

State of New York

**Spinal Cord Injury
Research Board**

**2006
Annual Report**

May 2, 2007

**New York State Spinal Cord Injury Research Board
Roster of Members
As of December 31, 2006**

Allen L. Carl, M.D.
Albany Medical Center

David A. Carmel
New York Stem Cell Foundation, and
Alliance for Stem Cell Research

Deborah A. Hrustich, M.D.
Albany-Troy Neurosurgical Associates, P.C.

Barbara S. Koppel, M.D.
Metropolitan Hospital Center

Lorne Mendell, Ph.D.
State University of New York
at Stony Brook

Paul Richter
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Executive Summary

In July 1998, landmark legislation was enacted to establish the Spinal Cord Injury Research Trust Fund. The purpose of the fund is to assist leading researchers with ongoing and new efforts to find a cure for spinal cord injuries. This legislation also established the New York State Spinal Cord Injury Research Board (SCIRB). The Board consists of 13 members appointed by the Governor and legislative leaders. The New York State Spinal Cord Injury Research Board was first convened in August 1999. The Board is primarily responsible for administering a research grants program, financed by the Spinal Cord Injury Research Trust Fund, to support proposals from leading scientists, physicians and other experts who are dedicated to finding a cure for spinal cord injuries. The Board is to report annually to the Governor and Legislature on its grant-related activities, the status of Board-supported research and on the Trust Fund.

The Board is grateful to the Governor and the Legislature for the funding to support its mission.

The salient accomplishments of the Board and program in 2006 follow:

- On January 25, 2006, the Spinal Cord Injury Research Board issued a Request for Applications (RFA) to generate proposals for Collaborations to Accelerate Research Translation (CART), and Innovative, Developmental or Exploratory Activities (IDEA) projects. Obligations resulting from these multiyear awards total more than \$4.6 million. The application deadline was July 19, 2006. Twenty-four applications were received, and ten were recommended by SCIRB for approval. One application was tabled for a vote at the next regular meeting, as a conflict of interest prevented a vote. The estimated contract start date was April 1, 2007.
- Dr. Stacie Bloom, Manager, External Relations, New York Academy of Sciences, made a presentation to the Board at the October 25 meeting entitled, "Proposed 2007 Investigator Symposium at the New York Academy of Sciences." The presentation was in preparation for a planned New York State conference on spinal cord injury research for both lay and scientific audiences.
- On November 30, 2005, the Spinal Cord Injury Research Board issued an RFA for Postdoctoral Fellowships, Mentored Research Scientist Development Awards, and Mentored Clinical Scientist Development Awards. Obligations resulting from these multiyear awards total approximately \$2 million. The application deadline was May 17, 2006. Thirteen applications were received and 11 were recommended for funding by SCIRB, with an estimated contract start date of April 1, 2007.

- During 2005, the Board voted to name the Center of Research Excellence (CORE) after Christopher Reeve, its late member, and Paul Richter, a current member, in recognition of their major contributions to spinal cord injury research. The Commissioner of Health officially approved these recommendations in 2006. Appropriate legal steps are being taken to rename the CORE in honor of these distinguished pioneers in advancing spinal cord injury research.
- The Spinal Cord Injury Research Board issued a Request for Proposals (RFP) on August 15, 2005, seeking proposals from qualified organizations to convene scientific panels (sometimes referred to as study sections) and conduct technical merit peer reviews of spinal cord injury-related research applications for the period May 1, 2006 through April 30, 2011. Constella Group, LLC, was the successful bidder.
- A Board meeting was held on Wednesday, October 25, in Albany, with a videoconference site in New York City. A high priority was placed on filling the four vacant Board positions, and an introductory package was developed to inform potential candidates of Board responsibilities.
- After four years of service, the Board's executive secretary, Martin D. Sorin, Ph.D., assumed other duties and was replaced by Ms. Bonnie Brautigam on October 1, 2006. At the same time as that leadership transition, two other changes were implemented. First, the program was integrated into the Wadsworth Center's Office of Research Guidance, where it now benefits from the existing administrative infrastructure. Second, Wadsworth created a centralized Contracts Unit to assist the program in tracking and execution of contracts, amendments, and payment of vouchers. The position of Associate Accountant was vacated in January 2006 and filled in October 2006.
- As of December 31, 2006, it is estimated that a balance of reappropriated, unencumbered funds of negative \$1.5 million remains in the Spinal Cord Injury Research Trust Fund.
- During 2006, a total of 13 papers, one textbook chapter, and 13 abstracts stemming from 26 SCIRB-funded projects active in 2006 were published by 11 principal investigators (Appendix III). Twenty-three of these contracts began in 2006. Additionally, three patent applications were filed (Appendix IV).

Highlights of research accomplishments resulting from grants active in 2006 include:

- Researchers from the CORE in spinal cord injury, under the leadership of Burke/Cornell Research Institute Principal Investigator Rajiv Ratan, M.D., Ph.D., are working on five research objectives leading to clinical trials of acute and chronic spinal cord injury treatments. Through these efforts, the Center has identified 100 potential drug candidates already approved by the U.S. Food and Drug Administration (FDA) that may be useful in the treatment of spinal cord

injury. Resveratrol has been shown to reduce neurological injury when delivered prior to spinal cord injury in a rabbit model. Results from the CORE work suggest that Resveratrol may also be beneficial in axonoprotection and neural regeneration. Dosage and post-injury application are undergoing testing. Other protein biological compounds are under investigation for neuroprotection and neurorestorative properties. Studies on cellular repair and inhibition of type-2 astrocytes are revealing new targets for promoting endogenous repair and enhancing the usefulness of transplanted cells. Studies to develop robotic technologies for sensory-motor locomotion treatment are being conducted in rats and humans.

- Previous research has shown that implanted stem cells in the spinal cord injury zone do not have long-term access to growth factors needed for developing efficiently into useful spinal cord cells. Sally Temple, Ph.D. (Albany Medical Center), has generated Esco stem cells permanently producing high levels of the growth factor IGF-1. Dr. Temple's laboratory, in collaboration with Rensselaer Polytechnic Institute, is developing stem cells and slow-release microbeads that could deliver growth factors beneficial to the spinal cord-injured patient. These growth factors could reduce scar formation and promote circuit repair, and the stem cells could repair the spinal cord tissues.
- Micro-magnetic resonance imaging (MRI) can provide the mechanism to study the timing of events following spinal cord injury. The impact of growth factor administration and infusion of neural stem cells can also yield important insights into their potential role in repair and recovery. Since stem cells are likely to migrate through cord regions peripheral to the lesion, Alexandra Joyner, Ph.D., and her laboratory are attempting to acquire such images not only at the lesion site, but also extending over much of the spinal cord. They are developing micro-MRI hardware and image acquisition methods. In combination, these MRI developments have permitted three-dimensional visualization of the entire spinal cord *in vivo*.
- In the majority of spinal cord injuries, a subset of nerves survive injury. However, these surviving nerves may not function properly or may undergo degeneration following the initial injury. The myelin coat is necessary to protect nerves from degeneration, and, without myelin, nerves that survive the initial injury may die during the injury aftermath. Six months post-injury, the Cognato laboratory (SUNY at Stony Brook) located and electrically mapped functional sensory axons that had regenerated beyond the injury site and found them to be chronically demyelinated. This finding emphasizes the need to restore normal conduction properties to regenerated axons after spinal cord injury. Recent data indicate that: a newly discovered laminin receptor, dystroglycan, contributes to oligodendrocyte differentiation; laminins influence oligodendrocyte development by modulating Fyn regulatory mechanisms; and dysregulation of Fyn-signaling mechanisms may contribute to myelination failure in laminin-deficient *dy/dy*

mice. The laboratory also identified a possible means to reduce the number and activation of microglia, resulting in protection from cell death.

- Damage to the spinal column at or below thoracic-level 11 often causes injury to the *cauda equina*. Such injury can lead to loss of muscle function in the legs and bladder (paralysis), loss of sensation, and sometimes chronic burning pain. About one in every five patients admitted to the hospital with spinal cord injury actually has *cauda equina* injury. A surgical rat model was developed to implant nanospheres containing a combination of slow-releasing molecules to help nerve growth and reduce scar tissue. A new behavioral method was devised to assess the impact of the procedure. The injury does not affect leg movements, but does cause weakness in the rat tail. The Calancie laboratory (SUNY Upstate Medical University) discovered that when placed on a rope (one-inch diameter) that is then rotated, rats use not only their fore- and hindpaws to hang on, but also wrap their tails around the rope in the direction opposite to the rope's twisting. An angular scale was devised to measure this tail movement, using videotape records of the tail tip by attaching a small reflective foam ball to the tail tip. This process will provide quantitative measures of loss and recovery of tail function upon delivery of the implanted molecules. Following these rat studies, dog, and then human studies are planned.

The Board appreciates the opportunity to serve the citizens of New York State by studying such an important public health problem and, with the abiding support of the State's residents, anticipates continued progress and success in achieving its mandates.

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**STATE OF NEW YORK
SPINAL CORD INJURY RESEARCH BOARD
2006 ANNUAL REPORT**

I. Introduction

The Spinal Cord Injury Research Board of the New York State Department of Health and the Spinal Cord Injury Research Trust Fund were authorized by legislation signed in July 1998, under Chapter 338 of the Laws of 1998. This law added Title IV (Sections 250 through 251) to Article 2 of the Public Health Law and Section 99-f to Article VI of the State Finance Law. A technical amendment to the provisions of the State Finance Law was enacted in December 1999, under Chapter 612 of the Laws of 1999. The Board's enabling legislation is found in Appendix I to this document.

The Board's major responsibilities are:

- To develop general policies and procedures for soliciting and selecting meritorious proposals to be recommended for funding to the Commissioner of Health. To meet this responsibility, the Board must undertake the following tasks:

- 1) Develop grant mechanisms and establish award amounts to:
 - stimulate creativity in the investigation of spinal cord injury (SCI);
 - encourage development of new research programs in SCI; and
 - maximize the unique resources and strengths available in New York State for furthering the Board's mandate.
 - 2) Identify research areas of emphasis to:
 - address existing knowledge gaps or under-explored topics; and
 - ultimately lead to the cure of SCI.
 - 3) Establish objective merit and programmatic review processes to:
 - identify projects with the greatest potential to impact SCI and its effects positively; and
 - foster the entry of new neurologic and neuroscience investigators into areas or disciplines with potential to reverse the consequences of SCI.
- To report annually to the Governor and the Legislature on the administration of the Spinal Cord Injury Research Trust Fund and the progress of Board-sponsored research programs.

This report summarizes the Board's eighth year of operation and progress to date in fulfilling its mandate.

II. Board Organization and Membership

The Board is comprised of 13 members. The names, affiliations and brief biographies of appointed members follow. The composition of the Board's membership is approximately one-third basic science researchers, one-third clinicians and surgeons, and one-third spinal cord-injured persons. Members serve four-year terms. Four Board seats are currently vacant. The Department of Health liaison and Secretary to the Board is Bonnie Jo Brautigam, and the Department's attorney to the Board is David Quist, Esq.

A. Spinal Cord Injury Research Board Members (as of December 31, 2006)

Allen L. Carl, M.D., Albany Medical Center

Dr. Carl came to Albany after medical training at SUNY-Buffalo, post-graduate experience in orthopedics at NYU-Bellevue Hospital in New York City and spine injury training at the University of Toronto. He has been on the staff at Albany Medical Center for approximately 20 years, and is a professor of orthopedic surgery and pediatrics. Spine disease and disorders became his primary area of interest as he perceived it was one of the last frontiers for innovative medical development. His interest in contributing to the knowledge base in the field of the spine prompted his association with an academic medical center. Locally, he has collaborated with Rensselaer Polytechnic Institute, General Electric, and the State University of New York at Albany. From these successful associations, surgical navigation, new metal implants for scoliosis correction, and spinal fusion have been developed. Under the direction of Dr. Carl, spinal cord injury biomechanics as a model for neurological repair has been implemented and studied in his research laboratory for the past six years through generous donations from the Jeffrey Schneider Spinal Cord Research Program.

David A. Carmel, New York Stem Cell Foundation, and Alliance for Stem Cell Research

Mr. Carmel is a graduate of Harvard University (B.A., with highest honors in sociology) and the Stanford University Graduate School of Business (MBA). While vacationing in Mexico in 1999 just before entering Stanford, he sustained a spinal cord injury in a diving accident which left him paralyzed from the chest down. Since graduating from Stanford, Mr. Carmel has served as a White House Fellow in the Department of the Treasury and an advisor to Pfizer, Inc., on Medicare issues, and has helped raise \$25 million to place Proposition 71 (California's Stem Cell Research and Cures Initiative) on the ballot.

Deborah A. Hrustich, M.D., Albany-Troy Neurosurgical Associates, P.C.

In addition to serving as a neurosurgeon at Albany-Troy Neurosurgical Associates, Dr. Hrustich holds simultaneous medical staff appointments at Albany Medical Center, St.

Peter's Hospital, Albany Memorial Hospital, St. Mary's Hospital and Samaritan Hospital. She is a member of several medical societies, including the New York State Medical Society, the American Medical Society, and the New York State Society of Surgeons.

Barbara S. Koppel, M.D., Metropolitan Hospital Center

Dr. Koppel is Chief of Neurology at Metropolitan Hospital and Professor of Clinical Neurology at New York Medical College. In addition, she holds appointments at Terence Cardinal Cook Hospital, Catholic Medical Center of Brooklyn and Westchester Medical Center. She is the author of numerous journal articles, book chapters and abstracts.

Lorne Mendell, Ph.D., State University of New York at Stony Brook

Lorne Mendell is a Distinguished Professor at SUNY-Stony Brook whose laboratory focuses on the functional effects of neurotrophins in pain and segmental reflex pathways. Specifically, his laboratory is involved in research on the physiology of neurotrophins, and their action in modifying well-delineated circuits in the intact and injured spinal cord, including sensory input and motor output. His laboratory is investigating the effects of neurotrophins on nociceptors and nociception in rats. In previous work the group determined that administration of the neurotrophin nerve growth factor (NGF), known to be normally upregulated in skin during inflammation, produces hyperalgesia. The group is also studying the basis for the peripheral component of this hyperalgesia. Another focus in his laboratory is the action of neurotrophins such as NT-3 and BDNF on spinal reflexes and pathways in the neonatal rat. Dr. Mendell is the author of numerous journal articles and a past president of the Society of Neuroscience.

Paul Richter, Spinal Cord Society

Mr. Richter is responsible for the 1998 legislation that created the Spinal Cord Injury Research Board. He was a State Trooper Zone Sergeant when he was shot three times, leaving him with a spinal cord injury.

Robert D. Trotta, Esq., Davis and Trotta, Attorneys-at-Law

Mr. Trotta is a graduate of Hobart College in Geneva, New York, and Syracuse University College of Law. He has been admitted to the New York State Bar, and worked with Davis and Trotta since 1966. He also served in the Dutchess County Public Defender's Office (1968-1981), was Town Attorney for the Town of Northeast for 16 years, and School Attorney of the Webutuck (New York) Central School District for three years. He became interested in finding a cure for spinal cord injury after his son, David, was paralyzed from the neck down as the result of a motorcycle accident.

David S. Whalen, Esq.

Mr. Whalen is Senior Assistant Counsel with the New York State Office of Court Administration. He has been in private legal practice, and is the founder and past president of the Capital District Chapter of the Spinal Cord Society. Mr. Whalen is spinal cord-injured.

Jonathan R. Wolpaw, M.D., Wadsworth Center, New York State Department of Health

Dr. Wolpaw is a board-certified neurologist who has worked at the Wadsworth Center for 24 years. He received a medical degree from Case Western Reserve University in 1970, and then completed a residency in neurology at the University of Vermont and a fellowship in neurophysiological research at the National Institutes of Health (NIH). He is Chief of Wadsworth's Laboratory of Nervous System Disorders and a professor in the Department of Biomedical Sciences of the University at Albany's School of Public Health. Dr. Wolpaw's major research interest is developing and using operant conditioning of spinal reflexes as a new model for studying learning and memory in the vertebrate nervous system. These methods are now being applied to the study of spinal cord injury and to development of new treatment methods. He is also designing electroencephalograph-based brain-computer interface technology as a new communication and control channel for those with severe motor disabilities. He is the author of numerous journal articles and holds several NIH grants.

B. Board Membership Changes in 2006

The following Board members resigned during this reporting period:

Moses V. Chao, Ph.D., Chair, Professor of Cell Biology and Physiology, and Neuroscience, Departments of Cell Biology (Skirball); and Physiology and Neuroscience, Skirball Institute Program of Molecular Neurobiology, New York University School of Medicine

Mary E. Hatten, Ph.D., Laboratory of Developmental Neurobiology, The Rockefeller University, New York City

Gerard M. Kelly, Executive Director, United Spinal Association

III. Major Activities of the Board

A. Meeting Schedule

The Board is mandated to meet twice per calendar year. During 2006, a Board meeting was convened on Wednesday, October 25, at the Wadsworth Center's David Axelrod Institute, New Scotland Avenue, Albany, with a videoconference site at the Health

Department's Regional Headquarters at 90 Church Street, New York City. No members of the public requested to speak at the meeting.

