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**NINTH INTERNATIONAL
SYMPOSIUM ON NEURAL REGENERATION**

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**International Symposium
on Neural Regeneration**

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NINTH INTERNATIONAL SYMPOSIUM ON NEURAL REGENERATION

*The Asilomar Conference Center, Pacific Grove, California
December 12–16, 2001*

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The symposium is sponsored by the U.S. Department of Veterans Affairs (Medical Research Service and the Rehabilitation Research and Development Service), the Paralyzed Veterans of America (Spinal Cord Research Foundation), the National Institutes of Health (National Institute of Neurological Disorders and Stroke), the Christopher Reeve Paralysis Foundation (a merger of the American Paralysis Association and the Christopher Reeve Paralysis Foundation), and the Eastern Paralyzed Veterans Association.

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Scientific Program

WEDNESDAY—DECEMBER 12, 2001

Afternoon Session

- 3:00–6:00 P.M. Arrival of Participants and Check-in
6:00 Dinner
7:30 Welcome and Keynote Address
KEYNOTE SPEAKER:
Marc Tessier-Lavigne (Stanford University, CA)
Molecular studies of axon guidance, branching, and regeneration

THURSDAY—DECEMBER 13, 2001

Morning Session

TARGETED AXONAL GROWTH/REGENERATION IN THE PNS

CHAIRMAN: RONALD OPPENHEIM (WAKE FOREST UNIVERSITY, NC)

- 8:15 A.M. CHAIRMAN'S INTRODUCTION
8:30 **Wolfram Neiss** (University of Cologne, Germany)
The misdirection of reinnervation after peripheral nerve suture and chances of its therapy
9:00 **Roger Madison** (Durham, NC, VAMC/Duke University)
Preferential motor reinnervation and PNS regeneration
9:30 **Michael Laskowski** (University of Idaho)
Reinnervation of muscle: Cues that guide synaptic choices
10:00 Break

- 10:30 **Christian Krarup** (University Hospital, Copenhagen, Denmark)
The influence of the near-nerve environment on the rate of elongation in the cat
- 11:00 **William Snider** (University of North Carolina, Chapel Hill)
Roles of growth factor signaling in axon development and regeneration
- 11:30 **Julia Terzis** (Eastern Virginia Medical School)
Neural regeneration following end-to-side coaptation: A basic science and clinical review
- Noon Lunch
- 1:00 **Poster Session 1**
- 5:00 **FEATURED SPEAKER**
Paul Letourneau (University of Minnesota)
Guiding growth cone navigation: Stop and go in complex environments
- 6:00 Dinner

*Evening Session***HUMAN CELLS FOR TRANSPLANTATION**

CHAIRMAN: MAHENDRA RAO (UNIVERSITY OF UTAH)

- 7:15 P.M. CHAIRMAN'S INTRODUCTION
- 7:30 **Steven Goldman** (Cornell University Medical Center, NY)
Isolation and induction of adult neural progenitor cells
- 8:00 **Evan Snyder** (Harvard Medical School/Children's Hospital, Boston)
The biology of neural stem cells may make them uniquely suited for neural regenerative processes
- 8:30 **Melissa Carpenter** (Geron Corporation, Menlo Park, CA)
Neural specific differentiation of human embryonic stem cells
- 9:00 **Ian Duncan** (University of Wisconsin)
Myelinating cells for transplantation and myelin repair: Comparative aspects of human versus animal cells

FRIDAY—DECEMBER 14, 2001*Morning session***NEURON-GLIA INTERACTIONS AND SYNAPTIC PLASTICITY**

CHAIRMAN: PLATON KOSTYUK (BOGOMOLETZ INSTITUTE OF PHYSIOLOGY, KIEV, UKRAINE)

- 8:15 A.M. CHAIRMAN'S INTRODUCTION
- 8:30 **Ian Whishaw** (University of Lethbridge, Canada)
Modification of skilled movement: Fixed circuits versus plastic changes
- 9:00 **Bryan Kolb** (University of Lethbridge, Canada)
Neurotrophic factors, synaptic plasticity, and recovery from cortical injury

- 9:30 **Michela Matteoli** (University of Milan, Italy)
Block of glutamate-glutamine cycle between astrocytes and neurons inhibits epileptiform activity in hippocampus
- 10:00 Break
- 10:30 **Bruce Ransom** (University of Washington School of Medicine)
Glial modulation of neural excitability mediated by extracellular pH
- 11:00 **Harald Sontheimer** (University of Alabama, Birmingham)
Glial regulation of perisynaptic glutamate in health and disease
- 11:30 **Harry Goshgarian** (Wayne State University, MI)
Activity-dependant plasticity in rat phrenic nucleus after reversible hemispinalization of cervical spinal cord by a cooling device
- Noon Lunch
- (Breakout lunch presentation for interested attendees by Dr. Donald Fink of the FDA: *Regulatory issues of clinical trials*; Arlene Chiu of the NIH NINDS: *Stem cell research—NIH policy update*)
- 5:00 **FEATURED SPEAKER**
Larry Benowitz (Harvard Medical School/Children's Hospital, Boston)
A purine-sensitive switch regulates the axon growth program of CNS neurons
- 6:00 Dinner

*Evening Session***SIGNALING PATHWAYS IN NEURAL PLASTICITY**

CHAIRMAN: JOSEPH NEARY (VA MEDICAL CENTER, MIAMI, AND UNIVERSITY OF MIAMI)

- 7:15 P.M. CHAIRMAN'S INTRODUCTION
- 7:30 **Joseph Avruch** (Harvard Medical School, MA)
Signal transduction through the Ras GTPase
- 8:00 **Bruce Gold** (Oregon Health Sciences University)
FKBP-52/Hsp-90 steroid receptor chaperones and ERK signaling in the regulation of nerve regeneration
- 8:30 **Lisa McKerracher** (University of Montreal, Canada)
Overcoming growth inhibition in the CNS: Use of Rho antagonists
- 9:00 **Seth Finklestein** (Harvard Medical School, MA)
The role of growth factors in repair and recovery from brain injury and stroke

SATURDAY—DECEMBER 15, 2001*Morning Session***NEUROGENESIS IN THE ADULT**

CHAIRMAN: PASKO RAKIC (YALE UNIVERSITY, CT)

- 8:15 A.M. CHAIRMAN'S INTRODUCTION

- 8:30 **Jeffrey Macklis** (Harvard Medical School, MA)
Induction of neurogenesis in the neocortex of adult mice
- 9:00 **Theodore Palmer** (Stanford University, CA)
Microenvironments and neural stem cells in the adult brain affiliation
- 9:30 **Marla Luskin** (Emory University, GA)
Factors controlling the proliferation and migration of neuronal progenitor cells in the postnatal mammalian forebrain
- 10:00 Break
- 10:15 **Anne Calof** (University of California, Irvine)
Bone morphogenetic proteins: Multifunctional regulators of neurogenesis
- 10:45 **Jack Parent** (University of Michigan)
Injury-induced neurogenesis in the adult mammalian forebrain subventricular zone
- 11:15 **Pasko Rakic** (Yale University, CT)
Adult neurogenesis: Promises and controversies
- 11:40 ROUND TABLE: **Chaired by Pasko Rakic** (Yale University, CT)
- Noon Lunch
- 1:00 **Poster Session 2**

*Afternoon Session***EMERGING TOPICS IN REGENERATION**

CHAIRMAN: OSWALD STEWARD (UNIVERSITY OF CALIFORNIA, IRVINE)

- 4:15 P.M. CHAIRMAN'S INTRODUCTION
- 4:30 **John McDonald** (Washington University, St. Louis)
Stem cells: From beginnings to clinical trials
- 5:00 **Marie Filbin** (Hunter College of City University of New York)
Overcoming inhibitors of regeneration in myelin
- 5:30 **James Fawcett** (Cambridge University Centre for Brain Repair, UK)
The glial scar and glial boundaries
- 6:00 **Michael V. Sofroniew** (UCLA School of Medicine, CA)
Astroglial cell roles in protection, repair, and neurogenesis
- 6:30 Symposium Banquet

SUNDAY — DECEMBER 16, 2001

Departure of Participants

POSTERS

Presenters for posters numbered P1–P25: mount posters after 4:00 PM on Wednesday, December 12, and dismount posters before noon on Friday, December 14. Poster authors in this group are requested to be present at posters from 1:00–3:00 PM on Thursday, December 13.

Presenters for poster numbers P26–P51: mount posters after 1:00 PM on Friday, December 14, and dismount posters before 11:00 PM on Saturday, December 15. Poster authors in this session are requested to be at posters from 1:00–3:00 PM on Saturday, December 15.

Poster display numbers correspond to poster abstract numbers.

- P1** Specificity and Time Course of Sensory Neuron Addition in Juvenile Rat
P.B. Farel
- P2** A Gradient of Adaptability in Premotor Neural Centers Generating Reflex Eyelid Responses Following Hypoglossal-Facial and Facial-Facial Anastomosis in Cats
A. Gruart, M. Streppel, O. Guntinas-Litchius, D.N. Angelov, W.F. Neiss, E. Stennert, and J.M. Delgado-García
- P3** Electrodiagnostic Criteria to Predict Nerve Regeneration
M.L. Kropp, G. Phongsamart, and J.J. Wertsch
- P4** Loss of NeuN Immunoreactivity Following Peripheral Nerve Injury Is Not Associated with Cell Loss or Atrophy
C.B. McBride, L.T. McPhail, J. McGraw, and W. Tetzlaff
- P5** Axonal Regeneration Through Nerve Coaptations is Enhanced by Degradation of Chondroitin Sulfate Proteoglycan
D. Muir, C.A. Krekoski, D. Neubauer, and J. Zuo
- P6** Differential Migration of Peripheral Nerve Fibroblasts and Schwann Cells in an Oriented Three-Dimensional Collagen Matrix
E.E. Sabelman, M. Hu, D. Kim, and V.R. Hentz
- P7** Locomotor Differences Between Strains of Rats—Implications for Models of Central Nervous System Disease and Regeneration
A.A. Webb and G.D. Muir
- P8** Grafts and aFGF Restore Partial Hindlimb Functions in Adult Paraplegic Rats
Y.-S. Lee, I. Hsiao, J. Yu, R.T. Robertson, and V.W.-H. Lin
- P9** Changing Neural Activity Influences the Restoration of Locomotory Function After Complete Spinal Cord Transection
L.M.F. Doyle and B.L. Roberts

- P10** Enriched Environments Improve Goal-Directed Reaching Following Neonatal Cervical Spinal Cord Injury in Rats
P.S. Diener
- P11** Transplantation of GFP-Positive Mouse Olfactory Mucosa-Derived Ensheathing Glia into Rat Spinal Cord
L. Lewis, E. Au, E. Flynn, J. Liu, W. Tetzlaff, and A.J. Roskams
- P12** Cellular Plasticity in the CNS of Adult Dysmyelinated Rats
J.M. Kwiecien, K.H. Delaney, J. Wang, S. Jiang, M.P. Rathbone, D.L. Kirkham, and L.C. Doering
- P13** Reducing the T Cell Response to Spinal Cord Injury Decreases Posttraumatic Degeneration and Behavioral Deficit
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- P16** Effects of Glial Restricted Precursor Cells on Acute Spinal Cord Contusion Injury
C.E. Hill, C. Proschel, J.C. Gensel, C.A. Tovar, M. Mayer-Proschel, M.D. Noble, M.S. Beattie, and J.C. Bresnahan
- P17** Ablation of Reactive Scar-Forming Astrocytes After Spinal Cord Injury in Transgenic Mice
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H. Higashimori and R.C. Carlsen
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V.W-H Lin, I. Hsiao, K.H. Kim, and W. Brown

Ninth International Symposium on Neural Regeneration

Introduction

The Ninth International Symposium on Neural Regeneration will be held at the Asilomar Conference Center in Pacific Grove, California, from December 12–16, 2001. The meeting will be cosponsored by the U.S. Department of Veterans Affairs (Medical Research Service and the Rehabilitation Research and Development Service), the Paralyzed Veterans of America (Spinal Cord Research Foundation), the National Institutes of Health (National Institute of Neurological Disorders and Stroke), the Christopher Reeve Paralysis Foundation, and the Eastern Paralyzed Veterans Association.

The keynote speaker for this year's symposium will be Marc Tessier-Lavigne from Stanford University, California. Featured talks will be given by Paul Letourneau from the University of Minnesota and Larry Benowitz from Harvard Medical School and Children's Hospital, Boston. Following the format of preceding neural regeneration symposia, the program is divided into six sessions, including (1) Targeted Axonal Growth/Regeneration in the PNS, chaired by Ronald Oppenheim; (2) Human Cells for Transplantation, chaired by Mahendra Rao; (3) Neuron-Glia Interactions and Synaptic Plasticity, chaired by Platon Kostyuk; (4) Signaling Pathways in Neural Plasticity, chaired by Joseph Neary; (5) Neurogenesis in the Adult, chaired by Pasko Rakic; and (6) Emerging Topics in Regeneration, chaired by Oswald Steward. The abstracts of the speaker presentations for each of these sessions are given on the following pages.

The International Neural Regeneration Symposia began in 1985 and have been held biennially since that time. The 2001 symposium will be the ninth meeting in this series. Dr. Roger Madison recently took over the directorship of the symposium. Dr. Fredrick Seil, from the VA Office of Regeneration Research Programs in Portland, has been directing the meetings since 1985. He retired in July 2001, and Dr. Madison was selected by Veterans Affairs Central Office to take over the responsibility of all aspects of the symposium.

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. These International Neural Regeneration Symposia have 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 10th International Symposium will be held in 2003.

Roger Madison, Ph.D.

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Abstracts of Oral Presentations

1 **The misdirection of reinnervation after peripheral nerve suture and chances of its therapy**

*W.F. Neiss, M. Streppel, M. Barham,
D.N. Angelov, and O. Guntinas-Lichius*

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After surgical repair of an injured peripheral nerve, the motor axons sprout, skeletal muscle fibers rereach, motor endplates reconstitute, and the musculature is reinnervated. This is the simplest case of neural regeneration in neurobiology. Nerve sutures to achieve this end have been clinically routine for over a century. Nerve grafts or artificial nerve conduits, made from silicone tube or more sophisticated materials, have been devised to bridge traumatic nerve gaps. However, despite all these possibilities, the functional outcome of surgical repair of a peripheral nerve in man is often disappointing or even dismal. Using sequential double-fluorescent retrograde labeling of motoneurons before lesion and after regeneration or simultaneous triple labeling of three different nerve branches after lesion and repair of the main nerve trunk in rats and cats, we have quantified the time course and extent of reinnervation and its morphological accuracy. In addition, electromyographic (EMG) recordings and motion analysis allow correlating recovery of structure and impaired regain of function. With this methodological approach, we are assessing how the results of peripheral nerve reconstruction can be improved. So far, we have tested different techniques for surgical nerve repair, the Ca-entry-blocker nimodipine and other drugs, growth permissive matrix proteins, manipulation of afferent input of motoneurons, and delayed nerve suture. Although most of these manipulations do influence—some even quite profoundly influence—this or that parameter of peripheral nerve regeneration, so far none of these approaches has yielded a major improvement of final outcome—better function. Further work is required. Once true

regeneration of long central nervous system (CNS) tracts will be reality, the misdirection of reinnervation in the spinal cord will most likely cause the next level of severe problems for paralyzed patients. This study was supported by the Deutsche Forschungsgemeinschaft (DFG), Köln Fortune, Jean-Uhrmacher-Foundation, Bayer AG, Boehringer Ingelheim, and COST B10 “Brain Damage Repair.” The work is supported by Acciones Integradas Hispano-Alemanas and by grants FIS-SS 01/0194 and La Caixa 00/032-2000 to J.M.D.-G.

2 **Preferential motor reinnervation and peripheral nervous system regeneration**

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Preferential motor reinnervation (PMR) refers to the proven ability of regenerating motor axons in the rat femoral nerve to preferentially, albeit incompletely, reinnervate a distal terminal nerve branch to muscle vs. skin (cutaneous). Because regeneration in the peripheral nervous system (PNS), while robust, is often inaccurate, an understanding of how this accuracy might be improved would be of fundamental interest to basic and clinical neuroscience. In addition, if such discriminatory choices are not successfully made at the level of the terminal nerve branch, progressively finer discriminations might be rendered impossible because of the lack of appropriate end organ choices available at the termination of the tributary nerve. PMR was originally shown to take place in the rat femoral nerve; however, we have recently demonstrated the existence of PMR in the nonhuman primate following median nerve regeneration. This finding in the nonhuman primate suggests that PMR may play a role during human peripheral nerve repair. Our current working hypothesis is that PMR is made possible by the molecular memory of Schwann cells for the type of axon with which they were previously associated

(i.e., motor or cutaneous). To elucidate some of the potential molecular targets that might be responsible for PMR, we are using high-density oligonucleotide microarrays (Affymetrix) to look at differential gene expression in the terminal nerve branches of the rat femoral nerve during nerve regeneration, and the emergence of PMR. We are using an RNA amplification protocol to obtain gene expression profiles of individual nerve branches using as little as 10 ng of total RNA as the starting material. We can obtain anatomical indices of PMR in the same animals by the use of standard retrograde tracing techniques to quantify the number of motor neurons that have regenerated axons into each of the terminal nerve branches. This work was supported by the Office of Research and Development (Medical Research Service and Rehabilitation Research and Development Service) of the Department of Veterans Affairs and the National Institutes of Health.

3 **Reinnervation of muscle: Cues that guide synaptic choices**

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Motor neurons form an orderly topographic map on the surface of muscles. This map is detectable in the embryo and is partially restored following denervation. To understand the mechanism for the development of the topographic map and its restoration upon reinnervation, we cocultured slices of E-15 rat embryonic spinal cord with muscle membranes. We found that caudal spinal neurites grow selectively on membranes derived from caudal muscle. Rostral neurites prefer rostral membranes. We next explored the role of the Eph A-ephrin A subfamily of kinases in neuromuscular topography, analogous to that found in the retinotectal system. All five ephrin A genes are found in developing muscles. Overexpression of ephrin A5 or deletion of the genes for ephrin A2 and A5 disrupted the topographic map. In vitro studies revealed that selective growth of neurites on muscle membranes was disrupted by Eph A fusion proteins or pretreatment of membranes with phosphoinositol phospholipase C (PI-PLC). These results strongly implicate the Eph A-ephrin A subfamily of tyrosine kinase receptors in the formation of the neuromuscular map and imply a role for reestablishment of the map follow-

ing denervation. This work was supported by the National Institutes of Health.

