

Abstracts of Poster Presentations—Session 1

P1 MICROGLIAL CELL ACTIVATION AND MACROPHAGE INFLUX IN EXPERIMENTAL RETINAL DETACHMENT AND REATTACHMENT

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We have previously demonstrated both microglial proliferation and an influx of macrophages following experimental retinal detachment. In this study we sought to determine the extent of this reaction at various times following detachment and reattachment. Experimental detachments were created by infusing fluid between the neural retina and retinal pigmented epithelium. Vibratome sections of retina were exposed to biotinylated Griffonia simplicifolia isolectin B4 overnight at 40C. Streptavidin, conjugated to a fluorochrome, was subsequently added overnight at 40C. The sections were viewed using a BioRad 1024 confocal microscope. Isolectin B4 labels both infiltrating macrophages and microglia, but the 2 cell types often could be distinguished by morphology and/or location. In control retina, lightly labeled microglia reside in the inner and outer plexiform layers. At 1 day following detachment, the labeling became more robust with microglia appearing to migrate within the retina. Labeled cells, presumed to be macrophages, also were observed between degenerating photoreceptor outer segment segments. The number of lectin-labeled cells continued to increase at 3, 7 and 28 days of detachment, and these cells were distributed throughout the retina. In retinas reattached for 28 days following a 3 day detachment period, and hence undergoing outer segment regeneration, labeled cells were still observed throughout the retina with

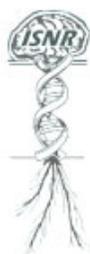
the largest population occurring in regions of continued photoreceptor disruption. Finally, similar results were observed in samples from human detachments using biotinylated Ricinus communis agglutinin I. Other studies have shown that activated microglia may have a detrimental effect on the recovery of neurons following injury. Since both activated microglia and macrophages accumulate in retinal areas undergoing degeneration or with poor recovery, these cells may contribute to the photoreceptor degeneration and cell death associated with detachment.

P2 AXON REGENERATION IN THE MATURE RAT OPTIC NERVE: ISOLATION OF THE KEY MOLECULAR SIGNALS

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In adult mammals, retinal ganglion cells (RGCs) normally fail to regenerate their axons after optic nerve injury. However, macrophage activation in the eye, resulting from either lens injury or intravitreal zymosan injection, enhances RGC survival and enables these cells to regenerate their axons well beyond an optic nerve crush site¹⁻³. We describe here three molecules that act together to stimulate axon regeneration in RGCs. One is a previously unknown 14 kDa macrophage-derived protein (MDP14) that we isolated using gel filtration chromatography, bioassays, and sequencing. Its effects on RGCs require two other molecules: a low molecular weight growth factor that is constitutively present in the vitreous fluid and increased [cAMP]_i. In cell culture, cAMP caused a binding site for MDP14 to appear on the RGC outer membrane. A MDP-AP fusion protein bound to live RGCs in low nanomolar concentrations



only when [cAMP]_i was elevated, and this binding was competitively inhibited by unlabeled MDP14. Although MDP14 binding depends on cAMP only, its biological activity required the presence of the small, vitreous-derived factor as well. We identified the latter as D-mannose using chromatography and mass spectrometry, and showed that its effects on RGCs are highly specific, cAMP-dependent, and unrelated to energy metabolism or glycoprotein synthesis⁴. *In Vivo*, preliminary data indicate that MDP14, D-mannose and cAMP together enhance RGC survival and promote axon regenerating into the distal optic nerve of mature rats.

References: ¹Leon et al., J Neurosci 2000;20: 4615-4626. ²Yin et al., J Neurosci 2003;23: 2284-2293. ³Berry et al., J Neurocytol 1996; 25:147-170. ⁴Li et al., J Neurosci 2003;23: 7830-7838.

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P3 AXON REGENERATION IN THE RAT OPTIC NERVE: ESSENTIAL ROLE OF THE NOGO RECEPTOR

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The failure of lesioned axons to regenerate in the mature CNS is commonly attributed to inhibitory proteins of the glial scar and of myelin. The latter include Nogo-A, OMgp, and MAG, all of which signal, at least in part, through the Nogo receptor, NgR. Deletion of the MAG or Nogo genes, or suppressing the function of NgR or its downstream effectors, have resulted in modest regeneration *In Vivo*, raising the question of the overall significance of NgR to CNS regeneration⁽¹⁾. In mature mammals, although axons that arise from retinal ganglion cells (RGCs) normally fail to regenerate through the optic nerve after injury, factors released from activated macrophages can significantly reverse this situation⁽²⁻⁴⁾.

Using this paradigm, we tested the hypothesis that suppression of NgR will enhance mature CNS regeneration *provided neurons' intrinsic neuronal growth program is reactivated*. NgR functioning was altered by transfecting RGCs with adeno-associated viruses (AAV) expressing either wild-type NgR (NgR-wt) or a dominant-negative mutant (NgR-dn)⁽⁵⁾. Transfections were highly selective to RGCs and highly efficient. Expression of NgR-dn greatly enhanced the ability of RGCs to regenerate their axons through the injured optic nerve, but only when these cells' growth program was activated. Overexpression of NgR-wt prevented RGC regeneration, and caused axons to retract back to the unmyelinated nerve head even when the growth program was activated. These results show that successful regeneration requires both deactivating NgR function and reactivating neurons' intrinsic growth program.

References: ¹Wolf, Neuron 38:153-56(2003); ²Fischer et al. Invest Ophthalmol Vis Sci 12: 3943-54 (2000); ³Leon et al., J Neurosci 20: 4615-26 (2000); ⁴Yin et al., J Neurosci 23: 2284-93 (2003); ⁵Domeniconi et al., Neuron 35:283-90 (2002).

Support: NIH EY05690 and DA15335, BLSI, DFG.

P4 RETINAL DIFFERENTIATION OF HUMAN NEURAL—AND MESENCHYMAL—STEM CELLS

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We have previously reported that transplanted human neural stem cells (HNSCs) display extensive migration and positional incorporation into the aged rat brain, which is associated with an improvement in cognitive function (Qu et al., 2001). We have recently also succeeded to produce neural cells from human mesenchymal stem cells (HMeSC). Because of the multipotency of the stem cells, we hypothesized that these stem cells could be differentiated into retinal cells that may be

used to treat retinal degeneration. In the current study, to investigate whether HNSCs are capable of differentiating into retinal cells in vitro, we treated the cells with transforming growth factor-beta3 (TGFb3, 10-100 ng/ml) before or during differentiation under a serum-free condition. After five days of in vitro differentiation, the stem cells were fixed and double-immuno-fluorescent-stained with anti-opsin, anti-calbindin, anti-protein kinase C (Chemicon), anti-syntaxin (Sigma) and anti-human glial fibrillary acidic protein (GFAP, Research Diagnostics). We found opsin-, calbindin-, protein kinase C- and syntaxin-positive in HNSCs treated with the factors but not without the treatment, indicating the differentiation of HNSCs into photoreceptor, horizontal bipolar and amacrine cells. These retinal markers immunopositive cells were observed not only in the HNSCs treated with these factors during differentiation but also in the cells treated with these factors before differentiation, indicating that these factors are capable of altering cell fate before differentiation. We also transplanted TGF-b3-treated HNSCs and HMeSCs into the rat vitreous cavity. The donor cells migrated and differentiated into opsin-positive cells in the host retinal cell layer. This fact greatly increases the possibility of producing retinal cells from human stem cells, although we have yet to confirm the physiological function of opsin-expressed cells or investigate whether other retinal cell markers are expressed by differentiated human stem cell. Nonetheless, our results suggest that a new and effective treatment for retinal degenerative diseases may be established with stem cell transplantation.

P5 DELAYED TRANSPLANTATION OF LENTIVIRAL-TRANSDUCED OLFACTORY ENSHEATHING GLIA OR SCHWANN CELLS INTO MODERATE SPINAL CORD CONTUSION INJURY

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The olfactory system is unique in the adult mammalian central nervous system (CNS), as its neurons are replaced throughout adult life. Replacement involves axonal extension from the peripheral nervous system into the usually inhibitory environment of the CNS. Olfactory ensheathing glia (OEG) may facilitate this extension, and as such are promising candidates for transplantation into spinal cord injuries, where a primary aim of research is to encourage extension of damaged axons past the inhibitory lesion site toward their targets. Previous studies have shown that transplanted OEG exert beneficial effects in spinal cord injury when transplanted within a week of injury (Plant et al. 2003, J. Neurotrauma 20:1-16). Our aim was to examine their effects with an interval of two weeks between injury and transplantation. Control groups received transplanted Schwann cells, injected vehicle or no treatment. The injury model employed was contusion, produced with the NYU Impactor. Our study is the first to employ a permanent lentiviral vector-based method of labeling to track the cells after transplant into a contusion lesion. It was found that the volume of tissue present at the lesion site after a recovery period of four months was significantly greater when OEG or Schwann cells were transplanted, as compared to no treatment, or an injection of vehicle. No significant difference was found





between OEG and Schwann cell-transplanted groups. Transplanted OEG were shown to survive for at least four months after transplant, and were found in areas containing extracellular matrix molecules including laminin-1 and collagen IV. In conclusion, when transplantation is delayed until 14 days after contusive spinal cord injury, OEG are still beneficial in reducing cavitation, and appear to provide a source of growth-promoting and adhesive molecules such as laminin-1 and collagen IV.

