

**14<sup>th</sup> INTERNATIONAL SYMPOSIUM ON  
NEURAL REGENERATION (ISNR)  
Dr. Roger Madison, Director**

December 7<sup>th</sup>-11<sup>th</sup>, 2011  
Asilomar Conference Center  
Pacific Grove, CA  
USA

*Hosted by:*

International Symposium on Neural Regeneration  
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(Office of Research and Development)

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or by calling (919) 286-0411 x7691.



# **THE FOURTEENTH INTERNATIONAL SYMPOSIUM ON NEURAL REGENERATION**

**December 7-11, 2011**

Asilomar Conference Grounds  
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Pacific Grove, California 93950

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## **ACKNOWLEDGEMENTS**

The symposium is sponsored by the US Department of Veterans Affairs (Biomedical Laboratory Research and Development Service, Office of Research and Development), the Paralyzed Veterans of America (Education Foundation), the National Institutes of Health (National Institute of Neurological Disorders and Stroke), California Institute for Regenerative Medicine, International Society for Neurochemistry and Mission Connect (A Project of TIRR Foundation).

# INTRODUCTION

The Fourteenth International Symposium on Neural Regeneration will be held at the Asilomar Conference Center in Pacific Grove, California from December 7-11, 2011. The International Symposium on Neural Regeneration began in 1985, and has been held on a biennial basis since that time. The primary sponsor has been the Department of Veterans Affairs, with the NIH continuously co-sponsoring the symposia since 1987. Long term generous support has also been given by the Paralyzed Veterans of America. Current ISNR funding has also been received from the California Institute for Regenerative Medicine, the International Society for Neurochemistry and Mission Connect, which is a project of TIRR Foundation. The generous support of all of our sponsors is gratefully acknowledged.

The keynote speakers for this year's symposium will be Mark Tuszynski from the University of California, San Diego and the San Diego VA Medical Center. Featured talks will be given by Hunter Peckham from Case Western Reserve University and the Cleveland VA Medical Center; Colin McCaig from the University of Aberdeen; Dwight Bergles from the Johns Hopkins School of Medicine; Stefan Heller from Stanford University School of Medicine and Ed Boyden from Massachusetts Institute of Technology. Following the format of preceding neural regeneration symposia, the program is divided into six sessions, including: 1) Brain Machine Interface, chaired by Doug Weber; 2) DEBATE – The future of functional recovery is robotics not regeneration, moderated by Dave Shine; 3) Stem Cells – The promises and challenges, chaired by Itzhak Fischer; 4) Translational Approaches, chaired by Naomi Kleitman; 5) ISN Symposium on Neurochemistry and Neurobiology of Repair, chaired by Jacqueline Bresnahan; and 6) Route 28 Summit Presentations – Novel ways to exploit stem cells for recovery of CNS function, chaired by Theo Palmer. The abstracts for the speaker presentations as well as 87 poster presentations are given in the program on the following pages.

The primary purpose of the symposium is to present current work in neural regeneration, especially in those areas of research in which there has been some notable recent progress or in which some particularly interesting issues have been raised. A secondary purpose is to foster an atmosphere that is both stimulating and conducive to a free interchange of ideas among investigators, or between seasoned investigators and students. The International Neural Regeneration Symposium has become an established, regularly occurring event with high attendance by both students as well as internationally recognized experts in the field of neural regeneration.

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Director, International Symposium on Neural Regeneration  
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# 14<sup>th</sup> International Symposium on Neural Regeneration

## Scientific Program

### WEDNESDAY – DECEMBER 7, 2011

**3:00 PM** Arrival of participants and check-in

**6:00 PM** DINNER

**7:30 PM** Welcome and Keynote Address

**Keynote Speaker:** Mark Tuszynski  
University of California, San Diego  
San Diego VAMC  
San Diego, CA - USA  
*“Combinatorial Strategies for Regeneration After Spinal Cord Injury”*

### THURSDAY – DECEMBER 8, 2011

#### **Session One: 8:15 am**

Brain Machine Interface (BMI)

8:15 AM Chair: Doug Weber  
University of Pittsburgh  
Pittsburgh VAMC  
Pittsburgh, PA - USA

8:30 AM Andrea Kubler  
University of Wurzburg  
Germany  
*“Out of the frying pan into the fire – BCI faces real world application”*

9:00 AM Bradley Greger  
University of Utah  
Salt Lake City, UT - USA  
*“Micro-electrodes in and on the cerebral cortex for decoding and encoding information”*

9:30 AM Chet Moritz  
University of Washington  
Seattle, WA - USA  
*“Leveraging neural plasticity for the treatment of paralysis and other movement disorders”*

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- 10:00 AM Jennifer Collinger  
University of Pittsburgh  
Pittsburgh VAMC  
Pittsburgh, PA - USA  
*“Is BCI technology ready for prime time? The science is mature and the consumers are willing - what are we waiting for?”*
- 11:00 AM Featured Speaker  
**Hunter Peckham**  
Case Western Reserve University  
Cleveland VAMC  
Cleveland, OH - USA  
*“The Role of Neural Prosthesis and Neural Stimulation In the Restoration of Function”*
- 12:00 Noon LUNCH
- 1:00 PM Featured Speaker  
**Colin McCaig**  
University of Aberdeen  
Scotland  
*“Regulating neural cell behaviour with extracellular electrical signals”*
- 2:00 PM **Viewing: Poster Session 1**
- 6:00 PM DINNER
- Session Two: 7:15 PM – 9:00 PM (or beyond)  
DEBATE – Proposition: The future of functional recovery is robotics not regeneration
- 7:15 PM Moderator: Dave Shine  
Baylor College of Medicine  
Houston VAMC  
Houston, TX - USA
- Affirmative: Vivian Mushahwar  
University of Alberta  
Edmonton, Alberta, Canada
- Chet Moritz  
University of Washington  
Seattle, WA - USA
- Doug Weber  
University of Pittsburgh

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Pittsburgh VAMC  
Pittsburgh, PA - USA

Alan Harvey  
University of Western Australia  
Crawley, Western Australia

Negative: Hunter Peckham  
Case Western Reserve University  
Cleveland VAMC  
Cleveland, OH - USA

Linda Noble-Hauesslein  
University of California, San Francisco  
San Francisco, CA - USA

Jerry Silver  
Case Western Reserve University  
Cleveland, OH - USA

Arthur Prochazka  
University of Alberta  
Edmonton, Alberta, Canada

## **FRIDAY – DECEMBER 9, 2011**

### Session Three: 8:15 AM

Stem Cells – The promises and challenges

8:15 AM Chair: Itzhak Fischer  
Drexel University  
Philadelphia, PA - USA

8:30 AM Mathew Blurton-Jones  
University of California, Irvine  
Irvine, CA - USA  
*“Can Neural Stem Cells be used to treat Alzheimer disease?”*

9:00 AM Itzhak Fischer  
Drexel University  
Philadelphia, PA - USA  
*“Transplanting neural stem cells to reconnect the injured spinal cord”*

9:30 AM Nicholas Maragakis  
Johns Hopkins School of Medicine  
Baltimore, MD - USA

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*“Scientific and Clinical Challenges in using Stem Cells for Investigating and Treating Amyotrophic Lateral Sclerosis”*

10:00 AM Gary Steinberg  
Stanford University School of Medicine  
Stanford, CA - USA  
*“Neural Stem Cell Therapy for Stroke: Underlying Mechanisms and Clinical Translation”*

10:30 AM BREAK

11:00 AM Featured Speaker  
**Dwight Bergles**  
Johns Hopkins School of Medicine  
Baltimore, MD - USA  
*“Fate and function of glial progenitors in the mammalian CNS”*

12:00 Noon LUNCH

1:00 PM Featured Speaker  
**Stefan Heller**  
Stanford University School of Medicine  
Stanford, CA - USA  
*“Inner ear sensory hair cells from stem cells”*

Session Four: 7:15 - 9:00 PM

Translational Approaches – “Lost in Translation”

7:15 PM Chair: Naomi Kleitman  
NIH/NINDS  
Bethesda, MD - USA  
*“Implications Of Replications: Improving The Quality Of Preclinical Research”*

7:45 PM David Howells  
University of Melbourne  
Melbourne, Australia  
*“Avoiding bias at the bench”*

8:15 PM Dan Lammertse  
Craig Hospital  
University of Colorado, Denver  
Denver, CO - USA  
*“The Challenges of Translation: A Clinical Perspective”*

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8:45 PM Jan Nolta  
University of California, Davis  
Davis, CA - USA  
*“Working toward cellular therapies to treat Huntington's disease”*

**SATURDAY – DECEMBER 10, 2011**

Session Five: 8:15 AM

ISN Symposium on Neurochemistry and Neurobiology of Repair

8:15 AM Chair: Jacqueline Bresnahan  
University of California, San Francisco  
San Francisco, CA - USA

8:30 AM Linda Noble-Haesslein  
University of California, San Francisco  
San Francisco, CA - USA  
*“Matrix metalloproteinases as modifiers of injury and recovery processes after spinal cord injury”*

9:00 AM Herb Geller  
NIH/NHLBI  
Bethesda, MD - USA  
*“The Sour Side of Sugars: Chondroitin Sulfate Signaling in Axonal Guidance”*

9:30 AM Roman Giger  
University of Michigan  
Ann Arbor, MI - USA  
*“Identification and functional characterization of novel receptors for inhibitory chondroitin sulfate proteoglycans.”*

10:00 AM Klaus Nave  
Max Planck Institute for Experimental Medicine – Germany  
*“The role of myelinating glia in preserving axon function”*

10:30 AM BREAK

11:00 AM Featured Speaker  
**Ed Boyden**  
Massachusetts Institute of Technology  
Cambridge, MA - USA  
*“Optogenetics: Tools for Controlling Brain Circuits With Light”*

12:00 Noon LUNCH

1:00 PM **Viewing: Poster Session 2**

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Session Six: 4:15 PM – 6:00 PM

Route 28 Summit Presentations – Novel ways to exploit stem cells for recovery of CNS function.

4:15 PM      Chair: Theo Palmer  
                  Stanford University  
                  Palo Alto, CA - USA

**Symposium Summary:** James Guest and John Steeves

6:30 PM      Symposium Banquet

7:30 PM      Awards Presentation (Banquet Hall)

**SUNDAY – DECEMBER 11, 2011**

Departure of Participants

### **O-1 Sprouting, Regeneration and Relay Formation After SCI: Realistic Targets for Translation?**

*M. Tuszynski<sup>1,2</sup>, P. Lu<sup>1</sup>, E. Rosenzweig<sup>1</sup>, L. Alto<sup>1</sup>, K. Kadoya<sup>1</sup>, A. Blesch<sup>1</sup>, J. Brock<sup>1</sup>, L. Havton<sup>3</sup>, M. Beattie<sup>4</sup>, R. Edgerton<sup>5</sup>, G. Courtine<sup>6</sup>, J. Bresnahan<sup>4</sup>*

<sup>1</sup>*University of California – San Diego,*

<sup>2</sup>*Veterans Administration Medical Center – La Jolla,* <sup>3</sup>*University of California-Irvine,*

<sup>4</sup>*University of California – San Francisco,*

<sup>5</sup>*University of California – Los Angeles,*

<sup>6</sup>*University of Zurich*

Substantial progress has been made in the last 30 years in understanding fundamental responses of the nervous system to injury, and in elucidating mechanisms limiting central axonal regeneration. Accordingly, different experimental approaches target different aspects of the degenerative/regenerative response. Some therapeutic discovery efforts aim to minimize the early effects of injury and to reduce secondary injury (neuroprotection). Other efforts focus on improving the nervous system's ability to recover from injury, targeting either: 1) enhancement of functional reorganization of the nervous system (e.g., through rehabilitation or electrical stimulation), 2) post-injury pharmacological modulation of spinal neurotransmitter systems, 3) enhancement of intraspinal compensatory collateral sprouting, 4) promotion of true axonal regeneration into and beyond a lesion cavity, and 5) formation of novel circuitry across a lesion site, i.e., neuronal relay formation. Several of these approaches overlap; for example, rehabilitation or electrical stimulation can modulate transmitter systems and axonal sprouting.

This talk will provide an update on SCI research that focuses on structural aspects of neural repair. We will attempt to demonstrate that enhancement of endogenous sprouting, induction of bridging axonal regeneration, and formation of novel relay circuits all constitute

distinct and intriguing mechanisms for enhancing outcomes after spinal cord injury. The translational relevance of these approaches will be discussed.

### **O-2 Out Of The Frying Pan Into The Fire - BCI Faces Real World Application**

*A. Kubler*

*Department of Psychology, University of Wuerzburg, Germany*

Brain-Computer Interfaces (BCIs) have been investigated for more than 20 years. Many BCIs use non-invasive electroencephalography as a measurement technique and mostly sensorimotor rhythms (SMR) or event related potentials (e.g., P300) as an input signal. Since the early days of BCI in the late 1980's not only data processing has improved, but also stimuli presentation, feedback modes and training protocols have been varied and a plethora of applications have been developed and refined. These applications are facing the challenge of being transferred from the research laboratory into real life situations to serve motor-impaired people in their homes as assistive technology. Evaluation studies are carried out at the patients' bedside and their home environment. Recently BCI control was integrated into commercial assistive technology ICT product and the usability of the first prototype was evaluated in terms of effectiveness, efficiency and user satisfaction. High performance with the BCI and high overall satisfaction of users and AT-experts, contrast with the fact that none of them could imagine the use of the device in daily life without improvements. However, an increasingly better understanding of the factors influencing BCI performance renders such improvement likely in the near future.

### **O-3 Micro-Electrodes In And On The Cerebral Cortex For Decoding And Encoding Information**

*B. Greger*

*Department of Bioengineering,  
University of Utah, Biomedical Polymers  
Research Building, 20 South 2030 East, Salt  
Lake City, UT 84112-9458*

We are investigating the ability of micro-ECoG grids to record multiple independent neural signals from the cortical surface for use in neural prosthetic applications. This study describes the classification of spoken words using surface local field potentials (LFPs) recorded on subdural micro-ECoG grids. Data recorded from these micro-ECoG grids supported accurate and rapid classification of spoken words. Furthermore, electrodes spaced only millimeters apart demonstrated varying classification characteristics and strong correlations with different words, suggesting that cortical surface LFPs may encode information with high temporal and spatial resolution. Arrays which penetrate into the parenchymal of the brain are being used by several groups to provide control signals for neural prosthetic applications. We have also been investing these devices focusing on fine finger movements and providing sensory information through micro-stimulation. Using neural signals recorded from primary motor cortex we have been able to classify individual finger movements with a high degree of success. We've been able to evoke subjective perceptions using micro-stimulation. These results further support the micro-electrode technology as a promising technology for both motor and sensory neural prosthetic applications.

#### **O-4 Leveraging Neural Plasticity For The Treatment Of Paralysis And Other Movement Disorders**

*C. T. Moritz*

*Departments of Rehabilitation Medicine  
and Physiology & Biophysics, University of  
Washington School of Medicine, Seattle, WA,  
USA 98195*

Brain-machine interfaces (BMI) and neuroprosthetic technology have the potential to dramatically improve quality of life after paralysis resulting from spinal cord injury, stroke or traumatic brain injury. We have recently demonstrated that brain activity can be used to control Functional Electrical Stimulation (FES) delivered to muscles and reanimate simple movements of an otherwise paralyzed wrist<sup>1</sup>. Monkeys rapidly learned to modulate the activity of individual neurons in motor areas of the brain in order to control the timing and magnitude of FES delivered to temporarily paralyzed wrist muscles. In addition to direct muscle stimulation, another promising neuroprosthetic approach is intraspinal stimulation. This technique has shown promise in the lumbar spinal cord with success in reanimating coordinated lower extremity movements, including weight bearing and stepping in animal models. We recently quantified the hand and arm movements evoked by cervical spinal stimulation<sup>2</sup>. Movements of the digits, wrist and arm were readily evoked by intraspinal stimulation in the cervical cord of sedated monkeys. Due to the ease of activating complex functional movements from a small number of stimulating sites, intraspinal stimulation may be an ideal method for reanimating paralyzed limbs under direct control of a BMI. In addition to directly restoring movements, brain-triggered spinal stimulation may also aid in guiding recovery and promoting regeneration after injury to the brain or spinal cord. Recent work demonstrated that pairing activity recorded within the brain with stimulation delivered a short distance away lead to durable increases in connectivity based on Hebbian mechanisms. We are currently testing whether synchronizing brain activity with intraspinal stimulation below an incomplete spinal lesion leads to functional recovery. Such a regenerating BMI may have the capacity to direct synaptic strength in spared

pathways, either alone or in collaboration with stem cell therapies.

References

1. Moritz, C. T.\* , Perlmutter, S. I., Fetz, E. E. (2008) Direct control of paralyzed muscles by cortical neurons. *Nature*, 456, 639-642.
2. Moritz, C. T.\* , Lucas, T. H., Perlmutter, S. I., Fetz, E. E. (2007) Forelimb movements and muscle responses evoked by microstimulation of cervical spinal cord in sedated monkeys. *Journal of Neurophysiology*, 97(1), 110-120.

Supported by a National Institutes of Health EUREKA Award (NIH/NINDS 1R01NS066357) and Ruth L. Kirschstein NRSA (NIH/NINDS F32NS5101), an American Heart & Stroke Association Scientist Development Grant (AHA 09SDG2230091), the University of Washington Royalty Research Fund (#4417), and the Bayley Family Foundation, and an NSF ERC in Sensorimotor Neural Engineering (EEC-1028725).

**O-5 Is BCI Technology Ready For Prime Time? The Science Is Mature And The Consumers Are Willing- What Are We Waiting For?**

*J.L. Collinger*

*Human Engineering Research Laboratories, Department of Veterans Affairs, Pittsburgh, PA; Department of Physical Medicine and Rehabilitation, University of Pittsburgh, Pittsburgh, PA*

Brain-computer interface (BCI) technology can take many forms but all have the same goal of translating command signals from the brain to functional control signals for an external device. Researchers have been successful in developing and testing BCI systems to control spellers, computer cursors, and even robotic arms. Much of this research has been conducted in non-human primates or with able-bodied individuals. Potential consumers of BCI technology are eager to take advantage of this technology to assist with activities of daily living and improve their quality of life. Each type of BCI system has advantages and disadvantages, as well as unique scientific, technical, and regulatory challenges

to bring the product to market. In this talk, I will discuss the progress that has been made in motor-based BCI research as well as perceived and real limitations to translating this technology to the clinic. The consumer population should be included as an important contributor to the development process. In a recent survey of veterans with spinal cord injury, we found that restoration of bladder/bowel function and walking ability were important priorities of the entire group. For those with tetraplegia, restoration of arm and hand function was the most important. Clearly integration of BCI and other technologies, such as functional electrical stimulation, will be critical to delivering a product that meets the priorities of the end users. Independent operation was reported to be the most important BCI design characteristic and training time was the least important. Interestingly, even though more than 70% of respondents indicated that non-invasiveness was a very important design characteristic, more than half indicated that they would definitely, or very likely, consider having surgery to implant BCI electrodes. Individuals with other disabilities will likely have different priorities and should also be included in pre-market technology development.

**Disclaimer:** The views expressed herein are those of the authors and do not reflect the official policy or position of the Department of Veterans Affairs, Department of the Army, Department of Defense, or the United States government.

**O-6 The Role of Neural Prosthesis and Neural Stimulation in the Restoration of Function**

*P. H. Peckham*

*Case Western Reserve University and Veterans Affairs Medical Center, Cleveland, OH*

Major advances have been made over the past decade in use of neuroprostheses for restoration of motor function. These advances have been built on the fundamental science of

neural excitation and the technologies for implantable stimulation and control. The technologies have advanced to provide operational systems that function in the human body for decades, and include implantable electrodes, stimulation devices, and sensors. The neural interface between the excitable tissue and the delivery electrode are enabling evermore powerful control and precision in the delivery of stimulation, not only providing for excitation but also blocking of neural activity (e.g. for annihilation of pain or spasticity) and selective stimulation. Distributed implantable systems and brain control interfaces are currently in development that will provide greater flexibility in implementation and performance.

The clinical manifestations of these findings are the availability of systems that have been created, and are being developed, to restore function. These include many areas of the body for people with spinal cord injury, including hand grasp, standing and walking, breathing, and bladder and bowel control. Several patients have benefited from systems that enable more than one function (e.g. hand function and bladder control). The presentation will provide examples of both upper and lower extremity neuroprostheses to restore full arm mobility and levels of standing and ambulation. Several areas where future advances are likely to meet clinical challenges will be discussed.

### **O-7 Electrical signals control multiple cell behaviours.**

*C. D. McCaig*

*University of Aberdeen, Scotland*

There is a long history of the use of electrical stimulation in medicine. For instance the Romans used the discharge from electrical fish to treat a number of pathologies, including gout and migraine. More recently, we have become aware that many tissues generate their own electrical signals which are present

generally in the extracellular spaces, for minutes, hours, even days. A host of basic behaviours such as the cell cycle, cell division, cell shape, cell migration and cell growth may all be controlled by these small electrical signals during normal development.

Following damage in several systems, steady electrical signals rapidly re-appear and again seem to regulate a range of coordinated cell activities. In epithelial tissues such as skin and cornea, there is direct evidence for electrical regulation of the axis of cell division, the rate of cell proliferation and the direction of cell migration. These events need to be coordinated for successful wound healing. Since there is evidence that these electrical signals may be the earliest to appear at a wound and that they may override coexisting chemical signals, they could act as a master regulator signal to kick start an integrated array of coordinated cell behaviours. Similar electrical signals have been measured following injury to the central nervous system and spinal cord. The mechanisms underlying the generation of these signals and the varying mechanisms by which electrical signals direct neuronal cell division, neuronal and glial cell migration, nerve guidance and epithelial cell migration will be explored.

#### **Background References**

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Zhao, M., Song, B., Pu, J., Wada, T., Reid, B., Tai, G., Wang, F., Guo, A., Walczysko, P., Gu, Y., Sasaki, T., Suzuki, A., Forrester, J.V., Bourne, H., Devreotes, P., McCaig, C.D. & Penninger, J. (2006). Electrical signals control wound healing via phosphatidyl -3 kinase  $\gamma$  and PTEN. *Nature* 442, 457-460.

McCaig, C.D., Rajniecek, A.M. & Song, B. (2009). Electrical Dimensions in Cell Science. *J. Cell Sc.* 122, 4267-4276.

### **O-8 Can Neural Stem Cells be used to treat Alzheimer disease?**

*M. Blurton-Jones*

*Department of Neurobiology & Behavior, University of California at Irvine, Irvine, CA 92697-1705*

Alzheimer disease (AD) is the leading cause of age-related dementia, affecting over 5 million people in the US alone. Unfortunately, currently approved therapies are largely palliative. Thus, there is a critical need to identify and test novel approaches to treat this disorder. Recently, we examined the effects of neural stem cell (NSC) transplantation in a transgenic model of AD. Our studies revealed that haplotype-matched murine NSCs can improve learning and memory in aged 3xTg-AD mice. Interestingly, NSCs have no effect on the underlying beta-amyloid or tangle pathology. Instead, the mechanism underlying improved cognition involves a robust enhancement of hippocampal synaptic connectivity, mediated by brain-derived neurotrophic factor. Although these initial findings suggest that NSC transplantation could provide a promising therapeutic approach for AD a great deal of additional work is needed. For example, NSC transplantation fails to modify the underlying beta-amyloid pathology. Long-term efficacy may therefore require combinatorial approaches that also target beta-amyloid or tangle pathology. To that end we have begun testing the use of NSCs to deliver disease-modifying proteins such as neprilysin. Our experiments reveal that NSC-mediated delivery of neprilysin can dramatically reduce beta-amyloid pathology in aged 3xTg-AD mice. On-going studies will determine whether this approach provides additional long-term cognitive benefits. Experiments aimed at identifying and testing candidate human NSCs lines are also being actively pursued.

### **O-9 Transplanting neural progenitors to reconnect the injured spinal cord**

*I. Fischer and J. Bonner*

*The Spinal Cord Research Center, Department of Neurobiology and Anatomy, Drexel University College of Medicine, Philadelphia, PA 19129*

Spinal cord injury (SCI) is characterized by cell death and loss of connectivity resulting in permanent functional deficits. Transplants of neural progenitor cells (NPC) have a potential to replace neurons and promote connectivity, but significant challenges remain regarding neuronal integration and functional connectivity. To address these issues we developed a relay model of SCI repair where graft derived neurons are used to reconnect injured dorsal column sensory axons with the denervated dorsal column nuclei (DCN). Neuronal and glial restricted precursors (NRP/GRP), expressing the human placental alkaline phosphatase (AP) marker, were transplanted into a C1 dorsal columns injury. A week later, BDNF-expressing lentivirus was injected into the DCN to guide graft axons to the intended target. We used tracing methods and immunocytochemical analysis by light and electron microscopy to demonstrate that NRP-derived neurons are capable of establishing afferent and efferent synaptic connections with the injured host at the site of injury and the DCN, respectively. Specifically, we observed anterogradely traced sensory axons regenerating into the graft, and robust growth of graft-derived AP-positive axons along the neurotrophin gradient into the DCN. We then used stimulus evoked c-Fos expression to test synaptic activity and found that host axons formed active synapses with graft neurons at the injury site. We also observed reproducible electrophysiological activity in the DCN, with a temporal delay predicted by the relay model, providing evidence for the presence of a functional synapse at the grafting site and the conduction of action potentials by graft axons to the DCN target. This study demonstrates the ability of NPC to form a neuronal relay across the injured spinal cord and provides the

framework for the restoration of sensory and possibly motor activity.

### **O-10 Scientific and Clinical Challenges in using Stem Cells for Investigating and Treating Amyotrophic Lateral Sclerosis**

*N.J. Maragakis*

*Johns Hopkins University Department of Neurology, Baltimore, MD*

Amyotrophic lateral sclerosis (ALS) is the most common form of adult motor neuron disease in which there is progressive degeneration of both the upper motor neurons in the cortex and the lower motor neurons in the brainstem and spinal cord. Because modeling ALS has been largely limited to the use of the mutant SOD1 animal model of familial ALS, a significant proportion of ALS biology has gone largely unstudied.

“Stem Cells” from a variety of different sources are excellent tools for investigating and potentially treating a variety of neurological disorders. The relatively new discovery for the creation of induced pluripotent stem cells (iPSC) has also allowed the generation of pluripotent stem cells from skin fibroblasts.

This technique is particularly powerful for several reasons. 1. It allows for the development of neural tissues which cannot be readily sampled from living patients. 2. Investigators can develop stem cell lines from patients with sporadic ALS. 3. Stem cell lines can be derived from groups of patients with common features thus allowing for comparisons of various disease phenotypes. 4. iPSC allow for the differentiation into several neural subtypes—each of which has a unique role in disease onset and progression. 5. Cell lines can be used to study potential pathways possibly involved in ALS pathogenesis. 6. Candidate drugs can be screened in specific assays using iPSC-derived neural subtypes.

However, with the promise and potential that comes with the broad array of stem cells as

tools comes the challenge of investigating differences amongst these stem cell types with regard to the methods by which they are generated, heterogeneity of the tissues from which they are derived, differences in their immunogenicity, and potential tumorigenicity. Furthermore, educating the public about these challenges and limitations as well as their promise is the responsibility of the scientific community.

