plasticity. In addition, the design of this RCT will test methods for clinical research that are applicable to future trials of biological interventions for SCI. Funded by NIH/NICHHD UO1 HD37439.

CNTF ENHANCES THE REGENERATION CAPACITY BUT NOT THE SURVIVAL OF RETINAL GANGLION CELLS IN ADULT HAMSTERS

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Ciliary neurotrophic factor (CNTF) has recently been shown to promote the axonal regrowth of retinal ganglion cells into a peripheral nerve graft following an intracranial or intraorbital transection of the optic nerve. It is unclear whether the enhancement of axonal regrowth by ciliary neurotrophic factor application correlates to the enhancement of survival of retinal ganglion cells and/or the upregulation of expression of growth-associated protein-43 mRNA in retinas. The present study evaluated the regenerative capacity of retinal ganglion cells following intraorbital transection of the optic nerve (~1.5 mm from the optic disc) and the attachment of a peripheral nerve to the ocular stump of the optic nerve. In addition, we have determined the survival of retinal ganglion cells and the expression of growth associated protein-43 mRNA in ciliary neurotrophic factor-treated retinas following optic nerve transection. The results showed that in the ciliary neurotrophic factor-treated retinas, the number of retinal ganglion cells which had regrown axons into a peripheral nerve is about 4 times more than the control. In the axotomized retinas, ciliary neurotrophic factor initiated sprouting of axon-like processes at 14 and 28 days post-axotomy and upregulated the expression level of growth associated protein-43 mRNA at 7, 14 and 28 days post-axotomy. However, ciliary neurotrophic factor did not prevent the death of axotomized retinal ganglion cells. We suggest that one possible mechanism for the axonal regeneration of axotomized retinal ganglion cells by ciliary neurotrophic factor could be mediated by the upregulation of growth associated protein-43 gene expression and not by increasing the pool of surviving retinal ganglion cells after axotomy.

BCL-2 DOES NOT ENHANCE THE ABILITY OF RETINAL GANGLION CELLS TO EXTEND AXONS IN VITRO

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Retinal ganglion cells (RGCs) lose the ability to regenerate their axons

during early postnatal development. Their ability to regenerate is enhanced by bcl-2 overexpression, leading Chen et al. (1997; Nature 385:434-9) to propose that bcl-2 acts as an intrinsic genetic switch that controls axon growth. As axotomized RGCs quickly die, however, an alternative hypothesis is that bcl-2 promotes RGC regeneration by enhancing survival. To distinguish between these possibilities, we purified and cultured E19 and P8 RGCs in serum-free medium that promoted the survival of the majority of cells at both ages. While both E19 and P8 RGCs were capable of axon extension in response to BDNF and CNTF, the E19 RGCs extended axons at about 0.6 mm per day, a rate up to 10 times higher than the P8 RGCs. When bcl-2 was overexpressed in the purified RGCs using an adenoviral vector, the majority of cells survived but did not extend axons or dendrites in medium lacking neurotrophins. The P8 bcl-2-expressing RGCs continued to extend axons up to 10 times more slowly than E19 RGCs in response to BDNF and CNTF. These results show that surviving RGCs do not extend axons by default, and that bcl-2 is not sufficient either to induce axon growth or to enhance neurotrophin-stimulated axon growth in vitro. Remarkably, despite equivalent survival in vitro, P8 RGCs have drastically less regenerative ability than do E19 RGCs. Experiments in progress should distinguish whether this decrease is the result of an intrinsic aging program or is signaled by target innervation. Taken together, these results raise the question of whether bcl-2 expression enhances RGC regeneration in vivo by enhancing survival.

OPTIC NERVE REGENERATION IN THE LIZARD *CTENOPHORUS ORNATUS* - A MODEL SYSTEM FOR EXPLORING CENTRAL NERVE REPAIR

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Anamniotic vertebrates such as the fish and frog have provided valuable insights into events underlying successful optic nerve regeneration after crush lesion, a procedure which severs all axons and leaves the meningeal sheath intact as a conduit for regrowth. In these species, growth cones form and cross the lesion site by approximately one week to reach the visual centers by between 2-4 weeks. A retinotopic map is reformed and consolidated within the optic tectum with the return of vision. By contrast, in Amniotes such as birds and mammals, growth cones fail even to penetrate the lesion site and blindness persists. Reptiles are Amniotes and phylogenetically intermediate between the fish and frogs and the birds and mammals. We have shown that in the ornate dragon lizard, *C. ornatus*, optic nerve regeneration is transitional between the fish and frogs and the birds and mammals. Growth cones cross the lesion site and enter the optic tectum within a similar time-frame to that in fish and frogs (Beazley, et al., J. Comp. Neurol., in press). Axons form a crude transient retinotopic map between 5-7 months, coincident with the time that the projection is at its most robust. However, the map subsequently degrades and retinotopic order is lost concomitant with a diminution in the projection. As a consequence, animals remain blind via the experimental eye (Stirling, et al., Vis. Neurosci. 16:681-693, 1999; Beazley, et al., J. Comp. Neurol. 377:105-121, 1997). The lizard thus provides the first model in which regrowth of optic axons is dissociated from retinotopic map formation within visual centers. Optic nerve regeneration in the lizard also exhibits a number of other unusual features (Beazley, et al., J. Comp. Neurol., in press). In addition to the instability of the retinotopic map, regenerating axons display inaccuracies by entering inappropriate regions of the visual pathway such as the unoperated optic nerve and the ipsilateral optic tectum. Furthermore, some regenerating axons exit the visual pathway altogether and invade the tectal and anterior commissures, the olfactory nerve and non-visual nuclei such as the nucleus rotundus. In addition, a high degree of variability is seen between individuals. Taken together, the dysfunctional regeneration seen in the lizard suggests that a number of strategies will be necessary to restore vision in mammals following optic nerve lesion. These include approaches that will encourage not only the regrowth of axons across the lesion site and into the optic tract, but also strategies that encourage regenerating axons to remain within the visual pathway as well as to reform stable retinotopic maps. Funded by a National Health & Medical Research Council of Australia Program Grant (99329) and the Neurotrauma Research Program of Western Australia.

MOLECULAR CHANGES DURING OPTIC NERVE REGENERATION IN THE LIZARD *CTENOPHORUS ORNATUS*

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In our companion abstract (Beazley et al., this meeting), we describe dysfunctional optic nerve regeneration in the lizard *Ctenophorus ornatus*. Regenerating axons reach the visual centers by 1 month, form a crude, transient retinotopic map between 5-7 months but with a subsequent breakdown of order that is observed for up to 1-2 years. Axon regrowth is thus dissociated from retinotopic map formation and animals remain functionally blind. Here we have investigated a number of molecular changes during optic nerve regeneration in the lizard and have maintained our comparative approach by undertaking some parallel studies in the goldfish, a species in which retinotopic maps are consolidated and functional vision is restored. Immunohistochemical staining has shown that whereas a number of molecules are transiently upregulated in the goldfish, they remain permanently upregulated in the lizard. We examined the Eph-receptor tyrosine kinase ligand Ephrin A2, a molecule implicated in the formation of retinotopic maps during development of the visual system. In goldfish, Ephrin A2 is transiently re-expressed as a gradient across the optic tectum over a time-course that coincides with the ingrowth of axons and the consolidation of the retinotopic map. By contrast, in lizard, Ephrin A2 remains upregulated for up to 1 year after optic nerve crush. We also examined two markers for axonal growth. Gefitin is a neuronal intermediate filament protein that is expressed in actively regenerating axons in goldfish between 2-6 weeks, coincident with regrowth along the visual pathway and reformation of the retinotopic map. By contrast, in lizard, expression is biphasic. An early peak coincides with axon regrowth and a second wave occurs concomitantly with the formation of the crude transient retinotopic map and is maintained thereafter. The growth associated protein GAP-43 is also expressed transiently during optic nerve regeneration in the goldfish. However in the lizard, expression is upregulated in the long term. Furthermore, staining patterns confirmed our previous observations that regeneration is inaccurate with axons not only growing and arborizing in inappropriate tectal laminae but also exiting the visual pathway altogether. We have also examined the NMDA receptor NR1 which in goldfish is transiently expressed during the time that terminals display synaptic plasticity during consolidation of the retinotopic map. In lizard, however, NR1 remains upregulated for up to one year. Taken together, our findings support our view that Ephrin A2 may function similarly in goldfish and lizard to reestablish a retinotopic map but in lizard appears insufficient to maintain the map. Furthermore, in the absence of forming stable retinotopic connections, regenerating optic axons appear to be held within the penultimate phase of regrowth and tectal cells continue to express a receptor indicative of synaptic plasticity. Funded by a National Health & Medical Research Council of Australia Program Grant (99329) and the Neurotrauma Research Program of Western Australia.

PC12 CO-CULTURE WITH MATURE ASTROCYTES EXPRESSING HUMAN L1: EFFECT ON NEURITE EXTENSION

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While neurons of the CNS are capable of regrowth in the proper environment, *in vivo* regeneration does not occur, possibly due to a lack of appropriate molecular elements on surrounding cell surfaces. We tested whether a monolayer of primary, mature rodent astrocytes transfected with the neural cell adhesion molecule L1 could enhance neurite extension by co-cultured PC12 cells. Monolayers of astrocytes were derived from two-day postnatal rats and cultured for five weeks. They were liposomally transfected in three experimental groups: 1) L1, 2) β -gal, and 3) mock transfected, two days prior to co-culturing. A β -galactosidase assay showed that approximately 2-3% of cells were expressing foreign protein. PC12 cells were differentiated with NGF and neurites were removed prior to co-culturing. Measurements were taken three days after adding PC12 cells to the monolayers using immunofluorescence to visualize neurofilament. PC12 cells grown on monolayers of L1 transfected astrocytes grew significantly longer neurites than the control groups, with an average length of 131 μ m (n=109). Those grown on β -gal transfected monolayers extended neurites with an average of only 82 μ m (n=100, $P < 0.0001$ vs. L1), and on mock transfected monolayers, the average neurite length was 84 μ m (n=101, $P < 0.0001$ vs.