The meeting agenda and approved minutes are available by request from the Board's Executive Secretary.

B. Bylaws

There were no changes to the bylaws in 2006. The bylaws are found in Appendix II.

C. Renaming the Center of Research Excellence

In August 2003, the New York State Department of Health shepherded the formation of the Center of Research Excellence (CORE) in Spinal Cord Injury. In 2005, the Board voted to rename the CORE, first for Christopher Reeve, and then for Paul Richter. During 2006, the Commissioner of Health approved both actions. Appropriate legal actions are being taken to rename the CORE after these distinguished contributors to the Board's mission.

D. Institute of Medicine Report

The following excerpt is taken from the Institute of Medicine (IOM)'s 2005 Report, Spinal Cord Injury: Progress, Promise, and Priorities:

An estimated 11,000 spinal cord injuries occur each year in the United States, and 247,000 Americans are currently living with a spinal cord injury. The newly discovered potential for central nervous system (CNS) regeneration and repair has opened up numerous therapeutic targets and opportunities. Many current avenues of research suggest that a concerted research effort on spinal cord injuries could result in important gains in restoring function and improving quality of life.

Recognizing this wealth of new opportunity, the New York State Spinal Cord Injury Research Board asked the Institute of Medicine (IOM) to examine future research directions in spinal cord injury. The IOM was asked not just to advise New York State on its research program, but to look more broadly at research priorities for funders of spinal cord research, including federal and state agencies, academic organizations, pharmaceutical and device companies, and nonprofit organizations. To accomplish this task the IOM appointed a 13-member committee with expertise in basic and clinical neuroscience research, trauma surgery, health care, biomedical engineering, clinical research methods, and research management.

This report by the IOM Committee on Spinal Cord Injury provides a broad overview of the current status of spinal cord injury research, examines the research and infrastructure needs, and provides recommendations for advancing and accelerating progress in the treatment of spinal cord injuries with particular attention to issues regarding translational research. The committee also addresses the contributions that the New York State program can make to complement the scientific efforts of other state, federal, and private supporters of research in this area.

The following table is a summary of the IOM committee's recommendations to strengthen New York State's Spinal Cord Injury Research Program and implementation progress to date for each Board-approved goal.

IOM Spinal Cord Injury Implementation Plan

IOM Recommendations	Proposed Actions	Progress Report
8.1: Build and Strengthen New York State’s Research Infrastructure		
<p>* Develop and sustain a vigorous recruitment and training effort for fundamental and translational research; the number of investigators should be increased progressively over the next three years with the goal of at least doubling the number of researchers focused on fundamental and translational studies of spinal cord injuries.</p>	<p>Issue a request for applications (RFA) for postdoctoral fellowship and career development awards to recruit new and established researchers from the fields of neuroscience, neurology, and bioengineering in the State and out-of-state to do SCI research.</p> <p>Create new three-year endowed faculty associated professorships. Seek funds from either reappropriated monies or the “Contingency Fund” to support the professorships.</p>	<p>The Board issued an RFA for Postdoctoral Fellowships, Mentored Research Scientist Development Awards, and Mentored Clinical Scientist Development Awards. Although RFAs for postdoctoral fellowships have been distributed previously, this was the first time an RFA was released for Mentored Development Awards.</p>
<p>* Establish a coordinated statewide research network that encourages collaborations among individual investigators and inter-institutional research efforts; the Board should convene a statewide meeting of investigators and relevant stakeholders to plan a research strategy and coordinate research efforts.</p>	<p>Enlist the help of Board-funded principal investigators (PI’s) to establish a Statewide network for encouraging individual and inter-institutional collaborations among SCIRB, NIH, the Reeve Foundation, and other State SCI researchers. The Board has already held two statewide PI meetings. Use the next meeting to strategize, if possible.</p>	

IOM Recommendations	Proposed Actions	Progress Report
<p>* Cultivate formal linkages with researchers, programs, and biopharmaceutical companies in the region to forge partnerships for basic, translational, and clinical research.</p>	<p>Establish a regional SCI Research Consortium with NJ, CT, MA and MD, including state, private (e.g., United Spinal) and VA programs. Hold Consortium PI meetings.</p> <p>Issue an R43-44-like RFA for pharmaceutical and biotechnology firms in the region led by NY firms. Explore a partnership with NYSTAR.</p>	
<p>* Establish regional core laboratory facilities.</p>	<p>Establish a Consortium-financed animal (rat) SCI model core facility. Consult with Dr. Lawrence Sturman on his experience in the establishment of the shared New York City Structural Biology Center.</p>	
<p>8.2: Develop a Regional Clinical Trials Center</p>		
<p>* Develop and coordinate multicenter clinical trials to examine therapies for the treatment of spinal cord injuries.</p>	<p>For now, CORE will be doing preliminary chronic SCI work in New York. Then, multi-site trials will be set up, possibly to include facilities in nearby states.</p>	
<p>* Sponsor a clinical trial of decompression as an early intervention and clinical trials of other therapies to be used during the acute phase of a spinal cord injury by using the special opportunities offered by New York City's geographic location and the unique resources of its trauma centers; and manage a clinical trials clearinghouse.</p>	<p>Issue a request for proposals (RFP) to select a treatment facility to conduct a trial of acute phase therapy (the State Health Department cannot limit bidders to one region). NIH already manages a national clinical trials clearinghouse.</p>	

IOM Recommendations	Proposed Actions	Progress Report
8.3: Restructure Research Funding and Oversight Processes		
<p>The New York State Spinal Cord Injury Research Board should work with the State of New York to reduce administrative burdens, improve the approval and grant distribution processes, and establish a rapid-response funding mechanism to capitalize on new research ideas.</p>	<p>Continue ongoing efforts to expedite approvals of RFAs and contracts.</p> <p>Continue to research federal and state approaches for more expeditious processing of solicitations and research contracts, and enlist the assistance of politically active members of the Board to find a legislative solution to funding and administrative issues.</p> <p>Work to establish a mechanism to capitalize on new research ideas.</p>	<p>Contractor training was provided in January 2006 to improve contractor reporting and invoicing procedures. In October 2006, Wadsworth Center created a centralized Contract Unit to assist the program in tracking and execution of contracts, amendments, and voucher payments. The vacant Associate Accountant position also was filled. Additional training was provided to program and Contract Unit staff on the latest procedures for obtaining approval of draft solicitations (RFAs) and initiating contracts for execution. Each of these efforts was a building block to improve the funding and oversight processes.</p>

8.4: Ensure Independent Evaluation		
The New York State Spinal Cord Injury Research Board should establish an independent external review panel that meets periodically to rigorously assess the program's efforts toward its stated mission to cure spinal cord injuries.	Solicit external reviewers from among the Constella contracted application review team to perform an evaluation five to seven years from 2005. Target RFAs to areas identified by IOM.	
In addition, the following changes have been recommended by the executive director to facilitate Board effectiveness:		
Complete outstanding annual reports.	Annual reports for 2003 and 2004 to be completed by July for Board approval.	Reports for 2003, 2004 and 2005 have been completed and submitted to the Governor and legislative leaders.
Fill Board vacancies.	Continue to propose nominees and closely monitor the approval process. Continue to press for full Board complement of 13 members.	
Encourage timely member arrival to Board meetings.	To make best use of meeting time, remind members that tardiness unduly delays official Board business and voting.	
Increase number of Board meetings.	Add a third meeting per year.	

E. 2005 Spinal Cord Injury Research Board Annual Report

At its October 25, 2006 regular business meeting, the Board approved the 2005 Annual Report, drafted by program staff, with enhancements, including a summary of researchers' accomplishments.

The 2005 Annual Report, setting forth the status of funds appropriated for spinal cord injury research and the progress reflected in the results of Board-funded spinal cord injury research efforts, has been distributed to the Governor and leaders of the State Senate and Assembly.

F. Presentation to the Board

“Proposed 2007 Investigator Symposium at the New York Academy of Sciences” – Dr. Stacie Bloom, Manager, External Relations, New York Academy of Sciences

Dr. Stacie Bloom introduced Rashid Shaikh, New York Academy of Sciences (NYAS) director of programming, and Shari Dermer, NYAS conference manager. Dr. Bloom stated the objective of organizing a spinal cord injury conference and described her organization as qualified to do so. The NYAS is the third oldest scientific association in the country, with a membership of about 26,000. The group convenes interdisciplinary groups on various cutting-edge topics and is an effective disseminator of scientific information through published annals and Web-based e-briefings. Dr. Bloom expressed interest in participating with SCIRB in a conference on spinal cord injury research for both a lay and a scientific audience. Dr. Dermer indicated a three-day conference for 100 to 150 participants would cost about \$150,000. The Board agreed to look into various options of contracting with NYAS for such a conference in 2007.

IV. Granting Activities for Research in Spinal Cord Injury

A. Peer Review of Research Applications Request for Proposals (RFP)

The Spinal Cord Injury Research Board issued an RFP on August 15, 2005, seeking proposals from qualified organizations to convene scientific panels (often referred to as study sections) and conduct technical merit peer reviews of spinal cord injury-related research applications for the period of May 1, 2006 through April 30, 2011. This process calls for recruitment of skilled professionals who have experience with peer review procedures, arranging peer review meetings, scoring research applications and other requirements listed below. The selected contractor performs the following functions:

- receive applications and screen for compliance with instructions;
- perform scientific and technical merit peer review of research applications;

- implement and monitor procedures for an established and systematic application review process to ensure ethical standards of conduct and high-quality research;
- provide quarterly progress reports and an annual interim written report presenting a strategy to best allocate available funds;
- provide support services, such as conference management and travel logistics; and
- ensure compliance with requirements for conflict of interest disclosure, use of human subjects, and ethical use of animal standards.

Bidders' proposals were due on December 1, 2005, with a contract start date of May 1, 2006. The awardee was Constella Group, LLC. Since the inception of this contract, the Constella Group has organized two successful peer reviews for the program (see Sections B and C, below).

B. RFA for Postdoctoral Fellowships, Mentored Research Scientist Development Awards and Mentored Clinical Scientist Development Awards

The intent of the Postdoctoral Fellowship Awards is to support the continued training of basic or clinical investigators with exceptional potential for making significant contributions to cures of SCI and SCI-induced paralysis. The awards are for a two-year period. The objective of the Mentored Research and Clinical Scientist Development Awards is to underwrite the transition of neuroscientists and neurologists into spinal cord injury research careers at New York institutions. The awards are for a three-year period. The Board expects that outcomes of supported activities will benefit subsequent research and/or education efforts. To fulfill this vision, applications may address any topic or issue related to spinal cord injury, with any investigative approach appropriate to the application topic.

The Mentored Research Scientist Development Award (K01-like award) provides support for an intensive, supervised career development experience in one of the biomedical, behavioral, or clinical sciences, leading to research independence. The candidate must be able to demonstrate the need for a three-year period of supervised research, as well as the capacity and/or potential for highly productive independent research. The proposed career development experience must be in a research area new to the applicant and/or one in which an additional supervised research experience will substantially add to the research capabilities of the applicant. The candidate must provide a plan for achieving independent research support by the end of the award period.

The purpose of the Mentored Clinical Scientist Development Award (K08-like award) is to support the development of outstanding clinician research scientists. This mechanism funds specialized study for individuals with a health professional doctoral degree committed to a career in laboratory or clinical research. Candidates must have the potential to develop into independent investigators. The award supports a three-year period of supervised research experience that may integrate didactic studies with

laboratory- or clinically based research. The proposal must have intrinsic research importance, as well as serving as a suitable vehicle for learning the methodology, theories, and conceptualizations necessary to become a well-trained independent researcher.

On November 30, 2005, the Spinal Cord Injury Research Board issued an RFA for Postdoctoral Fellowships, Mentored Research Scientist Development Awards, and Mentored Clinical Scientist Development Awards. The application deadline was May 17, 2006. Thirteen applications were received, and 11 were recommended for funding by SCIRB, with an estimated contract start date of April 1, 2007.

C. 2007 CART/IDEA RFA

In 2005, work began on an RFA to be issued in January, 2006 for a Collaborations to Accelerate Research Translation (CART) grants mechanism to foster translation of results from basic (preclinical) research into the next research phase by supporting synergistic partnerships among scientific disciplines and/or organizations. This mechanism is expected to contribute to more rapid translation of basic science findings to potential therapeutic applications or clinical research through novel or innovative treatment strategies.

The collaborative partnerships must facilitate expansion of the body of knowledge/expertise applied to research problems in spinal cord injury. The CART mechanism is expected to encourage experts from other fields to bring their knowledge to bear on problems in spinal cord injury research. By supporting interactions and cooperation, and facilitating cross-disciplinary research, it is anticipated that creative solutions to intractable problems in spinal cord injury treatment may be developed.

The intent of the Innovative, Developmental or Exploratory Activities (IDEA) Awards is to support novel scientific approaches to spinal cord injury research that, although as yet untested, hold out significant likelihood of leading to breakthroughs or new avenues of investigation. Researchers are also encouraged to explore new concepts, to challenge existing paradigms, or to address overlooked gaps in knowledge.

The IDEA research grant allows established researchers to enter the spinal cord injury field, and gives existing spinal cord injury researchers the opportunity to try new methods and approaches to investigate the problems of spinal cord injury (e.g., implantable nanobiotechnological devices to create new neuromotor replacements for nerves damaged by spinal cord injury).

Upon project completion, the PI should have: (1) opened a new area of investigation, (2) satisfactorily tested a novel or innovative hypothesis, or (3) produced viable data for preparation of a full-scale research application to the SCIRB program or another funding agency. It is the intent of the Board that successful IDEA project PIs also be eligible to apply for CART awards.

The application deadline was July 19, 2006. Twenty-four applications were received, and ten were recommended by SCIRB for approval. One application was tabled for a vote at the next regular meeting, as a conflict of interest prevented a vote at the October 25, 2006 meeting. The estimated contract start date was April 1, 2007.

D. Research Accomplishments Associated with Existing Awards

Previously unreported highlights of research accomplishments related to 26 active SCIRB grant contracts include the following:

- **Rajiv Ratan, M.D., Ph.D., Burke Medical Research Institute “Center of Research Excellence,” 5/1/04-4/30/09**

Partnering institutions in 2006 include:

- Acorda Biotechnology, Inc.
- Baylor School of Medicine
- Burke Medical Research Institute
- Helen Hayes Rehabilitation Hospital
- Hunter College of the City University of New York
- Massachusetts Institute of Technology
- Rutgers University
- University of California, San Francisco
- University of Rochester
- Weill Medical College of Cornell University

The goals of this CORE award are to:

- harness the power of contemporary neuroscience to the development of rational, multimodality therapies for spinal cord injury;
- develop an interventional strategy that is effective despite the heterogeneity of spinal cord injury; and
- develop an interventional strategy that is safe and can be rapidly moved to testing in humans.

Since the initiation of CORE funding, the work of the consortium (composed of laboratories from the ten institutions above) has coalesced around these mandates.

Through exploration of five research aims, CORE has identified 100 potential new drug candidates already approved by the U.S. Food and Drug Administration (FDA) that may be useful in the treatment of spinal cord injury. One of these, Resveratrol, has been shown to reduce neurological injury when delivered prior to spinal cord injury in a rabbit model, and results suggest that it may also be beneficial in axonoprotection and neural regeneration. Dosage and post-injury application are currently being investigated. Two other candidate compounds of interest, Daidzein and Methoxyone, are being explored. Plans also are in place for testing of protein biological compounds for neuroprotection and neurorestorative properties.

Studies on cellular repair and inhibition of type-2 astrocytes are revealing new targets for promoting endogenous repair and enhancing the usefulness of transplanted cells. In an effort to identify the optimal cell for promoting regeneration, CORE transplanted glial-restricted precursor (GRP) cells and the derived astrocytes. Researchers found that GRP cells do not promote repair, despite being the ancestors of all the spinal cord glia. Type-1 astrocytes suppress scarring, promote regeneration of dorsal column axons, rescue neurons of the red nucleus, promote extensive behavioral recovery after rubrospinal tract lesions, and cause massive reorganization of tissue structure. However, transplanting type-2 astrocytes does not promote neuron regeneration. Type-2 astrocytes are generated in abundance in spinal cord injury, and CORE has discovered a new regulatory pathway that selectively inhibits generation of these cells, and provides new targets for promoting endogenous repair and enhancing the usefulness of transplanted cells.

Studies to develop robotic technologies for sensory-motor locomotion treatment are underway in rats and humans. The goal is to enable a study of the sensory-motor therapy that works best and most complements/synergizes other treatments (e.g., pharmacological, cellular, surgical). Rat and human studies are ongoing to develop adjustable partial body weight support and balance control, as well as guidance and assistance to achieve appropriate limb movement. Through the rat studies, CORE has learned that passive body weight support does not promote optimal recovery and that active use of limbs may be required. The human studies are applying lessons learned in development of lower limb robotic training devices and upper extremity robots for stroke recovery. Evidence from these studies suggests that much apparent coordination of the limbs in unimpaired gait originates from the passive dynamics of the periphery and is not controlled by the neuromuscular system. This finding suggests that small amounts of muscle activity may be required to achieve interjoint coordination, and that muscular weakness may not be a critical limitation. Moreover, neural activity underlies locomotion, but it need not be sophisticated – simple oscillatory networks may do most of the work.