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P6 GENE TRANSFER TO OLFACTORY ENSHEATHING GLIA AND TRANSPLANTATION TO THE INJURED RAT SPINAL CORD

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This study combines olfactory ensheathing glia transplantation with ex vivo adenoviral vector based neurotrophin gene therapy. Primary OEG were transduced with AdV vectors encoding the bacterial marker gene b-galactosidase (LacZ), brain-derived neurotrophic factor (BDNF) or neurotrophin-3 (NT-3), and subsequently implanted into adult Fischer rats directly after unilateral transection of the dorsolateral funiculus. Implanted animals received a total of 2×10^5 OEG that were transduced with a neurotrophin-encoding AdV vector, or subjected to either no or AdV-LacZ transduction (controls). At 4 months after injury, lesion volumes were smaller in OEG implanted rats, and significantly reduced in size if implanted with neurotrophin-encoding AdV vector-transduced OEG. All OEG grafts were filled with neurofilament-positive axons, and AdV vector-

mediated expression of BDNF by implanted cells significantly enhanced the regenerative response of the rubrospinal tract (RST). Behavioural analysis revealed that OEG-implanted rats displayed better locomotion during horizontal rope walking than unimplanted controls. Recovery of hind limb function was further improved following implantation of OEG that were transduced with a BDNF- or NT-3-encoding AdV vector. Hind limb performance during horizontal rope locomotion did directly correlate with lesion size, suggesting that neuroprotective effects of OEG implants contributed to the level of functional recovery. Thus, our results demonstrate that genetic engineering of OEG not only resulted in a cell that was more effective in promoting axonal outgrowth but could also lead to enhanced recovery after injury, possibly by sparing of spinal tissue.

P7 IDENTIFICATION OF SECRETED FACTOR-BASED MECHANISMS EMPLOYED BY OLFACTORY ENSHEATHING CELLS IN PROMOTING NEURAL REGENERATION

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The glia of the olfactory system, Olfactory Ensheathing Cells (OECs) provide a permissive and promotive environment for newly generated olfactory receptor neurons to mature and extend their axons towards their target, the olfactory bulb. Since OECs are able to do so in the adult system, there has been considerable interest in using them to promote axonal repair and regeneration in other regions of the adult nervous system, in particular the spinal cord. These studies, coupled with functional and behavioural analyses, have met with some success however the underlying mechanisms by which OECs mediate axonal growth and repair remain largely unknown. We are attempting to elucidate some of these mechanisms by identifying secreted factors produced by OECs that facilitate axonal elongation and neuronal survival. To this end, conditioned media samples from

OECs cultured under various conditions are being assayed for neurotrophic and neuroprotective activity. We have found that OECs lose their potency for promoting neurite outgrowth in embryonic dorsal root ganglia and neuronal survival with age in culture, which could have a profound influence on the behaviour of OECs when transplanted. The difference in biological activity between the various samples of conditioned media, coupled with proteomic techniques, will allow us to identify the functionally relevant secreted factors produced by olfactory ensheathing cells. By identifying these factors, we hope to develop more effective strategies for utilizing OECs in the treatment of neural dysfunction.

P8 OLFACTORY ENSHEATHING CELLS TRANSPLANTED INTO PHOTOCHEMICALLY INJURED SPINAL CORD CAUSE AN EARLIER, INCREASED AND SHORTED-TIME OF THE INFLAMMATORY REACTION

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Olfactory ensheathing cells (OECs) cultures were obtained from olfactory bulbs of adult female Sprague-Dawley rats. A cellular suspension of OECs (180.000 cels/12µl) or DMEM alone were transplanted into the spinal cord 30 minutes post-injury. Locomotor skills were evaluated through the open-field BBB score during the first 14 days post-operation (dpo). At 3, 7 and 14 dpo, five animals of each experimental group were perfused with a 4% paraformaldehyde solution, and transverse sections of the lesion block were cut and stained against COX-2, VEGF, IL-1β, iNOS, GFAP and tomato lectin. Functional

results showed that OECs transplanted animals displayed higher locomotor outcomes than DMEM injected animals in the BBB scores during all the follow-up. Histological results exhibit that reactivity against COX-2, VEGF, IL-1β, iNOS and LEC was increased in OECs compared to DMEM transplanted animals during the first week post-surgery. However, these levels were reversed at 14 dpo. Moreover, OECs transplanted animals showed a higher density of blood vessel profiles in the ventral gray matter. Therefore, our results show that acute transplantation of OECs into the injured spinal cord improve locomotor skills during the first 14 dpo, probably mediated by an early, higher, and shorter time of the inflammatory response, as well by increasing angiogenesis.

P9 ACUTE TRANSPLANTATION OF OLFACTORY ENSHEATHING CELLS OR SCHWANN CELLS PROMOTES FUNCTIONAL RECOVERY AFTER SPINAL CORD INJURY IN THE RAT

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We have investigated the neurological and electrophysiological outcome, glial reactivity and spared spinal cord connectivity promoted by acute transplantation of olfactory ensheathing cells (group OEC) or Schwann cells (group SC) after a mild thoracic injury in the rat spinal cord. The animals were subjected to a photochemical injury of 2.5 minutes irradiation at the T8 spinal cord segment. Thirty minutes after the injury, a suspension containing 180000 OECs or SCs was injected at the injury site of each animal. A control group (group DM) received injections of the vehicle medium (DMEM F-12) alone. During 3 months post-surgery, the animals behavioural





skills were assessed with the open field-BBB scale, inclined plane and thermal algesimetry tests. Motor (MEPs) and somatosensory evoked potentials (SSEPs) were performed to evaluate the integrity of spinal cord descending and ascending pathways and the lumbar motoneuronal excitability was evaluated by H reflex responses. Finally, the animals were perfused and transverse section of the injured spinal segment were analyzed. Glial fibrillary acidic protein (GFAP) and chondroitin sulfate proteoglycan (CSPG) expression were quantified immunohistochemically, and the preservation of corticospinal and raphespinal tracts was evaluated. Both the OEC and SC transplanted groups showed significantly better results in all the behavioral tests than the DM group. Furthermore the OEC group had higher MEPs amplitudes and lower H responses than the other two groups. At the injury site, the area of spared parenchyma was larger in the transplanted than in the control rats. The OEC animals had reduced astrocytic reactivity and CSPG expression in comparison to the SC and DM groups. Taken together, these results indicate that OEC or SC transplantation have potential for restoration of injured spinal cords. However, OEC grafts are more interesting candidates due to their superior capability to reduce glial reactivity, promote neuroprotection and improve functional recovery.

P10 IN VIVO INTERACTIONS BETWEEN RAT SENSORY AXONS AND MOUSE PERIPHERAL OLFACTORY ENSHEATHING CELLS FOLLOWING DORSAL RHIZOTOMY

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Olfactory ensheathing cells (OECs) may support axonal regrowth, and thus might be a viable treatment for SCI; however, little is known about OEC-host interactions, primarily due to difficulty in identifying grafted cells. We have transplanted OECs from the nasal mucosa of

mice expressing GFP in all cell types into immunosuppressed rats with cervical or lumbar dorsal rhizotomies. OECs were deposited either into the dorsal root ganglion (DRG), into intact or injured dorsal roots, or into the dorsal columns via the dorsal root entry zone (DREZ). OECs injected into the DRG or dorsal root migrated centripetally, and migration was more extensive in the injured root than in the intact root. These peripherally-deposited OECs migrated within the PNS but did not cross the DREZ; similarly, large- or small-caliber primary afferents were not seen to regenerate across the DREZ. OEC deposition into the dorsal columns via the DREZ resulted in a laminin-rich injection track: due to the pipette trajectory, this track pierced the glia limitans at the DREZ. OECs migrated centrifugally through this track, but did not traverse the DREZ; axons entered the spinal cord via this track, but were not seen to reenter CNS tissue. We found a preferential association between CGRP-positive small- to medium diameter afferents and OEC deposits in injured dorsal roots as well as within the spinal cord. In the cord, OEC deposition resulted in increased angiogenesis and altered astrocyte alignment. These data are the first to demonstrate interactions between axons and peripherally-derived OECs following dorsal rhizotomy.