4 **The influence of the near-nerve environment on the rate of elongation in the cat**

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The outgrowth of axons following Wallerian degeneration occurs mainly in the peripheral nervous system but is abortive in the central nervous system. It is also considered to be dependent on the integrated actions of a number of different cell types and associated humoral and structural factors in the distal nerve stump. We have further studied the effect on regeneration, as expressed by functioning nerve fibers, of (1) destroying cells in the distal nerve stump by freezing injury and (2) the dependence of recruitment of such cells from the nerve environment by shielding the distal nerve from the environment by a Silastic tube. Physiological longitudinal in vivo studies of regeneration are usually carried out by following the recovery of the muscle response as nerve fibers reinnervate the target. This method, however, does not allow evaluation of the rate of elongation along particular segments of the regenerated nerve. To measure the rate of elongation through the frozen section of nerve, we chronically implanted electrodes on the inside of Silastic tubing around the tibial and sciatic nerves in cat. Wallerian degeneration was obtained by crushing or sectioning of the proximal tibial nerve, and these injuries were combined with freezing for 20 to 40 mm distal to the site of section or crushing. The rate of elongation after crushing was 3.3 mm/day, and after sectioning, it was 2.5 mm/day. At freezing for 20 mm distal to sectioning, the rate was 50% reduced, whereas no effect of freezing occurred distal to crushing. However, when freezing for 30 mm distal to crushing, the rate was markedly reduced through the frozen section but accelerated distal to this area. At 40-mm freezing, the rate of elongation was reduced by more than 90%, and fibers were not found to traverse the frozen section during the observation period of 60 days. When recruitment of cells from the environment was prevented, the rate and extent of elongation were markedly affected at freezing over long distances. However, when contact between the nerve and the environment was preserved, regeneration could take place of over

distances of frozen nerve of more than 70 mm. These findings would support that degenerated and ischemic nerve attracts angiogenesis and that cells recruited through newly formed vessels support nerve regeneration. These findings indicate that the cellular environment of the endoneurium is of central importance for regeneration to occur. Other studies in the monkey (Krarup, Archibald, Madison, *Ann Neurol*, in press) would indicate the even a temporary delay in regeneration may have severe impact on the final level of recovery.

5 Roles of growth factor signaling in axon development and regeneration

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Neurotrophins are among the most powerful known regulators of axon growth. Nevertheless, the precise roles of neurotrophins and neurotrophin signaling in regulating axon growth during development and during regeneration remain unresolved. To assess the roles of neurotrophin signaling in regulating axon growth during development, we have crossed BAX null mice with neurotrophin-deficient mice to separate morphological versus survival effects. We have shown that all dorsal root ganglia (DRG) neurons survive neurotrophin deficiency when BAX is mutated but that peripheral axon growth of nerve growth factor (NGF) and NT3-dependent populations is arrested around embryonic day 13. Furthermore, the spinal cord projections of NT3-dependent neurons are deficient because of the lack of appropriate regulation of the transcription factor ER81. To address the signaling mediators responsible for these neurotrophin effects, we have transfected constitutively active and dominant inhibitory Trk effectors into cultured BAX null neurons. In agreement with previous results, we find that the Trk effectors Ras, Raf, PI3-K, and Akt are all required for normal axon extension. However, each of these mediators is associated with a different morphological effect in gain of function experiments, with Raf mediating axon elongation and PI3-K and Akt mediating increases in axon caliber. Finally, we have investigated growth factor signaling dependence in a "conditioning lesion" model of regenerative axon growth in adult DRG neurons. We find that in contrast to the situation with embryonic axon growth, inhibition of MEK and PI3-K has little effect on the

regenerative mode of growth. However, addition of the JAK phosphorylation inhibitor, AG490, almost completely abolishes regenerative growth. We conclude that growth factors and growth factor-signaling mediators are required for normal axon growth at all stages of development but that the relative importance of neurotrophin versus cytokine signaling is different between the developmental and regenerative growth modes. This study was funded by the National Institutes of Neurological Disorders and Stroke (NINDS), R01-NS31768.

6 Neural regeneration following end-to-side coaptation: A basic science and clinical review

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End-to-side neural coaptation has been used as early as the 19th century. However, the technique fell into disfavor. Viterbo reignited interest in 1992. Since that time, many investigators attempted to elucidate the mechanisms of nerve regeneration operable at this type of coaptation both in the laboratory and in the clinic. The technique involves the attachment of a denervated nerve trunk to a donor nerve in an end-to-side fashion, which seems to stimulate collateral sprouting from the axons of the intact donor nerve. Whether mechanical damage is a prerequisite for collateral sprouting to occur is still a controversial issue. The concept of the end-to-side coaptation is of great interest to restorative surgeons because it can spell improved prognosis for paralyzed patients by allowing reinnervation of a second muscle target without downgrading the original target. The purpose of this report is to review information over the last 10 years concerning this procedure and to speculate on the interactions that transpire between the axons of the donor nerve and their microenvironment. Neural regeneration involves multiple interactions between the cells of the microenvironment and the actions of multiple neurotrophins. It is these factors, which probably provide the stimulus, along with the action of the Schwann cells, macrophages, and other cells, for regeneration in the end-to-side repair. Clinically, we have used end-to-side coaptation in the following specific situations: (1) to enhance a denervated muscle target without loss of donor nerve function

and (2) to deliver additional motor fibers to a partially innervated muscle target. We have had measurable success with this type of repair in brachial plexus injuries as well as in the facial paralysis patient.

7 Isolation and induction of adult neural progenitor cells

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Neural precursor cells remain ubiquitous in the adult vertebrate brain, including that of humans. These cells persist within both the ventricular subependyma and the dentate gyrus of the human forebrain and include distinct populations of neuronal progenitors and uncommitted precursors. In addition, a distinct population of nominally glial progenitor cells remains dispersed throughout the subcortical white matter. Each of these progenitor cell types may be identified, specifically extracted and enriched from the adult brain, based upon their expression of fluorescent transgenes driven by cell-specific promoters. At first glance, these appear to represent distinct cell populations. However, once removed from the tissue environment, each may generate multiple cell types, suggesting that each of these progenitor phenotypes may be plastic in its autonomous lineage potential and directed to its *in vivo* fate by virtue of local environmental signals. Our ability to specifically harvest these cells from the human central nervous system (CNS) has allowed us to assess their lineage potential, functional capacity, and engraftment efficacy upon transplantation. In addition, it has allowed us to rationally design strategies for inducing resident progenitor cells *in vivo*. On this basis, we have found that each adult progenitor phenotype can mature to functional competence *ex vivo*, can successfully integrate upon xenograft, and can be induced *in vivo* by exogenous delivery of cognate neurotrophic cytokines. As such, neural progenitor cells may provide a cellular substrate for neuronal and oligodendrocytic replacement in the damaged adult brain and spinal cord.

8 The biology of neural stem cells may make them uniquely suited for neural regenerative processes

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It has become well accepted that neural cells with stem-like features exist in the rodent central nervous system (CNS) and may be of value for the study of development and, in transplantation paradigms, for gene transfer and cell replacement. More recently, it has been determined that such insights might extend to cells from the human CNS. Neural stem cells (NSCs) can be isolated from the human fetal telencephalon. *In vitro*, these self-renewing cells give rise to all fundamental neural lineages. Following transplantation into germinal zones of the newborn mouse brain, emulating their rodent counterparts, they participate in aspects of normal development. This includes migration along established migratory pathways to disseminated CNS regions, differentiation into multiple developmentally and regionally appropriate cell types, and non-disruptive interspersions with host progenitors and their progeny. Readily genetically engineered *ex vivo*, human NSCs (hNSCs) are capable of expressing foreign transgenes *in vivo* in these disseminated locations. Their secretory products can cross-correct a prototypical genetic metabolic defect in neurons and glia *in vitro*. hNSCs can replace specific deficient neuronal populations. hNSCs may be propagated by both epigenetic and genetic means that are comparably safe and effective. In helping to link human NSC biology to the body of rodent work, these observations encourage investigations into the feasibility of hNSC transplantation for a range of disorders. hNSCs have been observed to emulate their rodent counterparts in rodent models of spinal cord dysfunction (contusion, motor neuron degeneration), brain tumors, and infarction. They seem capable, as well, of integrating into the nonhuman primate CNS. When a clone of hNSCs is implanted intraventricularly to allow its integration into cerebral germinal zones of Old World monkey fetuses, the NSCs distribute themselves into two subpopulations: one contributing to corticogenesis by migrating along radial glia to temporally appropriate layers of the cortical plate and differentiating into lamina-appropriate neurons or glia and the other remaining undifferentiated and contributing to a secondary germinal zone (the subventricular zone) with occasional members interspersed

throughout brain parenchyma. An early neurogenic program allocates the progeny of NSCs either immediately for organogenesis or to undifferentiated pools for later use in the “postdevelopmental” brain. Harnessing this biology for repair of the primate CNS seems feasible as suggested by preliminary results in adult primate models of nigral dopamine deficiency, spinal motor neuron degeneration, and possibly cerebellar Purkinje cell death. Funding was supported by NINDS, March of Dimes, Project ALS, and A-T Children’s Project.

9 Neural specific differentiation of human embryonic stem cells

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Embryonic stem (ES) cells isolated from blastocysts are immortal and have the capacity to differentiate into mesoderm, ectoderm, and endoderm, in vitro and in vivo. To date, ES cells have been isolated from mouse, monkey, and human blastocysts. Although similarities exist between cell lines derived from different species, such as immortality and pluripotency, many differences exist as well. For instance, mouse and human ES (hES) cells require different growth conditions for the maintenance of the pluripotent state. In addition, the requirements for differentiation to specific phenotypes may be different. Because human ES cells remain karyotypically and phenotypically stable in long-term culture, they may provide an excellent source material for cell therapies. To realize the potential of human ES cells for cell therapies, we have focused on the evaluation of culture conditions that maintain pluripotency and control differentiation. Using human ES cells maintained in feeder-free culture conditions (Xu et al., 2001), we have induced differentiation into the neural lineage with the use of several paradigms. Neural progenitors could be identified in these populations by positive nestin, PS-NCAM, or A2B5 immunoreactivity. Neuronal cells underwent maturation in culture to express additional neuron-specific antigens, such as MAP-2 and neurofilament. These neurons responded to neurotransmitters and could fire action potentials. These data indicate that hES cells could provide an abundant cell source for the neural progenitor cells and mature neurons for therapeutic and drug research and development uses.

10 Myelinating cells for transplantation and myelin repair: Comparative aspects of human versus animal cell

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While the successful transplantation of myelinating cells into animal models of myelin disease has suggested that such an approach may have therapeutic value in humans with focal myelin disorders, the discovery of suitable cells from the human central nervous system (CNS) that could be used for such repair has not yet been achieved. As can be expected, much of the knowledge of the development of myelinating cells of the CNS has come from in vitro studies of the rodent CNS. The lineage of the oligodendrocyte and its growth factors and mitogens has been well defined in culture. Most recently, it has been shown that oligodendrocytes can be derived in vitro from mouse embryonic stem (ES) cells. A considerable amount is now known about the in vivo myelinating potential of such cells and of cells at different stages of the oligodendrocyte lineage following their transplantation. We now use neural stem cells grown as neurospheres as our starting point for the production of purified populations of oligodendrocyte progenitors (OPs), grown as a free-floating collection of cells called oligospheres. While numerous oligodendrocytes and OPs can be derived from human neurospheres, the production of such pure populations of human OPs has not been achieved. Likewise, the myelinating potential of the human OPs that have been isolated, when transplanted into animal models, remains to be shown. The growth factors and mitogens for human OPs appear different from those that have been known for animal OPs. We have now determined that human ES cells can give rise to neurons and astrocyte in vitro and in vivo, but production of significant numbers of oligodendrocytes from these cultures remain elusive. Nonetheless, these cells remain as one of the most promising sources of cells for human myelin repair.

11 Modification of skilled movement: Fixed circuits versus plastic changes

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Skilled movements, movements used for reaching and grasping with a hand, are highly conserved, originating with the first terrestrial mammals. Their evolution is closely related to food handling, and movement details are characteristic of orders, families, and species. These movement characteristics suggest that skilled movements are specified genetically and mediated by relatively fixed neural circuits. Lesion studies support this notion, indicating that many cortical and subcortical neural circuits mediate the movements and that, following injury, mainly compensation and not true recovery mediate improved performance. Nevertheless, skilled movements are plastic in that they can be adapted to different uses and compensatory circuits are plastic in that compensatory movements appear rapidly after motor system injuries. Neuron-glia changes underlying normal plasticity and compensatory process of skilled movements are widespread within motor circuits and in other circuits that influence the motor system. Funding is provided by Canadian Institutes of Health Research (CIHR), National Science and Engineering Research Council (NSERC), and The Canadian Stroke Network.

12 Neurotrophic factors, synaptic plasticity, and recovery from cortical injury

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Evidence is accumulating that at least partial functional restitution is possible after cortical injury. This functional improvement is associated both with compensatory changes in the remaining, intact neural circuitry, as well as with the generation of new circuitry. Although these morphological changes sometimes can occur spontaneously after injury, various factors can stimulate them, including experience and neurotrophic factors such as nerve growth factor (NGF) and fibroblast growth factor-2 (FGF-2). Both NGF and FGF can stimulate synap-

tic change in both the normal and abnormal brain and both can be used to stimulate functional recovery in the cortically injured brain. Several factors complicate the use of these factors, however. First, there is an interaction between the administration of FGF-2 and sensory-motor experience or other behavioral therapy. For example, behavioral therapies may act, in part, via their action in stimulating the endogenous production of trophic factors. Thus, combining behavioral therapies with pharmacological administration of compounds to increase the availability of trophic factors may lead to enhanced functional outcome. Recently, we have shown that the combination of treatments, such as the infusion of FGF-2 when animals are receiving intense training (i.e., therapy), appears to be more effective in stimulating functional recovery after cortical injury than either treatment alone. Second, the timing of the administration of neurotrophic factors may either enhance or retard recovery depending upon the timing of administration. Third, the age at administration appears to be critical. There are times during development when FGF-2 may interfere with normal brain development, whereas at other times, it is a potent stimulant of functional recovery. The work is funded by the Natural Science and Engineering Research Council of Canada.

13 Block of glutamate-glutamine cycle between astrocytes and neurons inhibits epileptiform activity in hippocampus

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In the central nervous system (CNS), neurons are metabolically coupled to astrocytes. The key site of this neuron-glia interaction is the synapse, which is the most specialized structure responsible for transmitting and processing information between neurons. Among different functions, astrocytes play a major role in removing synaptically released glutamate and in converting it to glutamine, which is then delivered back to neurons as a source for the

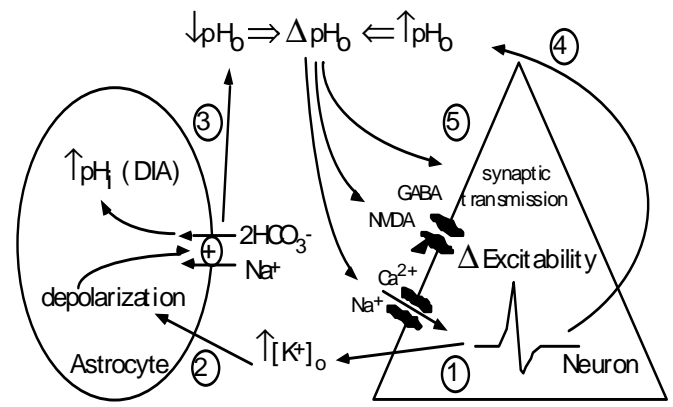
synthesis of neurotransmitter. We have investigated the functional relevance of the glutamate-glutamine cycle between astrocytes and neurons in supporting recurrent epileptiform activity, which occurs spontaneously in cultured CNS neurons and in bicuculline-treated brain slices. We demonstrated that pharmacological treatments, resulting in either the block of glutamine production by astrocytes or the inhibition of glutamine uptake by neurons, suppress or markedly decrease the frequency of spontaneous epileptiform discharges both in primary hippocampal cultures and in bicuculline-treated hippocampal slices. Impairment of the glutamate-glutamine cycle also affect mEPSC amplitude and frequency, without affecting neuronal responsiveness to glutamate. These data suggest that the inhibition of epileptiform activity produced by the impairment in the glutamate-glutamine cycle may be achieved through a reduced replenishment of glutamate into synaptic vesicles and point to an important role for the neuron-astrocyte metabolic interaction in sustaining episodes of intense rhythmic activity in the CNS. This study was supported by the Italian Ministry of University and Scientific and Technological Research (MURST-PRIN 2000) and by European Community (QLGR3-CT-2000-01343) to M.M.

14 Glial modulation of neural excitability mediated by extracellular pH

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Astrocytes are diffusely distributed throughout the nervous system, and nearly every neuron has immediate astrocyte neighbors with which it shares a common, extremely narrow, extracellular space (ECS). Because of the minute amount of fluid in this compartment, its ionic and solute composition can fluctuate significantly with neural activity. About 10 years ago, I proposed a functional interaction between astrocytes and neurons based on activity-induced changes in $[K^+]_o$ and $[H^+]_o$ (or pH_o). It was viewed as a negative feedback loop, whereby active neurons would signal to nearby astrocytes the extent of their activity, in the form of graded changes in $[K^+]_o$, and astrocytes would respond by producing graded degrees of extracellular acidification to dampen neuronal excitability (figure). This feedback system could adjust local brain excitability in relationship to ongoing activity. The status of



this hypothesis will be reviewed with special emphasis on the nature of the astrocyte-induced extracellular acidification and on the manner in which pH changes can alter neural excitability. Discharging neurons increase $[K^+]_o$ (1). Depolarized astrocytes (2) cause depolarization-induced-alkalinization (DIA) via $Na^+HCO_3^-$ cotransport leading to ECS acidification in proportion to the increase in $[K^+]_o$ (3). Activity in neurons can also cause extracellular alkaline shifts (4). The final change in pH_o (Δ pH_o) is the integral of glial acidification and neuronal alkalization. Finally, the change in pH_o, in a feedback fashion, alters neuronal excitability by modulation of ligand-gated and voltage-gated channels, and by affecting synaptic transmission (5). This work was supported by grants from the National Institutes of Health (NS15589) and the Eastern Paralyzed Veterans Association.

15 Glial regulation of perisynaptic glutamate in health and disease

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Astrocytes extend processes into the perisynaptic space of essentially all excitatory synapses in the brain. This space is narrow, and consequently, glutamate released at synapses can readily reach concentrations of hundreds of micromoles in the synaptic and perisynaptic space. Astrocytes express two potent Na^+ -dependent glutamate transporters, GLAST and GLT-1, which are believed to regulate perisynaptic glutamate concentrations. The role of these transporters in preventing spillage of glutamate from the synaptic space and ensuing excitotoxic

neural injury has been established through knockout studies. It is currently not known if astrocytic transport of glutamate out of the synaptic cleft also contributes to the signal received by the postsynaptic cell. We used double electrode patch-clamp recordings in rat cerebellar slices to search for a contribution of glial cells to normal postsynaptic activity. We show that Bergman glial cells respond to parallel fiber stimulation with an inward current that is largely because of glutamate transport and that can be inhibited by THA. Inhibition of glutamate transport into Bergman glial cells, either pharmacologically with THA or through depolarization of single Bergmann glial cells, was sufficient to enhance postsynaptic firing frequency by eightfold. This effect was fully reversible and suggests that the termination of postsynaptic currents requires removal of glutamate via glial transporters. We next examined the effect of acute and chronic injury to the expression of glutamate transporters in brain. We show that GLAST and GLT-1 are down-regulated at sites of injury, as for example induced by cortical or hippocampal lesions, and that the down-regulation correlates with proliferation of glial cells. Interestingly, neoplastic proliferation of glial cells (glioma) similarly leads to a disappearance of glutamate transporters from the cell surface. These findings suggest that conditions that induce cell proliferation of glial cells cause the disappearance of functional glutamate transporters. This in turn may contribute to increases in extracellular glutamate and possibly to excitotoxic neural injury.