L1). There was no statistically significant difference in growth on β -gal and mock transfected monolayers ($P=0.83$). Lack of observed increase in neurite length on β -galactosidase transfected monolayers indicates that enhanced PC12 neurite extension is most likely a specific effect of L1. The observed effect is possibly due to a homophilic interaction between L1 on the PC12 cell surface and the transfected monolayer. To further evaluate this, the authors present results from L1 antibody blocking assays to examine the specificity of L1 effect on PC12 neurite extension. The neurite extension assay described offers a convenient, rapid method for screening the effect of induced L1 expression by astrocytes. These results are consistent with previous studies implicating L1 as a growth enhancing factor for neurite extension, and a candidate for use in *in vivo* gene therapy to induce CNS regeneration.

POTENTIATION OF NEURITE OUTGROWTH BY ADENOSINE ANALOGS IN PC12 CELLS

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While investigating the biochemical mechanisms controlling neurite outgrowth, we observed a potentiation of NGF-induced neurite outgrowth by inclusion of 100 μ M ATP in the medium. Significant differences in neurite expression were noted at concentrations as low as 20 μ M of ATP. The PC12 cells (obtained from ATCC) were cultured on Falcon Primaria culture flasks in RPMI medium supplemented with 10% horse serum, 5% fetal calf serum, 1% penicillin/streptomycin and 5% CO₂. Subsequent experiments demonstrated a similar potentiation of NGF-induced neurite outgrowth by inclusion of 100 μ M ATP, ADP, AMP, cyclic AMP and adenosine, suggesting that this enhanced outgrowth was not dependent upon the presence of a high energy phosphate bond. This conclusion was supported by inclusion of nitro-blue tetrazolium and dipyrindamole, which had no effect on the potentiation. The P2 receptor antagonist suramin inhibited the response, suggesting that these receptors were responsible for mediating the potentiation of neurite expression. This potentiation was also inhibited by calmodulin antagonists, trifluoperazine and haloperidol, but not by CamK-II inhibitors KN62 and KN93. These results suggest that stimulation of purinergic receptors will enhance neurite expression *in vitro* and stimulation of these receptors may also have a role *in vivo*. Future experiments will investigate the biochemical mechanism of this potentiation of NGF-induced neurite outgrowth.

NERVE FIBER GROWTH ADJACENT TO FOREBRAIN STAB INJURY AFTER ABLATION OF SCAR-FORMING REACTIVE ASTROCYTES IN ADULT TRANSGENIC MICE

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To investigate roles of reactive astrocytes after brain injury, we generated transgenic mice expressing herpes simplex virus thymidine kinase (HSV-TK) from the mouse glial fibrillary acid protein (GFAP) promoter (Cell 93:189, 1998; Neuron 23:297, 1999). In adult transgenic mice, 7 days of continuous subcutaneous treatment with the antiviral agent ganciclovir ablated proliferating reactive astrocytes adjacent to stab injury in the forebrain. Neural parenchyma depleted of astrocytes was not repopulated by GFAP-positive astrocytes for at least 35 days, and compared with controls exhibited a prolonged, 25-fold greater density of CD45-positive leukocytes, including ultrastructurally identified monocytes, macrophages, neutrophils and lymphocytes. Astrocyte ablation prevented blood brain barrier (BBB) repair, and preceded substantial neuronal degeneration that could be attenuated significantly by chronic glutamate receptor blockade. Wound margins depleted of astrocytes exhibited a pronounced increase in local neurite outgrowth. These findings demonstrate roles for reactive astrocytes in regulating leukocyte trafficking, repairing the BBB, protecting neurons and restricting nerve fiber growth after injury in the adult CNS. Supported by Action Research, The Wellcome Trust, and MRC.

DIFFERENTIAL EXPRESSION OF CALPAINS BY CULTURED AND REACTIVE ASTROCYTES

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Calpains are calcium-dependent cysteine proteases found in many mammalian cell types and known to cleave a variety of intracellular substrates. There

are two major calpain isozymes: one activated by micromolar concentrations of calcium (μ -calpain) and the other by millimolar calcium concentrations (m-calpain). Presently, little is known about the role of calpains in astrogliosis that results from glial reactivity to brain injury. This study was designed to investigate the presence of calpains in cultured rat cortical astrocytes and to determine the pattern and time course for calpain expression in astrocytes in the adult rat brain under injury conditions. The *in vitro* presence of calpains was examined using both Western blot analysis and immunohistochemistry for both major isozymes. The results showed the expression of both calpains in cultured astrocytes, with μ -calpain being the predominant isoform. This suggests a role for calpains in astrocyte proliferation, differentiation or migration during development. Systemic injection of kainic acid and a scalpel wound penetrating the cerebrum and hippocampus comprised the two injury models. Animals from both models were sacrificed at 1, 3, 5, 7 and 12 days postlesion, followed by transcardial perfusion with 4% paraformaldehyde, frozen sectioning and immunohistochemical analysis for both calpains. The mechanical injury model did not produce detectable amounts of astrocytic calpains at any time interval. In the excitotoxic model, increased m-calpain expression was detected in astrocytes beginning at 3 days, increasing to a maximum at 5-7 days and declining thereafter. Astrocyte expression of m-calpain was predominantly cytoplasmic and largely colocalized with glial fibrillary acidic protein. Increased *in vivo* expression of m-calpain throughout the hippocampus in astrocytes responding to an excitotoxic injury suggests that this isozyme may play a role in the generalized hypertrophic response of astrocytes to neuronal damage. Further studies will investigate whether the excitotoxin-induced expression of calpain results from increased synthesis or posttranslational modification of the enzyme, and determine the role of glutamate and calcium in these events.

SIGNAL TRANSDUCTION PATHWAYS RELATED TO REACTIVE GLIOSIS

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ATP, which is released upon tissue injury, can induce characteristics of gliosis both *in vitro* and *in vivo*. These trophic effects include astrocytic process elongation, increased expression of glial fibrillary acidic protein (GFAP) and proliferation. Fibroblast growth factors (FGFs) are also increased after injury, and synergistic interactions between extracellular ATP and FGFs have been observed in astrocytes. We are now investigating the signaling pathways underlying these trophic actions in primary cultures of rat cortical astrocytes. ATP and FGF stimulate extracellular signal regulated protein kinase (ERK), a key component of a signal transduction cascade that is crucial for cellular differentiation and proliferation. Although both ATP and FGF activate ERK, they do so by different pathways. For example, FGF recruits cRaf-1, the first kinase in the ERK cascade, but ATP does not. In astrocytes, ATP stimulates metabotropic P2Y purinergic receptors, members of the G protein-coupled receptor superfamily. P2Y receptors are linked to the ERK cascade by protein kinase C (PKC) because down-regulation or inhibition of PKC blocks ATP activation of ERK. Although P2Y receptors are also coupled to the phosphatidylinositol-specific phospholipase C (PI-PLC)/calcium pathway, signaling from P2Y receptors to ERK does not involve this pathway because inhibition of PI-PLC or chelation of intra- or extracellular calcium does not block ATP activation of ERK. A calcium-independent PKC isoform may be a pathway component because ATP stimulates translocation of PKC δ but not the calcium-dependent PKC γ . The diacylglycerol needed to activate PKC δ may come from the hydrolysis of phosphatidylcholine (PC) because ATP rapidly stimulates phospholipase (PLD) activity and choline formation. PC hydrolysis appears to be upstream of ERK because inhibition of PC hydrolysis blocks ATP activation of ERK. These observations suggest that P2Y purinergic receptors in astrocytes are coupled independently to PI-PLC/calcium and ERK pathways and that PLD and PKC δ are key components of the ERK pathway. Inhibition of MEK, the direct upstream activator of ERK, blocks ATP- and FGF-induced mitogenic signaling, thereby indicating the importance of the ERK cascade in mediating trophic actions of extracellular ATP and FGF. These studies suggest that components of ATP and FGF signaling pathways may provide novel targets for therapeutic intervention in gliosis. This work was supported by the Department of Veterans Affairs.

COMPARISON OF VIRAL VECTORS: EFFICACY IN HUMAN BRAIN NEUROGLIA

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Herpes viruses (HSV), Adeno (Ad) and Adeno-associated viruses (AAV), and Lentiviruses are viral vectors currently under investigation for gene delivery. These vectors differ in their tropism, ability to infect postmitotic cells, immunogenicity, duration of expression and efficacy of gene transfer. While each vector has been studied extensively on an individual basis, direct comparative data for CNS applications are rather scarce. Our study compares and characterizes the efficacy of these vectors to productively infect human neuroglial tissue *in vitro*. Our pilot results using GFP constructs validate this approach to gene delivery in a human primary culture system using fetal brain neuroglia. These cultures are grown as attached monolayers or cell aggregates in a chemically defined serum free medium, and contain abundant neurons that grow and differentiate for several months. Pilot results show that we can temporally follow the relative efficacy and cellular distribution of the viral vectors in the neuronal and glial populations using double and triple label immunofluorescent laser confocal microscopy. Among the several viral vectors we compared, the most promising in terms of length of expression and minimal or absent toxicity are the lenti and adenoviruses. Based on these preliminary data, we plan to test the *in vivo* benefits of using viral vector delivery in experimental models of cell transplantation and neurotrophic factor delivery as neuroregenerative therapies. We have developed and characterized a model based on human fetal brain xenografts stereotactically implanted in the brains of SCID mice. These neural grafts survive and differentiate for up to one year and constitute an optimal environment to study the long term efficacy of viral vector based therapy in the human CNS. This work was supported in part by a University of Pittsburgh Center of Excellence in Parkinsons Disease award to C.L. Achim

TRANSPLANTATION OF ASTROCYTES INTO SENSORIMOTOR CORTICAL LESIONS IN NEONATAL RATS AND EFFECTS ON LESION-INDUCED PLASTICITY