### **O-11 Neural Stem Cell Therapy for Stroke: Underlying Mechanisms and Clinical Translation**

*G. K. Steinberg*

*Department of Neurosurgery and Stanford Institute for Neuro-Innovation and Translational Neurosciences, Stanford University, Stanford, CA, USA*

Stroke is the leading cause of disability and the #2 cause of death in the Western world. Currently, no clinical therapy exists to restore neurologic function after stroke. Stem cell transplantation holds great promise for facilitating recovery after stroke. This presentation will discuss the mechanisms underlying behavioral recovery after experimental stroke such as replacement of neurons, immunomodulation, and secretion of trophic factors that promote endogenous plasticity including axonal sprouting, dendritic branching and neovascularization. Completed and ongoing clinical stem cell trials for stroke will be reviewed. The complex process of translating the experimental results into a Phase I clinical trial will be described.

### **O-12 Fate And Function Of NG2<sup>+</sup> Glial Progenitors In Health And Disease**

*D.E. Bergles*

*The Solomon H. Snyder Department of Neuroscience, Johns Hopkins School of Medicine, Baltimore, MD*

The mammalian CNS contains an abundant, widely distributed population of glial cells that expresses the chondroitin sulfate proteoglycan NG2 (CSPG4) and the alpha receptor for PDGF (PDGF $\alpha$ R). Although initially defined as a class of astrocytes, studies completed over the past two decades indicate that these cells represent a third class of macroglia, with properties distinct from astrocytes and oligodendrocytes. These NG2<sup>+</sup> cells serve as progenitors for oligodendrocytes (OLs) during early development, and are often referred to as oligodendrocyte precursor cells (OPCs). However, NG2<sup>+</sup> cells remain abundant in the mature CNS after myelinated tracts have been established, accounting for approximately 5% of all cells, and they retain the ability to proliferate throughout life. These cells undergo dramatic morphological changes and increase their proliferation following acute CNS injury and in neurodegenerative diseases; nevertheless, the contribution of these cells to regeneration and tissue repair remains uncertain. Over the past several years we have developed new lines of transgenic mice that have allowed us to manipulate gene expression within these progenitors, track their fate, monitor their dynamics on timescales of minutes to months *in vivo*, and selectively ablate these cells from the adult CNS. Genetic fate tracing studies using these mice indicate that NG2<sup>+</sup> cells are lineage restricted progenitors that either remain as progenitors or differentiate into oligodendrocytes, but do not transdifferentiate into astrocytes or neurons, a behavior that is maintained in neurodegenerative disease (amyotrophic lateral sclerosis). *In vivo*, time lapse imaging has revealed that NG2<sup>+</sup> cells are highly dynamic in the adult mouse cortex. They continually reorient their processes and migrate through tissue to sites of injury to participate in the formation of glial scars. Together, these results suggest that NG2<sup>+</sup> glial cells have prominent roles in regeneration and repair in the adult CNS.

### O-13 Inner Ear Sensory Cells From Stem Cells

S. Heller

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Despite elegant biophysical analyses, the molecular mechanisms that underlie our senses of hearing and balance are not fully understood. A major reason for this lack of knowledge is the paucity of material, a few thousand well-hidden sensory hair cells per inner ear, compared to, for example, the 140-150 million photoreceptor cells per retina. Our goal was to devise an efficient *in vitro* guidance protocol for generation of functional hair cell-like cells from a renewable source. This was achieved by utilizing, for the most part, known principles of early embryonic development and inner ear induction. Recapitulation of specific signaling events in the culture dish led to otic progenitor cells that were able to differentiate into clusters of nascent and maturing sensory epithelia, featuring cells with hair and supporting cell characteristics. Cytomorphological assessments revealed that hair cells generated in an *in vitro* environment grow typical hair bundles of single kinocilia and stereocilia of graded heights. Bundle-bearing cells responded to mechanical stimulation with currents that were reminiscent of transduction currents. Our guidance method offers a platform for molecular studies on hair cells, which are otherwise difficult to obtain in large numbers. Likewise, intermediate presumptively otic cell types, generated by applying inner ear developmental principals, are emerging as useful *in vitro* tools to study specific signaling pathways that function during inner ear development. The fact that *in vitro*-generated hair cell-like cells are functional shows that generation of replacement hair cells from pluripotent stem cells is feasible, a finding that justifies the development of stem cell-based

treatment strategies for hearing and balance disorders.

### **O-14 Implications Of Replications: Improving The Quality Of Preclinical Research**

*N. Kleitman*

*National Institute of Neurological Disorders and Stroke, NIH, Bethesda, MD.*

The 2009 International Symposium for Neural Regeneration included a session presenting the goals and results of the National Institute of Neurological Disorders and Stroke (NINDS) Facilities of Research Excellence in Spinal Cord Injury (FORE-SCI) replication contracts. This session invoked lively discussion and substantial interest in the importance and inherent difficulties of reproducing published observations in complex preclinical models. A review of the goals and the results of continuing NINDS FORE-SCI studies will be presented, highlighting findings from recent replications performed at the University of California, Irvine, and the Ohio State University contract sites. Conclusions drawn from these studies and the experience of performing such replications will be presented (Steward et al., *Exp. Neurology*, *in press*). In addition, other NINDS activities designed to improve the quality of NINDS-supported preclinical and clinical research through rigorous study design and transparent reporting (NOT-NS-11-023) will be discussed.

### **O-15 AVOIDING BIAS AT THE BENCH**

*D.W. Howells*

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Every year around 5 million people are affected by stroke, and the cellular and molecular pathophysiology of ischaemic brain

injury is, at least in animal models of the disease, well understood. However, while more than 700 interventions have published efficacy in animal stroke models, around 150 have been tested, and been found to be ineffective, in human stroke studies. If the animal models faithfully represent human pathophysiology (and what evidence there is suggests that this is, for the most part, the case) there are two potential explanations for the systematic failure of animal experiments to identify effective human neuroprotectants; either the clinical trials have been falsely negative, or the animal studies have been falsely positive. There are a number of potential reasons for the latter. In the pre-clinical development of stroke drugs we have usually failed to consider the impact of the risk factors such as age, hypertension and diabetes that define the bulk of the ischemic stroke population. For example, where efficacy has been reported in the context of hypertension, it is usually substantially lower. The interpretation of animal studies may also have exaggerated drug efficacy, distorting the selection of drugs for clinical trial and creating unreasonable expectations of clinical efficacy. Possible reasons for this might include systematic flaws in experimental design or conduct which have the effect of exaggerating efficacy, or a disproportionate publication of positive studies. Alternatively, clinical studies may have failed statistically to detect a true biologically significant therapeutic effect where one exists, perhaps through testing the wrong drug dose, in the wrong type of patients, or given at the wrong time, or recruiting too few patients.

### **O-16 The Challenges of Translation: a Clinical Perspective**

*D.P. Lammertse*

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The field of spinal cord injury (SCI) research is now more than 30 years into the era of clinical trials for interventions intended to improve neurological outcome, and yet we still have not achieved a consensus standard-of-care treatment. Motivated by an interest in improving the conduct of clinical trials in SCI as well as concerns over the growing availability of unproven cellular and other experimental therapies, the International Campaign for Cure of spinal cord injury Paralysis (ICCP) and others have published guidelines for clinical trial design and conduct (Anderson 2005, Fawcett 2007, Steeves 2007, Tuszynski 2007, Lammertse 2007). Others have commented on the pre-clinical evidence basis that would justify making the critical translational step to human trials (Steeves 2004, Kwon 2010). As the field moves forward, there is a growing need for improving the dialogue between pre-clinical and clinical scientists—ideally, the translational path is a two-way street: both bench-to-bedside and bedside-to-bench. This presentation will cover the clinician's perspective on the challenges inherent in the design and conduct of clinical trials in SCI with commentary on the necessary and sufficient evidence—both efficacy and safety, clinical and pre-clinical—that the Principal Investigator must consider when embarking on human subject research.

### **O-17 Working toward cellular therapies to treat Huntington's disease**

*J. A. Nolte*

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There is much interest in the use of Mesenchymal Stem Cells/Marrow Stromal Cells (MSCs) to treat neurodegenerative disorders, such as Huntington's disease (HD) and Amyotrophic lateral sclerosis (ALS). MSCs present a promising tool for cell therapy, and are currently being tested in FDA-approved Phase I to III clinical trials for many disorders. In pre-

clinical studies of neurodegenerative disorders MSCs have demonstrated efficacy in slowing disease progression. Proposed regenerative approaches to neurological diseases using MSCs include cell therapies in which cells are delivered via intracerebral or intrathecal injection. Upon transplantation, MSC in the brain promote endogenous neuronal growth, encourage synaptic connection from damaged neurons, decrease apoptosis, reduce levels of free radicals and regulate inflammation. These abilities are primarily modulated through paracrine actions. Therapies will capitalize upon the innate trophic support from MSC or on augmented growth factor support, such as delivering brain-derived neurotrophic factor (BDNF) into the brain to support injured neurons, using genetically engineered MSC as the delivery vehicles (*Joyce et al, 2010*). MSC engineered to secrete BDNF have had significant effects on reducing disease phenotype in transgenic HD mice (*Dey et al 2010*). We are using immune deficient mouse and large animal models to test the biosafety of human cell products that are therapeutic candidates for clinical trials. Phase 1 clinical trials to test the safety of MSC injection into the central nervous system to treat ALS, traumatic brain injury and stroke in humans are currently ongoing. The progress toward applying MSC-based cellular therapies to the treatment of Huntington's disease will be discussed.

### **O-18 Matrix Metalloproteinases As Modifiers Of Injury And Recovery Processes After Spinal Cord Injury**

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Of over 25 members of the matrix metalloproteinase (MMP) family, MMP-9 is one of the most studied in central nervous system injury. Nevertheless, we have yet to fully

appreciate its contributions to injury, wound healing events, and recovery. This is due to the complex functions of this protease that are context/time dependent, its expression in diverse cell populations, and an ability to engage other factors, having opposing or synergistic functions. This is exemplified in studies of MMP-9 in the context of barrier dysfunction, glial scar formation and leukocyte trafficking in the injured spinal cord. MMP-9 is expressed over the first week post injury with maximal expression within the first 24 hours after injury. It is localized to blood vessels in the acutely injured cord and contributes to early disruption of the barrier. In the chronically injured cord, MMP-9 is up regulated in astrocytes. Formation of an inhibitory glial scar is more prominent in spinal cord injured MMP-9 null mice relative to wild-type controls. *In vitro* studies confirm the specificity of MMP-9 directed migration of astrocytes. Finally, MMP-9 is integral to leukocyte recruitment and trafficking. This protease is utilized by neutrophils to transmigrate into the injured cord. Moreover, neutrophils constitute the primary source of MMP-9 in the acutely injured cord. Recent studies reveal a synergistic partnership between MMP-9 and SDF-1 in facilitating transmigration of monocytes into the injured spinal cord. That MMP-9 facilitates the trafficking of both neutrophils and monocytes is of considerable interest, as we have found that combinatorial strategies to reduce the infiltration of these leukocytes, enhances neurologic recovery. In summary, improved neurologic recovery in spinal cord injured MMP-9 null animals may be attributed to diverse, adverse roles for MMP-9, propagated by different cells types that contribute to the early injury response and direct aberrant wound healing.

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### **O-19 The Sour Side of Sugars: Chondroitin Sulfate Signaling in Axonal Guidance**

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Chondroitin sulfate proteoglycans are recognized as molecules which repel growing axons. Much of this repellent activity is abolished by treatment with the enzyme chondroitinase ABC, which digests the chondroitin sulfate glycosaminoglycan (GAG) chains, leaving the proteins intact.

These data support a primary role for CS GAG chains as active signaling molecules. CS GAG chains are heterogeneous, comprised of a series of sulfated disaccharides of glucuronic acid (GlcA) and N-acetyl-galactosamine (GalNAc), varying both in the length of the GAG chain and the disaccharide composition. Our data support 4-sulfation GalNAc of as being responsible for the signaling by CS GAG chains to neurons. We will discuss biochemical data and immunocytochemical data on the composition and localization of CS GAG chains from normal and injured brain, as well as functional data demonstrating the biological activities of CS GAG chains *in vitro* and anatomical data on the binding of CS GAG chains to brain. We will also address the signal transduction pathways used downstream of putative receptors for CS GAG chains.

### **O-20 Identification and Functional Characterization of Novel Receptors for Inhibitory Chondroitin Sulfate Proteoglycans**

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Following injury to the adult mammalian CNS, severed axons do not regenerate beyond the lesion site, often leading to permanent functional deficits. Multiple lines of evidence suggest that growth inhibitory molecules in CNS myelin (including Nogo, MAG, OMgp) and chondroitin sulfate proteoglycans (CSPGs) associated with glial scar tissue contribute to the growth inhibitory nature of the injured adult mammalian CNS tissue. CSPGs are a diverse family of extracellular matrix glycoproteins, members of which have been shown to function as potent inhibitors of neurite outgrowth *in vitro*. Enzymatic digestion of CSPG glycosaminoglycan (GAG) chains with chondroitinase ABC lyase (ChaseABC) promotes experience-dependent neuronal plasticity in the adult visual cortex and leads to improved behavioral outcomes following spinal cord injury *in vivo*.

Here we report on a novel interaction between select members of the Nogo receptor family (NgRs) and CSPGs. NgR1 and NgR3, but not NgR2, bind strongly to the glycosaminoglycan (GAG) chain of neural CSPGs. Soluble NgR1 and NgR3 bind in a ChaseABC-sensitive manner to postnatal-day (P)1 rat brain tissue sections. Soluble NgR1 and NgR3 bind strongly to optic nerve tissue sections of adult mice subjected to retro-orbital crush injury to the optic nerve. A much weaker binding of soluble receptors is observed to control (uninjured) optic nerve tissue. We have mapped the CS-GAG binding motif on NgR1 and show that it is distinct from the binding site of Nogo, MAG, and OMgp. A soluble form of the NgR1 GAG binding motif promotes neurite outgrowth of cerebellar neurons plated on a mixture of substrate adsorbed CSPGs.

Loss-of-function experiments revealed that NgRs are important for CSPG mediated inhibition of neurite outgrowth. Primary cerebellar granule neurons (CGNs) isolated from *NgR1*, *NgR2*, *NgR3* triple mutant (*NgR1,2,3*<sup>-/-</sup>) mice grow longer neurites on CSPGs than CGNs isolated from wild-type

controls. A similar release of inhibition was obtained with CGNs obtained from mice null for *RPTPσ*, a previously identified CSPG receptor. *NgR1,2,3*<sup>-/-</sup> triple null mice are viable into adulthood and show no obvious neurologic phenotype. To examine whether loss of all three NgRs leads to enhanced regeneration of severed retinal ganglion cell axons, adult mice were subjected to optic nerve injury. The combined loss of *NgR1*, *NgR2*, and *NgR3* leads to enhanced axonal regeneration following retro-orbital optic nerve injury as assessed by anti-GAP43 immunolabeling. The combined loss of the MAI receptors *NgR1* and *NgR2*, however, is not sufficient to promote axonal regeneration. Collectively, these results identify NgR1 and NgR3 as novel CSPG receptors, demonstrate functional redundancy among Nogo receptors, and provide unexpected evidence for shared mechanisms of MAI and CSPG inhibition.

### O-21 Oligodendrocyte Support Of Axon Function And Integrity

*K.-A. Nave\**, U. Fuenschilling, L. Supplie, F. Kirchhoff, A. Saab, I. Tzvetanova, C. Moraes, D. Mahad, J. Edgar, S. Beltan, J. Frahm., S. Boretius

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Oligodendrocytes maintain axon function and survival in myelinated fiber tracts. Specifically, we have hypothesized that myelinating glia provide trophic support, however the underlying mechanisms are not understood. Recently, we obtained evidence for a metabolic component of neuron-glia interactions by targeting a null mutation of the *Cox10* (protoheme IX farnesyltransferase) gene to myelinating glial cells. This gene encodes the hemeA-farnesyl transferase, essential for the assembly of the cytochrome *c* oxidase (COX) complex IV in mitochondria. In the absence of COX10, newly synthesized complex IV is unstable and rapidly

degraded. In the peripheral nervous system, these mutants exhibit a novel neuropathy phenotype with dysmyelination, axonal degeneration, muscle atrophy, and paralysis. Surprisingly in the CNS, depletion of mitochondrial respiratory functions from mature oligodendrocytes did not cause demyelination, axonal degeneration, or secondary inflammation in the CNS. In contrast to cultured oligodendrocytes, which die upon inactivation of complex IV, post-myelination oligodendrocytes survive in vivo by aerobic glycolysis. Using magnetic resonance spectroscopy, we found brain lactate levels increased, but only in mice exposed to volatile anaesthetics that inhibit respiration. This demonstrates that glycolysis products can be efficiently shuttled out of oligodendrocytes and utilized in the normal appearing white matter. In conditional mouse mutants lacking NMDA receptors from oligodendrocytes, we find that glial support of axonal energy metabolisms is significantly compromised. We suggest that oligodendroglial-axonal metabolic coupling is likely to serve a physiological function in myelinated tracts, and that its perturbation contributes to axonal degeneration (Supported by an ERC Advanced Investigator Grant).

### **O-22 Optogenetics: Tools For Controlling Brain Circuits With Light**

*E. S. Boyden*

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Depts. of Biological Engineering and Brain and  
Cognitive Sciences, Massachusetts Institute of  
Technology, Cambridge, MA*

Understanding how different kinds of neuron in the brain work together to implement sensations, feelings, thoughts, and movements, and how deficits in specific kinds of neuron result in brain diseases, has long been a priority in basic and clinical neuroscience. In order to determine how different kinds of neurons in the brain work together to implement brain

functions, and to assess the roles that specific sets of neurons play within neural circuits, it would ideally be possible to drive or quiet the activity of defined neurons embedded within an intact neural network. Over the last few years, we have developed a number of genetically-encoded tools that enable the electrical activity of neurons to be activated or silenced by pulses of light. These molecules are microbial opsins, seven-transmembrane proteins adapted from organisms found throughout the world, which react to light by transporting ions across the lipid membranes of cells in which they are genetically expressed. Beginning with the light-gated inward cation channel channelrhodopsin-2, adapted from the green alga *C. reinhardtii* and the light-driven inward chloride pump halorhodopsin, adapted from the archaeon *N. pharaonis*, we have mined genomic resources from across the tree of life to discover reagents that support high-performance, multiple-color, control of the electrical activity of defined neural substrates in the brain. Recently for example we demonstrated that molecules from different kingdoms of life enable powerful, multi-color silencing of different sets of neurons. We have also discovered molecules that enable 100% shutdown of neural activity in awake behaving mice and non-human primates, in response to light. We have also developed microfabricated light sources capable of delivering light into complexly-shaped, 3-D, distributed neural circuits. These tools are enabling the causal assessment of the roles that different sets of neurons play within neural circuits, and are accordingly being used by hundreds of groups to reveal how different sets of neurons contribute to the emergent computational and behavioral functions of the brain. These tools are also being explored as components of prototype neural control prosthetics capable of correcting neural circuit computations that have gone awry in brain disorders, and we recently have begun to conduct pre-clinical tests of the safety and efficacy of these molecules in non-human primates.

Presenters for posters numbered P-1 to P-46: Mount posters from 3:00-6:00 p.m. on Wednesday, December 7 and dismount posters from 12:00 – 1:00 p.m. on Friday, December 9. Poster authors in this group are asked to be at their posters from 2:00 – 4:00 p.m. on Thursday, December 8.

Presenters for posters numbered P-47 to P-88: Mount posters after 2:00 p.m. on Friday, December 9 and dismount posters after 8:00 p.m. on Saturday, December 10. Poster authors in this group are asked to be at their posters from 1:00 – 3:00 p.m. on Saturday, December 10.

Please do not mount or dismount posters during speaker presentations. Poster display numbers correspond to poster abstract numbers.

### **SESSION ONE**

- P-1** Impaired Degeneration And Regeneration Of Peripheral Motor Axons Of Mice Heterozygously Deficient For The Myelin Protein P<sub>0</sub> Gene  
*M. Moldovan, M. Rosberg, J. Vikeså, F. C. Nielsen, C. Krarup*
- P-2** Does The Canonical Wound Healing Model Help To Explain The Pathogenesis Of Compression Neuropathies?  
*M. Lin; P. Hahn; J. Kang; D. Frump; J. Jung; T. Chao; R. Gupta*
- P-3** Efficacy Of C3 Peptide To Promote Peripheral Nerve Regeneration Depends On The Mode Of Application.  
*K. Haastert-Talini, T. Hettwer, A. Rohrbeck, I. Just, C. Grothe*
- P-4** Peripheral Nerve Repair: An In Vivo Investigation of Structurally Enhanced Nerve Guidance Conduits  
*W. Daly, M. Abu-Rub, B. Breen, D. Zeugolis, C. O'Connell, L. Yao, T. Windebank, A. Pandit*
- P-5** Mmp-3 Inhibition Blocks Degradation of The Neuromuscular Junction After Traumatic Peripheral Nerve Injury  
*T. Chao; D. Frump; N. Nassiri; J. Jung; P. Hahn; T. Mozaffar; R. Gupta*
- P-6** Sensory Neuron Responses to Peripheral Tissue-Damage: Implications for SCI Secondary Conditions  
*J.C. Petruska, C.H. Hill, K.K. Rau*
- P-7** Aspartate Aminotransferase Is Elevated In Rat Dorsal Root Ganglion Neurons During Regeneration Following Sciatic Nerve Crush  
*B. Bolt, Z. Zhang, S. Alothman, K.E. Miller*

- P-8** Vesicular Glutamate Transporter 2 Expression Is Altered In Dorsal Root Ganglion Neurons During The Regenerative Phase Following Sciatic Nerve Crush  
*S. Alothman, Z. Zhang, B. Bolt, K.E. Miller*
- P-9** Alteration of Glutaminase In Rat Dorsal Root Ganglion Neurons During The Regenerative Phase Following Sciatic Nerve Crush  
*Z. Zhang, S. Alothman, B. Bolt, K.E. Miller*
- P-10** An Immunohistochemical Analysis of Various Growth-Associated Proteins In Regenerating Adult Rat Retinal Ganglion Cells  
*A.R. Harvey, C.E. Humphries, A.T. Julian, K.L. Hoath, M. Hellström, Y. Hu, M.A. Pollett*
- P-11** Patterns From Noise: A Data-Intensive Retrospective Study of Thoracic SCI Laboratory Records From 1992-2003 (N = 1400)  
*A. R. Ferguson, C. F. Guandique, A. W. Liu, J. L. Nielson, J. C. Bresnahan, M. S. Beattie*
- P-12** Signals From Noise: Reflex Variability Analysis As A Diagnostic Tool In Spinal Cord Injury  
*J.J. Cragg, L.M. Ramer, A.P. van Stolk, and M.S. Ramer*
- P-13** Evaluation Of Conduction Through Motor Pathways: Using Evoked Potentials In Non-Human Primates  
*F. Benavides, D. Tovar, A. Santamaria, L. Guada, J. Guest*
- P-14** Correlates of Motor Dysfunction After Spinal Cord Injury In Rats  
*G. Stein, J. Ankerne, O. Semler, S. Angelova, M. Ashrafi, L. Eisel, R. Harrach, G. Schempff, K. Wellmann, F. Wirth, O. Ozsoy, U. Ozsoy, E. Schönau, A. Irintchev, D. Angelov*
- P-15** Neuromuscular Plasticity In The Rat Forelimb After High Cervical Spinal Cord Injury  
*E.J. Gonzalez-Rothi, R.A. Federico, A. Daly, A. Rombola, B.E. O'Steen, K.V. Vandenborne, P.J. Reier, D.D. Fuller, M.A. Lane*
- P-16** In Vivo Testing Of Candidate Genes To Promote Neuron-Intrinsic Growth Ability And Axon Regeneration In The Injured Spinal Cord  
*M.G. Blackmore, Z. Wang, P. Zheng, C. Shields, V.P. Lemmon, J.L. Bixby*
- P-17** Allogeneic And Autologous Schwann Cell Grafts In A Porcine SCI Contusion Model: Immune Response And CNS Integration  
*A. Santamaria, F. Benavides, L. Guada, G. Athauda, H. Levene, J. Solano, J. Guest*

- P-18** Hot ‘n’ bothered: the role of TRPV1-positive sensory neurons in autonomic dysreflexia  
*L. M. Ramer, A. P. van Stolk, J. A. Inskip, M. S. Ramer, J. D. Steeves, A. V. Krassioukov*
- P-19** Myelin Gene Regulatory Factor Knockout Delays Functional Recovery From Spinal Cord Injury  
*G. J. Duncan, J. R. Plemel, B. J. Hilton, J. Liu, J. K. Kramer, J. Kim, S. Mao, and W. Tetzlaff.*
- P-20** *In Vivo* Real Time Observations of Immune Cells And Neurons In Traumatic Spinal Cord Injury In The Mouse  
*T. A. Evans, D. S. Barkauskas, A. Y. Huang, J. Silver*
- P-21** The Presence of Cd4<sup>+</sup> T Cells Is Required For Nt-3-Induced Axonal Sprouting In The Injured Spinal Cord  
*Q. Chen and H.D. Shine*
- P-22** Characterizing The Effect Of Neurotrauma On Spinal Cord And Dorsal Root Ganglion Vasculature  
*M.A. Crawford and M.S. Ramer*
- P-23** Autonomic And Sensorimotor Recovery From Cervical Contusion Injury Is Boosted By Midbrain Periaqueductal Gray Stimulation.  
*I.D. Hentall, A. Vitores, M.M. Carballosa-Gonzalez*
- P-24** Skin-Derived Precursors Differentiated Into Schwann Cells (Skp-Scs) And Transplanted Eight Weeks Post Spinal Cord Contusion Improve Recovery Of Motor Function And Bladder Pathology  
*P. Assinck, S. Dworski, J.S. Sparling, G.J. Duncan, D.L. Wu, Y. Jiang, J. Liu, C.K. Lam, B.K. Kwon, F. D. Miller, W. Tetzlaff*
- P-25** Nerve Regeneration And Improvement In Urodynamics And External Urethral Sphincter Activity In Complete Spinal Cord Transected Rats Treated With A Peripheral Nerve Graft, Acidic Fibroblast Growth Factor, And Chondroitinase ABC  
*Y.-S. Lee, H-H. Jiang, M. DePaul, C.-Y. Lin, M. S. Damaser, V. W. Lin, and J. Silver*
- P-26** Early and late behavioral and electrophysiological changes in lower urinary tract function following mid-cervical spinal cord injury  
*T. Martin – Carreras, G. Grossl, D.D. Fuller, P.J Reier, M.A. Lane, D.J. Hoh*
- P-27** Integration of Microchannel Neural-Electrode Interfaces Into Dorsal And Ventral Roots For Bladder Control After Spinal Cord Injury