16 Activity-dependent plasticity in rat phrenic nucleus after reversible hemispinalization of cervical spinal cord by a cooling device

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Although it has been known for sometime that spinal cord injury results in pronounced alterations of the neuronal and glial cytoarchitecture caudal to the injury, determining which component of the injury causes the plasticity has been difficult. For instance, it is not clear if the plasticity observed in the phrenic nucleus after C2 spinal cord hemisection from our earlier studies is caused by the multifactorial mechanisms induced by the physical trauma to the spinal cord (i.e., edema, ischemia, local tissue hypoxia, etc.) or the more specific interruption of the descending respiratory drive onto phrenic moto-

neurons. The present study differentiates between “injury-induced” and “activity-dependent” plasticity in the phrenic nucleus by using a cooling device that allows reversible block of the inspiratory drive to phrenic motoneurons without injury to the spinal cord at the C2 level. Thus, we were able to differentiate between the plasticity induced by blockade of synaptic activity in the phrenic nucleus from the trauma-induced plasticity caused by a C2 spinal hemisection that would also interrupt descending drive. The surgical exposure of the ventral surface of the spinal cord at C2 and the construction of the inexpensive cooling device have been described in detail (Exp Neurol 1996;141:102–112). The device was applied to the spinal cord for 4 hours while bilateral diaphragm EMG recordings confirmed paralysis of the hemidiaphragm ipsilateral to the block and normal activity contralaterally. The temperature at the surface of the spinal cord was maintained between 7° and 10° during cold block. After the cooling device was removed, function returned to the ipsilateral hemidiaphragm. Ultrastructural morphometric analysis of the phrenic nucleus of cold block animals as compared to controls revealed a significant increase in (1) the number of dendrodendritic appositions, (2) the number of multiple synapses, and (3) the length of symmetric and asymmetric synaptic active zones (Exp Neurol 1997;147:299–310). The above changes are similar to the changes induced in the phrenic nucleus 4 hours after C2 hemisection. Thus, injury is not a requirement for this type of morphological plasticity in the phrenic nucleus, but rather, the induced changes are activity-dependent and are caused by interruption of the descending respiratory drive to the phrenic nucleus. The work was supported by National Institutes of Health grant HD 31550.

17 Signal transduction through the Ras GTPase

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The Ras protooncogenes, Ha-Ras, Ki Ras, and N Ras are the founding members of the small GTPase family. First identified as a retroviral-transforming

agent, mutant GTPase deficient forms of Ras occur in nearly 30% of all human tumors. The wild-type cellular Ras polypeptides function downstream of cell surface receptors, primarily RTKs and guanine protein-coupled receptors (GPCRs), to control cell fate, i.e., proliferation, differentiation, and survival, as well as a variety of differentiated functions. Ras signaling involves a GTP-induced conformational change that creates a binding site for Ras "effector" proteins. On binding to Ras-GTP at the surface membrane, these effectors are functionally activated and mediate aspects of Ras biologic program. The first Ras effectors to be identified were the family of Raf kinases, which control the mitogen-activated protein kinase (MAPK) pathway. The Ras-dependent activation of cRaf1 is a complex multistep process involving other protein kinases (PAKs and possibly Src-like kinase) and 14-3-3 proteins. The Type 1 PI-3 kinase catalytic subunits also bind to Ras in a GTP-dependent manner, and Ras-GTP augments PI-3 kinase activity; in contrast to Raf. However, Ras-GTP is a secondary regulator of Type 1 PI-3 kinases, as those lipid kinases are recruited to the membrane through adapter subunits that interact directly with RTKs or bg subunits. PI-3 kinase plays an important role in cell survival, mediated in a large part through the multiple antiapoptotic actions of PKB, whose activation is Ptd Ins 3,4,5P₃-dependent. A third group of Ras effectors is a family of guanine nucleotide exchange factors active on RalA. Ras-GTP also recruits a number of noncatalytic proteins, such as AF-6, Rin and NORE, whose role in Ras biology is less well defined. We have been interested in NORE1, a member of a small gene family that includes the tumor suppressor RASSF1A. Nore exists in a constitutive complex with a Ste-20 related protein kinase; Ras-GTP recruits this complex to the cell surface. Targeting of the NORE/kinase complex to the membrane independently of Ras promotes apoptosis. Overexpression of constitutively active Ras induces apoptosis in several cell backgrounds; the apoptotic response to Ras-GTP can be blocked entirely by interference with the Ras recruitment of the NORE/kinase complex. These findings identify a new Ras regulated signaling pathway that controls cell survival in the presence of constitutively active Ras. The physiologic function of this pathway awaits characterization of knockout models.

18 FK506/Hsp-90 steroid receptor chaperones and ERK signaling in the regulation of nerve regeneration

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Systemic administration of the immunosuppressant drug FK506 dose-dependently accelerates functional recovery by increasing the rate of nerve regeneration following a peripheral nerve injury. The mechanism is distinct from that underlying immunosuppression, which is mediated by binding to the immunophilin FK506-binding protein-12 (FKBP-12) and subsequent calcineurin inhibition. First, nonimmunosuppressant (noncalcineurin-inhibiting) FK506 derivatives are orally effective in speeding nerve regeneration. Second, the neurotrophic activity of FK506 is retained in hippocampal neurons from FKBP-12 knockout mice. Third, nonimmunosuppressant FK506 derivatives that do not bind FKBP-12 increase neurite elongation in SH-SY5Y cells and accelerate nerve regeneration. Thus, binding to FKBP-12 is not necessary and orally active non-FKBP-12 neuroimmunophilin ligands may be useful for the treatment of human neurological disorders without any potential side effects resulting from FKBP-12 binding (such as calcium regulation and cardiomyopathy). In contrast, neurotrophism is blocked by an antibody to FKBP-52, a component of steroid receptor complexes. Furthermore, FK506 increases ERK phosphorylation (activation) and the selective MAP kinase inhibitor, PD98059, blocks its neurotrophic activity. Moreover, classes of compounds (e.g., geldanamycin and radicicol) that target other chaperone components of steroid receptor complexes (i.e., Hsp-90) are also neurotrophic. Geldanamycin and radicicol are known to reduce activation of steroid response elements via their capability to disrupt steroid receptor complexes. A novel model is thereby proposed to account for the neurotrophic activity of these different classes of compounds (i.e., neuroimmunophilin ligands, Hsp-90 binders, and steroid hormones). It is hypothesized that the neurotrophic activity of these compounds is mediated by a "gain-of-function" involving disruption of the steroid receptor/FKBP-52/Hsp-90 chaperone complex leading to activation of extracellular signal-regulated kinase (ERK) pathway of the mitogen-associated (MAP) superfamily. Taken together, these findings reveal that components of steroid

receptor complexes are potential targets for the development of new drugs to treat neurological disorders.

19 Overcoming growth inhibition in the CNS: Use of Rho antagonists

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Rho family GTPases are important in the regulation of axon growth and regeneration. We have investigated the use of Rho antagonists to overcome growth inhibition and promote axon regeneration in two different models of CNS injury: optic nerve crush in adult rats and spinal cord injury in adult mice. We have tested C3 toxin from *C. botulinum* to inactivate Rho. When we applied C3 to crushed rat optic nerves, retinal ganglion cells axons were able to cross the lesion to extend in the distal optic nerve. Axons that extended past the lesion took a twisted course, typical of regenerating axons. To test C2 in injured spinal cord, we applied C3 to the site of injury in a fibrin gel. Behavioral recovery was followed for 1 month and assessed by the BBB scale. Two days before perfusion of the animals with fixative, the corticospinal tract (CST) axons were anterogradely labeled with WGA-HRP, and then the spinal cord and brains were removed. The application of Rho antagonists to injured spinal cord promoted both short-term and long-term functional recovery of locomotion. We have determined that one short-term consequence of C3 application is neuroprotection. In animals examined 1 month later, both local sprouting and long-distance regeneration of CST axons was observed. Analysis of mRNA levels by *in situ* hybridization in motor cortex of treated animals showed that Rho inactivation elicited GAP-43 expression. These studies indicate that inactivation of Rho is a successful strategy to promote axon regeneration and functional recovery after spinal cord injury. Funding was supported by Canadian Institutes of Health Research (CIHR).

20 The role of growth factors in repair and recovery from brain injury and stroke

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Polypeptide growth factors are likely to play several important roles following brain injury or stroke. In the acute phase, such factors may protect against cell death. In the recovery phase, these factors may enhance new neural sprouting and synapse formation and may promote the proliferation, migration, and differentiation of endogenous progenitor cells. The expression of many growth factors is up-regulated following brain trauma or stroke. Moreover, these factors can be delivered exogenously as drugs, perhaps in combination with cellular therapies. Our laboratory has focused primarily on basic fibroblast growth factor (bFGF, FGF-2), a factor with pluripotent effects on brain cells. In animal models of stroke, the acute administration of bFGF reduces cell death, while the delayed administration of bFGF enhances neurological recovery when given after cell death has occurred. In recent studies, we have examined the coadministration of bFGF and neural and umbilical cord blood stem cells in models of stroke recovery. Data from these studies will be discussed in our presentation. Funding was provided by the National Institutes of Health (NSP01-10828) and the ViaCell, Inc.

21 Induction of neurogenesis in the neocortex of adult mice

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Neurogenesis does not normally occur in postnatal mouse cortex. Is this because of limitations of endogenous precursors' potential or the lack of signals for neuronal differentiation and/or survival? Prior results from our lab show that in regions of adult mouse cortex undergoing synchronous apoptosis of projection neurons, nearby cells up-regulate genes that guide transplanted neuroblasts or precursors to undergo directed migration, differentiation, synaptic integration, and re-formation of long-distance projections. To ask whether we can direct the fate of endogenous precursors in mature cortex, we have examined their differentiation when exposed *in situ* to these signals, without transplantation. In one set of experiments, we induced degeneration of cortico-thalamic neurons in layer VI and examined fates of dividing cells in cortex, via bromodeoxyuridine

(BrdU) and immunocytochemical markers of progressive neuronal differentiation. We found that precursors can be induced in situ to differentiate into mature neurons in a layer- and region-specific manner. BrdU+ newborn cells express NeuN, a mature neuronal marker, exclusively in regions of cortex undergoing targeted neuronal death; the newborn neurons survived at least 28 weeks. We observed 97 ± 69 new neurons/mm³ in experimental cortex, vs. zero in controls. Early on, subsets of BrdU+ precursors expressed Doublecortin, a protein exclusively expressed in migrating neurons, and Hu, an early neuronal marker. The retrograde label "FluoroGold," injected into thalamus, labeled "newborn," BrdU+ neurons, showing that newborn neurons can form long-distance corticothalamic connections. Other experiments from our lab suggest that these results are generalizable to other populations of projection neurons. Together, these results demonstrate that endogenous precursors can be induced in situ to differentiate into cortical neurons, survive for many months, and form appropriate long-distance connections in the adult mammalian brain. This suggests the possibility of neuronal replacement therapies that do not require transplantation of exogenous cells. Ongoing experiments are investigating the molecular mechanisms underlying this induced neurogenesis, whether this induced cortical neurogenesis can be modulated to increased levels, whether the new neurons differentiate precisely into the mature neuronal phenotype they are replacing, and whether newborn neurons join or form functional circuitry in the adult brain.

22 Microenvironments and neural stem cells in the adult brain affiliation

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The adult brain is limited in its ability to repair after injury, but some areas display a slow and continuous neuronal replacement. The hippocampus is one such structure where neural precursors proliferate within a discrete zone underlying the dentate granule cell layer. Daughter cells born in the "subgranule zone" then migrate into the granule cell layer where they differentiate into granule cell neurons. This process starts within a unique vascular microenvironment where endothelial and neural precursors proliferate within tightly packed clusters.

This architecture suggests that endothelium and/or angiogenic signaling may influence neurogenesis. Initial analysis of these clusters in vivo shows that the vascular endothelial growth factor (VEGF) receptor, Flk-1, is expressed. In addition, VEGF can directly stimulate the proliferation of neural precursors cultured from the adult hippocampus. The role of vascular microenvironment in adult neurogenesis and the potential utility of induced angiogenesis as a framework for modulating neurogenesis are under investigation.

23 Factors controlling the proliferation and migration of neuronal progenitor cells in the postnatal mammalian forebrain

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We have performed experiments to determine whether the adult mammalian forebrain has endogenous progenitor cells with the capacity to generate new neurons. To investigate this possibility, we analyzed the distribution and phenotype of newly generated cells in the forebrain of adult rats following the intraventricular infusion of brain-derived neurotrophic factor (BDNF) in combination with the cell proliferation marker bromodeoxyuridine (BrdU). BrdU-immunoreactive cells were present in widespread but well delineated regions of the forebrain, lining both the lateral and third ventricles. New cells were also found in particular regions of the forebrain parenchyma, including the septum, striatum, thalamus, and hypothalamus. The newly generated cells only appeared in regions expressing TrkB, the high affinity tyrosine kinase receptor for BDNF. However, the BrdU-positive cells were not TrkB-positive. A substantial percentage (27 – 42 %) of the newly generated cells in the parenchyma expressed a neuronal phenotype. Thus, the adult brain has a greater capacity to generate new neurons than previously recognized. Only a low percentage of the BrdU-positive cells were GFAP-positive, and very few expressed nestin, a marker of undifferentiated cells. A high incidence of the BrdU-positive cells were present in pairs, suggesting that the subventricular zone may not be the only source of newly generated cells in the adult forebrain. Increased numbers of newly generated neurons were also observed in the rostral migratory stream

(RMS) and olfactory bulb (OB), structures known to acquire new neurons throughout life. In the RMS and OB, greater than 95% of the newly generated cells were neurons. Collectively, these studies suggest that it may be possible to promote the proliferation of endogenous progenitor cells for the replacement of neurons lost because of injury or disease. This work was supported by a grant from the National Institute of Deafness and Other Communicative Disorders of the National Institutes of Health (R01 DC03190).

24 Bone morphogenetic proteins: Multifunctional regulators of neurogenesis

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Studies of the olfactory epithelium (OE) model system have shown that high (10–20 ng/ml) concentrations of BMPs 2, 4, or 7 inhibit neurogenesis, by decreasing numbers of proliferating progenitor cells and blocking production of olfactory receptor neurons (ORNs), the differentiated neurons of the OE. BMPs do this by causing neuronal progenitors to rapidly lose MASH1, a transcription factor known to be essential for production of ORNs; disappearance of MASH1 is due to proteasome-mediated degradation of preexisting protein. These studies have revealed a novel mechanism of BMP action, whereby induced degradation of an essential transcription factor results in premature termination of a neuronal lineage (Shou et al. *Nature Neurosci* 1999;2). Subsequent investigations have shown that OE stromal cells, which are known to be required for neurogenesis in long-term cultures of OE neuronal progenitors, express *Bmps*. Importantly, blockade of BMP action in such long-term cocultures also blocks neurogenesis. Furthermore, at low concentrations (0.1 ng/ml), BMP4 (but not BMP7) actually stimulates neurogenesis. Interestingly, the effect of low-dose BMP4 is not due to stimulation of neuronal progenitor cell proliferation, but rather to BMP4 promoting the survival of newly-generated ORNs (Shou et al. *Development* 2000;127). Thus, BMPs exert both positive and negative

effects on neurogenesis, depending on ligand identity, ligand concentration, and target cell type. Our current studies are focused on analyzing the diversity of BMP action in the OE, as well as other regions of the nervous system, in vitro and in vivo; on identifying the receptors that mediate opposing actions of BMPs; and on extending our understanding of how BMPs and other growth factors interact to regulate neuron number during development and regeneration. This study was supported by grants from the March of Dimes (FY00-660) and National Institutes of Health (DC03583 and HD38761) to ALC.

25 Injury-induced neurogenesis in the adult mammalian forebrain subventricular zone

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Accumulating evidence of persistent neural stem cells and neurogenesis in the adult mammalian brain suggests that the brain maintains a potential source of cells for neuronal renewal after injury. However, only a limited number of investigations have addressed the question of how endogenous neural precursors are influenced by brain insults. One region in which injury has been shown to increase neural precursor proliferation is the forebrain subventricular zone (SVZ), a persistent germinative region that generates olfactory bulb neurons throughout life. We examined the effects of acute injury on adult rat forebrain SVZ neurogenesis using experimental models of two common neurological disorders, epilepsy, and stroke. BrdU pulse labeling studies showed that systemic chemconvulsant-induced status epilepticus (SE) in adult rat, which produces diffuse limbic and extralimbic brain injury, stimulates rostral forebrain SVZ neural precursor proliferation. Using “pulse-chase” BrdU labeling and stereotactic injection of retroviral reporters, we found that prolonged seizures increased neurogenesis throughout the SVZ-olfactory bulb pathway and accelerated the migration of neuroblasts to the olfactory bulb. A subset of neuronal precursors in the migratory stream exited prematurely and migrated aberrantly into forebrain

cortical regions, although few neuroblasts differentiated into mature neurons and survived for up to 5 weeks. Using a similar approach, we induced focal cerebral infarcts in adult rat by transient middle cerebral artery occlusion (tMCAO) to examine the effects of focal injury on SVZ neurogenesis. Within 2 weeks of tMCAO, cell proliferation increased in the ipsilateral SVZ and it expanded. Immunostaining for immature neuronal markers alone or in combination with BrdU labeling showed that many of the neural precursors appeared outside of the SVZ-olfactory bulb in regions of striatum and cortex near the ischemic injury. Chains of neuroblasts extending from the SVZ in close proximity to astrocytes suggested that focal injury induced ectopic migration of SVZ neural precursors. The majority of BrdU-labeled cells expressed neuronal, but not glial, markers. These findings indicate that different forms of diffuse or focal brain injury exert remarkably similar effects on endogenous neuronal precursors in the adult rat forebrain SVZ. The similarity of neural precursor responses to diverse brain insults suggests common underlying mechanisms modulating progenitor cell proliferation and migration after injury. Progress in understanding these mechanisms should advance brain repair strategies with the use of both transplanted and endogenous neural stem cells. The funding is from NIH (NINDS), grants NS02006 and NS42143.

