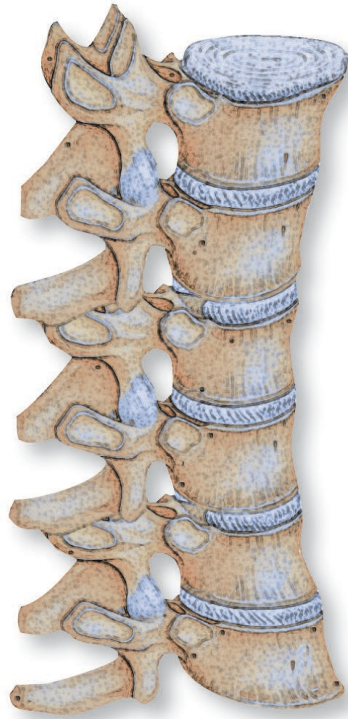




Department  
of Health



SPINAL  
CORD  
INJURY  
RESEARCH  
BOARD

**Annual Report**

January 1, 2018 to December 31, 2018

## I. INTRODUCTION

Spinal cord injury (SCI) was once thought of as incurable. The basic science carried out by researchers in this field, much of it accomplished in New York State, has served as an important stimulus for the clinical trials now underway in fields as diverse as neuro-rehabilitation, axon growth, cell biology and robotics. Although it is not yet possible to reliably repair the human spinal cord, there are new treatments that improve the lives of SCI patients, and continued scientific explorations offer hope for doing more.

SCIs contribute to significant disability, illness and death in the United States. Each year, approximately 1,100 New York residents suffer traumatic SCIs<sup>1</sup> joining the nearly 288,000 people living in the United States who have SCI.<sup>2</sup> The personal and economic costs to these individuals, their families and society are immense.

Most frequently, these injuries are caused by motor vehicle accidents, falls, sports injuries, or acts of violence. SCI results in an abrupt change in the quality of life for those affected. Injuries to the spine near the head can result in quadriplegia, with the loss of motor control, sensation and function of the arms, legs, bowel, bladder, chest, abdomen and diaphragm. Injuries to the lower spine can result in loss of sensation and movement in the lower body, and loss of bowel and bladder control. Both types of injuries can result in significant chronic pain.

In addition to societal and individual costs incurred for medical care and through loss of productivity, there are significant costs for home and vehicle modifications, equipment purchase, medications and personal assistance services. The National Spinal Cord Injury Statistical Center reported that first-year costs for an individual with SCI range from approximately \$359,783 to more than \$1,102,403, with annual costs thereafter ranging from approximately \$43,700 to \$191,436<sup>2</sup>. These expenses are borne by the individuals, their families and society at large.

The New York State Spinal Cord Injury Research Board (SCIRB) was created in 1998 to solicit, review and support proposals from leading New York State researchers in their efforts to find a cure for SCI. The Spinal Cord Injury Research Trust Fund (Trust Fund) was established to fund this research. It is financed primarily by a portion of surcharges on moving traffic violations, because motor vehicle accidents are the leading cause of SCI, followed by falls.<sup>2</sup> The SCIRB and Trust Fund are authorized by Title IV (Sections 250 through 251) of Article 2 of the Public Health Law and Section 99-f of Article 6 of the State Finance Law.

The SCIRB first convened in August 1999. The SCIRB is required to report annually to the Governor and Legislature on funds appropriated for SCI research and the progress of the SCIRB in terms of the results of its SCI research efforts.

<sup>1</sup> New York State Department of Health, Bureau of Occupational Health and Injury Prevention, 2016 data

<sup>2</sup> "Spinal Cord Injury Facts and Figures at a Glance." *National Spinal Cord Injury Statistical Center*. University of Alabama at Birmingham, 2018. Web. 10 September 2018. <https://www.nscisc.uab.edu/>

The SCIRB's mission and goal is to:

1. Seek major advances toward a cure and not simply incremental gains or incremental improvements for SCI patients
2. Support research that tests novel hypotheses and/or advances innovative research approaches that could move the field of SCI research significantly forward toward discovering a cure for SCI.

The SCIRB's mission is to stimulate high-quality, innovative SCI research that will help promote treatment and cure for SCI, including methods for reversing paralysis or restoring function caused by injury, or for minimizing or preventing damage occurring during acute phases of injury. To achieve this mission, the SCIRB advises the New York State Department of Health Extramural Grants Administration Program regarding funding opportunities for competitive research awards to support New York State scientists and their collaborators from a variety of biomedical disciplines.

The SCIRB is responsible for advising the Commissioner of Health on research proposals from leading New York State researchers in their efforts to find a cure for SCI. Information about the SCIRB can be found at: <https://www.wadsworth.org/extramural/spinalcord>.

The SCIRB appreciates the opportunity to serve the citizens of New York State by focusing on this important public health problem while stimulating economic growth through scientific research, investigation and discovery. The SCIRB looks forward to providing additional financial support for such highly meritorious SCI research in the coming years.

## **II. SCIRB ORGANIZATION AND MEMBERSHIP**

The SCIRB is comprised of 13 members appointed by the Governor and legislative leaders (see [Appendix 3](#)). There is one vacancy to be filled by the Temporary President of the Senate. The current composition of the SCIRB includes seven researchers, three clinicians and two spinal cord-injured persons. Members serve four-year terms.

## **III. SCIRB OPERATIONS**

In fiscal year 2018-19, \$8.5 million was programmed to support SCI research. Meetings are announced at least two weeks in advance whenever possible and are open to the public. Meeting agendas are posted on the Wadsworth Center's web site at: <https://www.wadsworth.org/extramural/spinalcord/meetings>.

A recording of each meeting is available via the Department of Health's public web site <https://www.health.ny.gov/events/webcasts/archive/> for 30 days after a meeting, opening the proceedings to a wide audience.

All SCIRB meeting agendas and approved minutes are available by request from the SCIRB's Executive Secretary.

The SCIRB held two meetings in 2018 (see [Section IV](#), below).

No changes were made to the SCIRB's bylaws in 2018. The bylaws can be found at <http://www.wadsworth.org/extramural/spinalcord/advisory-board/statutes-bylaws>.

### **NYS SCI Research Opportunities**

By the end of December 2018, the SCIRB had recommended funding to nineteen (19) different NYS Institutions and thirty-nine (39) Principal Investigators. These research projects span across a variety of disciplines including Biophysics, Endocrinology, Engineering, Immunology, Molecular Medicine, Neurobiology, Neurosurgery, Orthopedics, Pathology, Pharmacology, and Physical Therapy.

The SCIRB issues the following recurring request for applications (RFAs):

| <b>Name of RFA</b>   | <b>Number of Awards</b> | <b>Description</b>  |
|--|-------------------------|---|
| Individual Predoctoral and Postdoctoral Fellowships in Spinal Cord Injury  | 29                      | The purpose of this RFA is to support promising fellows during their mentored training and research period under the guidance of outstanding faculty PI/sponsors. The integrated program of research and training should enhance the individual's potential to develop into a productive, independent researcher.   |
| Projects to Accelerate Research Translation (PART) and Innovative, Developmental, or Exploratory Activities (IDEA) in Spinal Cord Injury | 41                      | <ul style="list-style-type: none"> <li>• The purpose of the PART award is to foster the translation of results from basic (preclinical) research into the next research phase. These awards are expected to contribute to rapid movement of findings to potential therapeutic applications or treatment strategies.</li> <li>• The purpose of the IDEA award is to support new methods and approaches to investigate the problems associated with SCI. IDEA projects are self-contained research projects.</li> </ul> |
| Translation Research Projects (TRP) in Spinal Cord Injury  | 4                       | TRP in Spinal Cord Injury are designed to build on a proven hypothesis and previously-completed early translational work. Successful applications are likely to capitalize on collaborative approaches between research institutions, businesses and regulatory consultants or agencies, and to result in the development and commercialization of products, technology, tools, treatments and therapies for SCI.   |

### **Clinical Trials**

As new treatments for spinal cord injured persons move from the laboratory to the clinic, they will undergo clinical trials to ensure the treatment is safe for patients. SCIRB funding contributes to this important work and the investigators listed below reported that their projects were undergoing clinical trials in 2018.

- Maria Knikou, P.T., Ph.D., City University of New York, *Activity-Dependent Transspinal Stimulation in SCI*, ClinicalTrials.gov Identifier: NCT03669302
- Rajiv R. Ratan, M.D., Ph.D. and Dianna E. Willis, Ph.D., Winifred Masterson Burke Medical Research Institute, *Combined Robotic Training and tDCS in Chronic SCI*, ClinicalTrials.gov Identifier: NCT03555838

## IV. MAJOR ACTIVITIES OF THE SCIRB

### ***Business Meetings***

At its February 5, 2018 meeting, the SCIRB recommended funding for two (2) predoctoral and five (5) postdoctoral fellowship awards from the “Individual Predoctoral and Postdoctoral Fellowships in SCI (Round 3)” RFA, for a total of \$1.2 million. These are three-year awards and contracts began in September 2018; one (1) organization declined funding due to receiving funding elsewhere. A tabular summary of this procurement can be found in [Appendix 1](#).

The SCIRB also approved, at its February meeting, the following three (3) RFAs for release in 2018/2019:

- “Individual Predoctoral and Postdoctoral Fellowships in Spinal Cord Injury (Round 4),”
- “Projects to Accelerate Research Translation (PART) and Innovative, Developmental or Exploratory Activities (IDEA) in Spinal Cord Injury (Round 4), and
- “Translational Research Projects in Spinal Cord Injury (Round 3).”

At its October 15, 2018 meeting, the SCIRB met and recommended funding for seven (7) awards from the “PART and IDEA in Spinal Cord Injury” (Round 3) RFA for a total of \$4.2 million. These are three- and two-year awards respectively. A tabular summary of this procurement can be found in [Appendix 1](#).

### ***Previously Recommended SCI Research Contracts***

By January 2018, four (4) PART contracts and seven (7) IDEA in Spinal Cord Injury (Round 1) contracts completed their first year. The scientific progress resulting from these multiyear awards can be found in [Appendix 2](#).

By March 2018, the Institutional Support for Spinal Cord Injury Research in New York State (Round 6) contracts completed their first year. This opportunity made SCI research funds available to organizations located within New York State that demonstrated a current notice of funding award or renewal from a peer-reviewed SCI research project conducted by a principal investigator employed at their organization. Twenty (20) five-year awards were approved to provide additional support for SCI research projects through the purchase of laboratory supplies, salaries, equipment and other customary expenses necessary to support research efforts. The scientific progress resulting from these SCI funded projects can be found in [Appendix 2](#).

Also, in March 2018, five (5) Individual Predoctoral and Postdoctoral Fellowships in Spinal Cord Injury Research (Round 1) contracts completed their second year. The scientific progress resulting from these three-year awards can be found in [Appendix 2](#).

In May 2018, two (2) contracts from the Translational Research Projects in Spinal Cord Injury (Round 2) began. The scientific progress resulting from these multiyear awards can be found in [Appendix 2](#).

In June 2018, three (3) PART and eleven (11) IDEA in Spinal Cord Injury (Round 2) contracts began. The scientific progress resulting from these three- and two-year awards, respectively can be found in [Appendix 2](#).

By August 2018, two (2) Translational Research Projects in Spinal Cord Injury (Round 1) contracts completed their second year. The scientific progress resulting from these five-year awards can be found in [Appendix 2](#).

By September 2018, eight (8) of the nine (9) Individual Predoctoral and Postdoctoral Fellowships in SCI (Round 2) contracts completed their first year. The ninth awarded contract from this Round was cancelled because the fellow was no longer employed by the contracted organization. The scientific progress resulting from these three-year awards can be found in [Appendix 2](#).

By the end of 2018, three (3) Collaborations to Accelerate Research Translation (CART) contracts completed their third year and two (2) IDEA contracts received no cost extensions and completed their final year. The scientific progress resulting from these three- and two-year awards, respectively, can be found in [Appendix 2](#).

### ***NYS SCI Research Symposium***

The NYS SCI Research Symposium took place in New York City at the Carson Family Auditorium of Rockefeller University, on October 16 and October 17, 2018. The goal of the symposium was to highlight recent advances and developments in basic and translational SCI research. The symposium featured presentations of the research supported by the Program. The program book and abstracts are available at <https://www.wadsworth.org/extramural/spinalcord/resources>. In addition, attendees heard about new discoveries from a group of leading external investigators from the U.S. and abroad. This event was hosted by the SCIRB and NYS DOH and sponsored by the Craig H. Neilsen Foundation.

