The Eleventh International Symposium on Neural Regeneration

December 14-18, 2005
Pacific Grove, California

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OFFICE OF THE INTERNATIONAL SYMPOSIUM ON NEURAL REGENERATION

The Eleventh International Symposium on Neural Regeneration

Hosted by:

U.S. Department of Veterans Affairs
International Symposium on Neural Regeneration
(Biomedical Laboratory Research and Development Service)
(Office of Research and Development)

Co-sponsored by:

Paralyzed Veterans of America
(Education Foundation)

National Institutes of Health
(National Institute of Neurological Disorders and Stroke)
(Office of Rare Diseases)

Christopher Reeve Paralysis Foundation

United Spinal Association

Shapiro Spinal Cord Research Foundation

The Eleventh International Symposium on Neural Regeneration

December 14-18, 2005

Asilomar Conference Grounds
800 Asilomar Boulevard
Pacific Grove, California CA 93950

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David Gardiner (University of California, Irvine, CA)
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Acknowledgements

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Introduction

The Eleventh International Symposium on Neural Regeneration will be held at the Asilomar Conference Center in Pacific Grove, California from December 14-18, 2005. 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, Eastern Paralyzed Veterans Association (now United Spinal Association), and more recently the Christopher Reeve Paralysis Foundation. The 2005 symposium will be the eleventh meeting in this series and is the second to also be sponsored in part by a generous donation from the Shapiro Spinal Cord Research Foundation. The generous support of all of our sponsors is gratefully acknowledged.

The keynote speaker for this year's symposium will be Jerry Silver from Case Western Reserve University. This year the keynote address is given in honor of Christopher Reeve who passed away in 2004. Featured talks will be given by John Steeves from the University of British Columbia, Miguel Nicolelis from Duke University, Steve Goldman from the University of Rochester Medical Center, Ed Wirth III from Geron Corporation, Jeff Lichtman from Harvard University, and Paul Martin from The Ohio State University. Following the format of preceding neural regeneration symposia, the program is divided into six sessions, including: 1) Autonomic Dysfunctions and the Long-Term Effects on the Aging SCI Population, chaired by Kimberly D. Anderson; 2) Growth/Plasticity and Functional Outcome, chaired by Harry Goshgarian; 3) Intrinsic Mechanisms Underlying the Initiation of Nerve Regeneration, chaired by Larry Benowitz; 4) Increasing Inflammation Is Beneficial for Neural Repair and Outcome (debate), chaired by Keith Crutcher; 5) Convergent Signaling Regulating Axon Growth, chaired by Marie Filbin; and 6) Emerging Topics, chaired by Michael Sofroniew. The abstracts for the speaker presentations as well as 113 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. The 12th International Symposium is being scheduled for December 2007 at the Asilomar Conference Center.

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Scientific Program

Wednesday, December 14, 2005

3:00 PM Arrival of participants and check-in
Poster Session I set-up (pins provided)

6:00 PM Dinner

7:30 PM Welcome and Keynote Address
Jerry Silver
Case Western Reserve University—Cleveland, Ohio, USA
"Regeneration Beyond the Glial Scar"

Thursday, December 15, 2005

Session One: 8:15AM
Autonomic Dysfunctions and the Long-Term Effects on the Aging Spinal Cord-Injured Population

8:15 AM Chairperson's Introduction
Kimberly D. Anderson
University of California, Irvine—California, USA

8:30 AM Video: SCI patient speaking about daily function

9:00 AM **Michael D. Craggs**
Royal National Orthopaedic Hospital, NHS Trust—Middlesex, England
"Repeated autonomic dysreflexia episodes stimulated by bladder and bowel dysfunction: Implications for the aging SCI population"

9:30 AM **Stacy Elliott**
University of British Columbia—Vancouver, BC, Canada
"Autonomic dysreflexia with sexual activity: Do the benefits outweigh the risks?"

10:00 AM **Christopher Mathias**
Imperial College of Science, Technology and Medicine——London, England
"Hypotension and hypertension in SCI: Short- and long-term implications"

10:30 AM Break

11:00 AM Featured Speaker
John Steeves
University of British Columbia/International Collaboration on Repair Discoveries—Vancouver, BC, Canada
"Guidelines for the conduct of SCI clinical trials"

12:00 Noon Lunch

1:00 PM Viewing: Poster Session I

5:00 PM Featured Speaker
Miguel Nicolelis
Duke University–Durham, NC, USA
"Computing with neural ensembles"

6:00 PM Dinner

Session Two: 7:15PM
Growth/Plasticity and Functional Outcome

7:15 PM Chairperson's Introduction
Harry Goshgarian
Wayne State University School of Medicine—Detroit, MI, USA

7:30 PM **John H. Martin**
Columbia University—New York, NY, USA
"Bypassing spinal injury to promote motor function"

8:00 PM **John Houle**
Drexel University College of Medicine—Philadelphia, PA, USA
"Combinatorial treatment strategy to overcome astroglial barriers and promote functional axonal regeneration after spinal cord injury"

8:30 PM **Gwendolyn L. Kartje**
Edward Hines, Jr. VA Hospital—Hines, IL, USA
"Recovery and structural plasticity after stroke and Anti-Nogo antibody therapy in adult and aged rats"

9:00 PM **Blair Calancie**
SUNY Upstate Medical University—Syracuse, NY, USA
"Neurophysiologic testing for predicting outcome after acute spinal cord injury: Simple, well-tolerated and accurate...but is it useful?"

Friday, December 16, 2005

Session Three: 8:15AM
Convergent Signaling Regulating Axon Growth

8:15 AM Chairperson's Introduction
Marie Filbin
Hunter College—New York, NY, USA

8:30 AM **David Van Vactor**
Harvard University—Cambridge, MA, USA
"Signaling mechanisms that control axon guidance decisions"

9:00 AM **Zhigang He**
Harvard University—Cambridge, MA, USA
"Signaling mechanisms for myelin inhibitors"

9:30 AM **Toshihide Yamashita**
Chiba University—Chiba, Japan
"Cell signaling cascades regulating axon growth inhibition"

10:00 AM Break

10:30 AM **Britta Eickholt**
King's College—London, England
"PI 3-kinase signaling controlling axon growth and guidance"

11:00 AM Featured Speaker
Steve Goldman—University of Rochester Medical Center, Rochester, NY, USA
"Isolation, induction and use of adult human neural progenitor cells"

1:00 PM Poster Session I Poster Removal

1:30 PM Poster Session II Set-up (pins provided)

5:00 PM Featured Speaker
Ed Wirth III
Geron Corporation—Menlo Park, California, USA
"Clinical trials in SCI: How can we link imaging to functional outcome?"

6:00 PM Dinner

7:15 PM Featured Speaker
Jeff Lichtman
Harvard University—Cambridge, MA, USA
"Watching nerves grow and retract in fluorescent mice"

Session Four: 8:15 PM-9:45 PM
DEBATE: Increasing Inflammation Is Beneficial for Neural Repair and Outcome

8:15 PM Chairperson's Introduction
Keith A. Crutcher
University of Cincinnati Medical Center—Cincinnati, OH, USA

In Favor **Howard E. Gendelman**
University of Nebraska Medical Center—Omaha, NE, USA

Jonathan Kipnis
University of Nebraska Medical Center—Omaha, NE, USA

J. Regino Perez-Polo
University of Texas Medical Branch—Galveston, TX, USA

Opposed **Phillip G. Popovich**
The Ohio State University–Columbus, OH, USA

Lynne C. Weaver
Robarts Research Institute—London, ON, Canada

V. Hugh Perry
University of Southampton—Southampton, England

Saturday, December 17, 2005

Session Five: 8:15AM
Intrinsic Mechanisms Underlying the Initiation of Nerve Regeneration

8:15 AM Chairperson's Introduction
Larry Benowitz
Children's Hospital/Harvard Medical School——Boston, MA, USA

8:30 AM **Richard Ambron**
Columbia University—New York, NY, USA

"Intrinsic kinase pathways regulate the excitability of regenerating nociceptive neurons following nerve trauma: Role in chronic pain"

9:00 AM **Mike Fainzilber**
Weizmann Institute of Science—Rehovot, Israel
"Retrograde injury signaling in lesioned nerve"

9:30 AM **Jeffery Twiss**
Al duPont Hospital for Children—Wilmington, DE, USA
"Regulation of protein synthesis in regenerating axons"

10:00 AM **Gennadij Raivich**
University College, London—London, England
"Switching regeneration and sprouting on and off: Role of Jun and MEK"

10:30 AM Break

11:00 AM Featured Speaker
Paul Martin
The Ohio State University—Columbus, OH, USA
"New roles for glycosylation in neuromuscular development, regeneration and disease"

12:00 Noon Lunch

1:00 PM Viewing: Poster Session II

Session Six: 4:15PM
Emerging Topics

4:15 PM Chairperson's Introduction
Michael Sofroniew
University of California—Los Angeles, CA, USA

4:30 PM **Candace Floyd**
University of California—Davis, CA, USA
"17B-estradiol is neuroprotective in spinal cord injury in post- and pre-menopausal rats"

4:45 PM **Damien Pearse**
University of Miami, FL—Miami, FL, USA
"Gene transfer of constitutively-activated MEK or ERK at the neuronal soma by adeno-associated viral vectors to induce axonal regrowth across Schwann cell bridges implanted into the completely transected rat thoracic spinal cord"

5:00 PM **Yimin Zou**
University of Chicago—Chicago, IL, USA
"Axon guidance cues along the rostral-caudal axis of the spinal cord"

5:30 PM **Molly Shoichet**
University of Toronto—Toronto, ON, Canada
"Neural tissue engineering and drug delivery strategies for spinal cord injury repair"

6:00 PM Break

6:30 PM Symposium Banquet

Sunday, December 18, 2005

Departure of Participants

Poster Presentations

Presenters for posters numbered **P1-P58:** Mount posters from 3:00-6:00 p.m. on Wednesday, December 14, and dismount posters from 1:00-1:30 p.m. on Friday, December 16. Poster authors in this group are asked to be at their posters from 1:00-3:00 p.m. on Thursday, December 15.

Presenters for posters numbered **P59-P113:** Mount posters after 1:30 p.m. on Friday, December 16, and dismount posters after 8:00 p.m. on Saturday, December 17. Poster authors in this group are asked to be at their posters from 1:00-3:00 p.m. on Saturday, December 17.

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

Session One

P-1 Characteristics of acute spinal cord injury patients admitted to a level I trauma center in southern California
K. D. Anderson, F. D. Westhout and M. E. Linskey

P-2 The cellular inflammatory response after human spinal cord injury
J. C. Fleming, M. D. Norenberg, D. A. Ramsay, G. A. Dekaban, A. E. Marcillo, A. D. Saenz, W. D. Dietrich and L. C. Weaver

P-3 Preservation of vestibulo-spinal reflexes in incomplete spinal cord injury
S. Wydenkeller, M. Liechti, R. Müller and A. Curt

P-4 Spinal cord regeneration capabilities may be enhanced by aggressive acute management of spinal cord injuries
F. Castro-Moure

P-5 Interaction of Nogo-A antibody treatment and locomotor training on neuronal reorganization and functional recovery after an incomplete spinal cord injury
R. M. Ichiyama, I. Maier, L. Schnell, I. Lavrov, G. Courtine, M. E. Schwab and V. R. Edgerton

P-6 Polyethylene glycol administration after moderate spinal cord injury decreases lesion size and improves locomotor recovery
M. R. Detloff, E. Lavik, L.C. Fisher, R. Langer and D. M. Basso

P-7 Correlation of the use of computerized animal activity monitoring with BBB open field test to assess recovery of locomotor function in a rodent spinal cord injury (SCI) model
S. Tian, J. Gonzales and L. M. Lichtenberger

P-8 Therapeutic neuromuscular stimulation therapy improves recovery of locomotion after incomplete spinal cord injury in adult rats
J. V. Lynskey, A. Belanger, T. Kanchiku, G. Venkatasubramanian, M. Mukherjee, A. Thota, J. Abbas, and R. Jung

P-9 Environmental enrichment promotes recovery of forelimb movements and supraspinal pathway plasticity after cervical spinal cord injury in adult rats
J.V. Lynskey, M. McAtee, H. N. Dai, E. Iarikova, F. P.T. Hamers and B. S. Bregman

P-10 Combined strategies to increase plasticity and recovery of function after cervical spinal cord injury in adult rats
H. Dai, M. McAtee, K. Mansfield, T. Finn, L. MacArthur and B. Bregman

P-11 Fractalkine (CX3CL1)/fractalkine receptor (CX3CR1) interactions influence motor and sensory recovery after spinal cord injury
E. McDaniel and P. G. Popovich

P-12 17B-estradiol is neuroprotective in spinal cord injury in post- and pre-menopausal rats
P. Chaovipoch and C. L. Floyd

P-13 Corticospinal damage exacerbates locomotor deficits in rats with cervical dorsal column lesions
S. G. Kanagal and G. D. Muir

P-14 Forced exercise and transplantation affect behavioral recovery from cervical contusion spinal cord injury
H. Sandrow, J. S. Shumsky, M. A. Sabol, I. Fischer and J. D. Houle

P-15 Neuropathic pain in a rat cauda equina/Conus medullaris injury and repair model
A. J. Bigbee, T. X. Hoang and L. A. Havton

P-16 Functional and anatomical reinnervation of the rat lower urinary tract in a cauda equina/Conus medullaris injury and repair model
T. X. Hoang, V. Pikov and L. A. Havton

P-17 MINOCIN®, a semisynthetic derivative of tetracycline, improves functional outcome after spinal cord contusion injury in adult male rats
Y. Xie, D. P. Stirling, J. Liu, S. V. Shi, D. Sutherland, B. K. Kwon, J. D. Steeves and W. Tetzlaff

P-18 Intermittent food restriction starting at time of spinal cord injury improves functional recovery
W. T. Plunet, C. K. Lam, J. H. T. Lee, J. Liu and W. Tetzlaff

P-19 Protective effects of Motoneuronotrophic Factor (MNTF) in spinal cord injury
M. S. Kindy, J. Yu, P.-C. Chan-Yu, D. Ko and W. Ko

P-20 Spinal neural circuitry function after injury: Differential recovery in an intersegmental spinal reflex and locomotion
K. E. Tansey, N. Ortiz, P. Gerety, C. G. Smith and B. R. Botterman

P-21 Step training and OEG transplantation improve weight-bearing hindlimb plantar stepping and step kinematics in adult paraplegic rats
M. D. Kubasak, H. Zhong, D. L. Jindrich, R. R. Roy, V. R. Edgerton, A. Ramón-Cueto and P. E. Phelps

P-22 The neural pathway underlying the expression of crossed phrenic activity following spinal cord hemisection in the neonate rat
Y. Huang, M. B. Zimmer and H. G. Goshgarian

P-23 Increased close appositions between corticospinal tract axons and spinal sympathetic neurons after spinal cord injury in rats
L. P. Schramm, E. J. Kim and B. Pan

P-24 Limited recovery of reaching kinematics in rats after cervical dorsolateral funiculotomy
S. K. Stackhouse, J. Nicolai, M. Sabol, B. E. Keeler, B. Kilby, K. Horn, M. Murray and J. S. Shumsky

P-25 Activity-regulated cytoskeletal-associated (Arc) protein upregulation following C2 hemisection in rats
W. J. Alilain, D. Barkmeier and H. G. Goshgarian

P-26 The phosphodiesterase inhibitors Pentoxifylline and Rolipram induce hemidiaphragm recovery following cervical spinal cord hemisection
K. Satkunendrarajah and H. G. Goshgarian

P-27 Alginate-based anisotropic scaffolds as guiding structure for directed axonal regrowth in the injured spinal cord
P. Prang, R. Müller, A. Eljaouhari, K. Heckmann, W. Kunz, T. Weber, C. Faber, M. Vroemen, M. Caioni, U. Bogdahn and N. Weidner

P-28 Fibrin-based drug delivery scaffolds for treatment of spinal cord injury
S. E. Sakiyama-Elbert and S. J. Taylor

P-29 Human neural stem cells overexpressing Olig2 promotes locomotor recovery in rats with contusive spinal cord injury
B. G. Kim, D. H. Hwang, E. J. Kim and S. U. Kim

P-30 Delayed transplantation of adult neural stem cells promotes remyelination and functional neurological recovery after spinal cord injury
S. Karimi-Abdolrezaee, E. Eftekharpour, J. Wang, C. Morshead and M. G. Fehlings

P-31 CGRP and GAP43 increase and colocalize in cervical dorsal horns of allodynic rats following SCI and stem cell transplantation
M. Y. Macias, M. C. Bacon and S. N. Kurpad

P-32 Transplantation of adult neural stem cells into the spinal cord of Shiverer mice induces remyelination and restores the normal localization of axonal K+ channels
E. Eftekharpour, S. Karimi-Abdolrezaee, J. Wang, C. Morshead and M. G. Fehlings

P-33 Human embryonic stem cell-derived oligodendrocyte progenitors induce neurite branching and neuron survival in vitro and following transplantation into spinal cord injury
J. R. Faulkner, G. Nistor and H. S. Keirstead

P-34 Advances in utilizing neural precursors in spinal cord injury
A. C. Lepore, S. S. W. Han, J. F. Bonner, B. Neuhuber, T. M. Connors, C. Tyler-Polsz, A. L. Barshinger, S. A. Swanger, Y. Liu, P. Walczak, M. P. Daniels, J. W. M. Bulte, M. S. Rao and I. Fischer

P-35 Formation of new oligodendrocytes in an NG2-reactive zone in the contused rat spinal cord
R. Tripathi and D. McTigue

P-36 Axonal degeneration induces NG2+ oligodendrocyte progenitor cell proliferation and oligodendrocyte generation
F. Sun, D. McTigue, J. C. Bresnahan and M. S. Beattie

P-37 Transplanted astrocytes derived from embryonic glial precursors promote robust axon growth and functional recovery after spinal cord injury
J. E. Davies, C. Huang, C. Proschel, M. Noble, M. Mayer-Proschel and S. J. A. Davies

P-38 Lamina propria and olfactory bulb ensheathing cells behave differently following transplantation into the injured spinal cord
M. W. Richter, J. Liu, W. Tetzlaff and A. J. Roskams

P-39 Survival, migration and differentiation of bone marrow stromal cells grafted in the moderately contused adult rat thoracic spinal cord
R. D. S. Nandoe, A. Hurtado, A. Grotenhuis and M. Oudega

P-40 Mouse and human skin-derived precursors integrate, promote axonal regeneration and produce myelin after transplantation into rat spinal cord contusion injuries
J. S. Sparling, J. Biernaskie, J. Liu, A. M. Choo, C. K. Lam, A. K. Wong, D. Sutherland, I. A. McKenzie, F. D. Miller and W. Tetzlaff

P-41 Early death of Schwann cells following transplantation into the contused adult rat spinal cord: A quantitative analysis of cell survival, mechanisms of cell loss and host Schwann cell invasion
C. E. Hill, A. Hurtado, B. Blits, B. A. Bahr, M. B. Bunge, P. M. Wood and M. Oudega

P-42 Boost injection of Schwann cells promotes delayed axonal regeneration and myelination into poly (D,L-lactic acid) macroporous guidance scaffolds grafted into the transected adult rat thoracic spinal cord
A. Hurtado, V. Maquet, R. Jérôme and M. Oudega

P-43 Gene transfer of constitutively-activated MEK or ERK at the neuronal soma by adeno-associated viral vectors to induce axonal regrowth across Schwann cell bridges implanted into the completely transected rat thoracic spinal cord
B. Blits, G. Joseph, D. H. Mawson, J. Louro, S. Matheny, D. Benson, L. Lin, L. F. Parada, M. B. Bunge, D. D. Pearse

P-44 Modification of the glial scar following spinal cord injury
V. J. Tom and J. D. Houle

P-45 A novel animal model for chronic compression of the ventrolateral spinal cord
T. K. Chu and G. D. Muir

P-46 Identification and characterization of a specific subpopulation of angiogenic spinal microvessels binding the Griffonia simplicifolia isolectin B4 following traumatic spinal cord injury (SCI)
R. L. Benton, D. R. Minnillo, T. Hagg and R. Whittemore

P-47 Phosphorylated Bcl-xL in spinal cord: Are apoptosis regulation and neuronal differentiation related?
D. Cittelly, O. Nesic-Taylor and J. R. Perez-Polo

P-48 Sparing or sprouting of corticospinal, raphespinal and coerulospinal fibers after cervical hemisection in primates
E. Rosenzweig, H. Yang, P. Lu, G. Courtine, D. Jindrich, H. McKay, T. Bernot, L. Havton, V. R. Edgerton and M. Tuszynski

P-49 Receptor-mediated transport of LIF across blood-spinal cord barrier is upregulated after SCI
C. M. Cain, Y. Yu, M. Barron, R. Yemane, H. Tu, A. J. Kastin and W. Pan

P-50 Immunological demyelination prevents astrogliosis following spinal cord injury
F. Cloutier, I. Sears-Kraxberger and H. Keirstead

P-51 Effects of ROCK inhibition with Y27632 on astrocytes
C. C. M. Chan, A. K. Wong, J. Liu, J. D. Steeves and W. Tetzlaff

P-52 Neuroprotection of ChAT neurons and reduction of nNOS expression in spinal cord repaired rats
Y.-S. Lee, C. Y. Lin, R. T. Robertson, J. Yu, I. Hsiao and V. W. Lin

P-53 Enzymes of the glutamine cycle in the rat dorsal root ganglion following spinal injury and methylprednisolone treatment
K. E. Miller, K. M. Edwards and R. Schechter

P-54 Retroviral lineage mapping of NG2-expressing progenitor cells demonstrates their role in early gliosis following spinal cord injury
D. L. Sellers and P. J. Horner

P-55 Deletion of STAT3 attenuates astrocyte reactivity after crush spinal cord injury
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P-56 Cellular localization and temporal distribution of TNF-α following cervical/spinal cord injury
S. M. Schaal, W. D. Dietrich and D. D. Pearse

P-57 Biochemical profiles of mid-cervical adenosine A1 receptors after cervical spinal cord hemisection in adult rats
R. S. Saharan, T. Kizy and K. Nantwi

P-58 Acute injury is required for Neurotrophin-3 induced axonal plasticity in the spinal cord
Q. Chen and H. D. Shine

Session Two

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M. Goldman

P-60 Developmental neuroapoptosis: A promising new model for studying neural recovery potential
J. W. Olney

P-61 CK2 inactivation of serum- and glucocorticoid-inducible kinase via PP2A diminishes the anti-apoptotic effect of CK2 in rat hippocampus
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P-62 Upregulation of TNFα transport in mice after traumatic brain injury
Y. Yu, C. G. Pick, A. J. Kastin, M. Barron and W. Pan

P-63 Intrastriatal transplantation of cultured human-derived neural progenitor NT2N cells expressing Nurr1 at two weeks after middle cerebral artery occlusion produces stable behavioral recovery
K. Hara, N. Matsukawa, T. Yasuhara, L. Xu, G. Yu, D. C. Hess, Y. I. Yoon, S. U. Kim and C. V. Borlongan

P-64 Delayed intrathecal anti-Nogo-A antibody treatment improves functional recovery in rats after stroke
S.-Y. Tsai, T. M. Markus, E. M. Andrews, J. L. Cheatwood, J. R. Rosales, R. G. Farrer, A. K. Mir, M. E. Schwab and G. L. Kartje

P-65 Transplantation of NT2N cells, encoded by Nurr1 gene for accelerated neuronal differentiation, promotes robust amelioration of Parkinsonian symptoms in the 6-hydroxydopamine lesion rat model
T. Yasuhara, N. Matsukawa, K. Hara, L. Xu, G. Yu, Y. I. Yoon, S. U. Kim and C. V. Borlongan

P-66 No Nogo-A in fish, but Nogo-66 may affect fish axon growth
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P-67 Axonal regeneration in the Drosophila adult CNS
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P-68 Nerve regeneration in a genetic model organism
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P-71 Retinal detachment in mice deficient in GFAP and Vimentin: Reduced glial hypertrophy and increased ganglion cell remodeling
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M. E. Vargas, S. J. Singh and B. A. Barres

P-73 A local mechanism mediates NAD-dependent protection of axon degeneration
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P-74 Laceration of the spinal cord causes less demyelination and remyelination than contusion injury in rat and monkey
M. Siegenthaler and H. S. Keirstead

P-75 Hyperactivity of Na+/K+ pump in regenerated mature myelinated axons
M. Moldovan and C. Krarup

P-76 Thrombospondin-1 production and release is regulated by purinergic signaling in astrocytes: Implications for CNS development and repair
M. D. Tran and J. T. Neary

P-77 Bioinformatics analysis results in conflicting predictions for the M278T AQP4 SNP
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P-79 Anti-inflammatory drugs promote neurite outgrowth in the presence of myelin-associated growth-inhibitory molecules
E. Holmberg, M. Gross and S. Doolen

P-80 Immunomodulation in experimental models of neurodegenerative diseases
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P-81 Differential EGFP expression in the Lys-EGFP-ki transgenic mouse distinguishes between microglial macrophages and hematogenous macrophages in spinal cord injury
G. A. Dekaban, L. Mawhinney, T. Sharma and C. F. Simedrea

P-82 Dysfunctional astrocytes and SCI-induced neuropathic pain
O. Nesic, J. Lee, K. M. Johnson, Z. Ye, G. C. Unabia, C. E. Hulsebosch and J. R. Perez-Polo

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O-1

Regeneration beyond the glial scar

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My current research focuses on the role of inhibitory extrinsic and intrinsic factors in blocking severed axons from regenerating along their proper pathways within the CNS of adult mammals. We are particularly interested in studying the cellular and molecular interactions that occur between the "growth cones" that form at the end of the cut axon and the various types of reactive glial cells that lie along the presumptive routes and at the lesion site of several different fiber systems. We focus on the spinal cord.

We now know that a variety of cell surface and extracellular matrix molecules that positively or negatively regulate axon growth during embryonic development are also made in the adult by normal, mature astroglia. However, following injury, astroglia change dramatically and alter their interactions with growing axons especially in the vicinity of lesions that open the blood brain barrier. Thus, following injury in the adult CNS, so-called reactive astroglia near the lesion, but not those farther away, actively block rather than promote sustained axonal elongation. We have concentrated our efforts over the past several years learning whether a particular family of boundary molecules that astroglia make during normal development , the proteoglycans, are also critical after injury. Indeed, the sulfated forms of proteoglycans appear to be major players in creating developmental as well as regenerative boundaries. It is our hope that understanding the mechanisms that negatively impact axon growth and guidance in the adult will suggest regeneration strategies for altering or overcoming inhibitors that are made in excess. To this end we have developed a microtransplantation technique which enables us to gently inject fully adult neurons into the white matter tracts of the adult CNS, without causing the formation of reactive astroglial associated inhibitory molecules. Remarkably, the adult nerve cells can regenerate their axons with high efficiency and at high rates of speed, challenging long held beliefs that this is impossible. Many unanswered and provocative questions remain about the potential for neuronal circuit restoration in the regenerated adult CNS and our lab is now in a strong position to begin answering at least some of them.

Indeed, we have recently been able to promote robust functional regeneration into the adult rodent spinal cord of severed sensory roots using a combinatorial strategy that maximally stimulates an intrinsic growth response in the sensory neurons while simultaneously removing inhibitory proteoglycans from the cord with a bacterial enzyme (ie., chondroitinase) that removes the inhibitory sugar chains from proteoglycans. Other labs around the world are now showing that the use of chondroitinase combined with various cellular bridging techniques and an enhancement of the neurons intrinsic growth response can restore motor function in previously paralyzed adult rats and cats. It is highly conceivable that such therapeutic strategies may be useful in restoring both sensory and motor function in paralyzed humans.

O-2

Repeated autonomic dysreflexia episodes provoked by bladder and bowel dysfunction: Implications for the aging spinal cord injured-population

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In the United States alone there are nearly 0.25 million people with a spinal cord injury (SCI) and of these most have suffered on average for over 30 years.

Perhaps the most debilitating and stigmatising problems for a person with a SCI are those of bladder and bowel dysfunction. For example, following a suprasacral lesion then hyperreflexia of the bladder, exacerbated by sphincter dyssynergia, leads to incontinence, poor voiding and the likelihood of recurrent urinary tract infections. Modern treatments aim to lower bladder pressure and provide efficient bladder emptying. However, for many patients with lesions above T6 there is the serious added complication of autonomic dysreflexia (AD) caused principally by over-distension of the bladder and bowel. Medically, the symptomatic triad of AD comprises high blood pressure, bradycardia, and sweating with hot flushes and occasional headaches. AD is considered to be a life-threatening condition and sometimes presents as a medical emergency.

Following the introduction of better management of the urinary tract in SCI, especially the use of antibiotics for controlling urinary tract infections, the incidence of renal failure and its associated mortality has almost disappeared. Consequently, most people with a SCI can now expect a near normal life span, but this longevity brings with it the added complications of aging. We know little about the affects of aging on conditions like AD, but it is to be expected that with deteriorating bladder and bowel function as age increases then some people with SCI might expect to have more serious cardiovascular consequences of unremitting AD.

This talk will present, inter alia, an overview of bladder and bowel dysfunction, and their aberrant autonomic pathways, in causing AD. It will address the presumed mechanisms of AD, its symptomatology and the management required to minimise any adverse cardiovascular changes resulting from lifelong episodes of AD.

O-3

Acute and chronic autonomic issues associated with impaired sexual function in the aging SCI population

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This discussion will review the role of the autonomic nervous system in the physiology of sexual activity of both able-bodied and spinal cord injured (SCI) persons (Elliott 2003). Cardiovascular risks with sexual activity are minimal in able-bodied persons, except in those with cardiovascular disease, although orgasm does result in acute rise in blood pressure (BP). This rise can increase to potentially life-threatening levels in men and women with high level SCI due to autonomic dysreflexia (AD). We took 13 subjects (cervical n = 8, thoracic n = 5), instrumented them with an ECG and a beat-to-beat blood pressure (BP) monitoring system (Finometer, Netherlands), and applied vibrostimulation to evoke ejaculation. This was repeated twice, on and off sildenafil citrate. At ejaculation during the nonmedicated trials, the cervical group had significant bradycardia (- 5 – 10 beats/min) and an increase in arterial BP (+ 70 – 90 mmHg) relative to resting conditions, whereas the thoracic group had significant increases in both heart rate (HR) (+8 – 15 beats/min) and mean arterial pressure (+25 = 30 mmHg). Sildenafil citrate had no effect on the change of HR or mean arterial pressure in either group (Sheel et al 2005). However, 50% of cervical and 60% of

thoracic SCI men had dysryhthmias (defined by ECG as any variation from normal sinus rhythm) at rest and over 80% of these men developed dysryhthmias at ejaculation that persisted during recovery (Clayton et al unpublished). It is our clinical experience that arterial BP often goes higher than 200 mmHg and diastolic may follow up to 130 mmHg for a temporary (minutes) time, although ejaculation can also provoke malignant AD (Elliott and Krassioukov 2005).For others, repeated ejaculation can also attenuate symptoms of AD resulting in a false sense of security as we have documented symptoms of AD do not correlate with objective BP rise (Elliott et al, unpublished). Normal aging effects and accelerated aging with SCI related CV problems on sexuality will be discussed. Dr. Elliott will also speculate on several issues with sexuality, including the long term effects of intermittent hypertension on brain and genital vasculature, the potential for reduction of nitric oxide sources (required for genital arousal) from repeated AD causing endothelial damage and the resultant long term effects this may have on erectile function, as well as the psychodynamic effects of such risks on sexual motivation after SCI.