26 Adult neurogenesis: Promises and controversies

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In contrast to the high rate of neurogenesis in many vertebrates, unambiguous evidence for new neurons in normal adult mammals has so far been confined to the dentate gyrus and olfactory bulb, which serve as important model systems from which we can learn how to introduce new neurons into more resistant brain structures. To gain insight into regulation of neurogenesis in primates, we have used ³H-TdR autoradiography and BrdU immunohistochemistry to examine more than 100 classes of neurons in 35 regions in the macaque monkey brain, ranging in age from embryonic 25 days to 17 years postnatal. With the exception of granule cells in the olfactory bulb and dentate gyrus, each structure acquired its neurons during a specific developmental time win-

dow, which was unrelated to the final quantity of neurons in a given structure. To understand the regulation of neuronal production during development as well as the inhibitory mechanisms that prevent it in adulthood, we have used retroviral gene transfer methods and the application of various transcription factors both in vivo and in vitro. These studies indicate that the species-specific size of a given structure is determined early in the proliferative zones by genes controlling cell production (i.e., onset, cycle length, symmetric/asymmetric mode of division, and the rate of apoptosis) and the allocation of post-mitotic cells regulated by attractive and repulsive molecules. Overcoming the primate's brain resistance to the acquisition of functionally competent new neurons will require an understanding of why neurogenesis ceases at the end of specific developmental time windows and why there are regional variations in this phenomenon. Furthermore, the studies of the generation of "new neurons" following various experimental manipulations, including directing differentiation of stem cells along neuronal lineages or their transplantation to replenish normally nonrenewable neurons, should apply stringent criteria for identification of cell proliferation and their identity to assure scientifically sound advances in this promising field.

27 Stem cells: From beginnings to clinical trials

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Long regarded as impossible, spinal cord repair is entering the realm of doable therapeutics. However, this complex task will require multistage interventions, some easier to achieve than others. For example, it will be easier to limit the progression of injury than to repair subsequent damage. Of repair strategies on the horizon, harnessing the potential of neural stem cells already present in the central nervous system (CNS) or placed there by transplantation appears promising. In fact, using stem cells to remyelinate the damaged cord is an immediate clinical target. Dysmyelination is an important contributor to disability after spinal cord injury because it incapacitates axons that remain intact at the level of injury. Thus, remyelinating those bridging axons would likely improve some important functions, such as bladder and bowel control, limb movement,

or respiration. I will describe our early success with transplanting embryonic stem (ES) cells into the injured rodent spinal cord, focusing on remyelination. This work will be discussed within the context of newly developed concepts in regeneration and with regard to its potential for translation into clinical trials. Finally, current clinical trials in neural transplantation will be discussed. This work was funded by NIH NINDS NS39577 and NS40520, the National Football League Charities, and the Keck Foundation.

28 Overcoming inhibitors of regeneration in myelin

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Although adult CNS axons do not regenerate after injury, elevation of cAMP either with db cAMP or with neurotrophins can overcome inhibitors of regeneration in myelin to encourage regrowth both in vitro and in vivo. Recently, we have shown that at least one downstream consequence of this cAMP effect is the up-regulation of arginase I (Arg I), a key enzyme in polyamine synthesis. Inhibiting polyamine synthesis from arginine blocks the cAMP effect on regeneration. Conversely, overexpression of Arg I or exogenous-added polyamines is sufficient to overcome inhibition by a myelin-specific inhibitor, MAG (myelin-associated glycoprotein), and by myelin in general. Importantly, while MAG and myelin promote neurite outgrowth from young dorsal root ganglion (DRG) neurons, they become inhibitory as DRGs mature. This switch in response occurs sharply at P4, at the same time when endogenous levels of both cAMP and Arg I drop spontaneously and precipitously. Moreover, overexpressing Arg I in older DRGs blocks that switch. Arg I and polyamine synthesis represent more specific targets than direct manipulation of cAMP for potential therapeutic intervention to encourage regeneration after spinal cord injury. On the other hand, we have shown that neurons from mice treated subcutaneously with the phosphodiesterase inhibitor, rolipram, are not inhibited by MAG or myelin. Rolipram may also represent a possible therapy for spinal cord injury.

29 The glial scar and glial boundaries

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The glial scar inhibits axon regeneration after central nervous system (CNS) injury. Various lines of evidence suggest that the main inhibitory molecules in the glial scar are chondroitin sulfate proteoglycans. Of these molecules, neurocan, NG2, versican, brevican, and phosphacan have been shown to have axon growth inhibitory properties and are up-regulated after CNS injury. In addition to up-regulation of the core proteins, their glycanation is increased, associated with an up-regulation of one of the chondroitin sulfate sulfotransferase enzymes. The proteoglycans are produced not only by astrocytes, the main cell type of the mature glial scar, but also by oligodendrocyte precursors that are recruited and activated in CNS injuries and by invading meningeal cells. Treatments that have been successful in partially removing inhibition from glial scar tissue are chondroitinase ABC, killing of dividing cells with cytosine arabinoside and killing all CNS glia with ethidium bromide. Regeneration has been achieved in brain and spinal cord models. In addition to the inhibitory molecules produced by the glial scar, regenerating axons are stopped by barriers at which they are unable to grow from one glial cell type onto another. Thus, invading meningeal cells produce a barrier that few axons will cross. Another form of barrier exists at Schwann cell-astrocyte boundaries. These barriers can be modeled in vitro, where axons are unwilling to grow from astrocytes onto meningeal cells or from Schwann cells onto astrocytes. The ability of axons to cross these barriers can be enhanced by treatments affecting cAMP and Rho. This work was funded by the International Spinal Research Trust, Wellcome Trust, MRC, Action Research, and IFP.

30 Astroglial cell roles in protection, repair, and neurogenesis

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Astroglia and related cell types such as central nervous system (CNS) astrocytes, enteric glia, satellite cells, and subsets of Schwann cells are among the

most numerous cells in all regions of the vertebrate peripheral nervous system (PNS) and CNS. Astroglia in all parts of the CNS and PNS reacts to injury by hypertrophy, production of growth factors and cytokines, and in some cases proliferation. The functions of reactive astroglia are not well understood. A common feature of these cells is the expression of the intermediate filament protein glial fibrillary acidic protein (GFAP), through which they can be targeted with the use of transgenic techniques. Recent findings using a strategy for the con-

ditionally regulated ablation of reactive astroglia in the CNS and PNS of adult transgenic mice indicate that these cells exert important neuroprotective and anti-inflammatory functions. This ablation strategy also presents a model to study the roles of reactive astroglia in tissue repair and axon regeneration in both the CNS and PNS. Lastly, the selective ablation of GFAP expressing radial glia during development or in the adult, presents a model to test the newly hypothesized roles for these cells in neurogenesis.

Abstracts of Poster Presentations

P1 Specificity and time course of sensory neuron addition in juvenile rat

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In the frog (St Wecker and Farel. *J Comp Neurol* 1994; 342:430–438) but not the mouse (Berg and Farel. *Brain Res Dev Brain Res* 2000; 125:21–30), juveniles add neurons to dorsal root ganglia (DRG) as they grow to adult size. Neurons are added by late differentiation of immature neurons rather than neurogenesis. In the rat, total neurons in DRG L4–L6 increased ($p < 0.01$) from $31,864 \pm 1,569$ (mean \pm SD, $n = 5$) in neonates (6.4 ± 0.5 g) to $43,482 \pm 3,862$ ($n = 16$) in rats older than P100 (422 ± 45 g). Adult values ($41,538 \pm 3,109$, $n = 5$) were approximated by P50. The neuron number was determined by methods validated by 3-D (dimensional) reconstructions (Popken and Farel. *J Comp Neurol* 1997; 386:8–15). The distribution of neuron sizes was determined in neonate and 100D rats. Size estimates were made of each neuron that met the criteria for counting (600 to 1,200 observations per DRG). The proportion of neurons whose size fell below the mean was significantly greater ($p < 0.01$) for adults (55%) than for neonates (45%). These data suggest that added neurons serve functions associated with smaller sensory neurons. If added neurons are characterized by particular biochemical properties, then the proportion of neurons with those properties should be greater in adults than in neonates. The percentage of calcitonin gene related peptide (CGRP) immunoreactive and IB4-binding neurons increased from approximately 30% in P5 rats to over 50% in P100 rats in DRG L3 and L4. In contrast, the percentage of substance P immunoreactive neurons was about 35% in both P5 and P100 rats. In the frog, nerve injury in juveniles accelerates the neuron addition normally seen during juvenile development (Boyer and Farel. *J Comp Neurol* 1999; 410:171–177). This finding raises the possibility that immature neurons normally com-

pleting differentiation as the animal grows may be recruited to compensate for losses caused by injury or disease. The invaluable assistance of Harry (Guy) McCrae, Carolyn Suitt, and Elizabeth Deans is gratefully acknowledged. This work was supported by NIH grant NS37524.

P2 A gradient of adaptability in premotor neural centers generating reflex eyelid responses following hypoglossal-facial and facial-facial anastomosis in cats

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The blink response is a well-known experimental model for studying kinetic and frequency-domain properties of reflex motor responses. The model was used here to follow the adaptability of hypoglossal and facial motoneurons (and of their corresponding premotor circuits) to the de novo reinnervation of the orbicularis oculi (OO) muscle. Cats were prepared for the chronic recording of eyelid and tongue movements, with the search-coil in a magnetic-field technique, and of the EMG activity of the OO muscle. Three types of anastomosis were done: (1) a hypoglossal-facial anastomosis, (2) a buccal-zygomatic anastomosis, and (3) a zygomatic nerve section, and suture following 180° rotation of its proximal branch. Recordings of reflex blinks were done up to 1 year following the anastomosis. Unitary recording of axotomized hypoglossal motoneurons was done in two animals. Results indicate that hypoglossal motoneurons are unable to adapt their discharge properties to the motor needs of eyelid responses. Lid movements evoked by hypoglossal motoneurons oscillated at frequencies one-fourth of those

evoked by OO motoneurons. Facial (buccal) motoneurons were able to respond to corneal stimuli and, more interestingly, seemed to be susceptible of a (weak) adaptation to eyelid motoric, but without a change in their buccal-shaped oscillatory properties. The section and suture of the ophthalmic nerve altered the normal performance of air-puff evoked blinks but did not affect the performance of newly acquired motor responses. Some plastic compensatory changes were induced in the retractor bulbi system, an additional eyelid motor system not directly affected by the anastomosis. A noticeable hyperreflexia was evident at ipsi- and contralateral sides to the lesioned nerve. A gradient of adaptability seems to be involved in the different eyelid performance following each experimental anastomosis. This work was supported by grants FIS-SS 01/0194 and La Caixa 00/032-2000 to J.M.D.-G.

P3 Electrodiagnostic criteria to predict nerve regeneration

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In peripheral nerve injuries, the electrodiagnostic evaluation can be critical in determining expected prognosis and successful management strategies. The presence or absence of axon continuity as demonstrated on electromyography (EMG) by motor unit analysis can be critical in determining whether surgical intervention is used. Nerve conduction studies (NCS) are affected by many variables and viewed as a secondary in predicting nerve regeneration. We have noted that a decrease in spontaneous activity and the presence of a small compound motor action potential (CMAP) may precede the development of reinnervation motor unit potentials (MUPs). We present the case of a 50 year-old male referred with a 6-week history of left wrist drop. No forearm radial motor activity was seen clinically. Initially, no MUPs were found on EMG with 4+ spontaneous single-fiber discharges in all forearm radial-innervated muscles, suggesting no axon continuity. Surprisingly, conduction studies revealed a preserved radial sensory response and a small dispersed extensor indicis proprius (EIP) CMAP with elbow radial nerve stimulation but absent with more proximal sites of stimulation. Surgical intervention

with multiple tendon transfers would typically be suggested when EMG does not demonstrate axon continuity at 6 weeks, but conservative management was chosen because of the small preserved EIP CMAP. Serial electrodiagnostic monitoring revealed a decrease in spontaneous potentials at 10 weeks followed by return of a MUP at 12 weeks. This correlated with NCS, demonstrating progressively more proximal sites of stimulation. Electrodiagnostic criteria of a preserved CMAP when there is no evidence of axon continuity noted on EMG along with the phenomena of spontaneous activity decrease may play an important role in predicting nerve regeneration.

P4 Loss of NeuN immunoreactivity following peripheral nerve injury is not associated with cell loss or atrophy

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The monoclonal antibody NeuN has been used extensively as a specific neuronal marker within the central nervous system (CNS). Little is known about the NeuN antigen or how it responds to traumatic injury. In the present study, we analyzed changes in NeuN immunoreactivity following axonal injury to three neuronal populations (facial motoneurons (FMN), sciatic motoneurons (SMN), and rubrospinal neurons (RSN)) of rat and mouse. To identify the injured populations, we applied the retrograde tracers fluororuby or fluorogold to the nerve stump at the time of injury. Transection of the buccal branch of the facial motor nerve lead to a dramatic decrease in NeuN immunoreactivity within 3 days after injury, a response maintained for several days. Retrograde labeling revealed that the decline was not a result of cell loss or atrophy, because the injured neurons appeared robust and healthy. A similar phenomenon occurred following sciatic nerve transection. However, the loss of NeuN immunoreactivity was delayed compared to FMN, taking 2 weeks to manifest. Unlike peripheral nerve injury, lateral hemisection of the cervical spinal cord did not result in a loss of NeuN immunoreactivity in injured RSN, even at 2 weeks after injury. This may reflect differential regulation

of NeuN to injury in peripheral and central neuronal populations. The loss of NeuN immunoreactivity following peripheral nerve injury may have resulted from loss of target-derived signals. We are presently examining whether NeuN immunoreactivity is under control of trophic pathways. Glial-derived neurotrophic factor (GDNF) and/or brain-derived neurotrophic factor (BDNF) will be placed on the injured nerve stump of the facial nerve to assess its capability to attenuate the loss of NeuN immunoreactivity. The results of this study call for caution in interpreting the response of injured neuronal populations when NeuN immunoreactivity is used to identify them. This research was funded by a grant from the British Columbia Neurotrauma Fund (BCNTF). LTM was funded by a studentship provided by the BCNTF.

P5 Axonal regeneration through nerve coaptations is enhanced by degradation of chondroitin sulfate proteoglycan

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Our past work indicates that growth-inhibiting chondroitin sulfate proteoglycan (CSPG) is abundant in the nerve sheaths and interstitium. We tested to determine if degradation of CSPG by chondroitinase enhances axonal regeneration across the site of peripheral nerve injury and repair using three nerve injury models: (1) nerve crush (axonotmesis), (2) transection (neurotmesis) with primary repair, and (3) interpositional grafting using freeze-treated (acellular) nerve segments. Rats in each condition received the same injury and/or repair bilaterally, and then chondroitinase ABC was injected into the distal nerve on one side while the other side received vehicle only. Acellular nerve grafts were treated with chondroitinase or vehicle for 16 h before implantation. Four days after injury and repair, the nerves were removed and examined. Chondroitinase-dependent neoepitope immunolabeling showed that CSPG was thoroughly degraded throughout the chondroitinase-treated nerve segments. Axonal regeneration into the distal nerves and grafts was assessed by growth-associated protein 43 (GAP-43) immunolabeling. Axonal regeneration

after crush injury was unaffected by the chondroitinase treatment. In contrast, after neurotmesis and repair, axonal ingress into the chondroitinase-treated nerves and grafts was markedly accelerated and the total number of axons was increased several-fold compared to controls. On the basis of these results, we conclude that chondroitinase treatment, by removing inhibitory CSPG, creates a more permissive substratum at the site of nerve coaptation, provides more latitude for the growth of axonal sprouts, and improves the capability of the sprouts to access basal laminae in the graft or distal nerve. This suggests the potential of chondroitinase as an adjunctive therapy to improve the outcome of primary nerve repair. In addition, combined with the low immunogenicity of acellular grafts, pre-treating cryostored donor grafts with chondroitinase may be a significant advancement for nerve allografting. This study was funded by the National Institutes of Health.

P6 Differential migration of peripheral nerve fibroblasts and Schwann cells in an oriented three-dimensional collagen matrix

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We are developing a multicomponent artificial nerve graft (ANG) as an alternative to an autograft for traumatic loss of peripheral nerve. An ANG is typically composed of an outer tubular conduit, an internal matrix of oriented collagen, and cultured autologous Schwann cells (SCs). The conduit limits penetration of inflammatory cells into the region of axonal regrowth and facilitates microsurgical reanastomosis. The rapid rate of migration of fibroblasts compared to SCs on 2-D (dimensional) substrates is used to concentrate SCs for experimental ANGs. We hypothesize that the same process of differential migration of an initially mixed cell inoculum within a 3-D scaffold will result in depletion of fibroblasts in the interior, restoring the initial anatomy of perineurial fibroblasts surrounding an enriched deep SC population. Short segments of rat sciatic nerve were cultured intact overnight to stimulate SC proliferation, then trypsin and collagenase

were digested to yield mixed fibroblasts and SCs. Cells were combined with bovine Type I collagen formed into cylindrical bundles of 100- μ m-diameter strands; this microgeometry encourages axonal elongation when implanted into a nerve gap and provides high surface area for radial cell migration. Hematoxylin and eosin (H&E) histology after 6 weeks culture in Dulbecco's Minimum Essential Medium (DMEM) plus 10% fetal bovine serum and pituitary extract showed mainly fibroblasts populating a 2- to 3-cell-deep capsule surrounding a core containing both SCs and fibroblasts. Cell-mediated contraction of the matrix into roughly spherical shape collapsed the original collagen microstructure. Repeat experiments are underway with the use of cells from human sural and radial nerve cultured under tension to prevent contraction. Specimens will be examined by confocal microscopy after staining with Texas Red-conjugated antibody to growth-associated protein (GAP) 43 for SCs and FITC-anti-THY 1.1 for fibroblasts. Self-ensheathment of the ANG is expected to eliminate several assembly steps and reduce overall diameter for better fit into constrained sites in hand and craniofacial surgery. This work was supported by the VA Rehabilitation Research and Development Merit Review Project B588-3RA and Palo Alto Rehabilitation Research and Development Center.