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Following unilateral aspiration lesions of the sensorimotor cortex in neonatal rats, there is formation of new neuroanatomical pathways from the opposite unablabeled sensorimotor cortex. Since functional recovery after neonatal lesions is thought to be due to neuroanatomical remodeling, we are interested in evaluating the changes in lesion-induced plasticity that result from transplantation of neuronal and glial cell types. In the present study we investigate the effects of grafted astrocytes on lesion-induced plasticity. Primary astrocyte cultures were prepared from postnatal day 2 rats according to the method of McCarthy and de Vellis (1980). Isolated astrocytes were fluorescently labeled with DiI, then grown in Matrigel® matrix until time of transplant. Postnatal day 5 rats received a sensorimotor cortical aspiration lesion followed by an astrocyte graft. Examination at one week and two weeks post-grafting showed survival of labeled astrocytes located along the walls of the lesion cavity with few cells migrating to other distant brain regions. At 8 weeks of age, one group of animals received pressure injections of the anterograde tracer biotinylated dextran amine (BDA) into the unablabeled sensorimotor cortex. Animals were euthanized 1 week later and brains were processed histochemically for detection of BDA-labeled cells and fibers. Corticothalamic and corticopontine fibers were counted and compared to control animals receiving a lesion alone. Astrocytic effects on the remodeling of pathways from the intact cortex are now being analyzed using electrophysiological methods. Supported by the US Department of Veterans Affairs.

BRAIN-DERIVED NEUROTROPHIC FACTOR REGULATES SYNAPTIC PLASTICITY AND STRUCTURE IN THE DEVELOPING CEREBELLUM

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An increasing body of evidence supports the notion that neurotrophins can influence synaptic transmission and activity-dependent synaptic plasticity. However, it is not clear if such effects reflect neurotrophin regulation of

synaptic structure. Here we have investigated the potential role of BDNF (brain-derived neurotrophic factor) in the cerebellar plasticity in mice with a targeted gene disruption of BDNF. This mouse is ataxic and exhibits cerebellar abnormalities including abnormal foliation, layering and stunted Purkinje cell dendrites. Electrophysiologic studies of the parallel fiber-Purkinje cell synapse revealed normal basal neurotransmission but a significant decrease in PPF (paired-pulse facilitation), a form of short-term synaptic plasticity. Immunohistochemical and ultrastructural studies were undertaken to analyze associated changes in synapse morphology. The density of synapses in the molecular layer of BDNF $-/-$ mice was less than in $+/+$ animals. Furthermore, preliminary results suggest alteration in the synaptic vesicle distribution in BDNF $-/-$ mice. Hence, neurotrophins remain promising candidates for molecules that bridge the gap between synaptic activity and synaptic morphology and therefore may be important mediators of metaplasticity.

GRAFTING OF ENCAPSULATED BDNF-PRODUCING FIBROBLASTS INTO INJURED SPINAL CORD WITHOUT IMMUNE SUPPRESSION PROVIDES A PERMISSIVE ENVIRONMENT FOR AXONAL GROWTH

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Cellular therapies for treatment of spinal cord injury often evoke strong immune responses which limit their usefulness. Alginate encapsulated fibroblasts, genetically modified to produce BDNF (BDNF/FB), may provide an effective strategy for delivery of therapeutic products to the injured spinal cord. The alginate encapsulation provides an immunological barrier between the encapsulated cells and the host immune system. This allows the grafting of genetically engineered non-autologous cells, in the absence of immune suppression, for delivery of a neurotrophin product through diffusion from the capsule into the injured spinal cord. We examined (1) survival of encapsulated BDNF/FB grafted into a partially hemisectioned cervical adult rat spinal cord, (2) expression of the transgene *in vivo*, using the reporter gene β -galactosidase, (3) the immune response generated against the capsules *in vivo* by immunostaining for ED-1 to identify microglia and macrophages and (4) whether the capsules improved the environment in or around the transplant region. We found that alginate encapsulation allowed survival of the cells and expression of the reporter gene for at least one month post-injury in the absence of immune suppression. ED-1 staining revealed a moderate level of inflammation in the transplant area. The encapsulated BDNF/FB provided a permissive environment for host axonal growth as indicated by immunostaining for CGRP, MAP-2, neurofilament and 5-HT. We conclude that alginate encapsulation provides a protective barrier for transplanted cells, allows for a therapeutic product to be secreted at the injury site, and appears to provide a permissive environment for axonal growth, all in the absence of immune suppression. Supported by NIH grants NS24707, EPVA, VA and MCP Hahnemann University.

RECOVERY OF FORELIMB AND HINDLIMB USE FOLLOWING THE GRAFT OF FIBROBLASTS GENETICALLY MODIFIED TO PRODUCE BDNF IN THE INJURED ADULT RAT SPINAL CORD

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Grafting genetically engineered fibroblasts (FB) that express the brain-derived neurotrophic factor (BDNF) into a partial cervical hemisection (HX) promotes regeneration of rubrospinal and other axons and prevents death and atrophy of red nucleus neurons. In the present study, we demonstrate that these rats show recovery of forelimb and hindlimb use. Rats were randomly divided into 3 groups: HX+FB/BDNF, HX+FB and HX alone. A subtotal HX was created on the right side of the cord at C3-4 by aspirating the lateral funiculus, sparing most of gray matter and dorsal column. Rats were tested weekly starting 1 wk post-surgery for 8 wks. The motor tests included: 1) cylinder test that measures forelimb usage, 2) horizontal rope walking to evaluate posture and balance as well as limb functions and 3) swim task that measures limb functions independent of weight support. Our results show that the performance of FB/BDNF rats was superior to that

of either the unmodified FB or HX alone group. In the cylinder test, FB/BDNF transplant rats' forelimb usage improved, beginning the 1st wk post-surgery and reaching a plateau by 3-4 wks. The same extent of recovery was not observed in the FB or HX alone group. The FB/BDNF rats made fewer errors (slips and falls) on the horizontal rope than either of the groups. The swim task showed a similar pattern of results. Open-field (BBB) and narrow beam walking tests revealed no significant functional deficits among the groups, demonstrating the limitations of these tests for effects of transplants on partial lesions. The recovery of function in the FB/BDNF group was due to the presence of the graft, since a 2nd lesion rostral to the original lesion at the end of the 8 wk period nearly abolished the recovered functions for all tasks in the 3 groups of rats. These results show that rats receiving BDNF-producing FB recovered forelimb and hindlimb functions and suggest that the regenerating rubrospinal axons contributed in part to the recovery. Supported by NIH, EPVA, VA and MCP Hahnemann University.

FETAL TRANSPLANTS AND NTFs AID AXONAL REGENERATION AND FUNCTIONAL RECOVERY IN ADULT RATS AFTER CHRONIC CERVICAL SPINAL CORD INJURY

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Transplants (TP) of fetal spinal cord tissue improve skilled forelimb function following acute cervical spinal cord injury in neonatal rats. Neurotrophic factors (NTF) increase axonal growth in adult rats after immediate or delayed injury and TP. We examined here the effect of delayed TP and NTF administration on recovery of skilled forelimb function in adult rats following cervical overhemisection (HX). Adult rats were pre-trained to a reaching task and then underwent HX at the C4 level, transecting the entire right side of the spinal cord and the dorsal columns bilaterally. Two weeks later, E14 cervical spinal cord tissue was placed in the lesion site. Rats received NT-3 (n=13), BDNF (n=14) or saline (SAL) (n=9) via osmotic minipumps for an additional two weeks. Five weeks after TP, animals were filmed during a forelimb reaching task and locomotion. All behavioral analysis was done blind with regard to the NTF treatment; comparisons were made to lesion only animals (HX). Qualitative and quantitative assessment of reaching, posture and locomotion were performed. Neuroanatomical tracing and ICC were used to examine the growth of axonal pathways within the transplant. Cervical HX disrupts forelimb use bilaterally in reaching and locomotion. NTF treated rats performed better on the reaching task than HX animals. The quality and quantity of reaching were improved in both left and right forelimbs. PEAK analysis revealed HX rats had impaired forelimb and digit extension during locomotion compared with animals receiving NTFs. We observed increased length and density of both supraspinal and segmental axons within TP of rats given NT-3 or BDNF than in the SAL treated animals. These results indicate that TPs and NTFs can enhance axonal regeneration and recovery of skilled forelimb function as well as patterned locomotion in adult rats after cervical hemisection. Supported by NIH grants NS 27054 and HD 07459.

NEUROTROPHIN-PRODUCING GRAFTS ENHANCE OLIGODENDROCYTE PROGENITOR PROLIFERATION AND SCHWANN CELL MYELINATION IN THE CONTUSED RAT SPINAL CORD

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We have previously shown that fibroblast grafts producing neurotrophin-3 (NT-3) or brain-derived neurotrophic factor (BDNF) placed in the contused rat spinal cord stimulate myelination by oligodendrocytes. In the present study, proliferation of oligodendrocyte (OL) progenitors and invasion of Schwann cells was examined after spinal contusion injury and transplantation of NT-3- and BDNF-producing fibroblasts. Total cellular proliferation in injury control animals (non-transplant recipients) was highest during the first week post-injury and declined thereafter. Proliferation of OL progenitor cells, labeled with an anti-NG2 antibody, was stimulated by spinal cord injury, but did not peak until 2-4 weeks post-injury. Schwann cell myelin was detectable within the injured spinal cord by 14 days post-injury and increased continuously up to 5 months post-injury (latest time examined). In spinal cords of animals receiving NT-3- and BDNF-producing grafts, OL progenitor proliferation was elevated compared to those receiving control β -galactosidase grafts. These new progenitor cells were located both inside the grafts and in the host

white matter. Schwann cell myelination (detected with a P0 antibody) was also enhanced by the NT-3/BDNF grafts. Enhanced P0-positive myelin was observed both in the host white matter and within the fibroblast grafts. These findings demonstrate that cells of the oligodendrocyte lineage proliferate in response to spinal cord injury and exogenous neurotrophins. In addition, NT-3 and BDNF grafts stimulate migration and myelination by Schwann cells. Thus, NT-3 and BDNF may be beneficial to the injured spinal cord not only by enhancing neuron survival and regeneration but also by elevating the number of potential myelinating cells. Supported by the Christopher Reeve Paralysis Foundation.