- D Chew, E Delivopoulos, N Granger, S Mosse, I Minev, L Zhu, S McMahon, M Craggs, N Jeffery, N Donaldson, S Lacour, J Fawcett*
- P-28** Optimized acellular Grafts Support Functional Recovery after Cervical Spinal Cord Injury  
*Z. Z. Khaing, W. J. Alilain, J. Silver, and C. E. Schmidt*
- P-29** Environmental Enrichment Enhances Retrograde Transport of Polysynaptic Virus In The Dentate Gyrus After Traumatic Brain Injury  
*C. Burger, R. Zamanskaya, W. Liu, T.-J. Chang, J. Liu*
- P-30** Analysis of Gene Expression Following A Localized Traumatic Brain Injury Reveals A Bias Toward Increased Cell Death Locally And Cell Survival Remotely  
*T. E. White, G. D. Ford, A. Gates, M. LaPlaca, B. D. Ford*
- P-31** Training of The Impaired Forelimb After Traumatic Brain Injury Enhances Hippocampal Neurogenesis In Mice Without Corpus Callosum  
*S. Wu, M. Neumann, W. Liu, C.C. Lee, R. Zamanskaya, L. Noble-Haeusslein and J. Liu*
- P-32** Using Multimodel Therapies To Improve Outcome Following Traumatic Brain Injury  
*D. Bingham, T. Lam, J. Lee, T.J. Chang, C. Burger, C. Sun, S. Wu, J. Shi, S. Massa, R. A. Swanson, J. Liu.*
- P-33** Stroke Affects Functional Activation During Spatial Tasks  
*S. Badurek, W. Liu, C. Burger, C. Lee, R. Saloner, J. Liu*
- P-34** Reduction of Neuroprogenitor Cells In Mice Impedes Post-Stroke Functional Recovery  
*C. Sun, H. Sun, S. Wu, T.J. Chang, R. Saloner, S.G. Kernie and J. Liu*
- P-35** Experimental ischemic and hemorrhagic strokes induce memory impairment and reduce hippocampal functional recruitment during spatial navigation  
*C.C.J. Lee, Y. Wang, W. Liu, C. Sun, S. Badurek, S. Wu, C. Burger, J. Liu*
- P-36** Unexpected Neuronal Loss After Spinal Cord Injections In Primates  
*E. S. Rosenzweig, G. Courtine, J. H. Brock, Y. S. Nout, A. R. Ferguson, J. L. Nielson, S. C. Strand, R. Moseanko, S. Hawbecker, R. R. Roy, H. Zhong, J. L. Weber, M. S. Beattie, L. A. Havton, V. R. Edgerton, J. C. Bresnahan, O. Steward, and M. H. Tuszynski*
- P-37** Long-Distance Axonal Growth, Connectivity and Functional Recovery After Complete Spinal Cord Transection: Cell-Intrinsic Mechanisms Overcome Inhibition Of The Adult Lesioned Spinal Cord

- J.H. Brock, P. Lu, Y. Wang, L. Graham, K. McHale, M. Gao, D. Wu, , L.A. Havton, A. Blesch, B. Zheng, J.M. Conner, and M.H. Tuszynski*
- P-38** Daily Acute Intermittent Hypoxia Restores Respiratory Plasticity And Breathing Capacity Following Cervical Injury  
*S. Vinit, J. Terada, P.M. MacFarlane, G.S. Mitchell*
- P-39** Molecular Mechanisms involved in Recovery of Respiration after Upper cervical Spinal Cord Injury: Potential Therapeutic Strategies.  
*K. D. Nantwi, and L. P. Singh*
- P-40** Intraspinal grafts of neural progenitors improves respiratory function following mid-cervical contusion injury in adult rats  
*D.E. Sanchez, K. Salazar, L.M. Mercier, B.E. O'Steen, P.J. Reier, M.A. Lane*
- P-41** Changes In Interneuronal Activity In The Cervical Spinal Cord Of Adult Rats Following Spinal Cord Injury (SCI)  
*V.M. Spruance, A.M. Rombola, B.E. O'Steen, M.A. Lane, P.J. Reier*
- P-42** High Cervical Spinal Cord Injury Results In Altered Distributions of Medullary Inspiratory And Expiratory Neuronal Activity  
*L.M. Mercier, D.F. Ryczek, K-Z Lee, B.E. O'Steen, D.D. Fuller, P.J. Reier, M.A. Lane*
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**P-1 Impaired Degeneration And Regeneration Of Peripheral Motor Axons Of Mice Heterozygously Deficient For The Myelin Protein P<sub>0</sub> Gene**

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Mice with a heterozygous knock-out of the myelin protein P<sub>0</sub> gene (P<sub>0</sub><sup>+/-</sup>) are models of Charcot-Marie-Tooth disease. The mice are indistinguishable from wild-types (WT) at birth; however, from about 7 months of age, they develop a slowly progressing demyelinating neuropathy. Although conduction slowing is known to be associated with a reduction of compound muscle action potential (CMAP) amplitude, the relationship between demyelination and axonal loss remains poorly understood.

The aim of this study was to compare degeneration and regeneration of peripheral motor axons in early symptomatic P<sub>0</sub><sup>+/-</sup> and age matched WT.

Right sciatic nerves were lesioned at the thigh. Changes of the tibial nerve at ankle were investigated by electrophysiological, histological and molecular methods. In vivo motor nerve electrophysiology was carried out by conventional conduction studies and axon excitability studies using threshold tracking. The overall motor performance was investigated using Rotor-Rod. To map differences in degeneration, we compared gene expression profiles in the distal stump of degenerated nerves using Affymetrix genechips. To evaluate regeneration we monitored the recovery of motor function after crush, and then compared the fiber distribution by histology.

In both P<sub>0</sub><sup>+/-</sup> and WT axonal degeneration triggered a change of gene

expression related to axon guidance and neuroinflammation. At 7 days after lesion we found that on the lesioned side, a series of genes (Dnm3, Paxip1, Aspa, Tmod1, Frzb, Slitrk6, Thy1, Met, Sytl2, Sema3e, Fbn2, Akap6, Snord52 and Gnb4) were up-regulated less in P<sub>0</sub><sup>+/-</sup> as compared to WT, whereas on the unlesioned side the expression of these genes did not differ. CMAP recovery during regeneration appeared delayed in P<sub>0</sub><sup>+/-</sup>, in agreement with reduced excitability and poor performance at Rotor-Rod after one month. Together, these data suggest that both axonal degeneration and regeneration are impaired in the presence of mutant Schwann cells heterozygously deficient in the P<sub>0</sub> gene.

**P-2 Does The Canonical Wound Healing Model Help To Explain The Pathogenesis Of Compression Neuropathies?**

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Chronic nerve compression (CNC) injuries such as carpal tunnel syndrome cause patients significant morbidity with the ensuing loss of sensation and motor function. Previous studies have demonstrated changes in Schwann cell form and function with limited early axonal pathology during the progression of CNC injury. Recent advancements from our group have prompted a reinterpretation of compressive neuropathies as being an example of a wound-healing model with the associated phases of inflammation, angiogenesis, cellular proliferation, and connective tissue remodeling. Here we demonstrate CNC injury induces a progressive and significant increase in ECM components including fibronectin, laminin, and collagen type IV at the level of mRNA transcription and protein translation. Furthermore, we were able to localize their up-regulation to the site of CNC injury relative to controls supporting the hypothesis that CNC

injury does induce ECM remodeling. At later time points when the neural scar is well established, functional assessment of time-integrated blood flow dynamics using laser speckle imaging technology demonstrates significant reduction of neurovascular flow at the site of compression. Current experiments with alpha-2 laminin deficient and Desert hedgehog knockout mice will further elucidate the role of fibroblasts and Schwann cells within the basal lamina of the ECM following CNC injury. By considering the pattern of ECM expression with our quantitative and qualitative findings in two different animal models, the data supports that CNC injury induces a biologic response which mimics canonical wound healing and leads to up-regulation of ECM components with eventual scar formation. This pathway promotes tissue repair by modifying structural support of the peripheral nerve after CNC injury and thereby restricts the capacity for functional tissue and nerve regeneration with surgical management alone after this remodeling has occurred. As such, this work provides the basis for the development of adjuvant treatment to improve functional recovery for compression neuropathies.

**P-3 Efficacy Of C3 Peptide To Promote Peripheral Nerve Regeneration Depends On The Mode Of Application.**

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We have recently shown that one-time administration of C3<sup>156-181</sup> peptide derived from the Rho-inactivating C3 transferase (C3bot) from *Clostridium botulinum*, promotes peripheral nerve regeneration (PNR) (Huelsenbeck et al, Neurotherapeutics 2011).

Soluble C3<sup>156-181</sup> was applied into a sciatic nerve crush injury or the epineurial sutures after nerve autotransplantation. Here, we analysed the effects of C3<sup>156-181</sup> on PNR through a 10 mm epineurial pouch filled with PuraMatrix<sup>TM</sup> hydrogel containing 8 nmol C3<sup>156-181</sup>/kg. Regarding the previous positive results, we hypothesized that sustained release and thus prolonged presence of the C3<sup>156-181</sup> would increase axonal and functional regeneration in comparison to application of phosphate buffered saline (PBS) or C3bot in PuraMatrix. 10 mm nerve autotransplantation served as positive control (left sciatic nerves of adult rats; n=7/group).

The 10 mm epineurial pouch was prepared by stripping-off nerve fascicles through two epineurial windows. PuraMatrix formulations were then injected as replacement. During 12 post operational weeks, we performed functional tests; weekly: Static Sciatic Index (SSI)-Evaluation (motor task), Pinch-Test (mechanosensitivity), bi-weekly: non-invasive electrodiagnostic measurements (motor function). By end of the observation period, invasive electrodiagnostic measurements, lower limb muscle weight analysis and histomorphometrical analysis was performed.

Preliminary data demonstrate that autotransplantation is still the superior condition. SSI-evaluation did not show any significant recovery in presence of C3bot and C3<sup>156-181</sup>. Recovery of mechanosensitivity and functional axons (non-invasive electrodiagnostics) in the C3<sup>156-181</sup> group only slightly precedes the PBS and C3bot groups. The lower limb muscle weight did recover to the lowest level in the C3<sup>156-181</sup>.

In the previous study (Huelsenbeck et al, Neurotherapeutics 2011), effects of C3<sup>156-181</sup> were significantly stronger than those of C3bot or vehicle only, while here both groups show less regeneration than under control conditions. Further data analysis will demonstrate if indeed, prolonged availability of C3<sup>156-181</sup> at the lesion

site impairs PNR in contrast to the one-time administration.

**P-4 Peripheral Nerve Repair: An In Vivo Investigation of Structurally Enhanced Nerve Guidance Conduits**

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This study investigates the use of extruded collagen fibres (EGFs) as an intraluminal filler, to improve existing nerve guidance conduits (NGCs). It is hypothesized that EGFs increase the surface area available for cell adhesion, enhancing cell migration, and providing topographical guidance cues for regenerating axons. This study involves the characterisation of the EGFs *in vitro* and *in vivo* as a platform for cell migration and topographical guidance of axons. The EGFs were fabricated in a multi-step process. EGF structure was analysed using standard surface characterisation techniques. Neuronal interaction and cell migration, on the EGFs, was assessed using rat PC12 cells and 3T3 fibroblast cells respectively. Following *in vitro* characterization, 18 EGFs were enclosed within a collagen NGC, and implanted in a rat sciatic nerve model for 16 weeks. 16 weeks post implantation, simultaneous retrograde tracing and nerve morphometry analyses were carried out. *In vitro* assessment of the neural interaction, of the EGFs, showed a significant increase in neurite length and higher alignment versus control collagen fibres (n=3, p<0.05) and 3T3 cells successfully migrating across the fibres. 16 weeks post implantation, successful nerve

regeneration was seen across a 10 mm nerve gap. Initial histological results showed similar levels of regeneration to that of a hollow conduit. However retrograde tracing results showed a significant increase in targeted nerve regeneration in all EGF groups versus the autograft group. In conclusion: EGFs show the ability, *in vitro*, to increase aligned nerve growth. *In vivo*, the addition of EGFS, did not significantly affect the level of nerve regeneration but the addition of EGFS within the conduit showed a significant increase in targeted nerve regeneration. This targeted nerve regeneration is a critical step to improve current NGCs.

**P-5 Mmp-3 Inhibition Blocks Degradation of The Neuromuscular Junction After Traumatic Peripheral Nerve Injury**

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Traumatic peripheral nerve injuries often produce significant functional deficits despite optimal medical management. The loss of targets for reinnervation leads to a down-regulation of trophic factors guiding regenerating axons with the subsequent end-organ atrophy of the neuromuscular junction (NMJ). It is known that NMJ assembly and maintenance depends highly on the interaction between agrin and its receptor, muscle-specific kinase (MuSK). The NMJ matures during development under the direction of signaling mechanisms initiated with binding of agrin to MuSK. Agrin is degraded by matrix metalloproteinase 3 (MMP-3) produced by the perisynaptic Schwann cell. The current study focuses on assessing the NMJ after long-term denervation injury to simulate the clinical scenario. Specimens from both wild-type and MMP-3 knockout mice were harvested at 3 days, 7 days, 14 days, 1 month, 4 months (n=4 all time points) post-injury. Immuno-

histochemical analysis was performed to define the integrity of all three components of the NMJ after denervation. Agrin levels were quantified to determine if destabilization of the NMJ corresponds to a decrease in agrin. Specimens from normal muscle contained Ach receptors with round profiles and multiple perforations consistent with the normal phenotype. In contrast, denervated muscles demonstrated significant attenuation of Ach receptor profiles. Surprisingly, denervated muscles from MMP-3 animals contained receptor profiles with areas greater than contralateral specimens. Western blot data confirmed a decrease in agrin after denervation in wildtype mice. Conversely, agrin isoforms were upregulated following denervation in MMP3 knockout mice. The ability of the Ach receptor to resist destabilization after prolonged denervation secondary to the elimination of MMP-3 and the ensuing persistence of agrin at the NMJ provides an exciting novel opportunity to improve functional outcomes after traumatic nerve injuries.

**P-6 Sensory Neuron Responses to Peripheral Tissue-Damage: Implications for SCI Secondary Conditions**

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Spinal cord injured individuals frequently and repeatedly experience tissue damage secondary to the SCI itself (pressure ulcers, bowel impaction/colitis, lacerations, etc.). In spinal intact individuals such repeated tissue damage often leads to neuropathic pain. In the SCI population, such conditions below the level of the injury may not lead to pain

because of the loss of ascending transmission, but this does not prevent the effects on sensory and spinal neurons and spinal circuitry. Further, because SCI often includes loss of descending pain modulation, the effects of tissue injury on the nervous system may be worse than in the spinal-intact condition, but occur subliminally (clinically and experimentally).

We recently discovered that damage to peripheral tissues leads to many of the same responses in dorsal root ganglion (DRG) sensory neurons innervating that tissue as occur with direct injury to a peripheral nerve. There was strong and sustained expression of genes related to axonal regeneration. Injury of peripheral nerves is a standard method for inducing axonal growth in the injured spinal cord, and the process exhibits a conditioning response (i.e., growth after 2<sup>nd</sup> injury is greater than after single). Accordingly, the response of DRG to repeated tissue damage was synergistically-enhanced over the response to single tissue damage not only for regeneration-related genes, but also those linked to chronic pain. Repeated tissue damage induced changes in DRG that mimicked those induced by nerve-injury models of chronic pain, whereas a single bout of tissue damage had little effect.

These findings may have implications for the consequences of post-SCI conditions and their treatment standards. For the research and clinical communities to develop and implement effective therapies, particularly activity-based therapies which rely on proper sensory input, it is important that the status of sensory neurons and spinal circuitry below the level of the injury be addressed.

**P-7 Aspartate Aminotransferase Is Elevated In Rat Dorsal Root Ganglion Neurons During Regeneration Following Sciatic Nerve Crush**

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The sensory neurons of the dorsal root ganglia (DRG) are glutamatergic, utilizing glutaminase (GLS) in the glutamine cycle for glutamate production (Miller et al., *Pharmacol Ther* 130:283–2011). Aspartate aminotransferase (AST) is a key enzyme linking the neuronal TCA cycle with the glutamine cycle for synthesis of glutamate. We have previously demonstrated that AST is expressed in large ( $>800 \mu\text{m}^2$ ), medium ( $400\text{-}800 \mu\text{m}^2$ ) and small ( $100\text{-}400\mu\text{m}^2$ ) DRG neurons corresponding to A $\beta$ -, A $\delta$ -, and C-fiber neurons, respectively. During the acute phase of adjuvant induced arthritis, there is an increase in AST in small neurons in rat DRG. The fate of AST expression in the regenerative phase following peripheral nerve injury is unknown. We hypothesize that AST would be elevated in the DRG following sciatic nerve crush. In the present study, we evaluated changes in AST immunoreactivity in DRG neuronal cell bodies at 14 days following sciatic nerve crush. Adult Sprague Dawley rats were anesthetized, the sciatic nerve was exposed mid-thigh, and the nerve was crushed. Control rats received a skin incision mid-thigh. After 14 days, rats were anesthetized and transcardially perfused with fixative. L4 DRG's were processed for AST immunohistochemistry. DRG sections were observed and photographed with Olympus BX5 epifluorescence microscope and SPOT camera. Image J (NIH) was used to evaluate AST immunoreactive intensity. We observed a qualitative elevation in AST expression in all neuronal cell sizes. Using quantitative image analysis, we observed a statistically significant increase in AST immunoreactivity of small sized DRG neurons after nerve crush. We suggest that this increase is part of the regenerative process of C-fiber neurons. Other times points are needed for evaluation to

determine if this also occurs in medium and large sized neurons.

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**P-8 Vesicular Glutamate Transporter 2  
Expression Is Altered In Dorsal Root  
Ganglion Neurons During The Regenerative  
Phase Following Sciatic Nerve Crush.**

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Dorsal root ganglion (DRG) neurons are glutamatergic using glutamate as a neurotransmitter in the spinal cord and, in a subset of DRG neurons, releasing glutamate into peripheral tissue (Miller et al., *Pharmacol Ther* 130(3):283-309, 2011). DRG neurons express either vesicular glutamate transporter (VGLUT) 1 or 2 for the release of glutamate (Brumovsky et al., *Neuroscience*147(2):469-90, 2007). Previous studies have suggested alterations in glutamate metabolism in DRG neurons in the regenerative phase following peripheral nerve injury (Porcellati and Thompson, *J Neurochem* 1(4):340-7, 1957; Johnson, *Experientia*, 32(2):184-6, 1976), but it is unknown if there are changes in VGLUT expression. In the present study, we evaluated changes in VGLUT2 immunoreactivity in DRG neuronal cell bodies at 14 days following sciatic nerve crush. Adult Sprague Dawley rats were anesthetized, the sciatic nerve was exposed mid-thigh, and the nerve was crushed with a hemostat. Control rats received only a surgical incision. After 14 days, rats were anesthetized and transcardially perfused with picric acid-paraformaldehyde fixative. Lumbar 4 DRG's were removed and processed for immunohistochemistry using primary antiserum: mouse anti-VGLUT2 (1/2000, NeuroMab). DRG sections were observed and photographed using an Olympus BX5 epifluorescence microscope and SPOT camera.

Image J (NIH) was used to evaluate VGLUT2 immunoreactive intensity. VGLUT2 was found primarily in small to medium sized (100-800 $\mu\text{m}^2$ ) DRG neurons, although some larger neurons were VGLUT2 immunoreactive. At 14 days following nerve crush, VGLUT2 immunoreactivity was elevated in small DRG neurons (100-400 $\mu\text{m}^2$ ) and diminished in medium sized DRG neurons (400-800 $\mu\text{m}^2$ ). There was no change in large neurons (>800 $\mu\text{m}^2$ ). Our data indicate that glutamate metabolism (VGLUT2 expression) is altered in DRG neurons during regeneration. Since VGLUT2 is used for glutamate exocytosis, it will be important to determine the fate of VGLUT2 synaptic vesicles during the regenerative process. Funded by NIH AR047410 (KEM).

**P-9 Alteration of Glutaminase In Rat Dorsal Root Ganglion Neurons During The Regenerative Phase Following Sciatic Nerve Crush**

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Glutamate is the major neurotransmitter in nervous system and the integrity of the glutamate-glutamine cycle is important in maintaining or restoring neuronal function. After peripheral nerve insult, the glutamate-glutamine cycle is challenged or compromised. It has been reported that there is an elevation of glutamate at the site regenerating nerve fibers, but the source of the elevated glutamate has not been studied. Glutaminase (GLS) is the enzyme responsible for the conversion of glutamine to glutamate. Previous studies from our laboratory using inflammatory models show that GLS is elevated in dorsal root ganglion (DRG) neurons in the acute phase of inflammation, followed by a decrease in soma GLS due to axonal transport to the peripheral site of injury. In the current

study, we hypothesized that GLS expression would be altered in DRG neurons after sciatic nerve crush and the alteration might contribute the regeneration of the nerve. Unilateral sciatic nerve crush was induced in rats at midthigh level and rats with skin/muscle incision were used as controls. At 14 days after sciatic nerve crush, rats were transcidentally perfused with picric acid-paraformaldehyde and lumbar<sub>4,5</sub> DRGs were collected. GLS was localized by immunofluorescence and evaluated by image analysis with Image J (NIH). Neurons were separated by cell size: small (100-400 $\mu\text{m}^2$ ), medium (400-800 $\mu\text{m}^2$ ), large (>800 $\mu\text{m}^2$ ). These sizes often correlate with C-fiber, A $\delta$ -fiber, and A $\beta$ -fiber neurons, respectively. After 14 days, there was a significant decrease in GLS in DRG neurons. Analysis based on neuronal size showed that the major decrease in GLS expression level occurred in small-sized (<400 $\mu\text{m}^2$ ) cells. These results help further characterize the expression of GLS after nerve injury. Additional information is needed to understand the temporal expression pattern of GLS and to determine the role of GLS in the regeneration process at the periphery. Funded by NIH AR047410 (KEM).

**P-10 An Immunohistochemical Analysis of Various Growth-Associated Proteins In Regenerating Adult Rat Retinal Ganglion Cells**

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The rat visual system is often used to study the mechanisms associated with regenerative responses in the adult central nervous system (CNS). We examined the expression of a panel of key components of various cell signalling pathways, including phospho-Akt, phospho-CREB, arginase-1,

phosphorylated ribosomal protein S6 (p-S6), the neurotrophin receptor TrkB, growth associated protein GAP-43, the immediate early gene c-Jun, and the anti-apoptotic protein bcl-2. We attempted to discern differences between regenerating retinal ganglion cells (RGCs) and viable, non-regenerating RGCs. In anesthetized (ketamine/xylazine) young adult rats, the left optic nerve (ON) was cut and a segment of autologous peripheral nerve (PN) grafted onto the cut end. Four and 11 days after surgery, the grafted eye was injected with saline (control) or ciliary neurotrophic factor (CNTF) and a cell permeant cAMP analogue chlorphenylthio-cAMP (CPT-cAMP) (Cui et al 2003; Park et al 2004). Surviving RGCs were visualized using anti- $\beta$ III-tubulin antibody, and regenerating RGCs identified by injecting fluorogold or fast blue into PN grafts 4 weeks after surgery. As expected there was increased RGC viability and axonal regeneration in eyes injected with CNTF and CPT-cAMP, associated with increased expression of c-jun and GAP-43. About 90% of regenerating RGCs expressed these markers; however there was variability in expression - some regenerating RGCs were not immunoreactive for c-jun, others negative for GAP-43, while some surviving but not regenerating RGCs could express high levels of these proteins. Similarly, there was increased expression of p-S6 (Park et al 2008), although only 30-40% of regenerating RGCs were immunoreactive, often the larger cells. Expression of other growth-associated proteins was seen in both surviving and regrowing RGCs, more frequently in the latter, but again expression was inconsistent. Taken together, the results suggest that individual adult RGCs can harness more than one potential growth promoting signalling pathway when initiating a regenerative response.

Cui Q et al (2003) *Mol Cell Neurosci* 22:49-61.

Park K et al (2004) *J Neurosci* 24:10806-15.

Park K et al (2008) *Science* 322:963-6.

**P-11 Patterns From Noise: A Data-Intensive Retrospective Study of Thoracic SCI Laboratory Records From 1992-2003 (N = 1400)**

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Spinal cord injury (SCI) produces complex symptoms including disturbances in mobility, sensory, and autonomic function. Despite their heterogeneity, these outcomes often co-vary in animal models, producing a constellation of symptoms that manifest across numerous outcome measures. This suggests that SCI represents a *multivariate syndrome* rather than a singular deficit that can be characterized by univariate analysis (t-test; ANOVA) of a single outcome. Our work uses multivariate information-processing approaches to enable integrative translation of stable outcome patterns in large heterogeneous SCI datasets. We focus on emergent patterns across many outcomes, rather than individual outcomes that may only detect species-specific or model-specific changes. Our approach requires large datasets and sophisticated statistics that are uncommon in basic SCI research. We are now assembling a database of raw historical SCI data collected at multiple research centers. Our goal is to develop a common infrastructure to enable large-scale data analysis and data-sharing for the SCI research community. As proof-of-concept, the present abstract reports the process required to assemble a database and perform multivariate pattern-detection from raw laboratory records from one laboratory. We manually curated 746,164 data points encoding 66 variables and multiple time-points, from >1400 subjects with thoracic contusion injuries. Outcome variables included locomotor performance, autonomic measures (blood pressure, heart rate, bladder function), and spinal histology. The goal was to

build a fully annotated record for each subject to enable multivariate syndromic knowledge-discovery. We then applied non-linear principal components analysis (CAT-PCA) using appropriate link functions for each outcome within the multiscale dataset. Despite data heterogeneity, we were able to identify consistent syndromic features in this diverse archive, representing distinct therapeutic targets from the intersection of histology, locomotor, and autonomic outcomes. Through this data-intensive approach we hope to provide decision support for translational comparisons and bench-to bedside testing of experimental therapeutics. Support: NS067092, NS069537, AG032518, NS038079, NS31193

**P-12 Signals From Noise: Reflex Variability Analysis As A Diagnostic Tool In Spinal Cord Injury**

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Simple, non-invasive and quantifiable measures are essential to assess outcomes of spinal cord injury (SCI) to track spontaneous changes and evaluate the efficacy of treatment. Current clinical methods suffer from their semi-quantitative nature and operator inconsistencies. The result is an inability to objectively detect small but potentially significant changes. Here, we have evaluated the variability in the monosynaptic H-reflex as a novel method to assess changes in spinal circuitry following SCI. The H-reflex is a widely-used clinical electrophysiological tool; it manifests as a compound muscle action potential upon electrical stimulation of a motor nerve. As striking as day-to-day and subject-to-subject differences is the variability of H-reflex size during an individual recording session. The usual procedure for reporting H-wave data is to

generate an *average* response from a number of trials: what is lost is information contained *within* H-reflex variability. We have analyzed both time-independent and time-dependent variability to determine the effects on the H-reflex of complete spinal transection, as well as of more selective lesions using a serotonergic neurotoxin, 5,7-dihydroxytryptamine. We used classic measures of time-independent variability (such as variance, the difference of an individual H-reflex from the mean) and time-dependent variability (using spectral analysis). If variability arises due to effects of random noise on H-reflex circuitry generated by descending and local neurons, then the variability is not time-dependent. If the injected noise instead has some underlying trend, then the variability is time-dependent or ‘fractal’. Fractal properties occur in several biological systems and may be functionally/prognostically relevant. H-reflex ‘fractalness’ is diminished in people with SCI, but has never been examined in an animal model, precluding eventual dissection of underlying mechanisms. These studies of H-reflex variability will set the stage for development of a novel, non-invasive tool for unraveling SCI-induced changes following SCI.