O-4

Hypotension and hypertension in spinal cord injury: Short- and long-term implications

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In spinal cord injuries (SCI), descending autonomic pathways are disrupted. Depending on the level and the completeness of the lesion there are cardiovascular aberrations in response to various stimuli. Particular problems occur in SCI with cervical and high thoracic injuries, where either the entire or a large proportion of the spinal sympathetic outflow is separated from cerebral control. Also, there are afferent pathways with input directly into the spinal cord, with abnormal responses resulting from stimuli beneath the level of the lesion. This overview will concentrate on hypotension and hypertension, two major abnormalities affecting cardiovascular autonomic function in SCI. They contribute to considerable morbidity and may result in death.

Hypotension results from the inability to activate sympathetic pathways in response to baroreceptor activation. Thus, in high lesions orthostatic hypotension is a particular problem, especially in the early stages of the disease, after prolonged recumbency, and following administration of drugs which normally have mild hypotensive effects. Symptoms of hypotension include dizziness, visual impairment, cognitive deficits and syncope, mainly the result of impaired cerebral perfusion. Other effects include diminished renal perfusion with oliguria. Hypotension also may occur in other situations as in response to hypoglycaemia. It also may result from overheating, as thermal dysregulation in SCI is caused by impaired sudomotor autonomic function.

Hypertension, often in association with autonomic dysreflexia, can be a major problem because of the initiation of spinal reflexes and other mechanisms that increase spinal sympathetic activity; the absence of cerebral control contributes further. Hypertension may result from stimulation of visceral, cutaneous or skeletal muscle receptors; a wide range of stimuli from a blocked catheter to an anal fissure may cause it. Sequelae include intracerebral bleeds, resulting in both morbidity and mortality.

There are thus short-and long- term implications that need to be considered in prevention and management of hypotension and hypertension in SCI, especially with increasing age and in response to interventional and therapeutic processes designed to repair the spinal cord.

O-5

Development of valid spinal cord injury (SCI) clinical trial guidelines

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In February of 2004, The International Campaign for Cures of spinal cord injury Paralysis (ICCP) supported and funded the first international clinical trials workshop on spinal cord injury (SCI) in Vancouver. One hundred invited participants attended from a variety of disciplines and backgrounds including: acute spinal injury units, rehabilitation centres, pharmaceutical and biotechnology companies, basic science research labs, government agencies, non-governmental organizations and foundations, as well as representatives of the SCI community. The main achievement of the meeting was to bring this varied team together and introduce them to the progress in clinical trials and the complexities involved in effective clinical trial design. A report of this meeting has been published (Steeves J, Fawcett J, & Tuszynski M. 2004. *Spinal Cord 42: 591-597*).

Another outcome of the ICCP Clinical Trials Workshop in Vancouver was a vote by the participants to establish a working panel to bring forward more detailed guidelines for how to conduct valid SCI clinical trials. With funding from the ICCP Partner Organizations and administrative support from ICORD, the long-term objectives were to delineate appropriate outcome measures, statistical power, and best practices for all clinical SCI trial protocols. However, given the rising number of SCI therapeutic interventions, where both the benefits and risks are large, the ICCP Clinical Trials Guidelines Panel determined the initial focus should be on pharmaceutical drug interventions and cellular transplants directed to facilitating enhanced spinal function.

The international panel has now met four times to discuss a large number of clinical trial issues, as well as consider the merit of many options, including:

Degree and level of injury, timing of clinical intervention, statistical power needed to achieve a valid outcome.

Determination of appropriate clinical outcome measures for different interventions.

Participant selection criteria (inclusion / exclusion) and ethics. Trial Design, the control of potential confounding variables, standardization of adjunct treatments, and the organization of multicenter trials.

We are now presenting our initial drafts at a number of international meetings and at www.icord.org to solicit feedback and input from the SCI research community. A final document will be submitted for peer-review in the first half of 2006.

O-6

Computing with neural ensembles

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I will review a series of recent experiments demonstrating the possibility of using real-time computational models to investigate how ensembles of neurons encode motor information. These experiments have revealed that brain-machine interfaces can be used not only to study fundamental aspects of neural ensemble physiology, but they can also serve as an experimental paradigm aimed at testing the design of modern neuroprosthetic devices. I will also describe evidence indicating that continuous operation of a closed-loop brain machine interface, which utilizes a robotic arm as its main actuator, can induce significant changes in the physiological properties of neurons located in multiple motor and sensory cortical areas. This raises the hypothesis of whether the properties of a robot arm, or any other tool, can be assimilated by neuronal representations as if they were simple extensions of the subject's own body.

O-7

Recovery and structural plasticity after stroke and Anti-Nogo antibody therapy in adult and aged rats

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Stroke and spinal cord injuries (SCI) are devastating disorders that often result in paralysis. One unique therapy that may improve motor recovery after stroke or SCI is neutralization of the neurite inhibitory protein Nogo-A through enhancement of neuroanatomical plasticity from uninjured areas of the central nervous system. To study the effects of Nogo-A neutralization after stroke in the aging CNS, we combined a one-week delay anti-Nogo-A antibody treatment with an ischemic lesion (permanent middle cerebral artery occlusion) in aged rats. We found that following ischemic stroke and treatment with anti-Nogo-A therapy one week following stroke, aged rats demonstrated motor recovery on a skilled forelimb reaching task and developed new cortico-efferent projections from the opposite, unlesioned hemisphere. We also found an increase in dendritic plasticity in pyramidal neurons located in the contralesional cortex. These results support the efficacy of Nogo-A neutralization as a treatment for ischemic stroke in the aging nervous system, and widen the effective treatment window to at least one week following stroke. Furthermore, these findings implicate both axonal and dendritic plasticity from the unlesioned hemisphere as a mechanism for recovery.

O-8

Bypassing spinal injury to promote motor function

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Spinal cord injury (SCI) interrupts connections between the brain and spinal cord that transmit motor control signals and sensory messages, but typically leaves spinal circuits below the lesion intact. We have developed a novel strategy to route motor control signals around the lesion to contact intact motor circuits caudal to the injury; ie, to bypass SCI. Our approach is to disconnect a spinal nerve that exits the cord above the site of the injury and insert the cut end directly into the lumbar spinal cord caudal to the level of injury. Because the origin of the bridge is rostral to the injury, its connections with the brain are intact.

We have found in the rat that motor axons in the nerve bridge regenerate extensively into the spinal cord at the insertion site and terminate predominantly within the intermediate zone and ventral horn, especially within the motor nuclei. Using confocal microscopy with synaptic markers, we found that regenerating bridge axons synapse on interneurons and lumbar motoneurons. Surprisingly, synapses on motoneurons are predominantly located on the cell body or proximal dendrites. Electrical stimulation of the nerve bridge produces postsynaptic field potentials in the cord at the insertion point and elicits hind leg movements, thereby indicating functional connections. Regenerating bridge axons grow into the intact cord, as well as after acute or chronic SCI.
To begin to examine therapeutic potential, we compared hind leg motor functions in animals receiving a L2 hemisection and nerve bridge insertion with animals receiving hemisection alone. Animals with the nerve bridge had significantly less spasticity-like signs and muscle wasting and showed greater hind leg joint mobility than animals with hemisection alone.

As novel synaptic connections develop between the regenerating bridge axons and spinal networks, we propose that supraspinal control can be exerted on the spinal circuits via the motoneurons of the inserted nerve. Motoneurons in the bridge now become interneurons in a new circuit between the brain and the portion of the spinal cord isolated by injury. Supported by: Christopher Reeve Foundation, NYS Spinal Injury Board

O-9

Neurophysiologic testing for predicting outcome after acute SCI: Simple, well-tolerated, and accurate...but is it useful?

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Testing of neurologic function in persons with acute spinal cord injury (SCI) relies almost exclusively on the 'ASIA' clinical examination. To complement this approach, and in anticipation of upcoming clinical interventions, we conducted repeated electrophysiologic studies on persons with acute SCI.

We tested 229 subjects with acute traumatic SCI (76% male, 6.3 – 85 year range, mean = 39.8 $\pm$ 16.8 years; 24% female, 7.1 – 83.5, 40.5 $\pm$ 19.9). EMG was recorded from multiple arm, hand, leg and foot muscles. Subjects were asked to make a maximal voluntary isometric contraction in each muscle, after which reflex testing was conducted. Whenever possible, repeat testing was carried out for 1 or more years post-injury. Analysis was done from taped records, by an investigator who did not participate with data collection. Muscle recruitment was scored on a 0 – 5 scale, based on EMG signal magnitude. Maximum tendon response amplitudes were determined for taps to the wrist, patellar and Achilles tendons, bilaterally.

Only 33% of the study population was 'complete' at the time of initial testing. The presence of volitional EMG in a muscle from which no palpable contraction could be discerned was not uncommon. Within a given muscle, delays > 5 weeks from injury to recovery of recruitment were common, particularly in persons with incomplete SCI. An intrinsic muscle of the foot (abductor hallucis) proved to be a more sensitive indicator of lower limb motor recovery than muscles included in the ASIA examination. Persons with incomplete SCI typically showed enhanced tendon reflex amplitudes and 'spread' to ipsi- and contralateral muscles (i.e. the 'crossed-adductor' reflex following patellar tendon taps). A simple algorithm based on absolute reflex properties at the most acute examination time-point was very accurate in differentiating between complete and incomplete SCI.

For routine medical management of a patient with acute SCI, the type of testing described above is not warranted. However, our findings may be relevant to: 1) patient selection for planned trials, where risk and benefit must be carefully weighed; 2) timing of interventions; and 3) choice of outcome measures for maximizing study sensitivity. This study was supported by the NIH (NS28059; HD31240; NS365423), by SUNY's Upstate Medical University, and by the The Miami Project to Cure Paralysis (University of Miami).

O-10

Strategies to overcome astroglial barriers and promote functional axonal regeneration after spinal cord injury

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Regeneration after spinal cord injury (SCI) is limited partly because of the inability of axons to grow through the microenvironment adjacent to the injury site. After SCI, extracellular matrix (ECM) molecules and astrocytic processes accumulate at the lesion forming a physical and chemical barrier. We used a peripheral nerve (PN) graft model to direct regenerating axons towards a region of the injured cervical (C) spinal cord treated to modulate components of the ECM, to test whether a more permissive environment for axonal regrowth in the spinal cord could be created. Chondroitinase ABC (ChABC), collagenase, the DNA enzyme against xylosyltransferase (XT-1) or vehicle was applied directly into a C5 dorsal quadrant lesion of adult rats for 5 days prior to apposition of a PN graft designed to bridge across a C3 hemisection. Animals were observed for behavioral activity weekly and biotinylated dextran amine (BDA) was used to trace axonal outgrowth. Transection of the graft 2 days prior to sacrifice permitted testing of graft mediated functional recovery. We observed improved open field movement and wheel walking only in animals treated with ChABC and this level of activity was diminished after cutting the PN graft. Extensive axonal outgrowth was observed in ChABC-treated animals with a good correlation between animal behavior and the degree of axonal outgrowth. Axonal contact with spinal cord neurons was identified by dual labeling for synaptophysin and BDA, and the absence of Schwann cell migration from the graft indicated axonal extension independent of these cells. Currently we are employing more sensitive tests of fore limb activity to better assess the potential for functional recovery in this treatment paradigm. We also are employing forced exercise of spinal cord injured animals to determine how activity-dependent plasticity might play a role in repair after SCI. Supported by NINDS NS26380 and the Daniel Heumann Fund.

O-11

Signaling mechanisms that control axon guidance decisions

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Growth cone navigation relies upon active remodeling of actin and microtubule cytoskeletal arrays. Despite rapid progress in finding actin regulators downstream of various guidance receptors, little has been learned about signaling effectors that directly associate with microtubules. Here we identify the microtubule-associated protein Orbit/MAST as a component that cooperates with the Abelson (Abl) protein tyrosine kinase during axon guidance in the Drosophila embryo. At the midline, Orbit/MAST and Abl mutants exhibit identical phenotypes, suggesting a model where Abl acts a node to coordinate actin and microtubule dynamics downstream of Slit. Orbit/MAST displays strong genetic interactions with Slit and its Roundabout-family receptors, supporting this model. Orbit/MAST is expressed at high levels in the developing nervous system where it localizes to axons and growth cones. Higher resolution imaging of the Orbit/MAST ortholog CLASP in Xenopus growth cones suggests that this family of microtubule plus-end tracking proteins identifies a subset of dynamic microtubules that probe the actin-rich peripheral domain of the growth cone where guidance signals exert their initial influence on cytoskeletal organization.

O-12

Signaling mechanisms for myelin inhibitors

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One of the major obstacles for successful axon regeneration in the adult central nervous system (CNS), arises from inhibitory molecules in CNS myelin, which all signal through a common receptor complex on neurons consisting of the ligand-binding Nogo-66 receptor (NgR), and two transmembrane co-receptors, p75 and LINGO-1. However, p75 expression is only detectable in subpopulations of mature neurons in the adult nervous system, raising the question of how these inhibitory signals are transduced in neurons lacking p75 expression. In this study, we provide evidence for the existence of other functional homologue(s) of p75 that can mediate the inhibitory activities of myelin inhibitors. We further demonstrate that TROY, a newly-identified member of the TNF receptor family that is selectively expressed in the adult nervous system, can form a physical and functional receptor complex with NgR and LINGO-1 to mediate the cellular response to myelin inhibitors. Also, both over-expressing a truncated TROY lacking its intracellular domain and presence of a soluble TROY protein can efficiently block neuronal response to myelin inhibitors. Our results implicate TROY in mediating myelin inhibition, offering new insights into the molecular mechanisms of regeneration failure in the adult nervous system. The intracellular molecules downstream of the receptor complex will be discussed.

O-13

Cell signaling cascades regulating axon growth inhibition

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Axons of the adult central nervous system are capable of only a limited amount of regrowth after injury, and that an unfavorable environment plays major roles in the lack of regeneration. Some of the axon growth inhibitory effects are associated with myelin. Three myelin-derived proteins have been identified to inhibit neurite outgrowth in vitro. These proteins induce activation of Rho, which is one of the key regulators of cytoskeletal organization, in some neruons. Inhibition of Rho or Rho-kinase, downstream effector of Rho, promotes axon regeneration in vivo. These findings establish Rho and Rho-kinase as key players in inhibiting the regeneration of the central nervous system. I will review recent findings regarding the signaling mechanism of axon growth inhibitors and talk about our ongoing research. Our experiments suggest that several new candidate proteins may be axon growth inhibitors. These proteins activate not only Rho/Rho-kinase but also other signals to inhibit neurite outgrowth from some neurons in vitro. Inhibition of repulsive guidance molecule, one of these proteins, promotes functional recovery and axon regeneration of cortico-spinal tract after the spinal cord injury in rats. These findings suggest that agents that block the multiple signals elicited by these axon growth inhibitors may provide efficient tools that produce functional regeneration following injuries to the central nervous system.

O-14

PI 3-kinase signalling controlling axonal growth and guidance

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Cellular levels of PI(3,4,5)P3 are known to be controlled by the activities of PI 3-kinase and the lipid phosphatase PTEN, and have been linked to the determination of internal polarity and directional movement in several cell types. I will discuss two aspects of PI(3,4,5)P3 dependent signalling in neurons. Firstly, the involvement of PTEN in the growth cone collapse response mediated by the inhibitory axon guidance molecule Sema3A. Stimulation with Sema3A suppresses PI3K signalling which, we show, depends on the phosphatase activity of PTEN. PTEN is highly enriched in the axonal compartment and the central domain of sensory growth cones during axonal extension, where it appears to co-localise with microtubules. Following exposure to Sema3A, PTEN accumulates rapidly at the growth cone membrane suggesting a mechanism by which PTEN couples Sema3A signalling to the growth cone collapse.

Secondly, I will discuss isoform specific aspects of PI3K signalling. Eight distinct PI3K isoforms have been characterised in mammals; however, detailed information on their tissue distribution in embryonic and adult tissues is not available. I will present data on the analysis of the expression of the p110d isoform of PI3K, by insertion of a b-gal reporter gene into the mouse p110d gene locus allowing the independent production of the p110d protein and the b-gal enzyme from the same bicistronic mRNA. During mouse development, b-gal/p110d staining is almost exclusively detected in the nervous system with particular high levels seen in the dorsal root ganglia and the cranial sensory ganglia at stages concomitant with the extension and guidance of their axonal processes. Sensory neurons derived from mice expressing p110dD910A, a catalytically inactive form of p110d, show reduced outgrowth under limiting conditions, and increased responsiveness to Sema3A. In addition, I will present evidence for an involvement of p110d during neuronal regeneration following injury to the adult peripheral nervous system.

O-15

Isolation, induction and use of neural progenitor cells of the adult human brain

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In the adult human forebrain, neural stem cells persist within the forebrain ventricular zone, and give rise to a variety of more restricted progenitor phenotypes. The major progenitor pools of the human brain, each of which has now been isolated to purity, include ventricular zone neuronal progenitor cells, hippocampal neuronal progenitors, and parenchymal glial progenitor cells. Each of these phenotypes exists within a local environmental niche, which tightly regulates both the mitotic activity and derivatives of its resident progenitors. Within these niches, both neuronal and glial progenitor cells may reside as transit amplifying pools, by which lineage-biased progenitors expand to replenish discrete phenotypes. The largest such pool appears to be that of parenchymal glial progenitor cells. When isolated and transplanted into neonatal shiverer mice, whose brains lack myelin basic protein, these cells can mediate quantitatively substantial and geographically extensive myelination. Remarkably, whereas adult glial progenitor cells only generate oligodendrocytes and astrocytes within the normal white matter, upon removal from the tissue environment they expand to generate neurons as well as glia. Thus, an abundant pool of glial progenitors retains both multilineage capacity and mitotic competence in adult brain parenchyma, suggesting the potential for substantial cell replacement in the human CNS.

Besides implanting precursor cells for therapeutic benefit, one may also achieve this end by inducting endogenous stem and progenitor cells. In particular, progenitor cells in the adult ventricular wall may be induced to generate new neurons by over-expressing cognate neuronal differentiation agents, such as BDNF. We have noted that the concurrent suppression of astroglial differentiation by resident stem cells, accomplished by over-expressing the bone morphogenetic protein (BMP) inhibitor noggin, can potentiate the BDNF-mediated addition of new neurons to the adult rat neostriatum. The new neurons mature as medium spiny neurons, and successfully project to the globus pallidus, extending processes over several mm of normal adult striatum. The neurogenic effect of BDNF and noggin treatment is maintained and robust in the R6/2 mouse, a transgenic model of Huntington's Disease (HD). R6-2 mice treated with adenoviral BDNF and noggin exhibit both improved motor performance and longer survival than untreated controls, suggesting the potential therapeutic efficacy of this strategy for replacing medium spiny neurons lost to HD. Together, these experiments argue that as our understanding of the biology of adult neural progenitor cells becomes more extensive, our ability to target, induce and implant these cells for therapeutic purposes will become increasingly manifest.

O-16

Clinical trials in spinal cord injury: How can we link imaging to functional outcome?

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Radiological imaging plays a crucial role in diagnosing the severity of tissue damage following traumatic spinal cord injury (SCI). This information is also essential for appropriate planning of surgical decompression and stabilization, as well as predicting neurological and functional recovery. Although x-ray plain films and computed tomography (CT) are used to determine spinal column misalignment and instability, magnetic resonance imaging (MRI) has assumed an increasingly important role in the evaluation of spinal trauma. Routine MRI enables detailed evaluation of the spinal cord, nerve roots, intervertebral discs, and adjacent vascular structures. Typical MRI protocols include the acquisition of T1- and T2-weighted spin-echo images in both the sagittal and transverse planes. Additional images with gradient-echo weighting are also acquired frequently. These imaging sequences can reveal several important details that are associated with neurological impairment and functional recovery following SCI. Specifically, MRI can demonstrate the presence, location and extent of hemorrhage and edema in the spinal cord, as well as the degree of cord compression. More recently, advanced MR methods such as diffusion-weighted imaging (DWI), diffusion-tensor imaging (DTI), and functional MRI (FMRI) have shown promise for providing additional information about the anatomical and functional integrity of the spinal cord. For example, DWI/DTI can reveal continuity in white matter tracts by visualizing the anisotropy of water motion. This anisotropy is related to both the parallel arrangement of axonal components (e.g. neurofilaments) and to myelin. Accordingly, MRI may serve as an important surrogate outcome measure for clinical trials of therapies that are intended to mitigate tissue damage following acute SCI or to facilitate repair of the spinal cord at later stages. Utilization of MRI as a surrogate endpoint will likely require protocol-driven readouts by neuroradiologists and, to the extent possible, quantitative assessment of key features on the MR images.

O-17

Watching nerves grow and retract in fluorescent mice

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My colleagues and I use transgenic mice that express fluorescent proteins in neurons to monitor the behavior of axons in vivo (Feng et al. 2000). These mice provide an opportunity to make longitudinal studies of axonal branch trimming during the period of synapse elimination in development, as well as track the ways axons navigate during regeneration and remodel in advanced age and with disease. We find that peripheral motor axons have a remarkable ability to reestablish their original connections by reestablishing their original branching pattern in their former Schwann cell tube (Nguyen et al., 2002). In addition once axons reach their postsynaptic sites they show a marked preference for growth along the basal lamina that coincides with the location of postsynaptic acetylcholine receptors. This regeneration allows axons to recapitulate their original synaptic connectivity with greater than 95% accuracy following nerve crush. Errors that do occur seem to be due to axons invading the tube of an adjacent axon at a node of Ranvier. Such jumping into adjacent glial tubes may also explain the large arbors of surviving axons both in aging and in response to motor neuron loss in a mouse model of ALS (Schaefer et al., 2005). Regeneration of peripheral axons following nerve cut is far less accurate, presumably because axons do not find any of the original Schwann cell tubes. We have also attempted to watch axonal regeneration following damage to central axons in the dorsal columns of the spinal cord (Kerschensteiner et al., 2005). We find that both the proximal and distal segment of an axon undergoes an acute die-back from the site of the lesion. Although spinal dorsal column axons appear to make an attempt at regrowth, this is always misdirected. Axonal branch loss is also commonplace in the developing peripheral nervous system during the period of synapse elimination when motor unit branches are trimmed in an activity dependent way (Walsh and Lichtman, 2003; Keller-Peck et al. 2001, Kasthuri and Lichtman 2003; Buffelli et al., 2003). This loss is due to progressive axonal atrophy followed by a process of local fragmentation ("axosome shedding") and glial engulfment (Bishop, Misgeld et al., 2004). Newly engineered transgenic "brainbow" mice in which many different spectral variants of GFP are expressed in the same animal may allow us to track many axons and axonal organelles simultaneouses simultaneously in development and regeneration.

O-18

Debate: Increasing inflammation is beneficial for neural repair and outcome

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Recruitment of inflammatory leukocytes to the injured spinal cord is associated with the production of cytokines and proteinases that are involved in host defense and wound repair. However, whether "inflammation" contributes to, or interferes with, ultimate recovery of function is debatable. The topic to be debated in this session is stated in the following way:

Resolved: Increasing inflammation is beneficial for neural repair and outcome

In Favor

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T cells that recognize CNS antigens can confer protection against neuronal injury (spinal cord injury model and MPTP model of PD).

Injury to the CNS results in a systemic adaptive response that requires activated T cells and an integrated cascade of cytokines to insure proper repair of the damaged tissue.

Enhancement of "protective autoimmunity" may provide a novel means of enhancing functional recovery following CNS injury.

Spinal cord injury-triggered inflammation includes a molecular cascade with elements common to angiogenesis and neurogenesis that may enhance recovery.

Opposed

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T cells that recognize CNS antigens exacerbate the effects of CNS injury.

"Inflammation" needs to be defined in terms of specific cellular and molecular mechanisms that are adaptive or exacerbate injury.

Acute anti-inflammatory treatment can enhance anatomical and behavioral recovery following CNS injury.

Spinal cord injury-triggered angiogenesis and neurogenesis may result in "leaky" blood vessels and neurons with aberrant circuitry, which may produce persistent inflammation and pain sequealea.

O-19

Intrinsic kinase pathways regulate the excitability of regenerating nociceptive neurons following nerve trauma: Role in chronic pain

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Axotomy or an inflammation that affects the peripheral nervous system often induces a long-term hyperexcitability (LTH) in the somata of primary nociceptive neurons. The increased excitability enhances input to the CNS, thereby increasing awareness of the injury and promoting adaptive responses. Persistent LTH, however, is a cause of chronic pain. How does trauma in the distal region of an axon induce the changes in gene transcription that underlie the LTH? Axons contain a class of signaling proteins that are activated by nerve injury and then retrogradely transported to the soma and into the nucleus. Using the mollusk Aplysia californica, we found that nerve crush activates a cascade in the axon at the site of injury that begins with NO synthase and culminates in the activation of protein kinase G (PKG). The active PKG is then retrogradely transported to the cell soma where it contributes to the induction of the LTH. Blocking any step in the pathway prevents the appearance of the LTH. Surprisingly, no increase in active PKG occurred in other neuron types whose axons were damaged, suggesting that this pathway specifically communicates information regarding injurious events. Switching to the rat, we confirmed the activation and retrograde transport of PKG after injury to the sciatic nerve and found that this occurred primarily in C-type nociceptive neurons. Significantly, PKG was absent from motor axons. PKG was also activated and transported after injecting an irritant into the hindpaw to induce an inflammation. These findings affirm the importance of retrograde signaling pathways in regulating gene expression during nerve regeneration and indicate that the PKG pathway is a prime target for drugs to ameliorate pain.

O-20

Retrograde injury signaling in lesioned nerve

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The long axonal process of a neuron poses a problem when information on damage to the axon must be conveyed to the distant cell soma. It is known that injured peripheral nerve axons can signal to their cell bodies, and cause a "reprogramming" of the cell body for long range elongating outgrowth. We have recently discovered that the mechanism for this process is based on local synthesis of nuclear import proteins in the axon at the injury site. These proteins can then bind signaling proteins tagged with nuclear import signals, and transport them back to the cell body where they induce changes in the regenerative properties of the nerve. Here I will describe three aspects of our ongoing work: 1) Analyses of targeting elements in 3' UTR of axonal mRNA's encoding critical components of the system; 2) Proteomics approaches to identify the retrograde injury signaling molecules; and 3) Microarray efforts to identify target genes activated by the retrograde signals very early in the regenerative response. I will also describe some initial observations of differences in mechanisms of retrograde injury signaling between central versus peripheral projections of DRG neurons. Identification of retrograde injury signals and their target genes may allow the design of new approaches to enhance nerve regeneration.

O-21

Regulation of protein synthesis in regenerating axons

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In developing axons, growth cone turning in response to pathfinding stimuli requires local protein synthesis. In adult neurons, axonal protein synthesis is activated by nerve injury. Axonally synthesized proteins help to trigger growth cone formation (Verma et al., 2005), provide a means to retrogradely transport signaling proteins to the cell body (Hanz et al., 2003; Perlson et al., 2005), and help to maintain structure of the regrowing axon (Zheng et al., 2001). Regenerating sensory axons synthesize over 100 polypeptides by metabolic labeling (Willis et al., 2005), and cDNA arrays show more than twice as many individual mRNAs in these adult rat axons. The proteins encoded by axonal mRNAs are quite diverse and include cytoskeletal, chaperone, resident ER, transmembrane, anti-oxidant, and metabolic proteins. Translation of axonal mRNAs is regulated by at least two distinct means. For some transcripts, transport of the mRNA from cell body into the axonal compartment is altered by local sources of neurotrophins. Altering transport of these mRNAs into axons changes the amount of these transcripts that is available for the axonal translational machinery. For most axonal transcripts, NGF-dependent transport requires activation of the Ras-MAP kinase or PI3 kinase pathways. Furthermore, some transcripts show unique regulation depending upon local vs. bath application of neurotrophins. In contrast to effects of the growth promoting neurotrophins, expos-

ing axons to MAG or Sema3A alters transport of an overall different population of mRNAs into the axonal compartment. A surprising proportion of axonal mRNAs shows constitutive transport, suggesting that their translation rather than their transport is regulated. Consistent with this hypothesis, mobilization of axoplasmic Ca2+ in isolated axons stores leads to increases in translation of specific axonal mRNAs. (Supported by NIH grants, NS041596 and NS045880, and programmatic funds from the Nemours Foundation).

O-22

Switching regeneration and sprouting on and off: Role of Jun, Ras and MEK

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Axonal regeneration and sprouting are key elements in the repair of the injured nervous system. Nerve injury is known to trigger numerous molecular changes in the injured neurons and surrounding non-neuronal cells that play a pivotal regulatory function in successful target reinnervation, e.g. the upregulation of neuronal adhesion molecule alpha 7 beta 1 integrin (Werner et al., 2000), or cell death, e.g. due to the de novo expression of tumour necrosis factor (Raivich et al., 1998; 2002). However, we are particularly interested in the identification of endogenous substances that act as molecular switches controlling these processes in the living organism, such as jun, ras or MEK.

c-Jun is a component of the heterodimeric AP-1 transcription factor, and c-Jun is highly expressed in response to neuronal trauma. Mice lacking c-jun in neural cells of the nervous system (jun delta-N) showed severe defects in the axonal response, including perineuronal sprouting, lymphocyte recruitment, and microglial activation. C-Jun-deficient motorneurons were atrophic, resistant to axotomy-induced cell death, and showed reduced target muscle reinnervation. Expression of CD44, galanin, and alpha7 beta1 integrin, molecules known to be involved in regeneration, was greatly impaired, suggesting a mechanism for c-Jun-mediated axonal growth (Raivich et al., 2004).

To identify more upstream mechanisms, we are currently focusing on components of the mitogen-activated protein kinase (MAPK) cascade, including ras, MEK and ERK. Interestingly, neuronal expression of active ras or dominant negative MEK1 (MEK1dn) did not interfere strongly with peripheral regeneration, but did augment neuronal survival, and strongly enhanced the sprouting of motor axons into central white matter following peripheral facial axotomy. Moreover, a similar sprout-enhancing effect of ras and MEK1dn was also observed on descending corticospinal axons, following dorsal over-midline hemisection of the pyramidal tracts, allowing these axons to grow caudally, beyond the site of injury. These effects were particularly pronounced in ras/MEK1dn double mutants where anterogradely BDA-labeled corticospinal axons where observed to grow through the post-traumatic glial scar.

O-23

New roles for glycosylation in neuromuscular development, regeneration and disease

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Most of the proteins that are known to be important for the development of the neuromuscular synapse are glycosylated with carbohydrate structures that may mediate or modulate their function. Little is known, however, about the exact nature of these structures or their roles in neuromuscular development, disease, or regeneration. Our laboratory has identified several carbohydrate structures that are uniquely present at the neuromuscular synapse in skeletal muscle, including the CT carbohydrate antigen. The CT carbohydrate is made by the CT GalNAc transferase, which is itself localized to the neuromuscular junction in skeletal muscle. Transgenic overexpression of this glycosyltransferase in skeletal muscle causes the ectopic expression of a complex of synaptic proteins known to interact with dystroglycan, the principal glycoprotein target of this enzyme in skeletal muscle. CT transgenic mice demonstrate roles for this carbohydrate in muscle growth, axon guidance, and neuromuscular structure. Evidence suggests roles for follistatin and NCAM in the muscle growth and axon guidance phenotypes, respectively. In addition, CT overexpression in skeletal muscle stimulates the ectopic expression of utrophin, a synaptic homologue of dystrophin, the protein missing in Duchenne muscular dystrophy (DMD), and laminin alpha 4 and alpha 5, synaptic homologues of laminin alpha 2, the protein defective in congenital muscular dystrophy 1A (MDC1A). The ectopic expression of these molecules in CT transgenic mice inhibits the development of muscular dystrophy in mdx mice, a model for DMD, as well as in dyW mice, a model for MDC1A. Moreover, this therapeutic effect can be accomplished using gene therapy approaches both in mdx and dyW animals in way that bypasses the developmental effects on muscle growth and neuromuscular development. This work was supported by grants from the Muscular Dystrophy Association and the NIH (AR050202) to PTM.