THE REGENERATIVE EFFECTS OF COMBINED DEMYELINATION PLUS GROWTH FACTOR SECRETING SCHWANN CELL TRANSPLANTATION THERAPY IN THE INJURED CNS

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The development of successful therapies for CNS repair requires elucidation of the regenerative effects of individual treatments and combination treatments in a quantifiable *in vivo* system. We have recently developed a quantifiable model of adult spinal cord regeneration in which a defined region of complete and selective demyelination facilitates a predictable amount of axonal regeneration at a distance from an injury site; when more axons are severed, more growth cones are produced (Keirstead et al., 1998). The ratio of growth cones to severed axons is increased by 26.5% when a known promoter of regeneration, Schwann cells, is introduced into this model. Furthermore, the presence of stably integrated Schwann cells induces growth cones to grow beyond the region of demyelination into myelinated regions of the spinal cord (Keirstead et al., 1999). In order to investigate 1) whether the percentage of regenerating axons can be further increased and 2) whether growth factors may increase the number of growth cones beyond the region of demyelination, we have transplanted Schwann cell lines which have been genetically altered to secrete either of the growth factors NT-3, BDNF or GDNF into this *in vivo* model of CNS regeneration. The transplanted Schwann cells integrated stably into the host tissue and did not produce tumors, regions of necrosis or cavities. The transplanted cells redistributed themselves from the point of transplantation throughout the region of demyelination, which extended 6-8 mm through the dorsal column. The number of growth cones within the transplanted and demyelinated regions will be determined by an electron microscopic analysis of animals that have received differing severities of axonal injury. The presence or absence of growth cones beyond the transplanted and demyelinated regions will also be investigated.

SCHWANN CELL AND OLFACTORY ENSHEATHING GLIA IMPLANTATION IN THE CONTUSED ADULT RAT THORACIC SPINAL CORD

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It has been shown that a Schwann cell (SC) graft in the transected adult rat thoracic spinal cord promotes axonal growth into the implant. Moreover, a SC graft combined with olfactory ensheathing glia (EG) injected into the spinal cord stumps close to the graft-cord interfaces promotes axonal growth from the implant into the spinal cord tissue. Here we have transplanted SCs or EG into a 1-week old cavity resulting from a moderate contusion lesion using the NYU impactor on adult Fischer rat cord. Approximately 2×10^6 cells were gently mixed in 6 μ l of 1% fibrinogen solution and slowly injected into the cavity using a Hamilton syringe. Control rats were not injected or received the fibrinogen solution only. The rats were perfused 2-4 weeks after the contusion injury. In control rats that did not receive an injection, a large cavity was found at the site of impact in which some strands of nervous tissue were present. Staining for GFAP and neurofilaments revealed that this tissue contained low numbers of astrocytes and axons, respectively. After an injection of fibrinogen only, small cavities (200 μ m) and small numbers of astrocytes and axons and some laminin-positive profiles were found in the lesion site. In rats that received either SCs or EG, the transplants were well integrated with the surrounding nervous tissue. Many more astrocytes and axons as well as an abundance of laminin-positive profiles were found within the lesion site compared with control rats. ED1-positive cells (macrophages/microglia) were present in the lesion site. In addition, serotonergic

and noradrenergic fibers were found in the SC transplanted lesion site. The results indicate that SCs or EG mixed in fibrinogen and implanted into a 1-week contusion cavity reduce tissue loss, prevent axon loss and/or promote axonal regeneration in parallel arrays and enhance angiogenesis. Support from the CRPF, NIH/NS-09923 and the Miami Project.

TRANSPLANTATION OF OLFACTORY EPITHELIAL PRECURSOR CELLS INTO THE SPINAL CORD OF ADULT RATS

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The sensory neurons within adult mammalian olfactory epithelium are continually generated throughout life. Olfactory epithelia-derived precursor cells can be isolated from neonatal and adult mice, cultured *in vitro* and induced to differentiate into neurons, oligodendrocytes and astrocytes. We investigated the fate of these precursor cells when transplanted into the spinal cord and determined the effect of these transplants on functional recovery following spinal cord injury. Fifty to 100 thousand fluorescently labeled precursor cells were injected bilaterally into spinal cord at T11-T12. Immunocytochemical techniques were employed to determine the fate of the precursor cells. Preliminary results demonstrate that some of these transplanted cells differentiate into neurons within the spinal cord. Kinetic and kinematic methods were used to quantify the effect of precursor cell transplantation on functional recovery following spinal hemisection. Our results demonstrate that olfactory epithelial-derived precursor cells differentiate and survive long-term in the spinal cord and may prove valuable in promoting functional recovery following spinal cord injury. Supported by WCVM Interprovincial and Saskatchewan Neurotrauma Funds.

EVIDENCE FOR REPAIR OF THE DORSAL COLUMN-DORSAL COLUMN NUCLEI SENSORY PATHWAY AFTER CERVICAL SCI WITH SUBDURAL PERIPHERAL NERVE GRAFT BRIDGES

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Repairing sensory pathways is essential for improving function in humans with spinal cord injury (SCI). Using a dorsal hemisection lesion to completely transect the dorsal columns of the adult rat cervical 4-5 spinal cord segments, we bridged the dorsal column-dorsal column nuclei sensory pathway with 25 mm long subdural sciatic nerve autografts and stabilized them with fibrin glue immediately after SCI. At 2 months post-grafting, Fluorogold labeling of the middle of well-vascularized grafts uncovered C5 and C6 DRG neurons that regenerated axons into the grafts of all 4 rats. To determine whether acutely injured axons of forelimb DRG neurons reinnervate the brainstem and form functional synapses with dorsal column nuclei neurons, needle stimulating electrodes were inserted into the glabrous footpad of the forepaw ipsilateral to the graft of 4 rats at monthly intervals. Somatosensory evoked potentials (SSEPs) were recorded from the scalp with a needle electrode inserted percutaneously over the forelimb somatosensory cortex contralateral to the graft. SSEPs were elicited with constant current stimuli at a rate of 1.4 Hz. The pulse width was 200 μ sec and the intensity was 1.5-8mA (normal threshold=0.5 mA). One hundred stimuli were averaged per waveform and each waveform was replicated. SSEPs with normal latencies but smaller amplitudes were recorded from 3 of 4 grafted rats at 2 months, but not 1 month, post-grafting. SSEPs were recorded from all rats at 4 months post-grafting. SSEPs were absent or minimal in 2 injured rats at 4 months post-SCI. Transcutaneous evoked potentials (TEPs) of similar latencies and amplitudes could be recorded from the C5-6 dorsal columns of all injured rats and all grafted rats. No TEPs could be recorded from the C3-4 dorsal columns or the dorsal column nuclei of injured rats. TEPs were recorded from the grafts of 2 of 3 rats. Stimulation of the graft itself elicited potentials from the dorsal column nuclei of 2 of 2 rats and cortex contralateral to the graft of 3 of 3 rats after graft stimulation. SSEPs were recorded from the cortex of 2 of 3 rats and these disappeared after the graft was cut in 1 of 1 rat. These data suggest that acutely injured axons of forelimb DRG neurons regenerate from the C5 dorsal columns through subdural sciatic nerve graft bridges, reinnervate the brainstem and form functional synapses with dorsal column nuclei neurons. Supported by a

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GRAY MATTER INJURY AND REPLACEMENT: A MODEL BASED ON DAMAGE TO THE SPINAL CORD CENTRAL PATTERN GENERATOR FOR LOCOMOTION

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The goals of this project were to further investigate the role played by lumbar spinal cord interneurons in the generation of locomotor activity and to develop a model of spinal cord injury suitable for testing neuronal replacement strategies. Adult rats received one or two bilateral microinjections of kainic acid (KA) directly into lamina VII of the upper lumbar spinal cord. Descending motor pathways and hindlimb motoneurons were evaluated using transcranial magnetic motor evoked potentials (tcMMEP) recorded from gastrocnemius and quadriceps muscles. None of the rats showed changes in tcMMEPs following KA injection indicating that the descending pathways and motoneurons serving these muscles were intact. Locomotion was assessed weekly using the BBB 21 point locomotor scale. Rats which received one or two injections of KA bilaterally encompassing the L2 segment showed severe locomotor deficits with a mean BBB score of 4.5 ± 3.6 ($n=10$). Three rats that received only one injection bilaterally at L2 had a mean BBB score of 3.2 ± 2.0 ($n=3$). Rats with lesions rostral to the L2 segment were able to locomote and had a mean BBB score of 14.6 ± 2.6 ($n=4$). Histological examination revealed variable motoneuron loss limited to the injection site that did not correlate with BBB scores. We conclude that L2 interneuron loss induces lasting paraplegia independent of motoneuron loss and white matter damage, supporting suggestions that circuitry critical to the generator of locomotor activity (the CPG) resides in this area. This injury model appears to be ideal for assessing neuron replacement strategies, and will allow functional aspects of gray matter loss and replacement to be evaluated in the absence of white matter dependent deficits. To this end, we have initiated studies grafting neural precursor cells isolated from adult and embryonic rat subventricular zone into the KA injury site. Survival, differentiation and integrator of engrafted cells is currently being examined. Supported by the Norton Healthcare System Louisville, by the Kentucky Spinal Cord and Head Injury Research Trust (DSKM) and by NS26887, NS62349(SRW).