Investigations at CORE build on existing knowledge, and are uncovering new ways to address issues of curing evolving and stable SCI. This work holds promise that spinal cord injury treatment and recovery, and restoration of function may be realized.

- **Zaven Kaprielian, Ph.D., Albert Einstein College of Medicine, “Regulating Axon Guidance in the Vertebrate Spinal Cord,” 11/1/03-10/31/07 CART grant.**

Characterization of post-crossing commissural projections in chick embryos: The recently generated Ngn1taumCherry construct has made it possible to attempt a direct comparison of the post-crossing trajectories of dI1 (dorsal interneuron I; visualized with Math1tauGFP, green label) and dI2 (visualized with Ngn1taumCherry, red label) axons. These experiments revealed that dI1 and dI2 axons travel along similarly shaped, ILC-like (sigmoidal trajectory) paths. However, it was found that this strategy of co-electroporation of visual constructs led to a diminution of each of the signals. Researchers are attempting to overcome this problem by staggering the electroporation of

the Math1 and Ngn1 constructs by four to six hours. This strategy will also be applied in a further attempt to compare the trajectories of post-crossing segments of dI1 and dI2 axons by electroporating a newly generated Math1 τ Cherry construct, and tauGFP-labeled Ngn1 or Ngn2 reporter constructs. Importantly, co-labeling experiments have now been carried out that validate use of murine enhancer constructs to direct reporter protein expression to dI1 and dI2 neurons/axons in the embryonic chick spinal cord. Work has also been done to identify the locations of synaptic targets for dI1 and dI2 axons. These analyses revealed that dI1 and dI2 axons ultimately project to different regions of the embryonic cerebellum and midbrain and that subsets of both types of axons appear to terminate locally, within the spinal cord marginal zone.

Characterization of post-crossing commissural projections in mouse embryos: A novel confocal microscopy approach has now been developed that facilitates selective visualization of post-crossing segments of labeled commissural axons in these embryos. Consistent with observations in chick embryos, these analyses showed that most dI1 and dI2 axons project along ILC-like trajectories on the contralateral side of the floor plate in transgenic mouse embryos. However, it appears that dI1 axons extend further away from the ventral midline and execute their second turn into the longitudinal plane at a greater distance from the floor plate than their dI2 counterparts. Immunohistochemical labeling also showed that both dI1 and dI2 axons express the following cell surface guidance receptors: Robo1, Robo2, Npn2, NCAM, PSA-NCAM, EphB1-3, EphA4.

Manipulating guidance receptor expression on commissural axons in chick embryos: In follow-up experiments, researchers examined the consequences of introducing the DN Robo constructs into chick embryos 48 hours and 96 hours after electroporation. In these longer-term experiments, the labeled commissural axons remained confined to the contralateral margin of the floor plate and failed to extend away from the ventral midline. These observations suggest that Robo proteins are likely to be restricted to contralateral segments of commissural axons in wild-type chick embryos. Accordingly, new polyclonal antibodies were raised to the extracellular domains of chick Robo1 and Robo2, showing rather selective labeling of post-crossing segments of commissural axons in the embryonic chick spinal cord. As a first step toward assessing a potential autonomous cell requirement for the cytoplasmic domains of Robo proteins in commissural axon guidance, it was next asked whether the pathfinding of Math1 axons was disrupted in response to misexpressing DN forms of Robo proteins led by the CMV promoter. In these experiments, essentially all post-crossing Math1 axons failed to extend away from the ventral midline. Importantly, these axons also expressed GFP, indicating that they bear DN forms of Robo1/2. These observations are consistent with a cell-autonomous role for Robo cytoplasmic domains in commissural axon guidance. However, the misexpression of DN Robo on non-Math1-expressing neurons/axons also may have contributed to the pathfinding defects displayed by Math1 axons. Surprisingly, no significant pathfinding defects were observed as a result of misexpressing either the DN or full-length constructs. Consistent with the lack of significant phenotypes, it appears that only low levels of the truncated and full-length Robo proteins are expressed by the electroporated neurons/axons. To investigate the possibility that the Math1 enhancer is significantly less efficient than the CMV promoter, several strategies are

being explored aimed at increasing Robo protein levels expressed via electroporation of these particular constructs.

Investigators are completing analyses of commissural axon pathfinding in Math1/ngn1 double-knockout mouse embryos, a necessary extension of the originally proposed aim. Specifically, good progress has been achieved in identifying the location of the likely synaptic targets for dI1 and dI2 axons in both the spinal cord and brain. The results of these ongoing studies will provide a necessary framework for determining the components of the guidance systems that direct dI1 and dI2 axons to their ultimate targets.

- **Sally Temple, Ph.D., Albany Medical College, “Engineering Embryonic Spinal Cord Stem Cells for Spinal Cord Injury,” 1/1/06-12/31/09 CART grant.**

Spinal cord injury (SCI) is a complex problem, and researchers have found that therapies involving multiple interventions are likely to be most beneficial. Previous research here has produced a novel type of stem cell that has not yet been tested for SCI. These cells are derived from embryonic spinal cord (Esco cells) and are grown in tissue culture to generate many cells for transplantation. Esco cells are more promising than embryonic stem cells in two major ways: 1) they only produce nervous system cells and no other cell types; and 2) they do not produce tumors after transplantation into animals. Moreover, Esco cells efficiently generate many types of spinal cord neurons, including motor neurons and glia such as oligodendrocytes, known to be beneficial for SCI. Hence, it is important to establish the utility of Esco cells for SCI.

Previous studies have shown that implanted stem cells in the SCI do not have access to growth factors needed for efficient development into useful spinal cord cells. To be directed, the implanted stem cells must be provided with growth factors over a long period of time, i.e., weeks. Esco stem cells have been generated that are permanently producing high levels of the growth factor IGF-1.

Aim 1: To test the hypothesis that regeneration of cortico-spinal (CS) axons is improved by implanting Esco cells or Esco cells modified to produce neuroprotective factors into the injury zone. Researchers generated full-length IGF-1 over-expressing Esco cells and empty vector-transduced Esco cells as controls. The cells were transduced using lentiviral vectors; successfully transduced cells are recognized by GFP fluorescence incorporated into the vector. The cells survive the transduction process and appear healthy. Work is ongoing to examine how cortical cells, including CS neurons, respond when co-cultured with these cell lines. IGF-1 over-expressing Esco cells versus control cells have been implanted into SCI dorsal column-injured adult mice. The tissue is being processed to determine the effect on cortico-spinal tract under these two conditions.

Aim 2: To design and produce a biodegradable delivery system that will slow-release specific bioactive factors over a period of weeks to act directly on the SCI injury zone and implanted stem cells to guide their development and stimulate repair. Collaborators

at Rensselaer Polytechnic Institute have generated microspheres containing shh or chondroitinase versus regular microspheres. Microspheres were made of two materials – polyanhydride and PLGA. These microspheres were co-cultured with spinal cord progenitor cells to assess the effect of shh release in ongoing experiments. Initial tests show that the beads are sterile and non-toxic to neural cells, and the first set of chondroitinase-releasing microspheres have been implanted *in vivo* into the dorsal column of an SCI mouse model.

Stem cells and slow-release microbeads are in development that could be used to deliver growth factors beneficial to the spinal cord-injured patient. The growth factors could help reduce scar formation and promote circuit repair, and the stem cells could help repair the spinal cord tissues. Few treatments for the spinal cord-injured patient are available, and developing novel therapies based on stem cell biology could have high impact for these patients.

- **John Martin, Ph.D., Columbia University, “Bypassing Spinal Cord Injury to Promote Motor Function,” 1/1/06-12/31/09 CART grant.**

This project aims to:

- characterize axon outgrowth from the thoracic nerve bridge and intraspinal synapse formation in the cat;
- determine whether supraspinal motor centers can use the nerve bridge to evoke simple muscle contractions caudal to an acute spinal injury;
- determine whether the motor systems can use the nerve bridge to control complex voluntary movements caudal to an acute spinal injury; and
- determine whether the spinal nerve bridge is effective in transmitting signals for controlling voluntary movements after chronic spinal injury.

Experiments conducted during the first year of funding aimed to develop the cat spinal bridge model. All experiments addressed the first aim, focusing on the following three topics:

Development of the bridge model in the cat: Researchers established the surgical protocol for the bridge in which the thirteenth thoracic nerve (T130) was dissected to the abdominal wall and disinserted from the abdominal musculature. A laminectomy was performed at the second lumbar vertebra, and the cut end of the nerve inserted into the spinal gray matter to the level of the junction between the dorsal and ventral horns. Optimization of the surgery resulted in the absence of motor signs caudal to the surgical level one week after surgery. Several problems were encountered with these experiments. First, the cat is a more active animal than the rat, and the inserted nerve was not stable. The problem was overcome by routing the nerve over paraspinal musculature rather than tunneling through the muscle. Second, reactive tissues encapsulated the nerve in the periphery, making it impossible to identify the nerve several weeks after the initial surgery. This problem was overcome by passing the nerve through a thin silastic sleeve tethered to the insertion site by a suture, resulting in a stable bridge.

Characterization of regenerating bridge axons: The tracing methodology was developed, and after the survival period, the bridge nerve was dissected 1-2 cm proximal to the insertion site, crushed, and injected with the axon tracer, neurobiotin. A 24-hour survival period was determined to be suitable. In four of the cats in which nerve insertion was confirmed histologically and tracer injection was successful, outgrowth of axons was found in the adjoining gray matter. This finding documented that bridge axon regeneration can occur in the cat. Now, the magnitude and distribution of outgrowth will be characterized, and regenerating axons synapse with spinal cord neurons at the insertion site ascertained. These experiments will determine whether the amount of growth is similar to that experienced in the rat.

Physiological characterization of bridge axon synaptic effects: To determine the feasibility and stability of recording in the lumbar spinal cord, studies were conducted in anticipation of examining the physiological effects of electrical stimulation of the bridge nerve. These experiments were performed in control animals, without a bridge nerve and without spinal injury. Cats were anesthetized, and a laminectomy was made in the lumbar enlargement. Nerve cuff electrodes were implanted in several hind limb nerves to identify afferent inputs and antidromically to activate lumbar motoneurons from their peripheral axon. In the course of this work, it was possible to record the laminar distribution of evoked afferent input, single neuron activity in response to nerve stimulation, and extracellularly and intracellularly from motoneurons.

Plans for the upcoming year include:

- continuing to develop the model, to achieve a high success rate. Implantation of a stimulating nerve cuff on the bridge in place of the silastic tube will be attempted to determine whether stimulation evokes hind limb muscle contraction to allow access to growth of bridge axons non-invasively;
 - conducting immunocytochemical experiments characterizing axonal outgrowth and synapse formation with spinal neurons; and
 - generating a small inventory of animals with intact and hemisected cords, to conduct spinal electrophysiological experiments.
- **Jane Lin, Ph.D., New York Medical College, “Bystander Death in Traumatic Spinal Cord Injury,” 1/1/06-12/31/09 CART grant.**

During this first year of the grant, major effort was invested in building a good foundation to study spinal cord injury. Data was generated that critically substantiates preliminary findings described in the application. In particular:

- Quantification of mitochondria in processes of varying diameters demonstrates the clear need for transporting energy metabolites to locations where astrocytes conduct their “business dealings” with neurons. These “frontier” processes are simply not wide enough to contain mitochondria. Gap junction is unquestionably

the best candidate to handle both intracellular and intercellular energy trafficking because of its abundance, large pore size, and strategic location. However, by the same token, gap junction may pave the way to send cells toward secondary death during injury, as it provides a conduit to drain energy from bystanders and recruits them into the ever-expanding zone of trauma. Confirming the popular gap junction blockage data, of both these researchers and many others, in limiting injury propagation, an astonishing difference was repeatedly observed in organotypic spinal cord cultures in the death rate of well-coupled “inner” cells versus that of well-spread “outer” cells.

- Immunocytochemical follow-up of the preliminary study on carbenoxolone’s blockage of both cell death and NADH release has moved knowledge in bystander death one notch up. Not only does injury manifest itself via gap junction, injury also induces expression of the vehicle, gap junction protein Cx43, needed for its manifestation. In other words, injury rides on the passage laid out by gap junction to expand. Upon arrival at a new territory, injury induces more expression of the gap junction proteins it utilizes to expand further.
- In addition to the “vicious cycle” described above, detailed imaging following the cellular constituents in a population reveals that, “Astrocytes are geared to interact, even in the face of death.” Astrocytes send numerous processes to reach out to their neighbors. The repeated and long-lasting wrapping of fine processes around dying cells was seen. Hence, the strong drive of astrocytes to connect to their neighbors adds a third layer to the significance that gap junction contributes to bystander death. Manifestation of injury is further enhanced by the basic coupling instinct of willing participants.
- This extensive imaging experience also sparked innovative approaches to enrich the proposed study. With genetic and traditional cell culture techniques, astrocytes with or without Cx43 may be mixed at various ratios, and the coupling effect on bystander death may be titrated. Availability of the gene gun technology should allow generation of a variety of microenvironments to dissect parameters governing cellular interactions at the single-cell level.
- While continuing to use the popular weight-drop model, development of more refined paradigms for injuring the spinal cord was begun. This is expected to establish systems that go beyond the traditional pharmacological approach of simply reducing or enhancing injury. The EOC-20 microglia model holds promise for its clear-cut result, the cellular nature of the injury, and its great potential for *in vitro* manipulation (an injury method that can be “bottled” and used as a reagent). Eventually, the gene gun approach will be extended to live animals, as a controlled and easily traceable means to introduce injury and blocking agents.

- With all the systems developed, investigators will study the effect of gap junction blockers, pH, Ca²⁺, K⁺, and hypoxia perform the proposed NADH/GFP imaging, and characterize injury by size and TUNEL staining.
- **Alexandra Joyner, Ph.D., New York University School of Medicine, “Genetic and MRI Studies of Spinal Cord Stem Cells,” 1/1/06-12/31/09 CART grant.**

Aim 1: Use micro-MRI approaches in longitudinal studies to assess spinal cord injury and subsequent repair, and to monitor neural stem cells migrating to injury sites with and without addition of growth factors by using contrast agents targeted at sites of spinal cord injury, or that mark transplanted neural stem cells. It is proposed to use micro-MRI methods for longitudinal evaluation of mouse models of spinal cord injury. MRI imaging will permit repeated evaluations in individual mice and thereby establish a time-course of the events following cord injury. Modifications to this time-course in response to administration of growth factors can also be observed, hence providing important insights into their potential role in repair and recovery. As a key component of this evaluation, MRI methods will be used for marking and labeling neural stem cells *in vivo*, so that their response to cord injury can be evaluated and monitored. In order to enable the longitudinal studies as described, modification and improvement of MRI methods were proposed. It was determined that high resolution, three-dimensional images would be required to quantify lesion status and track cell movements. Since stem cells are likely to migrate through cord regions peripheral to the lesion, it was also aimed to acquire such images not only across the lesion site, but extending over much of the spinal cord.

With these imaging goals in mind, important progress was made in development of micro-MRI hardware and image acquisition methods. Specifically, a prototype MRI spine coil was designed and constructed with dimensions appropriate for imaging the cervical, thoracic, and lumbar regions of the spine concurrently. The use of this coil was demonstrated both in idealized water phantoms and in live mice. A three-dimensional image acquisition protocol was implemented that permits flexible prescription of image contrast. Significantly, this will allow empirical manipulation of images for delineation of the lesion site and visualization of the surrounding anatomy. To allow imaging in the thoracic and cervical regions of the cord with this protocol, a novel image gating protocol was implemented for the elimination of motion artifacts, which otherwise hinder the interpretation of image data. This motion gating technique has proved particularly beneficial in the cervical spinal cord, where motion-related artifacts were routinely found to result in image ‘voids.’ In combination, these MRI developments have permitted three dimensional visualization of the entire spinal cord *in vivo*. These results were demonstrated in uninjured wild-type mice. Consequently, researchers have nearly completed the technical components of this project necessary for achieving the imaging aims and will soon begin conducting MRI of mice with spinal cord injury.

Significant progress was also made in the area of magnetic cell labeling and tracking required for this project. In ongoing parallel research projects in the Turnbull lab, a number of advances have been made in this area. Specifically, investigators have characterized the effects of iron-oxide particle size for imaging neural cell migration in

the embryonic and postnatal mouse brain, showing that particles with mean diameters ranging from 1.5 to 4.5 microns can be used to analyze cell migration patterns. Significantly, neuronal progenitors in the embryonic mouse brain have been shown to migrate normally, even after internalization of 4.5 micron, polystyrene-coated iron-oxide particles, which can be detected by *in vivo* MRI. Finally, image analysis methods are in development to quantify the migration patterns of magnetically labeled cells, which will be critical for this project.