**P-13 Evaluation Of Conduction Through Motor Pathways: Using Evoked Potentials In Non-Human Primates**

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**Introduction:** Translation of spinal cord repair strategies from preclinical models to humans is aided when comparable non-invasive assessment methodologies can be applied. Trans-cranial electrical stimulation (TES) and trans-cranial magnetic stimulation (TMS) are alternate methods to assess the integrity of motor pathways in disease states. It is

particularly useful to be able to stimulate the motor cortex of one hemisphere. Theoretically, axonal repair of the corticospinal tract mediated via cell transplantation and subsequent remyelination could be detected with these techniques. Our objective was to establish reproducible methodology in monkeys using TES and TMS and to compare the utility of the two methods in order to obtain baseline data in ongoing experiments in which the unilateral medullary pyramid is lesioned. Then after neurological recovery plateau, the ability of a cell transplant to repair demyelinated axons is assessed. Lateralized cortical stimulation is highly desirable to test collateral sprouting after cellular therapy. We hypothesized that focalized cortical stimulation resulting in selective activation of the contra lateral limb muscles to the site of stimulation could be achieved with TMS rather than TES in anesthetized primates. **Methodology:** Primates were assessed under propofol anesthesia. **TES:** Scalp stimulation was obtained by subdermal placement of monopolar needle electrodes, trains with increasing intensity values were delivered in a range of 150V–300V. **TMS:** a focal eight-shaped coil used, single pulse stimuli were delivered increasing gradually the percentage of stimulation. **EMG:** needle electrodes were placed in the myotomes of fore and hind limbs bilaterally. Thresholds were assessed for each muscle and 6-8 consecutive stimulation trials were performed. Recordings were obtained with similar filtering/amplifying parameters. Stimulation arrays were optimized to isolate stimulation to the motor cortex of one hemisphere. **Conclusions:** Reliable TES and TMS methodology was established. TMS stimulation was more effective to selectively stimulate unilateral motor cortex in primates under anesthesia.

#### **P-14 Correlates of Motor Dysfunction After Spinal Cord Injury In Rats.**

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*Aims and Objectives.* Knowledge on the morphological grounds of motor deficits after traumatic spinal cord injury (SCI) is crucial for understanding the mechanisms of functional recovery. In the present study we looked for correlations between objective functional (single-frame video motion analysis, electrophysiological measurements) and morphological (lesion scar volume) parameters after SCI compression injury in rats. *Materials and Methods.* Adult female Wistar rats were subjected to graded compression of the spinal cord at midthoracic level (Th8). Recovery of locomotion was analyzed using video recordings of beam walking and inclined ladder climbing. Four functional parameters were used: the foot-stepping angle (FSA), the rump-height index (RHI) estimating paw placement and body weight support, respectively, the number of correct ladder steps (CLS) assessing skilled hindlimb movements and the locomotor rating score of Basso, Beattie and Bresnahan (BBB). *Results and Discussion.* Together with the enhanced H-reflex responses at frequencies between 0.1 and 5.0 Hz these parameters correlated with the scar volume (measured in Cresyl Violet-stained longitudinal sections

through the lesion site) showing significant differences between moderately and severely injured rats at 1-12 weeks after SCI. *Conclusion.* The use of these objective functional and morphological measures provides a time- and cost-efficient opportunity for versatile and reliable functional evaluations in both severely and moderately impaired rats combining clinical assessment with precise numerical measures.

**P-15 Neuromuscular Plasticity In The Rat Forelimb After High Cervical Spinal Cord Injury**

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Injury to the cervical spinal cord (cSCI) can lead to devastating consequences, including impaired upper extremity (UE) function. Although some capacity for functional improvement has been demonstrated, the extent of recovery is limited and the underlying mechanisms are ill defined. In contrast, functional recovery within the phrenic (respiratory) motor system is well characterized and the neural substrates associated with this plasticity have been studied in great detail using a well-established rodent model of cSCI, the lateral C2 spinal cord hemisection (C2Hx). The present work examines the muscular and neuroanatomical substrates underlying upper extremity dysfunction and recovery after C2Hx in adult Sprague-Dawley rats. Gross forelimb motor function was assessed prior to, and at 1- and 8-weeks post-injury using the limb-use asymmetry (cylinder) test.

Immunohistochemical techniques were used to assess average forelimb muscle fiber cross sectional area (CSA) and the neuroanatomical circuitry of the forelimb was assessed using retrograde transneuronal tracing techniques. Initial results indicate dramatic reductions in ipsilateral forelimb use ( $p < 0.01$ ) and muscle fiber CSA ( $p < 0.05$ ) at 1-week post-C2Hx. By 8-weeks post-injury, improvements in ipsilateral forelimb use ( $p < 0.01$ ) and increased muscle fiber CSA ( $p < 0.05$ ) were observed. Preliminary results from tracing studies in uninjured animals revealed first-order labeling of forelimb motoneurons in the lateral ventral horn of the cervical spinal cord as well as labeling of second-order interneurons. These initial results indicate substantial muscular phenotypic remodeling and modest recovery in forelimb function following cSCI, and ongoing studies are exploring the neuroanatomical circuitry associated with forelimb dysfunction and recovery after injury. The pattern of forelimb recovery appears to parallel the previously documented recovery of ipsilateral phrenic motor output following C2Hx. These experiments are the first to examine the combined muscular and neuroanatomical mechanisms underlying neuroplasticity and recovery of function following cSCI and may aid in identifying potential therapeutic targets for rehabilitation interventions.

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**P-16 In Vivo Testing Of Candidate Genes To Promote Neuron-Intrinsic Growth Ability And Axon Regeneration In The Injured Spinal Cord**

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Axon regeneration in the adult central nervous system (CNS) fails in part because many CNS neurons possess an intrinsically low capacity for rapid axon growth. Identifying and reversing neuron-intrinsic constraints on axon growth is critical to restore CNS function after axon tracts are disrupted by injury or disease. Axon growth ability declines dramatically as neurons age postnatally, likely due to the both downregulation of growth-promoting genes and upregulation of growth-suppressive genes within CNS neurons. Using a screening approach, we previously identified a number of developmentally regulated genes that either enhance or suppress neurite outgrowth when overexpressed in primary neurons in culture. To test gene function *in vivo* we are now using adeno-associated viral particles (AAV) to overexpress or knock down candidate genes in corticospinal tract (CST) neurons in adult mice, followed by transection of CST axons in the cervical spinal cord. Using virally-produced GFP as a tracer, we quantify axonal dieback and regeneration by transduced CST axons. We have tested a number of candidate genes, including cytoskeletal interacting proteins (doublecortin / DCX), regulators of small GTPases (NGEF / Ephexin), transcription factors (multiple members of the Kruppel-like family / KLFs), as well as previously identified regulators of axon growth (PTEN and SOCS3). We demonstrate effective gene overexpression, and, importantly, highly effective knockdown of candidate genes in CST neurons *in vivo*. Furthermore, we have identified a KLF-based gene manipulation that promotes CST regeneration in the injured spinal cord. By testing large numbers of candidate genes in an

*in vivo* model of spinal cord injury, both singly and in combination, these experiments will help clarify the neuron-intrinsic control of axon regeneration and identify new therapeutic avenues to promote axon regeneration in the injured CNS.

**P-17 Allogeneic And Autologous Schwann Cell Grafts In A Porcine SCI Contusion Model: Immune Response And CNS Integration**

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**Introduction:** Transplanted Schwann cells (SC) have reparative effects after SCI. An autologous approach to human application in SCI is limited by the time required to culture sufficient cells from donor nerve. We assessed whether allogeneic SC promote similar reparative effects to autologous SC with cell survival, axonal sprouting, myelination and inflammation as endpoints. **Methods:** Twenty Yucatan minipigs underwent T8 laminectomy and contusion. Autografts were produced from a superficial femoral nerve, SC were purified and labeled with GFP lentivirus at P1. Three volumes 50, 100 or 150  $\mu$ l of 200,000 SC/ $\mu$ l (P2) were injected at 21d to mimic preparation time in a human trial. Pigs survived 3d to six weeks. Tissue sections were stained with LFB-H/E and immunostained for several relevant markers. **Results:** In allogeneic and autologously transplanted pigs the lesion cavity showed extensive macrophage activity, astrocytes enclose cavities and extend processes into the epicenter. The largest injections caused secondary cavities and pressure extrusion along grey-white matter boundaries rostral and caudal to the injury epicenter. Abundant peripheral nerve fascicles entered the injured cord

especially in grafted animals. Marked lymphocytic and polymorphonuclear perivascular infiltration was present in allografts but also detected in some autografts, one animal showed large lymphocytic nodules related to the implant. Close apposition of astrocytes and grafted SC was present in all transplants. Demyelinated axons and autologous SC were aligned even at early post transplant times, and the number of ensheathed axons and axonal sprouts was greater in longer survivals. **Conclusions:** Survival of autologous transplanted SC was superior to allogeneic SC. Host SC response from nerve roots into the lesion was extensive. SC promoted restructuring of injury cavities via axonal sprouting and myelination. The unexpected finding of substantial lymphocytic activity including nodules in both allo and autografts is under further study.

**P-18 Hot ‘n’ bothered: the role of TRPV1-positive sensory neurons in autonomic dysreflexia**

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TRPV1 is expressed by a subpopulation of sensory neurons. It is activated by heat (>43°C), low pH, and capsaicin (the pungent ingredient in hot chili peppers), and has emerged as a promising target for the treatment of pain. Both pain and autonomic dysreflexia that develop in the wake of SCI have been attributed to maladaptive sensory plasticity. We hypothesize that TRPV1-positive afferents contribute to the development of autonomic dysreflexia (AD).

Wistar rats (300g) received a complete transection of the spinal cord at the third (T3) or tenth (T10) thoracic segment or sham injury (durectomy without SCI). After 1-12 weeks, dorsal root ganglia (DRGs) and spinal cord were harvested and analyzed immunohistochemically to examine soma size of TRPV1-positive afferents and density of their central projections.

TRPV1-positive nociceptors exhibited hypertrophy after T3 SCI. Nociceptor hypertrophy only occurred in DRGs below the level of SCI, and was more pronounced after high thoracic (T3) SCI. Hypertrophy was pronounced in DRGs far distal to SCI, and occurred in DRGs comprised of predominately somatic (L4,L5) and visceral (L6,S1) afferents. TRPV1-expressing nociceptors also appeared to sprout within the lumbar dorsal horn: the area occupied by TRPV1-positive axons was increased one month after SCI.

TRPV1-expressing nociceptors respond to SCI by undergoing somal hypertrophy and expanding their central terminals in the lumbar dorsal horn. Recent data from another laboratory demonstrates that this anatomical plasticity is accompanied by spontaneous activity in TRPV1-positive afferents caudal to SCI. We are currently using capsaicin to determine the contribution of TRPV1-positive afferents to the development of AD following high-thoracic SCI.

**P-19 Myelin Gene Regulatory Factor Knockout Delays Functional Recovery From Spinal Cord Injury**

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Spinal cord injury causes death of oligodendrocytes and subsequent demyelination

of axons. Myelin replacement occurs by the recruitment of oligodendrocyte precursors (OPCs) followed by their maturation into remyelinating oligodendrocytes. The role of this regenerative process and its contribution to functional motor recovery following spinal cord injury (SCI) has never been tested directly. To test the role of remyelination in the spontaneous recovery following SCI, we removed a transcriptional regulator, known as myelin gene regulatory factor (MRF) that is necessary for the maturation of OPCs into myelinating oligodendrocytes. To remove MRF we injected a cre-expressing adeno-associated virus 5 (AAV5) into the spinal cord of mice that were either heterozygous or homozygous for floxed MRF two weeks prior to a thoracic crush injury. AAV5 was capable of infecting OPCs, as indicated by co-labelling of virally expressed GFP with OPC markers PDGF $\alpha$ R and Olig2 at the time of injury. This suggests that MRF was removed in a subset of OPCs prior to SCI. After injury, floxed MRF infected with Cre-expressing AAV5 had a delayed behavioural recovery on the Basso Mouse Scale. Co-labelling of the mature oligodendrocyte marker CC1 and viral eGFP was assessed to determine if OPC maturation was impaired in adult mice by MRF knockout. The delayed behavioural recovery suggests an important role for MRF in the spontaneous motor recovery following spinal cord injury. It is also the first demonstration that MRF has a role in the CNS beyond regulating strictly developmental myelination.

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**P-20 *In Vivo* Real Time Observations of Immune Cells And Neurons In Traumatic Spinal Cord Injury In The Mouse**

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The *in vivo* character of immune mediated secondary axonal dieback after traumatic spinal cord injury is not known. Studies of interactions between DRG neurons and macrophages in culture show a cell-cell contact mediated interaction that causes a dramatic retracting response by the neuron (Horn and Busch et al. 2008). Although these cell types are in contact in tissue, this type of dramatic retraction does not occur. Time lapse multi-photon imaging methods provide a powerful tool for observing these cellular interactions in real time in intact tissue with minimal disruption and high resolution. Animals with genetically expressed fluorescent proteins allow us to observe cellular structures. Yellow fluorescent protein expressed in a neuronally restricted manner under the Thy-1 promoter (Feng et al. 2000) labels single axons in the living animal. Green fluorescent protein expressed in place of the fractalkine receptor CX3CR1 (Jung et al. 2000) allows for the observation of monocytic cells and microglia. Multi-photon *in vivo* imaging has been used to characterize axonal dieback from a dorsal column crush injury in these transgenic mice over a period of several days. We have observed infiltration and movement of CX3CR1 GFP positive cells in the parenchyma of the lesion and in the subarachnoid space. Axons with retraction bulbs die back from the lesion by a membrane blebbing mechanism, also seen in response to physical contact by a CX3CR1 positive cell. Undisturbed axonal retraction balls are static after lesion formation for long periods of time. Large, phagocytic, strongly CX3CR1 positive cells are also static after injury. Understanding the *in vivo* cellular interactions involved in this secondary axonal injury may lead to candidates for clinical treatment.

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 Jung S et al. 2000. *Mol Cell Biol* 20(11):4106-14.  
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**P-21 The Presence of Cd4<sup>+</sup> T Cells Is Required For Nt-3-Induced Axonal Sprouting In The Injured Spinal Cord**

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Previously, we reported that over-expression of Neurotrophin-3 (NT-3) promoted axonal sprouting in the injured rat spinal cord but only when acute inflammatory responses were present. Axonal sprouting was not observed when NT-3 over-expression was delayed 4 months after injury when the inflammation had subsided. We observed that activation of CD4<sup>+</sup> T cells in the spinal cord coincided with axonal sprouting suggesting that they might participate in the NT-3-induced neuroplasticity. To test this, we compared NT-3-induced neuroplasticity in nude rats lacking functional T cells, rats heterozygous for the mutation that had functional T cells, nude rats grafted with CD4<sup>+</sup> T cells, and nude rats grafted with CD8<sup>+</sup> T cells. After a unilateral lesion of the corticospinal tract (CST) NT-3 was over-expressed in lesioned side of the lumbar spinal cord by transducing motoneurons with an adenoviral vector carrying the NT-3 gene. Three weeks later we measured the number of axons that sprouted from the unlesioned CST into the denervated side toward the source of NT-3. The degree of sprouting was significantly greater in rats with functional T cells and in nude rats that were grafted with CD4<sup>+</sup> T cells compared to nude rats or nude rats grafted with CD8<sup>+</sup> T cells. Additionally, CD4<sup>+</sup> T cells were polarized

towards a Th2 phenotype when the CST was lesioned. These findings suggest that CD4<sup>+</sup> T cells activated by trauma-associated antigens mediate NT-3-induced axonal sprouting. Supported by the Christopher and Dana Reeve Foundation, the Craig H. Neilsen Foundation, Mission Connect, and the Department of Veterans Affairs.

**P-22 Characterizing The Effect Of Neurotrauma On Spinal Cord And Dorsal Root Ganglion Vasculature**

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Along with other groups, we have shown that macrophages invade dorsal root ganglia and the spinal cord after spinal cord and peripheral nerve injury. Additionally, recent findings show that macrophages guide endothelial tip cells in angiogenesis. We hypothesize, therefore, that areas of the nervous system subject to an influx of macrophages after injury will also display injury-induced angiogenesis. In a rat model of T3 complete transection spinal cord injury and using immunohistochemistry, we have found an increased capillary network density in L4 and L5 dorsal root ganglia 35 days after injury. We are also interested in describing the three-dimensional morphology of vascular networks in the nervous system before and after neurotrauma. To accomplish this, we are using the vascular casting technique. This method involves perfusing the animal with a fluorescent resin that hardens to form a cast after injection. The resulting casts are dissected out, imaged with confocal microscopy and analyzed with 3D software. We seek to describe these vascular changes to further understand spinal cord injury pathology and to uncover potential therapeutic

targets related to the angiogenic response after neurotrauma.

**P-23 Autonomic And Sensorimotor Recovery From Cervical Contusion Injury Is Boosted By Midbrain Periaqueductal Gray Stimulation.**

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Previously we found that a few days of intermittent electrical stimulation in the hindbrain's nucleus raphe magnus (NRM) improves sensorimotor and anatomical recovery in rats with thoracic contusion injuries, and that the midbrain's periaqueductal gray (PAG), a major NRM input, gives similar outcomes. NRM neurons are known to respond to sensory and chemical correlates of body trauma (e.g., pain, circulating cytokines) and to release growth-promoting substances (e.g., serotonin, TRH) essentially everywhere in spinal gray matter. We therefore suggest that the NRM is ipso facto a generalized repair center, with actions extending beyond sensorimotor systems or particular spinal segments. To support this idea, we expanded our studies to cervical injuries and autonomic outcomes. The rat's ventrolateral PAG received one week of stimulation (-30  $\mu$ A, 1 ms, 8 Hz, alternating stimulation and rest in 5-minute periods) for 12 hours daily, starting 1-2 hours after cervical (C5) weight-drop contusion. Five weeks post-injury, colon distension by balloon catheter (4 cc) under ketamine/xylazine anesthesia caused significantly less autonomic dysreflexia (rise in intracarotid blood pressure) in stimulated rats (n=11) than in controls with inactive stimulators (n=8): 7.6 mm Hg ( $\pm$ 1.4 s.e.m) versus 24.8 ( $\pm$ 0.9). Noxious pressure applied to forepaws also gave significantly lower pressor responses in stimulated rats: 3.3 mm ( $\pm$ 1.0), versus 6.4

( $\pm$ 0.14) in controls. Moreover, forelimb motor performance, measured weekly, was also significantly improved. Motor test scores, averaged over the last 4 weeks for stimulated versus control rats, were, respectively: forelimb hang-time 8.0 s ( $\pm$ 2.0) versus 3.3 ( $\pm$ 1.1); grip strength 440 g ( $\pm$ 6.7) versus 110 ( $\pm$ 2.0); inclined-plane stability 43.0° ( $\pm$ 1.88) versus 40.0 ( $\pm$ 0.93). In sum, PAG stimulation reversed both autonomic dysreflexia and forelimb motor deficits (P<0.05, all tests). Already proven safe as a neurosurgical procedure in humans (for drug-refractory pain), it represents a readily translatable potential treatment for acute spinal cord injury.

**P-24 Skin-Derived Precursors Differentiated Into Schwann Cells (Skp-Scs) And Transplanted Eight Weeks Post Spinal Cord Contusion Improve Recovery Of Motor Function And Bladder Pathology**

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Cell transplantation has emerged as a promising candidate therapy for spinal cord injury. Our laboratories have previously shown that Schwann cells differentiated from skin-derived precursors (SKP-SCs), when transplanted 7 days after contusion injury, promote histological repair and functional recovery in rats (Biernaskie et al. 2007). Here, we transplanted one million SKP-SCs at 8 weeks post T9/T10 contusion injury and allowed survival until week 29. Behavioral analysis shows that SKP-SC transplanted rats elicited higher BBB scores, which reached significance at week 19-21 post injury. By histology, we found surviving SKP-SC in all transplanted rats at 21 weeks post

transplantation. Ki67 immunoreactivity indicated very little (<0.03-0.05%) proliferative activity in this chronic stage. Cellular bands of SKP-SCs bridged the lesion sites in predominantly rostro-caudal orientation and showed good integration into the host spinal cord. SKP-SC and astrocyte processes interdigitated extensively at this host transplant interface which was crossed by numerous axons; and these SKP-SC bridges were filled with neurofilament positive axons running predominantly in rostro-caudal orientation. Immunoreactivity for serotonin transporter (SERT) or tyrosine hydroxylase revealed many axons growing into and through these SKP-SC bridges with some crossing the distal interface. Fewer axons were positive for CGRP or SP, markers of sensory fibers from the periphery. The numbers of P0-positive fibers were significantly higher in SKP-SC transplanted rats, indicating a stimulation of an endogenous Schwann cell repair response by SKP-SC transplantation. Interestingly, in a post hoc analysis, the media control treated animals show a significant increase in bladder wall thickness as compared to the SKP-SC treated animals, suggesting improved bladder function in the transplanted group. In summary, our results highlight the potential of SKP-SCs as a possible cell for autologous transplantation in the sub-chronic state of spinal cord injury.

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**P-25 Nerve Regeneration And Improvement In Urodynamics And External Urethral Sphincter Activity In Complete Spinal Cord Transected Rats Treated With A Peripheral Nerve Graft, Acidic Fibroblast Growth Factor, And Chondroitinase ABC**

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Relatively few studies have focused on central neuronal regeneration with the goal of fostering recovery of efficient micturition. The effects of peripheral nerve grafts (PNG) and acidic fibroblast growth factor (aFGF) combined with Chondroitinase ABC (ChABC) on bladder reflexes after complete spinal cord transection (ST) at T8 in adult rats were studied. Rats were divided randomly into six groups: (1) Sham control (laminectomy only), (2) ST only, (3) ST+PNG, (4) ST+aFGF+ChABC, (5) ST+PNG+aFGF, (6) ST+PNG+aFGF+ChABC. Two injections of ChABC were made near the rostral and caudal stumps of the spinal cord. Urodynamics and external urethral sphincter electromyogram (EMG) activity were recorded six months after injury. Anterograde tracing was used to evaluate axonal regeneration. Urodynamic data in the ST+PNG+aFGF+ChABC animals was markedly improved beyond that in the other four injury groups. The ST+PNG+aFGF+ChABC group also showed significant shorter time to void and significantly improved patterns of high frequency oscillations of detrusor contractions (bladder pressure tracings) during voiding than the other four injury groups. This indicates that the PNG+aFGF+ChABC group did not need to store large volumes of urine (less bladder incontinence and distention) to void and developed better coordination between detrusor and sphincter activity, which is closer to a normal pattern. The ST+PNG+aFGF+ChABC group also displayed higher amplitudes and bursting rates of sphincter EMG activity than the other injured animals. The improvement in urodynamics and EMG disappeared after spinal cord re-transection in the ST+PNG+aFGF+ChABC group, suggesting that supraspinal regeneration is critical for recovery. Anterograde tracing studies revealed more regenerated fibers in the distal end of the spinal

cord in the ST+PNG+aFGF+ChABC group. The improvements in bladder reflexes might have been due to newly formed supraspinal control via nerve regeneration.

**P-26 Early and late behavioral and electrophysiological changes in lower urinary tract function following mid-cervical spinal cord injury**

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Lower urinary tract (LUT) dysfunction is often a significant source of morbidity after chronic spinal cord injury (SCI), irrespective of the spinal level involved. The majority of experimental studies of post-SCI LUT function, however, have investigated only thoracic injury models, and have paradoxically shown recovery of normal LUT function behaviorally after injury. Few studies have shown abnormal LUT function electrophysiologically similar to that seen clinically, characterized by bladder-external urethral sphincter (EUS) dyssynergy, but only in thoracic injury models. Therefore, we propose a study that to the best of our knowledge is the first of its kind, by examining both early and late behavioral and electrophysiological changes in LUT function after experimental cervical contusion injury. Mid-cervical (C4-5) unilateral (n=24) or bilateral (n=24) contusion injuries were produced in adult Sprague–Dawley rats using the Infinite Horizon Impactor. Six naïve rats served as controls. LUT function was evaluated electrophysiologically in all rats via continuous transurethral cystometry (7.5 ml/hr) and EUS electromyography. SCI animals were assessed on post-injury days 7, 14, 28, and 56.

Behavioral assessment of bladder function was recorded by daily manual bladder expression and measurement of retained urine. Naïve animals demonstrated normal LUT behavior and electrophysiology. Unilateral injury animals demonstrated normal LUT behavior throughout, and normal electrophysiology at early time-points (post-injury days 7, 14), but abnormal electrophysiology at late time-points (post-injury days 28, 56). Bilateral injury animals demonstrated significant early LUT behavior dysfunction (<14 days post-injury) and concomitant abnormal electrophysiology (post-injury days 7, 14). After 14 days, bilateral injury animals recovered normal LUT behavior, but had persistent late abnormal electrophysiology (post-injury days 28, 56). Therefore, with this novel preclinical model, we demonstrate that despite recovery of LUT function behaviorally, abnormal changes electrophysiologically in LUT function are evident late after unilateral and bilateral cervical contusion injury, closely mirroring the clinical condition.

**P-27 Integration of Microchannel Neural-Electrode Interfaces Into Dorsal And Ventral Roots For Bladder Control After Spinal Cord Injury**

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A debilitating consequence of spinal cord injury (SCI) is loss of bladder fullness perception and micturition. The development of a hyper-reflexive bladder provides some relief of urine. However, the dyssynergia between detrusor and sphincter results in incomplete expression and potential infection. An effective way to restore self-directed micturition is artificially, through implantation of a Sacral Anterior Root Stimulator (SARS). The critical limitation is the inability to detect bladder fullness, and therefore, when expression is required. The project aims to use afferent information during bladder filling, to drive SARS output. This is to be accomplished through the design and implantation of recording electrodes contained within microchannels, onto the dorsal roots of rats. When optimised, these prostheses will provide indication for timely bladder expression in dogs suffering SCI, with the ultimate goal of translation to humans. To date a newly designed SARS hook electrode for the bilateral S2 ventral roots has been implanted successfully, with maintained efficacy, in six dogs with SCI. Work has focussed on identifying bladder afferent activity at acute and chronic implantation stages, as well as optimising device design in rat. We have electrophysiologically identified bladder afferents during artificial bladder infusion using a variety of microchannel devices. Bladder afferent action potentials are small in amplitude compared to cutaneous and muscle spindle, so the root must be 'teased' into fascicles and insulated within 100 µm microchannels to improve the signal output. Implants containing electrodes are fabricated from polydimethylsiloxane (PDMS). Results after four weeks of L6/S1 dorsal root

implantation show that DRR is inappropriate for the implantation procedure, with respect to axon survival and bladder morphology. At twelve weeks activity does return, suggestive of a regenerative response, providing prospect for 'regenerative' prosthetic design. The optimum scenario however, is keeping root-spinal cord continuity, with axon viability and bladder morphology greatly improved.