P11 TRANSPLANTATION OF OLFACTORY MUCOSA IMPROVES REINNERVATION AND VIBRISSAE MOTOR PERFORMANCE AFTER FACIAL NERVE REPAIR

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The occurrence of abnormally associated movements is inevitable after facial nerve transection. The reason for this post-paralytic syndrome is poor guidance of regrowing axons, whereby a given muscle group is reinnervated by misrouted axonal branches. Olfactory ensheathing glia has been shown to reduce axonal sprouting and to stimulate axonal regeneration after transplantation into

the spinal cord. In the present study, we asked whether transplantation of olfactory mucosa (OM) would also reduce sprouting of a damaged peripheral pure motor nerve. The adult facial nerve was transected and the effect of the OM placed at the lesion site analyzed with regard to the accuracy of target reinnervation, axonal sprouting of motoneurons and vibrissal motor performance. Accuracy of target reinnervation and axonal sprouting were studied using pre-/post-operative labeling and triple retrograde labeling of facial motoneurons, respectively. The vibrissal motor performance was monitored using a video-based motion analysis. We show here, that implantation of OM, compared to simple facial-facial anastomosis (FFA), (1) improved the protraction, amplitude, angular velocity, and acceleration of vibrissal movements up to 80% of the control values, (2) reduced the percentage of branching motoneurons from 76% to 39%, and (3) improved the accuracy of reinnervation from 22% to 49%. Moreover, we present evidence, that transplanted OM but not buccal mucous membrane (BMM) induced a sustained up-regulation of trophic factors at the lesion site. It is concluded that transplantation of OM to the transected facial nerve significantly improves nerve regeneration.

P12 COMPARISON OF THE EFFECTS OF IMPLANTED SCHWANN CELLS, OLFACTORY ENSHEATHING CELLS AND BONE MARROW STROMA CELLS ON AXONAL BRANCHING AND VIBRISSAE MOTOR PERFORMANCE AFTER FACIAL NERVE TRANSECTION IN THE RAT*

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A major reason for the insufficient recovery of function after motor nerve injury lies in the high number of axonal branches often reinnervating muscles with different function.

To improve therapy we tested whether implantation of Schwann cells (SC), olfactory ensheathing cells (OEC) or bone marrow stroma cells (BMSC) in a collagen-filled silicon tube between the stumps of a transected facial nerve would reduce the branching of transected axons. Two months after implantation, retrograde labeling was used to estimate the portion of motoneurons the axons of which had branched and projected simultaneously into 2 or 3 major branches of the facial trunk. After transection and suture of the facial nerve or entubulation in acellular collagen gel, 30%–35% of all motoneurons projecting along the zygomatic branch sprouted and sent at least one twin axon to the buccal and/or marginal-mandibular branches of the facial nerve. The suspension with OEC had no effect on axonal branching. Implantation of SC and BMSC, however, significantly increased the portion of motoneurons which branched to 49% and 54% respectively. The parallel biometrical analysis of vibrissae whisking showed that the increased axonal branching was accompanied by a declined motor performance (amplitude, angular velocity, angular acceleration). We conclude that transplantation of SC and BMSC to the lesioned facial nerve in rats affects axonal branching and vibrissae motor performance.

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P13 CORTICAL PLASTICITY AFTER SPINAL CORD INJURY AND TREATMENT WITH FETAL SPINAL CORD TRANSPLANTATION AND NEUROTROPHIN ADMINISTRATION

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We have shown that fetal spinal cord transplants and neurotrophic factors contribute to recovery of skilled forelimb function after cervical spinal cord injury. However, the anatomical substrate of this recovery is not fully understood. We hypothesized that plasticity occurs at both spinal and supraspinal levels following spinal cord injury and treatment.





62 adult rats underwent a right C4 spinal cord overhemisection (HX) lesion. Two weeks after injury, rats were sorted into the following delayed treatment paradigm: 1) HX only, 2) HX+transplant of fetal cervical spinal cord tissue into the lesion site (TP), 3) HX+TP+infusion of brain-derived neurotrophic factor (BDNF), or 4) HX+TP+infusion of neurotrophin-3 (NT-3). At four weeks, tracing was performed by injection of biotinylated dextran amine (BDA) into sensorimotor cortex (SMC) or right red nucleus (RN). Subsequently, image analysis was performed to quantify corticorubral (CR) fiber density, corticospinal (CS) fiber density, and the number of neurons in the SMC. Analysis of crossed CR fibers (cortical efferent fibers traced to the contralateral RN) demonstrated that treatment with either TP+BDNF or TP+NT-3 leads to significantly more crossed CR axons compared to control animals. Quantification of right dorsal-lateral CS fibers rostral and caudal to the cervical lesion demonstrates a significantly greater CS fiber density in animals treated with TP+NT-3 as compared to control. Retrograde tracing after injection of the right RN indicates that rats treated with TP+NT-3 have significantly more labeled neurons in the SMC as compared to control. Taken together these data suggest that the anatomical changes following treatment with TP and neurotrophins include reorganization of spared pathways at both spinal and supraspinal levels. This plasticity may contribute to the functional recovery observed after treatment of spinal cord injury with TP and neurotrophins.

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P14 SYNAPTIC PLASTICITY IN THE MOTOR CORTEX FOLLOWING SPINAL CORD INJURY

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After spinal cord injury (SCI), structural plasticity occurs at multiple levels of the central nervous system neuraxis and may contribute to functional recovery. The neural mechanism for the structural changes at the cortical level

is not fully understood. We examined the effect of an axotomy at the spinal level on synaptic structures in the motor cortex. We used western blot analysis of synaptic proteins in the motor cortex at different time points up to 4 weeks after a cervical overhemisection injury in adult rats. The expression of synaptophysin, a presynaptic marker, did not change significantly at any time point examined. However, PSD-95/SAP-90, a postsynaptic protein believed to serve as a structural scaffold at the excitatory synapses, increased its expression as early as 3 days after injury and reached a three-fold increase at 7 days. This increase persisted until 4 weeks, although the levels started to decline by 2 weeks post-injury. Gephyrin, another postsynaptic protein involved in structural scaffold at inhibitory synapses, also showed an increase at 7 days and 2 weeks but to a much lesser extent than PSD-95/SAP-90. These findings suggest that synaptic remodeling, especially at postsynaptic sites, occurs at the cortical level within days to weeks after SCI. This remodeling may occur at both excitatory and inhibitory synapses, but their extent seems to differ. Synaptic structures in the adult brain may provide another potential target for therapeutic intervention to enhance functional recovery after SCI.

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P15 GENETICALLY TARGETED ASTROCYTE SCAR ABLATION RESULTS IN LIMITED, LOCAL GROWTH OF CORTICOSPINAL TRACT AXONS AFTER SPINAL CORD INJURY

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After spinal cord injury (SCI), scar tissue formed by reactive astrocytes is thought to prevent axon regeneration. We used a genetic targeting strategy to ablate reactive astrocytes after SCI. Transgenic mice that express herpes simplex virus thymidine kinase (HSV-TK)

from mouse glial fibrillary acidic protein (GFAP) promoter were given the antiviral agent ganciclovir (GCV). Transgenic and non-transgenic mice received a bilateral lesion of the corticospinal tract (CST) at T9/T10. Non-transgenic mice exhibited dense astrocyte scars. Transgenic mice given GCV exhibited substantial ablation of scar-forming astrocytes. Areas depleted of astrocytes exhibited both tissue degeneration and increased local growth of nerve fibers as detected by immunohistochemistry of neurofilament M. CST fibers were assessed using biotinylated dextran amine (BDA) injected unilaterally into the sensory motor cortex. In nontransgenic mice, many large BDA-labeled retraction bulbs were evident proximal to the glial scar and no labeled fibers were observed within or distal to the lesion. Transgenic mice given GCV had fewer retraction bulbs, and areas depleted of astrocytes exhibited many fine BDA-labeled fibers. In some cases, finely branched CST fibers grew across and beyond the lesion for a limited distance.

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P16 REACTIVE ASTROCYTES PROTECT TISSUE AND PRESERVE FUNCTION AFTER CRUSH SPINAL CORD INJURY

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Reactive astrocytes are prominent in the cellular response to spinal cord injury (SCI), but their roles are not well understood. We used a transgenic mouse model to study the consequences of selective and conditional ablation of reactive astrocytes after crush SCI. Mice expressing a GFAP-HSV-TK transgene were given moderate crush SCI and treated with the antiviral agent ganciclovir (GCV) to ablate dividing, reactive, transgene-expressing astrocytes in the immediate vicinity of the SCI.