CNS STEM CELL DIFFERENTIATION AFTER ENGRAFTMENT INTO THE ADULT NORMAL AND CONTUSED RAT SPINAL CORD

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Proliferating populations of undifferentiated neural stem cells were isolated from the embryonic day 14 rat cerebral cortex or the adult rat subventricular zone (SVZ). These cells were pluripotent through multiple passages, retaining the ability to differentiate in vitro into neurons, astrocytes and oligodendrocytes, as assessed both morphologically and electrophysiologically. Treatment with PDGF or BDNF increased the number of neurons, T3 oligodendrocytes and CNTF astrocytes. Two weeks to two months after engraftment of undifferentiated, BrdU-labeled stem cells into the normal adult spinal cord, large numbers of surviving cells were seen. The majority of the cells differentiated with astrocytic phenotype, although some oligodendrocytes and undifferentiated, nestin-positive cells were detected; NeuN positive neurons were not seen. Labeled cells were also engrafted into the contused adult rat spinal cord (moderate NYU Impactor injury), either into the lesion cavity or the white or gray matter both rostral and caudal to the injury epicenter. Up to 1 month post-grafting, cells either differentiated into GFAP-positive astrocytes or remained nestin-positive; no neurons or oligodendrocytes were detected. These results show robust survival of engrafted stem cells, but a differentiated phenotype restricted to the astrocyte lineage. We suggest that neuronal induction in vitro prior to transplantation will be necessary for large numbers of these cells to differentiate into neurons or oligodendrocytes. Supported by NS26887 and the Kentucky Spinal Cord and Head Injury Research Trust.

INTRASPINAL GRAFTING OF EMBRYONIC SPINAL CORD STEM CELLS INTO ADULT RATS

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The abilities of neural stem cells to self-renew, differentiate into multiple cell types and be genetically modified by viral vectors make them desirable as transplants for spinal cord injury. The transplanted stem cells have the potential to provide a bridge for regenerating axons and to replace cells lost at the lesion site. We have previously shown that spinal cord (SC) stem cells isolated as neurospheres from E14 Sprague Dawley (SD) or Fischer 344 (F344) rats expand in the presence of EGF and bFGF, self-renew and maintain the ability to differentiate into neurons and glia in vitro for a long period. Exposure of these cells to serum in the absence of EGF and bFGF promotes differentiation of astrocytes whereas retinoic acid treatment promotes differentiation of several different neuronal phenotypes. The SC stem cells can be successfully modified by adenoviral vectors, will survive grafting into the immunosuppressed SD rat and non-immunosuppressed F344 rat and express the transgene for several months. We have now examined the ability of these stem cells to differentiate into multiple cell types when grafted into the injured SC and their permissiveness for host axon growth. Immunocytochemical analysis of the stem cells grafted with exogenous BDNF revealed that they remain multipotential in vivo and differentiated into neurons, astrocytes and oligodendrocytes. The stem cell grafts are permissive for growth by several populations of host axons as demonstrated by penetration of axons immunopositive for 5-HT and CGRP, originating from neurons in the brainstem and dorsal root ganglia. These results demonstrate that grafted SC stem cells may be used for cell and gene therapy in spinal cord injury to promote regeneration and recovery of function. Supported by NIH grant NS24707, PVA, EPVA, VA and MCP Hahnemann University.

THE EFFECTS OF C6-R CELLS ON EMBRYONIC SPINAL CORD STEM CELLS: CO-CULTURE STUDIES AND INTRA-SPINAL GRAFTING

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The C6-R is a radial glial-like cell line that contains the GFP transgene and exhibits a polarized morphology of long parallel processes in culture. Previous studies have shown that co-culturing of the C6-R cells with cerebellar granular cells promoted the migration of neurons and their neurites along the radial processes (Friedlander et al., J. Neurobiology. 37:291-304, 1998). In this study we examined 1) the properties of the C6-R cells when co-cultured with multipotential stem cell neurospheres, isolated from embryonic spinal cord in the presence of EFG and bFGF and 2) the behavior of the C6-R cells when grafted into injured spinal cord. Spinal cord stem cells plated with C6-R cells migrated out of the neurospheres along the radial-like processes of the C6-R cells. After four days of co-culturing, some of the stem cells were positively stained for the early neuronal marker TuJ-1, and exhibited long neurites that appeared oriented along the radial-like processes of the C6-R cells. When the C6-R cells were grafted into a partial hemisectioned spinal cord of adult rat, they showed good survival and integration into the lesion site. In addition, they were oriented along the rostral-caudal axis and migrated from the graft site to white matter. Current studies are examining the effects of grafting C6-R cells together with spinal cord stem cells in terms of their survival, migration and differentiation as well as possible recovery of function in the injured spinal cord. Supported by NIH grant, EPVA, VA and MCP Hahnemann University.

ENVIRONMENTAL SIGNALS INFLUENCING NEURONAL DIFFERENTIATION OF ADULT-DERIVED SPINAL CORD STEM CELL CULTURES

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The adult rat spinal cord contains FGF-2-responsive stem cells that are self-renewing and have the ability to generate cells that express antigenic properties of neurons and glia in vitro. Little is known about the molecular mechanism regulating stem cell differentiation along specific neuronal pathways. The specification of neuronal diversity in the CNS appears to be controlled by inductive signals secreted by embryonic organizing centers.

These signals apparently define neuronal fates by regulating the expression of cell-intrinsic determinants, many of which are transcription factors. Specific effects of sonic hedgehog (shh) and other factors influencing cultured spinal cord stem cell fate remain to be determined. Using spinal cord derived stem cell clones, we examined the effects of retinoic acid (RA), forskolin (FSK) and shh on the acquisition of neuronal phenotypes. We also examined the effects of RA and FSK on the pattern of expression of transcription factors such as Pax 6, nkx2.2, isl-1, isl-2, Hb-9 and Lim 3. These transcription factors may contribute to neuronal diversification and serve as markers for ventral neural progenitors and motor neuron subtypes. Exposure to RA and FSK promotes expression of neuronal phenotypes, and some of these cells were immunoreactive for GABA, calbindin, acetylcholine esterase and tyrosine hydroxylase. Choline acetyl transferase was also detected using RT-PCR. However, exposure to shh promoted cell proliferation and not differentiation. With regard to expression of various transcription factors, nkx2.2, Hb-9 and isl-1 was not influenced, whereas the expression of Pax 6, isl-2 and Lim 3 was either induced or repressed as compared to cells grown in FGF-2. Preliminary data suggest that progenitor cells can differentiate into multiple classes of neurons and that this differentiation can be modulated by environmental signals. Supported by a grant from the PVA Spinal Cord Research Foundation and contract NINDS NO1-NS-6-2348.

XENOTRANSPLANTATION OF *DROSOPHILA* EMBRYONIC NERVE CELLS INTO MAMMALIAN BRAIN: MOLECULAR GENETIC AND CLINICAL ASPECTS

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Transgenic *Drosophila melanogaster* stocks bearing foreign genes (*lacZ*, *gdnf*, *bdnf* and *ngf*) were selected. Constructs based on the CaSper vector, containing bacterial gene *lacZ* or other foreign genes, were made and microinjected into *Drosophila* embryos. Modern technique allows localization of *Drosophila* cells transplanted to a foreign brain by X-gal staining. The constructs containing genes coding for GDNF, BDNF and NGF were made and introduced into *Drosophila* genome. It was shown that embryonic nerve cells from *Drosophila* neuromutants survived in mammalian brains (rats, mice) for 10-12 days. Subsequently, they were attacked by macrophages. A combination of homografts and xenografts of embryonic nerve tissue was transplanted into rat brain. *Drosophila* cells prevented formation of glial scar and stimulated the vascularization of the graft area. The presence of *Drosophila* cells promotes survival and differentiation of homograft cells. This method has been successfully applied to the treatment of Parkinson's disease in humans.

STEREOTACTIC IMPLANTATION OF LBS-NEURONS INTO PATIENTS WITH BASAL GANGLIA STROKE: RESULTS OF A PHASE I TRIAL

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In an animal model of focal cerebral ischemic injury, implantation of hNT-neurons led to the amelioration of cognitive and functional motor deficits. In this first human trial of neural cell implantation for stroke, LBS-neurons (clinical grade hNT-neurons) were stereotactically implanted into the basal ganglia of 12 patients with focal ischemic brain injury. The subjects, aged 40 to 75, had strokes involving the basal ganglia from 6 months to 6 years prior to entering the study. All had stable neurologic deficits for at least 2 months prior to implantation. LBS-neurons were thawed and resuspended in buffer at a density of 33,333 cells/ μ l. The first four patients were treated with a total of 2 million neurons implanted at 3 sites within the basal ganglia along a single needle pass. The next 8 patients were randomized to receive either 2 million neurons as above or a total of 6 million cells implanted at 3 sites along each of 3 needle passes. Patients were treated with cyclosporin from one week prior to implantation to up to 8 weeks post implantation. Assessment of safety and efficacy was performed through observer dose-blinded

assessments using the NIH Stroke Scale (NIHSS), European Stroke Scale (ESS), Short Form 36 (SF36) and Barthel Index (BI). PET scans were performed at baseline and Month 6. Presently, 8 patients receiving 2 million cells and 3 patients receiving 6 million cells have outcome measures and PET scans through Month 6. Of these 11 patients, 8 (73%) had improvement in performance on the NIHSS and 5 (45%) patients improved on the ESS. NIHSS scores improved in 5 of 8 (63%) patients receiving 2 million cells and 3 of 3 (100%) patients receiving 6 million cells. For those patients that improved, mean improvement was -1.4 points on the NIHSS. ESS scores improved in 3 of 8 (38%) patients receiving 2 million cells and 2 of 3 (67%) of patients receiving 6 million cells. For those patients that improved, mean improvement was +5.9 points on the ESS. Of those patients that had no improvement or worsened, mean change was -1.3 points on the ESS and 1.0 points on the NIHSS. PET scans positively correlated with both outcome measures. No patients had adverse events related to the implantation of LBS-neurons.