**P-28 Optimized acellular Grafts Support Functional Recovery after Cervical Spinal Cord Injury**

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Peripheral nerve (PN) grafts have been used successfully as a bridge to support severed axons of the central nervous system (CNS) since the early 80's. However, the fresh PN grafts are not ideal for clinical translation because of limited availability, loss of function at the donor site, and the need for multiple surgeries. Recently, Dr. Schmidt's group has developed an optimized acellular (OA) graft from peripheral nerve. This decellularization technique is now used produce acellular grafts from cadaver nerve tissue for clinical use in peripheral nerve repair. The acellular grafts were shown to be non-immunogenic and to support axonal growth of severed axons after a complete thoracic cord injury in rodents. Here, we compared acellular grafts to PN grafts in their ability to support axonal growth in combination with chondroitinase ABC enzyme after cervical C3/C4 lateral hemisection injury. We assessed forelimb function of the animals using a cylinder paw preference test pre-surgery and bi-weekly thereafter for up to 22 weeks post injury. We found that immediately after injury, animals

in all groups lost the ability to explore with the affected limb during vertical exploration. In addition, we observed similar recovery profiles for animals with PN and OA grafts. At 22 weeks, we resected the grafts and saw loss of forelimb function for PN and OA grafts, 65% and 36% respectively. Anatomical track tracing and histological studies are underway to determine origin and extent of axonal growth into and out of the grafts. Our results indicate, for the first time, that acellular grafts can support CNS axon growth and support functional recovery to a similar extent to that of fresh PN. Our findings support the idea that acellular grafts can potentially serve as a clinically relevant option for repair therapies after spinal cord injury.

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**P-29 Environmental Enrichment Enhances Retrograde Transport of Polysynaptic Virus In The Dentate Gyrus After Traumatic Brain Injury**

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One of the long-term neurological sequelae associated with traumatic brain injury (TBI) is memory dysfunction. TBI is also one of the most potent risk factors for development of neurodegeneration. Enriched environment (EE) is known to improve function in experimental models of TBI. Although EE-elicited hippocampal neurogenesis has been well documented after TBI, EE-induced synaptic reorganization that might underlie the observed benefit in functional memory has not been explored. The current study sought to determine whether exposure to EE induced synaptic

reorganization of the hippocampal circuitry in adult mice subjected to TBI, which was produced by a controlled cortical impact (CCI) to the cortex above the hippocampus. BrdU was injected daily from four to seven days after CCI or sham surgery, then mice were transferred to either the institution standard cages (STD) or to the EE. Four weeks later, mice were injected with pseudorabies virus (PRV), a retrograde transynaptic neuronal tracer, into the entorhinal cortex, and euthanized 50 hours after the injection. Quantitative analysis was performed to estimate the number of retrogradely infected cells by the PRV in the hippocampus ipsilateral to CCI. We found that the brain regions infected by PRV were similar between the mice that underwent sham surgery and CCI, suggesting a preservation of circuitry connected to the entorhinal cortex and within the hippocampal trisynaptic circuit following TBI. When the third order of infection in the dentate gyrus (DG) was corrected by the counts in the second and first orders (DG/CA3/CA1), TBI-EE mice displayed a higher ratio of PRV infectivity compared to that of TBI-STD mice ( $p=0.065$ ) and EE produced a higher ratio overall ( $p<0.05$ ). BrdU data suggest more neurogenesis not only in animals exposed to EE compared to STD conditions ( $p<0.001$ ), but also in those subjected to TBI than sham ( $p<0.01$ ). The degree to which EE-induced DG neurogenesis affects transneuronal spread of virus is under further investigation.

**P-30 Analysis of Gene Expression Following A Localized Traumatic Brain Injury Reveals A Bias Toward Increased Cell Death Locally And Cell Survival Remotely**

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We used microarray and bioinformatic analysis to better understand how gene expression is modulated throughout the brain by localized traumatic brain injury (TBI). Adult Sprague-Dawley rats received unilateral controlled cortical impacts and were sacrificed 24 hours post-injury. mRNAs isolated from the brain tissues of these rats and naïve (control) rats were hybridized to the Affymetrix Rat Genome 230 2.0 GeneChip for microarray analysis. We identified genes that changed in expression by 2-fold or more on both the ipsilateral and contralateral sides of the brain. Ingenuity Pathway Analysis was used to identify the functions, canonical pathways, and networks most significant to the data set. Cell death was the most significant functional category common to both sides of the brain. Ipsilaterally, 90% of the 272 unique (unchanged on the opposite side) cell death genes that changed in expression showed increased expression; including known apoptotic factors, caspases 3, 4, and 7, cyclooxygenase, and several members of the s100 family. Several key transcription factors also showed increased expression; including FOS, MYC, CEBPB, CREBBP, and NFkB2. Contralaterally, 77% of the 94 unique cell death genes decreased in expression; including cell death promoting genes BAX and HSP90AA1 and the transcription factor SP1. Additionally, there are 279 cell death genes that change on both sides of the brain. 47 of these genes show differential expression. Cyclin D1 and STAT3 increase ipsilaterally and decrease contralaterally. While CD44 expression increases on both sides, its expression is 6.5 times higher on the ipsilateral side. The observed expression pattern for cell death genes suggests that cell death would be more likely to occur ipsilaterally and less likely to occur contralaterally. Extension of the bioinformatic analyses used in this experiment will be useful for identifying novel molecules that may be targets for preventing TBI related cell death.

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**P-31 Training of The Impaired Forelimb After Traumatic Brain Injury Enhances Hippocampal Neurogenesis In Mice Without Corpus Callosum**

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The corpus callosum, as the principal fiber tract interconnecting the left and right cortices, mediates interhemispheric crosstalk and spatial coupling between the limbs. Unilateral brain injury, such as stroke, is known to disrupt the balance between the two cortices as evidenced by an abnormally high interhemispheric inhibitory drive from M1<sub>intact</sub> to M1<sub>lesioned</sub> transmitted transcallosally. Our previous work showed that the deletion of homeobox gene *Emx1* not only led to the agenesis of the corpus callosum but also reduced hippocampal neurogenesis. The current study sought to determine whether lacking the corpus callosum affected the recovery of forelimb function and hippocampal plasticity following training of the affected limb in mice with unilateral traumatic brain injury (TBI). One week after TBI, produced by a controlled cortical impact to impair the preferred limb, *Emx1* wild type (WT) and knock out (KO) mice were subjected to the single-pellet reaching training task with the affected limb for a period of 4 weeks. Over the training period, all groups improved on the task as evidenced by a progressive increase in the successful rate in reaching and grasping the pellet (p<0.0001). Both TBI and *Emx1* deletion had overall adverse effects on the performance of the reaching task (two-way repeated ANOVA:

$p < 0.01$  and  $p < 0.05$ , respectively), although *Emx1* KO mice did not perform significantly worse than their WT counterparts (post hoc:  $p > 0.16$ ). Both TBI and *Emx1* gene deletion also had a strong effect on hippocampal neurogenesis ( $p < 0.001$  and  $p < 0.0001$ , respectively), demonstrated by a reduction in doublecortin (DCX)-expressing immature neurons, while limb training enhanced DCX expression ( $p < 0.005$ ). Our results suggest that lacking corpus callosum has a more pronounced impact on forelimb dexterity in uninjured mice as compared to those with TBI. Our findings also suggest that limb training enhances neuroplasticity after brain injury at functionally remote regions, including the hippocampus, which might have implications for promoting overall recovery of function.

### **P-32 Using Multimodal Therapies To Improve Outcome Following Traumatic Brain Injury**

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Traumatic brain injury is one of the leading causes of long-term disability in the young adults in Western world. To date current treatment of TBI focuses primarily on maintaining vital functions and reducing intracranial pressure. Therefore, new efficacious treatments are essential that target both the resulting physical and mental disabilities. Constrain induced movement therapy (CIMT) has been shown to be efficacious in human studies of stroke to improve physical recovery as well as induce cortical remapping. We proposed combining the CIMT with an anti-inflammatory treatment to reduce the secondary injury component of TBI as well as improve

functional outcome. Traumatic brain injury was induced by a controlled cortical impact (CCI) using the following parameters: 1.5 m/s strike velocity, 2.5 mm depth of penetration and a dwell time of 120 msec. Botox was injected into the unaffected forelimb immediately post CCI to discourage the use of the unaffected limb. Minocycline administered via bolus i.p. at 25mg/kg commenced at 24 hrs post injury for 12 consecutive days. A two-week physical therapy regimen consisting of daily exercise on ropes, grids and balls began at 5 days post TBI. All animals underwent behavioral evaluation 4 weeks and 8 weeks post injury. TBI induced impairment not only in motor but also in cognitive domains. Minocycline treatment led to a significant reduction in activated microglia in the peri-lesional area, however no significant reduction in lesion volume. Rats treated with minocycline also appeared to perform significantly better in the sticky tape removal test and the Morris Water maze when compared to vehicle treated controls. The combination of physical therapy and botox appeared to have no beneficial effect on functional outcome, possibly due to the confounding effect of botox on the contralesional forelimb. Our results suggest that delayed and chronic treatment with minocycline can reduce inflammation and improve outcome following TBI.

### **P-33 Stroke Affects Functional Activation During Spatial Tasks**

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Post-stroke impairment of short- and long-term memory is not uncommon, yet the underlying mechanism is unclear. The hippocampus is involved in memory function, but direct ischemic lesions strategic to the hippocampus/parahippocampus are rarely

observed in middle cerebral artery (MCA) stroke. Previously, we found that stroke by distal occlusion of MCA produced injury restricted to the parietal cortex and mild memory impairment. It is well known that the interplay of several brain regions is crucial for learning and memory. Recent advances also suggest that shared neuronal activation patterns define brain networks linking anatomically separate brain regions. Since the parietal cortex receives highly processed spatial information, the present study sought to investigate how ischemic injury in the parietal cortex disrupted multiple brain regions involved in spatial processing. Stroke was induced in rats by distal middle cerebral artery occlusion (dMCAO). Neuronal activation induced by spatial exploration was mapped by immediate early gene Fos in the motor and limbic systems and in the thalamic nuclei that receive input from sensory systems. In awake animals during resting state in home cages, regions such as the dentate gyrus were not silent, suggesting ongoing information processing. Contrary to those that remained in home cages, rats exploring a novel environment exhibited activation of all regions examined. However, a region-specific reduction in neuronal activation was observed in the hippocampus, caudal and rostral retrosplenial cortices and anterior cingulate cortices, medial and lateral entorhinal cortices, and in the anteromedial and anteroventral thalamic nuclei during spatial exploration in dMCAO animals compared to sham animals. Stroke-induced hypoactivation during spatial exploration was not observed in control regions, such as the periaqueductal gray, suggesting that they are not a part of the spatial recognition network. Structural connectivity among regions identified in this study and their relationship to the parietal cortex warrant further validation.

**P-34 Reduction of Neuroprogenitor Cells In Mice Impedes Post-Stroke Functional Recovery**

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The causal relationship between neurogenesis and post-stroke functional recovery has not been properly explored. The current study aimed to determine whether attenuation of neuroprogenitor cells affects the post-stroke functional outcome. Ischemic or sham stroke was induced by distal medial cerebral artery occlusion (dMCAO) in 10-week-old nestin- $\delta$ -HSV-TK-eGFP transgenic mice, in which expression of a truncated viral thymidine kinase gene and an eGFP gene was restricted to type-1 neural progenitor cells, enabling both conditional ablation by ganciclovir (GCV) and eGFP-tagging of these neuroprogenitor cells. GCV (200 mg/kg/day) or saline was continuously administered via osmotic pumps in mice from 6 weeks of age until euthanasia. Wild type mice were subjected to GCV and stroke as controls. We found that following 4 weeks of GCV treatment, both type-1 and type-2 progenitor cells were reduced by 70% in the dentate gyrus (DG) of sham mice. Although dMCAO induced proliferation of type-1 and type-2 progenitor cells, there was still a 60% reduction of DG progenitor cells in the presence of GCV compared to mice treated with saline. Surprisingly, mice with conditional ablation of neuroprogenitor cells did not exhibit an increase in lesion size. Transgenic mice subjected to stroke and GCV displayed impaired spatial learning and memory in the Barnes maze test compared to saline control or wild type mice subjected to GCV and stroke, suggesting that the observed functional impairment was a

consequence of inhibiting neurogenesis and was not related to the toxicity of GCV. However, there was no significant difference in post-stroke motor function between transgenic mice treated with GCV and those treated with vehicle. Our data suggest a contributing role of stroke-induced neurogenesis in the recovery of cognitive function.

**P-35 Experimental ischemic and hemorrhagic strokes induce memory impairment and reduce hippocampal functional recruitment during spatial navigation**

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Cognitive impairment is a frequent, but a commonly neglected consequence after stroke. Hippocampal dysfunction occurs following injury to remote brain regions such as the anterior thalamus or fornix. The current study aims to determine whether stroke involving sensorimotor cortex (SMC) or basic ganglia (BG) affects hippocampal activation during spatial navigation and subsequent learning and memory function. Ischemic stroke was induced by the occlusion of middle cerebral artery (MCA) using either the distal (dMCAO) or the intraluminal suture method (sMCAO). Intracerebral hemorrhage (ICH) was produced by injection of collagenase into the BG. Functional recruitment of hippocampal circuitry was determined by mapping/quantifying the Fos expression during spatial exploration of a circular arena 5 days after stroke or sham surgery. Spatial memory was assessed at 7-8 weeks after stroke or sham by the Barnes maze test. dMCAO primarily affected the SMC and

spared the hippocampus, while ICH produced exclusive damage to the internal capsule and striatum. sMCAO led to cortical and subcortical lesions with only minor cell loss in the CA1. Spatial exploration in sham significantly increased Fos expression in all subfields of the hippocampus. In contrast, hippocampal hypoactivation occurred in rats with either ischemic or hemorrhagic stroke in a region specific manner. Fos activation was not affected in regions that were not involved in processing spatial information during spatial exploration, indicating that the observed effects were region and task-specific rather than a generalized metabolic depression. Further, reduced hippocampal recruitment during spatial exploration in stroke rats predicted the magnitude of memory impairment assessed by the Barnes maze test.

**P-36 Unexpected Neuronal Loss After Spinal Cord Injections In Primates**

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Safe delivery of therapeutic substances to the nervous system is critical when translating candidate treatments to humans, but the difficulty and expense of primate models often limits translational testing to smaller species. As part of a program to study therapies for spinal cord injury, we injected candidate therapeutics into the spinal cord of Rhesus macaques following C7 spinal cord hemisection lesions. Test substances included various mixtures of adeno-associated viral vectors (encoding green fluorescent protein, brain-derived neurotrophic factor, or neurotrophin-3) and chondroitinase. We injected 5  $\mu$ l of test substance through glass micropipettes into spinal cord gray matter below the hemisection at each of 10 sites per monkey. Infusions were made at low rates (0.5  $\mu$ l/min) using a Nano-injector syringe pump (N=9 monkeys) or a Picospritzer at pressures under 20 dynes (N=7). Eight 'Uninjected' controls received lesions and either no injections (N=6), needle entry only (N=1) or saline injection (N=1; designated 'Control' *a priori*). Subjects performed a battery of functional tests and were sacrificed 6 months after SCI. Qualitative evaluation of spinal cord tissue labeled for Nissl substance, choline acetyltransferase, or neuron-specific nuclear protein (NeuN) showed a dramatic loss of spinal cord neurons in all subjects receiving injections of any substance (except saline) into the spinal cord. Stereological analysis of NeuN-labeled nuclei revealed 58 $\pm$ 6% loss (compared to the contralateral, intact side of the spinal cord), while the Uninjected group showed no neuronal loss. Injected animals had more severe motor deficits than Uninjected controls, and Principal Components Analysis revealed a strong relationship between the degree of neuronal loss and impairment of forelimb function. Studies are underway to determine the mechanisms underlying the neuronal loss. These results, not predicted by rodent models, raise important concerns about safety in several ongoing human clinical trials, and highlight the need to test delivery methods in non-human primates.

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**P-37 Long-Distance Axonal Growth, Connectivity and Functional Recovery After Complete Spinal Cord Transection: Cell-Intrinsic Mechanisms Overcome Inhibition Of The Adult Lesioned Spinal Cord**

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We grafted embryonic spinal cord neural cells from green fluorescent protein (GFP)-expressing rats into sites of adult spinal cord injury. Grafted embryonic neurons extended large numbers of axons into the adult spinal cord over remarkably long distances. Axons extended rostrally from a mid-cervical lesion site into the brainstem, and caudally over more than half the distance of the remaining spinal cord; axons of grafted neurons also exited the spinal cord and regenerated into ventral motor roots. Grafted neurons differentiated into multiple neuronal phenotypes, including motor neurons. Axons emerging from grafts formed abundant synapses of both excitatory and inhibitory phenotypes with host neurons. The density but not distance of axon outgrowth was enhanced by viral delivery of brain-derived neurotrophic factor (BDNF). Axonal growth was partly dependent on the mammalian target of rapamycin (mTOR) signaling. Grafted cells supported formation of functional

electrophysiological relays across sites of complete thoracic spinal cord transection, resulting in significant functional recovery. Spinal re-transection immediately above the graft implantation site abolished all recovery. These findings indicate that intrinsic neuronal properties can overcome the inhibitory milieu of the injured adult CNS to mount remarkably robust axonal growth resulting in formation of novel relay circuits that restore function. Supported by the Veterans Administration, NIH (NS09881), Wings for Life, Canadian Spinal Research Organization, the Craig Neilsen Foundation, the Swiss Institute for Research into Paraplegia, and the Dr. Miriam and Sheldon G. Adelson Medical Research Foundation.

**P-38 Daily Acute Intermittent Hypoxia Restores Respiratory Plasticity And Breathing Capacity Following Cervical Injury**

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Acute intermittent hypoxia (AIH) elicits a form of serotonin-dependent respiratory plasticity in phrenic nerve activity in anesthetized rats known as phrenic long-term facilitation (pLTF). After chronic C2 hemisection (C2HS), AIH elicits pLTF ipsilateral (Golder and Mitchell, 2005), but not contralateral to injury (Doperalski and Fuller, 2006). Since respiratory LTF is enhanced by repetitive exposure to AIH, we hypothesized that daily AIH (dAIH, 7 days; 10-5 min episodes of 11% O<sub>2</sub>, 5 min normoxic intervals) would restore the pLTF contralateral to C2HS and increase the tidal volume capacity in unanesthetized rats. Following AIH, no pLTF was observed in untreated rats contralateral to C2HS (7% ± 4% of hypoxic activity, 60 min post-AIH; n=6; n.s.). In contrast, robust pLTF was observed contralateral to injury in dAIH treated rats (33% ± 11% of hypoxic activity, 60

min post-AIH; n=8; p<0.05). Tidal volume (via plethysmography) is reduced following a C2HS (vs pre-injury values) during normocapnia (-29% baseline; p<0.05), hypercapnia (-32% baseline, p<0.05) and hypercapnia/hypoxia (-30% baseline, p<0.05). dAIH significantly improves tidal volume capacity (normocapnia: 83%, hypercapnia: 82% and hypercapnia/hypoxia: 85% of pre-injury values; p<0.05). This significant improvement is observed up to 2 weeks following the end of dAIH. dAIH increases serotonergic terminal size and 5HT<sub>2A</sub> receptor density near phrenic motoneurons contralateral to injury (p<0.05). However, pre-treatment with the broad spectrum serotonin receptor antagonist methysergide prior to AIH on each day of the dAIH protocol had no effect on tidal volume recovery, suggesting that the effects of dAIH do not require serotonin receptor activation. Thus, dAIH may be a useful therapeutic approach to increase breathing capacity, although its mechanism of action remains unknown. dAIH may have potential as a treatment for spinal injury or neurodegenerative diseases that lead to respiratory insufficiency. Supported by NIH HL69064, Craig H Neilsen Foundation.

**P-39 Molecular Mechanisms involved in Recovery of Respiration after Upper cervical Spinal Cord Injury: Potential Therapeutic Strategies.**

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Previous studies have demonstrated that functional recovery of respiratory activity in an animal model (HC2) of SCI can be pharmacologically induced. In this model, activity in the hemidiaphragm rendered quiescent by injury can be restored by systemic administration of theophylline via (1) activation of cyclic AMP-induced CREB-phosphorylation

or lithium chloride via (2) activation of the GSK $\beta$   $\square$  phosphorylation pathway. Our studies have identified molecular signals and neurotrophic factors (BDNF, GDNF, and Bcl2) in both pathways that may mediate recovery. Although it is also known that inflammatory signals are triggered following injury, it remains unclear whether inflammatory events/signals, may mitigate against recovery. The objectives of the present study are: (1) to identify inflammatory signals triggered after injury and (2) to assess whether early resolution of inflammatory processes by neurotrophic factors underlies recovery. Briefly, adult female Sprague-Dawley rats were subjected to a left C2 hemisection (HC2). HC2 rats were divided into three groups. Group 1 rats (n=6) were administered theophylline (20 mg/kg 3X daily for 3 days), Group 2 (n=6) received lithium chloride (85mg/kg daily for 3 days) and Group 3 (n=5) rats were sham-operated controls. All rats were prepared for assessment of molecular signals immediately after conclusion of drug administration. Spinal cord segments C3-C6 were excised and sagittally divided into ipsilateral and contralateral sections and stored @ -80<sup>0</sup> C prior to analysis. In experiments thus far, expression of BDNF, GDNF and Bcl2 correlate positively with recovery. Furthermore, expression of inflammatory genes such as TXNIP and IL-1 $\beta$  are decreased after drug treatment. This may suggest an early resolution of inflammation to unravel recovery processes. However, pro-inflammatory cytokine TNF-  $\alpha$   $\square$  levels remain up-regulated after injury and subsequent drug treatment. We propose that in SCI therapy, it may be beneficial to target inhibitory genes that may be persistently expressed in response to injury. This strategy may further improve recovery processes in SCI.

**P-40 Intraspinal grafts of neural progenitors improves respiratory function following mid-cervical contusion injury in adult rats**

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Mid-cervical spinal cord injuries (SCI) can impair breathing due to disruption of bulbospinal respiratory fiber pathways and loss of phrenic motoneurons (PhMNs) and pre-phrenic interneurons. Diminished diaphragm EMG (diaEMG) response is a consistent outcome following respiratory challenge (e.g. hypercapnia) and recent evidence suggests that gray matter injury is the basis for this deficit. This study determines whether restitution of gray matter integrity following injury at the level of the PhMN pool would result in improved diaphragm function. Lateralized C3/4 contusions were produced in adult female rats using the Infinite Horizon Impactor (200 kilodyne preset force). One week later dissociated E13.5-14 fetal spinal cord (FSC) tissue was injected into the lesion epicenter. Treated animals were compared with untreated controls. Upon recovering one month post-transplant, pseudorabies virus was applied directly to the diaphragm or injected into the site of transplantation, to establish connectivity between 1) donor neurons and the host phrenic circuit and 2) host and donor neurons. Animals were then left to recover for 24-72 hours following PRV delivery. Terminal EMG recordings were made in spontaneously breathing animals to assess ipsi- and contralateral diaphragm activity under baseline (eupneic breathing) and challenge (exposed to hypercapnia) conditions. All injured-untreated animals exhibited blunted ipsilateral EMG responses to respiratory challenge 5wks post-injury. In contrast, graft recipients showed elevated EMG activity during challenge. PRV delivered to the ipsilateral hemidiaphragm resulted in infection of the PhMN pool and second-order host interneurons, as well as labeling of a contingent of donor neurons. Intra-

graft injection of PRV resulted in labeling of host interneurons in the vicinity of the grafts. These data demonstrate that restoration of gray matter integrity by spinal interneuronal progenitors can ameliorate an aspect of post-cervical SCI respiration. Neuroanatomical findings suggest this functional change is mediated by bi-directional connectivity between donor neurons and host interneurons and PhMNs.

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**P-41 Changes In Interneuronal Activity In The Cervical Spinal Cord Of Adult Rats Following Spinal Cord Injury (SCI)**

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Cervical SCI can significantly compromise breathing with the greatest deficits arising from direct damage to the phrenic circuit - controlling diaphragm function. It is known from experimental studies using a lateral C2 hemisection (C2Hx) in the adult rat, however, that spontaneous recovery of diaphragm function can occur. Recent experiments have identified cervical interneurons that could contribute to respiratory neuroplasticity following lateralized hemisection lesions at C2 (i.e., C2Hx). However, the activity of these putative pre-phrenic interneurons following C2Hx is not well-defined. The present study compared normal activity patterns of cervical interneurons and motoneurons versus those seen under conditions of increased respiratory drive and/or following C2Hx. Adult female rats were divided into uninjured or C2Hx groups. Animals were then allowed to recover for 2 weeks post-injury. Uninjured animals were euthanized without experiencing any additional procedures or following respiratory challenge (10 minute

exposure to hypoxia). The rats were euthanized immediately thereafter and immunocytochemistry was used to examine c-fos expression in brainstem and spinal cord tissue. Transynaptic tracing with pseudorabies virus applied to the ipsilateral hemidiaphragm was used in a subset of animals to label phrenic motoneurons and pre-phrenic interneurons ipsi- or contralateral to injury. Very limited c-fos expression was seen in the cervical cords of naïve animals. Following exposure to hypoxia, however, there was an increase in motoneuron c-fos staining in the region of the phrenic nucleus, as well as interneuronal labeling in laminae VII and X. This pattern was consistent with PRV first- and second-order labeling. A similar increase in c-fos positive motoneurons and interneurons was observed after respiratory challenge at two weeks post-C2Hx. These results are consistent with initial electrophysiological recordings showing cervical interneurons that are responsive to hypoxia and may thus be responsive to conditions requiring increased respiratory drive and phrenic motoneuron output.

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**P-42 High Cervical Spinal Cord Injury Results In Altered Distributions of Medullary Inspiratory And Expiratory Neuronal Activity**

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Respiratory rhythm originates in the medullary rostral and caudal ventral respiratory column (rVRC/cVRC) neurons, which extend

axonal projections to spinal neurons controlling inspiratory and expiratory muscle activity. Interruption of those bulbospinal pathways results in altered patterns of ventilation. While various neuroplastic changes have been shown in spinal respiratory centers following cervical spinal cord injury (SCI), it is unknown whether medullary respiratory circuitry is also affected. Since supraspinal functional map changes have been reported in other motor systems following SCI, we tested the hypothesis that a high cervical lateralized hemisection (C2Hx) will alter representation of brainstem respiratory neuronal activity.

Adult, female rats received C2Hx, and at 2 or 12 weeks post-injury, electrophysiological mapping was performed of medullary neurons active during breathing. Electrodes were inserted into multiple VRC locations (spaced 200 $\mu$ m, and at depths  $\sim$ 500 $\mu$ m apart) to determine the respiratory activity, inspiratory or expiratory, at each site ipsilateral to the C2Hx. External intercostal EMG recordings obtained simultaneously defined the pattern of inspiratory activity. Phasic respiratory activity was confirmed audibly and by correlation with EMG signals. Maps from C2Hx animals were compared to those obtained from spinal-intact rats.