P-1

Characteristics of acute spinal cord injury patients admitted to a level I trauma center in southern California

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Introduction: The clinical characteristics of acute, traumatic spinal cord injuries (SCI) in a densely populated urban area need to be identified in order to properly target clinical research strategies and injury prevention programs.

Material and Method(s): A retrospective chart review was conducted to identify clinical characteristics of acute traumatic SCI patients admitted to the only Level I trauma center in Orange County, California during January 1, 1995 to December 31, 2004. The following ICD-9 codes were used as search criteria: 806.0-9, 839.0-5, 952.0-4.

Results: Over 200 SCI patients matching the ICD-9 codes were admitted to the trauma center during the 10-year period reviewed. The mean age was 35.7 years and the majority were males (77.6%). The primary cause of injury involved motor vehicles (52.8%), followed by gunshot wounds/violence (19.6%) and falls (20.1%). One-third of the cases were fatal, involving multiple injuries in addition to SCI. Of the non-fatal SCI cases, 51.6% were cervical lesions, 30.6% were thoracic lesions, and 17.8% were lumbar/sacral lesions. The majority of gunshot wounds occurred in males under the age of 30. The majority of falls occurred in people over the age of 40.

Conclusion: The characteristics for acute SCI patients admitted to the only Level I trauma center in Orange County, CA indicate a potential clinical research population consisting of a high number of cervical lesions, but also a large number of people sustaining injuries to multiple body parts. Injury prevention strategies should be targeted toward motor vehicle safety, conflict avoidance and fall prevention.

P-2

The cellular inflammatory response after human spinal cord injury

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Spinal cord injury (SCI) initiates a complex inflammatory reaction that expands tissue damage beyond the initial injury, a poorly understood event after human SCI. We have examined cellular inflammatory responses within the lesion in thirty-four human spinal cords with injury to death intervals from 1 h to 1 yr. Uninjured cord segments distal to the injury site and four uninjured human control cords were analyzed for comparison. Neutrophils, visualized with a monoclonal antibody (mAb) to á-defensins (defensin), were within petechial hemorrhages 1 h after SCI. Neutrophil defensins are secreted anti-microbial proteins. Neutrophils extravasated from blood vessels and infiltrated disrupted tissue adjacent to haemorrhagic areas 4 h after injury. Neutrophil numbers were greatly increased by 1-3 days after SCI, remaining increased up to 8 days. Neutrophils expressed myeloperoxidase. A few activated microglia and infiltrating monocyte/macrophages, detected with anti-CD68 mAb and myeloperoxidase, were found within 5 days of SCI. Later, macrophages were numerous and sometimes persisted for weeks to months. These cells did not express defensin and expressed little or no myeloperoxidase. T-lymphocytes visualized with anti-CD8 mAb, were inside blood vessels and petechial hemorrhages from immediately to 4 h after injury. T-lymphocytes were rarely in the cord 3-10 days after SCI but increased in number 3 weeks after injury, remaining so for at least 7 months. The histopathological severity of cord injury was semi-quantitatively determined in lesion epicenters stained with H&E. Injury severity increased abruptly at 1-3 days after SCI and remained constant for the time course studied. Oxidative reactivity detected with a mAb to gp91phox, a subunit of NADPH oxidase, was primarily associated with neutrophils and microglia. Proinflammatory matrix metalloproteinase-9 was also expressed by neutrophils. Overall the data demonstrate a pattern of activation and/or infiltration of neutrophils, microglia, and macrophages resembling that observed after SCI in rats and mice. Support Contributed By: CIHR & ONF of Canada, NINDS.

P-3

Preservation of vestibulo-spinal reflexes in incomplete spinal cord injury

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Patients suffering from incomplete spinal cord injury (iSCI) show an impairment of postural control with high danger of falls. By clinical means it is impossible to distinguish if this instability is primarily due to the sensorimotor deficit and/or disturbance of the vestibulospinal pathways.

In patients with iSCI galvanic vestibular stimulation (GVS) was applied to investigate the influence of the vestibular spinal descendent pathways on postural stability. Lateral body sway and electromyographic (EMG) responses in postural active leg muscles were evoked by bipolar binaural GVS (stimulation strength 4 mA, duration 400 ms). In eight iSCI patients and matched controls the centre of pressure (CoP) and soleus EMG responses during free standing on firm and compliant ground were measured. The impairment in postural control was assessed by the mean amplitude of voluntary CoP deflections during two minutes stand ing.

iSCI patients were significantly less stable than controls as assessed by the mean sway during standing. There was a close correlation between all CoP deflections and medium EMG amplitudes to GVS with the extent of postural instability. When correcting for the level of postural instability, as the latter is strongly affecting GVS responses, the latencies of the EMG and CoP responses, as well as the amplitudes of the early CoP deflections

and the short-latency EMG response, were not significantly different. The amplitudes of the later components (medium-latency EMG; the medium and late CoP deflections) were significantly larger in the control group.

The postural instability of the assessed iSCI patients could not be proved to be caused by a vestibulospinal deficit, but was rather due to the sensorimotor impairment. While the early responses to GVS were not affected the late responses were reduced and might represent impaired voluntary compensatory reactions to the induced body sway.

P-4

Spinal cord regeneration capabilities may be enhanced by aggressive acute management of spinal cord injuries

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Spinal cord injuries have devastating consequences for victims, families and society in general. Despite recent advances in spinal surgical reconstruction meaningful recovery remains a challenge. Attempts to limit secondary injury of the spinal cord after trauma have proved unsuccessful. Recent disappointing pharmaceutical trials have turn investigators away from research on this area.

Two physical manipulations have resulted in successful outcomes in similar clinical entities such as brain trauma. These manipulations are: 1-Acute decompressive surgery and 2-Transient cooling. These techniques have a long and controversial history as therapeutic tools for brain and spinal injury. The literature available for treatment of spinal cord injuries with decompressive laminectomies or early decompression and stabilization is reviewed to answer if this procedure may be useful to prevent morbidity and increase recovery of patients after spinal cord injury (SCI) as compared to the patients that received closed treatment. Moreover the role of acute local hypothermia of the injured spinal cord by application of local heat sinks is reviewed for both, experimental animals and human patients. The findings of this review suggest that early spinal cord decompression followed by local cooling may be beneficial after SCI. These improved techniques using new technology may result in anatomical tissue preservation of spinal cord. Preservation of spinal cord tissue viability may provide the means for further regenerative interventions in the future. Anatomical preservation may directly result in improvement of clinical outcomes of patients with SCI.

P-5

Interaction of Nogo-A antibody treatment and locomotor training on neuronal reorganization and functional recovery after an incomplete spinal cord injury

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After an incomplete spinal cord injury (SCI), rats treated with monoclonal antibodies IN-1, 11C7 or 7B12, raised against the Nogo-A specific domain, showed an increased regeneration as well as neural reorganization of descending circuitry projecting to the spinal cord. Independently, locomotor training has also been shown to induce neural reorganization of the spinal cord sufficient to mediate functional motor recovery. The goal of this study was to investigate the interaction of anti-Nogo-A treatment and locomotor training on motor performance after an incomplete spinal cord injury. Forty female Sprague-Dawley rats (200-250g) received a dorsal hemisection and midline transection at T8, eliminating the corticospinal tracts but sparing the ventral lateral funiculi. Rats received either 11C7 or IgG delivered intrathecally (Alzet Osmotic Pumps) for two weeks immediately post-lesion. One week after the lesion, each group was then subdivided into a trained and non-trained groups. Rats were trained 5 days/week, 20 min bipedally and 20 min quadrupedally on an inclined treadmill (up to 10% grade) for 8 weeks. Rats were tested in a modified grid-walk motor task as well as during bipedal and quadrupedal treadmill locomotion at different speeds. Analysis of 3-D locomotion kinematics and gait characteristics was performed. Lesion size and anterograde (BDA) corticospinal tract tracing were quantified. Preliminary behavioral results have shown a significant effect of training and anti-Nogo-A administration. Morphological results will also be discussed.

P-6

Polyethylene glycol administration after moderate spinal cord injury decreases lesion size and improves locomotor recovery

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After spinal cord injury (SCI), the blood brain barrier (BBB) is compromised causing immune cell infiltration and secondary damage at the lesion. Poly(ethylene glycol) (PEG) is a non-toxic, hydrophilic polymer known to repair cell membrane damage. We investigated if intravenous (IV) delivery of PEG immediately after SCI would limit disruption of the BBB, resulting in a smaller lesion and improved recovery. Adult female Sprague-Dawley rats had 1.1 mm T8 spinal cord displacements with the OSU device. Rats were assigned to: SCI control (SCI n=7) or 3 groups that received ringers solution IV (VEH n=7), 5% solution of either a 4 arm, 10kDa PEG (PEG; n=4) or PEG with a rhodamine tag for visualization (RhPEG n=4) in ringers solution. We assessed open field locomotion preoperatively and for 5 weeks after SCI. White matter sparing (WMS) and lesion size, BBB and astrocytes were analyzed via EC, endothelial barrier antigen (EBA) and GFAP staining, respectively, along the rostro-caudal extent of the lesion. The PEG group had greater locomotor recovery than SCI as early as 10 d postop ($p<.05$) and a faster rate of recovery. Functionally, 100% of the PEG group attained frequent to consistent stepping while 29-36% of controls failed to step at all. The PEG group had greater WMS ($p<.05$) and smaller lesion volume ($p<.05$) than controls. RhPEG had greater EBA labeling in the white matter of segments caudal to the lesion compared to SCI ($p<.05$). A similar trend was seen in the PEG group. There was no difference in GFAP labeling ($p>.05$); however, hypertrophic astrocytes were

only seen in SCI and VEH groups. Acute administration of PEG may promote greater functional recovery, alter the wound environment and minimize the immune response after SCI by fusing and sealing the affected BBB. Support Contributed By: NS43798-01 (DMB) and a generous gift from Richard D. and Gail Siegal (EL).

P-7

Correlation of the use of computerized animal activity monitoring with BBB open field test to assess recovery of locomotor function in a rodent spinal cord injury (SCI) model

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The Basso, Beattie and Bresnahan (BBB) open-field locomotor rating scale is accepted as the standard method to assess recovery of hindlimb function following spinal cord injury (SCI) in rats. In this study, we used the Micro System (Accuscan Instruments, Inc.) which measures the disruption of infrared beams caused by ambulatory and stereotypic movements of animals over time to confirm and extend the findings of Xu et al (Neuroscience Let 384: 271, 2005) that activity monitoring provides a useful index of the recovery of motor function post-SCI. There are several parameters recorded by this system including total activity (TACTV); ambulatory activity; stereotypic count; movement time, etc. SCI was induced using the Infinite Horizons Impactor (Precision Systems & Instrumentation) in 18 adult, female Sprague-Dawley rats at T10. BBB scores were assessed on 1, 2, 3, 5, 7 days post-SCI. Immediately after BBB examination, subjects were placed in the activity cages for 15 min. TACTV, which is the total number of beam breaks/15 min, was determined and compared with the BBB score. The correlation coefficient of TACTV ratio with BBB score was determined to be 0.9879 ($P<0.05$). Comparison studies at later time points are currently underway. These results suggest that activity monitoring may serve as a complimentary test for motor function after SCI either by itself or in combination with BBB scoring. Supported by NIDRR training grant ED H133P020003; US army grant PR 043199.

P-8

Therapeutic neuromuscular stimulation therapy improves recovery of locomotion after incomplete spinal cord injury in adult rats

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In the current study, we investigated the ability of short-term therapeutic neuromuscular stimulation (TNS) to improve recovery of locomotion and promote plasticity of dopaminergic supraspinal-spinal pathways believed to contribute to the control of locomotion after incomplete spinal cord injury (iSCI). Fifteen Adult Long Evans rats received either moderate T8 contusion spinal cord injuries (iSCI, n = 9) or laminectomy only (Sham, n=6). A subset of injured animals also underwent implantation of intramuscular stimulating electrodes into the biceps femoris and iliacus muscles at the time of injury (iSCI+TNS, n=5). Following one week of recovery the iSCI+TNS animals received 15 minutes of TNS therapy daily for 5 days. Recovery was assessed using the BBB locomotor scale and 3D kinematics. Anatomical plasticity was assessed using lesion reconstruction and immunohistochemistry. While, no significant difference in BBB score between two injury groups was observed, kinematic analyses revealed significant functional improvements. Interlimb coordination between right and left hindlimbs (hip, knee and ankle) and between hindlimb and forelimb improved towards normal in animals with TNS therapy. Additionally, TNS therapy improved intralimb coordination between the hip/knee, knee/ankle, and hip/ankle joints. This recovery can not be explained by differences in lesion size, as quantification revealed no effects of TNS on lesion volume. In addition, preliminary observations regarding the effect of TNS on dopaminergic axon density in the dorsolateral funiculus of the spinal cord are inconclusive. Overall, these results suggest that short-term daily FNS therapy can promote recovery of hindlimb movement and coordination during locomotion after iSCI in adult rats. The anatomical substrates mediating this recovery, however, remain to be determined. Supported in part by: R01 HD40335

P-9

Environmental enrichment promotes recovery of forelimb movements and supraspinal pathway plasticity after cervical spinal cord injury in adult rats

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After thoracic spinal cord injury in both animals and humans, rehabilitative strategies, such as treadmill training, are known to promote significant plasticity in lumbar spinal circuitry and recovery of locomotion. Little is known, however, regarding the effects of rehabilitative strategies on recovery of skilled limb movements and plasticity in descending supraspinal pathways that control them, after cervical spinal cord injury. Therefore, the current study was undertaken to investigate the effects of a rehabilitative strategy (enriched environmental housing) on recovery of forelimb movements, as well as plasticity of supraspinal pathways using a rodent model of cervical spinal cord injury. Following C4-5 over-hemisections and a 15 day delay, animals were housed in enriched environments for 10 weeks. Forelimb movements were evaluated during multiple activities impaired by cervical spinal cord injuries, including skilled target reaching, grooming, exploration, and locomotion. Anatomical reorganization was evaluated using anterograde tracing of corticospinal axons and immunohistochemistry for raphespinal axons, two pathways known to contribute to the control of forelimb motor

function. The results presented here indicate that increased post-injury experience and activity improved recovery of rhythmic alternating forelimb movements during locomotion, but not skilled, goal directed or automatic movements. Anatomically, enriched environment supported plasticity of corticospinal fibers, but not raphespinal fibers, in both neurologically intact and injured animals. These findings demonstrate that increased post-injury experience and activity can improve forelimb motor function after cervical spinal cord injury and support plasticity in long descending supraspinal pathways. Supported by NIH NS27054 and a grant from the Christopher Reeve Paralysis Foundation.

P-10

Combined strategies to increase plasticity and recovery of function after cervical spinal cord injury in adult rats

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Our previous studies have shown that rolipram, a phosphodiesterase inhibitor, promotes axon regeneration, lessens glial scar formation, and enhances functional recovery after spinal cord injury. We have also shown that increased post-injury activity in the form of enriched environment significantly improves proximal forelimb functioning after cervical spinal cord injury. The purpose of this study was to determine the combined effect of two interventions on neuronal plasticity after spinal cord injury: 1) elevating endogenous cAMP, a putative therapeutic for increasing neuronal plasticity and 2) post-injury activity, a putative therapeutic for activity-dependent recovery of function. Young adult rats received an over-hemisection at cervical (C4-5) spinal cord and either rolipram or saline vehicle solution was administered for ten days by mini osmotic pump. Shams received laminectomy alone. Animals were housed in either standard or enriched housing for four weeks. Animals in enriched housing were subjected to post-injury behavioral training daily. All animals were recorded on behavioral tests once a week for four weeks after injury. Animals receiving a combined treatment of rolipram and enriched housing after injury showed a greater density of raphe-spinal fibers at the lesion site and caudal to lesion and improved quality of forelimb use during locomotion. These data suggest that the combined treatment of rolipram and enriched environment improves unskilled locomotion and forelimb use and promotes plasticity of descending serotonergic fibers. Studies are currently underway to examine the effect that post-injury training has on the recovery of function in skilled tasks for reaching and locomotion. Supported by Christopher Reeve Paralysis Foundation and NIH NS 27054.

P-11

Fractalkine (CX3CL1)/fractalkine receptor (CX3CR1) interactions influence motor and sensory recovery after spinal cord injury

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Macrophages derived from resident microglia and monocytes dominate the acutely injured spinal cord. Both cell types can secrete neurotoxic factors and have been implicated in secondary degeneration after SCI. The macrophage response to SCI is thus an attractive target for therapeutic intervention; the challenge is to elucidate molecules that will allow fine manipulation of this response. Within the CNS, microglia are tonically regulated by fractalkine (FKN; CX3CL1) - a chemokine expressed predominantly on neurons. Both microglia and hematogenous cells express FKN receptor (CX3CR1). Endothelia may also express FKN where CX3CL1/CX3CR1 interactions can influence leukocyte adhesion and chemotaxis. Since disrupting FKN or CX3CR1 could impair neuron/microglia cross-talk and/or monocyte infiltration, we performed moderate spinal contusion injuries in wild-type (C57BL/6), CX3CR1 +/- and CX3CR1 -/- mice. The Basso Mouse Scale for Locomotion (BMS) was used to monitor locomotor recovery and von Frey hair and plantar heat tests were performed to monitor sensory phenotypes before and after injury. All mice were euthanized at 6 weeks post-injury. Locomotor recovery was significantly improved in both CX3CR1 +/- and -/- mice compared to wild-type mice. Morphometric analyses of the contusion lesions corroborated these findings, showing shorter lesions with increased myelin sparing at the impact site in mice deficient in CX3CR1. Patterns of sensory recovery (mechanical and thermal) after SCI were comparable between groups; however, knockout mice exhibited lower thresholds to mechanical and thermal stimuli prior to injury. These data suggest that normal sensory function is influenced by FKN/CX3CR1 interactions – presumably due to active neuron/microglial cross-talk. However, removal of this regulatory mechanism can be neuroprotective. Supported by NINDS NS37846.

P-12

17B-estradiol is neuroprotective in spinal cord injury in post- and pre-menopausal rats

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Evidence suggests that 17B-estradiol is neuroprotective in several models of central nervous system injury including ischemia, brain injury and, more recently, spinal cord injury (SCI). Since recent trends suggest that SCIs in elderly women are increasing, the effects of menopause on 17B-estradiol mediated neuroprotection in spinal cord injury is relevant. The objective of this study was to evaluate the effect of 17B-estradiol pre-treatment on motor function, neuronal death, and white matter sparing in post- and pre-menopausal rats. One week prior to crush SCI, 2 month-old or 1 year-old female rats were ovariectomized and implanted with a silastic capsule containing either 180ug/ml of 17B-estradiol or vehicle. Additional animals of each age were not ovariectomized. Crush injury was performed in the mid-thoracic region. The Basso, Beattie, Bresnahan (BBB) locomotor test was performed on post-SCI days 3,7,14 and 21. Spinal cord were collected and processed for histology on post-SCI days 1, 7, and 21. We found that 17B-estradiol administration to ovariectomized rats protected motor neurons, decreased apoptosis, increased white matter sparing, and improved recovery of hind-limb locomotion in both the post- and pre-menopausal rats as compared to ovariectomized rats administered vehicle. Also, endogenous estrogen in the non-

ovariectomized young rats was sufficient to reduce cell death, but endogenous estrogen in the older rats was not, suggesting that the native neuroprotection seen in young rats is lost in older animals. These data indicate that 17B-estradiol is neuroprotective in SCI and that the loss of endogenous estrogen-mediated neuroprotection seen in older rats can be attenuated with exogenous administration of estradiol. Supported by the Roman Reed Spinal Cord Injury Research Act.

P-13

Corticospinal damage exacerbates locomotor deficits in rats with cervical dorsal column lesions

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The purpose of this research was to investigate the effects of dorsal column (DC) transection and corticospinal tract (CST) transection on locomotion and skilled forepaw usage in the rat. The CST is an important descending motor pathway in humans, but its pattern of spinal synaptic contacts suggests that it plays a role in modulation of sensory information in most mammals, including rats. In addition, due to the location of the CST in rats, spinal CST transection invariably damages sensory axons in the DC. This confounds interpretation of behavioural deficits after such lesions in rats. In an attempt to differentiate between these deficits, we measured (1) ground reaction forces during overground locomotion, (2) errors during ladder walking and (3) ability to retrieve food pellets, after either DC transection (DC lesions, n=7) or DC+CST transection (DC+CST lesions, n=8). All lesions were inflicted at the 3rd cervical spinal segment. One week after surgery, both DC and DC+CST rats had measurable changes during overground locomotion, made more errors during ladder crossing and were less skilled at retrieving food pellets. Six weeks after surgery, DC rats continued to show changes during overground locomotion but ladder and reaching performance were similar to presurgical performance. In contrast, DC+CST rats continued to show differences in all 3 tests compared to presurgical performance. Thus corticospinal damage exacerbates locomotor deficits in rats with cervical dorsal column lesions. This data suggests that the CST's putative role in modulation of ascending sensory information may be crucial for functional recovery after DC lesions.

P-14

Forced exercise and transplantation affect behavioral recovery from cervical contusion spinal cord injury

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Since cervical (C) level contusion is a common human spinal cord injury (SCI), we selected a unilateral C4 contusion injury model to determine the potential of different interventions to promote recovery of forelimb function. Previously we have shown that exercise after SCI promotes plasticity within the spinal cord and hind limb muscles and that transplants of fibroblasts (Fb) genetically modified to release neurotrophic factors aided functional recovery after a cervical hemisection lesion. Adult female Sprague-Dawley rats (N = 12) received a contusion injury of 200Kdyne delivered by the Infinite Horizon spinal cord injury device (Precision Systems & Instrumentation, Lexington, KY). Nine days later Fbs expressing Brain Derived Neurotrophic Factor (BDNF) (N=6) or unmodified Fbs (N=6) were transplanted into the lesion epicenter. Animals received a standard immunosuppression regimen with Cyclosporine A before and after transplantation to promote graft survival. Forelimb use was increased through a forced exercise wheel system (Lafayette Inst., Lafayette, IN) in a subset of both transplant groups. Over a six week period a battery of behavioral tests were employed to assess recovery of function. Locomotion was evaluated using the qualitative forelimb BBB scale and the quantitative catwalk test that examines parameters of gait. The grip task measured the force generated by the injured forelimb and the cylinder and grid walking tests measured forelimb control. Behavioral results show some promising effects of the combination of exercise and Fb transplants on recovery of function compared to Fb transplant only recipients. The forelimb BBB, grip, and cylinder tests all displayed similar trends of improved activity beginning two weeks after grafting. Ongoing anatomical evaluation will address possible mechanisms by which spinal cord plasticity may lead to changes in functional capabilities of these animals. Supported by NINDS NS26380.

P-15

Neuropathic pain in a rat cauda equina/Conus medullaris injury and repair model

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Cauda equina/conus medullaris (CE / CM) forms of spinal cord injuries (SCI) in humans can result in motor and autonomic dysfunction, as well as neuropathic pain. Our laboratory has developed a rat model of CE / CM SCI in which the ventral roots are avulsed from the lumbosacral spinal cord at the CNS / PNS interface. Our neural repair strategy for this injury involves reimplantation of the avulsed roots into the conus, thereby promoting axonal regeneration and functional reinnervation of peripheral targets. We addressed whether this motor-only lesion and / or the neural repair strategy affects somatosensory pain. Groups included (1) laminectomy controls (Lam), (2) laminectomy + unilateral ventral root avulsion (VRA) at L6-S1, and (3) laminectomy + VRA + immediate reimplantation of both ventral roots into the ventrolateral white matter (VRA+Imp). Somatosensory tests for allodynia and hyperalgesia were performed at the plantar surface of the hind paws, relating to the L5 spinal segment, which is uninjured but adjacent to the injured L6 segment. Data were collected weekly up to 7 wks after injury, and compared to pre-surgical baseline values. Testing for allodynia showed a late onset reduction in the ipsilateral hind paw withdrawal threshold in response to a non-noxious stimulus in the VRA group relative to Lam. The VRA+Imp group, however, showed an early and persistent reduction in the hind paw withdrawal threshold, suggesting on-going allodynia. The hind paw withdrawal latency to a noxious heat stimulus was not different between groups at any time point, suggesting an absence of hyperalgesia. These data may provide insight into possible mechanisms of neural plasticity related to CE / CM SCI and repair, and warrant further examination relevant to nerve reimplantation strategies in the clinical setting. Supported by: Roman Reed Spinal Cord Injury Research Fund of California.

P-16

Functional and anatomical reinnervation of the rat lower urinary tract in a cauda equina/Conus medullaris injury and repair model

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Cauda equina and conus medullaris injuries typically result in loss of bladder control. These types of injuries may include avulsion of dorsal and ventral roots from the spinal cord. We investigated whether implanting avulsed ventral roots into the spinal cord can promote functional recovery of the lower urinary tract. Adult female rats underwent either a sham-operation (n=6), bilateral avulsion of the L5-S2 ventral roots (n=5), or bilateral avulsion of the L5-S2 ventral roots with an acute bilateral implantation of the L6 and S1 ventral roots (n=6). At 12 weeks postoperatively, we performed urodynamic recordings of bladder intravesical pressure and EMG activity of the external urethral sphincter (EUS). In addition, we injected Fluorogold into the bladder wall and Fast Blue into the EUS to retrogradely label neurons that may have reinnervated these peripheral targets. The sham-operated group showed voiding normal bladder contractions coincident with bursting EUS EMG activation resulting in efficient voiding, whereas in the avulsed group the bladder and EUS EMG activities were completely abolished. In contrast to the avulsed group, the implanted group showed a range of recovery in micturition behaviors, including some animals, which had recovered normal-appearing voiding contractions coincident with EUS EMG activation. Autonomic and motor neurons innervating the bladder and EUS were retrogradely labeled in the implanted and sham-operated groups but not in the avulsed group. Our findings suggest that an acute implantation of avulsed ventral roots into the spinal cord may be a promising surgical strategy to repair the lower urinary tract following conus medullaris and cauda equina injuries. Supported by: NIH/NINDS NS042719, The Paralysis Project of America

P-17

MINOCIN®, a Semisynthetic derivative of tetracycline, improves functional outcome after spinal cord contusion injury in adult male rats

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Neuroprotection and improved locomotor function after SCI have been shown in experimental studies with minocycline, a 2nd-generation tetracycline. However, the optimal route, dosage and effective time window of minocycline application have not yet been determined. In the present study, we assessed the efficacy of three doses of intravenously delivered MINOCIN® (an I.V. formulation of minocycline) administered one hour following a moderate contusion of the T9 spinal cord in adult male Wistar rats (OSU device). The spinal cord injuries and insertion of an intravenous drug port were performed blinded to the randomization of the animals into control (Ringer solution) and experimental groups that were intravenously injected with an initial low (10 mg/kg), medium (20 mg/kg), or high (40 mg/kg) dose of MINOCIN® diluted in 1 ml of Ringer, respectively. Thereafter, the animals were injected with half of the initial dose every eight hours throughout four days post contusion injury. BBB and BBB subscores for open field locomotion were given by 2 blinded observers. There were no significant differences of both BBB and BBB subscores between the low and medium dose groups versus controls. BBB scores of the high dose treated group were 13.3 +/- 1.3, 11.7 +/- 0.3, 12.9 +/- 0.9, and 12.1 +/- 0.5 on 21, 28, 35, and 42 days after contusion SCI, which were significantly different from 10.2 +/- 0.6, 10.50 +/- 0.3, 11 +/- 0.5, and 10.8 +/- 0.4 of the control group, respectively ($p<0.05$). Similarly, the BBB subscores of the high dose treated group were significantly higher than those of the controls ($p<0.05$). Thus only high dose of intravenously administered MINOCIN® delayed one hour following a moderate contusion SCI improved open-field locomotion. We are presently assessing tissue sparing in these animals, and in future, the effective time window of MINOCIN® treatment. Supported by: Spinal Research (ISRT.)

P-18

Intermittent food restriction starting at time of spinal cord injury improves functional recovery

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There is a growing awareness for the need of combinatorial treatments for spinal cord injuries in order to add neuroprotective as well as regeneration/sprouting promoting effects. However, there is concern over the practicality and safety of multiple treatments.Calorie restriction has been shown to be neuroprotective as well as stimulating BDNF expression. Here we investigated in adult male rats whether an intermittent food (IF) regimen starting one month prior to injury facilitates functional recovery/compensation after a C4 dorsolateral spinal cord crush. In the cylinder rearing task at 15, 23, and 31 days post injury the IF group had significant more use of their injured limb (use of both + use of injured limb) (56.8 +/- 7.3, 58.8 +/- 3.4, 52.3 +/- 5.3) compared to the ad libitum fed rats (32.3 +/- 5.2, 21.9 +/- 4.4, 30.4 +/- 4.2) ($p < 0.05$). Histology revealed reduced lesion size ($p < 0.05$) in the IF group compared to controls. Subsequently, a second experiment with IF regimen starting at the time of lesion (IF acute) resulted in significantly less injured forelimb errors ($p < 0.05$) than the ad libitum fed controls on the horizontal ladder, but no differences in the cylinder rearing task. Hence, intermittent food restriction starting at time of spinal cord injury, or prior to, improves functional recovery. The mechanism of the neuroprotective effects may be mediated by reduced inflammation resulting in reduced lesion size. Additionally, increased expression of BDNF in the spinal cord and/or the brain, along with reduced GFAP expression could contribute to the observed functional recovery/compensation. Most importantly intermittent food restriction is a safe, and simple, multifaceted treatment that could be clinically implemented immediately to improve functional recovery in human patients.

P-19

Protective effects of motoneuronotrophic factor (MNTF) in spinal cord injury

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Motoneuronotrophic factor (MNTF) is an endogenous neurotrophin, which is highly specific for the human nervous system and it is expressed rapidly during the first trimester of human fetus development of the complete nervous system. MNTF is a neurosignaling molecule that binds with very specific receptors to induce intracellular signaling events, causing desired effects by cascading phosphorylation. The specific functions of MNTF, as demonstrated in animal and in vitro studies, are embryonic stem cell differentiation into motoneurons, motoneuron maintenance and survival, motor axon regeneration with guidance, and reinnervation of target muscles and organs. When the Central Nervous System (CNS) and Peripheral Nervous System (PNS) are under attack caused by diseases, disorders or injuries, MNTF creates a protective and permissive environment for nerve regeneration and repair that are neuroprotective, anti-apoptosis, anti-oxidation, anti-inflammation, and anti-scar. MNTF can cross the blood brain barrier in an effective and efficient way via intravenous (IV) administration. MNTF was tested for efficacy in the spinal cord injury (SCI) mouse model. Mice were subjected to spinal cord impact and 14 days of recovery. The animals were injected intravenously with MNTF at 1 or 5 mg/kg dose levels immediately after the injury and every day for 14 days. The animals were examined for changes in lesion volume (LV) and behavioral recovery (BR) as measured by rota-rod treadmill test and open field score. IV administration of MNTF demonstrated changes in both LV and BR, which showed a dose dependent effect. MNTF at both 1 and 5 mg/kg showed a significant reduction in lesion volume, which translated to improvement in motor functions. These data suggest that MNTF is neuroprotective in the spinal cord following IV injection in the mouse model of SCI.