**Appendix 1**

**2018 Individual Predoctoral and Postdoctoral Fellowships in SCI (Round 3) Recommendations for Award**

| Organization   | Investigators   | Funding Mechanism       | Project Title   | Recommended Award |
|--|---|-------------------------|---|-------------------|
| Rensselaer Polytechnic Institute <sup>1</sup>  | <b>PI (Sponsor):</b> Ryan J. Gilbert, Ph.D.<br><b>Fellow:</b> Devan Puhl                | Predoctoral Fellowship  | Delivery of Neurotrophin-3 mRNA by Electrospun Fibers to Promote Axonal Growth  | \$135,600         |
| Research Foundation for SUNY Stony Brook   | <b>PI (Sponsor):</b> Prithvi K. Shah, Ph.D.<br><b>Fellow:</b> Pawan Sharma              | Predoctoral Fellowship  | Activity-dependent Closed-loop Neuromodulation of the Cervical Spinal Cord for the Recovery of Skilled Upper Limb Function After a Spinal Cord Injury                         | \$117,570         |
| Research Foundation of CUNY, The City College of New York  | <b>PI (Sponsor):</b> John Martin, Ph.D.<br><b>Fellow:</b> Lillian Yang, Ph.D.           | Postdoctoral Fellowship | Harnessing Activity-Dependent Competition to Repair the Corticospinal Motor System After Cervical Spinal Cord Injury  | \$184,734         |
| Winifred Masterson Burke Medical Research Institute <sup>2</sup>   | <b>PI (Sponsor):</b> Jason B. Carmel, M.D., Ph.D.<br><b>Fellow:</b> Qi Yang, Ph.D.      | Postdoctoral Fellowship | Combined Therapy of Forelimb Area Motor Cortex and Spinal Cord Epidural Stimulation to Improve Hand Function After Spinal Cord Injury and Identifying the Responsible Pathway | \$186,426         |
| Winifred Masterson Burke Medical Research Institute  | <b>PI (Sponsor):</b> Caitlin E. Hill, Ph.D.<br><b>Fellow:</b> Carolin Ruven, Ph.D.      | Postdoctoral Fellowship | Role of Ubiquitinated Proteins in Dystrophic Axonal Endings Following Spinal Cord Injury  | \$186,426         |
| Winifred Masterson Burke Medical Research Institute  | <b>PI (Sponsor):</b> Edmund R. Hollis, Ph.D.<br><b>Fellow:</b> Hisham Mohammed, Ph.D.   | Postdoctoral Fellowship | The Role of Intracortical Circuits in Motor Recovery from Spinal Cord Injury  | \$175,926         |
| Winifred Masterson Burke Medical Research Institute  | <b>PI (Sponsor):</b> Jian Zhong, Ph.D.<br><b>Fellow:</b> Christina Leila Torturo, Ph.D. | Postdoctoral Fellowship | Generation and Validation of a Transgenic Chain-amplifying Tracer (TCAT) Mouse Line   | \$172,926         |
| <sup>1</sup> This organization declined funding due to receiving funding elsewhere.<br><sup>2</sup> Dr. Carmel accepted a position at Columbia University where he continues this research |   |                         | <b>Total (7 awards)</b>   | \$1,159,608       |

**2018 PART/IDEA (Round 3) Recommendations for Award**

| <b>Organization</b>                                 | <b>Principal Investigator</b> | <b>Funding Mechanism</b> | <b>Project Title</b>   | <b>Recommended Award</b> |
|---|-------------------------------|--------------------------|--|--------------------------|
| Bronx Veterans Medical Research Foundation, Inc.    | William A. Bauman, M.D.       | PART                     | Abaloparatide to Improve Bone Mineral Density and Architecture in Chronic SCI              | \$826,939                |
| Icahn School of Medicine at Mount Sinai             | Ravi Iyengar, Ph.D.           | IDEA                     | Systems Therapeutics for Spinal Cord Injury  | \$342,914                |
| Icahn School of Medicine at Mount Sinai             | David F. Putrino, P.T., Ph.D. | IDEA                     | Virtual Reality to Reduce Pain in the Upper Extremities After Spinal Cord Injury           | \$357,639                |
| University of Rochester                             | Gail V.W. Johnson, Ph.D.      | IDEA                     | Transglutaminase 2 as a Therapeutic Target to Facilitate Recovery After Spinal Cord Injury | \$360,000                |
| Winifred Masterson Burke Medical Research Institute | Edmund R. Hollis, Ph.D.       | PART                     | Rehabilitation and Cortical Remodeling After Surgical Intervention for Spinal Cord Injury  | \$963,000                |
| Winifred Masterson Burke Medical Research Institute | Botir T. Sagdullaev, Ph.D.    | IDEA                     | Blood Flow Control and its Impairment in Spinal Cord Injury                                | \$357,566                |
| Winifred Masterson Burke Medical Research Institute | Jian Zhong, Ph.D.             | IDEA                     | Repetitive Transcranial Magnetic Stimulation as a means to Promote CST Axon Regeneration   | \$990,000                |
| <b>Total (7 awards)</b>                             |                               |                          |  | <b>\$4,198,058</b>       |



## ***Appendix 2***

### **Scientific Progress Resulting from Spinal Cord Injury Research Board-Funded Projects**

#### **Individual Predoctoral/Postdoctoral Fellowships (Round 3)**

**Contract Term 9/1/18-8/31/21**

**6 Awards, Procurement Total: \$1,024,008**

#### **1. Columbia University**

Jason B. Carmel, M.D., Ph.D., Qi Yang, Ph.D.

Postdoc: \$186,426

Combined Therapy of Forelimb Area Motor Cortex and Spinal Cord Epidural Stimulation to Improve Hand Function After Spinal Cord Injury and Identifying the Responsible Pathway

Introduction/Background: After spinal cord injury, there are usually spared connections between the brain and spinal cord beyond the site of injury. Their goal is to make these weak connections stronger in order to restore arm and hand function to people with cervical spinal cord injury. Recently, Dr. Carmel's laboratory has shown that pairing brain and spinal cord electrical stimulation can strengthen these connections in uninjured rats. In this study, they will test their hypothesis that pairing brain and spinal cord stimulation can promote recovery of forelimb function in rats with cervical spinal cord injury. In order to quantify the effect of this therapy, they will compare the performance of rats with paired stimulation against rats with sham stimulation on forelimb tasks. They will also test which connections between brain and spinal cord are strengthened by this therapy.

Progress towards specific aims and future directions will be reported in the 2019 SCIRB Annual Report.

Impact: The researchers' experiments have the potential to improve their understanding of spared connections after spinal cord injury and whether those connections can be recruited using a promising therapeutic approach. The training involved will position Dr. Yang to be a leader in the field of SCI research.

#### **2. Research Foundation of CUNY, The City College of New York**

John Martin, Ph.D., Lillian Yang, Ph.D.

Postdoc: \$184,734

Harnessing Activity-Dependent Competition to Repair the Corticospinal Motor System After Cervical Spinal Cord Injury

Introduction/Background: SCI produces weakness and paralysis because the connections between the brain and spinal cord are damaged. Most SCIs are incomplete and some undamaged axons connecting brain and spinal cord survive. In order to restore function, spared connections must be strengthened. In a rat model of SCI, Dr. Martin's laboratory has shown that patterned electrical stimulation of motor cortex for a 10-day period can produce recovery of movement and fine motor skills. This recovery

likely happens due to the growth and branching of existing brain to spinal cord axon connections. In this study, researchers will try to maximize the increase in connectivity and recovery by optimizing the pattern of stimulation. They will test the differences between high intensity, short duration phasic stimulation and low intensity, long duration tonic stimulation. They will compare axon growth and muscle responses after stimulation and compare stimulated axons to non-stimulated axons. They will also look for cellular and molecular mechanisms as to how stimulation can activate an axon growth program. Lastly, they will determine the pattern of stimulation that produces the most recovery in a cervical contusion model and whether stimulation combined with rehabilitation training produces any additional benefit.

Progress towards specific aims and future directions will be reported in the 2019 SCIRB Annual Report.

Impact: The completion of this project will optimize activity-based therapies for SCI and generate a systems-level understanding of how neural activity promotes motor recovery. Through the training experience, the Dr. Yang will learn a wide range of experimental approaches to become an effective SCI researcher, including aseptic surgery, immunohistochemistry, electrophysiology, viral approaches, and stereological and behavioral assessments.

### **3. Research Foundation for SUNY Stony Brook**

Prithvi K. Shah, Ph.D., Pawan Sharma

Predoc: \$117,570

Activity-dependent Closed-loop Neuromodulation of the Cervical Spinal Cord for the Recovery of Skilled Upper Limb Function After a Spinal Cord Injury

Introduction/Background: Permanent impairments of hand function greatly deteriorates the quality of life for people with a cervical SCI (cSCI). Gains in recovery of upper limb motor function with epidural stimulation (ES) are modest. In this project, conventional ES (continuous stimulation applied below the injury level) is used. The researchers' preliminary data demonstrate that a new biofeedback-based ES strategy that is applied to the cervical cord during an attempted movement (i.e., activity-dependent ES, aES) can assist in significant recovery of skilled hand movements in adult rats. Their objective is to determine if aES applied above as well as below the cSCI, to activate the entire cervical cord, will promote skilled upper limb function after a cSCI in adult rats. cSCI adult rats will go through an extensive aES therapy, applied at C3-C8 or C6-C8 segments of the spinal cord in their home-cage as well as during supervised skilled motor training.

Progress towards specific aims and future directions will be reported in the 2019 SCIRB Annual Report.

Impact: This project will assist in developing a bio-feedback based neuroprosthetic home-therapy program. The research team will learn if cES is more successful when the whole cervical cord is activated, and they will better understand the role of propriospinal neurons in the recovery of hand function after a cSCI. The training involved will position Pawan Sharma to be a leader in the field of SCI research.

**4. Winifred Masterson Burke Medical Research Institute**

Caitlin E. Hill, Ph.D., Carolin Ruven, Ph.D.

Postdoc: \$186,426

Role of Ubiquitinated Proteins in Dystrophic Axonal Endings Following Spinal Cord Injury

Introduction/Background: SCI is a devastating trauma that leaves patients paralyzed and with very little hope of recovery. Various axonal tracts in the spinal cord can regenerate when provided with a permissive environment and correct intrinsic signals. After SCI, axons fail to grow, and they form dystrophic endings instead. Researchers will explore how the dystrophic endings form and why they persist for years. Their preliminary data shows that the failed endings accumulated ubiquitinated proteins could play important roles in the injury response. Researchers will test the hypothesis that alterations in the Ubiquitin-Proteasome System (UPS) and accumulation of ubiquitinated proteins lead to the formation and stabilization of dystrophic endings after SCI.

Progress towards specific aims and future directions will be reported in the 2019 SCIRB Annual Report.

Impact: This project addresses an important barrier for SCI repair and has the potential to shift how researchers target dystrophic axonal endings. Their findings may lead to the development of new strategies that would benefit the population of underserved chronic SCI patients. In addition, this project will help Dr. Ruven get closer to her goal of becoming an independent SCI researcher, as it provides her with the opportunity to widen her technical repertoire, SCI knowledge, and to gain skills such as grant writing, presentation, critical thinking, and leadership.

**5. Winifred Masterson Burke Medical Research Institute**

Edmund R. Hollis, Ph.D., Hisham Mohammed, Ph.D.

Postdoc: \$175,926

The Role of Intracortical Circuits in Motor Recovery from Spinal Cord Injury

Introduction/Background: Spinal cord injury interrupts not only the transmission of ascending sensory and descending motor information within the spinal cord, but also disrupts the cortical sensorimotor networks that process this information. Cortical reorganization occurs after SCI and motor maps are shaped through rehabilitation, though, the underlying mechanisms remain unknown. Intracortical horizontal connections in primary motor cortex contribute to the plasticity of motor maps. However, the contribution of horizontal connectivity to recovery after SCI is unknown. The overall objective of this project is to identify the intracortical circuitry responsible for restoring skilled forelimb function.

Progress towards specific aims and future directions will be reported in the 2019 SCIRB Annual Report.

Impact: The expected findings will inform combinatorial strategies that target cortical plasticity in order to fully realize the effects of axonal sprouting and regeneration, cell transplantation, and rehabilitation. The training involved will position Dr. Mohammed to be a leader in the field of SCI research.

**6. Winifred Masterson Burke Medical Research Institute**

Jian Zhong, Ph.D., Christina Leila Torturo, Ph.D.

Postdoc: \$172,926

Generation and Validation of a Transgenic Chain-amplifying Tracer (TCAT) Mouse Line

Introduction/Background: SCI interrupts the connections between the brain and body's muscles and sensory organs. While injured axons cannot regenerate in the adult human spinal cord, such regeneration can be triggered by certain experimental interventions in mice, giving hope that new pro-regenerative treatments may eventually be developed for SCI patients. Researchers will test how regenerating axons in the spinal cord contact potential target cells, form synaptic connections or retract from inappropriate partners by formulating new methods that allow researchers to observe synapse formation inside the living spinal cord. Their project aims to create such a novel tool. Specifically, they will generate a genetically modified mouse line where synapses and postsynaptic cells contacting regenerating axons will be labeled with a strong fluorescent signal *in vivo*, allowing the mapping of existing and newly formed spinal cord motor circuitry across several consecutive synapses.

Progress towards specific aims and future directions will be reported in the 2019 SCIRB Annual Report.

Impact: Data from their research will be correlated to measures of movement recovery, which will be very useful as a basis for the development of novel treatment to increase the formation of new synaptic connections in human SCI patients, through possibly new drugs or new strategies of rehabilitative training. The training involved will position Dr. Torturo to be a leader in the field of SCI research.

## **PART/IDEA in Spinal Cord Injury (Round 2)**

**IDEA Contract Term 6/1/18-5/31/20; PART Contract Term 6/1/18-5/31/21**

**Progress Reporting Period  
6/1/18-11/30/18**

**14 awards, Procurement Total: \$6,550,280**

### **1. Bronx Veterans Medical Research Foundation, Inc.**

Christopher Cardozo, M.D., Dongming Cai, M.D., Ph.D., Bin Zhang, Ph.D.

Sub-applicant: Icahn School of Medicine at Mount Sinai

IDEA: \$344,624

Role of Synj1 in Functional Recovery After Spinal Cord Injury

Introduction/Background: Apolipoproteins are specialized proteins that bind fats and transport them between cells. Apolipoprotein E (ApoE) is a protein of interest because genetic variations of ApoE are strong genetic risk factors for diseases such as Alzheimer's disease. Recent studies indicate that individuals who have one variant of the gene, known as ApoE4, have worse outcomes after a spinal cord injury because their function is poorer and their hospital stays are longer. The reasons for the negative effect of the ApoE4 variant are not known. Researchers will conduct studies in mouse models that carry the human ApoE3 or ApoE4 genes to determine how these genes alter lipid levels in spinal cord and to understand the role of synj1 in these changes. They will also identify mechanisms by which changes in lipid and synj1 levels in spinal cord tissues impair the recovery after SCI.