In naïve rats, inspiratory activity occurs predominantly in the rVRC, whereas expiratory activity is mostly present in the cVRC with each partitioned by an intermediate zone of activity overlap. By 2 weeks post-injury, a dramatic reversal occurred in which inspiratory activity recording sites were more frequently represented caudally and expiratory activity sites increased rostrally. At 12 weeks post-injury, rostral expiratory activity expanded caudally with shrinking representation of inspiratory activity in the cVRC. To our knowledge, these electrophysiological data are the first demonstration of changes in neural circuit phenotype associated with medullary respiratory pattern generation following SCI. These findings may reflect changes in

ventilatory behavior, as well as compensation in spinal respiratory motor function.

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**P-43 Synaptic Integration of Transplanted Cells With Phrenic Circuitry Following High Cervical Spinal Cord Injury In Adult Rat**

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Cervical spinal cord injuries frequently result in life-threatening respiratory dysfunction even at chronic post-injury times. Injuries rostral to C3-5 are especially devastating due to loss of bulbospinal inspiratory input to phrenic motoneurons (PhMNs), resulting in severe impairment of diaphragm function. Experimentally, this is best illustrated by a well-established lateralized C2 spinal hemisection (C2Hx) model in which injury results in paralysis of the ipsilateral hemidiaphragm. While some spontaneous diaphragm recovery has been demonstrated, the extent of improvement remains limited. To determine whether a cellular replacement approach can enhance this neuroplastic outcome, we first wished to determine whether donor neuronal progenitors can become integrated with the phrenic circuitry and mediate improved ipsilateral hemidiaphragm function. Accordingly, E13-14 rat fetal spinal cord tissue was grafted into acute C2Hx lesions of adult, female Sprague-Dawley rats. Lesion-only animals served as controls. A retrograde transsynaptic tracer – pseudorabies virus (PRV) – was applied to the ipsilateral diaphragm or injected directly into transplanted tissue, to assess synaptic integration between the host

neurons and transplanted cells. Terminal diaphragm EMG (DiaEMG) recordings were also obtained 24-72 hours after PRV delivery. Recordings were made during eupneic breathing (exposed to compressed air) or respiratory challenge (elicited by hypercapnia; 7% CO<sub>2</sub> delivered via nose-cone). DiaEMG in untreated animals revealed only limited activity ipsilateral, to C2Hx. Hypercapnia increased diaphragm activity, but ipsilateral output remained impaired. In contrast, transplant recipients showed improved muscle activity ipsilateral to injury during eupneic and challenged breathing. PRV tracing revealed connectivity between donor neurons and the host phrenic circuitry ipsilateral to injury. PRV injection into transplanted tissue revealed integration between donor neurons and 1) host spinal interneurons in surrounding gray matter and 2) medullary neurons in the ventral respiratory column, raphe and reticular nuclei. These results provide evidence for graft-mediated enhancement of post-C2Hx diaEMG activity that may involve a host-graft interneuronal relay.

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#### **P-44 Persistent Diaphragm Dysfunction Following Lateralized Cervical Contusion**

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Cervical spinal cord injury (SCI) compromises phrenic circuitry resulting in diaphragm paresis or paralysis. While research using a lateralized C2 hemisection model has revealed the potential for spontaneous recovery and anatomical plasticity, less is known about respiratory plasticity following more clinically

relevant contusion injuries at the level of the phrenic nucleus (C3-C5/6). The goal of the present study was to define the extent of PhMN compromise following lateral mid-cervical contusions and concomitant temporal changes in diaphragm EMG (diaEMG) activity and ventilatory function. Telemetric electrodes were bilaterally implanted in the diaphragms of adult female rats. One week later, all animals received a C3/4 contusion (Infinite Horizon, 150kD present force). Diaphragm EMG recordings were made simultaneously with ventilatory assessment (using whole-body plethysmography) in awake unanesthetized animals. Measurements were made pre-injury, daily during the first week post-injury, and weekly thereafter while animals were exposed to either normal (normoxic, normocapnic) or hypercapnic (7% CO<sub>2</sub>) air. Initial results demonstrate the feasibility of long-term telemetric recordings of diaEMG activity. Within the first two post-injury days, diaphragm activity increases while ventilation is relatively unaffected. Furthermore, diaphragm activity and overall ventilatory responses to respiratory challenge (hypercapnia) are dramatically attenuated compared with that seen pre-injury. By 1 week post-injury, the ventilatory response to challenge normalizes. Although there is some recovery of diaEMG responses to challenge, the extent remains significantly reduced relative to what occurs pre-injury. This deficit remains persistent at 4 weeks post-injury. Persistent diaphragm dysfunction in the presence of otherwise normal ventilatory patterns suggests significant compensation in other respiratory motor systems (e.g., intercostal circuitry). Ongoing studies are quantifying PhMN primary and secondary loss and examining the relationship between motoneuron loss and diaphragm dysfunction.

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#### **P-45 Chondroitinase ABC Treatment And Modest Exposure To Intermittent Hypoxia**

### Restores Hemidiaphragmatic Activity After Cervical Spinal Cord Injury

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Most spinal cord injuries (SCI) occur at the cervical level. This is particularly devastating because the phrenic motor neurons, which innervate the diaphragm, are located at this level. As a result, those afflicted with a cervical SCI usually have complications in breathing and can be dependent on a mechanical ventilator in order to survive, severely limiting their quality of life. To study these complications and ways to restore respiratory motor activity, our laboratory utilizes the lateral C2 hemisection (C2H) model of SCI. C2H severs the ipsilateral bulbospinal inputs to the phrenic nucleus and paralyzes the ipsilateral hemidiaphragm while sparing the crossed phrenic pathway which, although latent, serves as a potential anatomical substratum for respiratory plasticity. Additionally, acutely following C2H, there is a dramatic upregulation of the chondroitin sulfate proteoglycan (CSPG) containing perineuronal net (PNN) around the ipsilateral phrenic motor neurons. CSPGs and the PNN can severely inhibit plasticity and axonal regeneration, and thereby the means to restore function. Chondroitinase ABC (ChABC) can digest these inhibitory matrix molecules and we have shown that with enzyme administration alone, there is a modest return of hemidiaphragm activity after C2H. In other models of SCI, combining ChABC treatment with task-specific training can profoundly improve limb function. In the present study we hypothesized that combining ChABC treatment with a very modest exposure to intermittent hypoxia (IH), which can strongly drive respiratory motor plasticity (at higher exposure times), will further promote breathing recovery.

Here we show that this combination treatment of ChABC and modest IH exposure can induce robust respiratory motor function after C2H. Animals that received IH, with no ChABC displayed little to no recovery. Taken together, these results show that ChABC treatment combined with an IH regimen that is far more tolerable to the animal can augment recovery of respiratory motor function after SCI.

### P-46 Ipsilateral Inspiratory Intercostal Muscle Activity After C2 Spinal Cord Hemisection

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Upper cervical spinal cord hemisection (e.g., at C2) causes paralysis of the ipsilateral hemidiaphragm, however, the effect of C2 hemisection on the function of the intercostal muscles is not known. We hypothesized that ipsilateral intercostal muscles would be paralyzed after C2 hemisection and activity would return in a time-dependent fashion. Female, Sprague Dawley rats were anesthetized with urethane and inspiratory intercostal EMG activity was recorded in control rats, acutely-injured C2 hemisected rats, and at 1 and 16 weeks post C2 hemisection. Bilateral recordings of intercostal EMG activity showed that inspiratory activity was reduced immediately after injury and increased over time. EMG activity was observed first in rostral spaces followed by recovery occurring in caudal spaces. Theophylline increased respiratory drive and increased intercostal activity; in some cases inducing activity that was previously absent. These results suggest for the first time that there are crossed, initially latent, respiratory connections to neurons innervating the intercostal muscles similar to those innervating phrenic motor neurons.

**P-47 Generation of High Purity Neuronal Progenitor Cells And Oligodendrocyte Progenitor Cells From Blastocyst-Derived Human Embryonic Stem Cells And Human Induced Pluripotent Stem Cells**

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The discovery that human somatic cells can be reprogrammed to induced pluripotent stem cells (hiPSCs) which closely resemble human embryonic stem cells (hESCs) garnered tremendous excitement. While iPS lines pass gross tests of pluripotency, more stringent characterization indicates that most lines are partially rather than completely reprogrammed. The objective of our work was to: 1) determine the degree of equivalence between three human iPS and two blastocyst-derived human ESC lines and 2) determine whether differences have functional implications for the ability of lines to produce neuronal and oligodendrocyte progenitor lines via clinically relevant high purity differentiation protocols. Two blastocyst-derived hESC lines (H7 and CSC-14) and three hiPSC lines derived from adult human dermal

fibroblasts (Yamanaka-iPS-414C, Yamanaka-iPS-201B7, and Okano-iPS-WA29) were exposed to identical expansion conditions followed by differentiation into neuronal and oligodendrocyte progenitor cells. Proliferation rates and gross morphology were recorded during expansion. During stem cell expansion, all lines had similar rates of proliferation and gross morphology. Yield and purity of differentiated products were determined via cell counts and ICC profiling. ICC profiling indicated that both hESC lines and two of the three hiPSC lines produced differentiated products with a low percentage of contaminating cell populations. These two iPS lines matured faster than the hESCs. The third iPS line produced a very low yield of differentiated product, consisting primarily of undifferentiated neural cells. Despite the recently published differences between hiPSCs and hESCs, properly screened iPS lines are capable of producing differentiated products of purity sufficient for clinical applications and should be tested in vivo to determine if differentiated products have functional equivalence to those produced from hESC lines.

**P-48 Characterization Of High Purity Neuronal Progenitors Isolated From Human Embryonic Stem Cells**

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Human Neuronal Progenitors (hNPs) are difficult to obtain in large quantities from primary source. Neuronal Progenitors are more lineage-restrictive than Neural Progenitors,

since they do not differentiate into glial cells, but are rather committed to neuronal lineage. The availability of hNPs in high purity would greatly facilitate neuronal drug discovery and developmental studies, as well as cell replacement strategies for neurodegenerative diseases and conditions. Here we describe a method for producing hNPs in large quantity and high purity from human embryonic stem cells (hESCs) in a clinically compliant manner. The resulting population displays characteristic neuronal-specific markers, such as nestin, Pax6 and doublecortin. When allowed to spontaneously differentiate into neuronal subtypes in vitro, cholinergic, serotonergic, dopaminergic and/or noradrenergic, and medium spiny striatal neurons were observed. When exposed to media known to promote the propagation of glial cells, hNPs failed to form glial cells. These data demonstrate the lineage specificity of hNPs and their ability to produce a variety of different neuronal subtypes.

**P-49 Developing A Human Drug Discovery Platform For Huntington's Disease**

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The objective of this work is to develop a high purity human stem cell based drug discovery platform for Huntington's Disease (HD). HD is a neurodegenerative genetic disorder that results primarily in the loss of medium spiny projection neurons (MSN) of the striatum. Current animal models do not sufficiently recapitulate the complex cascade of

neurodegenerative events in the human. Current cellular models are complicated by primary extraction and purification methods or are confounded by contaminant progenitor populations. The need exists for high purity MSN and lateral ganglionic eminence progenitors (LGP) at multiple developmental stages for transplant studies as well as drug discovery and predictive toxicology assays. Genetically abnormal blastocysts donated by participants were thawed and an hESC line was derived and maintained in animal-free conditions and characterized. hUCI-HD1 is a karyotypically normal HD line carrying 44 CAG repeats. Undifferentiated hESC were grown on matrigel-coated flasks to subconfluence in a feeder-free system. Cultures were then transitioned from conditioned medium to a DMEM-F12 rich medium for neural induction. Cells were grown in suspension and passaged routinely in differentiation medium supplemented with growth factors until day 60, when they were replated and growth factors were withheld for final maturation to MSN by day 66. Stage-specific immunocytochemistry was performed at day 14 (GSH-2), day 25, day 42 and 45 (FoxP1) and day 66 (DARPP-32) to optimize growth conditions. Cultures were additionally matured to day 135 and electrophysiology was performed, demonstrating neuronal firing patterns and functional synaptic connections. During recording of spontaneous post-synaptic currents, NBQX/APV and GABAzine were used to block glutamatergic and GABAergic events, showing that the cells received both excitatory and inhibitory inputs. The early stage LGP will be used for studies of striatal development and the MSN will be used for transplant studies, drug discovery and predictive toxicology.

**P-50 High Purity Mouse Embryonic Stem Cell-Derived Progenitor Motor Neurons For Transplantation After Spinal Cord Injury**

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Embryonic stem cell (ESC)-derived motoneurons (MNs) and ESC-derived oligodendrocyte precursor cells (OPCs) have been shown to have therapeutic potential in preclinical models of spinal cord injury (SCI)<sup>1</sup>. Differentiation of ESCs into spinal MNs and OPCs can be achieved *in vitro* following exposure to retinoic acid (RA) and sonic hedgehog (Shh); however, this results in low purity MN and OPC cultures. Current protocols to increase MN and OPC purity are lengthy and technically challenging. *In vivo*, spinal cord MNs and some OPCs differentiate from progenitor motor neurons (pMN) in the ventral neural tube, which express the transcription factor Olig2. A subset of pMNs downregulate Olig2 and commit to the MN fate, while others will maintain Olig2 expression and become OPCs. Transgenic selection under control of the Olig2 gene regulatory elements (GREs) may therefore provide a simple and inexpensive method to enrich cultures for MNs and OPCs. In this study, we generated a transgenic mouse ES cell line (P-Olig2) that provides specific resistance to the antibiotic puromycin under the control of the Olig2 GREs. P-Olig2 ESCs were exposed to retinoic acid and purmorphamine, a Shh signalling agonist, using a 2<sup>7</sup>/4<sup>+</sup> induction protocol. First, ESCs were aggregated into embryoid bodies (EBs) for two days in suspension culture then transferred to gelatin coated well plates and induced with 2  $\mu$ M RA and 1.5  $\mu$ M purmorphamine for an additional four days. Puromycin was added during the final two days for selection. Markers for pMNs/OPCs (Olig2) and newly committed MNs (Hb9) were

analyzed using flow cytometry. Selection with puromycin led to an approximately 2-fold increase in the percentage of cells expressing Olig2. Similarly, the percentage of cells expressing Hb9 was increased approximately 3-fold. These results demonstrate that transgenic selection under Olig2 GREs can enrich cultures for MNs and OPCs.

References

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**P-51 The Effects of Human Motor Neuron Progenitor Transplants In A Mouse Model Of Spinal Muscular Atrophy**

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Infantile spinal muscular atrophy (SMA) is the most common and severe hereditary neurological disease in childhood, and is characterized by motor neuron loss. Children diagnosed with SMA demonstrate severe muscle weakness that makes it difficult for them to breathe, eat, and move, and >95% die within 2 years. There is currently no treatment that can change the course of the disease. This study set out to determine the histological, molecular, and functional effects following intraspinal transplantation of human embryonic stem cell-derived motor neuron progenitors (hESC-MNPs) into established models of  $\Delta$ 7SMA (*SMNdelta7;SMN2;Smn-/-*), and ALS (G93A SOD1) all of which are characterized by motor neuron loss. hESC-MNPs were transplanted into both models of motor neuron loss and their migration, engraftment, differentiation and

repair potential within the diseased or injured tissue was assessed. Our data demonstrates limited migration, stable engraftment, differentiation, sparing of endogenous tissue, and functional benefit as a result of transplantation, likely as a result of growth factor secretion. Currently, we are looking at the effect of hESC-MNP transplants on respiration and survival in  $\Delta 7SMA$  mice by transplanting in the upper thoracic region of the spinal cord, in addition to the normal lumbar transplantation site. hESC-MNPs represent a biological tool to investigate human motor neuron development, and provide a clinically relevant cell population for the development of therapies to treat injuries or diseases characterized by motor neuron loss.

**P-52 Adult Spinal Cord Radial Glia Display A Unique Progenitor Phenotype**

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Radial glia (RG) are primarily embryonic neuroglial progenitors that express Brain Lipid Binding Protein (*Blbp* a.k.a. *Fabp7*) and Glial Fibrillary Acidic Protein (*Gfap*). We used these transcripts to demarcate the distribution of spinal cord radial glia (SCRG) and screen for SCRG gene expression in the Allen Spinal Cord Atlas (ASCA). We reveal that neonatal and adult SCRG are anchored in a non-ventricular niche at the spinal cord (SC) pial boundary, and express a “signature” subset of 122 genes, many of which are shared with “classic” neural stem cells (NSCs) of the subventricular zone (SVZ) and SC central canal (CC). A core expressed gene set shared between

SCRG and progenitors of the SVZ and CC is particularly enriched in genes associated with human disease. Visualizing SCRG in a FABP7-GFP reporter mouse reveals an extensive population of SCRG that extend processes linearly through the white matter (WM), around the SC boundary and inwardly towards the SC gray matter, whose abundance increases in a gradient from cervical to lumbar SC. Confocal analysis of multiple NSC-enriched proteins reveals that postnatal SCRG are a discrete and heterogeneous potential progenitor population distinct from white matter astrocytes. SCRG become activated by multiple SC lesions, and, like CC progenitors, are also more heterogeneous than previously appreciated. Gene ontology analysis highlights potentially unique regulatory pathways that may be further manipulated in SCRG to enhance repair in the context of SC injury and disease.

**P-53 Anatomical & Functional Characterization Of Fenestrated Endothelium Formation During Revascularization In The Injured Spinal Cord**

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Spinal cord injury (SCI) results in immediate loss of microvascular function/structure at the injury site. A temporally specific adaptive angiogenic response occurs at the injury site over the first week post-SCI. These newly formed vessels exhibit abnormal structure and function, the effectors of which remain poorly understood. Previous work demonstrated the up-regulation

of plasmalemmal vesicle associated protein (PV-1) expression in neovessels in/around the injury site (Mozer et al., 2010). This is significant as PV-1 is hallmark of fenestrated endothelia found in endocrine and pulmonary tissue, where free passage of molecules and cells is desired. The goal of the current study was to more precisely identify the temporospatial appearance of these PV-1-expressing microvessels and to better determine their functional state. Adult female C57bl/6 mice (n=18) received a moderately severe contusive spinal cord injury. Mice were euthanized at 1,3, and 7 days post-SCI and spinal tissue was prepared for PV-1 immunohistochemistry. PV-1 expression patterns in serial sections representing one-half of the injury site (i.e. ~500µm) were reconstructed 3-dimensionally. Qualitatively, PV-1 expression peaks at 7 days SCI, consistent with the previous study. Interestingly, in earlier time-points, PV-1 is initially seen in radicular vessels, eventually repopulating affected gray matter only and then later spreads diffusely throughout the epicenter. Importantly, PV-1 is restricted to microvessels devoid of astroglial investment and is absent in focal areas containing significant inflammatory infiltrate. Further, caveolae formation appears to be elevated in PV-1-expressing microvessels, suggesting increased trans-endothelial transport potential. Lastly, using a novel model of *in vitro* CNS angiogenesis, PV-1 expression arises concomitant with vascular remodeling, suggesting that its expression is conserved during revascularization in various CNS areas. These data are significant as they suggest that angiogenesis post-SCI may be the consequence of ingrowth by “non-parenchymal” vessels, explaining the consistent dysfunction in these diffuse neo-vascular networks.

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#### **P-54 Thrombin Inhibits Adult Neural Progenitor Integration And Regeneration By Promoting Astroglialogenesis Via Id1.**

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The instructive cues that coordinate progenitor activation and fate specification early after injury remain largely unknown. Since thrombin sensing is a key stem cell trait, and blood extravasation is a hallmark of trauma, we examined the effects of thrombin on the proliferation and differentiation of adult neural progenitors (aNPC). Direct injection of thrombin, *in vivo*, increased endogenous aNPC proliferation 3-fold. Thrombin inhibition reduced proliferation and astroglialogenesis after spinal cord injury (SCI). Thrombin-treated aNPC cultures increased proliferation versus control cells, *in vitro*. Moreover, aNPCs became astroglialogenic when treated with thrombin and continued to proliferate. Thrombin-treated cells up-regulated Inhibitors of DNA-binding (Id) expression, which was demonstrated by shRNA to promote astrocyte formation and inhibit neuronal and oligodendroglial differentiation. When applied *in vivo*, transplanted aNPC integrated into the lesion, wrapped axons, and regenerated myelin rings with thrombin inhibition. Together, these data demonstrate thrombin acts as a blood-borne early-response factor capable of directing differentiation and scar formation in the post-injury niche. This work supported by NIH, The Paralysis Project,

Paralyzed Veterans of America and The Neilsen Foundation.

**P-55 Transplantation of Human Mpcs In Combination With Scar Reducing Decorin Following Acute And Chronic Spinal Cord Injury In The Rat.**

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Human mesenchymal precursor cells (hMPCs) isolated from bone marrow stroma of spinal cord injured patients were transplanted into the spinal cord of Nude (immunodeficient) rats both alone and in combination with the scar reducing compound decorin after acute (immediate) and chronic (long term) spinal cord injury (SCI). Functional recovery after highly purified human decorin infusion (core and non-core isoforms) via minipump was assessed using open field (BBB) and Catwalk gait analysis for up to 3mo post SCI. Morphological analysis of neuronal marker expression profiles, cyst size, tissue sparing, macrophage infiltration and extracellular matrix (ECM) deposition/glia scarring in and around the injury site was also assessed. Extensive analysis of hMPCs axonal growth stimulating potential *in vitro* using co-cultures of hMPCs with embryonic DRG explants revealed that neurite growth is markedly improved with hMPC co-culture but is not further enhanced in combination with either decorin isoform and there is no growth promotion of either decorin isoform with DRG alone. In acute and chronic SCI: functional

recovery is only significantly improved in animals subjected to cell transplantation alone or combined with decorin infusion, and there is prolonged survival of donor hMPCs after decorin infusion for at least 2mo post SCI, although numbers still decline over time. Decorin treatment appears to moderately enhance tissue sparing. In addition, there are reduced numbers of infiltrating macrophages with either core/non-core decorin isoforms. Neuronal expression profiles of proteins (RT97, GFAP, BetaIII Tubulin and CGRP) are apparent centrally within the lesion sites of many hMPC-treated, hMPC/decorin infusion-treated and even decorin only-infused (either isoform) animals. ECM expression and molecules associated with glial scar tissue (Collagen, neurocan, phosphocan) in decorin infused animals trends toward being slightly reduced compared to donor hMPC-treatment (alone). Decorin may be incorporated into combinatorial treatments to promote improved donor cell survival and reduced secondary damage after acute/chronic SCI.

**P-56 Self-renewal and multipotential properties of GFAP-expressing cell populations in the adult spinal cord**

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Neural Stem Cells (NSCs) cells can be found throughout the adult central nervous system (CNS), although neurogenesis is restricted to a few regions. In the forebrain subependymal zone (SEZ), neurons originate from NSCs expressing the astrocytic marker GFAP. In other regions of the adult CNS such as the spinal cord, cells showing NSCs properties can be isolated *in vitro*, but their identity remains elusive. Here, we used a transgenic inducible mouse (hGFAP-CreERT2 x Stop-RosaYFP) to label and fate map putative GFAP(+) NSCs populations in the adult SEZ and spinal cord

(SC), and compare their self-renewal and multipotential properties. Recombined SEZ NSCs could be identified based on their markers expression (i.e. Vimentin, Nestin), proliferative properties, and by their capacity to continuously give rise to olfactory neurons. In the SC, recombination occurred in the vast majority of parenchymal astrocytes as well as in a minor population of sub-ependymal, bipolar GFAP+ cells surrounding the central canal (CC). These cells expressed similar markers to SEZ-NSCs, while parenchyma GFAP+ astrocytes acquired these markers (i.e. Vimentin, Nestin) after injury. We next assessed the NSCs properties of these two cell populations in an *in vitro* neurosphere assay. Primary neurospheres were successfully obtained from both the intact and injured spinal cord, and in both conditions, ~20% of the spheres expressed the YFP reporter gene indicating their origin from GFAP expressing cells. These neurospheres were shown to be multipotent. Noticeably, while YFP+ neurospheres derived from intact SC could not be passaged for extended periods of time, those from the injury showed prolonged self-renewal properties. All together, our work reveals major differences in the identity of stem and progenitor cells in distinct regions of the adult CNS; our results further suggest that parenchyma reactive astrocytes may represent a minor population of NSCs that are recruited after spinal cord injury.

**P-57 Optimizing Recovery Of Overground Locomotion With Novel Robotic Gait Training Patterns Following SCI In Rats**

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In both clinical and experimental settings, robotic gait training has become a popular means to encourage neuronal plasticity in an effort to facilitate functional recovery

following neurological injury. However, previous works focus on improvements observed within the training devices, when improvements found in more normal, everyday activities should be the main goal. To address this, we train rats following a right cervical overhemisection injury with a robotic gait trainer (RRMPS, Robomedica Inc, Irvine CA) but assess recovery of overground locomotion with the CatWalk gait analysis system (Noldus, Wageningen, NE). Many measures of overground locomotion are dependent on walking velocity (short strides are taken gradually when walking slow; long strides are taken rapidly when walking fast). Therefore, we have developed a nonlinear regression analysis technique to parse out which changes in overground locomotion are due to neurological impairments and not simply a difference in walking speed. Several styles of robotic gait training were applied daily over a four week period. As is often done in the clinical setting, we applied the average pre-injury stepping patterns to one group of injured animals. This practice depends on precise alignment and timing to match the guiding forces with the animals walking. In contrast, training was also performed in viscous and negative viscosity fields. An untrained group, and a daily training without robotic forces (null field) group were used as controls. After four weeks of training, the pre-injury pattern and null field groups both exhibited less overground locomotor deficits than the untrained group. However, two weeks after the cessation of training the pre-injury pattern group maintained their improvements, while the null field did not. Results from the viscous and negative viscosity trained groups are promising, but statistically inconclusive at this time.

**P-58 Robotic Training In Spinal Rats Produces Novel Gait Patterns And Abnormally High Levels Of Glutamate And Glycine In The Lumbar Spinal Cord**

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After spinal cord transection, the generation of stepping depends on neurotransmitter systems entirely contained within the local lumbar spinal cord. Glutamate and glycine likely play important roles, but surprisingly, little is known about how the content of these two key neurotransmitters changes in order to achieve weight bearing stepping after spinal cord injury. We studied the levels of glutamate and glycine in the lumbar spinal cord of spinally transected rats. Rats (n=48) received spinal cord transection at five days of age and four weeks later, half were trained to step using a robotic treadmill system and the remaining half were untrained controls. Analyses of glutamate and glycine content via high-performance liquid chromatography (HPLC) showed training significantly raised the levels of both neurotransmitters in the lumbar spinal cord beyond normal. In the Trained rats, glutamate and glycine levels were significantly correlated with the ability to perform independent stepping. Immunohistochemical analyses showed that the expression of VGluT1 and GlyT2 around motor neurons was significantly greater in Trained versus Untrained rats. Training improved the ability to generate stepping at a range of weight support levels, but normal stepping characteristics were not restored by training. These findings suggested that training-induced adaptations in glutamate and glycine levels played a role in the generation of novel gait patterns following complete spinal cord transection.