AXONAL REGROWTH IN ACRYLAMIDE INTOXICATED MICE: ANALYSIS BY FILM MODEL AND MOLECULAR ORBITAL CALCULATIONS

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Acrylamide (AAM), N-methyl acrylamide (NMA), N-ethylacrylamide (NEA), and methacrylamide (MAA) damage the nervous tissue. After receiving acrylamides at 500 ppm concentration in the drinking water for 21 days, mice develop clinical symptoms: Skeletal muscles become atrophied and hind-limb spray reflexes appear to be exaggerated when the animals are quickly picked up by their tails. These clinical symptoms, however, can not be assessed quantitatively. Acrylamides also impair peripheral nerve regeneration. A film model, in which a proximal stump of the transected common peroneal nerve is sandwiched between two sheets of thin plastic film and maintained in vivo, enabled us to clarify the early outgrowth of the regenerating axons. We applied this film model to acrylamide intoxicated mice. The regenerating axons were retarded in growth in mice intoxicated with AAM followed by NMA, MAA, and NEA, and the degree of retardation showed 3.00, 2.25, 1.31 and 0.19, respectively. The axonal retardation seemed to be induced by the unusual accumulation of neurofilaments, the decrease in the number of tubules, and a refractory state of the axons to stimuli from migratory Schwann cells. To address a reason why the degrees of retardation were different among these four chemicals, the chemical structure of the acrylamides, especially their lowest unoccupied molecular orbit (LUMO) was analyzed by computer. The LUMO electric potential of AAM, NMA, MAA, and NEA was 0.166, 0.172, 0.189 and 0.255 eV, respectively. The electron potential (x) was the negative coefficient of the degree of retardation (y), as expressed by $y=0.054/(x-0.148)$. The structure and localization of LUMO were the same among the acrylamides examined. A pair of orbits was localized at nitrogen of the acrylamides and two pairs of the bigger orbits were at -C=C-CO. An electrical polarization exists in the carbonyl group. Hereby, received by the acrylamides in accordance with the LUMO electric potential, the electrons in situ, for example in the nervous tissue, might be attracted to the LUMO at carbon of the carbonyl group, and then move toward the LUMO at the acryl group.

A VISUO-MOTOR PARADIGM FOR ASSESSING THE RECOVERY OF SENSORY AND MOTOR COMPONENTS OF HAND FUNCTION FOLLOWING MEDIAN NERVE REPAIR

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Using histological and electrophysiological techniques, we have previously demonstrated that nerve guide tubes and growth factor treatments can improve the accuracy of motor neuron regeneration following surgical repair of rat femoral nerve. These data leave unanswered seemingly simple yet surprisingly complex questions such as "Is recovery of function improved?" To evaluate our surgical intervention's effect on primate hand function, we have developed an experimental paradigm capable of assessing the contributions of the median nerve's sensory and motor components that control the force exerted in a "precision-grip" task. Cynomolgous monkeys were trained to grasp a custom built manipulandum between the thumb and forefinger and exert finely graded grip forces. A computer monitor pre-

sented a visual display indicating the target level of force to be applied and gave continuous visual feedback indicating the actual amount of force being applied by the subjects. We evaluated the precision with which the subjects could control their grip force at three target levels, corresponding to delicate, moderate and firm grip forces. Application of a local anesthetic was used to produce "functional lesions" of the median nerve 1) at the level of the forearm, impairing both sensory and motor components or 2) at the base of digits I and II, impairing only the sensory component of the median nerve used in performing this task. The results indicate that: 1) although clearly compromised, the subjects were able to perform the task adequately following anesthetization of the median nerve at the level of the forearm; 2) the precision-reducing effect of digital anesthetization was greatest at the low target force, while 3) the precision-reducing effect of anesthetization of the median nerve at the level of the forearm was greatest at the high target force. Thus, as expected, both cutaneo-sensory feedback and voluntary motor control functions of the median nerve contribute to outcome of the precise control of grip force. This behavioral task will allow us to assess the functional recovery due to each of these components following performance of our experimental surgery. Supported by NIH NS 22404-13 (RM) and the Merit Review program of the U.S. Department of Veterans Affairs (RM).

INDUCTION OF PLASMINOGEN ACTIVATOR SYSTEM COMPANIES PERIPHERAL NERVE REGENERATION AND MICE LACKING INDIVIDUAL GENES SHOW DELAYED RECOVERY

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Plasminogen activator (PA)-dependent fibrinolysis has been demonstrated at the growth cones of regenerating sensory neurons in culture. These sensory neurons increase their tPA, uPA and uPA receptor (uPAR) mRNA levels up to 160-fold during their maximal rate of axonal regrowth. To determine if similar increases are seen in vivo, tPA, uPA and uPAR mRNA levels and enzymatic activity were studied during nerve regeneration. Knockout mice (tPA^{-/-}, uPA^{-/-} and plasminogen^{-/-}) were included in the studies to examine the effects of the absence of tPA, uPA and plasminogen on the rate of regeneration. Sciatic nerves were crushed in wild type (WT), tPA^{-/-}, uPA^{-/-} and plasminogen^{-/-} mice. Functional recovery was assayed by examining for the recovery of the toe spreading reflex and recovery of responses to pinch and pressure in the hindpaw distal to the crush. tPA, uPA and uPAR mRNA levels in the ganglia and nerve were evaluated by in situ hybridization at several time periods following injury in the crushed nerve and its normal or sham counterpart. Plasminogen-dependent PA activity and PA-inhibitor activity were investigated using gel zymography. In situ hybridization in sensory ganglia revealed that uPAR mRNA levels increase above sham levels by 1 day, uPA by 3 days and tPA by 7 days post-crush. These levels decrease towards pre-crush levels by 14 days following crush. In situ mRNA also increases distal to the crush site with uPA levels increasing by 8hrs post crush and tPA and uPAR mRNA levels by 7 days. Gel zymography indicated that PA-dependent enzymatic activity is increased up to four-fold in nerves that have been crushed when compared to the uncrushed counterpart; uPA activity is upregulated by 1 day post-crush and tPA by 3 days post-crush. The upregulation of the lone PA in either the tPA^{-/-} or the uPA^{-/-} mice was also apparent. There were no significant changes in PA-inhibitor activity between crush and sham following the injury. However, significant differences were seen in the rate of functional recovery between WT and knockout mice, some knockout mice showed delays in recovery up to 42% longer. These results suggest that PAs play an important role in facilitating timely nerve regeneration. Supported in part by NSF-IBN-9630458 and NIH-NS09818 (NWS) and T32-NS07083 and T32-HD07408 (LBS).

KINETICS OF FACIAL MOTONEURON (FMN) LOSS FOLLOWING FACIAL NERVE TRANSECTION IN SEVERE COMBINED IMMUNODEFICIENT (scid) MICE

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Four weeks after right facial nerve axotomy, FMN survival in C.B-17 (+/+) wild-type mice is 87% ± 3.0 of the unaxotomized left side control. In

contrast, facial nerve axotomy in C.B-17 (-/-) scid mice, which lack functional T and B lymphocytes, results in an average FMN survival of 55% ± 3.5 relative to the unaxotomized left side control, a 40% decrease in FMN survival 4 weeks post-axotomy, compared to wild-type. Reconstitution of scid mice with wild-type splenocytes containing T and B lymphocytes restores FMN survival in these mice to wild-type levels (Serpe et al., J. Neurosci. 19: RC7 (1-5) 1999). In this study we examined the kinetics of FMN survival by extending the post-axotomy time to 10 weeks. Surprisingly, FMN survival in wild-type controls and reconstituted scid mice also significantly decreased to approximately 60% of uninjured controls at 10 weeks post-axotomy. Scid mice showed a significant decrease in FMN survival by the end of the first week postoperative. In contrast, wild-type and reconstituted scid mice did not show any significant decrease in FMN survival until 4 weeks post-axotomy. These results may be due, in part, to the loss of target-derived neurotrophic factors (NTFs), with permanent target disconnection. Since T cells secrete NTFs, e.g., BDNF, it is hypothesized that, following facial nerve transection in wild-type controls, NTFs released from activated T cells initially support neuronal survival until target reconnection. Supported by NIH grants NS28238 (KJJ) and AI37326 (VMS).

TESTOSTERONE TREATMENT OF FACIAL NERVE TRANSECTION: EFFECTS ON GLIAL FIBRILLARY ACIDIC PROTEIN (GFAP) LEVELS IN THE HAMSTER FACIAL MOTOR NUCLEUS (FMN)

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Administration of testosterone propionate (TP) coincident with facial nerve injury accelerates the rate of recovery from facial muscle paralysis in the hamster. One of the mechanisms by which TP could augment peripheral nerve regeneration is through regulation of GFAP mRNA in the FMN. Axotomy alone induces increases in GFAP mRNA, with TP significantly attenuating the axotomy-induced increases in GFAP mRNA. In the present study, immunoblotting techniques were used to develop the studies of GFAP mRNA to the protein level. Castrated male hamsters were subjected to right facial nerve transection, with half of the animals receiving subcutaneous implants of 100% crystalline TP. The left FMN of each animal served as an internal control. Postoperative (po) survival times were extended from the previous study to include 4, 5, 7, 14 and 21d. Facial nerve transections alone increased the level of GFAP in all experiments by 7dpo, with some animals upregulating GFAP as soon as 4 dpo, relative to internal controls, in non-TP treated animals. Increased levels of GFAP remained elevated at 14 and 21 dpo, relative to internal controls, in non-TP treated animals. Peak effects of axotomy alone were seen at 7 dpo. As seen at the mRNA level, treatment with TP attenuates the axotomy-induced increase in GFAP levels. This was seen at 7dpo only, after which, TP lost its effect by 14 dpo. These results suggest that the regulatory actions of gonadal steroids on GFAP expression, manifested in parallel at the mRNA/protein levels. Supported by NIH NS28238 (KJJ).