Aim 2: Characterize neural stem cells in the adult spinal cord and determine how they respond to injury and growth factors by using genetic fate mapping with Gli1-CreER or Nestin-CreER and R26R-STOP-lacZ mice to mark, follow and characterize neural stem cells. Mating of the Gli1-CreER or Nestin-CreER and R26R-STOP_lacZ lines has begun to produce the mice needed for the fate-mapping studies. Additional CreER mouse lines have been tested to determine whether any mark cells in the adult spinal cord, in particular stem cells. Scientists have begun to characterize the expression of Gli1-lacZ in the normal spinal cord.

Aim 3: Determine the involvement of Gli1/2 activators and the Gli3 repressor in adult neural stem cell biology *in vivo* by analyzing the response of mice lacking each Gli transcription factor, specifically in adult neural stem cells. This project will begin after fate mapping studies (above) are completed.

- **Gordon Barr, Ph.D., Research Foundation for Mental Hygiene, Inc., “Gene Expression after Acute and Chronic Injury and Rescue,” 1/1/06-12/31/09 CART grant.**

Because about 40 percent of red nucleus cells undergo severe atrophy or die after a cervical lateral funiculus lesion and because the surviving but moderately atrophied neurons do not regenerate their axons, these microarray and Q-PCR studies will provide detailed information about the time course of expression of genes associated with neuronal cell death or atrophy, and a frustrated regenerative effort.

Chronically injured neurons do not regenerate as readily as do newly injured neurons, likely because they express different or attenuated patterns of regeneration-associated genes. Chronically injured axons do regenerate into peripheral nerve grafts but only when additional trophic factors are administered. This study will identify patterns of gene expression in chronically injured red nucleus neurons presented with a peripheral nerve graft and glial cell-line derived neurotrophic factor (GDNF) to compare the effects of grafts into chronic and acute lesions. Control animals will receive GDNF but will have no substratum for axonal regeneration. Results will suggest treatments for chronic injury.

These data will provide important information about the neural and genetic mechanisms of neuronal death, survival, axonal growth, and regeneration, and will offer insights into why different treatments for spinal cord injury may or may not work. The results can then direct the development of novel treatments for human spinal cord injury.

Preparation of tissue for assay

Investigators have prepared, sacrificed and harvested brainstems with the goal of identifying the temporal patterns of gene expression following cervical hemisection. Tissue retrieval includes sectioning the brainstem, identifying the nuclei and capturing, via laser microdissection, the axotomized neurons. The time points are: 4, 12 and 24 hours; 3, 7, 14, 30 and 42 days. The RNA from all operated animals and from unoperated control animals was sent to Dr. Barr for gene analysis. Animals will be prepared for Aim 2 and Aim 3 once the initial gene analysis is completed for the animals prepared for Aim 1.

Preparation of microarray assay

In anticipation of the arrival of tissue for the assays, two different platforms have been evaluated: Affymetrix and Codelink rat arrays. Each has advantages and disadvantages, and several hundred arrays have been performed, largely for other experiments. Moreover, the literature has been evaluated in those few studies in which both platforms were used. These studies, and in particular the large Microarray Consortium studies published recently in *Nature*, suggest that both platforms are reliable, able to be validated, and reasonably consistent with each other. Largely because of the better bioinformatics, the Affymetrix 230 rat array was chosen. Costs are approximately equal.

Next was purchased the NanoDrop ND-1000 UV-Vis spectrophotometer to allow quantitation of small sample volumes for total RNA. The machine with sample tissue was evaluated and found highly reliable and sensitive. Its use is essential in these assays.

Finally, the small tissue samples require amplification of RNA to provide sufficient samples for the microarray assays. Nugen's Ovation linear amplification method was tested, which, according to the literature, provides more reliable results for both microarray and quantitative real-time PCR assays. Several tissues samples were studied over the past month with the Affymetrix 230 arrays, and parallel experiments were conducted with amplified and non-amplified replicates of the same biological sample. Results show that the amplified samples are quite reliable and replicable, although with compression of the range of expression values. The reasons and implications of the compression are under discussion.

During the past six months, substantial progress was made toward the first Aim below. Surgeries have been completed with various survival times. Cells have been laser-dissected, and total RNA extracted from most of the animals. Assay conditions have been optimized. Completion of the first and largest Aim is fully expected in the coming months.

- **Victor Arvanian, Ph.D., State University of New York at Stony Brook, "Neurotrophins and Function of the Injured Spinal Cord," 1/1/06-12/31/09 CART grant.**

Most patients with a spinal cord injury (SCI) exhibit some continuity of white matter and thus are not "anatomically complete." The strategy of researchers here is to strengthen

synaptic effects of surviving descending fibers and to investigate whether this strategy will improve functional recovery in adult mammals after spinal injuries. This approach may significantly reduce the number of potential barriers to spinal cord repair that need to be overcome to restore function, and should be applicable to almost all patients with SCI. To study functions of survived connections in damaged spinal cords, synaptic transmission at survived fibers was recently studied following partial lesion of spinal cords in neonatal and adult rats.

Aim 1: To apply combined *in vivo* transgene delivery of neurotrophins (on top of injury) and NMDA receptor regulatory subunits (to lumbar motoneurons) to strengthen synaptic connections in the damaged spinal cord after incomplete spinal cord injury (contusion and hemisection) in adult rats.

There is very limited evidence about functional changes in synaptic transmission to individual motoneurons in damaged spinal cords. A new technique was recently developed and recorded intracellularly *in vivo* from individual motoneurons in a damaged spinal cord synaptic responses evoked by stimulation of the descending fibers across the injury region following total lateral hemisection, using models of acute and chronic SCI in adult rats.

During the reporting period, adult rats were used and control experiments performed to investigate effects of acute and chronic SCI (lateral hemisection) on the function of synaptic connection to individual L5 motoneurons at the sensory inputs (stimulation of dorsal roots; DR) and descending inputs (stimulation of ventrolateral funiculus fibers; VLF). It was found that in the acute SCI model, lateral hemisection induces interruption of monosynaptic transmission at the unilateral side, but polysynaptic responses at the contralateral side persist across the injury region. However, chronic SCI induces a blockade of polysynaptic responses at both unilateral and contralateral hemisection sides. This is the first known electrophysiological demonstration of synaptic responses blockade across the injury region following hemisection in a chronic SCI model.

Aim 2: To examine whether this combination treatment (transgene delivery of neurotrophins and NMDA receptor regulatory subunits) will establish functional synaptic contacts to individual motoneurons and improve functional recovery after spinal cord injury in adult rats.

In this ongoing study, hemisection at T8 was carried out in 40 rats. NT-3-secreting fibroblasts were implanted at the level of the lesion, and HSVnr2d amplicons were injected intrathecally just caudal to the lesion. The NMDA receptor antagonist ketamine was administered immediately and 24 hours after injury to minimize the NMDAR-mediated excitotoxicity. Controls received the same hemisection with ketamine only. Currently, behavioral assessments of these experimental animals are performed. After completion of behavioral testing, the above-mentioned experimental design will be used to evaluate synaptic transmission to lumbar motoneurons at the descending inputs in these animals.

Aim 3: To explore the properties of receptors mediating synaptic responses that reappear after viral delivery of NR2D and NT-3.

Neonatal rats. Recent studies revealed that transgene delivery of neurotrophin NT3 via engineered fibroblasts, combined with transient enhancement of the activity of glutamate N-methyl-D-aspartate (NMDA) receptors via NR2D-expressing herpes simplex virus type-1 (HSV-1) amplicon vector (HSVnr2d; US Patent 11/313,262 pending), is an excellent approach for strengthening synaptic connections with individual motoneurons in damaged spinal cords of neonatal rats. Within the frame of this work (Aim 3) it was found that prolonged administration of NT3 combined with enhanced activity of NMDA receptors in motoneurons (but not NT-3 alone) induced appearance of new functional connections in double hemisected neonatal spinal cords, and facilitated recovery of function in rearing and swimming behavioral tests.

- **Holly Colognato, Ph.D., State University of New York at Stony Brook, “Remyelination and Spinal Cord Injury,” 1/1/06-12/31/09 CART grant.**

Introduction: In the majority of spinal cord injuries, a subset of nerves survive injury. However, these surviving nerves may not function properly or may undergo degeneration subsequent to the initial injury. One reason this might occur is the loss of myelin, the protective fatty coating that normally surrounds nerves (myelin is also damaged or destroyed during spinal cord injury). Recently, it was discovered that, in addition to being required for transmission of normal nerve signals, the myelin coat is necessary to protect nerves from degeneration. Therefore, without myelin, nerves that survive the initial injury will die during the injury aftermath. Myelin is produced by specialized glial cells; however, it is currently unknown how to stimulate the survival of these glial cells or to reactivate myelin production following spinal cord injury. Several signaling mechanisms were discovered that control glial cell survival and myelin production during normal nervous system development. It will be ascertained whether these factors can stimulate glial cell survival and myelin production following spinal cord injury, and, if so, whether this stimulation can lead to enhanced recovery from spinal cord injury.

Aim 1: Demyelination and remyelination after spinal cord injury. Axon regeneration after experimental spinal cord injury can be promoted by combinatorial treatments that increase the intrinsic growth capacity of the damaged neurons and reduce environmental factors that inhibit axon growth. A prior peripheral nerve-conditioning lesion is a well-established means of increasing the intrinsic growth state of sensory neurons whose axons project within the dorsal columns of the spinal cord. Combining such a prior peripheral nerve-conditioning lesion with infusion of antibodies that neutralize the growth-inhibitory effects of the NG2 chondroitin sulfate proteoglycan promotes sensory axon growth through the glial scar and into the white matter of the dorsal columns. The physiological properties of these regenerated axons, particularly in the chronic SCI phase, have not been established. Here, researchers examined the functional status of regenerated sensory afferents in the dorsal columns after spinal cord injury. Six months post-injury, functional sensory axons that had regenerated beyond the injury site were located and electrically mapped. The regenerated axons showed reduced conduction

velocities, decreased frequency-following ability, and increasing latency to repetitive stimuli. Many of the axons that regenerated into the dorsal columns rostral to the injury site were chronically demyelinated. These results demonstrate that regenerated sensory axons remain in a chronic pathophysiological state, and emphasize the need to restore normal conduction properties to regenerated axons after spinal cord injury.

Aim 2: Modification of extracellular matrix signals. Investigators are testing the hypothesis that laminins enhance the survival and differentiation of oligodendrocytes through specific transmembrane receptors and signal transduction pathways. Here are described preliminary studies on the receptors and signal transduction mechanism by which laminin signaling occurs in the oligodendrocytes. Recent data indicate that: a newly discovered laminin receptor in this cell type, dystroglycan, contributes to oligodendrocyte differentiation; laminins influence oligodendrocyte development by modulating Fyn regulatory mechanisms; and dysregulation of Fyn signaling mechanisms may contribute to myelination failure in laminin-deficient dy/dy mice.

Aim 3: To determine the effect of macrophages/microglia on remyelination following spinal cord injury. It was previously proposed to modify the status of microglia and investigate their role in remyelination and regeneration after spinal cord injury. It was intended to use mice that can be made deficient in microglia to ask this question. These animals, upon delivery of ganciclovir (GCV), become effectively microglia-deficient. In the past several months, researchers have been experimenting with the delivery of GCV because, as reported in the literature, administration of GCV over several days can cause aplastic anemia, neuropathy and other adverse systemic effects. Based on literature reports, GCV crosses the blood brain barrier, so that CSF levels are about 30 percent of serum levels. GCV was recently delivered using a mini-osmotic pump directly into the brain parenchyma at the level of the hippocampus, which greatly decreased the risk of systemic effects. GCV was delivered for seven days. After the onset of GCV infusion, mice were injected unilaterally either with ketamine (KA) or with PBS. Five days later, neuronal survival was analyzed. The delivery of GCV alone had no effect on the tissue and did not elicit any microglial response. Injection of KA in wild-type animals receiving GCV did not alter the sensitivity of neurons to KA injury, nor did it affect microglial activation and migration/accumulation to the site of cell death. The administration of GCV in the CD11b-HSVTK mice dramatically protected the neurons from KA-induced death, and both reduced the number of detectable microglia and their activation. These results are in agreement with previous data here using MIF, which inhibited microglia activation and resulted in protection from cell death. The delivery of GCV still needs to be optimized, but preliminary data suggest that such an approach could effectively decrease the activation process of microglia.

- **Blair Calancie, Ph.D., SUNY Upstate Medical University, “Repair of Cauda Equina Injury,” 1/1/06-12/31/09 CART grant.**

Introduction. This project is designed to develop new methods to treat injury to the collection of nerve roots at the “bottom” of the spinal cord known as the *cauda equina*. Damage to the spinal column at or below thoracic-level 11 often causes injury to the

cauda equina. This can result in loss of muscle function in the legs and bladder (paralysis), loss of sensation, and sometimes chronic burning pain. About one in every five persons admitted to the hospital with spinal cord injury actually has *cauda equina* injury. Currently, there is no treatment for the nerve damage, and the chances for natural recovery is poor. Development of an effective treatment is planned, using a combination of molecules to help nerve growth and reduce scar tissue, which is delivered through tiny particles (nanospheres) that dissolve after being placed at the site of injury, slowly releasing their contents.

Progress. The surgical model has been finalized, largely through the efforts of Don Blaskiewicz, a fifth-year neurosurgery resident working on the project. EMG outcome measures have been refined, concentrating on different recording sites along the rat tail. Finally, an entirely new behavioral measure was developed that looks very promising. The injury does not affect leg movements, but does cause weakness in the tail. Dr. Blaskiewicz noticed that when placed on a rope (1" diameter) that is then rotated, rats use not only their fore- and hindpaws to hang on, but also wrap their tail around the rope in the direction opposite to that in which the rope is being twisted. An angular scale was developed to measure this tail movement, using videotape records of the tail tip (indicated by attaching a small reflective foam ball to the tip of the tail). It is believed this test will provide quantitative measures of loss, and recovery of tail function.

Future direction and impact. It is still early in the process, but progress has been good, and plans to begin dog studies within two years look feasible. Human studies could then follow after another two years.

Aim 1: Using an intrathecal scaffold of laminin-coated poly-HEMA, quantify motoneuron survival, axonal regeneration, tail-muscle reinnervation, and tail movement recovery in rats after intrathecal sacral nerve injury in response to the following interventions, individually and as a group:

- autologous Schwann cell transplantation;
- chondroitinase ABC (cABC; delivered via nanospheres); and
- glial cell-derived neurotrophic factor (GDNF; via nanospheres).

Much of the initial effort in this project has gone into the first component of this Aim. It is necessary to establish the number of motoneurons innervating intact tail muscles. Ultimately the method of Brushart was adopted, whereby a small crystal of label (i.e., not in solution) was placed directly on the cut/crushed nerve for several minutes. This approach seems to provide the optimal combination of labeling success without widespread labeling of all CNS elements in the distal spinal cord. A series of animals has been studied with low threshold stimulation to establish the minimum intensity to elicit responses pre- and post-crush.

Attention is concentrated on the tail, because this appendage is well-suited to study the role of regenerating motoneuron axons in mediating motor recovery after injury, without causing the animal undue suffering as a result of the injury. This means that ipsilateral hindlimb function is not disrupted, nor is bowel and bladder function affected, the latter presumably reflecting the presence of innervation from the contralateral (i.e., intact) spinal cord. Since rats with an acute *cauda* injury are incapable of deviating their tail towards the injured side, it is expected that this rope-walking paradigm will show a continual and profound deficit in angular tail movement. However, it is also expected that in the event of ipsilateral recovery of tail muscle innervation (due either to sub-total injury, or to regeneration of lower motoneurons across the injury region), partial recovery of tail rotation should be immediately evident with this novel behavioral analysis.

Aim 2: Develop methods to isolate, purify and expand populations of Schwann cells from dog peripheral nerve.

This Specific Aim was expanded considerably. The transplantation field has rapidly moved from the question of “Can cells be transplanted into the CNS” to expect answers to far more detailed questions, such as: “How many transplanted cells survive, and for how long?”; “Are transplanted cells capable of replication?”; “Do transplanted cells adopt the phenotype specific to the environment in which they are introduced?”; and so on.

To summarize, investigators have quickly moved beyond the question of “Can we purify and expand populations of dog Schwann cells?” The answer is an emphatic “Yes.” The much more difficult question has now been addressed of what happens to these cells after transplantation, particularly when associated with the different types of biomaterials that will be utilized in the guidance channels now being designed for implantation.

Aim 3: Develop a dog model of *cauda equina* injury and repair, in which segments of two adjacent lumbar ventral nerve roots are excised and repaired intrathecally, using the optimal strategies developed from Specific Aims 1 and 2.

No formal dog studies have begun at this point, nor were they expected to begin.