(Cell 93:189, 1998; Neuron 23:297, 1999). Moderate crush injuries in control mice caused focal tissue disruption and cellular degeneration, with mild and largely reversible motor impairments. Equivalent moderate crush injuries combined with ablation of reactive astrocytes caused substantial tissue disruption with pronounced cellular degeneration, inflammation and severe, essentially permanent motor deficits. These findings show that reactive astrocytes provide essential functions that protect tissue and preserve function after moderate SCI. Our findings suggest that identifying ways to preserve reactive astrocytes and augment their protective functions may lead to novel approaches to reducing secondary tissue degeneration after SCI.

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P17 THE ASTROCYTIC BARRIER TO REGENERATION AT THE DORSAL ROOT ENTRY ZONE IS INDUCED BY INJURY

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Several astrocytic and myelin-derived molecules have been identified which may contribute to regeneration failure in the adult spinal cord. A complete understanding of their role as barriers to regeneration has yet to be achieved. Here we investigated specifically whether the astrocytic barrier to regeneration at the dorsal root entry zone (DREZ) is intrinsic or induced as a result of injury. Dissociated trigeminal ganglia from GFP-expressing mice were micro-injected into the dorsal root ganglia (DRG) (C6–C8) of adult rats. One week post-injection, the spinal cord and the DRGs were





cryosectioned and immunohistochemistry for p75 (Schwann cells) BIII tubulin and P2X₃ (neurons), and GFAP (astrocytes) was performed. The level of GFAP (up-regulated in activated astrocytes) and p75 (up-regulated in activated Schwann cells) as indicators of the extent of glial reactivity at the DREZ due to the grafting procedure were quantified. In the absence of glial reactivity, (i.e. little injury) many transplanted axons crossed the DREZ. Increased expression of both GFAP and p75 were associated with a lower density of regenerating axons in the central nervous system portion of the dorsal root. Therefore, our findings indicate that there is an inverse relationship between the amount of axonal injury/glial reactivity at the DREZ, and the regenerative success of axons within the injury environment. These results clearly demonstrate that rather than being inherently prohibitive to regeneration, the astrocytic barrier is induced *de novo* following dorsal root injury. In addition, Affymetrix gene arrays were performed on DREZ tissue from rhizotomized rats, at 3 and 6 days post injury to identify potential candidate molecules responsible for this inhibition. Rhizotomy produced over 350 significant gene expression changes at 3 days and 300 at 6 days post injury at the DREZ. Work is ongoing to identify astroglial barrier associated candidates.

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P18 LONG-DISTANCE AXON REGENERATION AND FUNCTIONAL IMPROVEMENT FOLLOWING SUPPRESSION OF FIBROUS SCARRING IN SPINAL CORD INJURY

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The lesion scar has long been considered as a major impediment for axon regeneration in the injured CNS. In traumatic injuries of adult rat spinal cord and brain a lesion scar develops within 4–6 days that is composed of a basement membrane-rich fibrous scar in the lesion core and a glial scar in the perilesion area. The collagenous basement membrane serves as a scaffold to bind and enrich numerous axon growth inhibitory molecules such as chondroitin sulfate proteoglycans, semaphorins and ephrins which have been detected in the lesion zone. Here we describe a pharmacological scar suppressing treatment suitable for spinal cord injury that consists of the local application of (i) an iron chelator, which deprives a key enzyme of collagen biosynthesis (prolyl 4-hydroxylase) of its cofactor iron, and (ii) cyclic AMP to inhibit TGFβ-induced proliferation and collagen production of meningeal fibroblasts invading the lesion area. Following a Scouten wire knife transection at the level of Th8 of the dorsal corticospinal tract (CST) and dorsal columns in adult rats, the transient suppression of collagen biosynthesis and fibrous scarring in the lesion zone resulted in extensive long-distance regeneration of CST axons through the lesion area and for up to 2 cm into the distal cord. Anterogradely labeled CST axons regenerated within both grey and white matter and developed terminal arborizations in grey matter regions. In contrast to controls, animals receiving the scar suppressing treatment

showed significant functional improvement in locomotor behavior in the open field and in additional functional tasks that require coordination and fine motor control of the hindlimbs. We conclude that the lesion scar is a major impediment for axon regeneration in adult spinal cord and suggest that transient local inhibition of collagen biosynthesis and fibrous scarring is a potential therapeutic treatment for human spinal cord injury.

P19 TRANSPLANTS OF FIBROBLASTS GENETICALLY MODIFIED TO EXPRESS BDNF AND NT-3 PROMOTE RECOVERY OF BLADDER FUNCTION IN SPINAL CORD INJURED RATS

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Bladder dysfunction is a serious consequence of spinal injury. Because cellular transplants offer the possibility of repair, we transplanted fibroblasts into the injured spinal cord and studied recovery of bladder and hindlimb function and axonal growth in lower lumbar spinal cord. Female rats received a 25mm contusion injury at T8/9. Nine days post-contusion, fibroblasts genetically modified to produce BDNF and NT-3 (BN-group) or unmodified fibroblasts (OP-group) were transplanted into the injured spinal cord. The BN-group demonstrated significantly increased voiding volume per micturition for 2–4 weeks after transplantation, but both groups exhibited similar voiding patterns from 5–8 weeks, indicating an accelerated recovery in the BN-group. Cystometry measures at 8 weeks post-transplantation showed significant reduction in micturition pressure in the BN-group, with no differences in postvoid residual urine or bladder capacity. In addition, the incidence of detrusor hyperreflexia was significantly less in the BN-group, and bladder weight was significantly reduced. Hindlimb function, assessed by the BBB score, was also significantly improved in the BN-group compared to the OP-group. In sections from L6 spinal cord, immunoreactivity of CGRP and GAP43 in

dorsal horn, dorsal commissure and sacral parasympathetic nucleus was significantly lower in the BN-group than in the OP-group, suggesting reduced sprouting by C-fibers, which may explain the reduced detrusor hyperreflexia. Serotonin-positive fibers in ventral horn were more densely distributed in the BN-group, which correlates with functional recovery. In conclusion, transplants of fibroblasts genetically modified to express BDNF and NT-3 have the potential to improve bladder function as well as hindlimb function in spinal cord injury.

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P20 RECOVERY OF FUNCTION FOLLOWING GRAFTING OF HUMAN BONE MARROW STROMAL CELLS INTO THE INJURED SPINAL CORD

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Bone marrow stromal cells (MSCs) are multipotent adult stem cells that can be readily obtained from patients and therefore are attractive candidates for cell transplantation. We are developing transplantation strategies using MSCs to promote functional recovery after spinal cord injury. Adult human MSCs were purified and expanded in a high-throughput process and characterized by flow cytometry, ELISA and gene expression analysis. We found that MSCs produce and secrete therapeutic factors relevant for cell survival and axon growth. Testing MSC-conditioned media in an in vitro system resulted in significantly increased neurite outgrowth. We analyzed the ability of MSCs to promote behavioral recovery after contusion injury at T9-10 spinal cord of adult rats. Mild, moderate or severe contusions were generated; MSCs were grafted into lesion areas one week





following injury. All animals were immune suppressed with CyclosporinA starting 3 days before grafting and continuing for up to 11 weeks. Behavioral tests, including BBB (open field), a modified cylinder procedure, and a response to thermal stimulus, were performed. Animals grafted with MSC after mild or moderate contusions showed significant behavioral recovery compared to control groups. In animals with severe contusion injury followed by MSC grafting a trend toward improvement was observed. Neurofilament immunocytochemistry identified numerous axons passing through the injury site in animals with MSC grafts. Analysis of human cell markers revealed that many grafted cells were present at 2 weeks, but only few were detected at 11 weeks. These experiments indicate that MSCs can support axon growth and limited functional recovery even in the absence of long-term survival. As poor survival may have restricted the repair potential of these cells, we are currently investigating immune response and apoptotic activity in allogenic and syngeneic rat models. We anticipate that improving the survival of MSC grafts will increase functional recovery following spinal cord injury.

P21 THE BASSO MOUSE SCALE FOR LOCOMOTION (BMS) IS A MORE SENSITIVE INDICATOR OF RECOVERY THAN THE BBB SCALE IN MICE WITH SPINAL CORD INJURY

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Use of mouse models to explore spinal cord injury (SCI) and potential treatments is rapidly expanding given the availability of genetic mutants. However, the effects of these mutations and treatments on locomotor function have been difficult to assess due to a paucity of rating scales developed specifically for mice. One strategy has been to modify the rat BBB locomotor rating scale, but we found marked variability and disagreement between six expert raters using the BBB to assess mice with SCI ($p < .01$). Variability resulted from difficulty assessing attributes like toe clearance in

mice. Importantly, the pattern of locomotor recovery is different for mice. Therefore, we developed a new 10-point scale, specific for mice, based on visually detectable operational criteria. The BMS was tested on five strains of mice with mild, moderate or severe SCI or transection (TX) over 4–7 wk. The BMS predicted tissue sparing at the lesion epicenter ($p < 0.001$, $r^2 = 0.77$) and detected differences in locomotion between severe contusion and TX ($p < 0.05$). Novice BMS users had 62% accuracy with experts and their scores were highly correlated over a range of locomotor abilities ($r: .92-.99$, $p < 0.0001$). The BMS also detected strain differences in locomotor recovery after moderate SCI. These results indicate that the BMS is a sensitive, reliable and valid locomotor assessment tool for mice with SCI.