NIMODIPINE IMPROVES THE ACCURACY OF VIBRISSAE MUSCLE REINNERVATION AFTER FACIAL-FACIAL ANASTOMOSIS IN RATS

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Following facial-facial nerve anastomosis (FFA) in rats, all motoneurons survive and regenerate, but the reinnervation is random with pathological hyperinnervation e.g. of the vibrissae muscles of the whiskerpad, and the myotopic organization of the facial nucleus is completely lost. This misdirection of reinnervation severely impedes function. As nimodipine (Bay E9736) does not only accelerate neuronal sprouting, but also greatly reduces the hyperinnervation of the whiskerpad after FFA (J. Neurosci. 16:1041-1048, 1996), we tested with double labeling, whether this drug might reduce the misdirection of reinnervation and improve the functional outcome of regeneration. In 12 rats 100 µl of 1% Fluorogold (FG) were injected into both whiskerpads. 10 days later, when all facial motoneurons correctly innervating the vibrissae muscles, had been labeled by FG, microsurgical FFA of the right facial nerve was performed and subsequently 6 rats were treated orally with nimodipine (1000 ppm in food pellets) and 6 rats with placebo. 56 days

later, which is sufficient time for regeneration, 100 μ l of 1% Fastblue (FB) was injected bilaterally at the same spot as FG in all animals and 10 days later, after completion of FB-labeling, the frequency of whisking was analysed by video observation. Then the rats were killed by trans-cardial perfusion fixation and the numbers of FG-, FB- and double-labeled motoneurons were counted in 50 μ m serial vibratome sections. Normal rats had a spontaneous whisking frequency of 5.9 Hz. After FFA the whisking was reduced to 0.8 Hz with placebo, but still at 4.4 Hz with nimodipine. Neuron-counting revealed on the unoperated side a mean of 93.3% (placebo) and 94.4% (nimodipine) double-labeled neurons, which is sufficiently close to the theoretical value of 100% to prove the reliability of the labeling technique. On the side of FFA in placebo-treated rats only 11.6% (151 \pm 36 of 1302 \pm 41; mean \pm S.D., n = 6) of those motoneurons preoperatively labeled with FG were also postoperatively double-labeled with FB, i.e. had correctly reinnervated the original target. In the nimodipine-treated rats however 34.5% (406 \pm 66 of 1176 \pm 96) motoneurons were double-labeled; this difference is highly significant. In summary, nimodipine-treatment after nerve suture improved the morphological correctness and the functional outcome of muscle reinnervation. Supported by the Bayer AG and COST B10 "Brain Damage Repair".

ADAPTABILITY OF MOTOR AND PREMOTOR NEURAL CENTERS GENERATING UNCONDITIONED AND CONDITIONED EYELID RESPONSES FOLLOWING HYPOGLOSSAL-FACIAL AND FACIAL-FACIAL ANASTOMOSIS IN CATS

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The nictitating membrane/eyelid response is a well-known model to study the kinetic and frequency-domain properties of reflex and learned motor responses. The model was used here to follow the adaptability of hypoglossal and facial motoneurons and the corresponding premotor circuits to the de novo reinnervation of the orbicularis oculi (OO) muscle. Cats were prepared for the chronic recording of eyelid and tongue movements and of the EMG activity of the OO muscle. After control recordings, three types of anastomosis were carried out: i) a hypoglossal-facial anastomosis; ii) the proximal stump of the buccal branch of the facial nerve was anastomosed to the distal stump of the frontozygomatic branch; and iii) the ophthalmic branch of the facial nerve was sectioned, rotated 180 deg and resutured. Recordings of reflexively-evoked blinks were carried out up to one year following the anastomosis. Animals were also classically conditioned with both delay and trace procedures. Results indicate that hypoglossal motoneurons are unable to adapt their discharge properties to the motor needs of eyelid responses. Facial (buccal) motoneurons were able to respond to corneal stimuli and, more interestingly, seemed to be susceptible of a (weak) classical conditioning of the eyelid motor response. The section and resuture of the ophthalmic facial nerve branch altered the normal performance of air-puff evoked blinks, but did not affect the performance of new motor responses. Some plastic, compensatory changes were induced in the retractor bulbi system, an additional eyelid motor system not directly affected by the anastomosis. A gradient of adaptability seems to be involved in the different eyelid performance following each experimental anastomosis. Supported by the Acciones Integradas Hispano-Alemanas and COST B10 Brain Damage Repair.

ACCELERATED AND ENHANCED MUSCLE REINNERVATION WITH REDUCED COLLATERAL AXONAL SPROUTING DURING A DEFINITE DENERVATION PERIOD USING A DELAYED CROSS-ANASTOMOSIS PARADIGM

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To establish the influence of denervation time on the recovery of motor nerve, the rat facial nerve was transected and denervated for 0 to 224 days. Then, the freshly transected hypoglossal nerve was sutured to the predegenerated facial nerve (hypoglossal-facial nerve anastomosis, HFA). Using this nerve cross-anastomosis paradigm, we analyzed the nerve regeneration and muscle reinnervation 7 to 112 days post suture operation (DPSO). After HRP injection into the whiskerpad 931 \pm 27 hypoglossal neurons were labeled at 112 DPSO after immediate HFA. Following 14-112

days denervation, the number of labeled neurons increased to 138% (14 day delay), 154% (56 days) and 145% (112 days). In contrast, the reinnervation was poorer after 7 days denervation with 84% respectively 81% after long-term denervation of 224 days. The increase in amplitude of evoked electromyography wave after nerve suture correlated with the number of labeled neurons. After immediate HFA each regenerated motoneuron established on average 5.1 myelinated sprouts at 112 DPSO. The number of sprouts stayed constant after 14 to 112 days delayed suture, whereas the slower reinnervation after 7 or 224 days delay was accompanied by a massive sprouting of 9.1 respectively 8.1 sprouts per neuron. The muscles showed complete recovery after any denervation time. The muscle cross-sectional area continuously decreased with longer denervation time. This decrease only was significant after 224 days denervation (67% of the normal value). We conclude that motor nerve reconstruction achieves better functional results after a definite period of denervation when using a nerve cross-anastomosis paradigm. Supported by Köln Fortune Program (10/98) to OGL.

ABNORMAL SENSORY INPUT AS A RESULT OF INFRAORBITAL NERVE LESION CHANGES THE PROPERTIES OF THE REGENERATING FACIAL MOTONEURONS

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A lesion of the infraorbital trigeminal nerve (ION) reduces polyneuronal muscle innervation after cut and re-anastomosis of the buccal branch of the facial nerve (BBA) in the rat (Eur. J. Neurosci. 11:1369-1378, 1999). The whiskerpad of this animal receives only motor innervation from facial motoneurons. The polymodal sensory input is carried by the ION to the trigeminal complex, the second order sensory neurons of which establish synapses with the facial motoneurons. We hypothesized that an abnormal central sensory processing due to the ION lesion may cause the reduced sprouting of regenerating facial motoneurons. To disclose this issue further, we have combined EMG and morphometry, together with a study on the expression of AMPA GluR2/3 and synaptophysin with immunocytochemistry (ICC). One group of rats had BBA on the right side (BBA), the second had BBA and ION lesion, both on the right side (BBAipsi), and the third group had right BBA and left ION (BBAcontra). Forty-five days after BBA, vibrissae EMG revealed, that supramaximal stimulation of either facial nerve or ION caused an attenuated direct response but declined reflex response, compared to the left side. After BBAi we found increased direct and reflex response on the right side; finally in rats with BBAC the direct response had declined, whereas the reflex increased on the right side. At 17 days survival, measurements of the cell sizes of axotomized facial neurons labeled with FC prior the anastomosis, demonstrated that those sampled from BBA display on average significantly smaller cell sized compared with BBAi and BBAC. In addition to this, the ICC with synaptophysin showed decreased signal from the facial nucleus ipsilateral to the BBA lesion; the signal further decreased bilaterally after either one ION lesion combined with BBA. In axotomized neurons sampled from BBAipsi and BBAcontra, AMPA GluR2/3, normally down regulated after BBA, showed little, but significant increase of the ICC signal; whereas the opposite holds true for nonaxotomized motoneurons. Our results suggest that the altered central processing triggered by the sensory injury may have a certain importance upon regenerating facial motoneurons, taking in account the change of their specific membrane properties, modulated receptor expression, and pre-synaptic vesicle content. Supported by COST B10 "Brain Damage Repair" and the Jean-Uhrmacher Foundation.

ACCURACY OF FASCICULAR REINNERVATION AFTER GRAFT AND TUBE REPAIR OF 8 MM LONG GAPS IN THE RAT SCIATIC NERVE

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Misdirected reinnervation and muscle hyperinnervation are considered as some of the major phenomena hampering motor functional recovery after

peripheral nerve injuries. Sprague-Dawley rats were submitted to an 8 mm sciatic nerve resection, repaired by a nerve autograft (AG, n=10), a single (SIL, n=12) or fascicular silicone tube (SILD, n=8) and a poly (L-lactide-co-ε-caprolactone) resorbable guide (PLC, n=8). After 90 days follow-up, the peroneal and tibial branches were dissected above the injury and stimulated through a suction electrode while recording the compound muscle action potentials (CMAP) of proximal (gastrocnemius and tibialis anterior) and distal (plantar) muscles. The total amount of functional reinnervation (% of control CMAP amplitude) and the percentual contribution of tibial and peroneal reinnervation (% of sciatic CMAP amplitude) were measured. In other animals of each group (n=6), the peroneal nerve innervating tibialis anterior muscle and the tibial branches innervating lateral gastrocnemius and plantar muscles were labeled with DiI, Fast Blue and FluoroGold retrotracers respectively. The number of single and multiple labeled motoneurons were counted under fluorescence microscopy to obtain the percentage of motoneurons with simultaneous projections to different muscles. SILD group achieved the poorest levels of reinnervation in gastrocnemius, tibialis anterior and plantar muscles (43%, 22%, 11%) but the lowest percentage of misdirected functional reinnervation (5%, 10%, 0%). PLC guides allowed a similar amount of reinnervation (56%, 46%, 25%) to AG (62%, 51%, 22%) but lower misdirected reinnervation (25%, 38%, 26% vs 47%, 53%, 44%). Finally, SIL tubes showed lower reinnervation (52%, 35%, 14%) and higher percentage of misdirected reinnervation (42%, 42%, 41%). The percentage of motoneurons with dual or triple labeling due to multiple muscle projections was higher in SIL group (10% of total) than in PLC and AG groups (6%). We conclude that resorbable PLC guides offer a good balance between amount and accuracy of reinnervation in mid length gap repair in the rat and may be considered as an alternative to nerve autograft repair. Supported by CICYT grant and COSTB10 "Brain Damage Repair".