Progress Towards Specific Aims: Colonies of ApoE3 and ApoE4 mice have been expanded to have sufficient animals for their research project. Dr. Carlos Toro has been recruited and will complete the animal studies proposed and will provide histologic and biochemical data required for completion of studies.

Future Directions: Researchers anticipate that drugs that lower Synj1 in ApoE4 mouse brains may be very useful to improve function after SCI. Mechanistic studies may identify additional targets for future drug development.

Impact: This research project will provide a better understanding of the mechanisms responsible for the strong association of ApoE4 and poor outcomes after SCI. By identifying the molecular basis for adverse effect of ApoE4, the researchers will have a target for development of a new generation of drugs that might improve function of those with an SCI. The studies will also identify novel candidates for the development of drugs to improve function after an SCI.

### **2. Bronx Veterans Medical Research Foundation, Inc.**

Jill Wecht, Ed.D.

IDEA: \$344,887

Dose Effect of Norepinephrine Precursor (Droxidopa) on Blood Pressure and Cerebral Blood Flow Velocity in Hypotensive Individuals with Spinal Cord Injury

Introduction/Background: Hypotension and orthostatic hypotension (OH) are common clinical consequences of SCI, particularly in those with lesions above the thoracic 5 (T5)

level. Although low blood pressure (BP) is common in SCI in the neck and upper back, very few patients are diagnosed or treated for this condition. Part of the reason hypotension and OH are not treated in the SCI population may relate to the under-appreciated adverse consequences of sustained and episodic low BP. One of the most commonly prescribed medications to treat low BP is midodrine. However, in 2014 the FDA approved another medication, droxidopa (Northera). Researchers will test the effect of escalating dose of Droxidopa on seated systolic blood pressure in an open label trial. Researchers will determine the effect, compared to placebo, on supine systolic blood pressure and changes in cerebral blood flow and systolic blood pressure during a 70-degree head-up tilt maneuver.

Progress Towards Specific Aims: 20 potential participants have been screened for enrollment in the trial and three (3) participants have been scheduled for testing. Results are not yet available. An agreement has been reached between the Bronx VA Medical Center and Lundbeck, a global pharmaceutical company, which secures support for study medication and placebo to be shipped to the research pharmacist at the Bronx VA Medical Center.

Future Directions: Testing will begin on eligible participants in spring 2019 with a goal of completing ten participants by 6/1/19.

Impact: Droxidopa offers a potential therapeutic advancement over current pharmacologic intervention because of the limited side effects reported related to excessive increases in supine blood pressure. The results will be used to investigate the effects of droxidopa on long-term blood pressure and to determine if there are beneficial effects on parameters of cognitive function, mood and quality of life in hypotensive individuals with SCI.

### **3. Columbia University**

Jason Carmel, M.D., Ph.D.

IDEA: \$359,241

Combining 4-AP with Motor Training to Promote Forelimb Motor Recovery in Rats with Pyramidal Tract Injury

Introduction/Background: Recovery of arm and hand function remains a largely unmet need for people with cervical spinal cord injury. Researchers recently demonstrated that the drug 4-Aminopyridine (4-AP) is capable of exciting the connections between brain and spinal cord that control arm and hand movements and that are usually spared after injury. Researchers will test the hypothesis that combining 4-AP with motor training can strengthen these connections.

Progress Towards Specific Aims: Researchers have begun to work towards training the first cohort of eight animals on the knob supination task. Animals are slowly making progress towards attaining proficiency in the task. Once the animals reach baseline performance level, they are scheduled to undergo surgery to injure one half the motor pathway that will impair the fine motor function of their right paw.

Future Directions: Following recovery from the injury, animals will be randomly subdivided into three groups. One group will receive motor training only, one group will receive the drug only (4-AP) and the main experimental group will receive both the drug (4-AP) and training simultaneously for two weeks. Researchers will then investigate how

animals in each group recover supination function for the next four weeks. Finally, they will perform terminal physiological and anatomical experiments to determine the underlying causes for changes observed in the behavioral experiments.

Impact: Positive results would demonstrate that 4-AP, an FDA-approved drug which is safe in people with SCI, can make physical therapy more effective. The study will also determine why the changes occur, which is important for translating the proposed rat studies to people. Improved arm and hand function could improve the independence and quality of life for people with cervical SCI.

#### **4. Feinstein Institute for Medical Research**

Ona Bloom, Ph.D., Ann Spungen, Ed.D.

Sub-applicant- Bronx Veterans Medical Research Foundation, Inc.

IDEA: \$222,870

Impact of Walking on the Immune System of Persons with Chronic Spinal Cord Injury

Introduction/Background: SCI often results in paralysis and leads to drastic reduced mobility. Persons with chronic SCI are at a greater risk for many medical complications commonly referred to as metabolic syndrome. Finding treatments to help manage and lessen the impact of these medical complications is important to the health of persons with SCI. Persons with SCI are often unable to perform upright activity/exercise and do not have regular access to adaptive sports or gym equipment. Powered exoskeletons are a new type of technology and provide light-to-moderate physical activity. However, it is unclear if exoskeletal-assisted walking will provide health benefits like walking in able-bodied persons.

Progress Towards Specific Aims: Researchers are measuring if exoskeletal-assisted walking changes the immune system in persons with SCI, such as reducing inflammation or changing genes that are activated within white blood cells. Researchers have collected blood samples from 22 participants before and after 36 sessions of exoskeletal assisted walking (EAW).

Researchers performed a pilot study of samples from four (4) participants (pre and post EAW). Samples from three (3) non-SCI persons were also collected. RNA-Seq libraries were created and 14,882,422 RNA-Seq reads were collected. Data was trimmed, and transcripts were normalized using the recommended default parameters. Following normalization, differential gene expression analysis was performed using the gene specific analysis (GSA) algorithm using default parameters. Gene analysis was completed and researchers found that some signaling pathway genes were significantly enriched.

Future Directions: Researchers will evaluate EAW alone or in combination with other interventions as a physical activity that may promote health and wellness in more persons with SCI. Preliminary data from this study has been used to support a collaborative grant application which proposes a Phase II clinical trial to test the effects of long-term intermittent low oxygen therapy with or without EAW.

Impact: Exercise modalities for persons with SCI are limited. Identification of exoskeletal assisted walking as an intervention that promotes immune function would support future investigations of their utility not only as assistive mobility devices, but also as devices with therapeutic activity/exercise effects.

## **5. Icahn School of Medicine at Mount Sinai**

Hongyan Jenny Zou, M.D., Ph.D.

IDEA: \$360,000

Enhancing Axon Regenerative Capacity Through Epigenetic Regulation of DNA Methylation Dynamics

Introduction/Background: A major barrier for axon regeneration after SCI is a diminished axon regenerative capacity in Central Nervous System (CNS) neurons. This is partly because of failure of reactivating pro-growth genes after injury. Finding a way to turn on these genes is a worthy strategy for SCI, the important aspect of which is to induce a large repertoire of genes required to initiate the regenerative gene program as individual gene-based approaches yielded only limited success in axon regeneration. Recent studies from Dr. Zou's laboratory have indicated that modifying chromatin landscapes may set the stage for coordinated regulation for entire classes of injury response genes required for axon regeneration. They identified an upregulation of Tet methylcytosine dioxygenase 3 (Tet3) in sensory neurons of dorsal root ganglia (DRG) that are activated into a regenerative state. Tet is an enzyme that catalyzes DNA hydroxymethylation, a form of epigenetic regulation that influences chromatin structure and thereby gene expression. They constructed comprehensive mapping of DNA hydroxymethylation, the result of which points to major influences of Tet3 in regulating regenerative injury responses. Their analysis also predicted that HIF-1a (hypoxia inducible factor-1 alpha) might assist Tet3 in modifying DNA methylation patterns. They propose to test the central hypothesis that epigenetic regulation of DNA methylation dynamics by Tet3 and HIF-1a enhances axon regenerative capacity. They will use combined in vitro and in vivo studies to establish a link between axon regeneration phenotypes with underlying molecular and epigenetic mechanisms of these two factors in modifying DNA methylation and gene expression.

Progress Towards Specific Aims: Researchers performed bioinformatic analysis to identify candidate downstream target genes of Tet3 and HIF-1a. They focused on 161 genes that are differentially regulated in conditioned DRG and display differential 5hmC regions (DhMR). They found 56 genes (35%) contained HIF-1a binding motif. Gene ontology analysis found that they are enriched for genes that regulate biological processes which may be important for axon regeneration, e.g. regulation of anatomical structure morphogenesis. They cloned various constructs into viral vectors, which allow doxycycline-inducible overexpression or knockdown of Tet3 or HIF-1a. Additionally, progress was made towards designing a novel CRISPR-Cas9 Synergistic Activation Mediator (SAM) targeting Tet3.

Future Directions: In primary DRG neurons, they will test the effects of over-expression or knock-down of Tet3 and/or HIF-1a on neurite outgrowth and target gene expression.

Impact: This research will provide a framework to firmly establish epigenetics as a worthy strategy for mediating adaptive injury responses and axon growth plasticity in injured neurons after SCI.

## **6. Regenerative Research Foundation**

David Butler, Ph.D., Jennifer Morgan, Ph.D.

Sub-applicant: Marine Biological Laboratory

IDEA: \$311,921



## Developing Intracellular Antibodies Against Alpha-Synuclein as Potential Therapeutics in Spinal Cord Injury and Disease

**Introduction/Background:** Traumatic damage to the spinal cord causes a widespread loss of nerve cells which result in permanent deficits in movements and sensations. This research will develop effective approaches that target synuclein protein accumulation following SCI using novel antibody-based reagents. They will also study a new therapeutic target consisting of synuclein aggregation and neurotoxicity.

**Progress Towards Specific Aims:** Significant progress was made toward identifying anti-synuclein intrabodies that target synuclein to the proteasome for degradation. The sub-applicant, Marine Biological Laboratory, reported that synuclein aggregation promotes neurodegeneration after SCI and limits axon regeneration. Researchers have successfully subcloned lamprey DY-synuclein~GFP, FD~synuclein~GFP and Syn3-synuclein~GFP mammalian pcDNA3.1 expression plasmid and after sequence verification of these plasmids, verified their expression in the ST14A neuronal cell line. They will focus on optimizing intrabodies for future use in the *in vivo* lamprey SCI model and staff have been trained to conduct the *in vivo* assays that will be utilized.

**Future Directions:** Researchers will validate target engagement of bifunctional anti-synuclein mouse-PEST and human-PEST intrabodies in human iPSC derived neurons that overexpress alpha-synuclein. The Marine Biological Laboratory will determine the extent to which bifunctional anti-synuclein PEST intrabodies improve anatomical and functional recovery in their established *in vivo* lamprey SCI model.

**Impact:** Researchers are utilizing highly selective intrabodies for synuclein that have been engineered to direct synuclein to the proteasome for degradation using the cell's normal clearing process. These studies will contribute to the development of novel reagents which may be useful in clinical SCI treatments.

### **7. Regenerative Research Foundation**

Sally Temple, Ph.D., Larry Benowitz, Ph.D.

Sub-applicant: The Children's Hospital Corporation

IDEA: \$335,000

The Role of Zinc in Axon Regeneration Following Spinal Cord Injury

**Introduction/Background:** SCI cuts projections of nerve cells which disrupt communication between skin, brain and muscles resulting in functional deficits. These nerve cells do not regrow after SCI which makes the disability permanent. Utilizing mice with an optic nerve injury, researchers demonstrated an improved nerve cell regrowth by using chelating agents blocking free zinc accumulation. Their objective is to develop a novel zinc chelation approach that promotes nerve regrowth and behavioral recovery after SCI. To complete their objective, researchers will characterize zinc response, optimize zinc chelation and test nerve regrowth and behavioral function after SCI.

**Progress Towards Specific Aims:** Researchers made progress in optimizing zinc chelation and testing nerve regrowth and zinc was characterized in normal brain, spinal cord, dorsal root ganglia as well as SCI time-course spinal cord, dorsal root ganglia and brain tissues. Varying zinc levels were found in both spinal cord gray matter and dorsal root ganglia change after SCI. This data suggests sufficient chelatable zinc is present to potentially suppress axon regeneration and neuronal survival. Researchers are currently

working on the technical aspects, including axon labeling and breeding experimental mice. The team has also written the animal protocol which will allow them to begin the chelation experiments in animals.

Future Directions: Researchers will further characterize brain zinc changes in response to SCI and they will optimize systemic zinc chelation methods. At the same time, researchers will begin testing nerve regrowth data after SCI.

Impact: The demonstration of nerve regrowth mediated by zinc removal after SCI in mice could lead to development of therapeutic agents for clinical SCI.

## **8. Research Foundation for SUNY, SUNY Polytechnic Institute**

Janet Paluh, Ph.D., Philip Horner, Ph.D.

Sub-applicant: The Methodist Hospital Research Institute

PART: \$970,404

Healing the Contusion-Injured Spinal Cord Microenvironment with Nanotechnology- and Stem Cell-Assisted Modulation

Introduction/Background: Numerous cell therapy studies for spinal cord injuries, indicate that stem cells can improve the regenerative environment with potential to restore neural connectivity. Evidence for this comes largely through rodent studies that are proving to be effective models for multiple CNS injuries using human stem cells. Key challenges to assessment of cell therapies and optimization include retaining healthy transplanted cells at the site of injury as well as stimulating signaling cues for remodeling of the injury microenvironment to remove inhibition and promote rapid repair and regeneration. Nanotechnology and materials science coupled with stem cell biology will address these critical needs and accelerate the pace of therapies from models to clinic.

The researchers will optimize SCI treatments by testing neural stem cell progenitors, motor neurons and oligodendrocytes in defined type/ratio/number combinations under a novel single nanotechnology-based platform. This approach allows the researchers to deliver and retain cells at the SCI injury site while favorably modulating the glycobiology of the SCI site to promote rapid remodeling and integration of transplanted cells. The researchers microribbon strategy stems from nanotechnology and applies FDA approved biodegradable materials. The researchers established a reproducible hemicontusion model in the rat, focusing on C4-C5, and including several behavioral monitoring assays and post analysis of repair/recovery.