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P22 MICE WITH L1CAM DELETION HAVE DEFICITS IN HINDLIMB LOCOMOTOR FUNCTION: BEHAVIORAL AND HISTOLOGICAL COMPARISONS WITH MILD CONTUSION INJURY TO THE MID-THORACIC SPINAL CORD

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L1cam is associated with axon growth and development of the corticospinal tract (CST). We wished to determine if functional deficits found by genetic deletion of L1cam would be detected in locomotor tasks designed to assess recovery after spinal cord injury (SCI) in mice. Wild-type (wt; +/Y) and hemizygous (hz; -/Y) littermates [B6;129S-L1camtm1Sor] were evaluated in the open field using the Basso Mouse Scale (BMS), and tested for deficits traversing a grid. Uninjured hz mice had lower BMS scores and increased footfalls compared to wt. Anterograde and retrograde tracing confirmed the absence of CST axons in the thoracic and lumbar spinal cord of hz mice. Retrograde labeling

of neurons in the hypothalamus was also reduced, indicating that supraspinal systems other than the CST are compromised by L1cam deletion. We then examined the role of L1cam in recovery after SCI. Wt and hz littermate pairs received mild contusion injuries, destroying the central region of the thoracic cord, including the dorsal CST. After injury, wt and hz mice showed deficits in the BMS and grid walk tests. In the open field, both groups recovered to their respective pre-injury levels by 1 week, and then showed a decline in BMS scores from 14 to 28 days after SCI. In contrast, both groups improved on the grid walk test from 7 to 28 days post-injury, despite the confirmed absence of an intact CST. Thus, hindlimb locomotor function is impaired in the absence of L1cam, and further deficits on these tests are demonstrated following a mild SCI. However, the recovery of grid walk performance and decline of gross locomotor function are not dependent upon L1cam, and implicate functional plasticity of descending motor systems in the B6;129S mouse strain after a mild contusion.

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P23 SENSORY EVALUATION OF CONTUSIVE SPINAL CORD INJURY (SCI) AS A MODEL TO EVALUATE REGENERATIVE THERAPIES IN THE RAT

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Changes in sensory function and neuropathic pain are common sequelae of spinal cord injury (SCI). The current study aims to establish a testing regimen to investigate sensory plasticity and behavior in a severe contusive model of SCI (NYU impact device). For histological examination of intact systems, we will

utilize CTB-HRP and WGA-HRP tracing from the sciatic nerve to label ascending myelinated and unmyelinated sensory fibers, respectively, and to map their pattern of innervation. Activity in these intact systems will also be evaluated using peripheral noxious stimulation (electrical, mechanical and chemical) of the sciatic nerve and subsequent examination of neuronal excitation with c-fos staining in sensory nuclei/dorsal horn above the injury site. To investigate sensory plasticity after injury we have employed tests for cutaneous allodynia and thermal hyperalgesia. Significant increases in tactile allodynia in both hindpaws and in specific locations on the skin of the torso of the injured animals were observed. Mechanical hyperalgesia developed in both hindpaws, as evidenced by a decrease in paw withdrawal thresholds. The mechanisms responsible for these sensory changes will be assessed using a variety of spinal (dorsal horn) and sensory system markers. These include calcitonin gene-related peptide and substance P, to evaluate sensory fiber in-growth; and the inhibitory neurotransmitters gamma-aminobutyric acid, glycine and serotonin, which have been implicated in normal and aberrant sensory processing in various pain models. This contusive SCI paradigm will allow us to determine how regenerative strategies might alter nerve function and sensory responses, including the development of neuropathic pain. Supported by The Miami Project.

P24 THE BEHAVIORAL DEFICIT OBSERVED FOLLOWING UNCONTROLLABLE SHOCK IN SPINALIZED RATS DEPENDS ON DE NOVO PROTEIN SYNTHESIS

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Prior research has shown that the spinal cord is capable of instrumental (response-outcome)





conditioning (Grau et al., 1998, *Beh Neurosci*, 112, 1366). Spinalized rats that receive shock when one hindlimb is extended (controllable shock) exhibit an increase in flexion duration that minimizes shock exposure. Rats that receive shock independent of leg position (uncontrollable shock) do not exhibit longer flexion duration. Moreover, uncontrollable shock engages a destructive process that undermines learning for at least 48 hours. We have previously shown that as little as 6 minutes of uncontrollable tailshock produces this learning deficit and that the induction of the deficit depends on an NMDA-mediated process (Ferguson et al., 2002, *Soc Neurosci Ab*). In other paradigms, researchers have shown that long-term changes in behavioral plasticity frequently depend on gene expression and de novo protein synthesis. Given this, we hypothesized that the long-term consequences of uncontrollable shock on intraspinal plasticity may also depend on protein synthesis. Spinally transected rats ($N = 32$) received the protein synthesis inhibitor cycloheximide or saline intrathecally before exposure to uncontrollable shock (180, 1.5-mA, 80-ms tailshocks). Twenty-four hours later, rats were tested with controllable shock. Saline treated rats failed to learn the instrumental response. Cycloheximide treated rats learned normally, suggesting that the learning deficit does indeed depend on de novo protein synthesis within the spinal cord.

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P25 INSTRUMENTAL SPINAL LEARNING CAPACITY IS ALTERED BY CHRONIC MOTOR TRAINING IN SPINALLY TRANSECTED RATS

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Motor training can facilitate functional recovery after spinal cord injury (SCI) in animal models and in human SCI patients. Animal data suggest that successful motor performance is a function of the task being trained, and that learning-type phenomenon may underlie these behavioral improvements. The present experiments address whether training induced state-dependent changes in the spinal cord of spinally transected (ST) rats influences the capacity to perform an acute spinal instrumental learning task. **METHODS:** Rats were spinalized at postnatal day 5 (P5). Motor training began on P30, consisting of either stepping on a treadmill (Step-tr) or standing on one hindlimb (unilateral limb extension: ULE+, ipsilateral trained limb; ULE-, contralateral limb) for 30 min/d at 75% body weight support (BWS). Nontrained rats (Ntr) were controls. After 4 wk of training, rats received 30 min of response-contingent leg shock during which each subject received a shock to one hindlimb ankle flexor, the tibialis anterior (TA) whenever the ankle extended below a preset criteria. **RESULTS:** Step-tr and Ntr rats learned at similar levels, while ULE+ showed some deficit compared to Step-tr and Ntr. ULE- showed the overall greatest deficit in learning. **CONCLUSIONS:** These data support the concept that spinal changes induced by chronic motor training can alter the ability to perform a second motor task, and emphasize that state-dependent, plastic changes occur in the spinal cord as a result of chronic motor training, i.e., a unilateral motor training paradigm can significantly alter the way in

which the contralateral side interprets a stimulus and executes a motor task. A potential role for the GABAergic neurotransmitter system in this behavioral plasticity is discussed.

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P26 SENSORIMOTOR TRAINING AND TRANSPLANTS OF BDNF AND NT-3 FIBROBLASTS: THEIR EFFECT ON LOCOMOTOR RECOVERY IN SPINALIZED CATS

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Sensorimotor training is beneficial in promoting locomotor recovery in spinalized animals. The neuroprotective effects of brain derived neurotrophic factor (BDNF) and neurotrophin-3 (NT-3) are also known. We sought to determine whether the combination of both treatments would augment locomotor recovery in chronically spinalized cats. Animals received transplants of either a mixture of BDNF or NT-3 producing fibroblasts (modified) or of unmodified fibroblasts and were trained daily or biweekly. Treadmill evaluation every two weeks showed that untrained animals were unable to weight support and primarily exhibited dorsal-stepping. With training, animals regained plantar paw placement, weight support, and gait pattern similar to pretransection stepping. Step lengths in animals with modified transplants were consistently longer than for animals with unmodified transplants post-transection. In one transplant and trained animal, there was no significant difference between pre and post-transection step lengths ($p > 0.05$). In animals with unmodified fibroblasts, step lengths were always significantly shorter than pretransection. Interlimb coordination was also disrupted in the latter group. The correlation between hip and shoulder angles was retained in animals receiving modified fibroblasts, but lost in those animals with unmodified fibroblasts. Immunohistochemistry revealed calcitonin

gene related peptide (CGRP) and growth associated protein (GAP-43) immunoreactive fibers in the lesion and serotonin (5-HT) fibers within and caudal to the graft in animals with modified transplants, suggestive of axonal growth and sprouting through the injury site. Intraspinal microstimulation of the lumbar cord in animals with modified transplants also return the proportion of spinal fields to that reported for spinal intact animals. These results indicate that the combined treatment of sensorimotor training and modified transplant are efficacious in promoting locomotor recovery in chronically spinalized cats. We are currently examining the mechanisms by which this recovery may occur.