- **Dennis J. Stelzner, Ph.D., SUNY Upstate Medical University, “Local Release of Chondroitinase to Treat Spinal Cord Injury,” 1/1/06-12/31/09 CART grant.**

Axonal regeneration is limited by several molecules that are expressed after spinal cord injury (SCI), including components of the glial scar, in particular chondroitin sulfate proteoglycans (CSPGs). Neutralizing these inhibitory factors, such as removing the inhibitory side chains of CSPGs (GAGs) with an enzyme, chondroitinase ABC (cABC), can enhance axonal growth, but this therapy has not been completely successful, either because the enzyme loses its effectiveness, is not localized to the scar, or additional factors inhibit further regeneration. This project is designed to:

- 1) Fabricate nanoparticles made of a polymer (PLGA) into which cABC is incorporated, and in a size range less than a micron in diameter so that they can be easily injected (nanospheres).
- 2) Characterize the release properties of the nanospheres and determine whether and for how long the released cABC remains enzymatically active, by removing the GAGs and enhancing axonal growth in a cell culture model.
- 3) Determine whether cABC injections into SCI can remove GAGs acutely or after the glial scar has formed, and whether axonal regeneration and recovery of function follow this treatment.

It is hypothesized that other therapeutic agents incorporated into nanospheres can stimulate regeneration in future experiments and that release of several factors in a combined approach ultimately will prove most effective.

Significant progress has been made in regard to the three specific aims:

Aim 1: A different method was used to make the nanospheres so that their size range is better controlled, and experiments are beginning to determine how to limit the uptake of nanospheres by scavenger cells (macrophages) in culture and after injury.

Aim 2: Researchers here have begun to determine the effect of release of different amounts of cABC in a culture system, and, in an initial attempt, determined that loading too high a concentration of cABC into nanospheres results in toxicity not observed at the original level used.

Aim 3: In culture and after SCI, it was found that enzymatically active cABC is localized to a 2.5mm region around the injection site, is released for one month in culture and for at least two weeks in the injured spinal cord, removes GAGs from the CSPGs, and induces axonal growth into a SCI lesion by seven days after injury that increases significantly by one month post-SCI.

Another project, not proposed in the original application, needed to be carried out to characterize the response of short- and long-distance propriospinal tract (PST) axons to moderate spinal contusion injury, in anticipation of using propriospinal axons to test the effectiveness of cABC nanospheres in enhancing neural plasticity and axonal regeneration after SCI. Among the project findings was that short PST axons are severely damaged, but many of these PST neurons survive this damage for several weeks, and are in a position so that cABC nanosphere injections are likely to foster regeneration of these PST axons.

- **Xiang Yang Chen, Ph.D., Wadsworth Center, NYS Department of Health, “Using Reflex Conditioning to Restore Spinal Cord Function,” 1/1/06-12/31/09 CART grant.**

Spinal cord reflex pathways play important roles in many other motor activities, including walking. Spinal cord injuries lead to abnormal spinal reflexes, and these abnormal reflexes contribute to motor disabilities. Studies here over many years have shown that operant conditioning can change spinal cord reflexes in monkeys, rats and humans, including humans with partial spinal cord injuries. Furthermore, recent studies show that this reflex conditioning can improve locomotion in spinal cord-injured rats. This work indicates that spinal reflex conditioning could be an important new method for inducing and guiding spinal cord plasticity to help restore function after spinal cord injury. The central goal of this proposal is to develop further operant conditioning of spinal reflex as a new therapeutic approach and establish its clinical value for humans with spinal cord injuries.

In accord with these goals, investigators will define the capabilities, characteristics and long-term efficacy of this new treatment method by testing, in rats with well-defined spinal cord lesions, the effects on locomotion of carefully selected conditioning protocols. The clinical value of this new therapeutic approach will be established by testing in patients with partial spinal cord injuries whether appropriate reflex conditioning can improve locomotion, and whether this improvement persists.

Progress toward specific aims. Studies have been completed to assess the interaction of H-reflex conditioning and locomotion in normal rats and in rats with a defined spinal cord injury. The permanence of the reflex conditioning dependence on the CST was demonstrated. A human H-reflex conditioning protocol was begun and will be used in future studies to determine whether appropriate reflex conditioning can improve locomotion in patients with partial spinal cord injuries.

Aim 1: To define the capabilities, characteristics, and long-term efficacy of this new treatment method by testing in rats with well-defined spinal cord lesions the effects on locomotion of carefully selected conditioning protocols.

First, it was found that in normal rats in which the soleus H-reflex elicited in the conditioning protocol had been decreased by down-conditioning, H-reflexes elicited during the stance and swing phases of locomotion were also smaller. Similarly, in rats in which the conditioning H-reflex had been increased by up-conditioning, the locomotor H-reflexes were also larger. In these rats, soleus H-reflex conditioning did not appear to affect the length, duration or right/left symmetry of the step cycle. These results are described in a paper published in *The Journal of Neuroscience*.

Second, the interaction of H-reflex conditioning and locomotion was further explored in rats with spinal cord injury. It was found that mid-thoracic transection of the right lateral column (LC) of the spinal cord in rats produced a persistent asymmetry in the muscle activity underlying treadmill locomotion. The time from onset of the right soleus burst

(RBO) to onset of the left soleus burst (LBO) was shorter than the time from LBO to RBO. Because H-reflex up-conditioning can increase the strength of the right soleus burst, up-conditioning of right soleus H-reflex might reduce this LC transection-produced abnormality and therefore help improve locomotor function. Results indicated that up-conditioning of the soleus H-reflex improved locomotor function in LC rats. In LC rats exposed to H-reflex up-conditioning, muscle activity during locomotion became symmetrical. In contrast, in the LC rats that were not up-conditioned, the locomotor asymmetry persisted.

Third, a study was completed to determine whether the loss of the capacity for H-reflex down-conditioning after CST transection is permanent. Results indicate that one year after lateral column (LC) or dorsal ascending tract (DA) transaction, LC and DA rats can still decrease the H-reflex when exposed to the down-conditioning protocol. In contrast, one year after CST transection CST rats exposed to the down-conditioning protocol actually increase the H-reflex. These results indicate that CST transection permanently eliminates the capacity for H-reflex down-conditioning. The unexpected increases in the CST rats may be related to similar increases found in cSMC and DIN rats exposed to the down-conditioning protocol.

Aim 2: To establish the clinical value of this new therapeutic approach by testing in patients with partial spinal cord injuries whether appropriate reflex conditioning can improve locomotion, and whether this improvement persists.

Studies have begun to determine whether appropriate reflex conditioning can improve locomotion in people with partial spinal cord injuries and to establish the clinical value of this new therapeutic approach. As a first step, researchers are developing a human H-reflex conditioning protocol in healthy subjects, comprising six baseline, 24-30 conditioning, and four follow-up sessions. Sessions, which always take place at the same time of day, normally occur three times/week for baseline and conditioning, and then every two to eight weeks for follow-up. Subjects stand during soleus (SOL) H-reflex elicitation, and each session consists of three blocks of 75 trials. Subjects maintain a defined level of SOL background EMG for two to five seconds prior to H-reflex elicitation. During baseline, the H-reflex is simply measured. Then the subject is randomly assigned to the up-conditioning (HRup) or down-conditioning (HRdown) group, and henceforth receives visual feedback after each trial indicating whether the H-reflex was larger (HRup) or smaller (HRdown) than a pre-set criterion value. Good performance earns the subject an additional monetary reward. Follow-up sessions are the same as conditioning sessions.

Results to date indicate that most subjects gradually change H-reflex size significantly in the correct direction. These data show that H-reflex conditioning is possible in humans and that H-reflex conditioning does not require the several thousand trials/day typically completed by the monkeys and rats. Humans performing only 225 trials/day, three days/week display a comparable course of gradual reflex change.

- **Thomas Jessel, Ph.D., Columbia University, “Differentiation of Embryonic Stem Cells into Motor Neurons,” 1/1/06-12/31/10 CART grant.**

Background:

A rational approach to the design of embryonic stem (ES) cell-based therapies requires a better understanding of the difference in motor neuron vulnerability – a problem that in turn demands a sharper understanding of the basic molecular and developmental distinctions in motor neuron subtype.

The overall goal of this proposal is to define the normal developmental mechanisms that promote the diversification of motor neurons into specific functional subtypes, focusing on the specification of motor neuron columnar and pool identity; and to use this information to direct the differentiation of ES cells to selective motor neuron classes.

The normal pathways of motor neuron subtype differentiation in the vertebrate spinal cord have been explored, focusing on the extrinsic signals and intrinsic molecular pathways that direct post-mitotic motor neurons to their columnar and pool subtype identities. It was also examined how to direct the differentiation of mouse ES cells into highly defined motor neuron subtypes.

Progress:

Aim 1: *Defining co-factors that determine the Hox-sensitivity of spinal motor neurons.*

The organization of neurons into columns is a prominent feature of central nervous system structure and function, which leads to the establishment of topographic neural maps. This link is prominent in the developing spinal cord, where columnar sets of motor neurons innervate distinct targets in the periphery. It was found that sequential phases of Hox-c protein expression and activity control the columnar differentiation of spinal motor neurons. Hox expression in neural progenitors is established by graded fibroblast growth factor signaling and translated into a distinct motor neuron Hox pattern. Motor neuron columnar fate then emerges through cell autonomous repressor and activator functions of Hox proteins. Hox proteins also direct the expression of genes that establish motor topographic projections, thus implicating Hox proteins as critical determinants of spinal motor neuron identity and organization.

Aim 2: *Application of pathways of motor neuron specification to ES-cell derived motor neurons.*

Studies over the past few years have revealed that ES cells can respond to extrinsic signals that direct the normal pathway of motor neuron (MN) differentiation and can generate spinal MNs at high efficiency. The pathway of motor neuron generation from ES cells recapitulates the steps of motor neuron generation *in vivo* – ES cells grown as embryoid bodies undergo neuralization to induce neural tissue, caudalization to specify the spinal identity, and ventralization to specify the motor neuron identity (Renoncourt et al., 1998; Wichterle et al., 2002). Two key signaling molecules regulate the ES cell

differentiation process – retinoids are responsible for the neutralization and caudalization of ES cells, and hedgehog signaling is responsible for ventralization of spinal cells. ES cell-derived motor neurons generated *in vitro* acquire electrophysiological properties that resemble their embryo-derived counterparts; they develop appropriate ionic currents in response to neurotransmitters, and can receive synaptic inputs and fire repetitively at rates sufficient for functional muscle contraction (Miles et al., 2004). In addition, they can form functional synapses with cultured muscle cells. ES cell-derived motor neurons can also repopulate the embryonic and adult spinal cord *in vivo* (Harper et al., 2004; Wichterle et al., 2002). In an embryonic environment, ES cell motor neurons can extend axons into the periphery and form synapses with muscle targets (Wichterle et al., 2002).

Initial studies of the antero-posterior segmental identity revealed that most ES cell-derived MNs generated in the presence of retinoic acid and hedgehog attain the anterior cervical identity, manifested by the expression of Hox5 genes and by the lack of expression of more posterior markers (Hox8-10). During normal development, posterior neural tissue is induced by signals emanating from paraxial, lateral plate and presomitic mesoderm. Among these caudalizing signals retinoids impose cervical spinal identity, while members of FGF, GDF and Wnt families of signaling molecules are implicated in the induction of more caudal (thoracic and lumbar) positional identities.

Similarly to the effects observed in the developing embryo, retinoids present in MN differentiation medium induce the anterior cervical positional identity (Hox5+/Hox8-) of ES cell-derived motor neurons. Elimination of both exogenous retinoids and hedgehog signals from the serum-free differentiation medium is sufficient to permit generation of more caudal motor neurons expressing Hox8, a lower cervical/upper thoracic positional marker. The Hox8-positive motor neurons can be further caudalized by FGF and GDF signaling molecules to give rise to thoracic (Hox9+) and lumbar (Hox10+) MNs, respectively. The yield of motor neurons in these basal culture conditions is relatively low (~7%). Development of more efficient methods for generation of caudal MNs depends on detailed understanding of molecular mechanisms underlying neuralization, caudalization and ventralization in the absence of retinoids.

Here are described studies on the role of FGF and Wnt signaling molecules in the specification of Hox8+ MN identity. Further, it is demonstrated that Hox8+ motor neurons generated from ES cells *in vitro* attain the identities of several prominent motor neuron pools found in the posterior cervical region of the spinal cord. Finally, a screen was initiated to identify novel genes expressed in a motor neuron subtype-specific manner. The functional signature of differentially expressed genes might illuminate novel mechanisms underlying consolidation of motor neuron subtype identity and formation of motor pool-specific neural circuits in the developing spinal cord.

- **Maria Knikow, Ph.D., City University of New York, College of Staten Island, “Sensorimotor Control of Spinal Locomotor Centers in Human SCI,” 1/1/06-12/31/07 IDEA grant.**

Signals from the muscles of the hip region and from the skin of the foot sole convey information to spinal reflex circuits that are involved in walking. Based largely on findings derived from experimental studies conducted in animals, stepping over a treadmill while some body weight is removed is currently the main tool for walking re-training of patients with a spinal cord injury (SCI). Nonetheless, the mechanisms involved are largely unknown, so the effects of this intervention cannot be maximized.

Because establishment of a subcontract between the Research Foundation of City University of New York (CUNY) and the University of Louisville was delayed, the principal investigator started work on this project thereafter. Currently in development are the script files that will be used to acquire and analyze all experimental data and to accurately control the stimulators of the peripheral nerves of the legs during assisted walking in people with SCI. Further, a protocol was established to recruit people with a SCI, and the experimental protocol has been approved by the Institutional Review Boards of CUNY and the University of Louisville. The next steps in the project are to recruit potential subjects, test all equipment for proper function and arrange for patients to participate in experimental sessions.

The findings of this project will help better understand how the spinal cord functions after an injury so as to maximize locomotion re-training in people with impaired motor control of the legs by proper excitation of these receptors during assisted walking. The objective of this research project is to establish the contribution of the sensory feedback to activation of the leg muscles during body-weight-supported treadmill walking in people with a motor incomplete spinal cord injury (SCI). The overall hypothesis is that hip proprioceptors and cutaneous afferents of the foot sole modify functional spinal interneuronal circuits that form part of the locomotor generator promoting stepping in SCI. This hypothesis will be addressed with the following specific aims:

Aim 1 a: To characterize the expression of the soleus H-reflex, the flexion reflex and the muscle activation pattern as a function of the hip angle and the gait cycle.

Aim 1 b: To determine the functional role of spinal interneuronal circuits by establishing spinal inhibitory neuronal mechanisms (reciprocal Ia inhibition, group I non-reciprocal inhibition; presynaptic inhibition) as a function of hip position and gait cycle.

Aim 1 c: To quantify the effects of cutaneous afferents from the foot sole onto spinal reflex expression (H-reflex and flexion reflex) as a function of the hip angle and gait cycle.

- **Takeshi Sakurai, Ph.D., Mount Sinai School of Medicine, “Enhancement of Remyelination by Modulating RPTPa Activity,” 1/1/06-12/31/07 IDEA grant.**

The project goal is to obtain optimal myelination and node formation after spinal cord injury, structures crucial for supporting efficient nerve conduction. The intent is to characterize RPTPa involvement in the processes, mode of action and point of action, both at the cell level and animal level.

Aim 1. Where is RPTPa localized with respect to its candidate substrates during myelination in the PNS and CNS?

First, Western blotting analysis was performed of RPTPa protein expression levels using nerves and brains, but significant changes have not been observed in expression during the course of myelination. This suggests that either regulatory proteins/substrates of RPTPa may change their expression levels, and/or that specific localization of RPTPa may be important in the formation of the myelinated nerve structure. Therefore, a sub-aim was set to clarify localization of RPTPa during formation of myelinated nerves by immunohistochemistry, but unfortunately, antibodies tested so far do not lend themselves to immunostaining. Some gave signals, but in order to establish specificity of antibodies, tissues prepared must be from RPTPa knockout mice.

The third approach is to use lentivirus RPTPa construct to follow RPTPa localization in culture systems. As described below, lentivirus RPTPa fused with Venus-GFP protein was obtained and will be used for the analysis.

The other main objective of this aim is to identify potential substrates for RPTPa, and clarify their localization and relation to RPTPa expression patterns. Several candidates were available for potential substrates, and a 293-cell reconstituted system was used to study their phosphorylation status. When NrCAM, membrane protein involved in sodium channel clustering was transfected with RPTPa, phosphorylation of NrCAM was observed. Preliminary data suggest that src family kinases can phosphorylate NrCAM cytoplasmic region, and activation of src kinases can be modulated by RPTPa. Therefore, it is possible that a signaling pathway involving RPTPa has been identified.

During the course of studies using primary oligodendrocytes, it was realized that several tyrosine phosphatases are expressed in these cells involved in oligodendrocyte development. Among them, it was found that RPTPb, another receptor-type tyrosine phosphatase, plays a role in regulation of oligodendrocyte differentiation (submitted). Therefore, it is crucial to compare the phosphorylation patterns of these potential substrates using RPTPa knockout tissues, and sufficient numbers of animals must be obtained to perform these analyses.

Aim 2. What is the functional role of RPTPa during myelination?