P-20

Spinal neural circuitry function after injury: Differential recovery in an intersegmental spinal reflex and locomotion

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Locomotion recovers following spinal cord injury (SCI) but it is not clear if this is due to recovered function in neural circuitry spanning the level of injury or in circuitry below that level.

We have studied the recovery of locomotion and function in an intersegmental pain reflex whose neural circuitry spans the level of injury after SCI. The cutaneus trunci muscle (CTM) reflex produces a skin "shrug" in response to a pinch on a rat's back and is mediated by a 3 neuron circuit: dorsal cutaneous nerve (DCN) afferents, ascending propriospinal interneurons, and the CTM motoneuron pool. Recovery of stepping in the hindlimbs was evaluated by open field scoring (BBB) and by stimulation of a supraspinal locomotor center, the hypothalamic locomotor center (HLR). Rats underwent thoracic cord contusions and weekly evaluation with open field locomotor scoring (BBB). At 1 or 3 weeks post-injury, animals underwent electrophysiological testing of the CTM reflex and anatomical measurement of their SCI. At the same time points, similar animals underwent HLR stimulation to evoke hindlimb stepping.

At 1 week, BBB scores, CTM reflex function, and percent spared spinal cord tissue were correlated. At that time, only animals that stepped in the open field showed hindlimb stepping in response to HLR stimulation. At 3 weeks, BBB scores had increased but reflex function and anatomical measures did not change. At this point, animals that showed hindlimb stepping in the open field did not frequently do so with HLR stimulation. Open field locomotor recovery becomes dissociated from intersegmental reflex function, percent spared spinal cord tissue, and the ability to demonstrate hindlimb stepping in response to supraspinal locomotor center stimulation suggesting that early locomotor recovery after SCI is the result of plasticity within spinal neural circuitry below, and not across, the level of injury. These findings suggest that CTM reflex function and/or HLR evoked hindlimb stepping may be more sensitive assays of translesional repair in SCI than open field locomotor recovery.

P-21

Step training and OEG transplantation improve weight-bearing hindlimb plantar stepping and step kinematics in adult paraplegic rats

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Following a complete spinal cord transection at T8-9 in adult rats, we combined the therapies of olfactory ensheathing glia (OEG) transplantation and treadmill step training to promote recovery of hindlimb movement. Immediately following transection 20 rats received injections of OEG cells and18 control animals received injections of media. Eight sham animals were used as unlesioned controls. One month after surgery half of each group began a regimen of weight-bearing hindlimb treadmill step training 20 minutes/day, 5 days/week for 6 months. We report a significant difference in the recovery of the number of weight-bearing hindlimb plantar steps performed by all OEG (7±1.8 steps) versus all media animals (0±1.9 steps; P=0.002) at 7 months. However, treadmill step training failed to improve plantar stepping in media injected animals while OEG trained animals significantly improved between 4 and 7 months (4±1.5 to 9±2.2 steps; P =0.006). This significant improvement was not seen in OEG non-trained animals (2±1.6 to 6±2.8 steps, P=0.14). We analyzed step frequency using the Fast Fourier transformation and movement pattern using the Continuous Wavelet Transform and found significant differences between the OEG and media animals ($P < 0.05$). We measured the volume of the lesion site and found that OEG transplanted animals averaged 1.8mm3±0.3, a volume significantly less than the 3.3mm3±0.5 found in media-injected animals (P =0.035). We also ranked the gross morphological appearance of the lesion sites and determined that tissue sparing is associated with improved stepping. Moreover, regener-

ation of 5-hydroxytryptamine- and dopamine b-hydroxylase-positive axons was demonstrated in OEG-injected animals only. Together these data suggest that task specific training can enhance the restorative potential of OEG transplantation and that combining cell transplantation and rehabilitative therapies can improve functional recovery beyond that acquired with either therapy alone. Supported by the Christopher Reeve Paralysis Foundation and NIH.

P-22

The neural pathway underlying the expression of crossed phrenic activity following spinal cord hemisection in the neonate rat

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In adult rats, C2 spinal cord hemisection results in paralysis of the ipsilateral hemidiaphragm. Recovery of diaphragmatic function can be established by the activation of crossed phrenic pathways. In neonates, however, crossed phrenic pathways are functionally expressed in P0-P4 neonatal rats. Moreover, the functional and anatomical connections between the rostral ventral respiratory group (rVRG) and the phrenic motor neurons are different in neonates and adults. In adults, the crossed phrenic pathway has been identified as collaterals from crossed and uncrossed axons of the rVRG projecting down the spinal cord and crossing the midline to synapse with contralateral phrenic motoneurons. In neonates, the pathway which is responsible for spontaneous expression of the crossed phrenic activity is unknown. Therefore, this study was designed to delineate the pathway underlying the expression of crossed phrenic activity in neonate rats. WGA-HRP, a retrograde transynaptic tracer, was utilized to trace the source of the neurons which mediate the spontaneous crossed phrenic activity. Neonate rats (P0-P4) were hemisected under ketamine/xylazine anesthesia and WGA-HRP injected into the phrenic nerve ipsilateral to the hemisection. After two days, the rat was euthanized and the tissue prepared for cryostat sectioning and staining. The results show that the rVRG neurons which drive the crossed phrenic activity in neonates are unilaterally located on the side ipsilateral to the hemisection. There was no transynaptic labeling of propriospinal neurons in the spinal-hemisected neonatal rats indicating a monosynaptic projection to phrenic motoneurons. The present results suggest that rVRG neurons unilaterally project to the contralateral phrenic motoneurons and send crossing midline collaterals to ipsilateral phrenic motoneurons to mediate the spontaneous expression of crossed phrenic activity in the neonatal rat. These results are important to our complete understanding of the mechanisms which govern motor recovery in mammals following spinal cord injury. Support from NIH Grant HD31550

P-23

Increased close appositions between corticospinal tract axons and spinal sympathetic neurons after spinal cord injury in rats

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Treatments for spinal cord injury may promote new spinal cord synapses. However, the potential for new synapses between descending somatomotor and spinal sympathetic neurons has not been investigated. We studied rats with intact spinal cords and rats after a chronic, bilateral, dorsal spinal hemisection. We identified sympathetically-related spinal neurons by transynaptic, retrograde transport of renally-injected pseudorabies virus. We counted retrogradely-labeled sympathetic preganglionic neurons (SPN) and putative sympathetic interneurons (IN) that, under light microscopy, appeared closely apposed by anterogradely-labeled axons of the corticospinal tract (CST) and by axons descending from the well-established sympathetic regulatory region in rostral ventrolateral medulla (RVLM). Spinal sympathetic neurons that were closely apposed by CST axons were significantly more numerous in lesioned rats than in unlesioned rats. CST axons closely apposed 5.4% of SPN and 10.3% of IN in rats with intact spinal cords and 38.0% of SPN and 37.3% of IN in rats with chronically-lesioned spinal cords. Further, CST appositions in SCI rats consisted of many more varicosities than those in uninjured rats. SPN and IN closely apposed by axons from the RVLM were not more numerous in lesioned rats. However, RVLM axons apposed many more SPN than IN in both control and lesioned rats. Therefore RVLM sympathoexcitation may be mediated largely by direct synapses on SPN. Although we have not determined the functional significance of close appositions between the CST and spinal sympathetic neurons, we suggest that future studies of spinal cord repair and regeneration include an evaluation of potential, new, somatic-autonomic interactions.

P-24

Limited recovery of reaching kinematics in rats after cervical dorsolateral funiculotomy

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The rubrospinal system contributes to skilled reaching in animals. In the rat, the rubrospinal tract makes direct projections onto motoneurons of the forelimb musculature that controls the wrist and digits, and lesions to the rubrospinal system can impair forelimb function. Limb function in rats has been largely assessed by qualitative measures, for example deficits have been described in digit abduction, digit flexion during grasp, and forepaw pronation and supination (Schrimsher & Reier, 1992; McKenna and Whishaw, 1999). We used kinematic analysis to identify specific deficits in limb function such as velocity, position of the digits, and trajectory. Ten adult female Sprague-Dawley rats (225-250 g) were selected to receive a lesion of the right cervical dorsolateral funiculus based on their preference to use their right forelimb to reach for single pellets. Kinematic assessment was performed using a high-speed (500 frames/sec) digital camera recording system. Each animal's right forelimb wrist and digits were inked so that their movements could be digitized during frame-by-frame analysis using tracking software. Quantitative measures of peak wrist velocity, path-length ratio of digit trajectory, amount of pronation excursion of the forepaw, and the amount of digit abduction were made from the digitized movements. All measures

showed deficits in performance from baseline to 1-wk followed by limited recovery at 8-wks. Kinematic analysis of single pellet reaching thus enables quantitation of individual reaching elements, which may be used to assess the effects of interventions aimed at promoting recovery of forelimb function after spinal injury. Supported in part by Grant# 2378 from the PVA Research Foundation & P01 NS.

P-25

Activity-regulated cytoskeletal-associated (Arc) protein upregulation following C2 hemisection in rats

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A C2 hemisection in the spinal cord results in paralysis of the ipsilateral hemidiaphragm. This paralysis is a result of the interruption of the descending bulbospinal pathway from the rostral ventral respiratory group (rVRG) to the phrenic nucleus (PN), which innervates the diaphragm. It has been demonstrated that functional activity can be returned to the paralyzed hemidiaphragm through the activation of a latent crossed phrenic pathway. Previous studies in our laboratory have shown that synaptic changes occur in the PN following a C2 hemisection.

One possible mediator of synaptic plasticity and long term potentiation is the protein called activity-regulated cytoskeletal-associated protein (Arc). It has been shown by others that Arc mRNA is immediately transcribed and translated following the onset of synaptic activity. Furthermore, Arc mRNA is directed to recently activated excitatory post-synaptic sites through NMDA receptor activation. In the present study, we hypothesize that following C2 hemisection and the activation of the latent crossed phrenic pathway to restore activity, Arc protein is upregulated on phrenic motor neurons. Female rats received a left C2 hemisection and were assessed for complete paralysis of the ipsilateral hemidiaphragm.

Following this assessment the crossed phrenic pathway was activated through: 1) contralateral phrenicotomy in acutely hemisected animals, or 2) spontaneous activation, which occurs in chronically hemisected animals. Arc protein levels were evaluated through both immunocytochemistry and Western Blot analysis. Both analyses suggest that there was an upregulation of Arc protein in C2 hemisected animals and C2 hemisected animals with recovery compared to naïve and sham hemisected animals.

P-26

The phosphodiesterase inhibitors Pentoxifylline and Rolipram induce hemidiaphragm recovery following cervical spinal cord hemisection

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High cervical spinal cord hemisection results in the interruption of the descending respiratory drive from the medulla to the ipsilateral phrenic motoneurons, consequently leading to the paralysis of the ipsilateral hemidiaphragm. Previous studies have shown that chronic oral administration of theophylline can restore function to the quiescent phrenic nerve and hemidiaphragm ipsilateral to hemisection. The theophylline mediated respiratory recovery persists long after the animals have been weaned from the drug. Theophylline is a phosphodiesterase (PDE) inhibitor and an adenosine receptor antagonist. However, the cellular mechanisms underlying the recovery induced by theophylline are still not known. Since the PDE inhibitory characteristics of theophylline can increase the levels of cAMP and cAMP has been implicated in neuroplasticity, it is reasonable to postulate that theophylline may mediate recovery by elevating the levels of cAMP. In the present study, we tested whether chronic administration of rolipram (ROL), a selective type IV PDE inhibitor, and pentoxifylline (PTX), a general PDE inhibitor, can reproduce the recovery induced by theophylline in male Sprague Dawley rats. Following a left C2 spinal cord hemisection, ROL (2 mg/kg) or PTX (20 mg/kg) was administered chronically (3 times/day) for 3 days. Recovery of left phrenic nerve activity was assessed at 2, 5 or 10 days following the last administration of the drugBoth PTX and ROL induced a persistent recovery of the phrenic nerve ipsilateral to the hemisection. These results indicate that cAMP-dependent processes are involved in the respiratory plasticity that promotes functional recovery following hemisection. Therefore, agents that elevate the levels of cAMP may be therapeutically useful in promoting functional recovery following spinal cord injury. Supported by NIH Grant HD 31550.

P-27

Alginate-based anisotropic scaffolds as guiding structure for directed axonal regrowth in the injured spinal cord

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Appropriate target reinnervation and functional recovery after spinal cord injury depends on longitudinally directed regrowth of transected axons. To assess the capacity to promote directed axon regeneration, highly anisotropic alginate-based scaffolds were introduced into an axon outgrowth assay in vitro and rat spinal cord lesions in vivo. In an entorhino-hippocampal slic eculture model, alginate-based scaffolds elicit highly oriented linear axon regrowth and appropriate target neuron reinnervation. After implantation into acute cervical spinal cord lesions in adult rats, alginate-based gels integrate into the spinal cord parenchyma without major inflammatory responses, maintain their anisotropic structure and in parallel to findings in vitro induce directed axon regeneration across the scaffold. Furthermore, adult neural progenitor cells, which have been shown to promote cell-contact mediated axon regeneration, can be seeded into alginate-based scaffolds as a prerequisite to further improve the regenerative capacity of these artificial growth supportive matrices. Thus, anisotropic alginate-based scaffolds represent a promising strategy to induce directed nerve regrowth following spinal cord injury.

P-28

Fibrin-based drug delivery scaffolds for treatment of spinal cord injury

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Exogenously delivered neurotrophic factors show promise as potential therapeutic treatments for spinal cord injury. These factors can be delivered by tissue-engineered scaffolds, which can also serve as physical bridges across the lesion site during regeneration. We have developed a fibrin-based delivery system to provide sustained delivery of growth factors by slowing diffusion-based release from a fibrin scaffold. This system has been shown to promote neurite extension from embryonic chick dorsal root ganglia and stimulates axonal sprouting in injured rat sciatic nerve. Here, we evaluated the use of this delivery system to release neurotrophin-3 (NT-3) after rat spinal cord injury. Adult female rats were injured by the complete 2-3 mm ablation of the spinal cord (T9) by aspiration. The lesions were treated with Tris-buffered saline (TBS) injection or implantation of fibrin scaffolds containing NT-3 at varying doses with or without the delivery system. Spinal cord were explanted after 9 days and examined by immunohistochemistry for neurons, astrocytes, macrophages, oligodendrocytes, and neuronal subpopulations. TBS-treated animals developed a large cavity at the site of injury that was surrounded by a dense glial scar. All implanted fibrin scaffolds increased cellular infiltration of the lesion site and integration of the lesion with caudal and rostral segments of the cord. Controlled delivery of NT-3 at an optimal dose of 1000 ng/mL reduced glial scar formation especially at the border of the lesion with surrounding white matter. Controlled delivery of NT-3 at the optimal dose showed enhanced neural fiber sprouting into the lesion vs. NT-3 without the delivery system. Infiltrating neural fibers included those that stained positive for calcitonin gene-related peptide (sensory neurons) and choline acetyltransferase (primary motor neurons). This result demonstrates that the controlled fibrin-based delivery of NT-3 can be used to create a more permissive environment that may promote spinal cord regeneration.

P-29

Human neural stem cells overexpressing Olig2 promotes locomotor recovery in rats with contusive spinal cord injury

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Cell replacement therapy with stem/progenitor cells holds promise for improving functional outcome after various CNS injuries. Contusive injury to the spinal cord is complicated by a delayed loss of oligodendrocyte and demyelination. Transplantation of neural stem cells that are genetically modified to differentiate into oligodendrocyte lineage cells, therefore, could be a rational strategy to repair the injured spinal cord. We have previously established stable cell lines of immortalized human neural stem cells using a retroviral vector encoding v-myc. One of the cell lines, HB1.F3, was transduced with a retroviral vector carrying a full length coding region of bHLH transcription factor Olig2. Overexpression of Olig2 in F3.Olig2 cells resulted in a forced differentiation into oligodendrocytes in vitro, as evidenced by expression of cell type-specific markers including O4, galacto cerebroside and CNPase. F3.Olig2 cells also showed the evidence of myelin formation in vivo in shiverer mouse brain. F3.Olig2 cells were transplanted into rat spinal cord rostral and caudal to the epicenter 7 to 8 days after a T9 contusive injury. Both F3 and F3.Olig2 cells were found to migrate from injection sites to white matter, and F3.Olig2 cells more frequently positioned in the white matter as compared to F3 cells. Animals transplanted with F3.Olig2 cells showed enhanced recovery in open field locomotion compared to either PBS-injected control or F3 cells transplant group. The present study suggests that transplantation of oligodendrocytes derived from neural stem cells is an effective strategy to promote locomotor recovery in experimental animals with spinal cord injury.

P-30

Delayed transplantation of adult neural stem cells promotes remyelination and functional neurological recovery after spinal cord injury

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Spinal cord injury (SCI) results in loss of oligodendrocytes and demyelination of surviving axons causing functional impairment. Spontaneous remyelination is very limited, and thus cell replacement therapy is an attractive approach for myelin repair. In this study, we transplanted adult brain-derived neural precursor cells (NPCs) isolated from YFP transgenic mice into the injured spinal cord of adult rats at two and eight weeks after injury, which represent the subacute and chronic phases of SCI. A combination of growth factors (EGF, bFGF, PDGF-AA), minocycline and cyclosporineA immunosupression was used to enhance the survival of transplanted adult NPCs. Our results show the presence of a substantial number of surviving NPCs in the injured spinal cord up to ten weeks after transplantation at the subacute stage of SCI. In contrast, cell survival was poor after transplantation into chronic lesions. After subacute transplantation, grafted cells migrated more than 5 mm rostrally and caudally. The surviving NPCs integrated principally along white matter tracts surrounding the lesion site and displayed close contact with the host cellular profiles including axons and glial cells. Approximately 50% of grafted cells formed either oligodendroglial precursor cells (19%) or mature oligodendrocytes (33%). NSC-derived oligodendrocytes expressed myelin basic protein and ensheathed the axons. We also observed that injured rats receiving NSC transplants had improved functional recovery as assessed by BBB, grid-walk and foot print analyses. Our data provide strong evidence in support of the feasibility of adult NPCs for cell-based remyelinating therapies at the subacute stage of SCI. For chronic SCI, further investigations are needed to identify those factors required to allow survival and differentiation of engrafted adult NPCs.

P-31

CGRP and GAP43 increase and colocalize in cervical dorsal horns of allodynic rats following SCI and stem cell transplantation

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Introduction: Although transplantation of neural stem cells (NSC) in the injured spinal cord may improve functional outcome, we have consistently observed forelimb allodynia, the mechanism of which remains poorly understood. Such forelimb hypersensitivity may hinder the application of NSC treatment in SCI. In the present study, alterations of primary afferent pathways rostral to injury and tranplantation are investigated with GAP-43, which identifies sprouting neurites, and CGRP, a well-characterized nocioceptive neuron marker.

Methods: Reproducible, moderate spinal cord injuries (10g from 25 mm) were produced in 25 Sprague-Dawley rats using the NYU Impactor model. At post-injury day 8, animals were randomly selected to receive either C17.2 NSC, (9); GDNF transfected C17.2 NSC, (13); normal saline (NS), (3); or sham operated (3). BBB scoring assessed locomotor function/recovery and hotplate testing measured sensory responses. Animals survived 42 days post injury. C6-T1 spinal cords were processed for CGRP/GAP43 immunohistochemistry. Density of immunoreactivity (IR) was measured and characterized.

Results: Locomotor function was not significantly improved in NSC treated animals at any time period when compared to NS, $p > 0.05$. Significant forelimb thermal allodynia was observed following transplantation with both NSC populations, $p < 0.05$. GDNF transfection failed to show a significant motor or sensory effect when compared to native C17.2 NSC. Sham treatment resulted in no locomotor or sensory dysfunction. Significant bilateral axonal sprouting was demonstrated by increased GAP-43-IR in NSC ($p < 0.5$) but not NS treated cords ($p > 0.5$). Similarly, CGRP significantly increased and colocalized with GAP-43-IR further suggesting sprouting of nocioceptive primary afferent fibers, $p < 0.05$.

Conclusion: Nocioceptive afferent sprouting may represent aberrant changes in pain pathways, reflecting a primary mechanism of allodynic pain following NSC treatment of the injured spinal cord. Understanding these changes may direct potential treatments toward minimizing post-injury and NSC transplant allodynia.

P-32

Transplantation of adult neural stem cells into the spinal cord of Shiverer mice induces remyelination and restores the normal localization of axonal K+ channels

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Disruption of myelin due to trauma or disease results in changes in the function and molecular organization of axons. Transplantation of adult neural stem cell (aNSC) is a potentially attractive therapeutic approach for myelin repair. In this study, we investigated the potential of aNSC transplantation to induce remyelination and restoration of normal axonal K+ channel localization in spinal cord axons of shiverer mutant mice, which lack myelin basic protein (MBP) and central myelin. Adult NSCs from YFP-transgenic mice were transplanted into the spinal cords of shiverer mice. A combination of the growth factors bFGF, EGF and PDGF-AA was infused intrathecally to enhance the survival of transplanted NSCs. Six weeks post-transplantation, a substantial number of transplanted donor-derived aNSCs migrated within the host spinal cord tissue. These cells showed oligodendrocyte-like morphology and were well integrated along white matter tracts in close contact with the host axons. The majority of donor-derived aNSCs expressed markers for mature oligodendrocytes and MBP. Electron microscopic assessment confirmed the ability of these cells to form compact myelin. In contrast to non-transplanted shiverer mice, in which K+ channels show a dispersed distribution along axonal internodes, the transplanted segments of the shiverer spinal cord showed evidence of restoration of the distribution of K+ channels to normal juxtaparanodal location. This is the first study to show the ability of aNSCs to promote remyelination and reorganization of axonal membrane K+ channels in dysmyelinated CNS. Using adult NSCs may represent an important approach to achieve remyelination and functional repair of conditions associated with the loss or absence of myelin.

P-33

Human embryonic stem cell-derived oligodendrocyte progenitors induce neurite branching and neuron survival in vitro and following transplantation into spinal cord injury

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Human embryonic stem cells (hESCs) hold exciting clinical potential in part because they are pluripotent cells that are capable of self-renewal and have the capacity to develop into essentially any human cell type. Our lab has recently demonstrated the ability to direct hESCs to a high purity population of oligodendrocyte progenitor cells (OPCs). Recent data from our lab demonstrates that transplantation of hESC-derived OPCs into a contusion spinal cord injury (SCI) resulted in formation of compact myelin and recovery of locomotor function (Keirstead et al., 2005).

These OPCs may also enhance repair of surrounding neural tissue after SCI. A growing literature suggests that oligodendrocytes provide trophic support for neurons and axons.

In this study, we investigated whether transplanted hESC-derived OPCs influenced neuron survival and neurite outgrowth in vitro and in vivo. Our findings indicate that hESC-derived OPCs produce soluble factors that promote neuronal survival and enhance neurite outgrowth both in vitro and in vivo, and suggest that transplant-mediated functional recovery of spinal cord injured animals is due to multiple roles of transplanted cells.

P-34

Advances in utilizing neural precursors in spinal cord injury

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We are interested in the therapeutic potential of neural stem cell transplants and have focused our studies on the fate of the cells in the adult CNS and the development of effective grafting protocols to treat SCI. We have shown that neural precursors (NPC) composed of neuronal and glial restricted precursors (NRP and GRP, respectively) can be isolated from transgenic AP rats and used for reliable fate analysis. When grafted into the adult CNS, NPC survive for over a year, migrate and differentiate into distinct neural phenotypes, including neurons, while multipotent cells do not. Graft-derived neurons integrate into the host tissue, and project long axons in response to exogenous BDNF. EM analysis shows formation of synapses between graft and host neurons. Furthermore, when mixed NRP/GRP are grafted into the injured spinal cord they also show robust survival, migration and neuronal differentiation, most likely because of the supportive microenvironment provided by graft-derived astroglia. The clinical relevance of this grafting strategy has been addressed by examining minimally invasive delivery and tracking methods that prevent further damage and simplify translation. We have shown that NPC, delivered intrathecally by lumbar puncture (LP), can be targeted to cervical and thoracic injuries. After LP delivery, grafted cells accumulate at the injury site with few cells present elsewhere, suggesting that factors present at, or released from the injured tissue, play a role in cell adherence and/or homing. For noninvasive analysis NPC can be labeled in vitro with the superparamagnetic iron oxide contrast agent Feridex, transplanted into the rat spinal cord, and tracked ex vivo using MRI. This imaging procedure corresponded with AP histochemistry by showing graft migration of NPC up to 5mm. These studies define a framework for using NPC grafts in repair of SCI, while ongoing experiments examine the specific mechanisms associated with recovery of function.

P-35

Formation of new oligodendrocytes in an NG2-reactive zone in the contused rat spinal cord

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Demyelination and loss of oligodendrocytes (OLs) are serious pathological consequences of spinal cord injury (SCI). In the adult CNS, however, an endogenous population of oligodendrocyte progenitor cells (OPC) exists, which can proliferate and give rise to new myelinating OLs. Previous data from our laboratory showed a marked increase in OPC proliferation after spinal contusion. Hence, we hypothesized that OPC proliferation is associated with formation new OLs after SCI. To examine this, we used a clinically relevant model of moderate spinal contusion in rats. Rats received BrdU during the first week post-injury (pi) to label dividing cells and were perfused at 3, 7 or 14dpi. Proliferating OPCs (BrdU/NG2+), total number of OLs and the number of new OLs were counted along the rostral-caudal extent of the lesions. Overall, OPC proliferation was elevated throughout the injured spinal cords during the first 3dpi and BrdU+ NG2 cells were maintained at 14 dpi. In course of our study, we noticed the band of tissue located between the lesion and spared tissue, which exhibits astrocyte hypertrophy, was also highly reactive for NG2 by 7dpi. This reactive zone usually contained axons and little to no myelin. BrdU+ NG2 cells were especially prominent in this reactive band of tissue. We also saw elevated OL numbers in this same region at 14 dpi, the density of which was significantly greater than that in the spared tissue within the same cross-sections. Ongoing studies have revealed that many of the OLs in this reactive zone are BrdU+ and therefore are indeed new. Occasional new OLs are also noted in spared white matter, but only very rarely within lesioned tissue. Hence, we propose that proliferating OPCs may contribute to repair processes after SCI by generating new oligodendrocytes. This oligodendrocyte genesis is especially robust in the NG2-reactive zone bordering the lesion. Studies were supported by NINDS043494.

P-36

Axonal degeneration induces NG2+ oligodendrocyte progenitor cell proliferation and oligodendrocyte generation

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After spinal cord injury (SCI), oligodendrocyte (OL) apoptosis has been described in areas of axonal degeneration. However, whether these are causally related is unknown. We investigated this issue following unilateral dorsal rhizotomy, which produced axonal degeneration in the ipsilateral dorsal column; the spared side was used as a control. 30 Long-Evans female rats were sacrificed after 8 hour, 1d, 3d, 5d and 8d. OLs and oligodendrocyte progenitor cells (OPC) marked by CC1 and NG2 were identified as apoptotic by condensed/fragmented nuclei, cleaved caspase3 upregulation and p75 expression. However, apoptosis of OL and OPC was not seen post rhizotomy; instead, a significant increase of OLs was seen at day 5, following a dramatic increase of OPCs at day 3. NG2 and CC1 double labeled cells were rarely observed. This suggests a proliferation and then differentiation of OPCs to OLs in response to axonal degeneration. Further study with BrdU injections supported this conclusion by revealing many BrdU+ OLs at day 5, showing that they were derived from proliferating cells. Thus, the above evidence demonstrates that axonal degeneration alone does not result in OL apoptosis at least within 8 days; rather it induces an early proliferation and differentiation of OPCs to OLs. The earlier described OL apoptosis after SCI might have resulted from additional mechanisms such as excitotoxicity.

P-37

Transplanted astrocytes derived from embryonic glial precursors promote robust axon growth and functional recovery after spinal cord injury

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Several major goals in repairing adult central nervous system (CNS) injuries are to suppress scar formation, support robust axon growth across the injury site, enhance neuroprotection and promote functional recovery. We now report that transplantation of astrocytes derived from embryonic glial-restricted precursors (GRPs) is by itself sufficient to achieve all of these goals after acute transection injuries of the adult rat spinal cord. Transplantation of GRP-derived astrocytes (GDAs) into dorsal column injuries promoted growth of >60% of sensory axons into lesion centers, with 66% of these axons extending beyond injury sites at 8 days post injury. Acute transplantation of GDAs into rubrospinal tract injuries also promoted growth of RST axons for up to 3mm beyond injury sites by 5 weeks and a marked suppression of atrophy of axotomized red nucleus neurons. Grid Walk analysis of GDA transplanted RST lesioned rats revealed a striking70% recovery in locomotor function at 1 month over controls. GDA transplantation also induced a remarkable linearization of the injured tissue and dramatically reduced the initial scarring reaction. Thus, GDAs represent a novel and highly effective cell type with which to repair the damaged CNS.

P-38

Lamina propria and olfactory bulb ensheathing cells behave differently following transplantation into the injured spinal cord

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The olfactory neuraxis is unique in its ability to replace injured or dying neurons with new olfactory receptor neurons (ORNs) that extend an axon through the peripheral nervous system and into their target, the olfactory bulb, in the central nervous system. Olfactory ensheathing cells (OECs) are unique glia of the olfactory system that allow penetration of peripheral ORN axons during development and throughout life, a property that has prompted their use in spinal cord injury (SCI) treatment. Less developmentally mature OECs can be harvested from the lamina propria, an accessible source in the human, however most transplantation experiments to date have used centrally-derived and more mature olfactory bulb (OB) ensheathing cells. We have therefore compared the abilities of GFP+ mouse LP and OB OECs transplanted into a rat rubrospinal crush, to modify the lesion site and promote sprouting/regeneration. We have found that LP OECs exhibit a heightened ability to migrate within the injured spinal cord in comparison with OB OECs, which decreases cavity formation and increases sprouting and regneration of axons into the lesion site. These properties of LP OECs in the spinal cord correlate well with their developmental role of guidance, growth promotion, and support within the olfactory system.