**P-59 Dose Dependant Effects of BDNF on Locomotor Recovery In Paraplegic Rats**

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We sought to determine an effective dose of BDNF necessary to promote the recovery of stepping in adult spinal rats. Following a complete T10 spinal cord transection injury, adult rats were immediately treated with BDNF delivered to the lesion via intraspinal injections of adeno associated viral (AAV) constructs. Three different viral titers were used, low ( $5 \times 10^9$  genomic copies (gc)), intermediate ( $1 \times 10^{10}$ ) and high ( $2.5 \times 10^{10}$ ). Kinematic analysis of hindlimb locomotor function revealed that animals receiving the highest dose recovered treadmill and over-ground stepping. Animals treated with low AAV-BDNF could not step over-ground and required perineal stimulation for treadmill locomotion. Some rats receiving intermediate AAV-BDNF recovered the capacity for both over-ground and treadmill stepping. Plantar testing demonstrated that reduced thermal withdrawal latency, indicative of enhanced responsiveness to noxious heat, developed in all BDNF treated rats. Hindlimb spasticity was also observed in rats in the high treatment group. In terminal electrophysiological experiments rheobase (Rh) was determined in medial gastrocnemius (MG) and lateral gastrocnemius soleus (LGS) motoneurons. Motoneurons of rats receiving high BDNF titers were considerably more excitable (Mean Rh:  $5.4 \pm 1.8$  nA) than motoneurons in intermediate (Mean Rh:  $7.2 \pm 3.5$  nA) or low (Mean Rh:  $9.4 \pm 4.8$  nA) treatment groups. In addition, c-Fos expression was significantly elevated in lamina VI-VII of the L2 spinal cord of rats in the high treatment group suggesting activation of central pattern generator neurons with AAV-BDNF treatment. These results indicate that intermediate doses of AAV-BDNF may be sufficient for the recovery

of over-ground locomotion while reducing some of the adverse side effects (spasticity) observed at the highest dose. At present, it is not clear whether sensitization of nociceptive projections is necessary for the recovery of stepping, or whether this is unrelated and an adverse side effect of BDNF treatment due to the generalized hyperexcitability.

**P-60 Post-Injury Administration of An Oxidoreductant Confers Protection After Contusional Spinal Cord Injury In Adult Rats**

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The deleterious effects of oxidative stress in spinal cord injury (SCI) have been previously described and detoxification of reactive oxygen species (ROS) is a well-documented therapeutic target for conferring cellular protection. Efforts to develop highly efficacious pharmacological agents to reduce ROS have focused on the native defensive enzyme superoxide dismutase (SOD). Previous generation SOD compounds were poorly translated to clinical use based on their large size, minimal cell permeability, short circulating half-life, and low bioavailability in target tissues. Recently, a class of smaller SOD-mimetics have been developed which includes a very potent metalloporphyrin catalytic oxidoreductant, manganese (III)-tetrakis (*N*-ethylpyridinium-2-yl)porphyrin (MnTE-2-PyP), that is efficacious in scavenging a broad range of ROS including superoxide, hydrogen peroxide, and peroxynitrite. In this study we evaluated the effect of post-SCI administration of MnTE-2-PyP on tissue sparing and functional recovery. Twenty-four adult, female rats received a moderately severe crush SCI at T10 calibrated forceps. MnTE-2-PyP (1mg/kg) or vehicle (Hanks Buffered Saline Solution) was

administered by intraperitoneal injection at 30 minutes post-injury and then once per day for 7 days. Hind limb locomotion was evaluated using the Basso, Beattie, Bresnahan (BBB) scale and the Catwalk kinematic analysis once per week for 4 weeks post-SCI. At the conclusion of the behavioral evaluation, the spinal cord tissue was extracted and processed to evaluate numbers of ventral horn neurons and white matter sparing. MnTE-2-PyP-treated rats had significantly higher BBB scores and a greater average regularity index as compared to the vehicle group. Preliminary analysis of the histological markers indicates an increase in neuronal number and increase in white matter sparing in animals that received MnTE-2-PyP. These data suggest that post-SCI administration of this novel oxidoreductant is protective in SCI and is a promising candidate for future development as a therapeutic.

**P-61 Fibronectin Administration Inhibits Chronic Pain Development After Spinal Cord Injury**

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Chronic pain following spinal cord injury (SCI) is a highly prevalent clinical condition that is difficult to treat. Using both von Frey filaments and radiant infrared heat to assess mechanical allodynia and thermal hyperalgesia, respectively, we have demonstrated that a one-time intraspinal injection of fibronectin delivered acutely robustly inhibits the development of mechanical allodynia (but not thermal hyperalgesia) over an extensive observation period following spinal cord dorsal column crush injury. By applying various

fibronectin fragments as well as competitive inhibitors, these effects were shown to be dependent on the CS-1 motif of fibronectin. Furthermore, we found that acute fibronectin treatment diminished inflammation and blood spinal cord barrier permeability which, in turn, leads to enhanced tissue and fiber sparing as well as fiber sprouting. In particular, the reduction of 5-HT in the superficial dorsal horn, an important descending brainstem system in the modulation of pain, was reversed with fibronectin treatment. We conclude that treatment of SCI with fibronectin preserves sensory regulation and prevents the development of chronic allodynia, providing a potential therapeutic intervention to treat chronic pain following SCI.

**P-62 Every Move You Make: Consequences of A Stretching/ROM Therapy Post-SCI**

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A potential impediment to the translation of effective therapies for spinal cord injury (SCI) is that the conditions of recovery in animal models do not parallel those of patients. In contrast to rodent models that show high levels of activity within a few weeks of incomplete injuries, the majority of patients are largely immobile for weeks or months receiving only stretching and range-of-motion (ROM) therapies, which are almost universally delivered in some form. Stretching therapy is employed primarily to avoid peripheral tissue pathologies, and the influence, positive or negative, of stretching on spinal cord circuitry is completely unknown. We found recently that a daily stretching protocol resulted in a significant attenuation of locomotor function following mild-moderate T9 contusions (Caudle et al., 2011). Thus, in the current study we utilized an intensive, physical therapy-based stretching

protocol, administered daily to adult female SD rats with moderate (12.5g-cm) thoracic contusion injuries. We found that locomotor function was rapidly and dramatically reduced following even a single stretching session and that this drop in function accumulated over the first 4-5 weeks post-injury. Interestingly, the negative impact of the stretching protocol decreased at 6-8 weeks post-injury, and when stretching ceased at 8 weeks the majority of animals showed a robust recovery but with significant retained deficits compared to unstretched controls. These observations suggest that the spinal cord circuitry is particularly vulnerable to aberrant afferent input acutely post-injury and that every action (or inaction) and treatment applied to SCI patients should be considered to have consequences that may fundamentally change the trajectory of recovery. Supported by the Kentucky Spinal Cord and Head Injury Research Trust and by the NIH/NINDS (R01 NS052292) and NIH/NCRR (P20 RR15576).

**P-63 Comparison Of The Effects Of Rubrospinal Tract And Red Nucleus Lesions On Skilled Reaching.**

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We have recently shown that small spinal cord lesions that damage the rubrospinal tract (RST) while sparing the other fibre pathways running in the lateral funiculus selectively abolish the arpeggio movement in skilled reaching (Morris et al., 2011). On the other hand, lesions of the red nucleus (RN) have been shown to interfere with several aspects of skilled reaching including the arpeggio movement (Whishaw and Gorny, 1996; Whishaw et al., 1998). The RST is the main descending output of the RN. Deficits in arpeggio after RN lesions are therefore in line

with our recent findings. However, the additional deficits reported after RN lesions are difficult to reconcile with our recent results. The present study was designed to compare, in the same experimental setup, the outcomes of RN lesions with that of lesions to the RST. Long Evans female rats were trained to reach for single sugar pellets. After the completion of the training, the animals were subjected to either RN or RST lesions. Detailed movement analysis revealed that both types of lesions abolish the arpeggio movement. This finding supports the view that the arpeggio movement is under the control of the RST. RN lesions, however, creates additional deficits in the grasping action. The results are explained in terms of the involvement of the RN with a network of neural structures that are directly involved in motor control (e.g., motor cortex and cerebellum). In light of these anatomical considerations, it is not surprising that lesions to the RN have a greater impact on skilled reaching than RST lesions.

**P-64 PTEN Deletion Promotes Regenerative Sprouting In The Aged Rubrospinal Tract**

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The devastating motor, sensory, and autonomic dysfunction that occurs following spinal cord injury is largely the result of transected central nervous system (CNS) axons failing to regenerate. Previously Park et al. (Science 322, 963-966 [2008]) and Liu et al. (Nat Neurosci 13, 1075-1081[2010]) have shown that a major contributor to this regenerative failure is a diminished intrinsic capacity of adult CNS (retinal ganglion cellular and corticospinal) axons to grow, based largely on inactivity in the phosphoinositide 3-kinase

(PI3K)/Akt/mammalian target of rapamycin (mTOR) pathway which is negatively regulated by phosphatase and tensin homolog deleted on chromosome ten (PTEN). The rubrospinal tract (RST), which originates in the red nucleus of the midbrain and travels contralaterally in the dorsolateral funiculus of the spinal cord, is an alternative model of CNS axon regenerative failure. Here, we assessed whether PTEN deletion would promote axon regeneration in the RST with deletion occurring in aged (7-8 month old) mice. Floxed PTEN mice were injected with adeno-associated virus serotype 2 expressing Cre and GFP (AAV2-Cre) or GFP alone for control (AAV2-GFP) into the right red nucleus. Four weeks later, mice underwent a left dorsolateral crush at cervical level C4/C5. Six weeks later, mice were injected with biotinylated dextran amine (BDA) into the right red nucleus to anterogradely trace the RST. Two weeks later, mice were sacrificed. Based on independent analyses of BDA and GFP labelling, AAV2-Cre injected animals showed significantly decreased dieback and increased regenerative sprouting of rubrospinal axons through the lesion site in comparison to AAV2-GFP injected animals, for up to 100µm caudal measured from the middle of the lesion site. Our findings suggest that PI3K/Akt/mTOR activity is a significant determinant of rubrospinal regenerative potential, yet advanced age may play an important role in decreasing the ability of RST axons to regenerate long distances.

**P-65 Intra-Cortical Injection of AAV-shRNA Abrogates PTEN Expression In Cortical Motoneurons**

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Recent studies have shown that conditional genetic deletion of the tumor suppressor gene PTEN in cortical motoneurons enables them to regenerate their axons after spinal cord injury (Liu et al., 2010, *Nat. Neurosci.* 13, 1075-1083). The experiments utilized mice with a LoxP-flanked PTEN gene (PTEN<sup>ff</sup> mice), which allows local conditional genetic deletion of PTEN via injections of AAV-Cre. Here, our goal is to develop an intervention that would allow clinically-relevant proof of concept experiments in which PTEN is targeted at the time of, or after a spinal cord injury. Towards this end we developed, in conjunction with the University of Pennsylvania Vector Core, an adeno-associated virus (AAV) vector that expresses an shRNA specific for PTEN (AAV2/9-shPTEN) and the ZsGreen reporter gene. Adult Sprague Dawley rats received a single injection of 10<sup>10</sup> genome copies of either AAV2/9-shPTEN or AAV2/9-shLuc (control vector), into the sensorimotor cortex. Three weeks post-injection brains were stained for H&E and immunostained for PTEN and phosphorylated ribosomal protein S6 (pS6), a marker for activation of mTOR-dependent protein synthesis. Immunostaining for PTEN was absent in a region approximately 500 μm in diameter in AAV2/9-shPTEN injected brains in the same area exhibiting expression of the ZsGreen reporter. Neurons in the area of PTEN deletion showed increased immunostaining for pS6, indicating persistent activation of mTOR-dependent protein synthesis. There were no changes in immunostaining for PTEN or pS6 in AAV2/9-shLuc injected brains. Examination of H&E stained sections revealed hypertrophy of individual PTEN-deleted neurons, but no overt cytotoxicity or other pathology. Finally, during the 3 weeks after AAV2/9-shPTEN injection, we did not observe any adverse effects on normal rat behaviors including activity, eating and grooming. These findings represent a preliminary indication that AAV2/9-shPTEN can be used to efficaciously and specifically

inhibit PTEN expression in cortical neurons for prolonged periods of time.

**P-66 Conditional Genetic Deletion of PTEN in the Sensorimotor Cortex Does Not Lead To Evident Motor Impairments**

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Recent studies have shown that conditional genetic deletion of the tumor suppressor gene PTEN in cortical motoneurons enables them to regenerate their axons after spinal cord injury (Liu et al., 2010, *Nat. Neurosci.* 13, 1075-1083). The experiments utilized mice with a LoxP-flanked PTEN gene (PTEN<sup>ff</sup> mice), which allows local conditional genetic deletion of PTEN via injections of AAV-Cre. Analysis of brain sections from mice that received injections of AAV-Cre into the sensorimotor cortex at postnatal day 1 (P1) and survived for 1.5 years revealed hypertrophy of individual PTEN-deleted neurons and a disruption of cortical lamination. Our goal here was to assess whether deletion of PTEN and the resulting anatomical changes in the cortex disrupted motor function. PTEN<sup>ff</sup> mice received bilateral injections of AAV-Cre or AAV-GFP into the sensorimotor cortex at P1. At 6-8 months of age, mice were tested on a battery of motor function tasks including grip walk, ladder climb, grid walk, Basso Mouse Scale (BMS), rotarod and open field exploratory activity. Overall, motor performance of mice that received bilateral AAV-Cre or AAV-GFP injections was comparable to age-matched control mice. The only differences were that PTEN-deleted mice had impaired performance on the rotarod, although differences were not statistically significant. These findings represent a preliminary test of the safety of long-term

deletion of PTEN, and reveal that the absence of PTEN in cortical neurons and the resulting alterations in cortical morphology have no adverse effects on gross motor function.

**P-67 Long-Term Deletion of PTEN in the Sensorimotor Cortex Causes Neuronal Hypertrophy But Does Not Lead To Tumors Or Other Neuropathology**

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Recent studies reveal that conditional genetic deletion of the tumor suppressor gene PTEN in CNS neurons induces a capacity for robust axon regeneration. The experiments took advantage of mice with a LoxP-flanked PTEN gene (PTEN<sup>ff</sup> mice), which allows local conditional genetic deletion of PTEN via injections of AAV-Cre. Mature mice that received AAV-Cre injections into the sensorimotor cortex at postnatal day 1 (P1) exhibited robust regeneration of CST axons after spinal cord injury as adults (Liu et al., 2010, Nat. Neurosci. 13, 1075-1083). An important consideration for potential translation is that PTEN is a tumor suppressor gene. Here, we assess whether deletion of PTEN in the cortex causes tumors or other neuropathology. PTEN<sup>ff</sup> mice received intracortical AAV-Cre injections at birth and were allowed to survive for 1.5 years. Brains were stained for H&E and immunostained for PTEN and phosphorylated ribosomal protein S6 (pS6), a marker for activation of mTOR-dependent protein synthesis. Immunostaining revealed that many cortical neurons lacked PTEN including large pyramidal neurons in layer V (the cells of origin of the corticospinal tract). Some neurons in the injected area remained PTEN positive,

especially neurons in layers III-IV. Neurons lacking PTEN showed increased immunostaining for phospho-S6, indicating persistent activation of mTOR-dependent protein synthesis. Examination of H&E stained sections revealed substantial hypertrophy of neurons, especially pyramidal neurons in layer V and a disruption of normal cortical lamination, in part because neurons in layer III and IV were less tightly packed. Importantly, there was no evidence of tumors or other cellular pathology. These findings represent a preliminary test of the safety of long term PTEN deletion, and reveal that long term deletion causes no obvious neuropathology.

**P-68 3D Imaging Of CST Axons That Regenerate After Spinal Cord Injury Due To PTEN Deletion**

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A recent study has shown that deletion of *PTEN* in the sensorimotor cortex enables robust regeneration of corticospinal tract (CST) axons beyond a spinal cord lesion (Liu et al., Nature Neuroscience 13, 1075-1081, 2010).

Importantly, however, this study did not define the origin of regenerating axons, course, and degree of midline crossing for individual axons. To address these questions, we have employed a technique for imaging fluorescently labeled axons in unsectioned blocks of spinal cord (Ertürk *et al.*, Nature Medicine, in press). Mice homozygous for the floxed *PTEN* gene (*PTEN<sup>ff</sup>*) received bilateral intracortical injections of AAV-Cre at P1 to delete *PTEN* from cortical motoneurons. At maturity, mice received dorsal hemisection lesions at T12 to cut the dorsal and dorsolateral CST. After 2, 6 or 10 weeks, fluorescent mini-ruby BDA was injected into the left sensorimotor cortex to trace descending CST axons, and mice were transcardially perfused 2 weeks later. Un-sectioned spinal cords were cut into blocks and made optically clear for imaging with 2-photon microscopy. Here, we present initial observations. Rostral to the lesion, there is a great burst of growth emanating from the region of cut axon ends. Of axons extending caudally, many turn away perpendicularly at the lesion margin, some cross the midline, some extend into the lesion, and some continue farther into the caudal intact grey matter. Of those continuing into caudal grey matter, some have axonal swellings apposed to individual somata, suggestive of synaptic contacts. These results indicate that axons that regenerate due to *PTEN* deletion can originate near cut axon ends, extend both ipsilateral and contraletaral to cut parent axons, and that the lesion margin clearly remains a major barrier to regenerating axons.

**P-69 TNF $\alpha$ -Induced AMPA Receptor Changes Undermine Adaptive Spinal Plasticity**

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Recovery of function after spinal cord injury (SCI) is hindered by the expression of a host of neuroinflammatory agents. These agents can induce neuropathic pain states, drive excitotoxicity, and exacerbate cell death following injury. In doing so, they may also undermine adaptive plasticity in the spinal cord, a process essential for behavioral recovery. Among these inflammatory mediators, we have found that tumor necrosis factor alpha (TNF $\alpha$ ) plays a major role in stunting the potential for rehabilitation. We have recently shown that injury-induced release of TNF $\alpha$  acts to increase membrane expression of calcium-permeable AMPA receptors (CP-AMPA), and that this mechanism leads to glutamatergic excitotoxicity and cell death. We wondered if this same mechanism may be working to inhibit adaptive spinal plasticity as well. Using a high-throughput behavioral model of spinal learning, we were able to assess the role of TNF $\alpha$ , as well as how TNF might interact with CP-AMPA, in affecting spinal plasticity. Using spinally transected rat subjects, we have previously shown that TNF $\alpha$  is necessary and sufficient to create a spinal learning deficit. In the current set of experiments, we delivered intermittent nociceptive stimulation that is known to undermine spinal learning, and found that this regimen increased TNF $\alpha$  mRNA and protein expression. Further, treatment with a specific CP-AMPA antagonist (Naspm) prior to instrumental testing rescued the capacity for spinal learning in subjects that had been given exogenous TNF $\alpha$  or intermittent stimulation. These behavioral and biochemical findings indicate that TNF $\alpha$  may undermine adaptive spinal plasticity after injury by inducing changes in CP-AMPA expression. Confocal microscopy is now being employed to further assess the localization of possible CP-AMPA changes induced by intermittent stimulation.

**P-70 An *In Vitro* Study Of The Roles Of Cspgs On Neurons: Neurite Outgrowth Inhibition Or Neuroprotection?**

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Chondroitin sulfate proteoglycans (CSPGs) have long been known as a class of neurite outgrowth inhibitors enriched in the glial scar that prevent axonal regeneration after central nervous system (CNS) injury. Although many studies from different groups have shown that CSPGs inhibit neurite outgrowth *in vitro* using different types of neuronal cell culture systems, it is difficult to relate the concentrations used in culture to the situation *in vivo* after injury. It is also noteworthy that the chondroitin sulfate glycosaminoglycan chains attached to the core protein of CSPGs are highly negatively charged and would hence affect cell adhesion when used as a the cell culture substrate *in vitro*. Under these circumstances, even though obvious neurite outgrowth inhibition is obtained it may not represent the true neurite growth inhibitory activity of CSPGs. Here, using cerebellar granule neuron cultures, we evaluated the effects of different concentrations of both immobilized and soluble CSPGs on neuronal growth. For immobilized CSPGs, at concentrations higher than 4  $\mu\text{g/mL}$ , many cells failed to adhere, resulting in a dramatic loss of cells. Although a strong inhibition of neurite outgrowth was observed by measuring the neurite length of cells that adhered to the CSPG substrates, this could also be due to a lower cell density and/or poorer adhesion. A relative low concentration of CSPGs (1  $\mu\text{g/mL}$ ) showed no obvious difference in cell density compared to neurons plated on PLL, but neurite length was inhibited about 30-40 %. High concentration of soluble CSPGs (10  $\mu\text{g/mL}$ ), when added at the time of cell plating, also effected cell adhesion in

addition to neurite outgrowth inhibition. Cells often formed clusters in the CSPG-treated group because of poor adhesion. However, if cells had first been allowed to adhere on PLL for 1 day before soluble CSPG was added, there was no obvious cell loss but CSPGs significantly inhibited neurite outgrowth. Surprisingly, although a relatively low concentration of CSPGs (1  $\mu\text{g/mL}$  immobilized or soluble CSPGs) showed short term (24 hour) inhibition of neurite outgrowth, neurons growing on low concentration of CSPGs survived for longer times (when cultured for longer than 5 days) than those on PLL. Our results collectively suggest that (1) high concentration of CSPGs which would inhibit cell adhesion is not recommend to be used for neurite outgrowth assay; (2) although CSPG accumulation at the injury prevent axons from regeneration, a low level of CSPG presented in the extracellular matrix might play a beneficial role in neuronal survival.

**P-71 RPTP $\sigma$  DEMONSTRATES A COMPLEX PATTERN OF BINDING TO PROTEOGLYCAN FOLLOWING TRAUMATIC BRAIN INJURY**

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Receptor protein tyrosine phosphatase sigma (RPTP $\sigma$ ) is a type IIa receptor protein tyrosine phosphatase that plays an important role in neural development and after injury to the central nervous system. Recent reports have provided data for RPTP $\sigma$  as the neuronal receptor for chondroitin sulfate proteoglycans (CSPGs), major axon repelling components in

the glial scar. Targeting RPTP $\sigma$  may be an ideal approach to make surviving neurons overcome the barrier represented by CSPG in the glial scar, and reduce neuronal die back in the periphery of injury. We therefore sought to better understand the role of RPTP $\sigma$  in the response to traumatic brain injury (TBI) by using a RPTP $\sigma$  receptor affinity probe (RAP) assay. Controlled cortical impact injury of mild to moderate severity was performed over the left sensory motor cortex in mice. Brains were transcardially perfused and serially sectioned for RAP assay and immunohistochemistry at 1, 3, 7 and 28 days post injury (dpi). cDNA corresponding to the extracellular domain of mouse RPTP $\sigma$  was subcloned into the pAPTAG5 vector and transfected into BHK cells. Secreted RPTP $\sigma$ -AP fusion protein was purified by immobilized metal affinity chromatography (IMAC) column and used for the RAP assay on brain sections. In the uninjured mouse cerebral cortex, RPTP $\sigma$ -AP bound mainly to neuronal cell bodies with little or no binding to perineuronal nets. Some binding was also observed on meninges and choroid plexus. Following TBI, RPTP $\sigma$ -AP binding was transiently observed on the blood vessels located in the proximity to the impact core as early as 1 dpi, which persisted up to 7 dpi. RPTP $\sigma$ -AP binding to neurons as well as blood vessels was displaced by 0.5 M NaCl, suggesting the ionic nature of the interaction. The binding was also interrupted in the presence of EDTA, demonstrating the dependency on divalent metal ion for the interaction. In addition, RPTP $\sigma$ -AP binding was completely displaceable by heparin, and partially displaced with 15 times more concentrated CS, suggesting a comparatively weaker interaction with CSPGs. The labeling of blood vessels by RPTP $\sigma$ -AP following TBI spatio-temporally overlapped with increased perlecan expression following TBI, suggesting it to be the likely partner for RPTP $\sigma$  binding on the blood vessels of injured cerebral cortex. These results demonstrate a complex binding pattern of RPTP $\sigma$  to multiple

partners following TBI. [Supported by the Center for Neuroscience and Regenerative Medicine, Uniformed Services University Health Sciences, and National Heart Lung and Blood Institute, NIH]

**P-72 Reduction of CSPGs after Cortical Brain Injury Enhances Forelimb-Evoked Brain Activation**

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Removal of the axon-growth inhibitory chondroitin-sulphate-proteoglycans (CSPGs) around the glial scar after brain injury enhances neuronal sprouting and leads to some behavioral improvements. We assessed whether reductions in CSPGs results in improved brain activation, as assessed indirectly by immediate-early-gene c-FOS expression. Following controlled-cortical-impact-injury over the left, cortical forelimb region in adult, male rats, chondroitinase-ABC (1.5 $\mu$ l,48U/ $\mu$ l) or vehicle (n=6,8) was infused immediately and at 3-days post-injury into the injury site. At 7-days, all rats and a group of naïve rats (n=6) received right forepaw, electrical stimulation (3.5mA) for 60mins under medetomidine sedation (0.1mg/kg/hr). Rats were perfused-fixed 120mins after the end of the stimulation period and processed for c-FOS immunohistochemistry to determine sensory-motor cortex c-FOS+ cell counts. Four additional groups, naïve, injured, injured+vehicle and injured+chondroitinase were not stimulated (n=3/group). Injury resulted in increased numbers of c-FOS+ cells, even without forelimb stimulation in ipsilesional grey matter (GM), and bilaterally in white matter (WM) compared to naïve (P<0.05), possibly indicating abnormal brain activity. This effect was reduced by chondroitinase infusion in WM but not GM in non-stimulated, injured rats (P

<0.01). Forelimb stimulation in injured/injured+vehicle rats significantly increased ipsilesional-GM c-FOS+ cell density when compared to contralateral or naïve values and compared to unstimulated ( $P<0.05$ ), consistent with cortical activation. Ipsilesional-WM values were not different to either contralateral injured, stimulated-naïve or even non-stimulated injured rats, indicating no specific effect of brain activation. Forelimb stimulation in injured+chondroitinase treated rats resulted in >5-fold ipsilesional-GM c-FOS+ cell counts compared to stimulated-injured-vehicle rats ( $P<0.001$ ). WM values were also significantly increased bilaterally ( $P<0.01$ ). Importantly, both GM and WM values were significantly increased from non-stimulated, chondroitinase-infused rats, indicating that c-FOS expression was specific to brain activation. These data indicate that reduction of pericontusional CSPGs has a significant effect on sensorimotor function, and this might underlie functional recovery in this model.