The effects of overexpression and downregulation of RPTPa in several *in vitro* culture systems will be studied to gain insight into potential RPTPa function in formation of the myelinated nerve structure. An RPTPa lentivirus construct was obtained encoding RPTPa and shRNAs against RPTPa, for several *in vitro* myelinating cultures. Currently, shRNA lentivirus constructs are being built. Investigators have also attempted to establish several myelinating co-culture systems and had good experience with a DRG-Schwann cell co-culture system that reflects myelination in the peripheral nervous system (PNS). Myelination *in vitro* was obtained using cerebellar slice cultures that can be evaluated for degree of myelination.

Aim 3. How does modulation of the RPTPa activity affect myelinated nerves in the PNS and CNS *in vivo*?

It was decided to finish studies using cell cultures before making BAC transgenic mice of RPTPa. Since potentially suitable BAC clones were identified for overexpressing RPTPa under authentic regulatory sequence in the public domain, it is believed that BAC transgenic mice that overexpress RPTPa can be developed relatively quickly.

In terms of knockout analysis, the mouse colony is being expanded to supply sufficient numbers of RPTPa knockout mice and controls. In addition, new breeding pairs from an outside source are being introduced.

- **Kyonsoo Hong, Ph.D., New York University School of Medicine, “Sema3A-Induced Ca²⁺ Signaling in Xenopus Spinal Neurons,” 1/1/06-12/31/07 IDEA grant.**

The process of establishing functional neural connections relies on pathfinding by growth cones, the chemo-sensing cellular domain of neurites of either developing or regenerating neurons, to their correct target cells. Nerve pathfinding depends on extracellular guidance cues, i.e., substrate-bound or diffusible factors that act on growth cones. Intracellular Ca²⁺ signaling in growth cones is essential for regulation of growth cone responses to diffusible guidance factors. Researchers here previously reported that growth cone response to a gradient of the diffusible guidance molecule, netrin-1, depends on intracellular Ca²⁺ level ([Ca²⁺]_i) where a high increase of [Ca²⁺]_i results in attraction and a moderate increase results in repulsion of growth cones of young, developing, neurites of Xenopus spinal neurons. Recent studies by others consistently support these findings that changes in [Ca²⁺]_i brought about by changes in either the internal or external environment of the growth cones switch the direction of growth cone extension. Thus, understanding the underlying mechanism of intra-growth cone Ca²⁺ signaling in response to guidance signals may provide a basis for development of a therapy to promote neuronal regeneration in the injured adult central nervous system. *The goal of this project is to elucidate the cellular and molecular mechanisms of intracellular Ca²⁺ signaling induced by Semaphorin 3A (Sema3A), a diffusible guidance factor that plays an important role during nervous system development, and, due to its*

repellent action on growing neurites, also has been implicated as an inhibitor of nerve regeneration.

Both *in vivo* and *in vitro* studies have shown that semaphorins exhibit bi-functional action depending on the level of cGMP in growing neurites by an as-yet-unknown molecular mechanism. Recent studies revealed that cGMP induced by Sema3A causes growth-cone membrane hyperpolarization essential for transducing the repulsive signal in cultured *Xenopus* spinal neurons. Preliminary studies showed that a moderate increase in $[Ca^{2+}]_i$ is required during the Sema3A-induced repulsion. During the first funding period, researchers focused on the original Aim I, *the identification and determination of the function of Ca^{2+} conducting channels responsible for the early signaling events in response to the repulsive Sema3A signal in cultured *Xenopus* spinal neurons.*

It was found that the cGMP induced by repulsive Sema3A activates cyclic nucleotide gated cation channels (CNGCs) and mediates the repulsive turning response of *Xenopus* spinal neuron growth cones. Down-regulation of the endogenous *Xenopus* CNGA1 subunit (xCNGA1), either by treatment with antisense morpholino oligonucleotides or by overexpression of the xCNGA1 subunit deleted of its cGMP-binding domain, blocked CNG currents and converted Sema3A-induced growth-cone repulsion to attraction. Thus, these findings provide a new insight into cGMP-activated CNGCs during growth cone guidance and suggest that therapeutic conversion of Sema3A-induced turning by down-regulation of CNGCs may promote adult nerve regeneration.

The laboratory will continue to study the function of cGMP-activated CNGCs and their regulation during Sema3A-induced repulsion, as proposed in Aim I-2. Work will begin on the original Aim 2, the establishment of primary cultures of regeneration-competent mature *Xenopus* spinal neurons derived from embryonic stages 36 to 48, to examine the Sema3A-induced Ca^{2+} signaling in cultured mature *Xenopus* spinal neurons.

Further study of mature cultured neurons will allow investigation of whether Sema3A signaling changes occur in mature neurons. These studies will advance understanding of repulsive Sema3A signaling during development and nerve regeneration.

- **Mark Noble, Ph.D., University of Rochester, “Remyelination of SCI: Overcoming the Inhibitors,” 1/1/06-12/31/07 IDEA grant.**

Symptoms of spinal cord injury (SCI) primarily result from disconnection of neurons located above and below the injury site and also from destruction of the myelin sheaths required for impulse conduction in surviving neurons. Thus, successful regeneration of injured axons and restoration of lost neuronal connectivity are likely to be insufficient to restore neuronal function in the absence of repair of demyelinating damage. At the same time, myelin expresses molecules that can themselves inhibit neuronal regeneration. Thus, it is necessary both to promote repair of demyelinating damage and to override inhibitory signals that may interfere with neuronal regeneration.

In order to balance these competing needs, researchers here are identifying the genes expressed in oligodendrocyte progenitor cells (OPCs), and, in oligodendrocytes that might inhibit successful remyelination and/or inhibit neuronal regeneration. Two approaches are used to address this problem:

First, a microarray-based approach is used to find genes expressed in OPCs, and potentially regulated during oligodendrocyte maturation. The focus is on gene products expressed on the cell surface that have previously been implicated in regulation of cell migration, cell differentiation, or process outgrowth. In a parallel line of investigation, a candidate gene approach is used to study the function of Lingo-1 and its interaction partners in myelination. Lingo-1 has recently emerged as an important regulator of myelination. Lingo-1 is an axonal protein composed of leucine-rich repeats (LRRs) and an Ig-like domain. Lingo-1 is a potent inhibitor of oligodendrocyte differentiation and myelination (Lee et al., 2007). As such, it could have useful roles in causing the pool of progenitors to expand, but it also may be necessary to override Lingo signaling to obtain myelination.

To identify genes that may play an important role during maturation of oligodendrocyte progenitors, researchers first compared gene expression profiles of OPCs and the progenitor cells from which they are derived, the embryonic glial-restricted precursor (GRP) cell. Several members of the semaphorin gene family were found that are regulated during oligodendrocyte differentiation. Most notably, some members of the class 6 semaphorin subfamily (Sema6A, 6B, 6C, and Sema6D) and the class 5 semaphorin Sema5A are upregulated during oligodendrocyte differentiation. The microarray expression data for class 6 semaphorins was confirmed by q-PCR, and for Sema6B and Sema5A by Western blot analysis of GRP and O2A cell lysates. Class 6 and class 5 semaphorins are type 1 membrane proteins implicated in reverse-signaling, e.g., depending on the cellular context they may function as ligands for Plexin receptors or serve as receptors themselves. Second, to explore the role of these molecules in oligodendrocyte differentiation an RNAi-based strategy will be pursued coupled with markers specific for oligodendrocyte differentiation. In a parallel approach, advantage will be taken of Sema5A transgenic mice (which express Sema5A under the transcriptional control of the nestin promoter) to examine whether ectopic expression of Sema5A influences differentiation of oligodendrocyte progenitors. Also investigated will be the ability of OPCs, in which protein expression is reduced, to migrate on glial scar tissue.

To begin to address how Lingo-1 inhibits oligodendrocyte differentiation, first a full-length Lingo-1 was cloned using an RT-PCR approach, and a Lingo-ectodomain-Fc (Lingo-Fc) fusion protein is being generated. Lingo-Fc will be expressed in HEK293T cells and purified by protein A-affinity chromatography. To test the bioactivity of recombinant Lingo-Fc, fusion protein will be used to inhibit the differentiation of OPCs *in vitro*. Interestingly, GRPs do not express NgR1, a neuronal receptor for Lingo-1. Lingo-Fc, in an expression cloning approach, will be used to identify receptor candidates in the search for new binding partners of Lingo-1.

- **Wen-Biao Gan, Ph.D., New York University School of Medicine, “The Role of Microglia in Axon Regeneration Following Spinal Cord Injury,” 1/1/06-12/31/07 IDEA grant.**

Following spinal cord injury, injured axons do not regenerate past the lesion. The failure of axonal regeneration is at least partially due to the scar that forms at the injury site. It is therefore important to understand the sequence of events leading to scar formation and to prevent the detrimental effects of the scar on axonal regeneration in spinal cord injury. Specific aims in the project are to:

Aim 1: examine microglial response and glial scar formation following spinal cord injury in living mice with two-photon microscopy; and

Aim 2: determine the effects of blocking G-protein-coupled purinergic receptors or connexin channels on microglial response, scar formation and axon regeneration in vivo.

In the first year of the project, an experimental setup was established that allows stable and high-resolution imaging of microglia in the living mouse spinal cord with two-photon microscopy. It was found that microglia in the injured spinal cord rapidly extend processes to surround the damaged area, similarly to an observation in the cerebral cortex. Because ATP release from peritraumatic regions plays an essential role in attracting microglial processes (Davalos et al., 2005), the mechanisms that underlie persistent release of ATP upon injury were further investigated. An immediate increase of cytosolic calcium was found in astrocytic processes near the site of injury. This calcium increase persisted long after the injury occurred and may lead to high ATP levels in peritraumatic regions through a calcium-dependent ATP release mechanism. In addition, it was found that ATP is sufficient to attract microglial processes in culture. Together, these data suggest that upon injury, an increase in cytosolic calcium from the injured astrocytes maintains high ATP levels in peritraumatic regions. A positive feedback between ATP release and calcium influx may play an essential role in maintaining high ATP levels near the site of injury long after the injury has occurred. These studies also suggest that blocking calcium influx into astrocytes after injury may be important to prevent microglial response and reduce the extent of axonal injury associated with SCI. In the second year, researchers here will examine the effect of blocking G-protein-coupled purinergic receptors or connexin channels on calcium dynamics, microglial response, scar formation and axon regeneration as proposed. These investigations will provide the first glimpse at microglial response, scar formation and axonal regeneration following spinal cord injury, and will test treatments aimed at enhancing axonal regeneration.

- **Joseph A. Helpern, Ph.D. and Ray F. Lee, Ph.D., New York University School of Medicine, “*In Vivo* MR Microscopy of the Human Spinal Cord at 7 Tesla,” 1/1/06-12/31/07 IDEA grant.**

The ability of magnetic resonance imaging (MRI) at high magnetic field strengths (i.e., 7 Tesla) to detect small changes in tissue structure and function will provide unique information in patients with spinal cord injury (SCI). It is predicted that imaging will enable more specific classification of SCI by detecting lesions that previously were unseen by conventional MRI. It was proposed to build and test a radio frequency (RF) coil for spinal cord MRI at a high magnetic field strength of 7 Tesla. The most technically challenging aspect of MRI at 7 Tesla lies in development of strategies for RF coil construction and management of RF power deposition, which must remain within acceptable limits for humans. This coil design includes four loops and four strips, and was mainly driven by the linear anatomical presentation of the spinal cord. The coil is designed to transmit RF power with four strips, and receive the RF signal with four strips and four loops. Work in year 1 included the initial design phase, prototype construction and testing in phantoms. Over the past four months, researchers here have designed and constructed the first prototype and are bench-testing the coil performance. Once bench-testing is successfully completed, phantom imaging will begin on the 7 Tesla MRI to measure the performance of the coil by assessing its homogeneity and to monitor for "hot spots" in the transmission field. Very good homogeneity is important for the coil, and there should not be any "hot spots." The results of these tests will be analyzed and will drive further engineering design changes.

- **James Salzer, Ph.D., New York University School of Medicine, “*Role of Neuregulin 1 in Spinal Cord Remyelination*,” 1/1/06-12/31/07 IDEA grant.**

Introduction: Nerve fibers are covered by myelin, an insulating sheath that enhances conduction of nerve impulses and helps maintain the integrity of the nerve fibers. Functional recovery of the injured spinal cord thus requires not only regeneration of nerve fibers, but also formation of new myelin sheaths. Indeed, loss of myelin, or demyelination, results in progressive degeneration of axons, and is believed to be a significant contributor to disability in spinal cord injury. Enhanced remyelination is therefore an important therapeutic goal. Recent studies here suggest that a growth factor on the axon surface, termed neuregulin 1 (NRG1), promotes myelin formation in the brain and helps to control how much myelin is formed. NRG1 is made in several different forms, including one called type III that is attached to the axon surface. It is proposed to extend these studies to determine whether NRG1, particularly type III, also controls myelin formation in the spinal cord and might be useful to promote new myelin formation (remyelination) after injury. If so, NRG1 might be of value in serving two important, related goals: neuroprotection and enhanced impulse propagation.

Progress: Analysis of mice with reduced numbers of genes encoding the type III NRG1 isoform suggest that NRG1 protein levels in the spinal cord are stable, whereas the brains of these mice show reduced levels of NRG1, suggesting that regulation of NRG1 is under

complex regional control within the central nervous system. It was further found that myelination in the spinal cord of these mice is normal – unlike the brains, which have reduced myelin levels. Viruses were generated that can drive expression of NRG1 genes when injected into the spinal cord. These viruses will be used to assess the role of NRG1 in remyelination in the rat spinal cord.

- **Ben G. Szaro, Ph.D., SUNY University at Albany, “Gene Expression Profiling in Successful Spinal Reinnervation,” 1/1/06-12/31/07 IDEA grant.**

The objective of this grant is to identify genes that may be important for spinal cord regeneration by sampling from tadpole brains individual neurons that can actually regenerate an axon when the spinal cord is severed. The neurons under study are those that in humans send signals for movement from the brain to the spinal cord. Whereas in humans these neurons cannot regenerate an axon, they do so readily in the tadpole. A state-of-the-art, microsurgical laser is used to dissect these neurons from sections of brainstem harvested at various times after injury. First, researchers are localizing and quantitating the expression of individual genes known, from studies in other systems, to respond in characteristic ways to injury when axon regeneration is successful. Once these reference data are obtained, gene arrays will be used to study the expression of tens of thousands of genes at once to look for those correlating with successful regeneration. Finally, once these genes are identified, researchers will change their expression in transgenic frogs to determine those which actually play a major role in spinal cord regeneration. This year, a study was published that definitively demonstrated that the large, so-called reticular magnocellular neurons actually regenerate an axon when the spinal cord is cut. In addition, researchers have nearly finished characterizing the expression response of four separate genes known to respond to successful regeneration in other systems. Two of these (NF-M and GAP43) exhibit the same response to injury and successful regeneration seen in all other systems that regenerate, including mammalian peripheral nerve, frog and fish optic nerve, and lamprey spinal cord, further validating this approach. Another gene (musashi), because it is only expressed in proliferating neuronal stem cells, demonstrates that regeneration in frog occurs without replacing the damaged neurons with new cells, further establishing similarities between frogs and humans by the nature of their injury responses. These results are now being replicated in preparation for publication, while the final parameters are determined for sampling neurons by laser capture ahead of the microarray work. Pieces of DNA are in preparation that can be used to make transgenic frogs. Work will commence to knock-out or over-express candidate genes to test their function in transgenic animals. The impact of this work is to characterize at the molecular level what constitutes a successful injury response in a spinal cord that can regenerate, as well as identify potential targets for gene therapy approaches for treating spinal cord injury in humans.

- **Roman Giger, Ph.D. and S-S Sheu, Ph.D., University of Rochester, “Calcium Trap for the Myelin-Associated Glycoprotein Receptor,” 1/1/06-12/31/07 IDEA grant.**

Neurological deficits as a consequence of SCI are primarily the result of lost neuronal connectivity between neurons located proximal and distal to the injury site. While many neurons survive the consequences of SCI, they fail to grow fibers necessary to restore lost synaptic contacts. The main goal of this research project is to define mechanisms that inhibit axonal growth in the injured adult mammalian nervous system.

The focus is on characterization of the receptor for myelin-associated glycoprotein (MAG), a potent inhibitor of axonal growth. Understanding the mechanisms of growth inhibition will allow development of strategies aimed at promoting neuronal growth and restoring neuronal contacts lost as a consequence of injury.

***Aim 1:** To identify signal-transducing molecules in the NgR2-MAG receptor complex.*

For screening to identify candidate NgR2 co-receptors, a "calcium trap" was developed. As shown in a recent manuscript (Duan et al., 2006; submitted), imaging of calcium mobilization in primary neuronal cells is a sensitive and routine procedure. NgR2 was identified as a lipid-linked surface molecule and high-affinity receptor for MAG. Currently, it is not clear how NgR2, upon binding of MAG, signals inhibition across the neuronal cell membrane. One important second messenger that has been shown to mediate MAG responsiveness is ionic calcium (Ca^{2+}). MAG binding to its receptor(s) leads to a transient increase in cytosolic $[\text{Ca}^{2+}]_i$. Imaging of $[\text{Ca}^{2+}]_i$ mobilization is used at the single-cell level to detect the presence of candidate signal-transducing components in an NgR2 receptor complex. A cDNA library was generated (from dorsal root ganglion neurons), aliquoted into 200 pools and is being screened by co-transfection together with NgR2 into 293T cells. Twenty-four hours following transfection, cells are loaded with the calcium-sensitive dyes and then stimulated with MAG-Fc. Following MAG-Fc application, calcium mobilization is imaged. Screening of the library is ongoing. Control experiments in primary neurons (e.g., following MAG-Fc application) lead to Ca^{2+} mobilization, demonstrating that the assay has the sensitivity required to monitor MAG responses.