P-39

Survival, migration and differentiation of bone marrow stromal cells grafted in the moderately contused adult rat thoracic spinal cord

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Stem cells are defined by their capacity for self renewal and differentiation into different cell types. The bone marrow stromal cell (BMSC), a multipotent stem cell from the mesodermal germ layer, can differentiate into cells from the neural lineage, which has opened avenues for use in CNS repair. The application of BMSCs in vivo for repair of the injured spinal cord has sparsely been studied. Here, we have investigated survival of BMSCs in the contused rat thoracic spinal cord following grafting at different times after injury. We also investigated BMSC migration and differentiation into neural cells. BMSCs were harvested from 8 week-old female Sprague-Dawley rats by aspirating bone marrow from femurs and tibias according to a previously published protocol. Cells were infected with lentiviral vectors encoding for green fluorescent protein (GFP) prior to transplantation of 1 x 106 cells into the injured cord at 15 min, and 3, 7, and 21 days post injury. Rats survived for 15 min, 3, 7, and 28 days after transplantation. During the first 3 days after transplantation robust BMSC death was observed. During the following weeks, BMSC death continued, such that at 28 days after transplantation only a few living BMSCs could be recognized. At that time, the injury area contained cavities and mainly cellular debris and macrophages. We observed that grafted BMSCs migrated away from the injury site and integrated into the surrounding spinal tissue. We also found grafted (GFP-positive) BMSCs expressing Tuj1, indicating their differentiation into neurons. These Tuj1/GFP positive cells were located in the tissue nearby the contusion injury. In addition to these findings we also observed a high number of nestin-positive cells, which had formed an extensive network of immature glial cells at the injury site. This work was funded by Netherlands Organization for Scientific Research (NWO).

P-40

Mouse and human skin-derived precursors integrate, promote axonal regeneration and produce myelin after transplantation into rat spinal cord contusion injuries

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We have recently discovered neural crest-related precursors isolated from rodent and human skin referred to as SKPs (skin derived precursors; Toma et al. 2001; Fernandes et al. 2004). These SKPs can be pre-differentiated in vitro to assume Schwann cell properties (SKP-SCs). In vivo both naïve SKPs and SKP-SCs have been shown to produce myelin (McKenzie et al. 2005). Here, we tested the behavior of SKP-SCs and naïve SKPs isolated from YFP-expressing transgenic mice after transplantation of 8x105 cells into the lesion epicentre of adult rat spinal cord 7 days after contusion injury.

The transplanted SKPs and SKP-SCs survived well, filled the contusion sites and integrated into the host spinal cord. The SKPs and SKP-SCs stained negatively for astrocytic (e.g., GFAP) and neuronal/axonal markers (e.g., nestin, beta-III-tubulin, TH) and positive for p75, indicative of their Schwann-like properties. Both cell types attracted abundant growth of neurofilament/tubulin positive axons and some axons stained positive for TH/SERT, suggesting a likely brainstem origin. Naïve SKPs and SKP-SCs produced myelin around large axons. These myelin sheaths were positive for MBP and confocal microscopy revealed that the MBP was embedded in YFP positive cells extending along adjacent axons. Importantly, the YFP positive cells induced the expression and appropriate placement of the potassium channel Kv1.2 and the paranodal adhesion protein Caspr along the paranodes of newly formed myelin.

These results indicate that SKPs/SKP-SCs show a remarkable capacity to integrate, promote axonal growth and myelinate within the injured rat spinal cord. Both cell types also demonstrated improvement in locomotor function compared to control animals up to 9 weeks post-injury. Similar results were found in a group of contused rats implanted with naïve SKPs isolated from human skin. Given their accessible and ethically acceptable source, these cells may become attractive candidates for clinical autotransplantations.Financial support provided by: NeuroScience Canada Foundation, Canadian Institutes for Health Research, and the Michael Smith Foundation for Health Research.

P-41

Early death of Schwann cells following transplantation into the contused adult rat spinal cord: A quantitative analysis of cell survival, mechanisms of cell loss and host Schwann cell invasion

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Despite the ability of transplanted Schwann cells (SCs) to promote axon regeneration and remyelination, very little is known about how well SCs survive following transplantation into the injured spinal cord. Recently we have shown that labeled SCs from transgenic rats expressing alkaline phosphatase die early after transplantation and that their survival is enhanced by delaying transplantation. In the current study we examined the loss of SCs over time following delayed transplantation. Green fluorescent protein (GFP)-labeled SCs (2.5x105, 5x105, 1x106, 2x106) were transplanted into the lesion 7 days after a moderate contusion (10 g x 12.5 mm; NYU device injury) and the number of surviving SCs and the volume of the transplant were quantified (10 mins., 6 hrs, 7 days or 28 days). The results indicate an early and extensive loss of SCs, with only 5-15% of SCs surviving beyond 1 week. Examination of transplanted SCs for activation of calpain and caspase-3 implicate both necrosis and apoptosis in SC death within the first 3 days. Quantification of p75 staining, a marker expressed by SCs and commonly used to identify transplanted SCs, revealed that the majority of p75 staining at 28 days did not correspond with GFP-labeled cells, suggesting invasion of host SCs. The similarity in the extent of cell survival, and the time course and mechanism of cell death between SCs transplanted into the injured spinal cord and mesencephalic neurons transplanted into the striatum in models of Parkinson's disease (Emgard et. al., 2003), suggests that common mechanisms may be at play in the acute loss of transplanted cells in the CNS. Future studies will focus on enhancing the survival of transplanted SCs and assessing the effects of enhanced transplant survival on regeneration and myelination. (Support: ISRT NET-002; NIH grants 009923, 038665; and Lois Pope LIFE Fellowship to C.E.H.)

P-42

Boost injection of Schwann cells promotes delayed axonal regeneration and myelination into poly (D,L-lactic acid) macroporous guidance scaffolds grafted into the transected adult rat thoracic spinal cord

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We have demonstrated that axonal growth into a freeze-dried poly(D,L-lactic acid) macroporous scaffold seeded with Schwann cells (SCs) transplanted into the transected adult rat thoracic spinal cord is limited due to, at least in part, poor SC survival. Here we have investigated whether delayed grafting of additional SCs by injection into the scaffold would enhance axonal regeneration and myelination, and hind limb function. Scaffolds seeded

with SCs were implanted in the transected spinal cord of adult female Fisher rats. Prior to seeding, the SCs were lentivirally transduced to produce and secrete D15A, a bi-functional neurotrophin with BDNF and NT-3 activity, and to express green fluorescent protein (GFP), or with SCs expressing GFP only (control). Five weeks later, 5x105 SCs similarly transduced were injected in the center of the scaffold. Control rats received the scaffold with SCs but not the boost injection. At 8 weeks after the additional injection of SCs, the scaffolds were well integrated in the cord and had caused minor loss of spinal tissue at the graft-cord interfaces with no apparent differences between groups. The extra SCs survived within the scaffold and were closely related to neurofilament-positive axons. In rats that received an additional injection of D15A-SCs more myelinated axons were present within the scaffold (462 ± 167.4) than in controls (119 ± 86). Retrograde tracing revealed that axons did not grow from the scaffold into the caudal cord. Using the BBB-test, we observed a similar behavioral improvement in the hind limbs in all groups. Our results demonstrate that the poly(D,L-lactic acid) macroporous scaffold integrates well in the injured spinal cord. In addition, we demonstrate that axonal regeneration and myelination into a SC-seeded scaffold in the transected adult rat spinal cord can be enhanced via an additional injection of SCs. This work was supported by the Miami Project.

P-43

Gene transfer of constitutively-activated MEK or ERK at the neuronal soma by adeno-associated viral vectors to induce axonal regrowth across Schwann cell bridges implanted into the completely transected rat thoracic spinal cord

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Spinal cord injury (SCI) results in the disruption of axonal connections between supraspinal areas and their targets in the lower spinal cord, resulting in varying degrees of neurological dysfunction, including paralysis. A major goal of spinal cord repair is to overcome the inability of severed axons to regenerate by altering either the extrinsic environment of the injured spinal cord or the intrinsic growth ability of the injured neuron. Among therapeutic interventions, the delivery of a myriad of neurotrophic molecules has proven to be effective in promoting axon growth and restoring function after SCI. Several intracellular signal transduction pathways have been implicated in mediating the actions of the neurotrophin family of growth factors, one of which is the mitogen-activated protein kinase (MAPK) pathway. Experiments indicate that blockade of MAPK activation inhibits neurite induction, whereas constitutive MAPK activation produces neurite outgrowth. The activation of this common neurotrophin signaling pathway directly may therefore be a more efficacious means of promoting axon regeneration after SCI than neurotrophin administration.The current study investigated in a complete thoracic spinal cord transection, the combination of Schwann cell (SC) bridges, to modify the extrinsic cord milieu, and gene delivery of constitutively-activated kinases from the MAPK pathway, MEK and ERK, via adeno-associated viral vectors, to enhance the intrinsic regenerative capacity of reticulospinal neurons. Histological evaluation of grafted animals included examination of GFP-traced axons into and beyond SC bridges and identification of the activation of MAPK signaling intermediates within infected neurons of the reticular formation at 9 weeks after bridge implantation. Behaviorally, at endpoint, animals receiving MAPK activation and SC grafts demonstrated improvements in gross locomotor ability compared to SC-only grafted controls as measured using the BBB score. Targeting the MAPK pathway directly may offer an alternative approach to multi-neurotrophin delivery for SCI repair. Supported by The Christopher Reeve Foundation.

P-44

Modification of the glial scar following spinal cord injury

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The glial scar, which is rich in many known growth inhibitors, including chondroitin sulfate proteoglycan (CSPG), poses a major impediment to axonal regeneration following injury. Therefore, it should be beneficial to develop treatment strategies that limit CSPG formation or expression following both acute and chronic injury to provide for a more growth-permissive environment. In one experiment, a dorsal quadrant lesion was made in the cervical spinal cord. The lesion cavity was treated with gel foam saturated with chondroitinase ABC (ChABC) or vehicle immediately and every other day over 5 days. This paradigm resulted in limited CSPG digestion within the lesion site, but remaining intact CSPG suggested that this approach was not sufficient to completely degrade CSPG along the lesion border. To get a more sustained infusion of ChABC, we placed microspheres coated with ChABC into the lesion site immediately following injury.

After 5 days there was evidence of CSPG digestion along the lesion border, but the extent was not greater than with gel foam application. In another experiment, the dorsal quadrant lesion cavity was acutely treated with â-xyloside, which decreases CSPG synthesis, or vehicle. Animals also were given daily subcutaneous injections of â-xyloside or vehicle for 5 days. The â-xyloside-treated animals had less CSPG in the center of the lesion than in vehicle-treated ones. Therefore, â-xyloside may be another potential therapeutic tool to limit CSPG expression in the scar. We also assessed the ability of ChABC to modify a chronic glial scar. Four weeks after a dorsal quadrant lesion was made, ChABC-coated microspheres were inserted into the cavity every other day over 5 days. While microspheres were visible within the lesion cavity, there was no obvious CSPG digestion.

In this situation ChABC may not have been able to penetrate the dense network of the scar. Thus, it appears necessary to fur
ther manipulate the chronic scar in order to allow for better treatment dispersion. Supported by NINDS NS26380 and NIH T32 NS007440.

P-45

A novel animal model for chronic compression of the ventrolateral spinal cord

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Compression of the spinal cord accompanies many pathological conditions affecting the vertebral column of humans and animals. Fractures and luxations of the vertebral column, neoplastic growths invading the spine, and herniation of intervertebral discs can all lead to compression injury to the spinal cord. Despite the abundance of compression models currently available, one suitable for chronic compression of the ventrolateral cord is still lacking. Injury specifically directed to this area is of clinical relevance because of the contribution of descending axons in this region toward the maintenance of posture and locomotion. We present a novel animal model for chronic compression of the ventrolateral thoracic cord. Silicone implants of three different sizes were surgically inserted into the vertebral canal between thoracic vertebrae eight and ten, causing unilateral compression of the ventrolateral thoracic cord in Long Evans rats for up to six weeks. Histological examination of spinal cord tissue compressed by this method showed graded severity of injury with different sizes of implants. Increasingly large implants also resulted in progressively more severe motor deficits as evaluated by open-field locomotor scores, performance on the horizontal ladder, and ground reaction force analysis. Interestingly, more ventral placement of implants produced more severe spinal cord pathology and more severe locomotor deficits compared to more laterally placed implants of the same size.

P-46

Identification and characterization of a specific subpopulation of angiogenic spinal microvessels binding the Griffonia simplicifolia isolectin B4 following traumatic spinal cord injury (SCI)

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Following traumatic spinal cord injury (SCI), disruption and plasticity of the microvasculature within injured spinal tissue contributes to the pathological cascade associated with the evolution of both primary and secondary injury cascades. Conversely, preserved vascular function most likely results in tissue sparing and the degree to which subsequent functional recovery will result. Thus, a complete characterization of the vascular response to SCI is an important first step toward the development of therapies that may prove useful in stabilizing vascular structure and function. It has been difficult to identify subclasses of damaged blood vessels at the cellular level. Here, adult mice received an intravenous injection of the Griffonia simplicifolia isolectin B4 (GSA I-B4) at 1, 3, 7 or 14 days following a moderate thoracic contusion (50 kdyn, IH impactor). Vascular binding of I-B4 is first observed 3 days post-SCI, peaks by 7 days (when pathologic angiogenesis is maximal following SCI), with numbers of positive vascular profiles decreasing again by 14 days. Quantitative assessment of I-B4 binding shows that >80% of bound vessels occur within the evolving lesion epicenter. Ultrastructural analysis confirms luminal binding in vessels with intact endothelial cell (EC) layers, with labeled vascular profiles enriched in areas of significant inflammation and extracellular matrix disturbance. Further, this subpopulation of vessels does not appear to be overtly leaky, as few I-B4 bound vascular profiles demonstrate significant Evans blue extravasation, with many expressing a normal tight junctional phenotype (i.e. expressing claudin-5, occludin, etc.). Interestingly, preliminary evidence suggests the development of this altered endothelial phenotype is VEGF-independent. Current efforts are focusing on both identifying the molecular effectors responsible for the observed microvascular plasticity and determining the feasibility of exploiting this transient luminal EC phenotype as a therapeutic target and/or reporter of the efficacy of vascular-targeted therapy for SCI.

P-47

Phosphorylated Bcl-xL in spinal cord: are apoptosis regulation and neuronal differentiation related?

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Bcl-xL is an apoptotic regulator in adult spinal neurons and glia whose expression decreases after spinal cord injury (SCI). Bcl-xL overexpression has been shown to stimulate intrinsic axonal regeneration in the adult injured CNS. Bcl-xL phosphorylation can be induced by microtubule disruption and has been proposed to be pro-apoptotic in non-neuronal cells, probably by targeting Bcl-xL for degradation. However, the role of phosphorylation in Bcl-xL activity in non-dividing cells and in normal or injured CNS is unknown. To determine if phosphorylation correlates with SCI-induced decreases, we measured protein levels and subcellular localization of phosphorylated (P-ser62Bcl-xL) vs. non-phosphorylated Bcl-xL (Bcl-xL) in naive and contused rat spinal cord at T10. Western blot analysis of naive spinal cord showed Bcl-xL to be present in mitochondria, endoplasmic reticulum, nuclei and cytosolic extracts while P-ser62-Bcl-xL was only present in membranous organelles. During the first 24h after SCI, Bcl-xL levels decreased in all fractions but membrane bound-P-ser62-Bcl-xL did not change. As early as 15 min and up to 24h after SCI, P-ser62-Bcl-xL appeared in the cytosolic fraction. These results suggest that early after SCI, membranous bound-Bcl-xL is phosphorylated and released into the cytosol. Immunohistochemical analysis showed strong P-ser62-Bcl-xL staining in axons of both sham-treated and injured spinal cords. Interestingly, 24h after injury, P-ser-Bcl-xL exhibited a staining pattern characteristic of degenerating axons. Using an NGF-differentiated PC12 model of neurite-axon formation, we found P-ser62-Bcl-xL in the forming neurites and mitochondria. Furthermore, western blot analyses showed NGF-differentiation of PC12 to decrease Bcl-xL and increase P-ser-Bcl-xL levels. Thus, phosphorylated Bcl-xL might be involved in neuronal differentiation in vitro and in vivo. Phosphorylation of Bcl-xL after SCI could result from failed attempts at regeneration by injured axons. Supported by NINDS NS39161, TIRR Foundation and Sealy Smith Endowment Fund.

P-48

Sparing or sprouting of corticospinal, raphespinal and coerulospinal fibers after cervical hemisection in primates

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In non-human primates, lateral hemisection spinal cord injury at C5-6 causes a temporary deficit in gross aspects of treadmill walking, with recovery beginning 4 weeks post lesion. We investigated anatomical correlates of this recovery by examining the density of corticospinal (CST), raphespinal (5HT), and coerulospinal (TH) fibers caudal to the lesion site in 4 rhesus monkeys. CST axons were anterogradely labeled with injections of dextran-conjugated Alexa488 into the left motor cortex; 5HT and TH fibers were revealed with immunostaining. Hemisection was performed on the right side of the spinal cord. There was a complete loss of CST, 5HT, and TH fibers in white matter immediately caudal to the lesion. However, fibers from all three tracts were found in gray matter caudal to the lesion. CST labeling was found primarily in the intermediate zone of the gray matter (Rexed's laminae V, VI, VII), and to a lesser extent in the motor neuronal pool (lamina IX). 5HT labeling was found primarily in lamina IX. TH labeling was found in all portions of the gray matter. These fibers originated from axons descending in the intact side of the spinal cord and crossing the midline below the lesion.

Preliminary quantification of labeling density in the gray matter was performed with NIH Image software. CST, 5HT, and TH labeling densities caudal to the lesion were approximately 30%, 20% and 20%, respectively, of the density in the corresponding gray matter on the intact side of the cord. Stereological quantification of these results is underway.

Also underway are studies to determine, in the intact cord, the amount of CST, 5HT, and TH decussation and termination in the gray matter. The results should reveal if the present results reflect lesion-induced sprouting, the unmasking of circuits present in the intact primate spinal cord, or a combination of the two.

P-49

Receptor-mediated transport of LIF across blood-spinal cord barrier is upregulated after SCI

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The intrinsic environment of the spinal cord has limited regeneration capacity. Signals from the neurovascular interface, such as inflammatory cytokines from the peripheral circulation, play important roles to modulate the regenerative process. We have shown that leukemia inhibitory factor (LIF) can cross the normal blood-brain and blood-spinal cord barrier by a saturable transport system (Pan et al. 1999). In this study, we first showed that spinal cord injury (SCI) caused time- and region-dependent disruption of the blood-spinal cord barrier as indicated by increased uptake of 99mTc-albumin 10 minutes after intravenous injection. We then determined whether the uptake of 125I-LIF followed the same pattern. The lumbar spinal cord (injury site) had higher permeability to LIF than that to albumin; moreover, there was an increase in LIF uptake even when the barrier disruption had resolved. 125I-LIF uptake was suppressed by both excess non-radiolabeled LIF and a blocking antibody against LIFRa, whereas the uptake of 99mTc-Albumin by the injured spinal cord was not affected by the treatment. This indicates that the entry of LIF from blood to the injured spinal cord remains a specific process rather than non-selective leakage across the disrupted blood-spinal cord barrier, and that LIF receptor is involved in the saturable transport which is upregulated after SCI. At this time, the expression of LIF receptor (gp190) and the co-receptor gp130 was measured by immunofluorescent staining of spinal cord sections. After injury, there was increased expression of LIFR in endothelial cells as demonstrated by co-localization of LIFR immunoreactivity with anti-CD31 antibody. This is consistent with enhanced transport across the blood-spinal cord barrier, which is mainly composed of endothelial cells. The results suggest that increased LIF transport is part of spinal cord response to injury and could be important for CNS regeneration.

P-50

Immunological demyelination prevents astrogliosis following spinal cord injury

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The glial scar and axon growth inhibitors associated with myelin play important roles in the failure of axonal regeneration in the adult mammalian central nervous system (CNS). Our laboratory has previously demonstrated that immunological demyelination of the CNS facilitates long distance regeneration of severed axons following spinal cord injury. Given that demyelination itself represents an injury to the CNS, and astrocytes survive within regions of immunological demyelination, here we have evaluated whether immunological demyelination eliminates or attenuates injury-induced astroglial hypertrophy. Demyelination within the dorsal funiculus was induced with an intraspinal injection of anti-galactocerebroside antibodies plus complement proteins. 24 h after immunological demyelination animals received a stab or hemisection injury to the dorsal funiculus, and were sacrificed 7 days later. Astrogliosis extended several millimeters above and below the lesion, evidenced by astroglial hypertrophy and upregulation of glial fibrillary acidic protein (GFAP). However, astrogliosis was absent or severely attenuated within regions of immunological demyelination extending several millimeters cranial and caudal to the lesion. Immunohistochemical and electron microscopic analyses confirmed that astrocytes survived within regions of immunological demyelination, did not become hyper
trophic, and expressed the intermediate filament vimentin. These findings indicate that immunological demyelination suppresses that capacity of astrocytes to become reactive and suggest that immunological demyelination induces astrocytes to revert to a developmentally immature state. This paradigm provides a unique model in which to address the causes of reactive astrogliosis.

P-51

Effects of ROCK inhibition with Y27632 on astrocytes

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Using a rat dorsal column transection model of spinal cord injury we have shown previously that inhibition of Rho-kinase with Y27632 stimulated sprouting of long projection tract axons (corticospinal tract, CST, and dorsal column tract, DCT), and accelerated functional recovery/compensation when applied at high doses. However, regeneration of these axons was not observed and they typically failed to reenter the normal spinal cord after crossing the lesion site, possibly due to the astrocytic barrier forming at the edge of the lesion. Therefore, we hypothesized that in addition to its effects on the growth cones of cut axons, Y27632 treatment also affected non-neuronal cells in the injured spinal cord, resulting in undesirable side effects. In the present study, we focused on the effects of Y27632 treatment on astrocytes, which are a key component of reactive gliosis. In vitro, overnight treatment with Y27632 (1 to 50 ìM) caused the astrocytes to assume an activated morphology and to upregulate CSPG expression as indicated by CS56 immunostaining. Cortical neurite growth decreased on the extracellular matrix (ECM) deposited by Y27632-treated astrocytes compared to that on ECM from non-treated astrocytes. Thus Y27632-treated astrocytes might be a less permissive substrate for neurite growth than non-treated astrocytes. On the other hand, conditioned medium from drug-treated astrocytes promoted neurite growth. In vivo, we assessed astrocyte activation and CSPG expression in vehicle- and Y27632-treated rats 1 week after spinal cord dorsal hemisection injury. Preliminary results indicate that at the edge of the lesion cavity, close to the transected DCT (or sensory) tract, GFAP immunoreactivity (IR) increased in Y27632-treated animals compared to vehicle controls. In addition, Y27632-treated animals had elevated neurocan IR at the lesion edge and along the degenerating DCT. Thus Y27632 treatment may exert promoting as well as inhibitory effects on axonal regeneration in vivo. Supported by CIHR and the Canadian Neurotrauma Research Program

P-52

Neuroprotection of ChAT neurons and reduction of nNOS expression in spinal cord repaired rats

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We aimed to assess the survival of cholinergic neurons and protein expression of neural nitric oxide synthase (nNOS) in complete spinal cord (SC) transected adult rats that were treated with peripheral nerve grafts (PNG) and acidic fibroblast growth factor (aFGF). Rats were randomly divided to three groups: (1) sham control (laminectomy only) group, (2) transected group (SC transection at T8), and (3) repaired group (SC transection at T8, aFGF treatment, and PNG). The spinal cords of all rats were collected at six-month post surgery. Immunocytochemistry for choline acetyl transferase (ChAT) was used to evaluate cholinergic neuronal cell survival following the injury and treatment. Western blotting and immunocytochemistry for nNOS were used to detect the protein expression and location. When comparing with the transected group, the repaired group showed (1) higher numbers of ChAT positive neurons in ventral horn (VH) and intermediolateral (IML) column near the lesion site, (2) lower nNOS expression near the lesion site, (3) lower number of nNOS positive neurons in intercalated nucleus. We conclude PNG and aFGF treatments provide neuroprotective effect of ChAT neurons and reduce nNOS expression near the lesion site in a T-8 SC transected rat model. This work was supported by Veteran Affairs Rehabilitation Research and Development Service.

P-53

Enzymes of the glutamine cycle in the rat dorsal root ganglion following spinal injury and methylprednisolone treatment

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Introduction: Following injury, dorsal root ganglion (DRG) neurons respond with altered protein expression in response to the insult and in attempt to regenerate to their target. Almost all of these neurons are glutamatergic, but little is known about the glutamine cycle in the DRG after spinal injury. Purpose: In the present study, we evaluated glutamine cycle enzymes, glutaminase (GT) and glutamine synthetase (GS), with immunohistochemistry after seven days of spinal injury. Methods: The spinal cords of anesthetized male, Sprague-Dawley rats (250g) were transected at T5-T6. Controls consisted of rats with a sham surgery. One-half of all rats received methylprednisolone (MP) treatment (iv 41 mg/kg @ 0,2,4,6 h). After seven days, the L4 DRG was evaluated for GT and GS immunoreactivity (IR). Results: In controls, GS-immunoreactive satellite cells surrounded all DRG neurons. After spinal injury with and without MP, GS-IR increased in satellite cells compared to controls, predominantly in satellite cells surrounding large diameter DRG neurons. GT-IR was found in all DRG neurons. After injury with and without MP, GT-IR increased in many large and small diameter neurons. Summary and Conclusion: GS is important for shuttling carbon in the form of glutamine from glial cells for use in neuronal metabolic cycles (Miller et al., Brain Res 945:202. '02). The results of this study indicate that DRG satellite cells are responsive to spinal injury. This satellite cell reaction may be an important contributor to regenerating neurons that have increased metabolic requirements. GT in DRG neurons is used for the synthesis of glutamate for neurotransmission. Glutamatergic mechanisms are important for neurite outgrowth/sprouting and synapse development. Increased levels of GT may be one mechanism for the regeneration and sprouting of afferent axons after spinal injury. Supported by NIH AR47410 (KEM).

P-54

Retroviral lineage mapping of NG2-expressing progenitor cells demonstrates their role in early gliosis following spinal cord injury

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The role of spinal cord progenitor cells in neural repair is just beginning to be explored, with evidence of a role in remyelination and astrocyte genesis. The present experiments aimed to determine the relative role of proliferating progenitor cells vs. quiescent progenitors in gliogenesis in a model of spinal cord injury. Using the mitotic indicator bromodeoxyuridine (BrdU) and a retroviral vector, we found that, in the adult female rodent, endogenously dividing neural progenitors are acutely vulnerable in response to T8 dorsal hemisection spinal cord injury. We then studied the population of dividing cells in the injury epicenter by delivering BrdU or a retrovirus containing GFP under the control of the NG2-promoter at 24 hours after spinal cord injury. Animals were euthanized at five time points ranging from 6 hours to 9 weeks after BrdU delivery. BrdU incorporation was coincidently prominent in NG2 immunoreactive progenitors that matured into oligodendrocytes, and in a transient population of microglia.

Using a GFP+ chimeric mouse, we determined that 90% of the dividing cells in this early proliferation event originate from the spinal cord, whereas only 10% originate from the bone marrow. Fate mapping using an NG2-specific reporter virus revealed the progenitor cells were initially restricted to an astrocyte lineage but became bi-potent when born 1 week post-injury. Our results suggest that dividing NG2-progenitor cells are vulnerable to injury, but these cells are rapidly replaced by a separate, NG2-expressing population that participates in the formation of scar as well as myelin regeneration. Supported by the Christopher Reeve Paralysis Foundation, Paralysis Project Paralyzed Veterans of America, the Glaucoma Research Foundation and NSO46724.

P-55

Deletion of STAT3 attenuates astrocyte reactivity after crush spinal cord injury

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Reactive astrocytes are prominent in glial scar formation after spinal cord injury (SCI). This glial scar is an obstacle for axonal regeneration across the injury site, and is thought to be an obstacle to functional recovery. Here we examine the effects of manipulations of astroctye reactivity on behavior, tissue integrity and axonal regeneration after crush spinal cord injury. We focus on the STAT3 protein because it is proposed to regulate astrocyte reactivity. We used the Cre-loxP system to delete STAT3 selectively in astrocytes with the goal of attenuating astrocyte reactivity. Astrocytes deficient in STAT3 signaling exhibited reduced expression of GFAP and vimentin as well as disorganized glial scar formation after SCI. Preliminary results show that SCI moderate crush injuries in STAT3 deficient mice exhibited larger lesion size, as well as an increased inflammatory response, and a greater amount of demyelination. The presence of neurons near the lesion area in mice with STAT3 gene deletion indicates that astrocytes with attenuated reactivity may still be able to perform some protective functions for neurons. Mice with STAT3 deficient astrocytes exhibited less recovery of locomotion and motor coordination after SCI as compared to control mice. Taken together, these findings suggest that reactive astrocytes exert more protective functions than astrocytes with attenuated reactivity after SCI. We are currently examining axon regeneration after SCI in mice with attenuated astrocyte reactivity via deletion of the STAT3 gene.

P-56

Cellular localization and temporal distribution of TNF-α following cervical spinal cord injury

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Spinal cord injury (SCI) consists of instantaneous cellular and axonal destruction caused by mechanical trauma, and subsequent progressive tissue damage resulting from exposure of the surrounding tissue to excitatory amino acids, cytokines, and oxidative metabolites released from cellular debris or infiltrating immune cells. A major cytotoxic molecule present following SCI is tumor necrosis factor alpha (TNF-α), a proinflammatory cytokine responsible for initiating/maintaining inflammation and contributing to cell death. Determining the cellular source(s) of TNF-α after SCI will enable further elucidation of the mechanisms of its regulation and provide putative therapeutic targets.

To induce SCI, Fischer rats were given a moderate cervical contusion using the electromagnetic SCI device (0.95 mm displacement, 20 msec). After perfusion, cellular sources of TNF-α protein within and near the injury epicenter were examined at 1, 4, or 24 h post-injury. Cell specific markers (GFAP, astrocytes; GSA IB4, microglia/macrophages; NeuN, neurons; CC1, oligodendrocytes) were employed in combination with a TNF-α antibody using immunohistochemistry.

Throughout the 24 h period following cervical SCI, TNF-α distribution was localized to the ventral gray matter and a substantial area of the white matter. Levels of TNF-α diminished over time and distance from the injury epicenter. TNF-α was present in most CNS cell types post-injury. In white matter, oligodendrocytes represented the predominant TNF-α-positive cell type, while microglia, and to a lesser extent, astrocytes also stained positive. In gray matter, ventral motorneurons were TNF-α immunoreactive with increasing numbers of microglia exhibiting TNF-α at later times post-injury. Originally devoid of TNF-α immunoreactivity at 1 h post-injury, dorsal horn neurons began to exhibit TNF-α labeling at 4 h with greater numbers TNF-α-positive by 24 h. Deciphering the cell type(s) responsible for TNF-α production in the context of its receptors could ultimately lead to improved interventions for neuroprotection after cervical SCI. Supported by the Miami Project and NIH NINDS.

P-57

Biochemical profiles of mid-cervical adenosine A1 receptors after cervical spinal cord hemisection in adult rats

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In an animal model of spinal cord injury, a latent respiratory motor pathway can be activated by chemical and pharmacologic manipulations to restore function to a hemidiaphragm paralyzed by a left cervical (C2) spinal cord hemisection. Previous studies have shown that adenosine A1 receptors located in the phrenic motor nucleus (PMN) may be implicated in restoration of respiratory function after C2 hemisection. Although adenosine A1 receptors have been localized in the PMN, the biochemical profiles i.e., receptor numbers (Bmax) and affinity (Kd) are not known yet. The objective of the present study was to address this relevant question in order to ascertain any sub-cellular changes in adenosine A1 receptors after hemisection. Animals were anesthetized with ketamine (70mg/kg) and xylazine (7mg/kg) and subjected to a left C2 hemisection. Hemisected animals (n=5) were killed 24h after later by decapitation, the cervical spinal cord excised at C3-C5 and quickly frozen at -80C. Protein concentrations were determined by the Bradford 1 assay. Non-injured control animals were similarly prepared. Radioligand binding was conducted on prepared spinal cord membranes using [3H]-DPCPX. In noninjured animals (n=5), adenosine A1 receptors displayed characteristics compatible with a single binding site characterized by a Bmax of 256.00 + 32.13 (fmol/mg protein) and Kd of 2.89 + 0.45 (nM). In hemisected animals, adenosine A1 receptors displayed a higher Bmax 316.6 + 25.10. However, the Kd 2.72 + 0.72 (nM) was not altered. Our results suggest that C2 hemisection up regulates adenosine A1 receptors without significantly affecting affinity. The apparent changes in receptor number may be important in therapy with adenosinergic compounds. Supported by NIH (HD 35776).