Progress Towards Specific Aims: Researchers have refined their ability to generate size specific alginate microribbons and preload human stem cell derived neural therapeutic cells and microenvironment modifying chondroitinase ABC enzyme. In addition, they have engineered the microribbons for visualization in vivo and in vitro, tested cell long-term viability in microribbons and recovery, and validated shipping of the cell therapeutic products and their subsequent manipulation in rat models. They have generated MUSE cells that are known for their non-tumorigenic benefits, particularly when transplanting multipotent therapeutic cells.

Future Directions: Dr. Paluh's innovative microribbon strategy stems from nanotechnology and applies FDA approved biodegradable alginate biomaterial. The researchers have previously established a reproducible hemicontusion model in the rat, focusing on C4-C5, and including several behavioral monitoring assays and post

analysis of repair/recovery that are being applied in this research project. Rodents have proven to be effective models for evaluating human stem cells in CNS repair of injuries and for rapid and economical assessment of the injury microenvironment, transplanted cell survival and integration and restored neural connectivity linked to functional behavioral improvements.

Impact: To advance cellular regeneration and repair of SCI by providing a uniform platform for the SCI community capable of bridging benchtop to animal studies for rapid testing of multiple stem cell resources without error caused by loss of transplanted cells.

## **9. Research Foundation of CUNY, College of Staten Island**

Maria Knikou, P.T., Ph.D., Noam Harel, M.D., Ph.D.

Sub-applicant: Bronx Veterans Medical Research Foundation, Inc.

PART: \$898,595

Activity-Dependent Transspinal Stimulation for Recovery of Walking Ability After SCI

Introduction/Background: In individuals with SCI, locomotor training is commonly used to promote recovery of walking function. However, even after multiple locomotor training sessions muscle activity and leg coordination remains largely pathological. Thus, locomotor training alone may be insufficient to increase the excitability of spinal neural circuits. Noninvasive transspinal stimulation has the ability to alter both corticospinal and spinal neural excitability, and thus may augment the effects of locomotor training. A fundamental knowledge gap exists on neuroplasticity and improvements in walking ability when transspinal stimulation is combined with locomotor training and especially when the stimulation is delivered at different stimulation frequencies during the actual motor task of walking. In this research project, transspinal stimulation at low (0.3 Hz) and high (30 Hz) stimulation frequencies will be delivered during assisted stepping in individuals with SCI, and the results will be compared to a control group whom will receive the same number of locomotor training sessions without transspinal stimulation. Stimulation is synchronized to the step cycle and occurs during the stance phase to improve activity of spinal locomotor centers. Researchers expect that this therapeutic intervention will strengthen neuronal synapses resulting in improvements of walking function in people with SCI.

Progress Towards Specific Aims: For this randomized multi-site clinical trial, following IRB approval from both performance sites (College of Staten Island/CUNY and James J. Peters VA Medical Center), researchers submitted all documents necessary for approval by ClinicalTrials.gov. The research study now is deposited in the clinical trials website and can be found at [NCT03669302](https://clinicaltrials.gov/ct2/show/study/NCT03669302). Furthermore, they completed a total of 30 experiments in 16 healthy control subjects, completed training and experiments in two (2) persons with SCI, and are currently establishing eligibility and scheduling for five (5) persons with SCI.

Future Directions: The PI's plans for the next six (6) months is to continue establishing eligibility and enrollment of interested participants along with the Co-PI at the James J. Peters VA Medical Center, in order to complete an additional six (6) persons with SCI at both performance sites.

Impact: Transspinal stimulation is a noninvasive method that can be used to provide tonic excitatory inputs to the spinal neuronal circuits augmenting the effects of locomotor training, resulting in recovery of walking ability. Electrophysiological assessments and

clinical evaluations will provide the scientific evidence of this combined intervention, and may change the standard of care because of its noninvasive approach it can be implemented in different real-life clinical settings worldwide.

#### **10. Research Foundation of CUNY, The City College of New York**

John Martin, Ph.D., Sunil Agrawal, Ph.D.

Sub-applicant: Columbia University

IDEA: \$332,738

Robotic Rehabilitation to Promote Recovery of Forelimb Function after Cervical SCI in Rats

Introduction/Background: SCI disconnects the brain from the spinal cord, resulting in severe motor impairments. To cure paralysis or major weakness after SCI will require combining a biological intervention to repair the damaged nerve connections and rehabilitation to improve general motor functions and refine skills. This research will design a robotic system that can be used for rehabilitation of forelimb movements in rats after cervical SCI. Researchers will pair robotics with SCI in animal models with a focus on spinal repair. They will develop a robot-based system for forelimb rehabilitation of rodents with cervical SCI. A computer will control the system either to apply a boosting force to help carry the weakened arm to the object to be grasped or to apply a force that the animal needs to push against harder, to help build strength. Researchers will use this system to perform robot-assisted rehabilitation therapy in rats with a 4th cervical segment contusion injury.

Progress Towards Specific Aims: The Martin and Agrawal labs have met several times and have developed a detailed plan for the robotic training system. They have piloted the overall configuration of the system, which will provide body weight support for the injured rats, and attachment of the robot to the rat's forelimb. They have tested prototypes of all key elements of the system and developed the pellet reaching task that the rats will perform under robotic control.

Future Directions: The researchers plan to complete fabrication of the robotic training system during the next reporting period and will implement the system in intact animals. During the later phases of the project, they plan to develop the graphical user interface of the system and further develop assistive and resistive functions of the system. They also plan to implement the system in animals with a C4 contusion injury.

Impact: The novel robot rehabilitation system, in addition to facilitating and strengthening performance of visually-guided movements, provides a semi-automated and objective evaluation of movements in injured rodents. It can be used to screen the behavioral efficacy of emerging therapeutic strategies, rapidly and efficiently. This approach provides an unparalleled bridge between robot-based animal and human rehabilitation.

#### **11. University of Rochester**

Mark Noble, Ph.D.

PART: \$990,000

Acute Treatment with 4-aminopyridine Promotes Extensive Recovery from Traumatic SCI

Introduction/Background: Dr. Noble and his research team will conduct a detailed study of a new therapeutic approach to provide an exceptionally attractive candidate agent

within short times after SCI. They demonstrate that administration of clinically relevant dosages of an existing drug, 4-aminopyridine, already approved for other purposes (and thus less expensive to develop), promotes an extent of behavioral recovery after experimental SCI that is quantitatively and qualitatively better than achieved in studies on other candidate SCI treatments. These dramatic improvements are seen even though they do not initiate treatment until 24 hours post-injury, a 6-fold longer interval than reported even for drugs that provide less benefit.

Along with promoting behavioral recovery, their therapeutic agent decreases lesion size and cell death. Moreover, treatment for just 48 hours is also sufficient to reverse multiple injury-associated changes in gene expression even in the lesion center, the region of greatest tissue injury. This drug appears to be unique in also promoting recovery from traumatic injury to peripheral motor nerves, which frequently accompanies SCI. Treatment with their candidate agent also decreases skeletal muscle atrophy following peripheral motor nerve injury.

Progress towards specific aims and future directions will be reported in the 2019 SCIRB Annual Report.

Impact: Researchers will study two additional clinically critical aspects of SCI, which have received less experimental attention than damage to the spinal cord. This research will provide a detailed investigation of the potential of their approach as a new treatment to decrease damage in acute SCI.

## **12. Winifred Masterson Burke Medical Research Institute**

John Cave, Ph.D.

IDEA: \$360,000

Molecular Mechanisms Regulating Cell Adhesion in Reactive Astrocytes and Glial Scar Formation Following Spinal Cord Injury

Introduction/Background: Reactive astrocytes are a key cell type of scar tissue produced by SCI. The overall objective of this proposal is to establish the molecular mechanism by which the ZEB2 transcription factor protein and Zeb2os RNA transcript regulate expression of the Cadherin 1 (CDH1) cell adhesion protein in reactive astrocytes during glial scar formation.

Progress Towards Specific Aims: Researchers will establish that ZEB2 protein and Zeb2os RNA expression levels are elevated in reactive astrocytes following SCI. To this end, they have started histological analyses to define the spatio-temporal expression pattern of ZEB2 following SCI. They have also started in vitro experiments with post-natal astrocyte cultures to establish that Zeb2os expression levels control ZEB2 protein production.

Researchers will show that ZEB2 and Zeb2os are critical for regulating both CDH1 expression in reactive astrocytes and glial scar formation following SCI. To this end, they have begun to generate genetically engineered mice that will lack ZEB2 specifically in astrocytes.

Future Directions: They expect to have preliminary findings defining specific cell types that express ZEB2 in the spinal cord after injury as well as preliminary findings showing that the forced over-expression of Zeb2os in cultured astrocytes alters ZEB2 protein

levels. They also expect to have begun studies testing whether glial scar formation is disrupted in mice lacking one or both genetic copies of the ZEB2 gene.

Impact: Successful completion of this project will significantly advance their understanding of the molecular mechanisms that regulate glial scar formation following SCI as well as the development of therapeutic strategies to reduce glial scar size and improve functional recovery from SCI.

### **13. Winifred Masterson Burke Medical Research Institute**

Edmund Hollis, Ph.D., Roman Giger, Ph.D.

Sub-applicant: University of Michigan

IDEA: \$360,000

Immune-mediated Nervous System Repair

Introduction/Background: Nervous system injury causes a rapid immune response. Blood-derived immune cells infiltrate damaged neural tissue, both in the peripheral nervous system (PNS) and the CNS. In the injured PNS, immune cells contribute to clearance of damaged tissue and release factors that promote neurorepair. In marked contrast, the immune response triggered by a CNS injury has detrimental effects and fails to promote repair. The cellular and molecular make-up of the immune response triggered by PNS and CNS injury, at different post-injury time points, has not yet been described in detail, and is a focus of the researchers' experiments.

Progress Towards Specific Aims: Experiments for Aim 1A include a comparative analysis of immune cell types that accumulate in injured nervous tissue at different post-injury time points. Specifically, they aim to analyze the presence of immune cells in naïve mice and in mice at 1, 3, and 7 days following PNS and CNS injury. Their studies have largely been completed and revealed a highly dynamic and site-specific distribution of cells of the innate immune system.

Other experiments proposed (Aim 1B) include the grafting on specific immune cell types into neural injury sites to assess their pro-regenerative or detrimental effects. Using cell sorting techniques, they were able to isolate highly purified populations of immune cell types. They used purified cells for grafting experiments and demonstrated feasibility in the mouse. Many of the grafted cells survived the surgical procedures. Preliminary studies show that neutrophils (immune cells that respond first to injury) support neuronal regeneration.

Researchers have determined the optimal method for isolating the genetic material for their study (Aim 2A) of gene changes underlying regeneration in sensory neurons. They have been performing the experiments required to isolate this genetic material prior to sending it out for sequencing.

Future Directions: Experiments are focused on the identification of biochemical pathways activated in immune cell types under regenerative and non-regenerative conditions.

Impact: Their work is the first detailed description of the immune response triggered by PNS and CNS injury. This work fills an important gap in our knowledge and provides a platform to study the role of different immune cell types in the injured mammalian nervous system.

#### 14. Winifred Masterson Burke Medical Research Institute

Jian Zhong, Ph.D.

IDEA: \$360,000

Investigating Axonal mRNA Translation in CST Axon Sprouting and Regeneration

Introduction/Background: Dr. Zhong's research aims to elucidate the mechanisms of B-RAF mediated axon regeneration in the corticospinal axons after injury. Successful axon regeneration requires the rapid production, transport, and assembly of large amounts of cytoskeletal and membranous materials at the site of axon extension. Local axonal protein synthesis could be a limiting factor for axon regeneration in the CNS, so it is important to understand how it is regulated.

Progress Towards Specific Aims: This research will address two specific questions regarding local gene expression in the corticospinal tract: (1) whether axonal translation is engaged in regenerating CST axons as it is in regenerating PNS axons, and (2) which mRNA species are translocated in CST axons, at baseline and during regeneration. Progress has been made towards determining the regulation of axonal ribosomes by RAF – MAP kinase and PI3 kinase signaling in the CST and will characterize the translomes of regeneration-competent CST axons. For the translating ribosome affinity purification (TRAP) analysis, the research team has generated mice carrying the *Isl-kaBraf : Isl-EgfpL10 : fezf-CreER* alleles and started to collect tissues. Pilot data indicate a low expression of axonal EgfpL10 and ensuing low amounts of axonal ribosome-associated mRNA they can isolate from CST. The team has therefore expanded the breeding colony to >10 cages to ensure they will have valid samples for our analyses going forward.

To assess the role of PI3 kinase signaling in axonal transport, they are in the process of generating *Pten<sup>ff</sup> : Isl-EgfpL10 : fezf-CreER* mice and anticipate to begin breeding this line in 2019.

Future Directions: The research team will generate sufficient mice to carry out the planned TRAP experiment as planned. Dr. Fabricio Nicola will participate in the animal breeding and will receive training in carrying out the TRAP study as well as RNA seq data analysis. It is expected that the first TRAP experiment will begin in 2019.

Impact: A detailed understanding of the mechanisms that enable axons to extend in the injured mature spinal cord will be crucial to identify and overcome the bottlenecks that limit axon regeneration, and to develop therapeutic strategies to benefit SCI patients. Candidate genes that emerge as crucial for CST regeneration will guide the development of novel therapeutic strategies to facilitate axon regeneration in SCI patients.

## Translational Research Projects in Spinal Cord Injury (Round 2)

Contract Term 5/1/18-4/30/22

Progress Reporting Period  
5/1/18-11/30/18

2 Awards, Procurement Total: \$2,827,075

### 1. Health Research, Inc.

Johnathan Wolpaw, M.D., Gerwin Schalk, Ph.D.