P7 Locomotor differences between strains of rats—Implications for models of central nervous system disease and regeneration

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The goal of this investigation was to describe and differentiate the locomotor and behavioral abilities of a number of rat strains. Five adult female rats of each of five different strains were assessed with the use of endpoint, kinematic, and kinetic behavioral measurements. The rat breeds used comprised three outbred (Long-Evans, Sprague-Dawley, and Wistar) and two inbred (Fischer and Lewis) strains of rats. Briefly, a paw preference task was used to assess each animal's preference for using either the right, left, or both forelimbs for supporting themselves during rearing against a Plexiglas wall. The animals' locomotor abilities were assessed while rats

locomoted within a runway for a food reward. Either a horizontal ladder or multiple force platforms (for ground reaction force determination) were placed equidistant between the ends of the runway. Kinematic differences in hind-limb posture were determined from a videotape recorded during a runway locomotion. Our results indicate that individual animals demonstrate a particular limb preference for body support during rearing. All strains performed similarly while crossing the horizontal ladder. Ground reaction forces demonstrated that Fischer, Sprague-Dawley, and Wistar rats bore more weight on their hind limbs than on their forelimbs, whereas Long-Evans and Lewis rats bore approximately 50% of their weight on their forelimbs and 50% on their hind limbs. Forelimbs were used more for braking than propulsion in all strains. Hind limbs were used more for propulsion than braking in all strains of rats except Fischer rats. This strain did not use their hind limbs for braking whatsoever but seemed to generate higher braking forces with their forelimbs. Kinematic analysis indicated that hind limbs of Fischer rats were much more abducted compared to other strains. These findings are important to consider when comparing behavioral results within and between laboratories when one uses different strains of rats. This investigation was supported by the Western College of Veterinary Medicine (WCVN) Interprovincial and Saskatchewan Neuro-Trauma Funds.

P8 Grafts and aFGF restore partial hindlimb functions in adult paraplegic rats

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The purpose of this study was to evaluate the degree of functional recovery in adult rats with complete transected spinal cord following an experimental treatment regimen that included implantation of peripheral nerve segments and local application of acidic fibroblast growth factor (aFGF). Rats were randomly divided into five groups: (1) spinal cord transection; (2) spinal cord transection and aFGF treatment; (3) spinal cord transection and peripheral nerve grafts; (4) spinal cord transection, aFGF

treatment, and peripheral nerve grafts; and (5) sham control (laminectomy only). The BBB open-field locomotor test analyzed the locomotor behavior of all rats over the 6 months survival time. Immunohistochemistry for neurofilament, somatosensory-evoked potential (SSEP) and motor-evoked potential (MEP) were used to evaluate axon regeneration following the different treatments. The results show four principal findings: (1) Only the combination of peripheral nerve grafts and aFGF treatment improved hindlimb locomotor function after spinal cord transection. (2) The SSEP and MEP demonstrated electrophysiological evidences of both sensory and motor information crossing the damaged site only in the combined nerve grafts and aFGF treatment rats. (3) Immunostaining demonstrated neurofilament-stained axons grow through the graft area and into the distal end of the spinal cord only in the combined nerve grafts and aFGF treatment rats. (4) Retransection of recovered rats demonstrated that the improvement of hindlimb locomotor activity came from supraspinal control. These results demonstrate the capability of the repair strategy combining nerve grafts and the aFGF treatment to facilitate the regeneration of the spinal ascending and descending tracts following spinal cord injury. This work was supported by Veteran Assistance League foundation.

P9 Changing neural activity influences the restoration of locomotory function after complete spinal cord transection

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Immediately upon transection of the cord of the eel, *Anguilla anguilla*, the denervated body moves spontaneously in a manner similar to normal cruising locomotion, "spinal swimming." To determine whether such enhanced cord activity contributes to the rapid recovery of function seen in these animals, we analyzed the swimming performance of each fish before and on various occasions after a complete cord transection was made caudally. The fish ($n = 40$) were treated as follows: Group I—retained individually after surgery; II—retained after surgery in flowing water so as to provoke continuous swimming; III—injected daily after surgery with L-Dopa (3,4-dihydroxyphenylalanine, 50 mg/kg) intraperi-

toneally; IV—implanted during surgery with thin slices of Elvax, a slow-release polymer impregnated with D-2-amino-5-phosphonovaleric acid (APV), placed dorsal to the denervated cord, just caudal to the lesion; and V—given Elvax implants impregnated with vehicle alone. APV reduces, while L-Dopa elevates rhythmic motor activity. The tail beat frequency of all fish was markedly elevated by cord transection; the patterns of recovery displayed by Groups I, II, III, and V were indistinguishable (ANOVA, $p > 0.05$), and presurgery values were restored by 35 to 45 days after surgery. However, Group IV fish were different ($p < 0.05$), restoring normal values only after 60 days. Subsequent histological analysis of the regenerated tissue indicated that the regrowth of transected axons of Group IV fish was retarded. We conclude, therefore, that activity manipulation localized to the denervated target zone modifies functional recovery, whereas overall activity enhancement exerts no effect. This work was funded by a grant from Enterprise Ireland (SC/1998/121).

P10 Enriched environments improve goal-directed reaching following neonatal cervical spinal cord injury in rats

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These studies were designed to determine if exposure to an "enriched" environment following neonatal cervical spinal cord injury leads to (1) the development of *skilled* forelimb movements and associated postural adjustments and (2) remodeling of central nervous system (CNS) pathways that may underlie the development of such activity. Following an overhemisection to spinal cord segment C-3, 3-day-old rats were placed either in an environment that provided proprioceptive and tactile input to elicit forelimb movement and postural reactions (ES, $n = 25$) or a nonenriched home cage (NS, $n = 20$). From birth through maturity, reflex development, skilled forelimb use, and postural reactions were assessed in each rat (ES and NS). Rats exposed to the enriched environment not only accomplished *skilled* activities such as reaching but also more efficiently maneuvered within various complex environments. Rats not exposed to

enriched environments used compensatory movements when attempting identical tasks. This suggests that exposure to enriched environments improves upon the development of rats' skilled movements and postural reactions. Improvements in proximal control and skilled movement may correlate with reorganization of segmental ascending and descending projections within the nervous system. Potential neuronal adaptations within the brain and spinal cord are currently being investigated to suggest mechanisms that may underlie some of the skilled motor activities that ES rats achieved. This work was funded by the Christopher Reeve Paralysis Foundation, DA1-9802-2.

P11 Transplantation of GFP-positive mouse olfactory mucosa-derived ensheathing glia into rat spinal cord

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We have transplanted olfactory ensheathing glia (OEG), prepared from the epithelium of transgenic mice, which express green fluorescent protein (GFP), into the sites of spinal cord lesions. Our aim is to assess the efficacy of OEG in promoting regeneration when OEG are transplanted into lesioned corticospinal and rubrospinal tracts of both rats and mice immediately following spinal cord injury. The GFP labeling of the cells enables us to follow their migration patterns *in vivo*; to determine their survival kinetics, posttransplantation; and to later analyze posttransplantation changes in gene expression (by laser capture microdissection). We have determined the optimal culturing and passage conditions for purified OEG prepared from both neonatal and adult mice of this colony, and we have achieved consistent viability of 90% to 95% of these cells after cryopreservation. These GFP-positive OEG (cryopreserved and noncryopreserved) have been injected into unlesioned and lesioned spinal cords of immunosuppressed rats, and we have assessed survival rate and migration at 1 day, 7 days, and 28 days following transplantation. We have also assessed the effects of transplanting varied numbers of GFP-positive OEG (from approximately 25,000

to 75,000 cells per animal). Although some inflammatory cell infiltration is present at the injection site, we have determined it is possible to use subtractive digital microscopy to visualize the survival and migration of the OEG into gray and white matter tracts. Animals that receive OEG appear to have significantly reduced cavitation at the lesion site. In addition, processes expressing low affinity nerve growth factor receptor (i.e., p75-positive processes) from cells filling the cavity can be visualized accompanying neurofilament-positive axons coursing through the lesion site.

P12 Cellular plasticity in the CNS of adult dysmyelinated rats

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The Long Evans Shaker (LES) and Bouncer Long Evans (LE-bo) rats lack myelin in the central nervous system (CNS) but have life spans similar to that of a normal laboratory rat. The LES has a dysfunctional MBP gene with a large retrotransposon insertion in the noncoding DNA (deoxyribonucleic acid) sequence flanking the exon 3. The LE-bo phenotype may be related to an identical mutation in the myelin basic protein (MBP) gene, but the LES and LE-bo phenotypes are distinct. In a study of kinetics of glial cell proliferation in the spinal cord, the maximum suppression of mitotic activity is delayed in the LES, at 8 weeks of age, and in the LE-bo, at 16 weeks, and is never complete. Then mitotic activity increases and peaks at 16 to 20 weeks in the LES and at 28 weeks in the LE-bo. Ultrastructurally, severe dysmyelination and progressive accumulation of a membranous material in the perikaryon of oligodendrocytes coincide with futile attempts at myelination. Immature oligodendroglia is prevalent in both adult mutants. Although myelination is arrested in the LES at 40 to 69 weeks, it is unabated in the LE-bo up to 45 weeks of age. Widespread axonal sprouting is evident in the CNS of adult LES and LE-bo. The right thirteenth thoracic dorsal nerve root (T13) of an adult LES rat was cut and resutured to its point of entry in the spinal cord.

After 3 weeks, a crystal of DiI was placed on the end of a 2- to 3-mm stump of the right T13. Morphology of the DiI fluorescence at the level of T13 indicates abundant regeneration of central axons of the right T13 dorsal root ganglia (DRG) into the right dorsal horn. Neural cell cultures from the subependymal region of the lateral ventricle of an adult rat brain were established, and cells surviving in nutrition-depleted conditions formed floating neurospheres exposed to a combination of basic fibroblast growth factor (bFGF) and epidermal growth factor (EGF) mitogens. A 5- μ L suspension of neurospheres was injected into the spinal cord at the T13 level of a 16-week-old LES. After 4 weeks, some of the transplanted cells were associated with myelin sheets around adjacent axons. The LES and LE-bo rats are animal models suitable for studies on cellular and molecular mechanisms regulating regenerative processes in the adult CNS, including axonal regeneration and remyelination. This work was supported by the Canadian Myelin Research Initiative, the Multiple Sclerosis Society of Canada, the J.P. Bickell Foundation, and the Department of Pathology and Molecular Medicine and Faculty of Health Sciences at McMaster University.

P13 Reducing the T cell response to spinal cord injury decreases posttraumatic degeneration and behavioral deficit

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Injury to the central nervous system (CNS) is followed in all instances by posttraumatic degeneration, which leads to progressive tissue loss and often cystic cavitation. Cellular and humoral immune responses have been implicated as mediators of posttraumatic degeneration, and the expression of specific leukocyte chemoattractants has been shown to precede immune cell influx into the injured CNS. However, regulation of the cascade of proinflammatory molecule expression and immune cell recruitment into the traumatized CNS is poorly understood. Here, we show that the lymphocyte chemoattractant IP-10 is up-regulated after injury to the adult mammalian spinal cord and that antibody neutralization of IP-10 in injured animals reduces the inflammatory cell invasion that nor-

mally occurs after trauma. This treatment resulted in a significant reduction of posttraumatic tissue loss, assessed by morphometric analyses and neuronal cell counts. Furthermore, behavioral deficit following hemisection injury was significantly decreased, and progressively lessened over time, in mice treated with anti-IP-10 antibody as compared to control mice. We conclude that reduction of the robust T cell response to CNS injury significantly benefits tissue preservation and functional outcome following spinal cord injury. This work was funded by the Reeve-Irvine Research Center.

P14 The window of opportunity for treating spinal cord contusion-injury as revealed by In Vivo MRI

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Progressive tissue decay at the damage site is the pathologic hallmark of spinal cord (SC) injury. Understanding the stages of progression of pathology is crucial in developing strategies for repair. The evolution of pathology in the transection injury has been described. Longitudinal studies, ex vivo by histology (Kalderon N, Fuks Z, Proc Natl Acad Sci 1996; 93:11,179) and in vivo by MRI (magnetic resonance imaging), of the transection site in rat SC suggest that an intrinsic reparative repertoire is activated after injury. However, by the end of the third week postinjury (PI), the repair is aborted and chronic inflammation/decay ensue, yielding a widening cavity. These data point to the existence of a window of opportunity for developing strategies for preventing the onset of tissue decay; e.g., radiation therapy at this critical period can facilitate natural repair (Kalderon N and Fuks Z, Proc Natl Acad Sci 1996; 93:11,179). The focus now is on contusion, which is more similar to the human SC injury. Here we obtained by MRI a dynamic in vivo view of the progression of events at the lesion site after contusion. A moderate contusion injury in rat SC was performed with a New York University weight-drop device. T₂-weighted sagittal images of the contusion site were acquired within 6 to 60 days PI. A normal course of wound healing events seems to proceed through the third week PI. At first, the cord at the lesion site is swollen/edematous; during the

second week, the edema subsides, the lesion site is indistinguishable, and the cord is almost normal. Pointed inflammation/decay (high signal) is noticed during the third week PI; this is localized at the cord center flaring up radial-laterally a week later and expanding with time PI, yielding a widening chronic inflammation. These data correlate well with the transection data (Proc Natl Acad Sci 1996; 93:11179), suggesting a window of opportunity for preventing the onset of decay also in contusion SC injury. This work was supported by National Institutes of Health NS39375 and 1R24CA83084.

P15 Anatomical reorganization of cortical projections to red nucleus after chronic cervical spinal cord lesions

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Experimental conditions that alter the intrinsic capacity of mature central nervous system (CNS) neurons for growth and those that modify the environment at the injury site increase axonal regeneration and recovery of function. We have shown, for example, that neural tissue transplants (which provide a favorable terrain for axonal elongation at the injury site) and neurotrophic factors (which alter the neuronal capacity for regrowth after injury) increase regeneration after spinal cord injury. We have also shown that the regrowth of supraspinal axons contributes to the recovery of both skilled forelimb movement and locomotion. We hypothesize that the recovery of function after treatment with transplants and neurotrophic factors following spinal cord injuries in adult rats is not only due to supraspinal axon regeneration but also is due to anatomical reorganization of pathways both above and below the site of injury. For the extent of anatomical plasticity at supraspinal levels to be determined, right cervical over-hemisections (C4) were made in adult Sprague-Dawley rats. The lesion site was reexposed at 2 weeks and the glial scar removed. The following groups were prepared: hemisection (HX) only, HX + transplant (TP), and HX + TP + neurotrophic factors (NTF) (brain-derived neurotrophic factor, BDNF, or neurotrophin-3, NT-3). Two weeks after the second surgery, cortical efferents were labeled unilaterally by corti-

cal injections with biotinylated dextran amine (BDA) and rats were perfused one week later. Analysis of variance (ANOVA) revealed that hemisection alone significantly decreased the number of cortical projections to the red nucleus. Application of transplant plus neurotrophins significantly increased the number of corticorubral efferents. We suggest that anatomical reorganization at supraspinal level contributes to the recovery of function after spinal cord injury. This study was supported by NIH NS27054.

P16 Effects of glial restricted precursor cells on acute spinal cord contusion injury

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Recently, there has been an increased interest in cellular transplantation, particularly the transplantation of stem cells or precursor cells, as a means of treating spinal cord injury (SCI). Glial restricted precursor (GRP) cells are tripotential precursor cells that can differentiate along oligodendrocyte or astrocyte lineages. GRP cells offer a novel approach for studying SCI, as immature astrocytes can support axonal growth and oligodendrocyte precursors can remyelinate axons. This study examines 8 days after a 12.5-mm MASCIS (multicenter animal spinal cord injury study) SCI at T9-10 the effect of immediate GRP cell transplantation and assesses: (1) GRP cell survival and motility, (2) the effect of immunosuppression and GRP cell transplantation on the lesion cavity, and (3) the effect of GRP cells on scarring. Eight days after SCI, numerous Hoechst labeled GRP cells were observed within the injury site interspersed between the macrophages and in the white and gray matter surrounding the injury. In some cases, labeled cells were observed up to 5 mm from the injury. Animals treated with methylprednisolone and cyclosporine A, with and without transplants, had a reduced inflammatory response compared to injury alone. Further, there was a trend for a decreased lesion size and percent cavity filled with macrophages in the conditioned media and transplant groups. The injury site was

surrounded by GFAP+ (glial fibrillary acidic protein positive) astrocytes and was positive for chondroitin sulfate proteoglycan (CSPG). Transplantation of GRP cells did not appear to increase the extent of scarring. Thus, 8 days after transplantation, GRP cells remain viable, fill the lesion cavity, and retain their ability to migrate. This study was supported by National Institutes of Health (NIH), grant NS38079.

P17 Ablation of reactive scar-forming astrocytes after spinal cord injury in transgenic mice

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After spinal cord injury (SCI), astrocytes become reactive such that they markedly alter their gene expression, hypertrophy, and proliferate. The functions of reactive astrocytes after SCI are not well understood. Scar tissue formed by reactive astrocytes is thought to impede axon regeneration after SCI. In this study we investigated specific functions of reactive astrocytes after SCI using transgenic mice that express herpes simplex virus thymidine kinase (HSV-Tk) from the mouse glial fibrillary acidic protein (GFAP) promoter (Cell 1998;93:189; Neuron 1999;23:297). In these mice, dividing reactive astrocytes can be ablated by subcutaneous delivery of the antiviral agent ganciclovir (GCV). In transgenic and nontransgenic mice, a unilateral longitudinal stab injury was placed in the spinal cord adjacent and parallel to the mid-line, with the use of a tungsten wire knife. Nontransgenic mice exhibited dense glial scars that contained many BrdU-positive dividing astrocytes. Transgenic mice that were given GCV exhibited pronounced ablation of reactive, scar-forming astrocytes along the SCI. In these mice, the neural tissue that had been depleted of astrocytes exhibited (1) a markedly increased density of CD45-positive leukocytes, (2) substantial neuronal degeneration, and (3) a pronounced increase in local neurite outgrowth. These findings suggest that reactive astrocytes modulate leukocyte trafficking, protect neurons and restrict nerve fiber growth after SCI. This study was supported by the Christopher Reeve Paralysis

Foundation and National Institutes of Health-NRSA, NS07479.

P18 Grafted lineage-restricted neuronal precursors generate mature neurons in the adult spinal cord

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Multipotential neural stem cell grafts into non-neurogenic adult central nervous system (CNS) have largely resulted in incomplete differentiation or a bias toward glial phenotypes. To test whether grafting neuronal-restricted precursors (NRPs) isolated from the embryonic spinal cord may be a better source for neurons, we transplanted cells derived from an alkaline phosphatase transgenic rat into the intact adult spinal cord and assessed their ability to survive, migrate, and generate mature neurons. NRPs survived for up to 1 month post-transplantation and demonstrated the ability to migrate and extend processes. Immunohistochemical analysis revealed that grafted cells efficiently differentiated into mature neurons, as evidenced by staining for neuronal markers MAP2ab and NeuN and were positive for various appropriate neurotransmitter phenotypes. Moreover, grafted NRPs did not adopt astroglial, confirming the neuronal-restricted property in vivo. We conclude that the adult spinal cord environment can support the survival and differentiation of neuronal precursors but may lack the necessary cues that promote the transition from a multipotential neural stem cell to the neuronal precursor state. This work was supported by National Institutes of Health, NS24707 and NS37515, and Eastern Paralyzed Veterans Association (EPVA).