P27 ASSESSMENT OF FORELIMB STRENGTH AFTER CERVICAL SPINAL CORD INJURY IN MICE

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A large proportion of spinal cord injuries in humans are at the cervical level, but there are few tests to quantitatively assess forelimb motor function after cervical spinal cord injury in rodents. Here, we describe a simple and reliable technique for assessing forelimb grip strength over time. Female C57Bl/6 mice were trained on the Grip Strength Meter (GSM, TSE-Systems), then received a lateral hemisection of the spinal cord at level C5, C6, C7, or T1 and each forepaw was tested for four weeks. Prior to injury, there was no significant difference in the force exerted by either forepaw for all animals. On day 2 post-injury, for hemisections at C5, C6, and C7, the injured forepaw was completely unable to grip and there was a slight decrease in the strength of the noninjured paw compared to presurgical levels. A T1 injury did not alter the strength of the noninjured forepaw and only decreased the strength in the injured forepaw by less than half that exerted prior to injury. By day 7 post-injury, there were no longer any deficits in the strength of the non-injured forepaw for all cervical injury levels. Between days 7 and 14 there was a gradual spontaneous recovery of function in the





gripping ability of the injured forepaw for injuries C5–C7, but the force exerted was always less than half that of the noninjured forepaw. This pattern persisted throughout the remainder of the experiment. Histological staining of the spinal cord confirmed complete lateral hemisections. These studies provide evidence that the Grip Strength Meter is a sensitive behavioral measure of forelimb strength and can be used to define the cervical levels necessary for partial or full function of grip strength in the mouse.

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P28 EXERCISE COMPENSATES FOR DECREASES IN BDNF AND SYNAPTIC PLASTICITY IN THE INJURED SPINAL CORD

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In addition to protecting neurons against various insult types, BDNF promotes neuronal excitability and synaptic facilitation, required for neuronal function. We have shown that exercise increases levels of BDNF in the spinal cord (SC), and here we investigate the role of exercise in promoting synaptic plasticity in the injured SC. The SC of adult male rats was hemisectioned at a mid-thoracic level (T7–T9). One week after surgery, the rats were exposed to voluntary running wheels for 0, 3, 7, or 28 days. Taqman RT-PCR measured changes in gene expression levels, and protein levels were determined using ELISA or Western blots in the lumbar SC region. BDNF and synapsin I mRNA were reduced to about 80% of intact controls in the lesioned side SC at all time points examined. BDNF protein levels measured at 28 days were reduced to about 50% of controls. Exercise compensated for the reductions in BDNF with a progressive effect up to 28 days. Exercise increased levels of synapsin I, a downstream effector for the action of BDNF on synaptic plasticity, only

after 28 days. CREB, a transcription factor important for neuroplasticity and learning and memory under regulation of BDNF, measured at 28 days showed an injury-related decrease and exercise compensated for this decrease. These results are consistent with the concept that BDNF modulation induced by exercise can play a role in facilitating recovery of locomotion following spinal cord injury. These actions of exercise can be achieved by activating synaptic pathways under the regulatory role of BDNF.

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P29 EFFECTS OF CYCLING PHYSICAL THERAPY AND SEROTONERGIC AGONISTS ON HINDLIMB MOTOR FUNCTION AFTER THORACIC TRANSECTION OF THE SPINAL CORD

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After spinal transection, muscles innervated by neurons below the level of injury are paralyzed and atrophy because of disuse. Repeated exercise may preserve the ability of hindlimbs to generate motion which will be necessary for recovery of motor function. We are exploring the effects of an hour of daily (5d/wk) cycling on hindlimb muscles and movements in animals with spinal transection, because repeated activity enhances release of neurotrophic factor BDNF in muscle and spinal cord, axonal growth and recovery in spinal cord may be stimulated. Locomotor function is also coordinated by the central pattern generator, which is modulated by 5-HT supplied by brainstem neurons but is lost after transection. mCPP is a 5-HT_{2c} agonist known to modulate locomotor patterns. We compared three groups of adult rats. Group 1 (n = 18) spinal rats received no exercise and no drug treatment; group 2 (n = 20) rats received no exercise but were administered the serotonin agonist mCPP once a month; group 3 (n = 23)

rats received cycling exercise 5 days/wk and mCPP once a month. Hindlimb movement in an openfield situation (BBB) did not differ among groups. The BBB score was increased in rats receiving mCPP. The exercised animals that received the drug scored better than those that were not exercised. Exercised animals were also more likely to use plantar paw placement and dorsal stepping. These preliminary data indicate that cycle therapy has a positive effect on the hindlimb function after spinal cord transection, which is revealed by administration of a serotonergic agonist.

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P30 FUNCTIONAL ELECTRICAL STIMULATION AFTER SPINAL CORD INJURY PROMOTES CELL BIRTH AND NEURONAL DIFFERENTIATION OF TRANSPLANTED EMBRYONIC STEM CELLS

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Recent work in humans raises the possibility that activity-based restoration strategies may aid recovery from spinal cord injury (SCI). We have developed a rat model of these treatments, using functional electrical stimulation (FES), and have assessed the effects of such treatment on the injured spinal cord. In this model, electrodes are implanted bilaterally adjacent to the peroneal nerves, and electrical stimulation is applied for one hour, three times per day. In Experiment 1, rats' spinal cords were completely aspirated at thoracic level 9 (T9), and stimulating devices were activated 24 days post-injury. On post-injury days 31–35, rats received daily intraperitoneal injections of bromodeoxyuridine (BrdU), a marker of dividing cells. Rats were euthanized either two hours ('cell birth' group) or

one week ('cell survival' group) after the final BrdU injection. Both groups of FES-treated rats showed an increased number of BrdU-labeled cells, relative to control rats, in the lumbar spinal cord (which presumably received stimulation through the peroneal nerves). In the cell birth group, there was also an increase in the proportion of dividing cells expressing Nestin, a marker of tripotential progenitors. In Experiment 2, 40 days after T9 SCI, rats received both embryonic stem cell (ES) transplants and stimulation-device implants. Dissociated 4–/4+ B5 embryoid body cells were transplanted to sites rostral and caudal to the injury. Rats were euthanized 16 days later. Rostral to the lesion, in both control and treatment groups, over 25% of ES-derived cells expressed the neuronal marker NeuN. Caudal to the lesion, in control rats, neuronal differentiation of ES-derived cells was reduced relative to the rostral section of the spinal cord. FES, however, restored neuronal differentiation to the levels observed rostrally. These results demonstrate that FES increases endogenous cell birth and promotes neuronal differentiation of transplanted ES cells in the injured rat spinal cord.





P31 RANDOMIZED TRIAL OF WEIGHT-SUPPORTED TREADMILL TRAINING VERSUS CONVENTIONAL TRAINING FOR WALKING DURING INPATIENT REHABILITATION AFTER INCOMPLETE TRAUMATIC SPINAL CORD INJURY

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We will present the 6-month follow-up results of the first prospective, multicenter, randomized clinical trial (RCT) of a task-oriented walking intervention for subjects during early rehabilitation for an acute traumatic spinal cord injury (SCI). The experimental strategy applies basic neuroscience research and pilot clinical studies about use-dependent locomotor learning in the lumbosacral neural circuits and supraspinal neurons that coordinate leg movements. The intervention, body weight-supported treadmill training (BWSTT), allows physical therapists to systematically train patients to walk on a treadmill at increasing speeds typical of community ambulation with increasing weight bearing. The therapists provide verbal and tactile cues to facilitate the kinematic, kinetic, and temporal features of walking. Subjects (N = 146) from 6 centers were randomly assigned to a conventional therapy program for mobility versus the same

intensity and duration of a combination of BWSTT and overground locomotor retraining. Subjects had an incomplete SCI (American Spinal Injury Association grades B, C, and D) from C-4 to T-10 (upper motoneuron group = 111) or from T-11 to L-3 (lower motoneuron group = 35). Within 8 weeks of a SCI, subjects were entered for 12 weeks of intervention. The two single-blinded primary outcome measures are the level of independence for ambulation and, for those who are able to walk, the maximal speed for walking 50 feet, tested 6 and 12 months after randomization and adjusted for time from onset of SCI. Walking distance in 6 minutes, quality of life, and community reintegration were also evaluated. The trial's methodology offers a model for the feasibility of translating neuroscientific experiments into a RCT to develop evidence-based rehabilitation practices. By defining and testing a specific intervention, whether or not the results find BWSTT to be better than or equal to conventional physical therapy, the trial standardizes a training approach that can be incorporated into future studies of neural repair strategies.