P-58

Acute injury is required for Neurotrophin-3 induced axonal plasticity in the spinal cord

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Adenoviral vector-mediated over-expression of Neurotrophin-3 (NT-3) promoted axonal sprouting after acute spinal cord injury but not in the unlesioned spinal cord. This suggests that neuroplasticity is dependent upon processes associated with injury such as Wallerian degeneration (WD). To determine whether WD mediates the neuroplasticity, we delayed NT-3 over-expression until the processes of WD had resolved. An adenoviral vector carrying the NT-3 gene (Adv.NT-3) was delivered to lumbar region of rats at 4 months after unilateral corticospinal tract (CST) lesion by retrograde transport through the sciatic nerve. We measured the number of CST axons that arose from the intact CST, traversed the midline, and grew into the lesioned side of the spinal cord where the NT-3 was over-expressed. No spouting was measured when NT-3 over-expression was delayed 4 months. Since the processes of WD would have resolved within 4 months after injury, these data demonstrate that products of WD are a likely source of the co-inducing signals that support neuroplasticity. To investigate whether inflammatory processes associated with WD play a role, we immunosuppressed the animals with Cyclosporine (CsA) before lesioning the CST and delivering Adv.NT-3. The number of axons that crossed the midline was less in immunosuppressed animals compared to that of the control group and suggests that processes of inflammation associated with WD may be involved in NT-3-induced axonal plasticity. Further studies to identify the specific factors associated with WD will provide a basis for an effective treatment for chronic spinal cord injury. Supported by grants from the NIH, Christopher Reeve Paralysis Foundation, and Mission Connect of the TIRR.

P-59

Regulatory oversight of pre-clinical development for stem cell-based therapies

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A regulatory quality assurance department within the laboratory provides support, coordination and leadership of the pre-clinical studies. The main goal is to work in compliance with a pre-established plan and standardized procedures in accordance with FDA regulations. The regulatory officer's duties include: maintaining and over-seeing laboratory notebooks for all scientists, producing and maintaining Standard Operating Procedures (SOP's), and producing Study Protocols and Study Reports. This in turn yields reliable, repeatable, and auditable data and results. The importance of conducting the pre-clinical research under FDA compliance is that all of the data that is collected from the research can be used in submitting an IND package to the FDA due to its traceability. This in turn cuts down on the time-line of translation to the clinic as well as the total cost of the study, from the pre-clinical research through the clinical trials and ultimately FDA approval.

The following issues, and toxicity need to be completed. The basic cell to cell interactions need to be assessed; transplantation into a rat is very different from transplantation into a human. The termination of the treatment must be determined in the event of a serious adverse event. In addition, the unfamiliarity of the FDA with stem cell based strategies demands an increased degree of communication as compared to conventional approaches.

P-60

Developmental neuroapoptosis: A promising new model for studying neural recovery potential

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Neurons, in small numbers, commit suicide as a natural phenomenon during synaptogenesis, but can be made to commit suicide in very large numbers by exposing the developing CNS to various drugs that suppress neuronal activity, including ethanol, all anesthetic drugs and many anticonvulsants (Olney et al., TiPS, 2004). For example, a single large dose of ethanol causes massive deletion of neurons from many different regions of the infant mouse CNS. Interestingly, infants sustaining such losses have profound learning deficits at P30, but show substantial recovery thereafter, so that only mild residual deficits are detectable in adulthood. Thus, by mechanisms we do not understand, the nervous system is able to sustain very large neuronal losses during early synaptogenesis, then after an initial period of functional disability, overcome these losses and gradually assume a near-normal level of functionality. Discovering how neural elements either regenerate or reorganize themselves to compensate for such early losses is an intriguing challenge for regeneration neurobiologists. A key to understanding may be that the initial losses occur during a period of great plasticity and, although many neural systems lose a high percentage of their functional units, other neighboring units survive to immediately begin grappling with the restoration challenge. It must entail extensive and well coordinated inter- as well as intra-cellular signaling, involving yet-to-be determined communication channels, for a successful functional outcome. This is a remarkably versatile model for studying morphological and functional recovery, in that by varying the timing of drug exposure (early vs mid vs late synaptogenesis) different targeted neuronal populations can be deleted from the retina, spinal cord, cerebellum, specific cranial nerve nuclei, thalamus, cerebral cortex, caudate/putamen, nucleus accumbens, etc., thus providing an opportunity to evaluate how specific patterns of losses and recovery on an anatomical/molecular level correlate with specific patterns of behavioral impairments and recovery.

P-61

CK2 inactivation of serum- and glucocorticoid-inducible kinase via PP2A diminishes the anti-apoptotic effect of CK2 in rat hippocampus

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We have previously demonstrated that protein kinase CK2 mediates the neurotrophic effect of glial cell line-derived neurotrophic factor (GDNF) on dopamine neurons in rat substantia nigra. In the present study we examined the role and mechanism of the anti-apoptotic effect of CK2 in rat hippocampus. Results revealed that over-expression of the CK2 wildtype DNA (1.5 ug) significantly enhanced Bcl-2 and Bcl-XL mRNA levels in the hippocampus. Transfection of the wildtype CK2a DNA (1.5 ug) decreased, whereas transfection of the catalytically inactive CK2a-A156 mutant DNA (1.5 ug) increased the phosphorylation of serum- and glucocorticoid-inducible kinase (SGK), a protein kinase associated with memory formation and neuronal plasticity. Further, inhibition of phosphatase 2A (PP2A) activity by phosphotrienin infusion to the hippocampus (15 pg/ul) antagonized the inhibitory effect of CK2a DNA on SGK phosphorylation. Phosphotrienin also potentiated the enhancing effect of CK2a on Bcl-2 and Bcl-XL mRNA expression. These results together suggest that CK2 inactivation of SGK through PP2A diminishes the anti-apoptotic effect of CK2 in rat hippocampus. On the other hand, both low (0.4 ug) and high (1.2 ug) concentrations of brain-derived neurotrophic factor (BDNF) increased CK2 activity and Bcl-2, Bcl-XL mRNA levels in the hippocampus. Low, but not high, concentration of BDNF also increased SGK phosphorylation. The lack of an effect of high concentration of BDNF on SGK phosphorylation is probably due to the over-activation of PP2A. Experiments are undertaken to examine whether CK2 mediates the anti-apoptotic effect of BDNF, and how does SGK modulate the anti-apoptotic effect of BDNF. Study supported by research fund from the Institute of Biomedical Sciences, Academia Sinica, Taipei, Taiwan, ROC.

P-62

Upregulation of TNFα transport in mice after traumatic brain injury

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Tumor necrosis factor alpha (TNFα) has been implicated in the pathophysiology of acute brain injury. In addition to its in-situ production inside the brain, TNFα in blood circulation also causes pronounced neuroendocrine changes. The purpose of this study is to determine whether minimally traumatic brain injury (mTBI) affects the permeability of blood-brain barrier (BBB) in general and the transport system for TNFα specifically. Mice sustained mTBI on the right scalp overlying the parietal and temporal cortices, with pre-calibrated weight of 50 g and 70 g delivered from a height of 1 m. The mice were studied 1 d and 3 d after injury, along with their sham controls which received brief anesthesia by inhalation of isofluorane. Radiotracer uptake studies showed that the barrier disruption (reflected by permeability of albumin) by mTBI was negligible in any CNS regions studied. Uptake of TNFα (measured 10 min after intravenous delivery), however, showed a significant increase not only in the injured right hippocampus and subcortical area, but also in the contralateral cerebral (left cortex and subcortical region) and the hindbrain. This increase was present only at 1 d after mTBI, and was more pronounced in the 70 g weight group than the 50 g weight drop. Chronic alcohol ingestion, which is a common co-morbidity factor for mTBI, did not show additional effect on TNFα uptake. Taken together, the results indicate that mTBI caused a selective, transient upregulation of TNFα transport in the brain both ipsilateral and contralateral to the injury.

P-63

Intrastriatal transplantation of cultured human-derived neural progenitor NT2N cells expressing Nurr1 at two weeks after middle cerebral artery occlusion produces stable behavioral recovery

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The human embryonic carcinoma cell line NT2N.Nurr1, originally derived from Ntera2/D1 (NT2) and cloned after exposure to retinoic acid (RA), represents an efficacious source of donor cells for transplantation therapy in neural diseases. In our pilot studies, NT2N.Nurr1 cells display increased tendency to express neuronal markers in particular tyrosine hydroxylase, supporting Nurr1's role in dopaminergic differentiation thereby advancing the concept of grafting Nurr1-expressing cells in Parkinson's disease. Accumulating evidence now implicates Nurr1 in post-ischemic brain injury (Erdo et al., 2004). In the present study, we investigated the potential of transplanting NT2N.Nurr1 cells in ischemic rat model. Transient focal cerebral ischemia was carried out in adult Sprague-Dawley rats using the middle cerebral artery occlusion model. At 2 weeks post-ischemia, animals that showed stroke-induced behavioral deficits were stereotaxically transplanted with cultured NT2N.Nurr1 cells (200,000 viable cells in PBS) or vehicle into the ischemic striatum. At 2, 4, and 6 weeks post-transplantation, motor and neurological tests using elevated body swing test and Bederson test, respectively, revealed significant behavioral improvements in transplanted stroke animals compared to vehicle-treated stroke animals ($p < 0.05$). Immunohistochemical examination at 6 weeks post-transplantation revealed graft survival of 0.2%-3.1%. Initial data suggest elevated brain levels of neurotrophic factors in transplanted stroke animals. The present study advances transplantation of neural progenitor cells for facilitating behavioral recovery in animals with fixed stroke.

P-64

Delayed intrathecal anti-Nogo-A antibody treatment improves functional recovery in rats after stroke

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Stroke is the leading cause for neurological disability in adults. The only effective approved treatment to reduce neuronal loss and the resulting function loss for ischemic stroke is thrombolytic agents given within three hours after the onset of stroke. Unfortunately, thrombolytic treatment is often not possible, and rehabilitative strategies are most effective when given in an intensive, focused manner in the early stage after stroke. New strategies to improve functional recovery after stroke are in urgent need. It is believed that myelin-associated inhibitory proteins such as Nogo-A play critical roles in impeding regain of function after stroke. Our laboratory has reported that immediate or one week delayed intra-cerebroventricular treatment with anti- Nogo-A antibody resulted in improved functional recovery and formation of new neuronal pathways in the rat after stroke (Papadopoulos et al 2002, Seymour et al 2005). In the present study, we tested a more clinically accessible route for applying purified anti- Nogo-A antibodies. Long-Evans black hooded rats were trained to perform the forelimb reaching task and received stroke surgery (middle cerebral artery occlusion) to induce motor deficits of their preferred forelimb. One week after stroke, animals were randomly distributed to different experimental groups before receiving treatment. Anti-Nogo-A antibody or control antibody was filled in a mini-osmotic pump and given intrathecally to rats for two weeks. Recovery of function was examined using the skilled forelimb reaching test. Our preliminary results indicate that Nogo-A neutralization through the intrathecal route given one week after stroke improved recovery of function as compared to stroke only and stroke/control antibody animals.

Neuroanatomical tracing of relevant pathways underlying this recovery is underway. Support contributed by: Department of Veterans Affairs, REAP, VA Merit, MREP, Novartis Institute of Biomedical Research Basel, Swiss NSF, NINDS 40960

P-65

Transplantation of NT2N cells, encoded by Nurr1 gene for accelerated neuronal differentiation, promotes robust amelioration of Parkinsonian symptoms in the 6-hydroxydopamine lesion rat model

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NT2 cells are derived from human embryonic carcinoma with potencies of neuronal differentiation to NT2N cells after exposure to retinoic acid (RA) and mitotic inhibitors. Transplantation of NT2N cells was demonstrated to be feasible and safe in stroke patients. Recently, Nurr1, an orphan nuclear receptor, has been shown to be critical for dopamine expression during early development and maintenance of the dopaminergic neurons in the midbrain. In this study, NT2.Nurr1 cell lines were characterized in vitro and their therapeutic efficacy examined in vivo using the 6-OHDA lesion model. Expression of tyrosine hydroxylase (TH) along with other neuronal markers in cultured NT2 and NT2.Nurr1 cells were initially evaluated at 0, 1, 2, 3, and 4 weeks of RA treatment. In particular, MAP2 and TH expression of NT2.Nurr1 cells was induced as early as two weeks of RA treatment, which was much earlier than the four-week exposure required for NT2 cells. In parallel, a cohort of adult male Sprague-Dawley rats received stereotaxic 6-OHDA injection into the medial forebrain bundle and one week later intrastriatally transplanted with NT2N.Nurr1 cells or vehicle. Quantitative measures of spontaneous locomotor movements, using the Accuscan Versamax system revealed that the horizontal activity and stereotypy activity were significantly ameliorated by transplantation of NT2N.Nurr1 cells compared with vehicle-treated lesioned rats ($p<0.05$). Additionally, the number of apomorphine-induced rotations of rats receiving NT2-Nurr1 cells was significantly reduced, compared to vehicle-treated lesioned rats ($p<0.05$). Immunohistochemical analyses revealed that surviving transplanted cells (3.2±0.5%) and preserved host TH positive striatal fibers and nigral neurons (3.1±0.6 and 2.4±0.8, respectively, ratio to vehicle-treated lesioned rats) were recognized in NT2N.Nurr1 transplanted rats displaying improved behavior. These results suggest that enhancing neuronal differentiation in NT2N cells by genetic manipulation with Nurr1 likely contributed to the robust neuronal rescue and behavioral recovery of parkinsonian rats.

P-66

No Nogo-A in fish, but Nogo-66 may affect fish axon growth

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Reticulons (RTN) are a family of evolutionary conserved proteins with four RTN paralogs (RTN1, RTN2, RTN3, RTN4) present in land vertebrate. While the exact functions of RTN1-RTN3 are unknown, mammalian RTN4-A/ Nogo-A was shown to inhibit axon regeneration in the mammalian central nervous system (CNS). This inhibitory function is exerted by two distinct regions, one within the Nogo-A specific N-terminus and the other in the conserved reticulon homology domain (RHD), called Nogo-66. In contrast to mammals, fish are capable of CNS axon regeneration. We performed detailed analyses of the fish reticulon gene family to determine whether this regeneration ability correlates with the absence of the neurite growth inhibitory protein Nogo-A. Seven rtn genes were identified in zebrafish. Phylogenetic and syntenic relationships indicate that the identified fish rtn genes are orthologs of mammalian RTN1, RTN2, RTN3 and RTN4 and that several paralogous genes (e.g. rtn4 and rtn6) resulted from genome duplication events early in actinopterigian evolution. Accordingly, two sequences homologous to the conserved RTN4/Nogo RHD are present in zebrafish, rtn4 and rtn6. However, the N terminal region harbouring the major neurite growth inhibitory Nogo-A is absent from zebrafish. Yet, when exposed to the mammalian most inhibitory portion of Nogo-A (delta 20) fish growth cones collapse, showing that fish have a receptor for this inhibitory protein. This data correlates well with the success of regeneration in fish after injury. However, the function of the zebrafish homolog of Nogo-66 remains elusive. To examine if fish are sensitive to Nogo-66, mammalian Nogo-66 was used as a substrate for fish retinal ganglion cell axons. Indeed, fish RGC axons fail to elongate on mammalian Nogo-66. This result indicates that the fish axons respond to the inhibitory activity of mammalian Nogo66, probably via NgR receptors, and may conserve a similar inhibitory program as mammals. Still, they are able to regenerate in vivo either because mammalian and fish Nogo-66 differ in their substrate properties which we are currently analysing; or fish Nogo-66 is not expressed in cells in contact with the regenerating fish axons, an aspect that we are also studying. On the other hand we are trying to identify the unknown Nogo-A receptor, that allows fish axons to recognize the mammalian Nogo-A.

P-67

Axonal regeneration in the Drosophila adult CNS

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Because injured neural connections only regenerate to a limited degree in the central nervous system (CNS), neurological function often remains impaired after injury. One of the reasons for this is the intracellular "signalling state" of mature neurons which makes them interpret many extracellular molecules as inhibitory. Signalling events which can change the intrinsic state of damaged neurons may be useful targets to treat CNS injury. Drosophila melanogaster has been used as a model system to address ques-

tions related to brain development, functioning and pathology. Very few studies have investigated the processes controlling axonal regeneration after injury in the fruit fly. We have identified APP, a gene involved in the pathophysiology of Alzheimer's Disease, as a strong axonal outgrowth promoting molecule in the Drosophila brain. Using genetic interaction studies and mutational analysis we found that this capacity of APP requires interaction with the Abelson Tyrosine Kinase and the Jun N-terminal kinase pathways. Using a novel in vivo brain injury paradigm we showed that APP is upregulated in injured neurons. The lack of this response in mutant animals increases mortality after brain injury. One of APP's functions in these circumstances may thus be to increase outgrowth of damaged axons.

We have now developed a model which will allow a more detailed study of axonal injury responses in Drosophila. In Drosophila brain explants we cut specific axonal tracts and visualize their responses in time lapse studies. As in mammals, severed axons often form terminal bulbs soon after cutting. The distal axon end than undergoes degeneration and fragmentation, while the proximal stub shows limited regrowth. By overexpressing or knocking-down signalling genes in the injured neuronal population we will identify factors which promote the intrinsic regenerative capacity of injured neurons. Furthermore, this model will allow genetic and chemical screens for modifiers of injured neurite outgrowth.

P-68

Nerve regeneration in a genetic model organism

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The peripheral nervous systems of invertebrates have long been known to display regeneration following axotomy. The permissive environment of the invertebrate nervous system allows analysis of the phenomenon of regeneration in the absence of inhibitory influences present in mammalian systems such as myelin or glial tissue. Genes and pathways that promote regeneration may be more readily analyzed in such a simplified system. However, until recently, regeneration of neuronal processes has not been examined in genetically amenable invertebrates such as the fruit fly Drosophila or the nematode worm C. elegans. Analysis of regeneration in such genetic systems offers many advantages. The C. elegans nervous system contains 302 neurons, each of which has a simple and highly invariant morphology. The processes of single neurons can be seen in intact living animals using transgenically expressed fluorescent marker proteins such as GFP. Thus, the regeneration of a single process can be followed in vivo, and its genetic requirements tested.

We and our collaborators have recently shown that neuronal processes in C. elegans can regenerate after laser axotomy (Yanik et al., 2004). The microsurgery used a femtosecond laser, which concentrates light into very brief high energy pulses and with which we can physically sever single neuronal processes. Motor neurons and a class of sensory neurons both showed regrowth within 24 hours of surgery. We will report our progress in analyzing how regenerative capacity reflects intrinsic or extrinsic differences between different neuronal classes. We will also explore the requirements for genetic pathways implicated in regeneration from work in other systems.

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P-69

Absence of a fibroglial scar in the regenerating zebrafish spinal cord

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Following injury, severed axons of the adult mammalian spinal cord are unable to cross the lesion site due to the formation of a fibroglial scar. Reactive astrocytes increase the size and number of their processes and interact with infiltrating meningeal cells to physically impede growing axons and, by secreting numerous extracellular matrix proteins, also act as a potent chemical barrier to their growth. In contrast, descending spinal cord axons of fish, such as the zebrafish, recover after transection and grow through the lesion site to reconnect with distal targets. In the present study, we investigated whether a similar fibroglial interaction occurs in the regenerating fish cord. Under anaesthesia, the spinal cord was transected at the level of the dorsal fin using fine microscissors. At varying survival times animals were reanaesthetized, transcardially perfused and prepared for light or electron microscopy. After transection the resulting gap in the cord was rapidly filled with blood forming a connective matrix between the severed stumps and contained numerous lymphocytes and infiltrating meningeal cells (MCs). Later, as regenerating cord material bridged the gap, MCs relocated to the periphery of the new bridge of cord tissue and formed a rudimentary new meningeal sheath. The regenerating bridge comprised axons, ependymal cells and numerous longitudinally aligned GFAP+ve processes of astrocytes that were often closely apposed to MCs in the injury site. Collectively, these data suggest that astrocytes and MCs do not form a fibroglial scar after complete transection of the zebrafish cord; rather they appear to interact in a manner that promotes cord repair and points to instructive signaling between the developing bridge and MCs. Supported by a Health Research Board Fellowship Grant.

P-70

Reactive glial cells act as scaffolds for neurite growth following retinal detachment and reattachment

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As elsewhere in the CNS, injury to the retina initiates an activation of glial cells that results in their hypertrophy and the formation of glial scars. Using retinal detachment as a model system we observed that the radial glia in the retina, the Müller cells (MC), hypertrophy within the neural retina and subsequently grow into the newly created space between the retina and the underlying epithelial layer. Not all MC, however, are equally reactive. The most reactive MC, as indicated by an upregulation of intermediate filament proteins, appear to have different characteristics than their less reactive neighbors. Indeed, it is along these

reactive cells that newly formed horizontal and ganglion cell neurites grow. These neurites can extend great distances within the retina and into subretinal glial scars. When the retina is surgically reattached, MC redirect their growth to the vitreal surface of the retina and ganglion cell neurites now extend among these glial scars in the vitreous cavity. The temporal sequence of changes was determined in a feline model of detachment where it was shown that MC hypertrophy and neurite growth begins within days after detachment and continues as long as the retina remains detached. Subsequently we have identified neurites from horizontal and ganglion cells in both sub- and epiretinal glial scars removed from human retinas. These data indicate that reactive retinal glial cells form a permissive scaffold for neurite growth both within the retina and on either surface of the retina and thus may hold clues to factors that support this growth. Supported by NIH grant EY00888.

P-71

Retinal detachment in mice deficient in GFAP and Vimentin: Reduced Glial hypertrophy and increased ganglion cell remodeling

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Retinal detachment induces Müller glia cell (MC) hypertrophy. Structural and immunolabeling data suggest a prominent role for the intermediate filament cytoskeleton in this process. There also may be a relationship between MC hypertrophy and neuronal remodeling after detachment. Here we studied both of these events after detachment in wild-type C57Bl/6J mice and mice deficient in the intermediate filament proteins GFAP and vimentin (GFAP-/-vim-/-). The retinas were harvested at 7 or 28 days post-detachment. Immunohistochemistry was performed using antibodies labeling both glial and neuronal cell types. Images were collected by confocal microscopy. MC hypertrophy following detachment was greatly inhibited in the GFAP-/-vim-/- mice. Anti-S100 labeling revealed abnormal MC morphology in the attached GFAP-/-vim-/- retina, which was exaggerated after detachment. Extensive neuronal remodeling occurred in both GFAP-/-vim-/- and wild-type animals. However, ganglion cell reactivity, identified by anti-neurofilament labeling, was greatly exaggerated in areas showing damaged MC endfeet. It has already been demonstrated that MC endfeet are more fragile in this strain of mice (Lundkvist, et al. JCS 117, 3481-3488). These results demonstrate the importance of GFAP and vimentin to the hypertrophy of MC after retinal injury and suggests that MC play an important role in regulating ganglion cell remodeling. Supported by NIH grants EY00888, EY012983, NSF grant 0331697, and the Department of Defense.

P-72

Why is Wallerian degeneration so slow in the CNS?

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After axotomy, degenerating myelin is cleared by phagocytes in a process known as Wallerian degeneration (WD). WD is rapid in the PNS, occurring in days to weeks, but slow in the CNS, taking months to years. As degenerating myelin is strongly inhibitory to axonal regeneration, slow WD in the CNS may contribute to the failure of CNS axons to regenerate. To understand why WD is slow in the CNS, we are testing the hypothesis that anti-myelin antibodies are necessary for rapid myelin clearance. After PNS injury the blood-nerve barrier **breaks** down along the length of the distal nerve, but in the CNS only local blood-brain barrier breakdown occurs preventing antibody binding to the degenerating CNS myelin. To test whether antibodies mediate rapid myelin clearance, we measured the rate of WD in transected sciatic nerves of mutant mice that lack B-cells and are therefore unable to make antibodies. In wild type mice, most myelin debris is cleared within one week after axotomy. However, in the mutant mice, myelin clearance is significantly delayed by one week. These findings show that antibodies help mediate the rapid myelin clearance in the PNS and suggest that myelin clearance in the PNS can be divided into two phases: an early antibody-independent phase that is mediated by Schwann cells and a later antibody-dependent phase that is mediated by macrophages. We are currently investigating whether the antibodies that mediate rapid myelin clearance are preexisting natural antibodies or are newly-generated against degenerating myelin. CNS WD may be slow because it lacks these mechanisms, raising the question of whether rapid myelin clearance and axon regeneration in the CNS may be triggered by delivery of antibodies to degenerating CNS myelin.

P-73

A local mechanism mediates NAD-dependent protection of axon degeneration

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Axon degeneration occurs frequently in neurodegenerative diseases and peripheral neuropathies. Important insight into the mechanisms of axon degeneration arose from findings that the degeneration of transected axons is delayed in Wlds mice with the over-expression of a fusion protein with the NAD synthetic enzyme Nmnat1. Although both Wlds and Nmnat1 itself are functional in preventing axon degeneration in neuronal cultures, the underlying mechanism for Nmnat1 and NAD-mediated axon protection remains largely unclear. We demonstrate that NAD levels decrease in degenerating axons and that preventing this axonal NAD decline efficiently protects axons from degeneration. In support of a local protective mechanism, we show that the degeneration of axonal segments that have been separated from their soma could be prevented by the exogenous application of NAD or its precursor nicotinamide. Furthermore, we provide evidence that such Nmnat1/NAD-mediated protection is primarily mediated by their effects on local bioenergetics. Together, our results suggest a novel molecular pathway for axon degeneration.

P-74

Laceration of the spinal cord causes less demyelination and remyelination than contusion injury in rat and monkey

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Demyelination constitutes a significant pathologic feature of spinal cord injury (SCI). Histologic studies of SCI have shown varying degrees of remyelination carried out by endogenous oligodendrocyte progenitor cells (OPCs) and Schwann cells (SCs). We hypothesized that different models of SCI cause different amounts of demyelination and remyelination. It is important to identify the extent of demyelination and remyelination in different experimental models so as so understand the applicability of developing therapies that may target demyelination. There currently is no report quantifying demyelination or remyelination following laceration injury to the rat or monkey spinal cord. We quantitatively compared demyelination and remyelination in contused and hemisected rat spinal cords. The hemisected rat spinal cords were also compared to hemisected monkey cords in order to determine if demyelination and remyelination is similar between rats and monkeys. Our findings indicate that hemisected rat spinal cords show significantly less demyelinated and remyelinated axons as compared to the contused rat spinal cords, and that hemisected primate spinal cords similarly displayed very few demyelinated and remyelinated axons. This study suggests that remyelination strategies are most suited to contusive SCI.

P-75

Hyperactivity of Na+/K+ pump in regenerated mature myelinated axons

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Following regeneration or remyelination of adult axons the internodal length remains short. As conduction velocity recovers with the fiber diameter, the functional consequences of the increased number of nodes remain unclear. We found functional evidence in vivo that due to the increased number of nodes the greater Na+ load during action potential conduction drives the Na+/K+ pumps leading to hyperpolarization (Moldovan and Krarup, 2004; J Physiol 560). To further test this hypothesis, the aim of the present study was to develop a mouse model that allows combining electrophysiological methods with pharmacological manipulations. Sciatic nerves of 8 mice (18-20g) were crushed unilaterally ~1cm above the knee. The contralateral unlesioned nerves served as controls. Electrophysiological estimations of recovery were carried out monthly under anesthesia by stimulation at ankle and recording of the CMAPs from tibial innervated plantar muscles. After 5 month of regeneration the CMAP amplitude and number of motor units (by statistical method) recovered. In spite of the good recovery of distal motor latency (~80% of control), measurements of various excitability indices by threshold tracking showed marked functional abnormalities consistent with membrane hyperpolarization. Evidence of Na+/K+ pump involvement in hyperpolarization was obtained during acute experiments by investigating excitability changes during post tetanic hyperpolarization (after 5 minutes of 100 Hz stimulation) and the effect of ouabain (0.5 mM applied for 20 minutes) on resting membrane potential. Histological investigations, carried out at the completion of the experiments, showed that even the diameter of large fibers (> 6μm) recovered to ~90%, their internodal length was only ~35% of control. Thus our experimental data suggest that persistently shorter regenerated internodes lead to Na+/K+ pump hyperactivity that impairs function during activity and drains the energy reserves of the axons. This persistent abnormality may contribute to the poor clinical outcome of nerve regeneration.

P-76

Thrombospondin-1 production and release is regulated by purinergic signaling in astrocytes: Implications for CNS development and repair

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Emerging evidence indicates that thrombospondin (TSP)-1, an extracellular matrix protein, participates in synaptogenesis and remodeling (Christopherson et al., 2005; Lin et al., 2003), but little is known about the mechanisms regulating its expression and release in the CNS. We found that purinergic signaling stimulates production and secretion of TSP-1 from astrocytes. When primary cultures of rat cortical astrocytes were treated with extracellular ATP, TSP-1 expression was increased by about 30 fold in a time- and concentration-dependent manner. This increase in TSP-1 was attenuated by antagonists of P2 and P1 purinergic receptors or by apyrase, an ATP diphosphohydrolase that degrades ATP to AMP. Agonist studies with nucleotides and their analogs revealed that P2 receptors of the P2Y2/4 subtype mediated TSP-1 synthesis and release. P2Y receptors are coupled to several protein kinase cascades, and selective blockade of signaling by the mitogen-activated protein kinases (MAPKs) ERK, p38, and SAPK/JNK, or by protein kinase B/Akt, resulted in partial or complete inhibition of nucleotide-induced TSP-1 expression. These experiments indicate that multiple protein kinase pathways, particularly p38/MAPK and Akt, regulate expression of TSP-1. Studies with an in vitro model of CNS trauma, which stimulates release of ATP, demonstrated that TSP-1 expression increased in a time-dependent manner after mechanical strain. The strain-induced increase in TSP-1 was completely blocked by a P2 receptor antagonist, thereby indicating a key role for P2 receptors in TSP-1 expression after trauma. Our results reveal that TSP-1 expression is stimulated by activation of P2Y2/4 receptors coupled to protein kinase signaling pathways and suggest that purinergic regulation of TSP-1 production and release may be an important factor in cell-matrix and cell-cell interactions that occur during development and after CNS injury. [This work was supported by the Department of Veterans Affairs and the National Institutes of Health (NS046651 and NS045470).