P19 Lineage restriction of neural stem cells grafted into injured spinal cord

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Pluripotent neural stem cells grafted into the uninjured spinal cord differentiate only along glial lineages, despite their ability to form neurons, astrocytes, and oligodendrocytes *in vitro*. When the cells are grafted into the contused spinal cord, differentiation is retarded and only astrocytes are observed (Cao et al. *Exp Neurol* 2001; 167:48–58). Even more lineage restriction is observed following grafting into the kainic acid-lesioned spinal cord, as most cells remain undifferentiated (Magnuson et al. *Neuroreport* 2001; 12:1015–19). Significant neuronal and oligodendrocytic differentiation of stem cells engrafted into the lesioned spinal cord will require partial lineage restriction before engraftment, modification of the host environment to suppress the local microenvironmental signals that restrict lineage fate, and/or modification of the engrafted cells so that they cannot respond to those signals. When neuronal-restricted precursors (NRPs) were grafted into the uninjured spinal cord, mature neurons were found. However, in the contused spinal cord, NRP differentiation was retarded. Most cells remained undifferentiated and others expressed only immature neuronal markers. Preliminary data examining the potential effectors in the injured spinal cord that may be responsible for restricting neuronal differentiation of engrafted stem cells suggests that the bone morphogenetic proteins (BMPs) may be involved. This work was supported by the National Institutes of Neurological Disorders and Stroke NS38665 and RR15576.

P20 Neural repair in a rat cauda equina/conus medullaris injury model

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Cauda equina and conus medullaris injuries typically lead to a combination of pathologies with intramedullary damage and stretch, tearing, and avulsion of proximal and distal nerve roots. For experimental purposes, we have chosen to study the

effects of ventral root avulsions as a model for such injuries. Within this model, we also study the effects of implantation of avulsed ventral roots into the spinal cord upon neuronal survival and axonal regeneration. In adult rats, lumbosacral preganglionic parasympathetic neurons and motoneurons were prelabeled with fluorogold after an intraperitoneal tracer administration. With the rats under general anesthesia, a laminectomy and unilateral L5-S2 ventral root avulsions were performed. In some of the animals, an acute implantation of the avulsed L6 ventral root into the spinal cord was also performed. At various postoperative intervals, the rats were perfused intravascularly with a 4% paraformaldehyde solution. The lumbosacral portion of the spinal cord was removed and processed for morphological studies. Quantitative light microscopic analyses with the use of a modified physical dissector approach were performed. Following the lumbosacral ventral root avulsion injury, there was a progressive loss of both preganglionic parasympathetic neurons and motoneurons in the lumbosacral spinal cord. At 6 weeks postoperatively, about 70% of both groups of neurons were undetectable. However, following ventral root avulsions with also an implantation of an avulsed ventral root into the spinal cord, the vast majority of cells of both neuronal groups were preserved at 4 months postoperatively. The implanted avulsed ventral roots also demonstrated numerous small and large regenerating axons with varying degrees of myelination. This study was supported by the Valley Foundation, UCLA School of Medicine.

P21 Evidence for neuronal plasticity in adult mammalian retina: Results from studies of experimental retinal detachment and reattachment

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Using immunocytochemical labeling in conjunction with laser-scanning confocal microscopy, we have identified several plastic changes in neurons in the adult feline retina following detachment of neural retina from retinal pigmented epithelium. Rod photoreceptors withdraw their synaptic terminals from the outer plexiform layer (OPL) within 3 days

of a detachment. Following reattachment, some rods extend axons that grow into the inner retina as beaded processes that also label with antibodies to synaptic vesicle proteins. Cone photoreceptors do not withdraw their axons, but their complex synaptic endings undergo significant changes in shape, often losing their deep synaptic invaginations. Horizontal and rod bipolar cells (second-order neurons in the retina) extend neurites that grow beyond the OPL. Some of these appear to reach their synaptic targets (rod terminals), while others continue growing, usually along Müller's glia well into the outer or inner retina or even beyond the external limiting membrane. A subpopulation of ganglion cells (the third-order neurons in the retina) significantly increases its expression of growth-associated protein 43 (GAP-43). GAP-43 is normally found only in the inner plexiform layer of adult retina, but antibodies to this protein label entire ganglion cells after detachment. These cells also show increases in labeling with an antibody to neurofilament proteins and sprout new processes after detachment. Most of these changes are well underway within 3 days and are prominent by 7 days. These plastic changes may affect the return of normal vision when the retina is reattached. The National Eye Institute of the National Institutes of Health supported this research.

P22 Pax6 expression during optic nerve regeneration in zebrafish and lizard

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Pax6 is a master gene for eye development and is expressed during differentiation of retinal neurons; its continued expression at maturity may be linked to establishment and maintenance of synaptic connections. Nothing is known of the role of *Pax6* in the reestablishment of connections, for example, during optic nerve regeneration. We compared *Pax6* expression in the retina after optic nerve crush in zebrafish, in which a retinotopic map and vision was restored, with that in lizard, in which a crude retinotopic map was reestablished but failed to con-

solidate, leaving animals blind via the experimental eye (Stirling RV, SA Dunlop, LD Beazley. *Vis Neurosci* 1999;16:681–93). Unilateral optic nerve crush was performed under anesthesia (fish: MS222 0.2%; lizard: halothane, 4% in air with cooling) and terminal anesthesia (fish: MS222 0.4%; lizard: sodium brietal, 0.18 mg/10 g body wt). Eyes were sectioned to reveal the nasotemporal axis. In normal zebrafish and lizard, *Pax6* was expressed in cells of the retinal ganglion cell and inner nuclear layers. During optic nerve regeneration in zebrafish, expression decreased during axon outgrowth (0.25 mo, $p < 0.05$), increased during reestablishment and refinement of the topographic map (0.5 and 1 mo, $p < 0.05$), and declined to normal levels in the long term (4.5 mo, $p < 0.05$). In lizard, *Pax6* expression also decreased during axon outgrowth (0.5 mo, $p < 0.001$), but in contrast to zebrafish, declined dramatically during the time when a crude topographic map was present (5–6 mo, $p < 0.001$); in the long term, when the map had degraded, levels returned to normal (12 mo, $p > 0.05$). The elevated *Pax6* expression during successful topographic map formation in zebrafish and its absence during the time when a crude retinotopic map existed in lizard suggests a pivotal role for this master gene in the establishment and maintenance of appropriate synaptic connections. This study was funded by the National Health and Medical Research Council (NH&MRC) Australia and Neurotrauma Research Program, WA.

P23 Activity dependent mechanisms and failure to refine a topographic retinotectal map in lizard

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During optic nerve regeneration in the lizard *Ctenophorus ornatus*, axons reach the tectum forming a crude retinotopic map. However, the map fails to consolidate, all order is lost, and animals remain blind via the experimental eye. Mechanisms leading to a failure of map consolidation are unknown but may be related to inappropriate activity dependent signaling. We have used immunohistochemistry to examine cellular localization of the NMDA- and

AMPA-subtype glutamate receptors; in addition, we examined nNOS expression, an enzyme that synthesizes nitric oxide, a downstream intercellular signaling molecule involved in synapse stabilization and map consolidation. Unilateral optic nerve crush was performed under halothane anesthesia with cooling to minimize bleeding; terminal anesthesia was with 1% sodium brieval (0.2 ml, ip). In the optic tecta of normal animals, NMDA expression was weak and confined mainly to cell bodies, AMPA expression was strong and localized to cell processes, and nNOS was expressed on cell bodies and processes. When a crude topographic map existed, NMDA receptor expression was up regulated, especially within the cell processes, and AMPA receptors were expressed predominantly within cell bodies; nNOS expression increased dramatically in cell bodies and processes. After the map had degraded, NMDA receptor expression continued to increase, whereas AMPA receptor expression decreased to normal levels in cell bodies but declined to below normal in cell processes. nNOS expression was reduced to below normal in both cell bodies and processes. The data suggest that a failure to consolidate a retinotopic map is associated with abnormally high levels of NMDA expression, a deficiency in AMPA expression, as well as loss of downstream NO signaling. Funding was provided by the National Health and Medical Research Council (NH&MRC) Australia and Neurotrauma Research Program, WA.

P24 Reinnervation of the superior colliculus delays down-regulation of Ephrin-A2 in neonatal rat

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Although the adult mammalian optic nerve does not regenerate following lesion, in the neonatal rat, retinal ganglion cell (RGC) axons retain the capacity to grow across lesion sites in the brain. Following a brachial lesion at postnatal day (P) 2, some RGC axons together with corticotectal ones crossed the lesion to reinnervate the superior colliculus (SC) (Tan MML, Harvey AR. *J Comp Neurol* 1998;386:681–699). Here, we have used immuno-

histochemistry to examine the expression of the guidance cue ephrin-A2 following a brachial lesion. Rat pups were anaesthetized for surgery by inhalation with ether; terminal anesthesia was with pentobarbitone (0.1 ml/gm bw). Brachial lesions were performed by exposing the experimental SC and cutting the superior brachium mediolaterally at the rostral border of the SC. Normal animals showed a steady decrease in ephrin-A2 immunoreactivity between P5 and P31, with a low rostral to high caudal gradient being evident only at P5. By contrast, after brachial lesions were performed, values were significantly elevated rostrally at P5 and caudally at P12; moreover, a steep rostro-caudal gradient was present at both ages. By P31, values fell to normal levels. Once unilateral enucleation at P2 was done, levels were not significantly different from normal. Our results show that innervation, not denervation, triggered increased ephrin-A2 expression after brachial lesion. Funding for this study was provided by the National Health and Medical Research Council (NH&MRC) Australia and Neurotrauma Research Program, WA.

P25 Ephrin-A2 and Eph-A5 expression after optic nerve lesion in adult rats

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During development, the ligands ephrin A2-A5 form an ascending gradient across the rostrocaudal axis of the superior colliculus (SC). Correspondingly, an ascending gradient of Eph receptors A3 to A7 is found across the nasotemporal retinal axis. These molecules are thought to be instrumental in establishing topographic maps. We have shown in species that undergo successful optic nerve regeneration that a gradient of ephrin-A2 is up-regulated coincidentally with topographic map re-formation (Rodger J, Bartlett C, Beazley LD, Dunlop SA. *Exp Neurol* 2000;166:196–200), although it is unclear how the up-regulation is triggered. Here, we have used immunohistochemistry and semiquantitative RT-PCR to examine expression patterns of ephrin A2 and Eph A5 in adult rats in which optic axons do not spontaneously reinnervate the SC. Rats were anaesthetized (Fluothane inhalation and Ketamine 35 mg/kg with Ilium Xylazil-20 5 mg/kg, ip) and

the left optic nerve crushed to sever all axons. In normal rats, there is a weak rostrocaudal gradient of ephrin A2 in the SC and a strong nasotemporal gradient of Eph A5 in the retina. At 1 month after optic nerve crush, ephrin A2 immunoreactivity in the contralateral SC was elevated throughout compared to normal ($p < 0.05$) with higher values caudally than rostrally ($p < 0.05$). By 6 months, the rostrocaudal gradient was maintained, although it was not significantly greater than normal. A parallel up-regulation was seen in the ipsilateral SC. Comparable results were obtained with the use of RT-PCR. Within the retina, the number of retinal ganglion cells (RGCs) had decreased 15% to 20% of normal and the Eph A5 gradient was no longer apparent. The results suggest that, in adult rats, denervation is sufficient to induce reexpression of ephrin-A2 in the SC and that RGC death may trigger the loss of the Eph A5 gradient. We suggest that gradients of ephrin A2 would be available to regenerating axons following a peripheral nerve graft, although they are insufficient to restore an appropriate topographic projection (Thanos S, Naskar R, Heiduschka P. *Exp Brain Res* 1997;114:483–91). This study was funded by the National Health and Medical Research Council (NH&MRC) Australia and Neurotrauma Research Program, WA.

P26 Binding characteristics of chondroitin sulfate proteoglycan and laminin, and corresponding neurite outgrowth patterns in a standard generation and regeneration assay

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Neuronal growth cones are capable of sophisticated discrimination of environmental cues (on cell surfaces and in the extracellular matrix) to accomplish navigation during development (generation) and following nervous system injury (regeneration). Choices that growth cones make are commonly examined with the use of tissue culture paradigms in which molecules of interest are purified and substratum-bound. From observations of growth cone behaviors with the use of these paradigms, assertions are made about choices neuronal growth cones may make in vivo. However, in many cases, the binding, interactions, and conformations of these

molecules are not determined. In the present study, we investigated the binding characteristics of two commonly studied outgrowth regulatory molecules: chondroitin sulfate proteoglycans, which are typically inhibitory to neurite outgrowth during development and following nervous system injury, and laminin, which is typically outgrowth promoting for many neuronal types. Using a novel combination of radio labeling and quantitative fluorescence, we determined the precise concentrations of chondroitin sulfate proteoglycans and laminin-1 that were bound separately and together in a variety of choice assays. Using the same culture preparations, we studied nerve outgrowth behaviors and correlated them with the binding characteristics. The data support our working hypothesis that neuronal growth cones respond to the ratio of outgrowth promoting to outgrowth-inhibiting influences in their environment; i.e., they summate local molecular cues. The response of growth cones to these molecular combinations is most likely mediated by integrins and subsequent activation of signal transduction cascades in growth cones. This study was funded by National Institutes of Health (NIH) EY10545 and a grant from Kentucky Spinal Cord and Head Injury Research Trust (KSCHIRT).

P27 Development of an in vitro assay system for assessing growth of axons from cortical explants into spinal cord: Effects of age and neurotrophic factors

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We have developed a sensory-motor cortex/spinal cord organotypic coculture that involves slices of sensorimotor cortex and longitudinal slices of thoracic spinal cord from P3 and P7 rats. Cultures were treated with neurotrophic factors, including nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), or NT3 (50 ng/ml respectively). Two weeks later, tissues were fixed and DiI was placed on the cortex for 2 weeks to label any fibers that grew from the cortex to the spinal cord. The distance of growth past the cortex/spinal cord boundary was assessed quantitatively. There was minimal if any detectable growth of axons from cortex into spinal cord in cocultures from either P3

or P7 animals. When cultures from P3 rats were treated with neurotrophic factors, however, axon growth was clearly detectable. At similar concentrations, NGF, BDNF, or NT3 elicited almost similar greater axon growth. In cultures prepared from P7 rats, there was minimal if any axon growth, even when cultures were treated with neurotrophins. To assess whether growth failure in P7 cultures was due to a loss of growth potential by the neurons or a change in the tissue substrate, we prepared mixed-age chimeric cocultures. P7 cortex/P3 spinal cord chimera cocultures exhibited moderate axon growth when treated with neurotrophic factors, although the degree of growth was less than in P3 cultures. P3 cortex/P7 spinal cord cocultures exhibited minimal axon growth. These results document a useful model system for assessing factors that regulate corticospinal axon growth.

P28 An in situ model of axon regeneration in developing chicken brain stem-spinal projection

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As they mature, axonal tracts in the developing central nervous system (CNS) of mammals and birds lose the capability to regenerate severed axons. In the brain stem-spinal projection of the chicken, this transition to failed regeneration occurs around embryonic day 13 (E13). The transition to failed regeneration may result both from the emergence of inhibitory molecules in the environment of the spinal cord, as well as from a decline in the intrinsic capability of some brain stem neurons to elongate axons. To examine the contributions of environmental inhibition versus intrinsic neuronal factors to the developmental failure of axon regeneration, we have developed an in situ model of the chicken brain stem-spinal projection, in which the age of the brain stem and spinal cord can be varied independently. Young (E9) brain stem slices were cultured adjacent to either young (E9) or older (E17) spinal cord slices, and DiI crystals were placed in the brain stem to anterogradely label axons growing into spinal cord. Cultures were fixed and observed for growth of fluorescent axons into the spinal cord after 1, 2, 3, or 4 days. As expected, young brain stem neurons extended axons robustly into young

spinal cord, with an average of 400 labeled axons entering 200 μm into the spinal cord and 180 penetrating more than 1.5 mm. In older cord, axon regeneration was reduced but not abolished: the average number of labeled axons entering 200 μm into the cord was about 300, with 80 penetrating more than 1.5 mm. A comparison of axonal growth across 4 days suggests that axons grow more slowly in older cord. This data suggest that brain stem neurons from an age of permissive regeneration retain a moderate capacity for regeneration even when confronted with a spinal cord environment 4 days older than the time of transition to failed regeneration. This work was funded by the National Institutes of Health, grant HD19950, and the Minnesota Medical Foundation.

P29 Calpain inhibition following spinal cord injury

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Calcium-activated neutral proteases (calpain) substrates include a wide variety of cytoskeletal proteins, signaling proteins, and life and death proteins. Following spinal cord injury (SCI), maximal calpain activation occurs within 1 to 2 h and remains elevated for up to 24 h. Prolonged calpain activation contributes to the cell loss that occurs following the acute SCI. Several synthetic calpain inhibitors have previously been administered following SCI. However, none of the studies examined the capability of the drugs to inhibit calpain. Our results demonstrate that IV infusion of E64d, calpeptin, and MDL 28170, at concentrations used in previous studies, is not sufficient to inhibit calpain activity in the spinal cord. Direct microinjection of 100 to 500 nmol of MDL 28170 reduced calpain activity to approximately 30% of control levels and markedly attenuated spectrin breakdown following SCI in the rat. However, MDL 28170 and other synthetic calpain inhibitors are inadequate because of limited cell permeability, inhibition of other proteases, and short half lives. On the other hand, calpastatin, the endogenous calpain inhibitor, is highly specific but is not membrane permeable.

Overexpression of calpastatin in CHO (Chinese hamster ovary) cells attenuated calpain activity. By producing cell permeable fusion proteins of calpastatin with the protein transduction domain of tat, we propose to determine if calpain inhibition attenuates the secondary damage that occurs following SCI. The work was funded by the Kentucky Spinal Cord and Head Injury Research Trust (KSCHIRT).

P30 Traumatic injury activates the ERK/MAPK signaling pathway in cultured astrocytes: Role of extracellular ATP

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Gliosis is characterized by the hypertrophic and hyperplastic response of astrocytes to brain injury. Cellular proliferation and differentiation are mediated by a signal transduction cascade involving the mitogen-activated protein kinase (MAPK), termed extracellular signal regulated protein kinase (ERK). The goal of this study was to determine whether traumatic injury of astrocytes activates ERK and, if so, to identify the signal(s) that cause ERK activation. To address these questions, we used a well-characterized in vitro model of brain trauma (J Neurotrauma 1995;12:325–339). Primary cultures of rat cortical astrocytes grown on deformable membranes were subjected to rapid and reversible (60 ms) stretch-induced injury; 10 min after injury, cells were lysed and ERK activation was measured by protein kinase assays with an ERK-selective substrate and by probing immunoblots with an antibody that recognizes dually phosphorylated ERK1/2. We found that ERK activity was increased by mild, moderate, and severe stretch-induced injury. Maximum ERK activation was observed at 50 to 60 ms of injury and 7.5-mm deformation. Postinjury time course studies revealed significant ERK activation 1 min after injury; maximal ERK activity was sustained from 10 to 30 min and then declined gradually thereafter. ATP is released upon

tissue injury, and activation of astrocytic P2 purinergic receptors by ATP leads to ERK stimulation (J Neurosci 1999;19:4211–4220). Because ATP is released from astrocytes upon stretch-induced injury (J Neurochem 2000;74:1951–1960), we decided to investigate whether the ATP released after injury could activate ERK. To test this hypothesis, we added apyrase, an enzyme that hydrolyzes ATP to AMP, to cultures prior to injury. Under these conditions of ATP breakdown, injury-induced ERK activation was reduced by about 50% ($p < 0.05$). These studies demonstrate that ERK is activated after traumatic injury and suggest that ATP released by injury is one of the signals that triggers ERK activation. Thus, these results suggest a role for extracellular ATP, purinergic receptors and ERK/MAPK in the development of gliosis after brain trauma. This work was supported by the Department of Veterans Affairs.