Sub-applicant: Medical University of South Carolina

\$1,623,620

A Spinal Reflex Operant Conditioning System Suitable for Clinical Translation

Introduction/Background: Current rehabilitation for people with motor deficits due to SCI consists mainly of pharmaceutical and physical therapies. Functional recovery could be enhanced by targeted neuroplasticity therapies that produce long-term beneficial changes in the spinal cord. One of the first new therapies are spinal reflex operant conditioning protocols that modify abnormal reflex pathways and improve walking and other motor skills that use these pathways. These protocols require a complex software/hardware system that is usable only by highly trained experts. The goal of this project is to translate this cumbersome system into a simple system which is suitable for widespread use by clinical therapists.

Progress Towards Specific Aims: Researchers have developed algorithms that aid in the selection of nerve stimulation and muscle recording sites; automatically generate M-wave and H-reflex recruitment curves; and define the subject-specific stimulation and recording parameters used by the conditioning protocol. These algorithms have been tested and are being disseminated to the sub-applicant who will test each component in hopes of providing feedback that will optimize the new system.

Future Directions: Researchers will complete development of the algorithms that set-up the system for conditioning and they will begin the development of algorithms that control the conditioning sessions.

Impact: This new system will enable clinical therapists to participate in further development, evaluation and dissemination of operant conditioning protocols that produce targeted plasticity and can supplement therapies and enhance recovery for people with SCI or other chronic neuromuscular disorders.

Publications: Eftekhar, A., Norton, J.J.S., McDonough, C.M., & Wolpaw, J.R. (2018). Retraining Reflexes: Clinical Translation of Spinal Reflex Operant Conditioning. *Neurotherapeutics*, 15(3), 669-683. doi: 10.1007/s13311-018-0643-2.



## **2. University of Rochester**

Mark Noble, Ph.D., Christoph Proschel, Ph.D.

\$1,203,455

Pharmacological Treatment of Acute Spinal Cord Injury

Introduction/Background: This research is designed to provide promising new treatments and to identify and overcome factors that might limit success in clinical trials. His lab discovered that treatment with a repurposed drug in the acute/sub-acute injury period is able to bring rats with traumatic SCI from a state of complete paralysis one day post-injury to nearly complete recovery within two weeks. Researchers found that these benefits can be achieved even when treatment initiation is delayed until 24 hours post-injury, in striking contrast with other treatments that must be initiated within 2-3 hours post-injury to provide benefit. The ability to delay treatment initiation will allow for patients to be properly stabilized and evaluated without losing benefit of treatment and will enable more accurate determination of suitability for inclusion in a clinical trial. Furthermore, the use of an existing drug greatly decreases therapeutic development costs.

Progress towards specific aims and future directions will be reported in the 2019 SCIRB Annual Report.

Impact: The results of this research is expected to lead to a clinical trial with a powerful new therapy and enhance clinical trial design to increase the likelihood of success by investigating such problems as effects of age on treatment outcome and whether existing treatments can compromise efficacy of their new therapeutic approach.

## Individual Predoctoral/Postdoctoral Fellowships (Round 2)

Contract Term 9/1/17-8/31/20

Progress Reporting Period  
3/1/18-8/31/18

**8 Awards, Procurement Total: \$1,341,954**

**1. Bronx Veterans Medical Research Foundation, Inc.**

Noam Harel, M.D., Ph.D., Yu-Kuang Wu, P.T., Ph.D.

Postdoc: \$187,440

EMG Triggered Closed-Loop Stimulation for Spinal Cord Injury Individuals

Introduction/Background: Most individuals with SCI have residual nerve circuits. Researchers aim to strengthen those circuits to improve motor recovery after injury. To do this, they are attempting to pair electrical and magnetic stimulation with physical training targeted toward the connections between nerve circuits. The brain's intention to move a muscle can be read by recording surface electrical activity over target muscles (electromyography or EMG). In animal models of SCI, scientists have successfully used target muscle EMG to trigger spinal cord electrical stimulation pulses while the animals perform physical exercises. Using the body's own signals to trigger nerve stimulation is called "closed-loop stimulation". This might be an optimal method to coordinate brain and nerve activity, especially with the clinical advantage of being possible to combine with physical exercise training. However, whether EMG-triggered closed loop stimulation has the same amount of effect when applied non-invasively in humans is still unknown.

Progress Towards Specific Aims: Researchers established effective algorithms for EMG-triggered closed-loop neural stimulation, which included finalizing the hardware and software design of the EMG and obtaining IRB approval for human testing. Researchers initiated subject enrollment in January 2018 and recruited two able-bodied participants and one participant with SCI. The two able-bodied enrollees have completed the study and the SCI participant is currently undergoing the study. The study includes five 20-minute EMG triggered closed-loop sessions at 0.1 Hz. Relative to baseline, the most improvement after stimulation was hand movement without stimulation, followed by passive paired stimulation and EMG-triggered PNS.

Future Directions: Researchers expect to enroll and test an additional four subjects (two SCI and two able-bodied participants), to test if other SCI participants will have similar responses to stimulation.

Impact: EMG-triggered closed-loop stimulation is assumed to have the greater potential to stimulate residual nerve circuits in persons with SCI compared to passively receiving brain or peripheral nerve stimulation. This new approach could be a future therapeutic modality to combine EMG-triggered stimulation with physical/robotic exercise training to further strengthen the new circuits.

## 2. Columbia University

Sunil K. Agrawal, Ph.D., Dario Martelli, Ph.D.

Postdoc: \$193,482

Improving Locomotor Function After Spinal Cord Injury with a Perturbation-Based Balance Training

Introduction/Background: The goal of this project is to test safety and preliminary efficacy of an innovative locomotor training based on unexpected balance perturbations. During the first year of the project, the following aims were accomplished:

- The capabilities of the Active Tethered Pelvic Assist Device (A-TPAD) were augmented by developing a cable-driven solution to assist and perturb locomotion in SCI and a Virtual Reality (VR) system was integrated as a part of the perturbation-based gait training; and
- An experimental study was initiated to analyze the adaptation of the reactive and proactive strategies to control gait stability in a single training session in patients with SCI.

Progress Towards Specific Aims: A cable-driven solution has been developed to simultaneously assist and perturb locomotion. The A-TPAD can now apply at the same time perturbations and body weight support (BWS). Various VR platforms such as an infinite walker and a software to deliver pseudo-random oscillations of the visual field can be integrated in the dome that the researchers developed.

Their system was tested on a healthy participant to analyze the effects of different levels of BWS. Results highlighted that the system was able to apply the desired amount of vertical and waist-pull forces and the amount of BWS influence both normal gait and the recovery reaction. During this reporting period, a single session experiment was performed by two patients with SCI. Results showed that the participants were able to modulate the recovery reaction for different amplitude of the perturbations and adapt the walking pattern at the end of the training session.

Future Directions: Researchers plan to complete the single session study on patients with SCI. Moreover, they plan to increase the sample of healthy young participants to test the effect of different levels of BWS. Two scientific papers based on the results of the studies will be submitted. In addition, researchers will start to recruit participants for a multisession study.

Impact: The A-TPAD is the first system that can apply balance perturbations while providing BWS. This unique feature may be useful for implementing new gait training strategies. This award will equip Dr. Martelli with the scientific training and specific knowledge in SCI to pursue an independent career in this field. He will analyze and gain knowledge of the effect of visual perturbations and the prevalence of visuomotor adaptation during unperturbed overground walking or in response to continuous visual oscillations in a VR environment.

Presentations: Martelli, D., Kang, J., & Agarwal, S.K. (July 2017). *A Single Session of Perturbation-based Gait Training with the A-TPAD Improves Dynamic Stability in Healthy Young Subjects*. Presented at the IEEE International Conference on Rehabilitation Robotics (ICORR), London, United Kingdom.

Martelli, D., Kang, J., & Agarwal, S.K. (August 2018). *A Perturbation-based Gait Training with Multidirectional Waist-Pulls Generalizes to Split-Belt Treadmill Slips*. Presented at the 7th IEEE International Conference on Biomedical Robotics and Biomechanics (Biorob), Enschede, The Netherlands.

Publications: Martelli, D., Luo, L., Kang, J., Kang, U. J., Fahn, S., & Agrawal, S.K. (December 19, 2017). Adaptation of Stability during Perturbed Walking in Parkinson's Disease. *Sci Rep*, 7(1):17875. doi:10.1038/s41598-017-18075-6

Martelli, D., Xia, B., Prado, A., & Agrawal, S.K. (January 2019) Gait Adaptations during Overground Walking and Multidirectional Oscillations of the Visual Field in a Virtual Reality Headset. *Gait and Posture*, 67:251-256. doi:10.1016/j.gaitpost.2018.10.029

### 3. Cornell University

Chris B. Schaffer, Ph.D., Yu-Ting Chen, Ph.D.

Predoc: \$135,600

In Vivo Three-Photon Excited Fluorescence Imaging of Spinal Cord Neural Activity in Awake, Locomoting Mice After Spinal Cord Injury

Introduction/Background: SCI leads to dysfunctional central pattern generator (CPG) circuit that send out aberrant signals to the ventral motor neurons. The goal of this grant is to develop the capability to directly monitor the ensemble firing of CPG circuits in the spinal cord of walking mice after upstream injury. With the combination of three-photon excited fluorescence (3PEF) microscopy at 1320nm excitation source with genetically-encoded calcium indicator (GECI) GCaMP labeled CPG neurons, researchers are able to long-term depict the changes of firing patterns in CPGs along with functional recovery after SCI.

Progress Towards Specific Aims: The postdoc has successfully demonstrated the power of 3PEF imaging in the mouse spinal cord over 2PEF at 1320nm source. The results show that 3PEF enables 3-4 times better optical penetration depth at single cell/capillary resolution. The 1320nm source enables multicolor imaging in a single imaging session that allows for monitoring cell-cell interaction in health and disease states. The results also show that there are larger fluorescence changes ( $\Delta f/f$ ) when the animals walk versus a canonical resting state. Preliminary results show that there is a 5-10 times signal gains in the YFP labeled neurons in the mouse spinal cord after adaptive optic correction. They have made a significant progress on kinematic analysis for limb tracking for spine-fixed awake calcium imaging in mice.

Future Directions: Their research goal is to develop the capability to directly monitor the ensemble firing of CPG circuits in the spinal cord of walking mice after upstream injury. With the combination of three-photon excited fluorescence (3PEF) microscopy at 1230nm excitation source with genetically-encoded calcium indicator, GCaMP labeled CPG neurons, they will be able to depict long-term changes of firing patterns in CPGs along with functional recovery after SCI.

Impact: The new imaging approaches outlined in this research will offer great potential to directly visualize these locomotor circuit dynamics in adult, moving animals and assess their pathological progression after upstream SCI over time for potential therapeutic intervention.

#### **4. Feinstein Institute for Medical Research**

Ona Bloom, Ph.D., Jake Deckert, Ph.D.

Postdoc: \$168,414

Biomarkers in Adult and Pediatric Traumatic Spinal Cord Injury

Introduction/Background: The postdoctoral fellow will join an ongoing, longitudinal observational clinical study, largely funded by the Congressional Directed Medical Research Programs' (CDMRP) Department of Defense Spinal Cord Injury Research Program (SCIRP), to measure inflammatory and other substances in blood and the progression and extent of physical recovery within the same individuals, immediately after their SCI and then throughout the first year after SCI. Together, this data will enable researchers to determine biomarkers of spontaneous recovery in adults with SCI. The experience will provide the fellow with a skillset in SCI research, conduct of clinical research and experimental methods. Previous funding, supported by the NYS SCIRB, expanded immunological outcome measures and additional time points of biological data collection to enhance the information gained and impact of the study. The fellow will also in parallel, initiate a pilot study of biological responses in children with SCI, obtaining blood samples from patients treated at Cohen Children's Medical Center (CCMC), the only Level One Trauma Center currently serving children in NYC and Long Island. In addition to its training value to the fellow, this study will provide a completely novel data set in children with SCI, who tend to have better physical recovery than adults, leading to a greater understanding of SCI.

Progress Towards Specific Aims: Dr. Deckert completed the required Collaborative Institutional Training Initiative (CITI) training modules to enable his participation in human subjects research. He was subsequently added to the IRB protocols relevant to this fellowship.

Future Directions: The fellowship career goals and training development plan will allow Dr. Deckert to advance his skills and knowledge to become an independent scientist leading translational studies in traumatic spinal cord injury.

Impact: This training award will equip Dr. Deckert with the scientific training and specific knowledge in SCI to pursue an independent career in this field, and create new knowledge to advance the health and quality of life of adults and children living with SCI.

#### **5. Icahn School of Medicine at Mount Sinai**

Hongyan Zou, Ph.D., M.D., Shalaka D. Wahane, Ph.D.

Postdoc: \$176,550

Molecular mechanisms of neural repair after CNS injury

Introduction/Background: SCI is a debilitating disorder with no current therapies that allow for motor function recovery. Researchers propose to dissect the genetic and epigenetic mechanisms that hinder repair after injury. Microglia together with macrophages (cells of the myeloid lineage) form the first line of defense against CNS diseases, insult and trauma. Microglia activation may be pro-inflammatory or pro-repair – and thus can hinder or promote the wound healing process by releasing pro- or anti-inflammatory cytokines at the lesion site. Their laboratory has identified histone deacetylase 3 (HDAC3) as a major regulator of the innate immune response after SCI. Researchers propose that modulating HDAC3 activity within microglia may provide for cues to alleviate spinal cord injury and improve motor activity.