***Aim 2:** To use PC12 cells, stably expressing recombinant NgR2, as a source for biochemical purification of novel MAG receptor components.*

One limitation of biochemical studies is the lack of robust anti-NgR2 antibodies. To address this problem, in a collaborative effort with Christoph Rader at the National Cancer Institute, monoclonal antibodies were developed specific for the Nogo receptor family members NgR1 and NgR2 (see Hofer et al., 2006: In this study, anti-NgR2 was developed that recognizes mouse, rat and human NgR2, but does not cross-react with NgR1 or NgR3 from mouse, rat or human). As a control for the biochemical studies, affinity precipitation experiments with MAG-Fc from lysates of PC12 cell lines over-expressing NgR2 were found to contain large amounts of NgR2. More importantly, mice

were recently developed with a targeted deletion in the NgR2 locus (*NgR2*^{-/-}). In addition, these mice contain a knock-in of an eGFP reporter cassette that allows monitoring of NgR2 expression using anti-GFP immunohistochemistry. *NgR2*^{-/-} mice are viable into adulthood. Immunoprecipitation experiments with anti-NgR2 in wild-type and age-matched *NgR2*^{-/-} mice showed selective immunoprecipitation of MAG in wild-type but not in NgR2-deficient mice. Because of the initial success of these biochemical studies it was decided to pursue a large-scale approach to affinity-purify NgR2 binding partners from PC12 cells overexpressing NgR2 and from wild-type mouse brain extracts (using *NgR2*^{-/-} brain lysate as a control). These experiments are ongoing, and affinity-purified NgR2 binding partners will be analyzed using a proteomics approach.

- **Samie Jaffrey, M.D., Ph.D., Weill Medical College of Cornell University, “Novel mRNA Regulatory Pathways Underlying Axon Growth in SCI,” 1/1/06-12/31/07 IDEA grant.**

The objective of the proposed research is to characterize the molecular mechanisms that mediate axonal retraction, neurite outgrowth inhibition, and growth cone (GC) collapse in response to inhibitory stimuli. The limited regenerative capability of CNS neurons is attributed to their responsiveness to inhibitory molecules in myelin. Work in recent years has demonstrated that these inhibitory molecules increase RhoA activity in neurons causing growth cone collapse. However, the mechanism of RhoA activation remains elusive. In preliminary studies, it was found that RhoA mRNA translation in the growth cone mediates the collapsing effects of Sema3A. Sema3A is expressed in neuronal scar tissue and may contribute to preventing axonal growth in spinal cord injury (SCI). The mechanism of other collapsing proteins, such as myelin-associated glycoprotein (MAG) or Nogo-66, including whether they regulate RhoA translation, remains unknown. During the project period, the question of whether this mechanism is operative in regenerating axons was addressed. The data indicate that: (1) RhoA mRNA is localized to regenerating postnatal DRG axons; (2) RhoA mRNA is translated in regenerating axons; (3) MicroRNAs that are predicted to bind the RhoA mRNA are found in axons. Together, these data, along with other experiments, demonstrate substantial progress towards the goals of this project and identify a potentially critical mechanism that might underlie axonal regeneration in injured spinal cord axons.

- **Neeta S. Roy, Ph.D., Weill Medical College of Cornell University, “Development of Rodent-Human Chimeras for Study of Spinal Cord Injury,” 1/1/06-12/31/07 IDEA grant.**

Introduction: Bi-potential progenitors capable of giving rise to both oligodendrocytes and motor neurons are uniquely specific to the developing ventral spinal cord. Such progenitors would therefore be extremely useful for repair in acute SCI, where both oligodendrocyte and motor neuron loss play a major role in the neurological deficits observed in patients. These studies propose to determine the potential therapeutic utility for SCI repair by hES cell-derived bi-potential progenitors selected on the basis of pro-neural transcription factor Neurogenin 2 (Ngn2) expression. Cells selected on the basis of this specific promoter will be transplanted in both the developing neural tube and

injured adult rat spinal cord, and assessed for: (a) engraftment competence; (b) differentiation potential; (c) structural integration; and (d) impact on functional improvement in the injured rats.

Aim: Can hES cell-derived progenitors isolated on the basis of GFP expression driven by the SC-specific Ngn2/4.4kb promoter survive and integrate in the injured SC of adult rats? Will they differentiate as motor neurons, oligodendrocytes, or both, in the highly dynamic environment of the injured SC tissue? Will this translate into functional recovery of the injured animals?

Observations/results:

1. hES cell culture-derived Ngn2-expressing progenitors survive in the spinal cord of adult rats subjected to SCI. Survival has been observed for as long as three months post-transplant following daily Cyclosporin A injections.
2. At three months post-transplant, <10% of the cells differentiate as motor neurons. Most of the transplanted cell population remains as undefined Ngn2 or Ngn2/BIII-tubulin-expressing cells. No evidence of differentiation as oligodendrocytes has been observed at three months post-transplantation.
3. Animals subjected to SCI and transplanted with Ngn2-defined progenitors show marginally improved BBB scores at three months post-transplant as compared to untransplanted injured control animals.

Future aims:

- Study the impact of hES cell-derived progenitor transplant in adult rats subjected to SCI at longer periods post-transplant (six months).
- Determine the ability of these progenitors to integrate into and differentiate in the developing rat spinal cord for design of chimeric animal models for SCI study.

E. Publications Resulting from SCIRB-Funded Research

During 2006, a total of 13 papers, one textbook chapter, and 13 abstracts stemming from 26 SCIRB-funded projects active in 2006 were published by 11 principal investigators (Appendix III). Twenty-three of these project contracts started in 2006.

Also, three patent applications were filed (Appendix IV).

V. Program Operations

After four years of service, the Board's executive secretary, Dr. Martin D. Sorin, assumed other duties as of October 1, 2006, and was replaced by Ms. Bonnie Brautigam. Simultaneously with that leadership transition, two other changes were implemented.

First, the program was integrated into the Wadsworth Center's Office of Research Guidance, where it now benefits from existing administrative infrastructure. Second, the Wadsworth Center created a centralized Contracts Unit to assist the program in tracking and execution of contracts, amendments, and payment of vouchers. In addition, the position of Associate Accountant was vacated in January 2006 and filled in October 2006. The program anticipates increased stabilization, improved communication with the Board and contractors, and significant scientific advances as a result of these changes.

VI. Fiscal Status of the Spinal Cord Injury Research Trust Fund

As of December 31, 2006, cash deposits to the Fund totaled \$59.5 million. Interest on unexpended funds rose to more than \$3.8 million for a total of \$63.3 million in revenue, minus a 2002 cash sweep into the General Fund of \$13.5 million, leaving a total of \$49.8 million.

Disbursements include vouchers paid (\$21.7 million) and administrative costs (\$1.6 million) over the life of the Fund. Encumbrances, or the amount obligated for future expenditures from approved contracts (about \$28 million), combined for a total of \$51.4 million in liabilities.

It is estimated that the Trust Fund has a negative balance of \$1.5 million, including reappropriated, unencumbered funds (see Current Balance). This amount will be further reduced by estimated first-year obligations from the new CART/IDEA and PostDoctoral/Mentored Scientist/Mentored Clinician contracts scheduled to begin April 1, 2007 (\$2 million under Estimated First-Year New Encumbrances), leaving an estimated balance in the Fund of negative \$3.6 million.

BALANCE SHEET
SPINAL CORD INJURY RESEARCH TRUST FUND
FROM INCEPTION THROUGH THE YEAR ENDED DECEMBER 31, 2006
Fund Balance – as of 12/31/06

Estimated Assets	
Cash deposits	\$59,500,000
Less cash sweep	(\$13,500,000)
Interest earnings	\$3,836,398
Total Assets	\$49,836,398
Liabilities	
Disbursements	\$23,413,378
Encumbrances	\$28,008,659
Total Liabilities	\$51,422,037
Current Balance	(\$1,585,639)
Estimated first-year new encumbrances	\$2,035,040
Total Estimated Liabilities	\$53,457,077
Total Estimated Balance*	(\$3,620,679)

* **Note:** \$8.5 million was expected to be deposited for FY 06/07 in late March 2007.

Appendix I
Chapter 338, Laws of 1998, as Amended by Chapter 612, Laws of 1999

Title IV, § 250. Spinal Cord Injury Research Board.

1. A spinal cord injury research board is hereby created within the department for the purpose of administering spinal cord injury research projects and administering the spinal cord injury research trust fund created pursuant to section ninety-nine-f of the state finance law. The purpose of research projects administered by the board shall be neurological research towards a cure for such injuries and their effects. The members of the spinal cord injury research board shall include but not be limited to representatives of the following fields: neuroscience, neurology, neuro-surgery, neuro-pharmacology, and spinal cord rehabilitative medicine. The board shall be composed of thirteen members, seven of whom shall be appointed by the governor, two of whom shall be appointed by the temporary president of the senate, two of whom shall be appointed by the speaker of the assembly, one of whom shall be appointed by the minority leader of the senate, and one of whom shall be appointed by the minority leader of the assembly.
2. Board members shall be reimbursed for ordinary travel expenses, including meals and lodging, incurred in the performance of duties pursuant to section two hundred fifty-one of this title.
3. The terms of board members shall be four years commencing January first, nineteen hundred ninety-nine.
4. At the end of a term, a member shall continue to serve until a successor is appointed. A member who is appointed after a term has begun shall serve the rest of the term and until a successor is appointed. A member who serves two consecutive full four year terms shall not be eligible for reappointment for four years after completion of those terms.
5. A majority of the full authorized membership of the board shall constitute a quorum.
6. One member of the board shall be chosen by the governor to serve as chairperson.
7. Meetings of the board shall be held at least twice a year but may be held more frequently as deemed necessary, subject to call by the chairman or by request of a majority of the board members. Board meetings shall concern, among other things, policy matters relating to spinal cord injury research projects and programs, research progress reports, and other matters necessary to carry out the intent of this title.
8. Members of the board shall be indemnified pursuant to section seventeen of the public officers law.

Title IV, § 251. Powers and Duties.

The spinal cord injury research board created pursuant to section two hundred fifty of this title shall:

1. Formulate policies and procedures necessary to carry out the provisions of this title;
2. Solicit, receive, and review applications from public and private agencies and organizations and qualified research institutions for grants from the spinal cord injury research trust fund, created pursuant to section ninety-nine-f of the state finance law, to conduct research programs which focus on the treatment and cure of spinal cord injury. The board shall make recommendations to the commissioner, and the commissioner shall, in his or her discretion, grant approval of applications for grants from those applications recommended by the board.
3. Ensure that state funds, appropriated for spinal cord injury research are not diverted to any other use; and
4. Provide the governor and the legislature an annual report by January thirty-first of each year succeeding the year in which this title shall take effect setting forth the status of funds appropriated for spinal cord injury research and the progress of the Board in terms of the results of its spinal cord injury research efforts.

§ 3. Article 2, section 17 of the public officers law is amended by adding a new paragraph (m) to read as follows:

(m) For the purposes of this section, the term “employee” shall include the members of the spinal cord injury research board within the department of health.

§ 4. Notwithstanding any inconsistent provisions of law to the contrary, effective April 1, 1999, an amount not to exceed \$8,500,000 shall be annually transferred from the general fund out of the mandatory surcharges collected pursuant to subdivision 1 of section 1809 of the vehicle and traffic law to the spinal cord injury research trust fund held by the state comptroller pursuant to section 99-f of the state finance law which monies shall then be deposited to the credit of the spinal cord injury research trust fund pursuant to section 99-f of the state finance law. Each such payment shall be accompanied by a true and complete report in such form and detail as the comptroller shall prescribe. Nothing contained in this section shall be construed to authorize the transfer to the spinal cord injury research trust fund of any monies collected under section 1809 of the vehicle and traffic law that are otherwise authorized to be deposited to the credit of the criminal justice improvement account established pursuant to section 97-bb of the state finance law.

§ 5. Article VI of the state finance law is amended by adding a new section 99-f to read as follows:

§ 99-f. Spinal cord injury research trust fund.

1. There is hereby established in the joint custody of the state comptroller and the commissioner of taxation and finance a special revenue fund to be known as the "spinal cord injury research trust fund."

2. The fund shall consist of all monies appropriated for its purpose, all monies required by this section or any other provision of law to be paid into or credited to such fund, and monies in an amount not to exceed eight million five hundred thousand dollars collected by the mandatory surcharges imposed pursuant to subdivision one of section eighteen hundred nine of the vehicle and traffic law. Nothing contained herein shall prevent the department of health from receiving grants, gifts or bequests for the purposes of the fund as defined in this section and depositing them into the fund according to law.

3. Monies of the fund, when allocated, shall be available for administrative costs of the spinal cord injury research board established pursuant to title four of article two of the public health law and for funding spinal cord injury research projects administered by such board.

4. Monies shall be payable from the fund on the audit and warrant of the state comptroller on vouchers approved and certified by the commissioner of health.

§ 6. This act shall take effect January 1, 1999

Appendix II
NEW YORK STATE
SPINAL CORD INJURY RESEARCH BOARD
Bylaws

I. OFFICERS

1. The officers of the Spinal Cord Injury Research Board ("Board") shall be the Chair and Vice-Chair. The Chair is designated by the Governor. The Vice-Chair shall be selected by the Chair and shall serve for one year or until his or her successor has been selected.
2. The Chair may appoint a Board member to preside during the absence of the Chair and Vice-Chair from any meeting.

II. DUTIES

1. The officers of the Board shall perform the duties ordinarily associated with their respective offices.
2. The Chair shall be responsible for the general supervision of the work of the Board. The Chair shall represent the Board before the Governor, committees of the Legislature, or other public authorities, and may request any member or members to appear with him or her in his or her stead. The Chair shall preside at Board meetings.
3. The Vice-Chair, in the absence of the Chair, shall perform the duties of the Chair.

III. CODE OF ETHICS AND CONFLICT OF INTEREST

Section 1. Code of Ethics.

Members of the Board shall comply with Section 74 (Code of Ethics) of the Public Officers Law. No member of the Board should have any interest, financial or otherwise, direct or indirect, or engage in any business, transaction, or professional activity, or incur any obligation of any nature, which is in substantial conflict with the proper discharge of his or her duties as a Board member. Members should exercise their duties and responsibilities as Board members in the public interest of the inhabitants of the State, regardless of their affiliation with, or relationship to, any institution, organization, facility, agency, program, activity, category of provider, or interest group. The principles that should guide the conduct of Board members include, but are not limited to, the following:

- a) A Board member should endeavor to pursue a course of conduct that shall not raise suspicion among the public that he or she is likely to be engaged in acts that are in violation of his or her trust as a Board member.

- b) No Board member should permit his or her employment to impair his or her independence of judgment in the exercise of his or her duties as a Board member.
- c) No Board member should disclose confidential information acquired by him or her in the course of his or her duties as a Board member, or by reason of his or her position as a Board member, nor use such information to further his or her personal interests.
- d) No Board member should use, or attempt to use, his or her position as a Board member to secure unwarranted privileges or exemptions for himself or herself or others.
- e) No Board member should engage in any transaction as a representative or agent of the State with any business entity in which he or she has a direct or indirect financial interest that might reasonably tend to conflict with the proper discharge of his or her duties as a Board member.
- f) A Board member should not make personal investments in enterprises which may be directly involved in decisions to be made by him or her as a Board member or which shall otherwise create substantial conflict between his or her duty as a Board member to act in the public interest and his or her private interest.
- g) To preserve the public trust, Board members are prohibited during the tenure of their appointment from applying for or receiving support from the Spinal Cord Injury Research Trust Fund under Section 251 of the Public Health Law, or from having any role or interest (other than routine professional and collegial interest in the success of their institution or department) in proposals submitted for consideration by, or in research or proposals supported by, the Spinal Cord Injury Research Trust Fund.

Section 2. Conflict of Interest - Applications and other Pending Matters.

This section applies both to activities of the full Board and its committees.

- a) Absolute Disqualifications.
When a Board or committee member, or his or her family has an interest, financial or otherwise, whether as owner, officer, director, fiduciary, employee, colleague, consultant, or supplier of goods or services, in an entity, institution, organization, facility, agency or program (hereafter collectively referred to as "entity") whose application is before the Board or a committee of the Board for consideration or determination for a grant from the Spinal Cord Injury Research Trust Fund under Section 251 of the Public Health Law, that member shall (i) identify such interest to the Board or committee at any meeting when the application or request is to be considered, (ii) absent himself or herself from any portion of any meeting when such application is considered, and (iii) not participate in any vote of the Board or committee on such application. For purposes of this Article, "family" shall include a spouse, children, sibling, and any relative living in the member's household.

b) Disclosure and Possible Disqualification.