P31 Activation of the integrin subunits $\alpha7$ and $\beta1$ and Rho GTPase are required for laminin-1-mediated axonal guidance

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Activation of integrin receptors regulates the activity of kinases and small GTPases and induces actin filament reorganization. Laminin-1, an ECM glycoprotein that contains multiple integrin binding sites, is thought to play a role in guiding axons during development and following injury. We have used enolase-digested fragments of laminin-1 to isolate the integrin receptors mediating axonal guidance by laminin-1, and the intracellular mechanisms of action by which neuronal guidance is effected. Boundaries between polylysine and laminin-1 and the E1', E4, or E8 fragments were created on glass coverslips. The behavior of cerebellar granule neurons at the boundaries was categorized as turned or crossed. Approximately 20% of axons cross from the E8 fragment of laminin-1 to PLL, while 10% cross from PLL to the E1' fragment. Thus, laminin-1 possesses both positive (E8) and negative (E1') guidance cues. The percentage of neurites that crossed from laminin-1 and E8 to PLL and from

PLL to E1' increased significantly when $\beta 1$ integrin was blocked. Similarly, blocking the $\alpha 7$ -integrin subunit increased the percentage of axons that crossed from laminin-1 and E8 to PLL. Thus, the $\alpha 7\beta 1$ integrin mediates the positive neuronal guidance actions of laminin-1 and the E8 fragment of laminin-1. The rho family of GTP-binding proteins signal downstream of integrin receptors to regulate cytoskeletal reorganization. Inactivation of rho with C3 exoenzyme significantly increased the percentage of neurites that crossed from laminin-1 and E8 to PLL and from PLL to E1'. In addition, using Sindbis viral-mediated expression of both dominant-negative and constitutively active rhoA caused a significant increase in the percentage of neurites crossing from laminin-1 to PLL. Taken together, these studies demonstrate that neuronal guidance by laminin-1 involves a signal transduction pathway that includes the $\alpha 7\beta 1$ integrin and Rho GTPase. This work was supported by the Christopher Reeve Paralysis Foundation and the New Jersey Commission on Spinal Cord Research.

P32 Fractones, fractone-like structures, and vimentin meningo-gliial network in adult brain and spinal cord: A role in neurogenesis/gliogenesis and repair?

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It is widely recognized that connective tissue cells and the basal laminae (BL) separating these cells from neural cells (neurons and glia) are important in determining the fate (proliferation, differentiation, and migration) of new neural cells during development. But the function of connective tissue cells and BL in the adult is unknown. We hypothesize that meningeal cells, in the meninges and sheaths of blood vessels, influence or even drive cytotogenesis and morphological plasticity of the neural tissue throughout adult life via BL. Here, we have traced BL throughout the brain and spinal cord with the use of confocal microscopy with laminin as a marker and by transmission electron microscopy. In addition to subpial and perivascular BL, we have characterized new forms of BL in the subependymal layer of ventricles and central canal and in richly vascularized zones of brain and spinal cord. The subependymal BL, named "fractones" because

of their fractal structure, are extensions of perivascular BL through the neural tissue. Most fractones start within the subependymal layer at the tip of a perivascular fibroblast/macrophage network and terminate immediately underneath the ependyma. Another form of BL, visualized as thin processes through the neural tissue between capillaries, ensheathes fibroblasts and macrophages. These cells connect and anastomose a preexisting network of perivascular fibroblasts and macrophages. Adjacent to or included within the different forms of BL are vimentin immunoreactive cells: fibroblasts, macrophages, ependymocytes, tanycytes, and a subpopulation of astrocytes. These cells form a network investing the brain and spinal cord from their surface to the ependymal walls. Because adult neurogenesis/gliogenesis occurs primarily in the subependymal, perivascular, or subpial locations, we suggest that the network of vimentin immunoreactive cells and their adjacent BL form the anatomical basis for a regulatory system releasing and targeting cytokines and growth factors toward the neural tissue, leading ultimately to events of plasticity. The work was supported by the National Institutes of Health, grant NS 09140.

P33 Stimulation of regenerative cell body responses in chronically axotomized motoneurons by a second axon injury

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Motoneurons exhibit pronounced cell body responses to acute axonal injury, which are characterized by hypertrophy of their somata and the up-regulation of regeneration-associated genes (RAGs). The molecular triggers of these responses are unknown, and both "negative" as well as "positive" signals have been suggested, for example, lack of target-derived factors because of axonal interruption (negative signals) and/or factors released at the site of injury (positive signals). To test the hypothesis that endogenous factors released by nerve lesion contribute to regenerative cell body responses, we used a mouse model of chronic motoneuron injury. In the chronic axotomy model (10 weeks after

lesion), mouse facial motoneurons are disconnected from target (muscle) derived factors and exhibit a massive atrophy, resulting in a loss of countable cell bodies. In addition, the motoneurons fail to maintain their initial increase in expression of RAGs, such as growth-associated protein (GAP) 43 and α -tubulin. Reaxotomizing 10-week chronically axotomized facial motoneurons by resecting the neuroma dramatically reversed the atrophy of the cell bodies, increasing the percentage of identifiable cell bodies from 36% of contralateral to 79% of contralateral (C57 black) and from 28% of contralateral to 40% of contralateral (Balb-C) mice. Moreover, the re-injured motoneurons displayed a striking reinduction of RAGs. These results suggest that lesion-induced molecules play a major role as "positive" injury signals that stimulate regenerative cell body responses in axotomized motoneurons. Furthermore, previous studies in which this model was used overestimated the number of dying motoneurons. The reasons for the strain differences, however, remain enigmatic. This work was supported by the British Columbia Neurotrauma Initiative, Natural Science and Engineering Research Council (NSERC) and Medical Research Council (MRC).

P34 Transplantation of brain cells assembled around a programmable synthetic micro-environment

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Cell therapy is a promising method of treatment of hematopoietic disorders, neurodegenerative diseases, diabetes, and tissue loss caused by trauma. Some of the major barriers to cell therapy have been partially addressed, including identification of cell populations, in vitro cell proliferation, and strategies for immunosuppression. An unsolved problem is recapitulation of the unique combinations of matrix, growth factor, and cell adhesion cues that distinguish each stem cell microenvironment, and which are critically important for control of progenitor cell differentiation and histogenesis. Hence, we demonstrate a new approach in which cells, synthetic matrix elements, and controlled release technology are assembled and programmed, prior to transplantation, to mimic the chemical and physical microenvironment of developing tissue. We demon-

strate this approach in animals using a transplantation system, in which fetal brain cell survival and differentiation is controlled by preassembly of neotissues containing cells and nerve growth factor (NGF)-releasing synthetic particles. Funding is provided by. Research for this study was completed at Cornell University School of Chemical Engineering. Funding was provided by NASA Graduate Student Research Fellowship.

P35 A transparent multichannel conduit that allows for linear or gradient cellular seeding and real-time evaluation prior to nerve grafting

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Studies on nerve conduits for peripheral nerve regeneration have concentrated on the manipulation of various materials to avoid sacrificing native nerve needed for autograft repair. Optimally, tubularization repair designs should approximate closely the cytoarchitecture of the native peripheral nerve, as well as to provide proper cellular and molecular cues to entice and direct axonal regeneration. However, current combinatorial strategies with the use of cellular and substrate-matrix components lack the capability to specifically seed cells in a linear arrangement, as well as to monitor for cell survival and behavior inside these conduits prior to grafting. Here, we report the fabrication of an agarose-based multichannel conduit that allows for the controlled culture and evaluation of cellular elements seeded into longitudinally arranged channels. Conduits with 10 to 20 channels (60–170 μm in diameter, 10 mm in length) were made with the use of plastic fibers for casting into agarose-filled tubes. Different cell types, such as 3T3-fibroblasts, PC-12 cells, as well as freshly isolated myocytes and Schwann cells, attached and grew successfully inside the multichannel conduits. The transparent nature of the conduit permitted real-time evaluation of the cells. Cell attachment and elongation was observed as early as 2 h after seeding, and cells were able to survive equally well as 2-D (dimensional) sister cultures. Within hours, Schwann cells were observed to form a cable-like structure, and PC-12

cells to send neurites along the channels. In addition, it is known that directed axonal growth might benefit from gradient expression of growth-promoting molecules. To this end, cell density inside the channels was controlled by differential loading of individual channels to create a cellular gradient. Furthermore, gene transfer tools are currently being tested for their capability to create molecular gradients inside these conduits. The cellular and molecular potential of this conduit should allow for the creation of an improved nerve repair strategy. This study was internally funded by the Texas Scottish Rite Hospital for Children (TRHC), No. 05-00-479.

P36 Voluntary exercise induces a BDNF-mediated mechanism that promotes neuroplasticity

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The capacity of exercise to help functional recovery after a central nervous system (CNS) injury is becoming well established. We have investigated potential mechanisms by which exercise can promote changes in neuronal plasticity and function via regulation of neurotrophins. Rodents were exposed to voluntary wheel running for 3 or 7 days, and their lumbar spinal cord region and innervated soleus muscle were assessed for changes in brain-derived neurotrophic factor (BDNF), its signal transduction receptor, and downstream effectors for the action of BDNF on synaptic function. In another series of experiments, the soleus muscle was paralyzed via intramuscular botulinum toxin A injection to study the effects of reducing the mechanical output on the neurotrophin response to locomotion. Exercise induced the expression of BDNF and its receptor in the lumbar spinal cord region and soleus muscle. Levels of synapsin I, the growth-associated protein (GAP-43), and cyclic adenosine monophosphate (cAMP) response element-binding (CREB) were elevated in the spinal cord. In particular, the mRNA (messenger ribonucleic acid) and protein (total and phosphorylated) for synapsin I, a synaptic mediator for the action of BDNF on neurotransmitter release

and synaptic formation, increased in proportion to TrkB mRNA levels. CREB mRNA levels increased in proportion to BDNF mRNA levels in animals that exercised. CREB is required for some forms of memory, and BDNF is an essential element in its regulation. The mRNA for GAP-43, important for neurite outgrowth, neurotransmitter release, and learning and memory, increased in proportion to synapsin I levels. BDNF and synapsin I mRNAs were reduced below sedentary control levels in the spinal cord and soleus muscle of rats following paralysis of only the soleus muscle in one leg, suggesting that active muscle contraction is essential for maintaining normal levels of BDNF mRNA in muscle and the spinal cord. This work was supported by the National Institutes of Health (NIH) awards NS 389978 and NS 39522 and the Brain Injury Research Center (BIRC) award.

P37 Grafting of BDNF and NT-3 producing fibroblasts into the chronically injured cervical spinal cord shows limited recovery of function

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Ex vivo gene therapy is a useful tool for delivering therapeutic molecules to the injured spinal cord (SC) through the grafting of engineered cells. Brain-derived neurotrophic factor secreting fibroblasts (Fb/BDNF) rescue axotomized red nucleus (RN) neurons, elicit rubrospinal tract (RST) regeneration, and promote recovery of locomotor function when transplanted into an acute subtotal cervical hemisection (HX) (Liu Y, et al. *J Neurosci* 1999;19(11):4370–4387). NT-3 secreting fibroblasts (Fb/NT-3) elicit corticospinal tract (CST) regeneration and recovery when transplanted into an acute dorsal thoracic SC HX (Grill R, et al. *J Neurosci* 1997;17(14):5560–5572). We want to test whether grafting these cells into a chronic injury site will also have therapeutic effects. We grafted a mixture of Fb/BDNF and Fb/NT-3 into a complete unilateral cervical HX 6 weeks after the initial

injury and compared these rats to recipients that received unmodified fibroblasts or gelfoam. The transplants survived grafting for at least 12 weeks, and the combination therapy provided a permissive environment for axon growth as indicated by immunostaining for CGRP-, neurofilament-, and 5-HT-containing axons within the transplants (TP). Biotin dextran amine (BDA) tracing of RST axons showed a small number regenerated into the combination TP but not into control TP. There was however no growth into the host; thus functional contacts were not made. Combination TP recipients showed recovery from heat-induced hyperalgesia (plantar withdrawal test) not observed in control TP recipients, whereas no significant difference in recovery was observed between the two groups in any of a battery of motor tests (BBB, cylinder test, grid, rope test). We conclude that the requirements for robust long distance regeneration by chronically injured neurons are greater than those by acutely injured axotomized cells and therefore less recovery of locomotor function. Neurotrophic factors delivered by engineered fibroblasts may prevent the development of maladaptive responses to sensory stimuli perhaps as a result of inducing sprouting by dorsal root axons. This study was supported by ISRT, NS 24707, Eastern Paralyzed Veterans Association (EPVA), and Department of Veteran Affairs (VA).

P38 Adeno-associated virus mediated expression of BDNF in red nucleus prevents atrophy of axotomized rubrospinal neurons and stimulates GAP-43 and T α 1-Tubulin mRNA levels

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Rubrospinal neurons (RSN) undergo massive atrophy after axotomy in the cervical spinal cord, and some (40–80) RSN regenerate their axon if offered a peripheral nerve transplant. We have previously shown that brain-derived neurotrophic factor (BDNF) infusion into the vicinity of the red nucleus prevents this RSN atrophy, increases T α 1-tubulin and growth-associated protein (GAP)-43 expression, and stimulates RSN regeneration into a

peripheral nerve graft by threefold. However, the continuous BDNF infusion causes significant inflammation and cavity formation. Therefore, we used a single microinjection (glass capillary) of an adeno-associated virus (AAV) expressing BDNF, driven by the chicken beta actin (CBA) promoter into the red nucleus at the time of a C3/4 hemisection. We observed minor cavity formation at the site of the AAV injection, and the green fluorescent protein (GFP) reporter revealed infection of numerous glial cells as well as some RSN. At 2 weeks post-treatment/injury, the RSN size in the AAV-BDNF treated group (88.74 % \pm 4.96% of contralateral) was significantly ($p < 0.05$) larger compared to the GFP control virus (with the same CBA promoter) treated group (64.85% \pm 4.50% of contralateral). At three weeks, this prevention of atrophy was still significant: BDNF (75.17% \pm 0.84 %) control virus (58.94% \pm 2.72 %), $p < 0.05$. In situ hybridizations for GAP-43 and T α 1-tubulin mRNA (messenger ribonucleic acid) showed increased levels in AAV-BDNF-treated RSN as compared to the control virus group, at both the 2- and 3-week time points. Thus, it is feasible to treat the cell bodies of central nervous system (CNS) neurons with single microinjections of AAV-BDNF in order to prevent their degenerative changes after spinal cord injury. This study was supported by Canadian Institutes of Health Research (CIHR), Canada, and the Neurotrauma Foundation of British Columbia and Natural Sciences and Engineering Research Council of Canada (NSERC), Canada.

P39 Axonal regrowth across a spinal cord injury site is not enhanced by exposure to radiation

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Regrowth of axons across a spinal cord injury site has been reported by others to be enhanced by exposure of the injury site to radiation. One factor to which this enhancement is attributed is the suppression of glial (astrocytic) scar formation. The present study examined effects of radiation on axonal regrowth and the non-neuronal environment with the use of a well-established spinal cord injury/peripheral nerve graft (PNG) paradigm. The rostral

end of the PNG was placed in the right hemisection of the C3 spinal segment. An aspiration lesion cavity was created subsequently in the right dorsal quadrant of the C5 spinal segment (C5DQ). Seven or seventeen days later, the C5DQ was exposed to X rays (25 Gy), and the caudal end of the nerve graft was implanted into the C5DQ site either immediately following the irradiation procedure or 7 days later. Three weeks to one month later, a tracer, biotin dextran amine (BDA), was placed in the brainstem so that the pathway(s) of regrowing axons could be followed. One week later, the animals were sacrificed. Control animals were subjected to the same procedures but were not irradiated. Frozen sections (25 μ m) were processed to visualize the BDA-labeled axons at the injection site, at various levels of the cervical cord, and within the PNG, as well as at the spinal cord/PNG interface. In some cases a few labeled axons appeared to extend across the interface between the PNG and the spinal cord in the irradiated animals, but their numbers and length were very restricted. Examination of sections immunostained to visualize the astrocytic population revealed a well-defined, glial fibrillary acidic protein (GFAP)-positive glial scar in both groups. Thus in this model, exposure to radiation did not enhance regrowth or suppress glial scar formation. This study was supported by Grant 2014 from the Spinal Cord Research Foundation of Paralyzed Veterans of America.

P40 Neurogenesis in the adult hippocampus is attenuated following ablation of GFAP-positive radial astrocytes

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The subgranular zone (SGZ) of hippocampal dentate gyrus contains dividing cells that have neurogenic potential and give rise to new neurons in adult mammals. The cell biology of these neural progenitor or stem cells is not well understood. It has been proposed that neural stem cells in the adult forebrain express glial fibrillary acidic protein (GFAP) (Doetsch et al., 1999). In the development

of neocortex, GFAP-expressing radial glia are stem cells with neurogenic potential (Malatesta et al., 2000; Noctor et al., 2001). The SGZ of adult dentate gyrus contains GFAP-positive cells with the appearance of radial glia that have been reported to divide in adult rodents (Cameron et al., 1993). In this study, we examined the effects on BrdU (5-bromodeoxyuridine) labeling in adult hippocampus of ablating spontaneously dividing radial astrocytes in the SGZ of transgenic mice that express HSV-Tk from the GFAP promoter (Bush et al., 1998; Bush et al., 1999). Numerous dividing radial astrocytes were identified in adult SGZ by immunohistochemical double labeling for BrdU and HSV-Tk. Stereological analysis revealed that adult (3 to 9 months) transgenic mice given the antiviral agent ganciclovir (GCV, Roche) for greater than 35 days or its lipophilic elaidic acid ester prodrug E-GCV (Norsk Hydro) for greater than 15 days exhibited few BrdU labeled cells in the dentate gyrus in comparison with untreated mice or nontransgenic mice given GCV or E-GCV. These findings support the hypothesis that SGZ radial astrocytes are, or give rise to, stem or progenitor cells with neurogenic potential in adult dentate gyrus.