**Progress Towards Specific Aims:** The researchers have demonstrated that HDAC3 gene expression is upregulated in SCI microglia and that HDAC3 plays a central node in the upstream regulatory network of gene activation in SCI microglia and microglia activation-associated genes. From these findings, they are characterizing the neuroprotective effects of HDAC3 inhibition in SCI by examining glial scar composition, axon regrowth and behavioral analyses of SCI recovery after pharmacological inhibition of HDAC3 by the small molecule inhibitor RGFP966. Further, they are examining a combinatorial treatment of RGFP966 and intrathecal injection of AAV-BMP4 to improve axon regeneration after SCI.

**Future Directions:** They will be examining histone acetylation dynamics, assessing axon regrowth, and identifying cytokine profiles present at injury sites. They hope to shed light on the molecular mechanisms directly affected by the loss of HDAC3 activity and defining changing gene signatures under HDAC3 regulation. This may lead to a potential treatment of SCI with the combinatorial treatment of RGFP966 and AAV-BMP4

**Impact:** HDAC3 functions as the central node in regulating upstream regulators of all chromatin regulators and transcription factors changed in IAM microglia and macrophages. This provides strong evidence that HDAC3 may play a critical role in the regulation of immune response after CNS injury. A number of pro and anti-inflammatory cytokine genes are differentially expressed upon SCI. HDAC3 has known functions in immune response and control of inflammatory cytokine genes in multiple cell types. By blocking HDAC3 activity using a small molecule inhibitor RGFP966 shows improved scar formation, axon regeneration and wound healing as well as motor recovery in mice, while unaffected normal physiological functions of microglia and macrophages.

**Publications:** Kuboyama, T., Wahane, S., Huang, Y., Zhou, X., Wong, J.K., Koemeter-Cox, A.,... & Zou, H. (2017). HDAC3 inhibition ameliorates spinal cord injury by immunomodulation. *Sci Rep* 7, 8641.

## **6. Rensselaer Polytechnic Institute**

Ryan J. Gilbert, Ph.D., Anthony D'Amato, Ph.D.

Predoc: \$135,600

Estrogen Based Biomaterials Promote Astrocytic Growth Factor Production and Provide Neuroprotection Against Glutamate Excitotoxicity

**Introduction/Background:** 17 $\beta$ -estradiol (commonly known as estrogen) is a steroidal hormone that differentially affects all cells in the CNS. Estrogen is potentially neuroprotective against glutamate excitotoxicity and the research suggests that this molecule can promote changes in astrocyte phenotype to be more supportive of regeneration following SCI. This research focuses on developing a biomaterial with tunable estrogen release kinetics to modulate astrocyte reactivity following SCI and protect neurons from glutamate excitotoxicity.

**Progress Towards Specific Aims:** During this reporting period Dr. D'Amato is focused primarily on characterizing the estrogen release kinetics from their newly developed biomaterial. They verified that this new material will release estrogen with zero-order kinetics over approximately 1.4 years. They determined that the cell culture model originally proposed for this project is not feasible since the primary astrocyte cultures grown in the lab require serum proteins for growth. Serum proteins, however, have large

and varying amounts of estrogen in them. This has interfered with the ability to assess the efficacy of our new material in mitigating astrocyte reactivity in vitro.

Future Directions: Dr. D'Amato plans to develop a serum-free, primary astrocyte culture model that can be used to analyze the material's effects on astrocyte phenotype. They have also begun exploring the materials ability to counteract glutamate excitotoxicity in vitro with rat dorsal root ganglion (DRG).

Impact: This study has the potential to increase the regenerative potential of electrospun fiber scaffolds in treating SCI. Typically, electrospun fibers facilitate directed tissue regeneration across an SCI lesion site but fall short of promoting functional recovery. This study adds a bioactive compound to the contact-mediated guidance provided by the fibrous scaffold. This added component should increase the efficacy of fiber scaffolds as estradiol promotes beneficial phenotypic changes in a host of cells in the spinal cord. Aim 1 of this study led to the development of a new biomaterial guidance scaffold for nervous tissue regeneration that delivers a neuroprotective and potentially regeneration-promoting drug for extended periods of time (as long as 1.4 years).

Dr. Gilbert and Dr. D'Amato's research has been published and citations are listed under Rensselaer Polytechnic Institute's Institutional Support for Spinal Cord Injury Research (Round 6) progress.

## **7. University of Rochester**

Christoph Proschel, Ph.D., Michael Rudy, Ph.D.

Postdoc: \$176,454

Novel Pharmacological Approach to Promoting Myelin Preservation, Neuronal Regeneration and Preventing Muscle Atrophy Following SCI

Introduction/Background: A recent approach to preserve myelin and/or enhance repair of demyelinating damage to promote axonal regeneration and to rescue muscle from atrophy following contusion injury has been to repurpose drugs that may be able to enhance neuronal function. One such FDA-approved drug is 4-aminopyridine (4-AP). The research team has found that acute treatment of spinal cord injured rats with 4-AP results in significantly improved motorfunction after SCI. They are focused on the effects of 4-AP treatment on myelinating cells (Aim 1). In addition, a significant part of this research project is aimed at examining the treatment parameters for the acute use of 4-AP in the rat thoracic SCI model.

Progress Towards Specific Aims: Consistent with the researchers prior characterization, rat GDAsBMP exhibited an epithelial morphology with a comparatively low level of GFAP expression and higher AQP4 expression than GDAsCNTF.. In contrast, GDAsCNTF resemble hypertrophic, reactive astrocytes with a more extended appearance and high levels of GFAP expression. These findings are further supported by gene expression analysis using RT-QPCR of GDA-BMP and GDA-CNTF cultures. Having established these GDA-BMP cultures, they are now in a position to test the effect of 4-AP with and without concomitant astrocyte transplants on the functional recovery after contusion SCI using our rat model.

Final Report: Dr. Micael Rudy's fellowship award concluded in 2018 due to personal circumstances which required his return to Colorado. During his fellowship, Dr. Rudy became proficient in many new procedures and surgery techniques which helped him to

secure a post-doctoral position studying viral infections in the spinal cord at the University of Colorado.

Impact: Efforts to move this same drug to clinical analysis in other injury settings is ongoing (which would offer training in aspects of translational science not available in most postdoctoral training in a scientific laboratory). This project provided an opportunity to be trained in multiple aspects of neurotrauma research relevant to SCI and in the context of developing an intervention that increasingly looks like an excellent candidate for eventual analysis in the clinic.

#### **8. Winifred Masterson Burke Medical Research Institute**

Edmund R. Hollis, Ph.D., Yue Li, Ph.D.

Postdoc: \$168,414

Motor Learning Mechanisms During Rehabilitation from Spinal Cord Injury

Introduction/Background: During the learning of motor skills, motor maps are remodeled in an experience-dependent process driven by cholinergic input from the basal forebrain. It remains unknown whether similar cholinergic mechanisms underlie the recovery of corticospinal circuit function after SCI. The overall objective of the project is to determine the role of motor learning mechanisms in functional motor recovery and motor cortex reorganization during rehabilitation. To achieve this, the postdoctoral fellow, Yue Li, Ph.D., will test the central hypothesis that cholinergic input to corticospinal neurons is required for the functional integration of circuit changes after SCI. Dr. Li has expertise in survival surgeries, behavioral analysis, and tissue processing required for the research.

Progress Towards Specific Aims: Researchers have been refining chronic electromyography electrode implantation in mice for use with automated motor mapping. They performed troubleshooting on basal forebrain cholinergic neuron deletion by injection of p75-saporin. They have developed a recessed skilled pellet retrieval behavior that shows a more distinct learning curve in mice. They are developing the tools for imaging neuron structure and investigated the role of nicotinic and muscarinic receptors in motor learning by administration of corresponding inhibitors (Mecamylamine, methyllycaconitine, dH $\beta$ E and Atropine). Additionally, researchers are breeding transgenic mice (Thy1-ChR2,  $\alpha$ 7 conditional knockout and ChAT-Cre) for each aim of the study.

Future Directions: Researchers will study the role of nicotinic receptors in motor learning by using  $\alpha$ 7 conditional knockout mice, use saporin to investigate the effect of basal forebrain cholinergic neuron deletion on rehabilitation after SCI, and test the effect of optogenetic modulation of acetylcholine input to motor cortex on learning and rehabilitation.

Impact: This training award will equip Dr. Li with the scientific training and specific knowledge in SCI to pursue an independent career in this field and gain new knowledge on acute effects of cholinergic modulation on corticospinal recruitment during rehabilitation.



## **PART/IDEA in Spinal Cord Injury (Round 1)**

**IDEA Contract Term 1/1/17-12/31/18; PART Contract Term 1/1/17-12/31/19**

**Progress Reporting Period  
1/1/18-7/31/18**

**11 Awards, Procurement Total: \$6,264,035**

### **1. Bronx Veterans Medical Research Foundation, James J. Peters VA Medical Center**

Hesham Tawfeek, M.D.

IDEA: \$356,999

Role of T cells in Bone Loss After Spinal Cord Injury

Introduction/Background: Bone loss after SCI is a major clinical problem due to the high incidence, severity, resistance to treatment, and the subsequent bone fracture. This project investigates a possible role of T cells of the immune system in bone loss after SCI. Therefore, mice that have or lack T cells undergo sham (control) or SCI surgery and bone loss is measured after one, two, or five weeks after surgery. Additionally, changes in T cell subsets, factor secretion, gene expression, and T cell interaction with other bone cells are assessed. The studies may shed light into a possible interaction between the immune system and the skeleton and could have important implications for reducing the risk of bone fracture in SCI. In the first and second quarters of the study, researchers aimed to examine the role of T cells in bone loss after one and two weeks of SCI.

Progress Towards Specific Aims: Researchers have achieved significant progress toward addressing the role of T cells in bone loss after SCI. During this period, the mice underwent sham (control) surgery, their bone loss was measured, biochemical data was collected, and analysis started.

Future Directions: In the final six-months of research, biochemical analysis will be completed, and results will be analyzed. Dr. Tawfeek will use the promising data from this research to support future grant applications to further study the importance of promoting the immune function as a new strategy to reduce bone loss in patients with SCI.

Impact: The bone structure analysis results obtained from animals strongly suggest that T cells control bone loss after SCI. Results from the remaining analyses and experiments of year 2 would further assure these findings and reinforce the possible role of T cells in SCI-induced bone loss. If confirmed by other analyses, these findings could provide meaningful insight into the importance of promoting the immune function as a new strategy to reduce bone loss patients with SCI.

### **2. Cornell University**

Chris B. Schaffer, Ph.D.

IDEA: \$350,876

Imaging Neural Activity in the Spinal Cord of Awake Mice After Spinal Cord Injury

Introduction/Background: The goal of this research project is to develop the capability to directly image the patterns of neural activity in the spinal cord of mice before and after spinal cord injury. Such a capability would enable researchers to uncover the changes in activity patterns in the spinal cord neurons that control limb motion after a spinal cord injury, with the long-term goal of modulating those activity patterns to enable higher fidelity limb control.

Progress Towards Specific Aims: Dr. Schaffer is working to develop the capabilities to image neural activity patterns in genetically-defined sub-populations of neurons in the spinal cord during mouse locomotion. In addition, his lab is investigating how these activity patterns change after an upstream spinal cord injury. They demonstrated imaging at sufficient depth in the spinal cord to study the relevant neurons, demonstrated imaging of neural activity in the spinal cord (but in shallower, sensory-related neurons), and successfully targeted the fluorescent reporters of neural activity to a genetically defined sub-population of neurons involved in locomotion (which sit at depth in the spinal cord). They discovered that bringing these three things together is challenging, and they had difficulty achieving high enough signal to reliably measure calcium transients from neurons. Two strategies have been identified to address this: 1. Obtaining new reporter mice that more strongly expresses the calcium indicator, and 2: Using adaptive optics to correct for the aberrations associated with focusing into the cylindrically-shaped spinal cord.

Future Directions: The new reporter mice (obtained from Allen Brain Institute) are at Cornell and are being bred. In a proof-of-principle experiment, working with Prof. Chris Xu's laboratory, they have shown that improvements in signal strength by a factor of 5-10 are achievable in the spinal cord with adaptive optics. They have made excellent progress in the analysis software they developed for extracting limb motion from videos of spine-fixed mice.

Impact: This work will use the recently developed capability to image neural activity in the spinal cord of awake, spine-fixed mice moving on a treadmill. They will be able to investigate altered patterns of activity in a genetically defined set of spinal cord neurons after upstream spinal cord injury and correlate those changes with changes in hindlimb motion.

### **3. Research Corporation of Long Island, Inc., Northport VA Medical Center**

Victor L. Arvanian, Ph.D., D.Sci

Sub-applicant: Houston Methodist Research Institute

PART: \$935,867

Neuroplasticity Integrating Human Induced Neuralized Pluripotent Stem Cells (NiPSCs) in SCI Animals

Introduction/Background: Recent studies from the Center for Neuroregeneration, Department of Neurosurgery, Houston Methodist Neurological Institute, led by Philip J. Horner, Ph.D., revealed that Neural Progenitor Cells (NPCs), derived from NiPSCs can be reprogrammed to become neurons and oligodendrocytes with an ability for good survival and integration in the chronically injured spinal cord of adult rats.

Based on results of recent experiments, conducted in the laboratories of Victor L. Arvanian, Ph.D., D.Sci and Dr. Horner, we hypothesize that spinal electro-magnetic stimulation (sEMS) and exercise combined with transplantation of NiPSCs may improve

incorporation of NiPSC-derived neurons into host spinal cord and promote formation of new functional synaptic connections to neurons in the damaged spinal cord.