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**P-73 Transient Expression And Purification Of Aggrecanase (Adamts-4) From Hek293t Cells**

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Following spinal cord injury (SCI), chondroitin sulfate proteoglycans (CSPGs, e.g. aggrecan), are up-regulated and constitute a major component of an inhibitory extracellular matrix (ECM). The influence of this complex barrier must be overcome to promote neuronal regeneration. The aggrecanase ADAMTS-4, a member of the ADAMTS (A Disintegrin And Metalloprotease with ThromboSpondin motifs) family of proteases, is capable of degrading CSPGs. Our hypothesis is: *ADAMTS-4 can*

*effectively degrade CSPGs and thereby attenuate inhibition of sensory axons in a model of axon regeneration in vitro.* To address this hypothesis, we transfected Human Embryonic Kidney (HEK293T) cells with an ADAMTS-4-FLAG expression construct. The resulting aggrecanase, purified using FLAG-tag antibody affinity column chromatography, and analyzed using the Pierce BCA assay, SDS-PAGE, and Western blotting, will be used for activity assays *in vitro*. The results thus far show that ADAMTS-4 can be successfully produced by transfecting HEK293T with an expression vector. The expressed ADAMTS-4-FLAG fusion protein can be identified by its reactivity with antibodies to the FLAG epitope, and to the ADAMTS-4 protein itself. ADAMTS-4 was retained in the cell layer, and was not in the media. Once purification is complete, enzymatic activity will be assessed by the enzyme's ability to degrade purified aggrecan *in vitro*, and outgrowth assays on degraded aggrecan *in vitro* will be undertaken. Alone or in combination with other CSPG degradative proteins, ADAMTS-4 could become a useful therapy to selectively degrade CSPGs that inhibit neuronal outgrowth thereby promoting regeneration and recovery of function in SCI patients. [Support: NIH (R01 NS053470), the Department of Defense (W81XWH-10-1-0778; the UK Office of Undergraduate Research, and AMSTEMM)]. \* These authors contributed equally to this work.

**P-74 HEK293T Cells Produce Proteoglycans with Varied Sulfation Patterns, Express Multiple Carbohydrate Sulfotransferases, and are a Novel System for the Production of "Designer PGs"**

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Following spinal cord injury (SCI), the development of a glial scar and reactive

astrocytosis results in increased expression of chondroitin sulfate proteoglycans (CSPGs) and subsequent inhibition of neuronal regeneration. The sulfation pattern of CSPG-associated glycosaminoglycans (GAGs) plays a pivotal role in neurite inhibition. In the present study, we have developed a model to examine the biosynthesis of CSPGs with varying sulfation patterns. Human Embryonic Kidney (HEK) 293T cells were transiently transfected with the gene for bovine aggrecan (pBAGG71-28). Through G50-Sephadex column chromatography and DEAE column elution, several peaks were detected using a DMMB assay to detect total proteoglycan. The presence of aggrecan within these peaks was verified by dot blot with an antibody to aggrecan. The resulting differences in retention times for aggrecan were further correlated with increasing degrees of sulfation. This novel pattern of aggrecan sulfation implicates HEK293T cells as a promising model for the continuing development of our novel “Designer PGs” to study the inhibitory influence of sulfation on neurite outgrowth. Further, RT-PCR revealed the expression of carbohydrate sulfotransferases in HEK293T cells involved in the production of the predominant sulfation patterns of CSPGs. By targeting these individual carbohydrate sulfotransferases with siRNA, “Designer PGs” with varied sulfation patterns can be synthesized and then analyzed for their inhibitory effect on neurite outgrowth. We are currently testing novel neurite outgrowth assays to discriminate between various sulfation patterns, and testing their effect on neurite inhibition. This model for the biosynthesis of CSPG constructs will allow us to elucidate the mechanisms of CSPG mediated neurite inhibition, while targeting genes of human origin, and represents a clinically relevant approach for the development of novel gene therapies. [Support: NIH R01 NS053470 and Kentucky Spinal Cord and Head Injury Research Trust (#10-11A)]. \* These authors contributed equally to this work.

**P-75 Overlapping Yet Distinct Roles Of Matrix Metalloproteinases-2 And -9 In Modulating Angiogenesis In The Injured Spinal Cord**

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Here we investigate the roles of matrix metalloproteinases (MMP)-2 and MMP-9 in modulation of angiogenesis in the injured spinal cord. Immortalized brain-derived capillary endothelial cells (RBCEC4) express MMP-2. When exposed to a MMP-2 inhibitor, proliferation, migration and tube formation are reduced, thus implicating this protease in key events related to angiogenesis. To examine the role of this protease *in vivo*, MMP-2 knockout (KO) and wildtype (WT) mice were subjected to spinal cord injury. Endothelial cell proliferation was reduced in the KO relative to the WT. However, KO mice showed a transient increase similar to WT in anatomic measures of vascularity, including vascular density, area and length between 7 and 14 days followed by a marked reduction at 21 days. Subsequent decline in vascularity in the KO mice corresponded to an upregulation of MMP-9. To test the hypothesis that MMP-9 compensates for MMP-2 deficiency in supporting angiogenesis, RBCEC4 cells were exposed to tumor necrosis factor  $\alpha$  (TNF $\alpha$ ) to induce MMP-9 or were directly exposed to purified MMP-9. Either approach yielded similar findings in these activated cells. Using selective pharmacologic inhibitors of MMP-9 and -2, we found that endothelial proliferation was dependent on

MMP-2, while MMP-9 modulated tube formation. To determine if MMP-9 directed tube formation is time-dependent, RBCEC4 cells were subjected to a prolonged exposure to either TNF $\alpha$  or MMP-9. Such prolonged exposure led to vascular regression. Taken together, these data suggest distinct and overlapping roles of both these gelatinases in angiogenesis. Our findings further suggest that MMP-9, expressed during the time course of wound healing may not only support early angiogenesis but direct longer-term regression. As the injured spinal cord of the MMP-2 null shows a compensatory, prolonged expression of MMP-9, the decline in vascularity, may be attributed to this protease.

**P-76 AXONAL REGROWTH IS REGULATED BY MRNAS COMPETING FOR ZBP1.**

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mRNAs are actively transported into axons of cultured neurons, where the locally synthesized proteins play a role in axonal growth. Cues for localization of these mRNAs are inherent to the RNA, with 3'UTR structures bound by RNA binding proteins (RBP) typically being responsible for axonal targeting. We recently reported that the  $\beta$ -actin's 3'UTR is sufficient for axonal localization in peripheral nerve and spinal cord axons (Willis et al., 2011). Interestingly, both  $\beta$ -actin and GAP-43 mRNAs require ZBP1 for their axonal localization. However, this RBP is expressed at very low levels in adult neurons, which limits mRNAs

transported into axons. Further depleting ZBP1 from adult DRG neurons, either by allelic deletion or by introducing a dominant negative RNA, decreases axonal regrowth from injured sensory neurons, both in culture and in vivo. Conversely, overexpressing ZBP1 increases axonal outgrowth and transport of  $\beta$ -actin and GAP-43. These data suggest that endogenous axonally targeted mRNAs compete with one another for RBP binding and axonal transport. Consistent with this, injury-induced increase in GAP-43 transcription decreases axonal levels of  $\beta$ -actin with a commensurate increase in axonal GAP-43. Since the injury conditioning for transcription of GAP-43 can accelerate axonal regeneration, we asked if this shift in altered axonal mRNA levels of GAP-43 and  $\beta$ -actin might affect axonal growth. Indeed, selectively depleting or overexpressing locally synthesized GAP-43 vs.  $\beta$ -actin protein in DRG neurons generated distinct patterns of axonal regrowth. The effect of overexpression was only seen with axonally localizing transcripts; restricting GAP-43 and  $\beta$ -actin mRNAs to the cell body using the non-localizing 3'UTR of  $\gamma$ -actin mRNA had no effect on axonal growth. These studies indicate that axonally translated  $\beta$ -actin protein is needed for axonal branching while axonally translated GAP-43 protein is needed for rapid unilinear growth.

**P-77 The Pro-Synaptic Protein LAR Mediates Glial Scar Induced Axonal Regeneration Failure Following Spinal Cord Injury**

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Regeneration of damaged axons following spinal cord injury is complicated by

several distinct processes, with the chondroitin-sulfate proteoglycan (CSPG) rich barrier at the astroglial scar being a primary impediment. In the weeks that follow spinal cord injury, damaged axons attempt to advance into the lesion core only to become entrapped and stopped by the inhibitory environment. Using an in vitro assay for glial scar mediated inhibition and time-lapse microscopy, we showed that axons become stabilized in proteoglycan rich gradients. We recently showed that the LAR family phosphatase, receptor-protein tyrosine phosphatase-sigma (RPTP $\sigma$ ), a key modulator of synapse maturation, is a receptor for chondroitin sulfate proteoglycans. Given that all three LAR family members share 99% sequence homology, we tested whether LAR, a second family member, mediated over-adhesion and stabilization of growth cones leading to regeneration failure. We show that LAR is highly upregulated in the dystrophic, but not normal, growth cone. By utilizing specific small peptide inhibitors to the intracellular domains of LAR coupled with a cytosolic-localizing TAT domain, we showed that adhesion to CSPG substrate was diminished upon LAR inactivation. In addition, we show inactivation of LAR can allow a dystrophic, highly adhesive growth cone to advance into a CSPG gradient and eventually cross the barrier. Our results suggest for the first time that CSPGs in the lesion environment block regenerative growth by creating an abnormally high adhesive interaction with the substrate. Furthermore, our results show that LAR can be modulated to prevent the over-adhesion and stabilization of axons following spinal cord injury, potentially allowing for regeneration. Supported by NINDS NS025713 to JS.

**P-78 Bidirectional Remodeling Of  $\beta$ 1-Integrin Adhesions During Chemotropic Regulation Of Nerve Growth**

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Chemotropic factors in the extracellular microenvironment guide nerve growth by acting on the growth cone at the tip of extending axons. Growth cone extension requires the coordination of cytoskeleton-dependent membrane protrusion and dynamic adhesion to the extracellular matrix (ECM), yet how chemotropic factors regulate these events remains an outstanding question. We demonstrated previously that the repellent factor myelin-associated glycoprotein (MAG) triggers endocytic removal of the adhesion receptor  $\beta$ 1-integrin from the growth cone surface membrane to negatively remodel ECM adhesions during chemorepulsion (Hines et al., 2010). Here we report that the neurotrophin brain-derived neurotrophic factor (BDNF) positively regulates the formation of substrate adhesions in axonal growth cones during stimulated outgrowth and prevents removal of  $\beta$ 1-integrin adhesions by MAG. Treatment of *Xenopus* spinal neurons with BDNF rapidly triggered  $\beta$ 1-integrin clustering and induced the dynamic formation of nascent vinculin-containing adhesion complexes in the growth cone periphery. Both the formation of nascent  $\beta$ 1-integrin adhesions and the stimulation of axon extension by BDNF required cytoplasmic Ca<sup>2+</sup> signaling and integrin activation at the cell surface. Exposure to MAG decreased the number of  $\beta$ 1-integrin adhesions in the growth cone during inhibition of axon extension. In contrast, the BDNF-induced adhesions were resistant to negative remodeling by MAG, correlating with the ability of BDNF pretreatment to overcome MAG-inhibition of axon extension. Pre-exposure to MAG

prevented the BDNF-induced formation of  $\beta$ 1-integrin adhesions and blocked the stimulation of axon extension by BDNF. Altogether, these findings demonstrate the neurotrophin-dependent formation of integrin-based adhesions in the growth cone and reveal how a positive regulator of growth cone substrate adhesions can block the negative remodeling and growth inhibitory effects of MAG. Techniques for manipulating integrin internalization and activation state may be important for overcoming local inhibitory factors after traumatic injury or neurodegenerative disease to enhance regenerative nerve growth. *Supported by the US National Institutes of Health (NS067311).*

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Hines JH, Abu-Rub M, and Henley JR (2010) *Nat Neurosci* 13:829-837.

### **P-79 In Vivo 2 Photon Imaging Of CNS Injury In Nogo Deficient Mice**

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After CNS injury the ability to follow axon dynamics after injury is difficult due to the conventional methods used to track axons after injury. However, with the use of 2-photon imaging and mice with fluorescently labeled axons, it is now possible to examine the behavior of axons at a high spatial and temporal resolution. In this study, we examine the degeneration and regeneration profiles of YFP labeled ascending sensory axons in Nogo deficient mice. Single sensory axons were injured and imaged for several hours and reimaged days and weeks later. Preliminary data suggests that there are no significant differences in degeneration between the ascending and descending branches of single sensory axons. However, there is a location specific regeneration phenotype that suggests that not all injury locations will elicit the same

response. Studies are underway to understand the possible mechanisms underlying this location specific regenerative phenotype.

### **P-80 A Very Large Organelle in Sympathetic Neurons**

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Recently a large, non-membranous organelle, named the loukoumasome (“doughnut” body) was discovered within specific subpopulations of peripheral autonomic ganglion neurons. This proteinaceous organelle is found throughout the sympathetic chain with the highest proportions in the pelvic and stellate ganglia (innervating the pelvic organs and heart, respectively). Additionally, a small population of neurons in the superior cervical ganglion (innervating the pineal gland) also contains loukoumasomes. In all cases, the loukoumasome is restricted to neurons which co-express neuropeptide Y (NPY) and calbindin-D28K, indicating that target-derived factors common to these tissues may specify whether a neuron contains this organelle. The loukoumasome appears singly within a neuron in one of multiple possible conformations, which is dependent on its position within the cell. The three most common conformations identified are: toroidal when on the nuclear side of the trans-golgi network (TGN), twisted when on the boundary of the TGN, and rod shaped when peripheral to the TGN. Furthermore, the loukoumasome associates with other organelles, notably the primary cilium, an important signaling centre, and the poorly understood nematosome. The protein composition of the loukoumasome is also currently under investigation: the core is composed, at least in part, of the microtubule nucleating protein gamma-tubulin, as well as the centrosome

associated proteins myosinIIIb and cenexin, whereas the outer shell is composed of a yet undefined protein. Its presence in multiple subcellular compartments, interaction with the primary cilium and association of a motor protein implies motility though the dynamic behaviour of this organelle has yet to be defined. Characterization of all the cellular components of neurons facilitates the understanding of normal form and function of neurons and allows for the identification of the changes that occur upon injury and disease.

**P-81 CD8+ T Cell Granzymes Injure Axons By Dysregulating Axonal Ionic Homeostasis**

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Multiple sclerosis (MS) is a primary cause of neurological disability for young adults in Westernized countries. MS-related functional deficits correlate with axon loss and CD8+ T cell burden in human cases and animal models. Genetic deletion of CD8+ T cell target recognition ( $\beta$ 2-microglobulin, an MHC-I component) or cytotoxic effector functions (perforin, of the perforin-granzyme protease system) is neuroprotective in a MS animal model. These observations implicate CD8+ T cells as drivers of MS-related neurological deficits; however, mechanistic linkages between functional deficits, axon loss and CD8+ T cell activity remain elusive. CD8+ T cell granzyme proteases cleave proteins that possess specific amino acid recognition sequences. The axonal voltage-gated sodium channel isoforms, Nav1.2 and Nav1.6, and Nav-associated structural proteins ankryin G and spectrin contain bioinformatically predicted granzyme B (GzB) cleavage sequences. Dysregulation axonal

sodium and calcium electrophysiology which leads to axonal energy depletion and calpain protease activation is a demonstrated axon injury mechanism; and pharmacological inhibition of ion channels is neuroprotective in MS animal models. These findings lead to the unifying hypothesis: cytotoxic CD8+ T cells dysregulate axonal sodium and calcium gradients via a GzB-dependent mechanism that leads to local energy depletion, calpain activation and ultimately axon injury and loss. Western blot data demonstrates that purified GzB effectively and specifically cleaves Nav1.2, Nav1.6 and spectrin. Preliminary data implicates GzB activity as sufficient to dysregulate Nav1.6 electrophysiological function. Finally, imaging studies establish CD8+ T cells as competent to induce perturbations in axonal calcium gradients and injure axons, *in vitro*. Further experimental investigations are anticipated to demonstrate granzymes as sufficient to induce axonal sodium channel dysfunction and axonal ionic dysregulation as a mechanism by which CD8+ T cells injure axons. These findings offer a novel mechanism of neuroimmunological axon injury and may reveal novel therapeutic strategies for the targeted prevention of CD8+ T cell-driven neuropathology.

**P-82 Toll-Like Receptor 2 Activation Limits Axon Dieback *In Vivo* And Promotes Macrophage-Mediated Regeneration *In Vitro* Without Causing Concurrent Neurotoxicity**

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Zymosan-activated macrophages (ZAMs) promote regeneration of injured peripheral and central nervous system axons but also cause tissue pathology and cell death (Gensel et al., 2009). Here, we test the

hypothesis that the divergent effects of ZAMs are induced by the concurrent activation of toll-like receptor 2 (TLR2) and dectin-1 receptors. With the goal of uncoupling the beneficial and detrimental effects of zymosan activation to promote regeneration without toxicity, we compared the effects of activating macrophages *in vivo* or *in vitro* with selective agonists of TLR2 or dectin-1 receptors. All responses were compared to those achieved with zymosan activation alone. Intraspinal microinjection of dectin-1 agonists mimicked the effects of zymosan, i.e., activated macrophages colocalized with zone of focal necrosis, overt axon pathology and demyelination. In contrast, intraspinal injection of Pam2CSK4, a synthetic TLR2 agonist, elicited a robust macrophage response but with significantly less axon or myelin pathology than zymosan or dectin-1 agonists. *In vitro*, products released by zymosan or Pam2CSK4-stimulated macrophages increased axon growth from adult DRG neurons. Importantly, Pam2CSK4-activated macrophages promoted axon growth without also causing toxicity. In contrast, activation of macrophages via dectin-1 caused neurotoxicity. *In vivo*, Pam2CSK4 injection into the site of dorsal column crush injury significantly reduced macrophage-mediated axonal dieback. Collectively these data provide compelling evidence that activation of macrophage TLR2 can promote axonal regeneration without simultaneously eliciting neurotoxic effector functions.

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**P-83 Reparative Effects Of Raphe-Spinal Activity Mediated By 5-Ht7 Receptors, Camp Signalling Cascade, And Co-Released Neuropeptides**

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We propose that brainstem raphe nuclei are capable of orchestrating a spectrum of protective and reparative responses in traumatized CNS regions, involving widespread co-release of serotonin and neuropeptides such as thyrotropin-releasing hormone (TRH), substance P (SP) and galanin (Gal). We here report that stimulating the hindbrain nucleus raphe magnus (NRM) 3 days after thoracic contusion acutely increased cyclic adenosine monophosphate, which has several known restorative actions. The increase was blocked by the 5-HT<sub>7</sub> antagonist pimozone (1 mg/kg, i.p.). We further investigated this signal cascade: western blot analysis showed significant recovery to control levels of phosphorylated protein kinase A (p-PKA) in cervical, thoracic and lumbar segments after stimulation of the NRM, compared with injured non-stimulated animals, while PKA values were restored to control values in cervical and lumbar tissue. Although cAMP responsive element binding protein (CREB) was unchanged, stimulation significantly increased p-CREB levels throughout the injured cord. A high-content screening platform using cultured E18 hippocampal neurons was used to examine the effects of 5-HT and neuropeptides. Assay plates were treated with 3 or 30  $\mu$ M montirelin (TRH analogue), SP or Gal, or 1, 5 or 10  $\mu$ M pimozone, alone or in combination with 1 or 10  $\mu$ M 5-HT for 48 hours. Quantified images showed maximum inhibition of neurite branching in response to 10  $\mu$ M pimozone. Intact neurites showed a concentration-dependent increase in branching in response to all of the peptides; some increases in neurite length were also noted. We conclude that 5-HT enhances neurotrophic signaling pathways in injured tissue, which can be accentuated by peptides that are often co-released with 5-HT by midbrain and hindbrain raphe nuclei. These findings have pharmacotherapeutic implications,

as well as offer a basis for the restorative effects of raphe stimulation after spinal cord injury and traumatic brain injury that we report elsewhere.

**P-84 Polymer Mediated Delivery Of Xylosyltransferase-I Sirna To Suppress Glial Scar Glycosaminoglycan Synthesis**

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Glial scar mediated inhibition of axonal regrowth following spinal cord injury is attributed to increased glycosaminoglycan (GAG) chain production by reactive astrocytes, driven partially by the enzyme xylosyltransferase-I (XT-I). The bacterial enzyme chondroitinase ABC, although effective in alleviating scar-mediated inhibition by removing GAG-side chains from core proteins, has a short half-life limiting its bioavailability. The overall objective of this study is to provide sustained knockdown of XT-I using small interfering RNA (siRNA) and a non-viral delivery vehicle comprised of hyperbranched copolymer (pD-E). The specific objectives are to: (1) demonstrate efficient siRNA delivery into reactive astrocytes using pD-E, and subsequent knockdown of XT-I, (2) compare this effect to other commercially available delivery vehicles, and to (3) test whether this knockdown reverses the neurite inhibitory properties of reactive astrocytes. XT-I siRNA was used naked or complexed with Lipofectamine 2000 or pD-E. siRNA formulations were then incubated with Neu7 astrocytes, which overexpress GAGs. Astrocytes from the different siRNA treatment

groups were then assessed for GAG quantity and mRNA expression, and their conditioned media (CM) used in neurite outgrowth assays of dorsal root ganglia. There was a significant reduction in XT-I mRNA expression from cells treated with pD-E siRNA polyplexes. This change in gene expression correlates with neurite outgrowth assays, where CM from pD-E treated cells resulted in neurite lengths significantly higher than CM from untreated cells, and no increase in neurite lengths was observed with the other treatment groups. In summary, polymer mediated non-viral delivery of XT-I siRNA to reactive astrocytes provides a novel approach by which the glial scar inhibitory pathway can be targeted to facilitate axonal regrowth. This therapeutic approach is currently being investigated as part of an implantable collagen hydrogel in a rat lateral spinal cord hemisection model.

**P-85 Microtubule Stabilization Reduces Scarring And Causes Axon Regeneration After Spinal Cord Injury**

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Hypertrophic scarring and poor intrinsic axon growth capacity constitute major obstacles for spinal cord repair. These processes are tightly regulated by microtubule dynamics. Here, moderate microtubule stabilization decreased scar formation after spinal cord injury in rodents through various cellular mechanisms, including dampening of transforming growth factor- $\beta$  signaling. It prevented accumulation of chondroitin sulfate proteoglycans and rendered the lesion site permissive for axon regeneration of growth-competent sensory neurons. Microtubule stabilization also promoted growth of central nervous system axons of the Raphe-spinal tract and led to functional improvement. Thus, microtubule stabilization reduces fibrotic scarring and enhances the capacity of axons to grow.

**P-86 Single Filopodial Contact With Inhibitory Chondroitin Sulfate Proteoglycans Induces Behavioral Changes In Sensory Neurons *In Vitro***

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Chondroitin sulfate proteoglycans (CSPGs) are up-regulated in response to spinal cord injury (SCI), and consequently inhibit axonal regeneration. The majority of CSPGs produced after injury stem from reactive astrocytes of the glial scar. Axons attempting to regrow "evaluate" inhibitory molecules and make "decisions" about whether to continue to advance, stop, or turn. To grow beyond the glial scar and toward appropriate targets, neurons must overcome the CSPG-induced inhibitory milieu. The current study examined behavioral changes in the leading edge of regenerating

neurons, the growth cone, as they come into *first contact* with CSPGs *in vitro*, thus modeling primary interactions with the glial scar. Using analyses of time-lapse video images, growth cone properties such as morphology, filopodial length and number, approach velocity (to substratum adsorbed CSPGs), and approach angle were compared before and after first contact with CSPGs. Using this methodology, we report that growth cone velocity was significantly reduced following first contact by a single filopodia. We recently reported that single filopodial contact with CSPGs resulted in regulation of growth cone area as well, and the two characteristics may be interrelated. Collectively, these data are the first demonstrations of significant behavioral changes in growth cone behavior resulting from a single filopodial contact with CSPGs. This intriguing result represents a potential therapeutic target for regeneration and recovery of function following SCI. [Support provided by NIH/NINDS (NS053470); the Kentucky Spinal Cord and Brain Injury Research Center (#10-11A); the Department of Defense (W81XWH-10-1-0778; and the UK Office of Undergraduate Research].

**P-87 Meningeal/Fibrotic Scar Removal In Axolotl Spinal Cord Regeneration**

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In mammalian penetrating spinal cord injuries, such as ballistic wounds, functional recovery is inhibited by the production of glial scar basal lamina from reactive astrocytes reinforced by production of a fibrotic scar by invading reactive meningeal cells. In mammals the meningeal/fibrotic scar is a major obstacle to axonal regrowth. In salamander regeneration there is also a period of robust meningeal fibrosis that has been largely unmentioned in

traditional amphibian regeneration literature due to the transient nature of the fibrosis. During the regeneration of transected spinal cord in axolotls (the salamander *Ambystoma mexicanum*), the meningeal response causes the pia mater to thicken from a single cell layer to a layer of 4 to 5 cells and to invade the lesion site depositing masses of fibrillar collagen. Invading arachnoid cells are seen in association with amorphous matrix material. Reactive ependymal cells that reconstruct the spinal cord grow into and remodel deposited extracellular matrix by producing matrix metalloproteinases. This differs from a recent report in newts. Our lab has hypothesized that removal of the fibrotic scar in salamanders is dependent upon meningeal/ependymal communication at the level of growth factor and extracellular matrix interactions affecting the behavior of both types of cells. Coculture of reactive ependymal and meningeal material causes re-epithelialization of the reactive ependyma associated with restoration of the central canal *in vivo*. Determination of the composition of the salamander fibrotic scar is being made to provide a basis of comparison with the mammalian fibrotic scar. Electron microscopy, histochemical staining and antibody staining have been used to localize and characterize components of the salamander fibrotic scar. Collagen type I is heavily deposited in the lesion site, in the ventral-lateral areas of the regenerating spinal cord, a phenomenon which has been undocumented until now. The regenerative ependymal outgrowth is associated with this type I collagen.

**P-88 Age And Time Since Injury Are Associated With Larger Cortical Activation After Incomplete Spinal Cord Injury**

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Brain function has been studied as a determinant of clinical status after spinal cord injury (SCI). Influences on the brain are of therefore of interest in this context. The current study examined how age, time since injury, motor status, and gait velocity are each related to brain function after SCI. A total of 20 patients who met eligibility criteria for a study of mental imagery effects on physical therapy after SCI underwent functional MRI (fMRI) scanning at baseline. The main entry criteria were age >18 years, incomplete SCI (ASIA C or D), able to ambulate >10 meters with one person assistance or assistive device, and normal leg range of motion. During fMRI (3 Tesla), subjects alternated rest with 0.3 Hz right ankle dorsiflexion. Subjects had age 52.6 +/- 12.1 (mean+/-SD), were 85.8+/- 84.0 months post injury, neurological levels C1-L2, and were 17 ASIA C/3 ASIA D. Higher age correlated significantly with larger activation in left lateral postcentral gyrus, bilateral anterior cingulate, and bilateral medial pre-frontal cortex. Longer time since injury correlated significantly with larger activation in left lateral postcentral gyrus/inferior parietal lobule and left lateral frontal lobe. ASIA motor score and gait velocity did not correlate significantly with any features of brain function. Time has significant effects on the brain after SCI, as both increased age and increased time post-stroke were associated increased activity. The meaning of these activity increases is open to interpretation, but signals an increase use of neural resource in relation to voluntary movement. The location of these activation increases is of interest, and might indicate altered sensory processing (post-central gyrus) and increased error detection (anterior cingulate). The current results suggest determinants of brain function in subjects with incomplete SCI, factors that might be important to developing and perhaps individualizing spinal cord rehabilitation interventions.

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