P41 Analysis of protein expression in spinal neuronal cultures subjected to pulsed magnetic field stimulation

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Previous studies in this laboratory have demonstrated that pulsed electromagnetic fields (PMF) can enhance and influence neurite outgrowth along the direction of the induced current in vitro. We wish to determine the mechanisms by which PMF is able to influence neurite outgrowth. We hypothesize that the expression of cellular proteins involved in neurite outgrowth is affected by PMF stimulation. Dissociated dorsal root ganglion (DRG) sensory neuron cultures from 15-day rat embryos were established and placed into an incubator equipped with copper coil energized by a waveform generator driving a power amplifier. Control cultures were placed in an incubator equipped with a sham coil. After an initial 12 hours for sensory neuron

attachment, the coils were energized for 18 hours, followed by a postexposure period of 18 hours. Total incubation time was 48 hours. At termination, PMF-stimulated and unstimulated DRG cultures were harvested into a lysis buffer and total protein was extracted. After quantification, total protein was loaded into 96-well plates and ELISAs were run with the use of commercially available antibodies to α -1a, α -1b, *c-jun*, calbindin, calmodulin, GAP-43, MAP-2, NF-200, NF-68, SMI-31, SMI-32, and tubulin. The overall effect of PMF stimulation appears to be a decrease in expression for the proteins examined, with significant differences observed for NF-200, NF-68, and SMI-31. These proteins, which are involved in neurofilament organization, may be down regulated by PMF stimulation, resulting in the observed changes in neurite outgrowth under these culture conditions. This study was supported by American Paraplegia Society and Zablocki VA Medical Center.

P42 Modulation of extracellular matrix associated with a spinal cord lesion site

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Components of glial scar tissue associated with a spinal cord injury (SCI) have been proposed to be inhibitory to axonal regrowth. Collagen IV is a main component of the glial scar, and preventing the accumulation of collagen IV postinjury can lead to extensive axonal crossing of a brain injury site (Stichel et al. 1999). To determine if modulation of the extracellular matrix (ECM) can provide an environment more conducive to axonal growth, we added collagenase by catheter and osmotic minipump to a cervical level 5 dorsal quadrant lesion in adult rats. Five days later, the animals were sacrificed and immunohistochemical analysis of the injury site was performed. In saline-treated animals, collagen was seen at the border of the lesion site, as well as within blood vessel walls throughout the cord. In collagenase-treated animals, a decrease in collagen within the microvasculature surrounding the lesion site and the absence of a collagen border around the edge of the lesion were found. An increase in chondroitin sulfate proteoglycan (CSPG) occurred after collagenase treatment within the lesioned tissue—possibly because of the disruption of bound CSPG from the collagen matrix. A

decrease in the expression of glial fibrillary acidic protein (GFAP) also was noted at the lesion site in the collagenase-treated animals when compared to saline-treated animals. In situ DQ gelatin zymography showed an increase in MMP activity adjacent to the injury for both collagenase and saline treatments when compared to the contralateral cord. MMP activity following collagenase treatment appeared to be greater than in the saline controls. Monoclonal antibodies to MMP-2 and MMP-9 ameliorated nearly all DQ gelatin activity, indicating that at least these specific MMPs had been activated after SCI. This work was supported by the National Institutes of Health (NIH) grant NS26380.

P43 Identification of differentially expressed genes in denervated neurons during regeneration of transected spinal cord of european eel, *Anguilla anguilla*

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The regenerative capacity of the eel central nervous system offers the possibility of identifying factors that promote the survival and successful regeneration of axotomised neurons and permit functional recovery. Spinal cord samples were removed from the eel at different time points after complete spinal transection and regrowing axons were labeled with the fluorescent marker DiI. Axonal regrowth data were used to select an appropriate position and time point for sampling to obtain target tissue not yet reinnervated. Total RNA (ribonucleic acid) was isolated from spinal cord tissue from injured regenerating eels 15 days posttransection, at least 5-mm caudal to the injury site. Total RNA was also prepared from uninjured control tissue. Both RNA populations were then used to construct subtracted cDNA (complementary deoxyribonucleic acid) libraries in *Escherichia coli* by suppression subtractive hybridization. These libraries are enriched for genes that are up-regulated (forward-subtracted library) and down-regulated (reverse-subtracted library) after injury. A differential screening step was done in duplicate to eliminate false positive clones from each library. Forty-six candidate differentially expressed gene fragments were identified (26 forward and 20 reverse subtracted cDNAs),

which were subsequently sequenced. Databases were searched for homologies to known genes. Eighteen of the forward subtracted cDNAs showed strong homology to the same mitochondrial rRNA (ribosomal RNA) gene, and eight had no significant homology to any database sequences. Two of the reverse subtracted cDNAs were homologous to globin genes, one cDNA was identified as part of a reverse transcriptase gene, and the functions of seventeen have yet to be identified. The differential expression of several of these cDNAs has now been verified by semiquantitative RT-PCR. Selected differentially expressed gene fragments were digoxigenin (DIG)-labeled and used to probe a full-length cDNA library in bacteriophage lambda to isolate the full-length transcripts for further analysis. Funding was supported by Enterprise Ireland, grant SC/99/349 and the Higher Education Authority of Ireland.

P44 Microarray analysis of several regeneration models identifies many overlapping changes in mRNA levels

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Using a custom oligonucleotide microarray, we have identified many genes associated with regenerative activity in rat dorsal root ganglia (DRG) explants. Work in the Filbin lab has previously shown that treatment with neurotrophins and elevation of cyclic adenosine monophosphate (cAMP) levels overcome inhibitors of regeneration in myelin and promote regeneration in vivo. Furthermore, they have shown that this effect is transcription-dependent. Here we used differences in gene expression between regeneration permissive states and nonpermissive states to identify some of the genes associated with increased neurite outgrowth. In particular, P5 DRG explants, which show limited neurite outgrowth, were compared to those treated

with brain-derived neurotrophic factor (BDNF), nerve growth factor (NGF), glial cell line-derived neurotrophic factor (GDNF), and dibutyryl cAMP as well as P0 explants. We have also tested DRG explants of animals with and without a preceding sciatic nerve injury to examine the conditioning effects of such a lesion. Our microarray contains 5,086 spotted 65 to 75 mer oligonucleotides, including a large base set that covers >95% of known rat gene clusters as well as several hundred genes found previously to be associated with nerve growth. The genes found to be common between our experimental comparisons include both previously interrogated transcripts as well as novel mRNAs (messenger ribonucleic acids). Observed mRNA levels are confirmed by real-time RT-PCR. We believe that this approach will yield important new targets for understanding regenerative mechanisms and for providing effective treatments. This work was supported by the Spinal Cord Injury Project at the W. M. Keck Center for Collaborative Neuroscience, the National Institutes of Health (NS37060), and the Research Centers for Minorities Institutes at Hunter College.

P45 Microarray analysis of gene expression following spinal cord injury

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Damage to the spinal cord severs sensory and motor tracts, disconnecting flow of information to and from the periphery. A cascade of physiological and biochemical processes occur in the injured tissue, making the site toxic and nonpermissive for axon growth, but the precise boundaries and molecular mechanism of postinjury events are poorly understood. In the first hours, heat shock proteins and regulatory transcription factors are produced in response to trauma. Subsequently, 3 to 15 days after injury, invading macrophages and other immune cells from the periphery contribute to the toxic environment. Resident microglia and astrocytes become

active and cause scarring at and around the injury site 2 to 4 weeks postinjury, while phagocytosis and degeneration span the 1- to 4-week-period postinjury. Here, we use cDNA array technology to examine the changes in gene expression that occur in the spinal cord 7 days postinjury, allowing us to examine the molecular events associated with the inflammatory response and to discover factors that interfere with neuronal outgrowth at this time point. Clontech Glass Arrays were used to compare spinal cord tissue just rostral to a unilateral C3/4 lateral funiculus lesion with normal controls. We observed a number of predicted as well as entirely unexpected changes in gene expression, including nerve growth factor (NGF) receptor and protein kinase C (PKC) inhibitor expression. This hemisection model axotomizes the rubrospinal tract, and as a result, numerous red nucleus (RN) neurons atrophy and die and the remaining fail to regenerate. In future experiments, we will compare gene expression in the RN of lesioned animals and of animals receiving a transplant following the lesion, and correlate the changes with the ability of the RN neurons to be rescued and regenerate. In further analysis, we will use customized cDNA arrays representing a specific subset of genes to better understand the breadth of the genetic response to spinal cord injury (SCI). This work was supported by National Institutes of Health grants NS24707 and NS37515 and Eastern Paralyzed Veterans Association (EPVA).

P46 Changes in gene expression following the relesion of chronically injured rubrospinal neurons

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In a chronic model of spinal cord injury (SCI), the administration of neurotrophic factors (NTFs) to the original injury site, following the physical clearance of scar tissue, significantly enhanced axonal regeneration by supraspinal neurons into a peripheral nerve graft. One hypothesis is that there is a neurotrophic factor-linked up-regulation of gene expression in chronically injured neurons. The present series of experiments determined how secondary injury of the chronically injured spinal cord affects the expression of regeneration-associated

genes (RAGs) in neurons of the rubrospinal tract (RST). Adult female Sprague-Dawley rats were subjected to a right full hemisection lesion by aspiration of the C3 spinal cord. With in situ hybridization (ISH), RST neurons were examined for temporal changes in GAP-43 and β II-tubulin mRNA expression acutely (0.25, 0.5, 1.0, and 3.0 days) following SCI. This was done chronically (28 days) after injury or at different time intervals (0.25, 0.5, 1.0, 3.0, 5.0, or 7.0 days) following relesion of the chronic injury site. Animals subjected to aspiration of glial scar tissue from the chronic injury site had saline-treated gel foam added to the lesion cavity (to serve as controls for NTF-treated spinal cords for ongoing experiments). ISH was performed on fresh frozen cryostat sections with the use of 33P-labeled cDNA probes specific to GAP-43 or β II-tubulin. Results indicate that mRNA expression of these RAGs at 28 days after injury was not significantly different from levels expressed shortly after an acute C3 SCI. Relesion of the spinal cord induced an increase in GAP-43 mRNA levels as early as 0.5 days, without any significant change in β II-tubulin mRNA expression. GAP-43 mRNA remained elevated throughout the 7 postoperative days examined, but at no post-reinjury interval was an increase in β II-tubulin mRNA detected. Funding was supported by the National Institutes of Health, grant NS26380.

P47 Molecular consequences of enforced expression of homeobox-containing transcriptional repressor Msx1 in myoblasts

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Msx1 belongs to a conserved family of homeodomain-containing proteins that are known to repress transcription. The three homologs of the Msx gene (Msx1, 2, and 3) are abundantly expressed at sites of epithelial mesenchymal cell interactions in the embryo and during tissue regeneration in the adult. Murine myoblast cells stably transfected with expression vectors containing Msx1 cDNA (complementary deoxyribonucleic acid) in either sense (F31-c) or antisense (F3R1) orientation show contrasting phenotype. While F3R1 cells readily differentiate into myotubes in the medium containing low serum, under identical con-

ditions, F31-c cells fail to differentiate. To gain insight into how the exogenous expression of Msx1 alters the phenotype of F31-c cells and to identify potential downstream targets of Msx1, we compared the total repertoire of mRNAs from F31-c and F3R1 by the differential display (DD) method. We have identified and cloned four cDNAs that represent a set of mRNAs (messenger ribonucleic acids) that are up-regulated in F31-c cells. Two of these clones represent mRNAs encoding ribosomal proteins S23 and S24 while the third has homology to sequences in the murine *Tcp-1* gene. A fourth cDNA does not have appreciable homology to cDNA sequences deposited in the National Institutes of Health (NIH) GenBank. Since withdrawal from the cell cycle and enhanced expression of MyoD generally precede differentiation of myoblasts into myotubes, we examined regulation of the major cell cycle proteins and MyoD by western blot analysis. We discovered that the levels of Cdk2, 4, and 6; cyclins A and D; and the Cdk inhibitor p27 in both proliferating and serum-starved F31-c cells were similar to those in F3R1. However, although MyoD protein levels increased in both cell types cultured in differentiation medium, the levels of MyoD in serum-starved F31-c cells were two- to fourfold lower. We postulate that the reduced amount of MyoD is sufficient to permit reversible withdrawal of F31-c cells from the cell cycle but is inadequate to permit myogenesis. The studies were supported by grants from the Department of Veterans Affairs and the Department of Defense.

P48 Functional regeneration of chronically injured sensory afferents into adult spinal cord following neurotrophin gene therapy

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Lesioned axons within the dorsal roots fail to regenerate through the peripheral nerve transition zone and into the spinal cord. This regenerative failure leads to a persistent loss of sensory function. To induce axonal growth across this barrier, we used recombinant adenovirus to express fibroblast growth factor-2 (FGF2), nerve growth factor

(NGF), L1 cell adhesion molecule (L1), or b-galactosidase (LacZ) within the endogenous glia of the dorsal spinal cord 16 days postinjury. Expression of either FGF2 or NGF, but not L1 or LacZ, induced robust axonal regeneration into normal as well as ectopic locations within the dorsal spinal cord. This regeneration led to near normal recovery of thermal sensory function. Functional recovery and the majority of regenerating axons within the dorsal horn disappeared upon recutting of the sensory roots. Injections of adenovirus encoding NGF, but not FGF2, also resulted in extensive sprouting of noninjured sensory axons, which we previously demonstrated could cause hyperalgesia and chronic pain. Thus, neurotrophic factor gene therapy administered as late as 16 days postinjury may be a useful treatment to elicit recovery after dorsal root avulsion; however, the choice of neurotrophin is important to induce selective regeneration of damaged axons. This study was supported by Kentucky Spinal Cord and Head Injury Research Trust and NINDS/NIH NS38126.

P49 Long-term theophylline-induced functional recovery and alterations in adenosine A₁ receptor mRNA expression in C2 hemisectioned adult rats

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Previous studies from our laboratory have demonstrated that in an animal model of spinal cord injury, a latent respiratory motor pathway can be pharmacologically activated to restore respiratory muscle function to a hemidiaphragm paralyzed by a left upper cervical spinal cord hemisection. Recovery of respiratory muscle function can be induced by theophylline by antagonism of central adenosine A₁ receptors. Theophylline-induced recovery can be maintained for up to 30 days. We have recently shown that adenosine A₁ receptor expression is localized in phrenic motoneurons. It is unclear whether adenosine A₁ receptor mRNA expression is altered following long-term theophylline administration in hemisectioned rats. The objective of the present investigation was to simultaneously assess putative effects of long-term theophylline on respi-

ratory recovery and expression of adenosine A₁ receptor mRNA in hemisectioned rats. Adult female rats were subjected to a left C2 hemisection as previously described. The functional completeness of the hemisection was electrophysiologically verified before initiating oral theophylline administration. Administration of theophylline for 3, 12, or 30 days restored and maintained respiratory function in all cases (n = 18). Theophylline-induced recovery did not wane even when drug administration was stopped for 2 (n = 6), 7 (n = 6), or 30 (n = 5) days. Assessment of adenosine A₁ receptor mRNA expression by in situ hybridization and immunohistochemistry demonstrated that mRNA expression following 3, 12, or 30 days of theophylline treatment was not significantly different from sham-operated controls ($p > 0.05$). Although theophylline-induced recovery is mediated through adenosine A₁ receptors, the present results indicate that the maintenance of recovery may be mediated by other mechanisms.

P50 Nitric oxide synthase inhibitors prevent acute motor nerve conduction deficit in mice caused by a full thickness dermal burn

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Full thickness dermal burn injury is associated with peripheral nerve deficits at a distance from the burn. Our lab showed that an 18% body surface area (BSA) dermal burn injury in mice produced a significant decrease in motor nerve conduction velocity 6 h after the burn. Motor conduction velocity (MCV) remained significantly reduced through 14 days. Sensory CV (SCV), in contrast, was not significantly affected. The results suggest that dermal burn injury produces a systemic response that specifically affects motor nerve fibers. Plasma and urinary nitric oxide (NO) levels are known to increase after thermal injury. We tested the hypothesis that the MCV deficit is caused by NO overproduction. An 18% BSA third-degree burn was applied to the back of an anesthetized mouse. All experimental procedures followed the National Institutes of Health (NIH) *Guide to the Use and Care of Experimental Animals*. MCV and SCV were determined in the intact mouse by percutaneous stimulation of the sciatic nerve (Elias, KA et al.,

Peripheral neuropathy in transgenic diabetic mice: Restoration of C-fiber function with human recombinant nerve growth factor. *Diabetes* 47:1637–1642, 1998.) Administration of the nonspecific NO synthase (NOS) inhibitor, N-nitro-L-methyl ester (50 mg/kg, IP (intraperitoneal), or one of the inducible NOS (iNOS) specific inhibitors, N-immunoethyl-L-lysine (5 mg/kg, IP) or aminoguanidine (10 mg/kg, IP) to burned mice, prevented the reduction in MCV. Administration of D-nitro-L methyl ester (50 mg/kg, IP), an inactive form of the NOS inhibitor, did not prevent the MCV deficit. The results suggest that production of NO from iNOS following the burn contributes to a deficit specific to motor nerves. NO activates second messenger cascades that are known to influence voltage-gated ion channels. NO can also form nitrogen dioxide, which can directly oxidize sulfhydryl groups important for modulating channel gating. This project was supported by a grant from Shriners Hospitals for Children.

P51 Functional magnetic stimulation facilitates gastric emptying

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The objective of this study is to evaluate the effectiveness of functional magnetic stimulation (FMS) on gastric emptying (GE) in subjects with spinal cord injury (SCI) and those without SCI. This is a prospective before-after trial, and five able-bodied males and four with SCI were recruited in the study. A commercially available magnetic stimulator with a round magnetic coil was placed along the lower thoracic spine. The intensity of the magnetic stimulation was 60%, with a frequency of 20 Hz and a burst length of 2 s. The duration of stimulation was 20 min. The results showed that placing the magnetic coil at T9 of the spinous process in both normal and SCI subjects achieved accelerated GE. The mean plus or minus standard errors of means (SEM) of the GE half-time ($GE_{t/2}$) at baseline and with FMS were 36 ± 2.9 min, and 33 ± 3.1 min, respectively, for the able-bodied subjects, a 8.3% decrease in $GE_{t/2}$. The mean \pm SEM of the $GE_{t/2}$ at baseline and with FMS were 84 ± 11.1 min and $59 \pm$

12.7 min, respectively, for the SCI subjects, a 30% decrease in $GE_{t_{1/2}}$. In both able-bodied and SCI cases, FMS was shown to facilitate GE. This is especially prominent in patients with SCI. We con-

clude that FMS can be further developed into a noninvasive therapeutic modality for facilitating GE in subjects with and without SCI.

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