**Progress Towards Specific Aims:** For Aim 1, researchers have concluded that animals that received NiPSCs combined with chronic sEMS/exercise treatment exhibited better recovery of locomotor function and transmission compared with rats that received NiPSCs only. Effects were, however, less robust than expected. Researchers hypothesize that acute delivery of NiPSCs could have limited the rats function or engraftment due to the inflammatory and metabolic challenge. Researchers now anticipate that delayed implantation of NiPSC (when microglia/macrophage responses are attenuated) will be a better approach, and there is sufficient evidence that sEMS and exercise combined with transplantation of NiPSCs may improve incorporation of NiPSC-derived neurons into host spinal cord and promote formation of new functional synaptic connections to neurons in the host damaged spinal cord.

**Future Directions:** The administration of sEMS plus exercise treatments will begin on one group that received NiPSCs and they will receive NiPSCs implantation combined with chronic spinal electro-magnetic stimulation while under light isoflurane anesthesia. The second group will also be maintained under anesthesia and consist of NiPSCs only, but no sEMS/exercise will be administered.

**Impact:** The triple combination treatment of NiPSCs, sEMS and exercise carries the potential for developing a novel, feasible and effective translational set of treatments for acute and chronic contusion SCI.

**Presentations:** Arvanian, V.L., Petrosyan, H.A., Liang, L., Alessi, V., Ballas, N., Kondiles, B.R., & Horner, P.J. (July 2018). *Electro-magnetic stimulation and exercise training combined with implantation of neuralized-pluripotent stem cells (NiPSCs) immediately after spinal cord injury in rats*. Abstract presented at the Federation of European Neuroscience Societies (FENC) 11<sup>th</sup> Forum of Neuroscience, Berlin, Germany.

Arvanian, V.L., Petrosyan, H.A., Zou, C., Hu, W., Leone, C., Tesfa, A., & ...Sisto, S.A. (2018). *Combined administration of electro-magnetic stimulation (EMS) at spinal level and at leg muscles to modulate neurophysiological properties at spino-muscular circuitry in healthy and SCI humans*. Abstract presented at the Society for Neuroscience, San Diego, California.

Petrosyan, H., Liang, L., Tesfa, A., Fahmy, M., Zou, C., Sisto, S., & Arvanian, V. (2018). *Spinal electromagnetic stimulation induces modulation of M-wave and H-reflex responses and recovery of frequency-dependent depression of H-reflex in chronic spinal cord injured rats*. Abstract presented at the Society for Neuroscience, San Diego, California.

#### **4. Research Foundation for SUNY Stony Brook**

Sue Ann Sisto, Ph.D.

PART: \$989,199

Effects of Spinal Electromagnetic Stimulation and Locomotor Training on Motor Recovery and Walking in Incomplete SCI

**Introduction/Background:** The objective is to examine the potential effects of spinal electro-magnetic stimulation (sEMS) and Locomotor Training (LT) exercise on the spinal,

cortical circuits and the recovery of motor and physical function in adults with incomplete SCI. This stimulation will first be provided to healthy individuals to determine the ideal parameters and expectations for SCI testing and training.

**Progress Towards Specific Aims:** The researchers completed Aim 1 of their study with non-injured adults. The researchers have started Aim 2 and are currently collecting data using the same sEMS protocol of 3 sessions to compare data between SCI adults and non-injured adults (Phase II). The researchers moved towards Phase II, with three of ten people tested and three more people enrolled. The gathered data will allow for comparison between this phase and later phases as they investigate outcomes in SCI after five weeks of SEMS only versus a combination of sEMS and Locomotor Training (LT).

**Future Directions:** The researchers have submitted a change in the inclusion criteria to the IRB, to allow for recruitment of people with cervical lesions (C5 and below) while still requiring them to have incomplete injuries (AIS C and D). Once approved, they will have at least five more people who are candidates for enrollment, completing phase II of the study. This change will increase chances of enrollment and chances of increasing the generalizability of the results to a larger range of injury levels. They expect to finish phase II in the Fall and begin phase III and IV. They will next submit for IRB approval of phases III and IV which will allow to immediately transition into the final phases of the study.

**Impact:** Determination of neuroplastic capacity of the spinal cord with sEMS will improve sensory-motor and physical function.

**Preprint:** Arvanian, V. L., Petrosyan, H.A., Zou, C., Zaidi, M., Hou, W., Tesfa A., ...& Sisto, S.A. (2018) *Non-invasive spinal electro-magnetic stimulation (SEMS): a tool for evaluation and modulation of lower limb spinal-muscular transmission in healthy adults* (submitted to *PLoSone Journal*, July 2018). bioRxiv: 10.1101/382341.

## **5. Research Foundation for SUNY Stony Brook**

Irene C. Solomon, Ph.D.

IDEA: \$355,111

Therapeutic Potential of Mild to Moderate AIH on LUT and Respiratory Function in SCI

**Introduction/Background:** SCI results in reduction or loss of motor, sensory, and autonomic function below the level of the injury. Research aimed at enhancing functional recovery following SCI is essential, and exposure to acute intermittent hypoxia (AIH; single and repeated bouts (rAIH)) has been shown to elicit functional improvements in spinal motor systems in rats and humans following incomplete SCI. This project investigates the effects of mild to moderate AIH (SA1) and the therapeutic potential of rAIH exposure (SA2) to improve lower urinary tract (LUT) and respiratory motor dysfunction following SCI.

**Progress Towards Specific Aims:** Experiments assessing LUT and respiratory motor activities in response to single bout moderate AIH exposure in both uninjured (SCI sham) and moderate contusion SCI (1- and 4-week survival) rats have continued. Before AIH exposure, both 1- and 4-week SCI rats exhibited abnormal bladder behaviors (e.g., rhythmic non-voiding bladder contractions or increased bladder pressure without voids) with 1-week SCI rats exhibiting a greater degree of LUT dysfunction. While at

both 1- and 4-weeks post SCI, exposure to hypoxia during AIH often produced increased urine output either with or without functional bladder contractions, bladder activities following AIH exposure were markedly different at these survival times. At 1-week post SCI, bladder behaviors typically consisted of extended periods of elevated bladder pressure accompanied by small amplitude pressure fluctuations with continuous leakage while at 4-weeks post SCI, a long-lasting improved pattern of bladder activity characterized by fewer non-voiding bladder contractions, increased voiding volume, and emergence of a threshold-driven pattern of bladder contraction (characteristic of that seen in uninjured rats) was observed. In uninjured rats, few or no non-voiding bladder contractions were seen and a decrease in voiding frequency with increased voiding volume was also noted post-AIH. These observations suggest that moderate AIH is capable of producing a significant improvement of LUT function at 4-weeks (and in uninjured rats), but only a mild improvement at 1-week, post-SCI.

**Future Directions:** Complete the experiments proposed in specific aim 1 and begin experiments proposed in specific aim 2, which will implement and assess potential therapeutic benefits of a rAIH gas treatment protocol that is designed to induce spinal motor plasticity on LUT and respiratory motor function following mid-thoracic SCI.

**Impact:** They have implemented a promising non-invasive therapeutic approach to facilitate LUT and respiratory recovery following SCI. This therapeutic strategy, which can be integrated into clinical use in various settings (e.g., hospital, rehabilitation center, home), would exert a significant positive impact on quality of life in SCI patients.

**Publications:** Collins, W.F., Wang, C., Torpie, M.E., & Solomon, I.C. Impact of acute intermittent hypoxia (AIH) on lower urinary tract function following mid-thoracic spinal cord contusion injury. (2018). *Federation of American Societies for Experimental Biology Journal*, Experimental Biology Program No. 931.163; 32:913.13.

**Presentations:** Collins, W.F., Torpie, M.E., Wang, C., & Solomon, I.C. (2018). *Moderate hypoxia produces voiding in rats with spinal cord injury-induced severe urinary dysfunction*. Presented at the Society for Neuroscience, San Diego, California.

## **6. Research Foundation for SUNY, University at Albany**

Ben G. Szaro, Ph.D.

IDEA: \$359,738

Functional Analysis of Genes Implicated in Successful CNS Axon Regeneration

**Introduction/Background:** The tremendous progress made toward understanding why CNS axon regeneration fails in mammals has underscored the need for combinatorial therapeutic strategies. Studying animal models, such as the frog *Xenopus laevis*, where successful CNS axon regeneration occurs naturally can provide a rational basis for designing such strategies. Frogs are one of the best models for such studies, because they recover from SCI as tadpoles but not as frogs, and because they recover from optic nerve injury throughout life. Earlier studies have identified a gene [heterogeneous ribonucleoprotein K (hnRNP K)] that is required for regulating expression of structural proteins required to make axons, and additional genes that are differentially expressed in tadpole vs. frog hindbrain after SCI. The first objective tests whether hnRNP K and its regulators help determine success or failure of CNS axon regeneration. The second objective explores functions of differentially expressed genes between successful and unsuccessful CNS regeneration.

Progress Towards Specific Aims: The analysis of genome-wide data of genes differentially expressed in hindbrain during successful recovery from SCI (tadpole) vs. unsuccessful recovery (frog), along with data from successful CNS axon regeneration after optic nerve injury, identified core genes shared by the two regenerative tissues. Analysis of epigenetic pathway genes suggests differential modifications to chromatin structure are particularly relevant for successful vs. unsuccessful recovery from CNS injury.

Future Directions: Efforts during the next reporting period will be targeted toward identifying cell types in the injured hindbrain and retina that undergo changes in the differentially expressed genes.

Impact: Successful completion of these objectives will provide new information which could ultimately lead to new therapies for treating SCI in humans. Important questions will be answered such as, "Why CNS axon regeneration succeeds in some instances and fails in others?" Such information is key to developing rational approaches for CNS axons to regenerate in mammals.

Presentations: Belrose, J.L., Sammons, M.A., Gibbs, K.M., Szaro, B.G. (April 2018). *RNA-seq analysis of optic nerve and spinal cord injury in Xenopus laevis reveals core genes associated with successful CNS axon regeneration*. Poster/Abstract presented at the Northeast Regional Meeting of the Society for Developmental Biology, Woods Hole, Massachusetts.

Szaro, B.G. (April 2018). *Parsing Molecular Mechanisms of Successful CNS Axon Regeneration using Xenopus*. Invited lecture at the Eugene Bell Center for Regenerative Biology and Tissue Engineering, Marine Biological Laboratories, Woods Hole, Massachusetts.

## **7. Research Foundation of CUNY, College of Staten Island**

Maria Knikou, P.T., Ph.D.

PART: \$947,004

Transspinal-Transcortical Paired Stimulation for Neuroplasticity and Recovery After SCI

Introduction/Background: The focus of this research is to combine locomotor training with transspinal-transcortical paired associative stimulation that is delivered during the mid-stance phase of each step. Researchers anticipate that their paradigm will strengthen corticospinal neural connections enhancing recovery of motor function in people with SCI.

Progress Towards Specific Aims: Researchers completed a transspinal-transcortical paired associative stimulation (PAS) and Lokomat training sessions and experiments in people with motor incomplete SCI, motor complete SCI, and at rest in healthy control subjects. Recruitment of people with SCI and healthy control subjects is an ongoing process. The principal investigator along with her research team are arranging to give several in-service research seminars and talks to increase recruitment rate.

Future Directions: The principal investigator will continue establishing eligibility of interested participants with SCI and healthy control subjects, complete transspinal-transcortical PAS and Lokomat training in an additional participants with SCI and healthy control subjects, submit research for publication, continue reduction and statistical

analysis of acquired data, and present preliminary findings to NYC Neuromodulation Conference, Society for Neuroscience Annual Conference, and ASIA conference.

Impact: Transspinal-transcortical stimulation is a non-invasive method that can be utilized in combination with locomotor training to alter spinal and cortical neural excitability in people with SCI. Neurophysiological recordings and clinical tests performed before and after daily training will provide the scientific evidence for this novel intervention, that may change the rehabilitation approach to promote recovery of sensorimotor function in people with SCI.

Publications: Côté, M.P., Murray, L.M., Knikou, M. (2018). Spinal Control of Locomotion: Individual Neurons, their Circuits and Functions. *Frontiers in Physiology*, June 25; 9: 784. doi: 10.3389/fphys.2018.00784.

Murray, L.M., Tahayori, B., Knikou, M. (2018). Transspinal direct current stimulation produces persistent plasticity in human motor pathways. *Scientific Reports*, January 15; 8 (1): 717. doi: 10.1038/s41598-017-18872-z.

#### **8. The Trustees of Columbia University in the City of New York**

Ulrich Hengst, Ph.D.

IDEA: \$360,000

Pumilio 1 and 2 (Pum 1 and 2) Control Axon Regrowth by Shaping the Axonal Transcriptome

Introduction/Background: Axon regrowth following injury is controlled by cell intrinsic and extrinsic factors. One of the most important cell intrinsic determinants of axon growth is the production of new proteins. While the bulk of protein synthesis occurs in the neuronal cell bodies, the injured axons themselves are also able to produce proteins locally. In their previous studies, researchers identified a pair of proteins, Pum 1 and 2, that control which mRNAs are localized to and translated within axons. By modulating the abundance of these proteins in neurons, they have been able to greatly reduce or increase the ability of axons to grow.

In this research project, researchers will elucidate the mechanism behind these effects and determine which proteins' local production is responsible for the increased or reduced capacity of axons to regrow following injury.

Progress Towards Specific Aims: For Aim 1, researchers found that Pum2 regulates the intra-axonal translation of a specific group of proteins. When the expression of Pum2 in neurons is inhibited, protein synthesis in axons is significantly increased, and developing axons exhibit defects in growth and branching. Further, regeneration of axons deficient in Pum2 is severely affected. Together the results suggest that Pum2 prevents the axonal synthesis of proteins that inhibit axon growth and regeneration.

Future Directions: The researchers plan to continue investigations into how Pum1 and Pum2 control axon growth and begin to determine the role of Pum1 in shaping the local transcriptome in regenerating axons.

Impact: The research team uncovered previously unrecognized mechanisms for the control of intra-axonal protein synthesis. Interference with this mechanism changes the intrinsic ability of axons to grow after injury.