P32 COMBINED USE OF MATRIX DEGRADING ENZYMES AND NEUROTROPHIC FACTORS TO FACILITATE AXONAL REGENERATION AFTER SPINAL CORD INJURY

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The current experiments took advantage of the ability of a peripheral nerve graft (PNG) to guide axons toward a specific target region, the potential for modulation of the extracellular matrix to create an environment more amenable to axon invasion using matrix degrading enzymes, and the potential for neurotrophic factors to promote axonal growth. Chondroitinase modulation of the extracellular matrix associated with a cervical spinal cord injury (SCI) site created an environment where more axons extended from a PNG into the spinal cord compared to either saline- or collagenase-treated lesions. However, the length of

growth was limited to several hundred microns and many more axons remained in the PNG despite matrix modulation. Behavioral studies of vertical exploration and forelimb-hindlimb coordination were completed with significant differences found between the chondroitinase and saline treated animals. We then tested whether the addition of neurotrophic factors after chondroitinase treatment would increase the extent of axonal growth from a PNG into the spinal cord. Brain-derived neurotrophic factor (BDNF) was provided to the lesion site by transplantation of fibroblasts (Fb/BDNF) genetically modified to release BDNF. Cells were transplanted after chondroitinase treatment and just prior to apposition of the distal end of a PNG. Anterogradely labeled axons from brainstem neurons traversed the Fb/BDNF transplant and readily grew across the interface into the host spinal cord. Quantitative measures of the density and length of axonal growth after the combined treatment are in progress. These results demonstrate the importance of addressing both neuronal and environmental factors associated with a spinal cord injury in an effort to promote regeneration.

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P33 THE TENASCIN-C-DERIVED PEPTIDE VFDFNVLK REDUCES DEGENERATION AND INCREASES SPROUTING IN A RAT MODEL OF SPINAL CORD INJURY

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The peptide fragment VFDFNVLK derived from the fnD fibronectin repeat of tenascin-C has activity to promote neurite growth and guidance in cell culture. We therefore tested this peptide in rat model of spinal cord injury using an Alzet pump and cannula to deliver the peptide to the lesion site. Adult Sprague Dawley rats were subject do a dorsal hemisection on the right side at T8. There were

5 groups of animals: a) Intact no pump; c) Lesion, no pump; d) Lesion with pump and PBS (4 weeks); and e) Lesion with pump and peptide (1 or 2 weeks). All animals were allowed to survive approximately 1 month. One week prior to euthanasia, the animals were labeled with retrograde and/or anterograde tracers. For anterograde tracing, 10% BDA in TBS pH 8 was ionophoretically injected into the sensorimotor cortex. The number of labeled axons were counted at levels C7 and T6. For retrograde tracing 0.3–0.5 microliters of 2% Fast Blue in PBS was injected into the lesion side, one segment caudal to the lesion. The number of labeled cells was analyzed in the spinal cord, lateral ventricular eminence, red nucleus and the cerebral cortex. Counts were made on both the left and right side and then combined to control for the possibility that the lesion extended across to the left side. Retrograde tracing revealed that animals that received the peptide had increased numbers of labeled neurons in each of the four areas. Anterograde tracing revealed a similar increase in the number of labeled axons in the spinal cord. These data suggest that this peptide may have some efficacy to promote recovery of function following spinal cord injury.

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P34 REGROWTH OF SYMPATHETIC FIBERS AND REDUCTION OF HYPERTENSION FROM TAIL ARTERY MEASUREMENT IN SPINAL CORD REPAIRED RATS

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We aimed to assess blood pressure and sympathetic pathway in complete spinal cord (SC) transected adult rats that were treated with peripheral nerve grafts (PNG) and acidic





fibroblast growth factor (aFGF). Rats were randomly divided to three groups (5 animals in each group): (1) sham control (laminectomy only), (2) SC transection at T8, and (3) SC transection at T8, aFGF treatment, and PNG. The blood pressures of all rats were analyzed from tail artery at six-month post surgery. Immunohistochemistry for choline acetyltransferase (ChAT), tyrosine hydroxylase (TH), dopamine-beta-hydroxylase (DBH), phenylthanolamine-N-transferase (PNMT), and Fluorogold (FG) retrograde tracing were used to evaluate axon regrowth following the injury and treatment. When comparing with the transected group, the repaired group showed (1) significant lower blood pressure, (2) presence of TH, DBH, and PNMT labeled axons below the lesion site, (3) FG labeled neurons in hypothalamus, zona incerta, subcoeruleus nuclei, raphe pallidus, and rostral ventrolateral medulla (RVLM). We conclude PNG and aFGF treatments facilitate the regrowth of the sympathetic fibers in a T-8 SC transected rat model.

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P35 L1 EXPRESSION IS INCREASED SURROUNDING THE TRANSECTION SITE IN RATS LESIONED AT POSTNATAL DAY 5

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L1 is a cell surface protein expressed on developing axons and associated with axonal outgrowth, guidance, and fasciculation. Additionally, L1 is reported to be reexpressed on regenerating peripheral nerves. To determine if L1 is re-expressed on regenerating axons following spinal transection (ST) with and without step training, we analyzed spinal cord tissue from control untrained, control trained, ST untrained, and ST trained rats. Complete mid-thoracic spinal transection was performed at postnatal day 5 (P5) and treadmill

step training began at P40 and continued until P100. Animals were tested monthly using the robotic stepper. Video analysis demonstrated an improved stepping pattern in the ST trained animals. Immunocytochemical experiments showed that ST rats exhibited substantially higher levels of L1 expression directly rostral and caudal to the lesion site as compared to controls. Western blot analysis confirmed this increase in L1 expression around the lesion site. Novel patterns of L1 immunoreactivity also were detected in transected animals. Sections above the lesion demonstrated distinct L1-positive corticospinal tract axons whereas those below the lesion displayed increased L1 expression in a group of midline ventral funicular axons. The dorsal horn and adjacent lateral funiculus contained increased levels of L1-positive both above and below the lesion than seen in controls. No differences in the L1 expression patterns were found between ST trained and nontrained rats. These novel areas of L1 expression in ST animals suggest that axonal sprouting of ascending and descending pathways is detected up to 3 months after ST and implicate the reexpression of L1 as a marker of axonal regeneration in spinal cord injury.

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P36 NEOANGIOGENESIS IN AN ENSHEATHING CELL MATRIX: A TRINITY OF MECHANISMS TO PROMOTE SPINAL CORD REGENERATION

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Lamina propria-derived olfactory ensheathing cells (LP-OECs) from olfactory mucosa are prime candidates for autologous human transplantation to repair spinal cord injuries. Previously, we transplanted mouse LP-OECs expressing green fluorescent protein (GFP) directly into rat and mouse rubrospinal tract lesion sites and demonstrated that despite their minimal migration, they significantly

remodeled the lesion site, reduced cavity formation, promoted significant host Schwann cell invasion and formed a novel glial environment to promote regeneration. In this context, multiple axons sprouted across the lesion site into the distal cord, including serotonin- and tyrosine hydroxylase-positive axons, but rubrospinal axons responded less robustly. Here, we have extended this model to directly test the efficacy of transplanting GFP+ LP-OECs 1mm rostral and caudal to the rubrospinal tract lesion in a model where the spinal cord is also physically stabilized for the duration of the repair response, to test if this paradigm improves the potential for long tract regeneration. Angiogenesis is induced by LP-OEC transplantation in both rostro-caudally and directly transplanted models. New blood vessels form in a laminin-rich matrix in the presence of LP-OECs. Also, when LP-OECs are transplanted rostrocaudally, they migrate extensively and completely occupy the lesion site. This allows for the remodeling of the gliotic scar by shaping an environment for glial alignment, axonal sprouting and regeneration. By comparing rostro-caudal and direct transplant models, we hope to better understand the interaction of transplanted LP-OECs with the host environment. This will allow us to exploit the properties of OECs to direct and promote axon regeneration in the injured spinal cord.