When a Board or committee member, or his or her family member has (i) any of the above-noted interests in an entity the status of which might reasonably be affected by another entity whose grant application is before the Board or a committee of the Board, or (ii) when a member has any other interest or association which might reasonably be construed as tending to embarrass the Board or elicit public suspicion that he or she might be engaged in acts in violation of his or her trust as a Board member, the member shall disclose such interest or association at the time the application or other matter is formally considered by the Board or committee, so that the Chair and, if necessary, the Board or committee can then determine whether the member's participation in the discussion or the vote on the application by the Board or by the committee or on the other matter would be proper.

c) Procedure.

Prior to the discussion of a grant application, the Chair of the Board and the Chair of the Committee shall request that Board members and committee members disclose all actual or potential conflicts and, when appropriate, explain the conflicts. In the case of conflicts constituting Absolute Disqualifications, the members with such conflicts shall immediately leave the meeting and remain absent during the period when the application is under consideration. In the case of conflicts constituting possible disqualifications, the Chair of the Board or Committee shall rule upon such conflicts subject to appeal by motion to the Board or committee that may override the Chair's decision by the affirmative vote of a majority of those present, excluding those members who are the subject of the vote.

d) Disclosure of Committee Interests to Board Meetings.

When the Chair of any committee reports the Committee's deliberations and recommendations on a matter to the Board, the Committee Chair shall indicate in the report all interests or associations disclosed by the committee members and state how such members voted with respect to the committee's recommendations.

e) Compliance with Public Officers Law.

Members of the Board shall comply with Sections 74 and 78 of the Public Officers Law as amended and the following rules governing conflicts of interest:

i) No member shall receive compensation in return for services rendered in relation to matters before any State agency if compensation is contingent upon action or failure to act by such State agency.

ii) No member of the Board who is also associated with any firm or association in which he/she has a specific interest shall sell any goods or services valued in excess of \$25 to any State agency unless pursuant to competitive bid.

iii) No member of the Board shall accept any gift (in excess of \$75) under circumstances in which it could reasonably be inferred that the gift was intended to influence him/her as a member of the Board.

iv) Members of the Board shall avoid any action which might result in or create the appearance of a conflict of interest.

f) Violation of Provisions.

If any member knowingly and intentionally violates these provisions, the Board or its Chair shall refer the matter to the Commissioner of Health for appropriate action.

IV. EXECUTIVE SECRETARY

The Board shall request the Department of Health to designate a Department employee as the Board's Secretary.

The Secretary shall prepare and send official notices of actions of the Board and shall administer the daily business of the Board under the general direction of the Chair. The Secretary shall send a copy of the minutes of each meeting of the Board to each member of the Board ten business days prior to the next Board meeting. The minutes, as approved or corrected, shall serve as the official record of a meeting of the Board. Minutes shall be distributed or made available to the public after they have been approved by the Board. The Secretary shall make available records requested under the Freedom of Information Law and make announcements to the media and public of scheduled meetings as required by the Open Meetings Law.

V. MEETINGS OF THE BOARD

a) Regular Meetings.

The regular meetings of the Board shall be held at least two times per year but may be held more frequently as deemed necessary, subject to a call by the Chair or by request of a majority of the Board members, at a date, time and place approved by a majority of members, unless otherwise determined by the Board or by the Chair, who shall notify the Secretary at least ten business days in advance of the meeting.

b) Meeting Notification.

The Secretary shall notify each Board member of Board meetings and shall send an agenda to his or her usual address not less than ten business days before the meeting.

c) Quorum.

A majority (seven members) of the members of the Board (13 members) shall constitute a quorum for the transaction of any business or the exercise of any power or function of the Board and all matters requiring action shall be passed by a vote of a majority of the voting members of the Board. (A voting member abstaining from a vote shall be counted as present for the purpose of establishing a quorum.) Except as provided below, all meetings shall be conducted in accordance with Robert's Rules of Order Newly Revised, and a record of each vote shall be maintained. The normal method of voting shall be by

roll call. A roll call vote on any question shall be taken by ayes and noes, abstentions noted, and a record of how each member voted entered in the Minutes.

d) Open Meetings.

Meetings of the Board shall be noticed and conducted in accordance with the requirements of Article 7 (Open Meetings Law) of the Public Officers Law. Such meetings shall be open to the public except when otherwise provided by law. Guidelines for observers shall be adopted by the Board.

e) Public Comment Period.

At least some portion of every regular Board meeting shall be set aside for public comment.

f) Order of Business.

The order of business may be altered at the Chair's discretion or upon the request of a Board member. A portion of each Board meeting shall be set aside for the development of an agenda for the next Board meeting.

g) Absences.

Any member who fails to attend three consecutive meetings of the Board, unless excused by formal vote of the Board, shall be deemed to have vacated his or her position.

VI. COMMITTEES

a. Standing Committees

There shall be the following Standing Committee:

A Scientific Review Committee for the scientific and technical merit review of requests for proposals (grant applications).

The Chair of the Board shall appoint the members of Standing Committee and designate its Chair. In appointing members to the Standing Committee, the Chair will, to the extent practicable, ensure that the Committee comprises national or international experts of the highest scientific and technical caliber appropriate to spinal cord injury-related research while minimizing the potential for real or apparent conflict of interest. The term of committee membership shall be three years from the date of appointment. The Chair of the Board shall prescribe duties of the Standing Committee with approval by a majority of Board members.

b. Ad hoc Committees

The Board may, at any time, appoint a special committee on any subject. All such special committees not previously discharged by the Board shall be considered discharged one year following their appointment, unless the Board shall move to continue them.

c. **Committee Actions**

All committee matters requiring action or a formal recommendation shall be passed by a vote of a majority of the members appointed to serve on the committee.

When making a report to the Board, a committee should, in addition to reporting any recommendations of the majority of the committee, summarize any significant deliberations leading to such recommendations as well as opinions or recommendations of committee members who did not support the majority recommendations.

VII. PROPOSAL REVIEW PROCESS

The Board shall establish merit review procedures to be used by the Scientific Advisory Committee which are modeled after the National Institutes of Health or the National Science Foundation as appropriate to the granting mechanisms the Board establishes.

VIII. OFFICE OF THE BOARD

The official headquarters of the Board (at which the official copies of its Minutes, records, documents and other papers shall be kept) shall be at the offices of the Commissioner of Health at Albany, New York. The Secretary shall be responsible for the safekeeping of all Minutes, records, documents, correspondence and other items belonging to the Board. Every member of the Board and any other person duly authorized by a member shall have access at all times during the ordinary office hours of the Department of Health to all such Minutes, records, documents, correspondence and other items belonging to the Board; provided, however, that persons authorized by members shall not have access to records, documents, correspondence or other items that are exempt from disclosure or confidential under the Freedom of Information Law, the Personal Privacy Protection Law, or any other state or federal law. The Secretary shall designate some person to be in charge of all such Minutes, records, documents, correspondence and other items belonging to the Board during his or her absence from the office.

IX. AMENDMENT OF BYLAWS

These Bylaws may be amended by the affirmative vote of the majority of the voting members of the Board at any regular or special meeting, provided that notice of the proposed amendment has been given at a prior meeting and that a copy of the proposed amendment has been sent by the Secretary to each member of the Board at least ten business days prior to the vote.

Appendix III

Publications Resulting from Spinal Cord Injury Research Board-Funded Projects

C016882 SUNY at Stony Brook

Project Title: Neurotrophins and Function of the Injured Spinal Cord

Arvanian VL, H Manuzon, M Davenport, G Bushell, LM Mendell, and JK Robinson (2006) Combined Treatment with Neurotrophin-3 and LSD Facilitates Behavioral Recovery from Double-Hemisection Spinal Injury in Neonatal Rats. *J Neurotrauma* **23**, 66-74.

Arvanian VL, WJ Bowers, A Anderson, PJ Horner, HJ Federoff, and LM Mendell (2006) Combined delivery of neurotrophin-3 and NMDA receptors 2D subunit strengthens synaptic transmission in contused and staggered double hemisected spinal cord of neonatal rat. *Exp Neurol.* **197**, 347-352.

2006 Society for Neuroscience Abstracts:

Arvanian, Schnell, Horner, Bowers, Federoff, Schwab, Mendell. Combination treatment with NT-3, NMDA-2D subunits and Anti-Nogo-A antibody increases connections of transected lateral funiculus fibers to lumbar motoneurons.

Schnell, Arvanian, Horner, Bowers, Federoff, Mendell, Schwab. Morphological evaluation of fibers projecting to motoneurons following lateral hemisection and combination treatment with Nogo-A antibody, NT-3 and NMDA-2D subunits in adult rats.

Garcia-Alias, Arvanian, Schnell, Horner, Bowers, Federoff, Levine, Fawcett, Mendell. Recovery of hindlimb motor function after a lateral spinal cord hemisection in adult rat is enhanced by combined administration of NT-3, NMDA-2D subunits and Chondroitinase-ABC.

C017688 University of Rochester

Project Title: Mitochondrial Glutathione: Protection Against Spinal Cord Injury

Sheu SS and JJ Lemasters (2006) Special Issue: Mitochondria in Diseases and Therapeutics. *Biochimica et Biophysica Acta-Molecular Basis of Disease* **1762**, 139.

Sheu SS, DG Brdiczka, and DB Zorov (2006) Mitochondrial Contact Sites: Their Role in Energy Metabolism and Apoptosis. *Biochimica et Biophysica Acta-Molecular Basis of Disease* **1762**, 148-163.

Sheu SS, D Nauduri, and MW Anders (2006) Targeting Antioxidants to Mitochondria: A New Therapeutic Direction. *Biochimica et Biophysica Acta-Molecular Basis of Disease* **1762**, 256-265.

Sheu SS, MW Anders, and JL Robotham (2006) Mitochondria: New Drug Targets for Oxidative Stress-Induced Diseases. *Expert Opinion on Drug Metabolism and Toxicology* **2** (1), 71-79.

C018609 New York University School of Medicine

Project Title: Role of Neurotrimin in Spinal Motor Pathways

Grijalva I, X Li, A Marcillo, JL Salzer, and AD Levi (2006) Expression of neurotrimin in the normal and injured adult human spinal cord. *Spinal Cord* **44** (5), 280-286.

C018615 Albert Einstein College of Medicine

Project Title: Regulating Axon Guidance in the Vertebrate Spinal Cord

Abstracts:

Kaprielian Z, SR Kadison, SL Reeber, JE Johnson, F Murakami, and M Matise. Commissural Axon Pathfinding on the Contralateral Side of the Ventral Midline in the Developing Mouse Spinal Cord. 65th Annual Society for Developmental Biology Meeting held at the University of Michigan, Ann Arbor, June 17-21, 2006. *Dev. Biol.*, **295**, 339-341.

Reeber S, E Carlin, N Sakai, Y Nakada, P Parab, JE Johnson, and Z Kaprielian. Elucidating the Molecular Mechanisms that Control the Guidance of DI1 and DI2 Commissural Axons on the Contralateral Side of the Floor Plate in the Developing Chick and Mouse Spinal Cord. Abstracts of papers presented at the 2006 meeting on Axon Guidance, Synaptogenesis & Neural Plasticity 159.

Reeber S, E Carlin, N Sakai, Y Nakada, P Parab, JE Johnson, and Z Kaprielian. Using Genetic Markers to Visualize Commissural Axon Trajectories in the Developing Chick and Mouse Spinal Cord. Abstracts of papers presented at the 2006 meeting on Axon Guidance, Synaptogenesis & Neural Plasticity, 160.

C020922 Albany Medical College

Project Title: Engineering Embryonic Spinal Cord Stem Cells for SCI Repair

Capela A and S Temple (2006) LeX Is Expressed by Principle Progenitor Cells in the Embryonic Nervous System, Is Secreted into their Environment and Binds Wnt-1. *Developmental Biology* **291** (2) 300-313.

C020929 SUNY at Stony Brook

Project Title: Remyelination and Spinal Cord Injury

Abstract:

Nolin WB, Y Zhang, JM Levine, and SE Tsirka. Interactions Between the tPA/plasmin Proteolytic Cascade and the CSPG NG2. Society for Neuroscience Annual Meeting, 2006.

C020930 SUNY – Upstate Medical University

Project Title: Repair of Cauda Equina Injury

Abstract:

Calancie B, DJ Blaskiewicz, DJ Stelzner, and RC Young. Repair of the Cauda Equina Using a Hemi-Cauda Injury Model in the Adult Rat. Society for Neuroscience Annual Meeting, 2006 poster 762.6.

C020931 SUNY Upstate Medical University

Project Title: Local Release of Chondroitinase to Treat Spinal Cord Injury

Stelzner DJ and AC Conta (2006) Rapid and Delayed Loss of Propriospinal Tract Neurons Following Moderate Spinal Contusion Injury in Adult Rat. Revision to *J. Comp. Neurol.*

Chapters:

Conta AC and DJ Stelzner (2006) The Propriospinal System – to appear in “The Spinal Cord – A Textbook and Atlas of the Mammalian Spinal Cord Anatomy.” Watson C and Paxinos G, Eds., Elsevier.

Abstracts:

Conta AC, J Siebert, and DJ Stelzner. Injury-Induced Sprouting of Propriospinal Tract Fibers Following Moderate Spinal Cord Contusion Injury. 88.14 Oct. 14, 2006 Program and Abstracts, Society for Neuroscience Annual Meeting.

Osterhout DJ, PD Garma, A Au, TL Laabs, HM Geller, JM Hasenwinkel, and DJ Stelzner. Chondroitinase Release from Nanospheres Induces Axonal Sprouting after Spinal Cord Injury. 284.7 Oct. 15, 2006 Program and Abstracts, Society for Neuroscience Annual Meeting.

C020932 Wadsworth Center, New York State Department of Health

Project Title: Using Reflex Conditioning to Restore Spinal Cord Function

Chen Y, XY Chen, LB Jakeman, L Chen, BT Stokes, and JR Wolpaw (2006) Operant Conditioning of H-Reflex Can Correct a Locomotor Abnormality after Spinal Cord Injury in Rats. *J. Neuroscience* **26** (48) 12537-12543.

Chen Y, XY Chen, L Chen, AM Tennissen, and JR Wolpaw (2006) Corticospinal Tract Transection Permanently Abolishes H-Reflex Down-Conditioning in Rats. *J. Neurotrauma* (in press).

Abstracts:

Thompson AK, XY Chen, JS Carp, and JR Wolpaw. Operant Conditioning of the Soleus H-Reflex in Humans. Program No. 146.9. Society for Neuroscience, 2006.

Chen Y, XY Chen, JS Carp, RL Liu, A English, and JR Wolpaw. Recovery of EMG Activity after Sciatic Nerve Transection and Surgical Repair. Program No. 146.2. Society for Neuroscience, 2006.

Chen XY, L Chen, Y Chen, S Pillai, Y Wang, and JR Wolpaw. Effects of Chronic Sensorimotor Cortex Stimulation on Soleus H-Reflex in Rats. Program No. 146.5. Society for Neuroscience, 2006.

C020940 University at Albany, State University of New York

Project Title: Gene Expression Profiling in Successful Spinal Reinnervation

Szaro BG and KM Gibbs (2006) Regeneration of descending projections in *Xenopus laevis* tadpole spinal cord demonstrated by retrograde double labeling. *J. Brain Research* **1088** (1) 68-72.

C020941 University of Rochester

Project Title: Calcium Trap for the Myelin-Associated Glycoprotein Receptor

Giger R, T Hofer, W Tangkeangsirisin, MG Kennedy, RG Mage, SJ Raiker, K Venkatesh, H Lee, and C Rader (2006) Chimeric Rabbit/Human Fab and IgG Specific for Members of the Nogo-66 Receptor Family Selected for Species Cross-Reactivity with an Improved Phage Display Vector. *J. Immunol. Methods* (in press).

Appendix IV

Patents Resulting from Spinal Cord Injury Research Board-Funded Projects

C016882 SUNY at Stony Brook

Project Title: Neurotrophins and Function of the Injured Spinal Cord

US Patent filed 11/313,262 pending for “Prevention or treatment of deficits that arise in connection with diseases of, or injuries to, nervous system.” Briefly, it is proposed to use the viral vector for delivery of certain subtypes of neurotransmitter receptors in combination with neurotrophins, as a novel strategy for strengthening the preserved connections and repairing the partially injured spinal cord.

C019772 Burke Medical Research Institute (CORE Project)

Project Title: Center for Research Excellence in Spinal Cord Injury

US Patent filed 11/485,245 pending for “Rapid Multi-dimensional Display of Information from Databases and Other Sources Using Optimal Mouseover Methods to Effectively Fly Through Info-space.” Computer methodology to facilitate database usage. Rolf J. Martin, inventor.

Patent pending for Neural Network, used for candidate drug discovery. Bruce Kristal and Rolf J. Martin, inventors.