ASSESSMENT OF POLYIMIDE CUFF ELECTRODES FOR IN VIVO PERIPHERAL NERVE STIMULATION

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Functional electrical stimulation (FES) systems have been developed in order to artificially replace the central motor control and directly stimulate the intact peripheral nerves or muscles of spinal cord injured patients. Cuff electrodes are the most widely used type for nerve stimulation, but they can induce damage to the implanted nerve depending upon their physical properties. We have tested a new tripolar self-sizing spiral cuff electrode suitable for chronic peripheral nerve stimulation. The device is composed of a thin (10µm) and flexible polyimide insulating carrier (PI2611, DuPont), with the cuff part rolled to become a cylinder with a diameter of 2 mm and a length of 12 mm. Three circumneural platinum electrodes are arranged with 5 mm interelectrode distance. Cuffs were implanted around the sciatic nerve of two groups of ten rats each, one in which the polyimide ribbon was attached to an external plastic connector to characterize the in vivo stimulating properties of the electrode and one without connector for testing possible mechanical nerve damage by means of functional and histological methods. The polyimide cuffs induced only a mild foreign body reaction and did not change the nerve shape over a 2 month implantation period. There were no changes with respect to the intact contralateral limb in motor and sensory nerve conduction tests, nociceptive responses and walking track pattern during the 2 month follow-up and no morphological evidence of axonal loss or demyelination. By delivering single electrical pulses through the cuffs, graded recruitment curves of alpha motor nerve fibers innervating plantar muscles were obtained. Recruitment of all motor units was achieved with a mean charge density lower than 4 µC/cm² for a pulse width of 50 µsec at the time of implantation, as well as 45 days thereafter. These data indicate that the polyimide cuff electrode constitutes a stable stimulating device, with physical properties and dimensions that avoid nerve compression or activity-induced axonal damage.

ADVANCES IN NERVE REGENERATION RESEARCH USING PULSED MAGNETIC FIELDS

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Enhanced growth and directionality of dorsal root ganglia (DRG) neurites have been observed in chick embryo (reported by Sutton et al., in previous Symposia) and in pup embryo obtained from 15-day time-pregnant Sprague-Dawley rats (reported by Battocletti, et al. at the 1997 Symposium). The report presented here finalizes the DRG study. Preliminary results of an ongoing in vivo study of PMF applied to an injured portion of the spinal cord in the cat are also presented. In the DRG study, a novel, large, rectangular-shaped sheet coil was used to generate a rapidly-changing magnetic vector potential and to obtain a uniform and linearly-directed induced electric field of about 0.25 volt/meter in multiple DRG cell cultures, which was uniform in both direction and magnitude within a tolerance of 5%. The coil was energized by a Power Amplifier (Tecron Model 7570) driven by a Waveform Generator (Wavetek model 95), programmed to produce a train of pulses similar to that used in bone healing. It was demonstrated, both visually and statistically, that PMF-exposed DRG exhibited asymmetrical outgrowth parallel to electric field and enhancement of neurite length. DRG cultures not PMF-exposed had a characteristic radial pattern of neurite outgrowth. In the in vivo cat study, PMF was applied to an injured portion of the spinal cord using a Figure-8 coil, designed and positioned so that the primary magnetic vector potential was directed parallel to the spinal column. While anesthetized, a mid/hi thoracic laminectomy was performed to expose the dural sac of the spinal cord. A standard weight dropping procedure was used to administer a 100, 200 or 400 gm-cm drop on the exposed spinal cord. Cats were randomly divided into an experimental and control group by the primary investigator. Experimental specimens received PMF stimulation for four hours daily. Control specimens received no stimulation. Specimens were evaluated weekly using the Modified Tarlov Score. The investigator performing the evaluations was blinded as to which group each specimen belonged. Specimens were survived for twelve weeks or when the Tarlov score was at least 4, indicating consistent ambulation with both limbs and solid gait with minimal toe and belly drags. Preliminary analysis of the results indicates that the cats receiving the PMF recovered faster than the control group. The spinal cord was removed and sectioned for lesion volume analysis and axon counting. These evaluations were performed by individuals blinded as to which group the specimen belonged.

EVIDENCE THAT MULTIPLE CONNEXINS ARE EXPRESSED IN THE ENTERIC NERVOUS SYSTEM

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The enteric nervous system (ENS) is the intrinsic nervous system of the gastrointestinal tract. The ENS is the largest and most complicated division of the peripheral nervous system. Enteric glial cells, the supporting cells in the ENS, exhibit structural features which render them more similar to astrocytes in the CNS than Schwann cells, the predominant glial cell type in other regions of the PNS. Moreover, enteric glia, like astrocytes, exhibit extensive gap junctional communication forming a chemically and/or electrically coupled syncytium. Given the similarities between enteric glia and astrocytes, we hypothesize that enteric glia express a repertoire of gap junction proteins, termed connexins (Cx), similar to that of astrocytes, and dissimilar to the restricted expression of Cxs by Schwann cells. To test this assertion, we carried out an immunohistochemical study of Cx expression in the ENS. In cryostat sections of rat proximal colon, myenteric ganglia (which comprise the largest nerve plexus of the ENS) were identified using a mouse monoclonal antibody which detects the expression of glial fibrillary protein in enteric glia. Sections were co-labeled with polyclonal rabbit antibodies to specific rodent Cxs. To date, we have detected Cx33, 44, 45 and 46-like immunoreactivity in the ENS. These results indicate that the ENS, like the CNS, utilizes multiple Cxs to mediate gap junction communication. The results may explain, in part, the differential ability of ENS neuropil to integrate into host CNS tissue following transplantation, compared to tissue containing Schwann cells, which does not integrate in the CNS neuropil.

DEGENERATIVE AND REGENERATIVE CHANGES IN RAT UTERINE INNERVATION DURING THE ESTROUS CYCLE

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The mature peripheral nervous system undergoes structural reorganization in response to a variety of pathological conditions. However, neuroplasticity also occurs in the normal adult mammal. A dramatic example of nerve remodeling under physiological conditions is cyclical alterations of the nerve density in the uterus of the adult virgin rat (Zoubina et al., 1998). Studies using Protein gene Product 9.5 as a pan-neuronal marker show that during the normal estrous cycle density of the uterine innervation decreases at estrus and is restored thereafter. Immunostaining for markers selective for different nerve populations implied that changes are limited to the sympathetic component of the uterine innervation. In the present study we performed quantitative electron microscopic analysis of the uterine innervation during the course of the normal estrous cycle to confirm changes in the uterine nerve fibers on the ultrastructural level, and to determine whether degeneration or retraction accounts for the nerve remodeling in the cycling uterus. In the myometrium, the numbers of the axonal branches decreased by about 50% at proestrus-estrus ($P < 0.01$). Numbers of axons displaying features of degeneration (such as disruption and disintegration of the organelles and axolemma, marked alterations in density of axonal content and the presence of highly osmophilic dense bodies) significantly increased at these stages of the estrous cycle ($P < 0.001$), suggesting that neural degeneration plays a role in the observed decrease in the uterine nerve density. On the other hand, number of axons considered to be potentially retracting (intact varicosities with the increased organelle packing density and/or increased distance from the target) was low and did not change with the cycle. At metestrus and diestrus, growth cones were observed. These findings suggest that terminal axonal branches normally undergo cyclical degeneration and regeneration during the estrous cycle in mature virgin rat.

DYNAMIC INTERPLAY OF NEURAL SIGNALS IN HUMAN CURSOR RELATED CORTEX

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We continue to record robust signals from the motor cortex of our second cognitively intact locked-in patient eighteen months after implantation with the Neurotrophic Electrode. He has learned to use the frequency of neural firings to control cursor movements on a computer screen, though health problems have minimized his ability to do this. He is implanted in hand area 4 of the motor cortex. Recordings begin at post-operative day 11 and remain robust at day 549. Initially, neural activity is related to his face representation that spreads across the cortex in which the electrode is implanted. Thus, face movement initially results in activation of the neural signals, first involving mouth and tongue movements, then eye movements at month four, and eyebrow movements at month five. At month six, the subject lies quietly while driving the cursor across the screen, suggesting that cortical activity is now related to cursor movement. Later protocols performed near month 15 include recording eyebrow EMG signals that drive the cursor vertically down the screen while neural signals drive the cursor across a horizontal row of icons. To succeed, the brow EMG activity must be suppressed. This is achieved each time it is attempted, with learning curves showing improvement in performance. These results suggest that the neural signals are activated by attempts to drive the cursor and not by eyebrow movements. Examination of individual waveshape activity reveals phase relationships between waveshapes of opposite polarity. [Waveshapes have different polarities due to the unique configuration of the recording wires inside the electrode's conical tip. The wires are held in opposite polarities whereas the axons all have external positivity. During membrane depolarization, the axon nearest one wire will have an initial deflection that is opposite in sign compared to an axon close to the other wire.] Waveshapes with up-going polarities usually occur before down-going polarities during voluntary horizontal cursor movements. These phase relationships reverse when driving the cursor vertically down. This dynamic interplay of phase relationships hints at a possible means of changing the direction of cursor movement.