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P37 HEME OXYGENASE-1 LIMITS NEUTROPHIL INFILTRATION, STABILIZES THE BLOOD-SPINAL CORD BARRIER, AND ATTENUATES WHITE MATTER DAMAGE IN THE ACUTELY CONTUSED MURINE SPINAL CORD

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Heme oxygenase (HO) metabolizes heme to biliverdin, iron, and carbon monoxide. We have previously shown that HO-1, the inducible HO, is induced in glia/macrophages in the acutely injured spinal cord. In this study, we hypothe-

sized that HO-1 represents an important defense against acute secondary injury in the traumatized spinal cord by restricting neutrophils infiltration and limiting both the extent of barrier dysfunction and white matter damage. To test this hypothesis, we have compared neutrophil infiltration, barrier permeability, oxidative injury, and degradation of myelin basic protein (MBP) in HO-1 heterozygous knockout mice (KO) and their wildtype (WT) littermates at 24 hours post injury. Neutrophil infiltration, as determined by morphometric assessment of tissue sections within the lesioned epicenter, and barrier leakage to luciferase, defined by luminescence of cord homogenates, were significantly increased in the KO as compared to the WT mice. We next examined the expressions of 4-Hydroxy-2,3-noneal (HNE), a major product of lipid peroxidation, and malondialdehyde (MDA) modified proteins in the injured epicenter. There were significant increases in HNE and MDA modified proteins in the KO as compared to WT mice, based upon densitometric analyses of western blotting. Finally, we compared the degradation of MBP, an indicator of white matter damage, in cord injured KO and WT mice by western blotting. Densitometric analysis revealed significantly greater degradation of MBP in the KO as compared to the WT mice. Together these findings suggest that HO-1 is neuroprotective in acutely injured spinal cord by limiting early inflammatory events, stabilizing barrier function, and minimizing early oxidative damage.

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P38 AN ACCELEROMETER-MONITORED DROP APPARATUS FOR SPINAL CORD CONTUSION INJURIES

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For a feline experimental spinal cord injury model, a free-fall weight drop system was chosen to induce injury. Because of the ease of implementation and the wealth of information





about drop parameters, a miniature accelerometer was mounted on the impactor. Three drop heights have been tested: 20 cm (n = 12), 16 cm (n = 9), and 12 cm (n = 5). The weight in all tests was 25 grams. The known acceleration due to gravity (-1 G), made the system self-calibrating. Several physical parameters were computed from the acceleration data—velocity, displacement, force, stress, energy, power, strain, and momentum. The nature and efficacy of each individual weight-drop event was assessed from these parameters. The quality of design of the system was continually assessed and improved by observing the linearity of the velocity curve. The drop height was confirmed in the 3 test groups to be (mean \pm SD): 20.158 \pm 1.327 cm, 15.926 \pm 0.671 cm, and 12.215 \pm 0.590 cm, respectively. The calculated and nominal impact velocities were computed in the 3 test groups to be: 185.10 \pm 13.97 cm/s (198.06 cm/s), 169.20 \pm 11.08 cm/s (177.15 cm/s), and 147.85 \pm 13.41 cm/s (153.41 cm/s), respectively. The deformation of the spinal cord tissue was computed in the 3 test groups to be: 0.409 \pm 0.080 cm, 0.369 \pm 0.085 cm and 0.260 \pm 0.090 cm, respectively. The type of tissue impacted was ascertained from the shape of the stress-strain curve [soft tissue was characterized by low stress and high strain; bone was characterized by high stress and low strain]. To determine the severity of injury, the analysis of data acquired during the injury event was combined with measurements of somatosensory evoked potentials before and after the injury event; behavioral analysis for 12 weeks after injury; and histological evaluation of the spinal cords.

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P39 ONSET OF PATHOLOGY AT THE BLOOD-CORD BARRIER AFTER SPINAL CORD INJURY: BY THE RISE OF VCAM-1 LEVELS AT THE LESION SITE

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Progressive tissue decay at the damage site is the pathologic hallmark of spinal cord injury. This chronic degeneration is most notable in highly vascularized regions, in the grey matter, and manifested as widening cavitation. Further, the blood-cord barrier at the lesion site is compromised, it remains leaky. Longitudinal studies, by histology [PNAS(1996) 93:11179] and by In Vivo MRI, of the lesion site in rat spinal cord show that repair repertoire is activated after injury; however, by the 4th week postinjury (PI) the repair is aborted as chronic inflammation takes over. VCAM-1 on blood-brain barrier endothelium is one of the major mediators of leukocyte migration through the barrier during inflammation. Here, we quantitatively pinpointed the onset of chronic inflammation at the lesion site after contusion-injury in rat spinal cord (0,4,6,8, 11,14,19,21,28,34, 40,50 & 57 days PI) using VCAM-1 immunocytochemistry. Following injury the levels of VCAM-1 labeled cells at the lesion site significantly declined ($p = 0.01$); these returning to normal levels by day 4–6 PI. Significant increase ($p = 0.05$), above normal levels, in VCAM-1 expressing cells occurred during the 3rd week PI. The rise in VCAM-1 was first noticed on day 11 PI; by day 14 it was twice of the normal levels; and flaring up (X3 normal level) by day 19–21 PI, seen as a continuous band of several cell layers that is surrounding the lesion site. The data correlate with our In Vivo MRI study in which pointed inflammation was first noticed early in the 3rd week PI flaring up a week later and expanding with time PI. These data indicate a crucial role of the blood-spinal cord barrier in the onset of pathology after injury. These also help in identifying potential therapeutic strategies and the window of opportunity for their deployment for preventing the onset of tissue decay.

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P40 STEPPING AND STANDING PERFORMANCE AFTER LOCOMOTOR TRAINING OF RADIATION TREATED OR UNTREATED RATS WITH SPINAL CORD INJURIES

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Repair of structure and function in rat transected spinal cord (SC) can be facilitated by radiation therapy given within a critical time window, 2 to 3 wk postinjury (PI) [PNAS(1996)93:11179; BrainRes(2001)904:199]. In contusion-injured rat SC, tissue repair can be facilitated by radiation therapy provided a prior surgical manipulation (midline incision) of the lesion site is performed. Stepping and weight support can be partially restored by training of spinalized rats [BrainResRev (2002)40:2671]. Here, we examined the effects of hindlimb locomotor training on the recovery of standing and locomotion in contused rats that were either radiation-treated or untreated. A severe thoracic contusion was performed in 32 adult rats with an NYU weight-drop device followed 2h later by a midline incision. Untreated (control) and radiation-treated (20Gy/10 fractions, starting on day 12PI), were subdivided into 2 groups, trained (starting 1mo PI) and untrained. Daily 20 min training sessions of a combination of bipedal hindlimb locomotion and static weight support (WS) was given for 8 wk. At the end, all rats were tested using a robotic device and limb kinematics of stepping and standing were recorded (3D-video) and analyzed. Preliminary analyses suggest that training in the contused rats significantly ($p = 0.02$) improved the weight-bearing stepping capacity in trained (10/16) vs. untrained (6/16) rats. There was a trend suggesting a better motor function in the trained-irradiated rats, e.g., number of steps/30sec was greater (10 vs. 3.5, $p < 0.05$) and WS in standing was higher (54% vs. 43% body WS) than in the untrained control. Although these preliminary data indicate that training following repair induced by radiation therapy in injured SC may lead to a better motor recovery, further analy-

ses of the locomotion data are required to identify the kinematics of the specific phases of stepping that seem to account for the improved stepping.

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P41 ESTROGEN NEUROPROTECTION IN THE ACUTE SPINAL CORD INJURY MODEL

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Spinal cord injury (SCI) is a devastating neurological problem affecting approximately 11,000 Americans each year. Several agents have been proposed for the treatment of SCI; however, only methylprednisolone has limited efficacy. Estrogen is a multiactive neuroprotectant with antioxidant and anti-inflammatory properties and is found to attenuate calcium (Ca^{2+}) influx following neuronal injury. We examined the neuroprotective effects of estrogen in SCI and its potential to decrease post-traumatic injury and increase neuroregeneration. SCI was induced by the weight-drop method (40 g•cm injury). Treatment groups were: sham (laminectomy only), SCI plus vehicle, and SCI plus estrogen. Injured rats were treated with either 4mg/kg 17 B-estradiol (estrogen group) or dimethyl sulfoxide (vehicle group) at 15 min and 24h following injury. All rats were sacrificed at 48 h for analysis of tissue segments. Calcium influx was measured in all three treatment groups using Calcium Green 2-AM dye. Inflammation was examined immunohistochemically using double-labeling for ED-2 or OX-42 (antibodies against macrophages) and calpain. Tissue edema was measured by sampling wet and dry weights of SCI tissue samples. Lesion volume and demyelination were examined using Luxol fast blue staining. As compared to samples from vehicle-treated rat's, spinal cord samples from estrogen





treated rats showed less calcium influx, fewer inflammatory cells, decreased tissue edema, and a decrease in lesion volume and demyelination. Our preliminary data suggest that estrogen may be effective in decreasing

inflammation, infiltration of inflammatory cells, and lesion volume following SCI.

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