P-77

Bioinformatics analysis results in conflicting predictions for the M278T AQP4 SNP

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Background: Brain injury often involves cytotoxic edema where astroglia swell in response to an osmotic gradient. The aquaporin 4 (AQP4) gene encodes a water channel membrane protein in brain astrocytes. Mouse AQP4 knockout models demonstrate reduced cytotoxic edema. We hypothesize that SNPs in AQP4 in humans may affect response to edema. In the dbSNP database, only one coding non-synonymous SNP (M278T) is documented for human AQP4. Factors that predict whether SNPs will have phenotypic effects include their location, chemical nature, and degree of evolutionary conservation.

Methods: We first used the SIFT program based on evolutionary conservation to predict whether M278T would be tolerated. We next ran BLASTP on AQP4 to determine which sequences SIFT used for comparison. Then, since no crystal structure is available for AQP4, we used CLUSTALW to produce multiple sequence alignments of human AQP1 and AQP4 isoforms as well as mammalian AQP4 orthologues. Finally, we used the PolyPhen server to predict the effect of M278T based on structural characteristics.

Results: SIFT predicted that any amino acid change at position 278 would be tolerated. A BLASTP search revealed that most of the 22 human AQP4 homologues found were aquaporins, suggesting reliable comparisons. One multiple alignment showed no homologous region in hAQP1 for the hAQP4 region harboring M278T, while another indicated conservation with four other mammals over a 27-residue span including the variant. PolyPhen predicted M278T to be possibly damaging.

Discussion: Computational analysis results in conflicting predictions of the effect of the M278T AQP4 variant. To assess the functional role of this and other SNPs, we plan to use an assay based on the uptake of 3-O-methyl-D-glucose to measure cell volume. This assay may yield further insight into the structure and function of aquaporin 4 and its role in edema after brain injury.

P-78

Innate immune responses mediated by toll-like receptor (TLR)2 and TLR4 signaling are important for recovery after spinal cord injury

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Spinal cord injury (SCI) activates innate immunity (e.g., macrophages). Signaling through pattern recognition receptors, called Toll-Like Receptors (TLRs), is an evolutionarily conserved mechanism of triggering macrophages. TLRs have traditionally been associated with host defense to pathogens; however, recent data suggest that TLRs modulate immune responses to injury by responding to endogenous ligands (e.g., heat shock proteins). To examine whether SCI activates TLRs, in situ hybridization was used to map spatio-temporal patterns of mRNA expression following a mid-thoracic spinal contusion injury. Expression of CD14 (TLR4 co-receptor), TLR2, and IkB-á mRNA was increased throughout the injury site, with peak expression occurring 3-14dpi. Microarray analyses confirmed these data and provided insight to SCI-induced regulation of molecules downstream of TLR2 and TLR4 signaling. To examine the biological impact of TLR2 and TLR4 signaling in vivo after SCI, TLR4 deficient mice (TLR4d) and TLR2 knockout (TLR2ko) mice were used. Compared to SCI wild-type (wt) mice, functional recovery was impaired in TLR4d mice and TLR2ko mice. However, morphometric analyses of the lesion revealed distinct differences between these mice. Specifically, myelin sparing was reduced in TLR4d mice and was accompanied by a more robust and disseminated macrophage response compared to (wt) mice. Myelin sparing was not different between SCI wt and TLR2ko mice. However, deletion of TLR2 frequently produced unusual patterns of myelin loss localized to the ventromedial white matter. These lesions extended beyond the site of impact. Together, these data suggest a physiological role for TLR2 and TLR4 signaling of innate immune-cells after SCI. Supported by NINDS NS37846 and F31 NS51069.

P-79

Anti-inflammatory drugs promote neurite outgrowth in the presence of myelin-associated growth-inhibitory molecules

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After CNS injury, expression of myelin-associated molecules such as myelin-associated glycoprotein (MAG), oligodendrocyte myelin glycoprotein (OMgp), and Nogo inhibit axon elongation and regeneration. The anti-inflammatory COX-2 inhibitor NS-398 exerts neuroprotective effects in stroke models and after spinal cord injury. Administration of COX-2 inhibitors to animals prior to a contusion spinal cord injury produces decreased production of inflammatory cells, increased viable tissue at the lesion and improved locomotor recovery. (Resnick et al., 1998; Hains et al., 2001). In order to assess the ability of NS-398 to promote neurite growth in the presence of myelin-associated growth-inhibitory molecules, post-natal rat spinal cord explants or embryonic rat cortex explants were grown in vitro on laminin-coated membrane filters prepared with alternating stripes of myelin. Antibody staining confirmed the presence of inhibitory molecules including MAG, OMgp and Nogo within the myelin stripes. The ability of neurites to extend over the myelin stripes was evaluated after 3-5 days in culture. When control explants were grown in the presence of alternating stripes, neurite outgrowth preferentially extended into regions devoid of myelin. In contrast, neurite outgrowth from explants grown in the presence of NS-398 was significantly less selective for myelin-devoid regions and was able to

extend into regions containing myelin. In order to determine if this effect could be attributed to COX-2 inhibition, the effect of other COX inhibitors was also determined. The data presented here suggest that COX inhibitors play a multi-faceted role in promoting axon regeneration after CNS injury.

P-80

Immunomodulation in experimental models of neurodegenerative diseases

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HIV-1-associated dementia (HAD) and Parkinson's disease (PD) are highly divergent disorders based on etiology, epidemiology, pathogenesis, and pathobiology. Indeed, one is characterized by cognitive, motor, and behavioral dysfunction induced by viral infection and progressive immunosuppression and the other by profound loss of dopaminergic neurons in the subtantia nigra (SN) and termini in the caudate-putamen projections. However, both disorders are linked by chronic microglial activation and neuroinflammation. To explore the role of microglial neuroinflammation in pathogenesis and disease treatments, we mirrored the pathogenic features of HAD and PD by injecting severe combined immune deficient (SCID) mice with human HIV-1 infected monocyte-derived macrophages (MDM) or by injecting 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), respectively. Observations of HIVE SCID mice directly immunized with copolymer-1 (Cop-1) paralleled adoptive transfer of Cop-1 immune cells to MPTP recipient mice which led to T cell accumulation within the SN, suppression of microglial activation, and increased astrocyte-associated glial cell line-derived neurotrophic factor. These immunization strategies resulted in a significant protection of subcortical neurons and their projects (for HIVE) and the nigrostriatal dopaminergic pathway (for PD). Neuroprotection was T cell dependent and independent in SCID mice. Co-registration, combining single photon emission computed tomography (for DA transporter and DA receptors) with magnetic resonance imaging and spectroscopy (MRI/S) (for biochemical profiles), paralleled our histopathological results for survival of dopamine neurons. Naturally occurring CD25+CD4+ regulatory T cells were neuroprotective in MPTP-intoxicated mice. Aggregated a-synuclein (confirmed by atomic force microscopy) induced a microglial-specific biomarker profile following analysis of spectra obtained from surface-enhanced laser desorption-time of flight assays. Distinct proteomic signatures of neurotoxic and neurotrophic microglia following injury and activation were determined. The role of microglial neuroprotective activities was found to revolve around their abilities to positively affect intracellular transport, protein degradation, and neuronal physiology. The abilities to uncover microglial functions in animal and laboratory assays and improvements in monitoring glial functions provide insights in immunoregulatory therapeutic strategies for neurodegenerative diseases. Supported by R37 NS036126-08. No real or perceived conflict of interest.

P-81

Differential EGFP expression in the Lys-EGFP-ki transgenic mouse distinguishes between microglial macrophages and hematogenous macrophages in spinal cord injury

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Inflammation plays a large, controversial role in the secondary injury response to spinal cord injury (SCI). One obstacle preventing advancements in the field is the inability to clearly differentiate between the CNS-derived microglial macrophage (mMF) and the hematogenous monocyte/macrophage (hMF) at the SCI. mMF are normal residents of the CNS, whereas hMF exist in the meninges, as perivascular macrophages and infiltrate into the CNS in response to trauma or infection. These two cell populations are distinct in the non-activated state. However, in their fully activated form, these two cell populations are morphologically indistinguishable and share common markers. This makes it difficult to determine their different spatial and temporal roles in the inflammatory response to SCI. We examined whether the differential expression of enhanced green fluorescent protein (EGFP) by the lys-EGFP-ki transgenic mouse, created by Faust et. al. (Blood, 96: 719, 2000), distinguished between mMF and hMF in the SCI lesion. The lys-EGFP-ki mouse has the gene for EGFP inserted into its genome under the control of the lysozyme M promoter. Therefore, EGFP is specifically expressed in mature cells of the myelomonocytic lineage, including the hMF and neutrophils. We previously reported the lys-EGFP-ki mouse expresses EGFP in neutrophils and hMF in the SCI. In SCI mice treated with clodronic acid liposomes to deplete the hMF prior to SCI resulted only in the depletion of EGFP+ hMF. Presented here is a completed characterization (up to 6 weeks post injury) of this SCI model using an extended clodronic acid liposome treatment and bone marrow transplantion to further demonstrate that the EGFP under the control of the lysozyme M promoter does allow one to easily distinguish between hMF and mMF. This will allow us to more easily dissect the functional role of each of these macrophage populations in the inflammatory response to SCI.

P-82

Dysfunctional astrocytes and SCI-induced neuropathic pain

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Central neuropathic pain (CNP) following spinal cord injury (SCI) severely affects the quality of life of about 70% of SCI patients. As in the human patient population, the majority of rats develop significant allodynia (CNP rats) after moderate SCI. Using DNA microarray analysis, we found significantly increased expression of a number of genes associated with astrocytic activation in the spinal cords of rats that developed CNP. We also found that an elevated expression of astrocytic proteins GFAP, S100beta

and Aquaporin 4 (AQP4)persisted for at least 9 months throughout contused spinal cords consistent with the chronic nature of CNP (Nesic et al., 2005). Astrocyte functions - including glutamate uptake/release, free radical scavenging, ion/water transport, the production of cytokines and nitric oxide - may, if deregulated, contribute to the development of hyperexcitability of pain processing neurons in injured spinal cords. Using DNA microarray analysis, we found expression changes of the genes involved in regulation of astrocytic ion/glutamate transport along with increased expression of AQP4 and chronic cytotoxic edema, which indicate a disturbed ion/water/glutamate transport in CNP spinal cords. Therefore, we hypothesize that CNP development results, in part, from chronically activated and dysfunctional astrocytes, which impaired regulation of ion/water/glutamate transport contributes to the hyperexcitability of pain processing spinal neurons, and consequently to the development of a chronic pain condition.

P-83

Aberrant intraspinal sprouting and corresponding changes in synaptic cadherins associated with neuropathic pain

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Peripheral nerve injury is known to produce changes in primary afferent circuitry including ectopic discharge of injured and uninjured DRG neurons and altered neurochemical expression that lead to or contribute to neuropathic pain behaviors. Ligation of the lumbar L5 spinal nerve (Kim and Chung, 1992) is a well characterized and widely used model for tactile allodynia, in which normally innocuous light touch is perceived as painful. We have utilized a modified L5 spinal nerve transection (L5 SNT) model to characterize the differential regulation of two synaptic cell adhesion molecules, N- and E-cadherin, that are normally associated with peptidergic and non-peptidergic subpopulations of nociceptive C-fibers, respectively (Brock et al., 2004). Following L5 SNT, E-cadherin is rapidly down-regulated within 4 days, while N-cadherin is up-regulated beginning at 7 days post-injury. Interestingly, this increase in N-cadherin occurs concurrently with the aberrant sprouting of wheat-germ agglutinin(WGA)-labeled C-fibers from their normal terminations in the L4 superficial dorsal horn into the deafferented L5 superficial dorsal horn. Furthermore, N-cadherin immunolabeling co-distributed with that for the axonal growth associated protein, GAP-43, but not with markers of astrocytes or microglia. Because N-cadherin mediates axon growth, targeting and synaptogenesis developmentally, our data suggest that cadherins may be involved in the reorganization of dorsal horn circuitry that underlies the maintenance of neuropathic pain. Supported by NIH/NINDS: NS044868, NINDS NRSA: NS44718, Christopher Reeve Paralysis Foundation.

P-84

Targeting Chemokine CXCL10 in the immune response: A possible therapy for acute spinal cord injury

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Secondary degeneration, which leads to progressive tissue loss, occurs following primary insult to the adult mammalian spinal cord. The inflammatory response in secondary degeneration is closely related to several detrimental processes such as ischemia, cellular and tissue edema, oxidative damage, myelin degradation, and apoptotic changes, all of which contribute to an increase in lesion size following spinal cord injury (SCI). Chemokines have been implicated as mediators of secondary degeneration, and their expression has been shown to precede immune cell influx into the injured central nervous system (CNS). The chemokine CXCL10 is a potent T lymphocyte recruiter that acts by binding to the CXCR3 receptor. CXCL10 has been implicated in the pathology of several CNS disorders. We have previously illustrated that prophylactic anti-CXCL10 antibody treatment reduces the inflammatory response and decreases behavioral deficits following SCI (Gonzalez et al 2003). In order to assess the role of anti-CXCL10 antibody treatment as a therapy, we administered the treatment 1 hour following SCI. Anti-CXCL10 antibody treatment leads to a reduction in inflammation, tissue loss, neuronal loss and behavioral deficits following injury. Furthermore, we have evidence illustrating that following SCI CXCL10 is expressed by blood vessels and anti-CXCL10 antibody treatment leads to a significant increase in the formation of new blood vessels (angiogenesis). In conclusion, since revascularization occurs prior to nerve regeneration (Zhang and Guth, 1997), we believe our treatment likely acts as a neuroprotectant by establishing the proper environmental conditions necessary for nerve growth.

P-85

Myelin-associated glycoprotein(MAG) protects neurons from apoptosis via MEK/ERK pathway

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It is now well established that axons of the adult CNS are capable of only a limited amount of regrowth after injury, and that an unfavorable growth environment plays a major role in the lack of regeneration. Myelin associated glycoprotein (MAG), Nogo and oligodendrocyte-myelin glycoprotein (OMgp) have been identified as components of the CNS myelin that prevents axonal regeneration.Here we show the new signaling pathway elicited by MAG in the neruons. It protects neurons via MEK/ERK pathway from the apoptosis signal activated by MAG itself . MAG was internalized into cerebellar granular neurons and dorsal root ganglion neurons in a lipid-raft dependent manner. And the activation of ERK occurred in parallel with internalization of soluble MAG. The co-localization of the extracellular domain of p75NTR,

labeled with fluorescent MC192, and MAG in the endosome of neurons was demonstrated. Time-lapse video microscopy showed that soluble MAG and p75NTR were transported to cell body from the axon terminals after internalization. Although no cell death was induced by MAG in the cerebellar neurons, MAG induces the apoptosis of neurons in the presence of the selective inhibitor of MEK. Our data suggest that p75NTR forms a signaling complex that is transported from the axon terminal to the cell body.

P-86

The window of opportunity for repair by radiation therapy in spinal cord injury: The optimal starting time of the therapy

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Longitudinal studies, ex vivo by histology [PNAS (1996) 93:111791] and in vivo by MRI, suggest that natural wound repair processes are activated after injury. However, by the end of the 3rd wk postinjury the repair is aborted and chronic decay ensues yielding a widening cavity. These suggest the existence of a critical time-window for intervention to suppress/prevent the onset of tissue decay. Specific cell elimination within 2-3 wks postinjury, by radiation therapy of the lesion site, can prevent the onset of decay thereby facilitating some structural and functional repair in transected rat spinal cord [Brain Res (2001) 904:199]. The objective is to identify the conditions which would yield optimal facilitation of natural repair. Here we asked, what is the best time after injury for starting the radiation therapy? Data show that the critical time window for starting the radiation therapy is in between days 10 to 12 postinjury. Studies were performed in a completely transected rat spinal cord, and radiation ?consisting of 8 sequential daily dose-fractions of 2 Gy (total of 16 Gy)? was given starting either on day 8, 11, 13, 16, or 21 postinjury (n= 5-9/group). The effectiveness of these protocols in reducing chronic inflammation and/or promoting repair was examined in vivo by MRI 50-55 days postinjury and ex vivo by routine histology 2 mo postinjury. Quantitative data of tissue preservation show that starting the therapy on day 11 is significantly (ANOVA, Tukey' post-hoc) better than starting it on day 8 ($p=0.048$) or on day 16 after injury ($p=0.018$). Further, the in vivo data suggest that starting the therapy too early, e.g., on day 8 postinjury, is deleterious; it interferes with the essential wound healing processes, yielding a somewhat reduced chronic inflammation but a wound gap bigger than that of the untreated lesioned cords. Supported by NIH, NS39375 (NK)

P-87

Enriched environment combined with multimodal stimulation is associated with reduced CNS scar and less neuromotor deficit after traumatic brain injury in rats

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To determine whether exposure to multimodal early onset stulation (MEOS) combined with enriched environment (EE) after traumatic brain injury (TBI) would improve neurological recovery and to elucidate morphological correlates. Male Sprague-Dawley (SD) rats were subjected to lateral fluid percussion (LFP) brain injury or sham operation. Thereafter, 1/3 of the animals (injured and sham) was placed in standard housing (SH), 1/3 received EE-only, and 1/3 underwent EE+MEOS. EE consisted of serial cages with beddings, inclining platforms, and toys. EE + MEOS animals additionally underwent a standardized paradigm of multimodal stimulation including auditory, visual, olfactory, and motor stimuli. A standardized composite neuroscore (NS) test assessed acute post-traumatic neuromotor deficits (24h post-injury) and recovery on post-injury day (DPI) 15; recovery of cognitive function was assessed on DPI 11–15 using the Barnes circular maze (BCM). Assessment of neuromotor function 24h post-injury revealed identical neurological impairment in all lesioned animals. On DPI 15, reversal of neuromotor dysfunction was significantly better in EE+MEOS animals versus SH and EE-only groups ($p < 0,02$). Similarly, latencies to locate the hidden box under the BCM platform were significantly shortened in EE + MEOS animals on DPI 15 ($p= 0.003$). EE+MEOS animals had consistently the lowest lesion volumes (mm3) after staining serial brain sections for neuron specific enolase/NSE, caspase 3 active/C3A, and glial fibrillary acidic protein/GFAP. This first report on combined EE+MEOS post-traumatic treatment indicates that exposure to EE+MEOS can reduce CNS scar formation and reverse neuromotor deficits in rats after TBI. * Koeln-Fortune Program, University of Cologne (Germany)

P-88

db-cAMP treatment of the Red Nucleus promotes regenerative response of rubrospinal neurons but fails to improve forelimb usage

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The neuronal cell body response to axonal injury plays an important role in the failure of CNS neurons to regenerate. Adult mammalian CNS neurons fail to re-express a variety of genes and signaling factors after axotomy that are seen at increased levels in regenerating peripheral neurons or in developing neurons: e.g. cAMP. db-cAMP treatment has recently been shown to promote regenerative sprouting in a variety of CNS neuronal systems. Here, we tested the hypothesis that db-cAMP application to the vicinity of rubrospinal neurons enhances their regenerative cell body response and promotes their sprouting/regeneration across a site of spinal cord injury at the C3/4 level (crush of the dorsolateral funiculus in adult male Sprague Dawley rats). Osmotic minipumps delivered either 25 or 12 mg/ml of dbcAMP or PBS respectively at a rate of 12ml/24h for 14 days and were mounted at either the time of injury or one week prior to injury (pre treatment of db-cAMP). Biotinylated Dextrane Amine was injected into the vicinity of the red nucleus to trace the rubrospinal axons. While controls showed a retraction of the rubrospinal axons from the enlarging cavity at the crush site, several db-cAMP treated rats displayed a pronounced sprouting of rubrospinal axons into the site of injury and beyond. Behavioural analysis of spontaneous

forelimb usage in the cylinder test (Schallert) revealed no significant differences between the db-cAMP groups and the control groups. These data underscore the concept that cell body treatment with cAMP stimulates the growth propensity of these neurons, but that it may not be sufficient to elicit functional benefits. Supported by the Christopher Reeve Paralysis Foundation and CIHR of Canada.

P-89

The RNA-binding protein La is sumoylated in regenerating axons

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Previous work from our lab has shown that a surprising number of different proteins are synthesized in the regenerating axons of injury-conditioned dorsal root ganglion neurons [DRG] (Willis et al., 2005). By cDNA array hybridizations, axonal mRNAs include several transcripts encoding ribosomal proteins. The eukaryotic La/SSB protein functions as an RNA binding protein in the cytoplasm (Kenan and Keene, 2004) and its targets include ribosomal protein mRNAs (Horke et al., 2004; Crosio et al., 2000). By immunofluorescence La/SSB extends into DRG axons and shows a granular signal. Immunoblotting further confirms that axons contain this protein but also show that the majority of axonal La/SSB migrates at a higher molecular weight than expected due to a post-translational modification. Immunoblotting of axoplasm from sciatic nerve shows similar findings. Co-immunoprecipitation and immunofluorescence indicate that this shift is due to sumoylation of this RNA binding protein in the axons. Further, the sumoylated form of La/SSB coimmunoprecipitates with dynein and it accumulates on the distal component of the sciatic nerve after ligation. This suggests that sumoylated La/SSB is retrogradely transported. Transfected human La-GFP distributes throughout the axon reaching the distal growth cone similar to the endogenous rat La/SSB. Mutation of potential sumoylation sites in human La-GFP suggest that sumoylation of La is involved in axonal transport of this protein. These data suggest a model where La/SSB is rapidly transported into the axons, likely with mRNA cargo; upon reaching the distal axon, La/SSB is sumoylated, targeting the protein for retrograde transport that occurs at a much slower rate than anterograde movement of mRNAs.

P-90

Axonal chaperone protein mRNAs are translationally regulated by ER stress

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Localized translation of axonal mRNAs has recently been recognized as a critical step in initiating axonal regeneration (Hanz et al., 2003; Verma et al. 2005). Work from our lab has shown that axonally synthesized proteins help to maintain structure of distal regenerating sensory axons (Zheng et al., 2001). In addition to cytoskeletal elements, we have recently shown that several chaperones including resident ER chaperones are synthesized in regenerating axons (Willis et al. 2005). In non-neuronal systems translation of chaperone mRNAs, (including those identified in sensory axons) is directly activated as part of the unfolded protein response (UPR). Here we show evidence that axons have the potential for a localized UPR that includes selective activation of axonal mRNA translation. Treatment of isolated rat DRG axons with thapsigargin to release ER calcium stores results in a net increase in axonal synthesis of calreticulin , but leads to a decrease in total axonal protein synthesis. To more specifically test for axonal UPR, isolated DRG axons were treated with tunicamycin, which inhibits glycosylation to initiate ER stress. Preliminary results show that exposing DRG neurons to tunicamycin results in a specific increase in the level of the ER chaperone proteins. Polysomal fractionation of axonal RNAs harvested from intact DRG cultures showed a shift of axonal calreticulin mRNA to the polysome fraction indicating that it is being translationally activated by tunicamycin. On the other hand, axonal mRNA encoding the cytoplasmic chaperone protein HSP70 fractionates in the sub-polysome both before and after tunicamycin treatment. Taken together, these data argue that axonal chaperone protein mRNAs are translationally regulated by stimuli that selectively invoke ER stress. This suggests that translation of chaperone mRNAs in axons represents part of a localized UPR.

P-91

Axons of passage and endogenous remyelination

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Demyelination has been proposed to be one of the major contributing factors of the functional deficits after spinal cord injury. However, the nature of myelin deficits in surviving motor pathways that maintain their connection following trauma has not been determined. We hypothesize that by closely examining morphological and molecular indices of myelin in surviving axons, we will be better able to predict therapeutic strategies for repair of the chronically injured spinal cord. Here we examined rubrospinal tract axons (RST) which are heavily myelinated. Adult C57\bl6 mice received a moderate T9 contusion injury. After a period of 8 weeks mice received bilateral BDA (biotinylated dextran amine) or Fluoro-Ruby (tetramethylrhodamine dextran) injections into the Red Nucleus. The mice were sacrificed 10-11, 18-19 weeks post injury and the extent of demyelination or remyelination was examined by employing two methods.1) Pre-labeled, single RST fibers that crossed the lesion site were mechanically teased away (~0.1mm caudal) from whole spinal cords. Individual RST axons were mounted and immunolabeled for CASPR (contactin-associated protein) and internodal distances were measured for control and injured animals. Injured RST axons had a significantly different modal distribution of internodal distances. 2) BDA labeled axons were examined with electron microscopy. The myelin index was determined for the BDA labeled axons caudal the site of lesion. These data show that many axons of passage either

maintain a normal looking myelin profile or have been remyelinated. Together, the findings demonstrate that axons of passage are primarily myelinated/remyelinated with altered internode sizes. (Supported by UW Royalty Research Fund and NIH R01 NS46724-01A1)

P-92

Deletion of NF1 in neurons induces increased axon collateral branching in vitro and in vivo following injury

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Ras-mediated signaling pathways participate in multiple aspects of neural development and function. Loss of function in the Ras-GAP (GTPase activating protein) neurofibromin, a negative Ras regulator encoded by the Neurofibromatosis type 1 (NF1) gene, endows embryonic sensory neurons with neurotrophic factor independent survival and differentiation. Through use of a Synapsin I promoter driven Cre transgene, a mouse line was generated (NF1SynIKO) that lacks NF1 in the majority of CNS neurons. Explants from NF1SynIKO mice showed increased axonal length, maximum number of collateral branches per axon, and total length of collaterals, compared to WT DRGs. The robust branching properties observed in culture for NF1 deficient neurons were evaluated in vivo by testing the ability of NF1SynIKO mice to recover from unilateral dorsal rhizotomy. In contrast to the permanent sensory deficits observed in control mice after dorsal rhizotomy, neuron-specific NF1 mutant mice spontaneously recover proprioceptive function. This phenomenon appears to be mediated both by a cell autonomous capacity of spared NF-/- DRG neurons for increased collateral branching, and by non-cell autonomous contribution from the NF1-/- spinal cord. The transcription factor NFATc1 expression and activity is increased after deletion of neuronal NF1, and thus represents a likely molecular candidate for mediating increased axonal branching of NF1-deficient neurons. The present study indicates that neurofibromin may serve as a useful therapeutic target to increase the sprouting capacity of spared neurons after neural trauma.

P-93

Chondroitin sulfate proteoglycan glycosaminoglycan chain synthesis: A potential molecular target

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Chondroitin sulfate proteoglycans (CSPGs) are up-regulated in the CNS after injury and have been shown to participate in the inhibition of axon regeneration. The glycosaminoglycan (GAG) chains of these molecules are involved in the inhibition of axon growth and in binding growth factors and other molecules. At present, the expression patterns of chondroitin polymerizing factor (ChPF), a key enzyme for CSPG GAG chain synthesis is not known, and it is also not known if reducing the expression of key CSPG GAG chain synthesizing enzymes will result in increased axonal regeneration after injury. Using conventional RT-PCR, we have shown that ChPF mRNA is present in oligodendrocyte precursor cells, meningeal cells, astrocytes, axon growth-inhibitory astrocytic cell line Neu7, axon growth-permissive astrocytic cell line A7 and embryonic and adult brain. Using quantitative real-time PCR we have shown that ChPF is not up-regulated after treatment of cultured astrocytes with brain injury related cytokines TGF-á or TGF-â1. ChPF is also not up-regulated in the area surrounding a cortical stab lesion in an adult rat. Using a vector based short hairpin RNA we show the reduction of ChPF mRNA by 65% in Neu7 cells with the greatest reduction in mRNA at two days post transfection. In parallel, this reduction in mRNA levels decreases the level of CSPG GAG chains in the conditioned media (CM) of these cells as soon as three days after transfection as shown by western blotting with CS-56. This decrease in GAG chains in the CM was consistent with an increased ability of the CM to support axon growth. These results suggest that ChPF is a potential molecular target to decrease CSPG GAG chain synthesis and thus, facilitate axonal regeneration after injury.

P-94

Local down-regulation of myelin-associated glycoprotein permits axonal sprouting with chronic nerve injury

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This work was supported by grants to R.G. from the National Institutes of Health 5K08 NS02221 and 5R01 NS049203. Until recently, little has been known about the cellular and molecular changes after chronic nerve compression (CNC) injuries. Unlike acute nerve injuries, such as axonotomy and crush, recent studies have revealed that CNC injury triggers dramatic Schwann cell proliferation and concurrent apoptosis in the absence of signs of Wallerian degeneration. This robust Schwann cell proliferation is in a distinct spatial and temporal pattern, which is accompanied by an increase in the number of small un-myelinated axons in the area of the injury. These findings suggest that this local proliferation of Schwann cells may induce local axonal sprouting. Here, we use quantitative electron microscopic techniques to define the nature of this sprouting response, and explore whether the local sprouting is in response to down-regulation of expression of myelin associated glycoprotein (MAG) by proliferating Schwann cells. Axonal sprouting was observed without evidence of Wallerian degeneration in the outer region of CNC injured nerves with a noticeable increase in Remak bundles within this region of injury. Immunolabeling of teased nerve fibers and Western blot analysis of nerves from CNC injured animals revealed a local down-regulation of MAG protein within the zone of injury.

Moreover, local delivery of purified MAG protein intraneurally at the time of CNC model creation abrogates the axonal sprouting response. These data demonstrate that CNC injury triggers axonal sprouting and suggests that a local down-regulation of MAG within the peripheral nerve secondary to CNC injury is the critical signal for the sprouting response. While the reciprocal relationship between neurons and glial cells is maintained after injury, the initial response in CNC injuries is primarily Schwann cell-mediated which in turn secondarily affects neuronal function and creates an environment within the peripheral nerve which fosters regeneration.

P-95

Plasminogen activator is necessary for the crossed phrenic phenomenon

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In addition to its role in the vascular system, plasminogen activator (PA) is involved in neural development, excitotoxic cell death, and has been implicated in aspects of cerebral synaptic remodeling associated with cerebellar motor learning, visual cortex ocular dominance columns, and hippocampal and corticostriatal LTP. We have explored the possibility that PA may also play a role in synaptic plasticity in the spinal cord. The crossed phrenic phenomenon (CPP) describes respiratory functional plasticity that arises following spinal cord injury; whereby, phrenic motoneuron drive to the diaphragm is restored following activation of "functionally ineffective" medullary respiratory neuron synapses on phrenic motoneurons (PMN). Synaptic remodeling is thought to occur during the characteristic delay period following spinal cord injury before the CPP becomes functional. The mechanisms underlying this synaptic plasticity are not well-defined. Our ultimate aim is to understand the underlying molecular mechanisms of this functional recovery using a mouse model amenable to a molecular genetic approach, and ours is the first report of CPP in mice. Using electromyographic (EMG) recordings from the diaphragm, we examined the inter-operative delay time between spinal cord hemisection and contralateral phrenicotomy required for diaphragm response, as compared to animal death from asphyxia at zerotime. A critical 1-2hr window is required for this synaptic plasticity. In situ hybridization shows that uPA and tPA mRNAs are rapidly induced in C4-5 ventral spinal cord neurons in the ipsilateral phrenic nucleus compared to the contralateral PMN and sham controls, with elevated PA protein at 1hr. post-hemisection. This specific and concomitant induction of PA suggests a role in CPP spinal cord plasticity, which we have confirmed by the markedly reduced response of PA knockout mice to acquire the CPP by 6hr. post-hemisection. This suggests future potential therapeutic uses for PA in spinal cord injury. (Supported in part by NIH- R01NS-44129 and T32NS-07083)

P-96

GABA-mediated inhibition of crossed phrenic pathways

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Hemisection of the upper cervical spinal cord causes paralysis of the ipsilateral hemidiaphragm due to an interruption of the descending respiratory premotor pathways. Thus, the phrenic motor neurons which innervate the diaphragm become quiescent below the site of injury. Phrenic motor neurons receive both excitatory and inhibitory, tonic and phasic inputs. Recently, it was demonstrated that activation of serotonin 1A receptors in the dorsal horn results in the disinhibition of phrenic motor neurons. In other words, inputs arising from the dorsal horn inhibit phrenic motor neurons. Once this inhibition is removed phrenic motor activity increases and crossed phrenic activity becomes visible. The mechanisms underlying this disinhibition are unknown. Therefore, this study was designed to examine whether phrenic motor neurons are inhibited via GABA-mediated mechanisms. Sprague Dawley female rats were hemisected at the C2 spinal cord level. Following one week of recovery, rats were anesthetized with urethane, vagotomized, ventilated and paralyzed. Bicuculline (10ul of 10mM solution), a GABA antagonist, was applied directly to the cervical spinal cord while bilateral phrenic motor output was recorded. Bicuculline caused a significant increase in the phrenic burst frequency and burst peak amplitude in both control and hemisected rats. The resulting increase in respiratory drive activated crossed phrenic pathways in the quiescent nerve of hemisected rats. These results suggest that crossed phrenic pathways are inhibited via GABA- mediated mechanisms. Supported by NIH Grant HD31550

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The morphological effects of genetic dysmyelination on neuronal elements of the spinal cord: An immunohistochemical study

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Central nervous system (CNS) myelin suppresses the innate plasticity of axons and dendrites. However the extent to which different populations of neurons are sensitive to myelin remains unknown. The Long-Evans Shaker (LES) rat is a naturally occurring mutant which, by virtue of a mutation in the myelin basic protein (MBP) gene, fails to make CNS myelin in adulthood, and thus serves as an excellent model to study the consequences of dysmyelination. Here we examine neuronal elements (axons, dendrites, and synapses) within the uninjured lumbar spinal cord of the LE and LES rats. Discrete populations of primary afferent axons were visualized by their expression of CGRP, IB4-binding carbohydrates, and VGLUT 1. Descending monoaminergic axons were visualized by the expression of the transporter for the serotonin neurotransmitter (SERT), TH, and DâH. Interneurons were visualized via their expression of PKCã, NK-1, and substance P. Finally, dendrites and synapses were visualized by immunohistochemistry for MAP-2, and synaptophysin respectively. Computer-aided image analysis was used to measure axonal, dendritic and synaptic density in various regions of white and grey matter. Primary afferents were minimally affected by the lack of myelin, with only slight increases in axon density in LES rats compared to LE rats. The density of descending monoaminergic

axons, MAP-2-positve dendrites, and synapses in the LES rat were all increased, suggesting a greater susceptibility of these neuronal elements to myelin. In LES rats, the increase in synaptic density exceeded that of the increase in dendritic density, and indicates that the abnormal sprouting of axonal processes is accompanied by augmented synaptogenesis. These results allow for reasonable predictions of the consequences of dysmyelinating disorders such as multiple sclerosis. Since distinct neural populations respond differently to dysmyelination, this reinforces the notion that successful regeneration following CNS trauma such as spinal cord injury will require a combination of approaches. Supported by: MSFHR, and CRPF

P-98

The p75 receptor inhibits neural regeneration at the PNS:CNS interface

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The re-growth of injured axons is controlled at the level of the cytoskeleton partly by the activity of RhoA, a small GTPase that reduces actin turnover leading to growth cone collapse. The constitutive activity of the p75 receptor, or its stimulation by myelin-associated inhibitory proteins (MAIPs) results in RhoA activation. In contrast, neurotrophins, produced by reactive Schwann cells of the injured PNS, can block RhoA activation through their interaction with the p75 receptor. Injured primary afferents, most of which express the p75 receptor, can regenerate within the peripheral dorsal root but fail to re-innervate the CNS. To evaluate the possible contribution of the p75 receptor to regeneration failure, we examined primary afferent axonal regeneration into and within the CNS following cervical dorsal root injury in wild-type and p75 knockout mice (p75-/-). We show that injured axons can spontaneously penetrate the astrocytic boundary at the PNS:CNS interface and regenerate into the spinal cord of p75-/- mice, but not wild-type mice. Once within the CNS, p75-/- axons continued to regenerate for at least 28 days. Given that the initial barrier to regeneration into the CNS is formed by astrocytes rather than myelin, and Schwann cells in p75-/- mice cannot sequester neurotrophins (reactive Schwann cells express p75 under normal circumstances), spontaneous regeneration into the CNS may occur as a result of an increased availability of Schwann cell-derived neurotrophins to regenerating axons. Continued regeneration within the CNS in the absence of the p75 receptor is likely to arise through both increased peripheral neurotrophin availability and reduced MAIP signalling.

P-99

Mechanisms of behavioural and axonal plasticity following dorsal rhizotomy: The roles of Trk signaling

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Dorsal root injury (DRI) disrupts the flow of sensory information into the spinal cord. The resulting sensory dysfunction is typified by both the loss of normal sensation and the development of abnormal pain. Behavioural manifestations of DRI change over time following injury: mechanosensory deficits can spontaneously recover, and pain can worsen and resolve. Since dorsal roots do not regenerate back into the spinal cord, behavioural changes and/or recovery must be mediated by axons spared by DRI. Both primary afferent axons in adjacent segments and descending sensory-modulating axons sprout in the spinal cord following DRI, and plasticity of these systems may underlie sensory dysfunction and/or recovery. We have previously shown that myelin signaling antagonism both enhanced monoaminergic sprouting and attenuated cold hypersensitivity in rats with rhizotomy of the 7th and 8th cervical dorsal roots (C7/8 rhizotomy). Since rhizotomy-induced plasticity is presumably under the control of both growth-inhibiting and growth-promoting influences, we are now investigating the role of neurotrophins, known to be upregulated in the spinal grey matter following rhizotomy, in behavioural and anatomical plasticity following DRI. We have administered the pan-Trk inhibitor K252a and a TrkB-Fc chimera to inhibit activity of endogenous neurotrophins in rats with C7/8 rhizotomy. Both treatments altered the time course of behavioural recovery following C7/8 rhizotomy: the onset and recovery of cold hypersensitivity were delayed in TrkB-Fc-treated rats compared to human IgG-treated controls, while recovery of mechanosensation was accelerated in both K252a- and TrkB-Fc-treated rats. These behavioural changes may be accompanied by changes in primary afferent and descending monoaminergic axons in the spinal cord, and morphological changes in these populations of axons may be differentially neurotrophin-dependent. These data reflect the importance of optimizing plasticity in the injured spinal cord, since sprouting of spinal axons may contribute to both sensory dysfunction and its resolution following spinal deafferentation.

P-100

Immunohistochemical analysis of the postnatal development of sensory root entry zones

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Sensory axons enter the spinal cord at the dorsal root entry zone (DREZ), where an abrupt demarcation exists between the peripheral nervous system (PNS) and the central nervous system (CNS). Following injury to the dorsal root in the adult, sensory axons are unable to cross this PNS-CNS interface into the spinal cord. However, following injury the neonatal DREZ (within the first postnatal week) is permissive to regeneration. In addition to the DREZ, PNS-CNS interfaces also exist within cranial nerves, and the ability of some of these to permit successful regeneration depends on their glial composition. For example, the sensory root entry zone of the vagus (VDREZ) supports regeneration due to the presence of Schwann cell insertions into the brainstem, and the entry zone of the olfactory nerve is permissive to regeneration due to the presence of olfactory ensheathing cells. In order to understand the potential relationship between the glial environment of the PNS-CNS interface and the ability to permit regeneration, we characterized the glial elements of the DREZ, VDREZ and trigeminal root entry zone (TREZ), throughout development. We used immunohistochemistry to visualize the developing glial

elements of neonatal (postnatal days 0, 3, and 7), juvenile (postnatal day 14) and adult Long Evans rats. Antibodies for GFAP (identifying astrocytes), p75 (Schwann cells and neurons), RIP (mature oligodendrocytes), laminin (basal lamina), Ox42 (microglia) and NG2 (oligodendrocyte precursors and extracellular NG2 proteoglycan) were used. Our results suggest that the glial elements in the cranial root entry zones (the vDREZ and TREZ) develop more quickly following birth than the DREZ. In addition, the adult DREZ and TREZ have a more sharply demarcated PNS-CNS interface, whereas the interface at the VDREZ is less well-defined. These glial differences may play a role in determining the regenerative potential of PNS-CNS interfaces in different sensory root entry zones. Supported by: the National Science and Engineering Research Council of Canada

P-101

Two photon in vivo imaging of axonal regeneration at the dorsal root entry zone

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In vivo two-photon laser scanning microscopy (TPLSM) permits the direct observation of the dynamic behavior (both spatial and temporal) of regenerating axons and growth cones at greater depths and with less resultant photo-damage than that of confocal microscopy. In addition, TPLSM allows for the examination, in real time, of in vivo acute drug treatments on the behavior of regenerating axons as they approach, contact, and, given the appropriate conditions, cross the glial barriers to regeneration. The dorsal root entry zone (DREZ), the point at which sensory axons enter the spinal cord, serves as an excellent model system to characterize axonal growth as well as the glial contributions to regeneration failure. Using TPLSM, our primary objective is to image the in vivo growth of individual regenerating sensory axons en route to and as they contact the DREZ using a YFP mouse following both acute and chronic dorsal root injuries. We find that YFP expressing sensory axons are clearly distinguishable in the uninjured animals in both the peripheral and the CNS portions of the DREZ. Following injury, some regenerating axons approach the DREZ, but then appeared to turn and grow back towards the periphery. Experiments are currently underway to test if this barrier to regeneration is indeed a "physiological stop signal", by comparing the behavior and rate of the growth of sensory axons at the injured DREZ with those of a physical barrier (a nerve root ligature). In addition, we will image the growth of injured sensory axons across the DREZ following a conditioning injury or the application of a trophic factor. These studies will attempt to characterize the growth of injured sensory axons with a view to identifying therapeutic strategies not only for sensory axon injuries, but also for spinal cord and brain injuries in general. Supported by: Michael Smith Foundation for Health Research, and the Natural Sciences and Engineering Research Council of Canada.

P-102

SPARC is secreted by olfactory ensheathing cells to stimulate neurite outgrowth and CNS repair

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Olfactory ensheathing cells (OECs), are a unique glial cell that have been implicated in promoting regeneration both within the olfactory system, and following lesion in the injured spinal cord. However, little is known about how OECs promote regeneration. Purified cultures of Lamina propria OECs (LP-OECs) were used as a model system to elucidate mechanisms of OEC-mediated neurite outgrowth. Using an embryonic dorsal root ganglion (DRG) culture system, LP-OECs were found to promote outgrowth in co-culture and also with LP-OEC conditioned media (LP-OCM) alone. Conditioned media from passage 2 (P2) and passage 6 (P6) LP-OECs were assayed for biological activity using the outgrowth assay. The two LP-OCM samples were found to elicit different growth, where P2 media was found to have a more effective dose-response curve. To identify key secreted factors that underlie their difference in biological activity, P2 and P6 LP-OCM samples were analyzed by isotope coded affinity tags (ICAT) proteomics, to identify and quantify the constituent proteins. By correlating biological activity with relative quantity, SPARC (secreted protein acidic rich in cysteine) was identified as a candidate factor of outgrowth. Gain- and loss-of-function experiments indicate that SPARC is a key stimulator of outgrowth in LP-OCM. SPARC-mediated neurite outgrowth, however, appears to depend, at least in part, on the Schwann cells of the DRG explant, and the presence of laminin. LP-OECs prepared from SPARC null mice display a differential ability to stimulate repair in the lesioned spinal cord, and following lesion in the olfactory system. We therefore conclude that SPARC is a key secreted factor underlying the ability of OECs to promote CNS repair.

P-103

Localized expression of Akt increases growth cone area and complexity

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During development and neural regeneration, neurotrophins direct axonal growth and guidance to establish synaptic connections. The growth cones of neurons respond to extracellular neurotrophin gradients by localized control of the cytoskeleton through signaling cascades downstream of the neurotrophin receptors. Localized activity of phosphatidylinositol-3 kinase (PI-3K) is required for neurotrophin-mediated growth cone turning and neurite branching. To further investigate the signaling mechanisms involved in these observations, we tested whether overexpression of constitutively active Akt, the primary down stream effector of PI-3K, would induce changes in the morphology of

cultured sensory neurons, and whether the sub-cellular location of Akt activity differentially affects neuronal morphology. Adenoviral vectors were used to direct expression of either EGFP or myristoylated Akt in chick dorsal root ganglion neurons. The myristoylation sequences from either fyn (Mf) or src (Myr) was used to recruit the Akt transgene to the inside of the plasma membrane, where it is constitutively activated. However, whereas the Myr sequence recruits Akt to the plasma membrane uniformly, Mf selectively recruits Akt to lipid rafts. In mouse brain, over 90% of fyn is located in lipid rafts, microdomains concentrated at the neurite's leading edge and required for neurotrophin-mediated growth cone turning. We find that neurons expressing Akt throughout the cell (Myr) had significantly larger cell bodies, compared to controls at 48 hours. In contrast, neurons expressing Akt at lipid rafts (Mf) did not have larger somas, but did exhibit an expansion of growth cone area. To further examine this finding, we used biolistic transduction ('gene gun') to direct expression of either EGFP or an Akt/EGFP fusion protein in lipid rafts (Mf). We find that neurons over-expressing Akt/EGFP had significantly larger (230%) growth cones as compared to controls at 48 hours. Funded by grants from NIH, Mission Connect of the TIRR Foundation, and the Christopher Reeve Paralysis Foundation.

P-104

The role of galectin-1 in neuritic outgrowth from adult sensory neurons following nerve injury

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Galectin-1 (Gal1) is a 14.5 kDa protein that has the ability to bind beta-galactosides under reducing conditions. Gal1 has roles in a variety of essential cellular processes, including apoptosis, adhesion, migration, and cell proliferation, and is expressed in many tissues. In the nervous system, Gal1 has roles in axonal pathfinding to correct targets during development: in a previous study, we implicated Gal1 in the targeting of central branches of small dorsal root ganglion (DRG) neurons to their targets in the superficial dorsal horn. In addition, Gal1 appears to be important in the initiation of axonal regeneration following nerve injury. In the present study, we investigate whether intrinsic neuronal expression of Gal1 is required for typical neurite outgrowth from adult DRG neurons in vitro. We compare outgrowth from adult DRG neurons from mice lacking the Gal1 gene (Gal1-/- mice) with those from Gal1+/+ mice. Our preliminary results indicate that the mean length of neuritic extension from Gal1-/- neurons is reduced significantly relative to Gal1+/+ neurons in dissociated cell culture. Thus, it is likely that Gal1 acts as an intrinsic factor within injured sensory neurons to promote axonal elongation. In ongoing experiments, we plan to establish whether the mutant phenotype can be rescued by addition of Gal1, and to determine whether Gal1 and nerve growth factor (NGF) have additive, synergistic or competitive effects on neurite outgrowth.

P-105

Embryonic motor neurons display preferential outgrowth on normal, but not prelesioned, femoral nerve tissue sections

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We previously reported that E-15 ventral spinal cord explants preferentially extend neurites onto prelesioned adult sciatic nerve membranes or tissue sections compared to normal nerve (Soc. Neurosci. 245.6, 2003). We now explore outgrowth of ventral spinal cord on cryostat sections of terminal muscle or cutaneous nerve branches from normal or prelesioned femoral nerve. A total of 21 individual cultures grown on normal nerve sections resulted in 8 explants that extended neurites on both substrates, 12 explants that grew only on muscle branch substrates, and the remaining explant grew only on the cutaneous branch substrate. Comparing just the explants that grew on both types of substrates, there was a trend for the average number of neurite bundles/explant to be greater on the muscle compared to the cutaneous (13.1 2.4 vs. 8.3 0.6; $p=0.07$), and there was significantly more total outgrowth per explant on muscle compared to cutaneous substrates (5,2621,307 vs. 2,300437 microns; $p< 0.05$). There was no significant difference in the average length of neurite bundles per explant (35253 vs. 262 34). No significant differences were seen between the two types of substrates from prelesioned nerve branches in terms of number of bundles, total neurite outgrowth, or average length of neurite bundles. There was however, more extensive outgrowth on lesioned cutaneous substrates compared to normal cutaneous substrates (avg length/explant: 508 vs 262, $p<0.005$; total outgrowth/explant: 6,647 vs 2,300; $p<0.05$). No such significant difference was seen between normal and prelesioned muscle nerve substrates. These results suggest that in this model system, normal muscle nerve is a preferred substrate for neurite outgrowth compared to normal cutaneous nerve, and that enhanced outgrowth on prelesioned nerve substrates is nerve branch specific. (Supported by the Department of Veterans Affairs (RDM). RDM is a Research Career Scientist for the Department of Veterans Affairs.)

P-106

Motor neuron regeneration accuracy: Balancing trophic support from terminal nerve pathways

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Rat femoral nerve studies have shown that regenerating motor neurons preferentially reinnervate a terminal nerve branch to muscle as opposed to skin. This process, termed preferential motor reinnervation (PMR), is usually not evident prior to 3 weeks post-repair and has been interpreted as evidence that regrowing motor axons can differentiate between Schwann cell tubes in muscle versus cutaneous pathways (Brushart, J. Neurosci. 1993, 13:2730). Recent work from our laboratory has proposed an alternate hypothesis; that motor neurons assess the amount of trophic support available from nerve branches and preferentially retain their axons in the one with the relatively higher amount of trophic support (Exp. Neurol. 2004, 190:407; and 2005, 192:39); moreover we suggest a hierarchy of trophic support with muscle contact being the highest, followed by the length of the terminal nerve branch and/or contact with skin. In the current experiments all rats received parent femoral nerve repair. In the *classical*-PMR group (*C-PMR*, N=10) both nerve branches remained intact to their respective end-organs of muscle and skin; in the *short cutaneous, long-muscle* group (*SC-LM*, N=11) the cutaneous branch was ligated and capped while the muscle branch was left intact (allowing trophic support from muscle without the competing influence of contact to skin or a long cutaneous nerve branch). Two weeks following surgery significantly more motor neurons projected to the muscle branch (10729) compared to the cutaneous branch (4913) in the *SC-LM* group, but not in the *C-PMR* group (7623, and 11123). These results suggest that the time it takes for a motor neuron to differentiate the relative levels of trophic support is directly related to the relative differences between the two terminal nerve branches. A time course study is currently in progress. Support contributed by: Office of Research and Development, Department of Veterans Affairs (RDM). RDM is a Research Career Scientist.

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Manipulation of the microenvironment within multichannel biodegradable polymer nerve scaffolds

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Objective: To optimize the physical and chemical properties of biodegradable biocompatible polymer scaffolds.

Biodegradable polymers provide structure and support for regenerating nerve tissue in multichanel scaffold tubes. A variety of polymers and copolymers, were tested and compared. These included established polymers such as (poly(lactic-co-glycolic acid); PLGA) and novel polymers such as (poly(caprolactone fumarate); PCLF). These polymers were assessed in terms of criteria thought appropriate for improving the microenvironment for nerve regeneration within the scaffold conduits.

Toxicity of the polymers were initially tested with a cell proliferation MTS assay carried out on a number of cell types over a period of 72 hours. Dorsal Root Ganglia (DRG), Schwann cells, SPL201, and PC12 cells all failed to attach and spread on either PLGA or PCLF surfaces. However, direct attachment of a laminin derived peptide, or NCAM derived peptide via a biotin/strepavidin bridge increased cellular attachment and spreading in both PC12 cells and DRG. Interestingly if PCLF crosslinking was reduced, the resulting surface roughness promoted cellular attachment and spreading in all cell types tested.

These polymer scaffolds were also used as a reservoir for biologically active peptides for controlled release. The test peptide chosen was a 44mer derived from PEDF (pigment derived epithelial factor). PEDF 44mer encapsulated as a powder, or as an emulsion with polyethylene glycol (PEG) in PGLA scaffolds, promoted both neuroprotection and neurite outgrowth in DRG for up to a month in vitro. In vivo the scaffold conduits filled with either schwann cells or stem cells contribute to a microenviroment designed to promote and sustain neuronal regeneration.

P-108

Axon regeneration in multimuminal biosynthetic nerve repair

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Peripheral nerve damage is routinely repaired by autogenous grafts, that often lead to poor functional recovery and the sacrifice of healthy donor nerves. An alternative repair strategy is to use biosynthetic scaffolds to facilitate recovery of a damaged nerve. Unfortunately, many of the commonly used scaffold designs fail to mimic the natural fascicular structure of nerves. To address this shortcoming, we developed a biosynthetic nerve implant (BNI) that uses a hydrogel-based, transparent, multi-channel matrix as a 3-D substrate for nerve repair. Novel scaffold-casting devices were designed for reproducible fabrication of grafts containing either 7 or 14 micro-conduits, and further tested in vivo using a sciatic nerve injury model. At sixteen weeks postinjury, nerve defects repaired with empty tubes formed a single nerve cable. In sharp contrast, animals that received the multiluminal BNI showed multiple vascularized nerve cables within the available microchannels, better resembling the multifascicular anatomy of the normal nerve. Total numbers of myelinated axons per unit of area at mid-graft were increased 3-fold in the BNI compared to both the autograph and the PTFE-tube repair methods, and the number of unmyelinated regenerated axons per unit of area were also increased by approximately 30% in the BNI compared to the PTFE-tube method, and similar to the autograph repair method. Regeneration distal to the graph and target innervation were confirmed by electrophysiology, FluoroGold retrograde axon-tracing, and behavioral analysis. These findings support the notion that a multiluminal BNI can be used to promote and direct fasciculated nerve regeneration in peripheral nerve gap repair, and possibly in the injured spinal cord.

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Somatic gene transfer and differentially promoted peripheral nerve regeneration due to Schwann cells over-expressing different FGF-2 isoforms in vivo

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Artificial nerve grafts are needed to reconstruct massive defects in the peripheral nervous system when autologous nerve grafts are not available in sufficient amounts. Nerve grafts containing Schwann cells display a suitable substrate for long distance regeneration. We injected genetically modified Schwann cells into Silicone tubes bridging 15 mm gaps in adult rat sciatic nerves. The transplanted Schwann cells over-expressed different fibroblast growth factor-2 (FGF- 2) isoforms as 18-kDa-FGF-2 and 21-/23-kDa-FGF-2, respectively. Over-expression of different FGF-2 isoforms in vivo resulted in distinct regeneration promoting effects on sensory and motor recovery as revealed by functional tests. Furthermore, morphometrical evaluation displayed differential effects on axonal regeneration by over-expression of different FGF-2 isoforms in vivo. During the first 3 months of regeneration 18-kDa-FGF-2 mediated inhibitory effects on the grade of myelination of regenerating axons, whereas, 21-/23-kDa-FGF-2 mediated early recovery of sensoric functions and stimulation of long distance myelination of regenerating axons (Haastert, Lipokatic´et al., Neurobiology of Disease, in press). These results suggested promotion of peripheral nerve regeneration by FGF-2 isoform over-expression in vivo during the initial regeneration process (first 3 months). However, preliminary results after long-term observation (6 months) suggested an interference of 21-/23-kDa-FGF-2 over-expression in vivo with the maintenance of regenerated nerve tissue. Differential effects on regeneration of sensory and motor axons as well as differential time pattern for optimal regeneration promoting activity are also discussed for other neurotrophic proteins. The results contribute to the development of new therapeutic strategies in peripheral nerve repair. Grant information: Deutsche Forschungsgemeinschaft, Bonn, Germany (to C.G., Gr 857/15-3), International Neurobionic Foundation, Hannover, Germany (to C.G.), Kogge-Stiftung für veterinärmedizinische Forschung, Gießen, Germany (to K.H.)

P-110

Human embryonic stem cell-derived oligodendrocyte progenitor cell transplants remyelinate axons in a MHV model of Multiple Sclerosis

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Demyelination of intact axons is a prominent secondary degenerative process in many CNS disorders including spinal cord injury (SCI) and Multiple sclerosis (MS). One approach to treating demyelination is cellular replacement using oligodendrocyte progenitor cells (OPCs). We have previously shown that high purity oligodendrocyte progenitors can be derived from multipotent human embryonic stem cells (hESCs), and when transplanted into dysmyelinated shiverer mice these cells are able to survive and produce myelin. In addition, we have recently shown that transplantation of these hESC-derived OPCs into spinal cord injured rat's enhanced remyelination and improved locomotor function. Here we attempt to go one step further to illustrate the efficacy of hESC-derived OPCs to promote remyelination in a model of multiple sclerosis. Transplantation of these cells into spinal cords of MHV-infected mice, demonstrating complete hindlimb paralysis, resulted in progressive remyelination and an overall decrease in demyelination compared to controls. These data indicate that hESC-derived OPCs mediate remyelination in both SCI and MS models alike.

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Recovery of function after facial nerve repair: Combined functional and structural analyses

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A major reason for the poor functional recovery after peripheral nerve lesion is the collateral branching of axons at the lesion site and their regrowth to incorrect target muscles. Using a facial nerve transection paradigm in rats, we previously developed a novel approach to combat axonal misguidance – the application of neutralizing antibodies against neurotrophic factors to the injured nerve. We also investigated whether reduced collateral branching at the lesion site would lead to better functional recovery. To our disappointment, biometric analysis of vibrissae movements did not show any improvement in functional recovery suggesting that hyperneurotization of the vibrissal muscles and polyneuronal reinnervation of the motor end-plates - rather than collateral branching - might be the critical limiting factors. In support of this hypothesis, we found that motor end-plates with morphological signs of multiple innervation were more frequent in reinnervated muscles of rats which did not recover coordinated muscle activity after injury (51% of all end-plates) compared to animals with well synchronized motor performance (10%). Since polyneuronal innervation of muscle fibers is activity-dependent and can be manipulated, these findings raised hopes for clinically feasible and effective therapies. Accordingly, we have now shown that

active whisker training for 2 months after facial nerve injuryresulted in full recovery of vibrissal motor performance. Restoration of function was associated with a reduced degree of polyneuronal re-innervation of target musculature rather than with limited collateral axonal branching or altered representation area in the motor cortex. These findings have immediate potential for enhancing clinical rehabilitation strategies to restore function following periphery nerve injury. *Supported by the Köln Fortune Programm, the Jean-Uhrmacher Foundation and the DFG (AN 331/3-1, AN 331/5-1).

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Spinal nerve versus spinal root compression injury: Differential regulation of gene expression and correlation with functional deficits

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Direct damage to axons running in spinal nerves or nerve roots results in muscle paresis or sensory disturbances ranging from anesthesia to intense pain. Functional deficits due to the lack of successful regeneration into target muscles or spinal cord often persist. We have established a nerve compression injury model in adult rats to identify novel treatment strategies for improving axon regeneration and limiting the development of lesion-associated neuropathic pain. At segmental level L5 the nerve roots and corresponding spinal nerve, respectively, were crushed by application of a clip for 1 minute. Sham-operated animals served as control. Three days later the L5 spinal ganglion was removed and processed for RNA extraction. After purification, labeled RNA was loaded onto rat 230A Affymetrix microarray chips containing about 16,000 genes. Starting from day 3 after the operation behavioral testing was performed in another group of animals for a total of six weeks.

Extensive data analysis revealed a limited number of genes up- or down-regulated more than twofold after nerve root or spinal nerve lesion. RT-PCR confirmed the regulation of a number of neuronal growth factors, Schwann-cell derived secreted molecules and of various genes associated with the inflammatory response after nerve lesion. Behavorial analysis revealed an impairment of sensory and motor function. This complex response of dorsal root ganglia to nearby axonal crush lesions is partially identical to the gene expression profile obtained after distal peripheral nerve transection. However, significant differences, particularly after nerve root lesion, were observed which may explain the reduced growth rate of root axons when compared to the rapid regeneration of lesioned peripheral nerves in rodents (funded by MFF Tirol).

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Localization, regulation and functions of basic fibroblast growth factor in peripheral ganglia after axotomy

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Basic fibroblast growth factor (FGF-2) is rapidly up-regulated in peripheral ganglia after nerve lesion suggesting important functions of this neurotrophic molecule in peripheral axon regeneration. Axotomized sympathetic neurons exhibit FGF-2 in the nucleus and in perinuclear Golgi fields. Biolistic transfection of plasmids encoding FGF-2 isoforms fused to fluorescent proteins demonstrates nuclear targeting, but FGF-2 overexpression does not promote survival nor neurite outgrowth in sympathetic neuron culture. Treatment of adult dorsal root ganglion neurons with FGF-2 only marginally promotes axon elongation but significantly increases axonal branching. In response to a preconditioning lesion, i.e., transection of the sciatic nerve one week before culture, the axonal length of lumbar sensory neurons increases twofold when compared to non-lesioned control rats. This response is significantly enhanced by FGF-2 isoforms, but not by NGF, and blocked by the FGFR inhibitor SU5402. Treatment with FGF-2 leads to the rapid internalization and degradation of neuronal FGF receptor type 1 which is blocked by lactacystine, an inhibitor of the proteasome. Taken together, the present data provide evidence for neuronal synthesis and targeting of FGF-2 to the nucleus and Golgi apparatus of sympathetic and sensory neurons supporting a dual role for FGF-2 within the nucleus and in the secretory pathway. Upon release, direct neurotrophic effects of the 18kD and 23kD FGF-2 isoforms on adult axon growth are observed, which may be of therapeutic value in the treatment of peripheral nerve lesions. Moreover, peripheral neurons possibly become more sensitive to neurotrophic factor treatment if tyrosine kinase receptor degradation is blocked by inhibitors of the proteasome.

P-114

Chondroitinase-secreting astrocytes mitigate axon inhibition by chondroitin sulfate proteoglycans

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Chondroitin sulfate proteoglycans (CSPGs) are up-regulated by astrocytes in the glial scar following injury and contribute to failed regeneration. Both *in vitro* and *in vivo*, enzymatic GAG chain elimination by chondroitinases enhances the ability of axons to regenerate. Use of chondroitinases *in vivo* requires repeated injections due to protein lability, however, the use of cells engineered to secrete this bacterial protein should reduce damage caused by repeated injections. Therefore, we have engineered astrocytes to produce a modified form of the bacterial enzyme chondroitinase AC (astChAC), which can be secreted from mammalian cells. Cultures of U373 human astrocytoma cells were transfected with adenovirus encoding ChAC and subsequently induced with doxycyclin to secrete ChAC. Enzymatic activity of concentrated (25:1) conditioned media was confirmed using Western blot analysis. In control experiments, chicken dorsal root ganglion (DRG) neurons growing on laminin (25µg/ml) were inhibited by a mixture of CSPGs (Sigma, #CC117; 100 µg/ml) that was adsorbed to the culture dish in a striped pattern.

In contrast, DRG neurons treated with astChAC grew into and across CSPGs. Further, injection of astChAC into the lesioned spinal cord in rats showed active enzymatic activity, as identified by staining with antibody 3B3, and immunocytochemistry using anti-5HT and anti-neurofilament antibodies showed neurite growth into the scar. These studies *in vitro* and *in vivo* show that using endogenous astrocytes transfected to secrete CSPG-degrading enzymes diminishes the inhibitory character of the glial scar and provides a more clinically-relevant approach than contemporary methods. *Support: Kentucky Spinal Cord and Head Injury Research Trust Grants: # 0-8 (DMS) and #2-16 (GMS).*

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