Publications: Walker, C.A., Randolph, L.K., Matute, C., Alberdi, E., Baleriola, J., & Hengst, U. (2018). Abeta1-42 triggers the generation of a retrograde signaling complex from sentinel mRNAs in axons. *EMBO reports*, 19(7). doi: 10.15252/embr.201745435.

## 9. Weill Medical College of Cornell University

Anthony Sauve, Ph.D.

Sub-applicant: Winifred Masterson Burke Medical Research Institute

PART: \$890,241

NAD-Augmenting Agents to Enhance Neural Survival and Function Following Spinal Cord Injury

Introduction/Background: SCI is a debilitating condition that causes long-term health consequences, such as paralysis, loss of limb function, loss of work, and decline in quality of life. The current set of proven useful pharmacologic approaches to prevent long-term loss of physiologic and functional performance in people with SCI is limited. There is opportunity to intervene to prevent loss of spinal cord neurons and axons and potentiate preservation of function at the earliest receipt of medical care following SCI. A key factor for preservation of neurons and axons is the metabolic co-factor nicotinamide adenine dinucleotide (NAD), suggesting that one possible strategy of intervention is to preserve NAD levels in injured neurons. Researchers seek to evaluate nicotinamide riboside (NR), a nucleoside precursor of NAD<sup>+</sup>, and a variant of NR called dihydro-NR (DHNR) in treatment of SCI, using a rat thoracic contusion model of SCI.

Progress Towards Specific Aims: For Aim 1, in addition to synthetic activities, time dependent pharmacodynamic studies and dose response studies were performed in rats, with readouts of NAD levels in spinal cord to optimize dosing and to better understand the duration of effect of the respective compounds. Researchers improved upon the synthesis of dihydroNR for production of amounts required for SCI studies, which enabled more efficient production of both isotopically labeled dihydroNR and NR. The compounds will be used in follow-up to ongoing PD studies in rats and mice.

For Aim 2, researchers hypothesize that optimizing NR dosing and route delivery can improve preliminary data demonstrating that enhancing NAD<sup>+</sup> in the spinal cord and brain by pharmacological NR or NRH administration. This study will involve dose-response profile studies in spinal cord rats (injured and uninjured). Training of a postdoctoral researcher to perform spinal cord injuries is being conducted.

Future Directions: Completion of most of the preliminary phase of the studies (Aim 1) which were designed to develop dosing and duration of effect, as well as route of administration of the compounds. Full entrance to Aim 2 is expected to proceed in earnest, with initiation of a full injury study using vehicle, dihydroNR and NR all in parallel. Pharmacodynamic (PD) studies in injured rats will be completed to provide insights into drug action in lesioned animals.

Impact: The current studies confirm NR and dihydroNR can have extended action out to 8 hours post administration of compound, and this is likely to provide opportunity for single dose administration of compound for effective protection against spinal cord injury.

Publications: Dai, Y. & Hill, C.E. (2018). Transplantation of Adult Rat Schwann Cells into the Injured Spinal Cord. *Methods in Molecular Biology*, 1739:409-438. doi: 10.1007/978-1-4939-7649-2\_28.



Mitchell, S.J., Bernier, M, Aon, M.A., Cortassa, S., Kim, E.Y., Fang, E.F., ... & de Cabo, R. (March 6, 2018). Nicotinamide Improves Aspects of Healthspan, but Not Lifespan, in Mice, *Cell Metabolism* 27(3):667-676. doi: 10.1016/j.cmet.2018.02.001.

Wong, V.S.C., Picci, C., Swift, M., Levinson, M., Willis, D., & Langley, B. (February 28, 2018).  $\alpha$ -Tubulin Acetyltransferase Is a Novel Target Mediating Neurite Growth Inhibitory Effects of Chondroitin Sulfate Proteoglycans and Myelin-Associated Glycoprotein. *eNeuro*. 5(1). doi: 10.1523/ENEURO.0240-17.2018.

Zhang, N. & Sauve, A.A. (2018). Regulatory Effects of NAD (+) Metabolic Pathways on Sirtuin Activity. *Progress in Molecular Biology and Translational Science*, 154, 71-104. doi: 10.1016/bs.pmbts.2017.11.012.

## **10. Winifred Masterson Burke Medical Research Institute**

Kathleen Friel, Ph.D.

Sub-applicant: Massachusetts Institute of Technology

IDEA: \$359,000

Improving Hand Function in Chronic SCI with Combined Robot Training and Transcranial Direct Current Stimulation

Introduction/Background: SCI typically occurs in young adulthood and often results in incurable paralysis and disability that profoundly affects quality of life of the injured. Interventions for improving hand function yield modest results at best. The pilot data in chronic incomplete SCI, showed that transcranial direct current stimulation (tDCS) over the hand area of the brain, or a regimen of upper extremity robotic training, can improve voluntary movement control of the hand or arm respectively. Researchers aim to study the combination of tDCS and robotic training on functional outcome. Participants will undergo robotic hand training (multiple times per week for six (6) weeks), preceded by application of tDCS (or sham tDCS).

Progress Towards Specific Aims: For Aim 1, researchers have screened 46 individuals to determine eligibility for the combined transcranial direct current stimulation (tDCS) and behavioral training (robotics) residual dysfunction of the hand study. 20 participants have been enrolled, with an additional ten individuals expected to participate in the coming cohorts. Of the 20 enrollees, 16 have completed their follow-up assessments and 9 participants out of the 16 had evaluations post-intervention. At the one-month follow up after training, four patients had follow-up evaluations. Full data sets are comprised of complete baseline, post, and follow-up evaluations. Therefore, there are only four complete data sets captured in the study.

For Aim 2, data from the robotic device for the first two cohorts was extracted and prepared for final analysis. New analytical methods were discussed, and proof-of-concept analysis began.

For Aim 3, to identify and compare the neurophysiological mechanisms by Transcranial Magnetic Stimulation (TMS), in parallel with clinical and kinematic data collection of the first four cohorts, the neurophysiologic data was successfully collected and preliminary data processing for motor evoked potential quantification was performed to assess data quality.

Future Directions: The researchers expect to have completed the intervention period for nine full cohorts by the end 2018. The recruitment effort is expanding to include more organizations that provide resources for individuals with spinal cord injury. This inclusion may increase the dissemination of study information and thus increase recruitment. In addition, clinic hours are being expanded to accommodate more participants. Saturdays will be included as optional training day for future cohorts to increase the number of participants that can join the study.

Impact: The researchers hypothesize that incorporating tDCS, targeting hand muscles with reduced motor output in chronic incomplete SCI, in combination with activity-dependent plasticity, resulting from robotic training, will improve voluntary motor function greater than motor practice alone.

## **11. Winifred Masterson Burke Medical Research Institute**

Jian Zhong, Ph.D.

IDEA: \$360,000

Visualizing New Synapses and Their Activity in the Injured Spinal Cord

Introduction/Background: To overcome paralysis after SCI, injured axons must regenerate and then form synapses to reconnect with target cells. Very little is known about synapse formation in the injured spinal cord because there is no known way to observe such synapses as they form and mature. Their objective is to express a novel anterograde transsynaptic tracer in corticospinal motoneurons (CSMNs), and observe it moving from CSMNs to spinal interneurons as new synapses form after injury. If researchers can thereby prove that new synapses are formed, they can then determine whether these synapses are active when a SCI mouse moves. Upon completion of this project, new tools will be available to study synaptogenesis, and they will be able to devise ways to accelerate and improve synaptogenesis after SCI.

Progress Towards Specific Aims: For Aim 1, researchers have generated the transsynaptic tracer and, using mice, have tested its functionality *in vivo*. Furthermore, in mice that have undergone unilateral pyramidotomy, activation of B-RAF resulted in increased contralateral sprouting of intact corticospinal tract (CST) axons. This enhanced axon sprouting into the denervated side led to new synaptic connections. These new connections will be further studied in Aim 2.

For Aim 2, imaging the activity of newly connected interneurons in SCI, researchers have confirmed CST regeneration in kaB-RAF expressing mice as well as in repetitive transcranial magnetic stimulation (rTMS) treated mice. Researchers have generated 5 different transsynaptic tracer plasmid constructs: CreERT2-CTB, CreERT2-Tau, CreERT2-Prion, CreERT2- $\alpha$ Syn and CreERT2-TTC and generated adeno-associated viruses (AAVs) of these constructs as well as comparing their ability to label CST postsynaptic interneurons in non-lesioned reporter mice.

Researchers at Burke are collaborating with Researchers at Cornell University in Ithaca to establish a protocol for 3-photon excitatory fluorescent *in vivo* live imaging of axons and interneurons in the spinal cord. Initial test runs of imaging axons in the spinal cord have not yet yielded the resolution needed. However, research remains on track and hardware updates to the 3PEFM system are ongoing to improve signal-to-noise ratio.

Future Directions: Researchers will live-image calcium transients first in intact mice to optimize the machine setup, and then in mice subjected to unilateral pyramidotomy or

dorsal hemisection, to explore synaptogenesis of sprouting and regenerating axons in the post-injury spinal cord *in vivo*. After testing several tracers, researchers will generate a transgenic chain-amplifying tracer mouse line using the most successful tracer construct, to achieve *in situ* transsynaptic chain amplification labeling in postsynaptically connected neurons. The generation of this mouse line will be funded by a separate grant. These mice will overcome the problem of tracer dilution when moving from one neuron to the connected ones. Tracer dilution is a substantial problem with all the existing transsynaptic tracing approaches, except those based on neurotropic viruses (these are however neurotoxic – another serious obstacle.)

Impact: If successful, completion of Aims 1 and 2 will provide a crucial and long-awaited tool for the SCI research community to study new connectivity forming with regenerating axons in the injured spinal cord *in vivo*. Researchers will be able to document in detail how new synapses are formed in the adult injured cord. This will have a direct impact on the development of novel SCI treatment strategies.

## **Institutional Support for Spinal Cord Injury Research (Round 6)**

**Contract Term 3/1/17-2/28/22**

**Progress Reporting Period  
3/1/18-8/31/18**

**20 Awards, Procurement Total: \$4,850,000**

### **1. Albany Research Institute, Inc. – Albany Stratton VA Medical Center**

Funding was used to support the personal services of personnel who maintained the laboratory during a transition period and continued the research on the trophic roles of dorsal root ganglia (DRG) in spinal cord repair. The trophic role of DRG was examined on tissue samples of a chronic spinal cord disease, namely, Friedreich ataxia (FA). Three major observations during the last and prior periods of support: (1) In the pathogenesis of spinal cord disease in FA, failed trophic support by DRG is due to DRG hypoplasia; (2) The deficit is caused by a failure of DRG neurons to supply adequate amounts of neuregulin 1 type III (NRG1[III]). This protein and its receptor, ErbB2 provide signals to Schwann cells of dorsal spinal roots to myelinate and permit proper conduction of nerve impulses to the spinal cord beyond the dorsal root entry zones; (3) DRG a subject to inflammatory infiltration by monocytes, and inflammation causes ultimate destruction of DRG neurons and thereby removes any residual function. In the interpretation of chronic spinal cord injury following trauma, inflammation of DRG is likely the most important factor in failed trophic support of spinal cord repair.

### **2. Albert Einstein College of Medicine (AECOM)**

Funding was used to upgrade the 4D-spinning disk confocal microscope to solid state-lasers. The ongoing experiments rely heavily on the use of the confocal microscope, which enables them to image subcellular structures at very high resolution. The confocal microscope previously used two Ar-Ion gas lasers, however, these lasers were in critical need of replacement, with one unusable since the end of 2016. Funds from this award were also used to upgrade the imaging system from gas lasers to solid-state lasers, which are considerably more stable and energy efficient. This upgrade will ensure that the researchers can complete their proposed cell biology studies and characterize with high precision subcellular alterations occurring in neurons with fidgetin-like 2 depletions.

Funding was also used to supplement research being supported by an existing SCIRB CART award. The expanded study includes additional readouts for organ function recovery in SCI rats following treatment of FL2-siRNA. In their CART-funded project they are assessing recovery of locomotor and urogenital function. With this award they began determining the extent to which topic treatment of the spinal cord injured site with FL2-siRNA prevent the deterioration of bone health following SCI. Long bones from forelimbs and hindlimbs sham rats and from SCI injured FL2-siRNA treated rats were harvested at 7 and 14 days after injury. The bone histological and histomorphometric analyses are underway.

### **3. Bronx Veterans Medical Research Foundation – James J. Peters VA Medical Center**

Funding supported equipment being used in support of the research project entitled, “RNA editing alteration in spinal cord injury.” This project proposes to identify adenosine deaminases that act on RNA (ADAR2) substrates whose editing is altered by SCI as well as identify regulators of ADAR2 expression and function. These studies will advance researchers understanding of the neuropathology of SCI and spasticity. Most importantly, researchers will suggest possible targets for the development of novel antispastic drug therapy.

Funding also supported personnel in two projects entitled “Precision of body composition measurements in persons with spinal cord injury” and “Subcutaneous Injection and Ultrasonic Dispersion of Cefazolin into Chronic Pelvic-Region Pressure Ulcers in Persons with Spinal Cord Injury.” These projects are characterized as feasibility, or proof-of-concept, studies. As of this report, researchers feel strongly that the preliminary work supported by the NYS SCIRB on the outlined protocols has contributed to their understanding of the respective research questions and will lead to several extramural grant applications.

Presentations: Cirnigliaro, C.M., Myslinski, M.J., Asselin, P., Hobson, J.C., Lombard, A., Specht, A.,...& Bauman, W.A. (2018). *Identification of reference scores at the total hip to more reliably diagnose osteoporosis at the distal femur and proximal tibia in persons with spinal cord injury*. Presented at the International Society for Body Composition Research Annual Meeting, New York, New York.

Cirnigliaro, C.M., Myslinski, M.J., Asselin, P., Hobson, J.C., Specht, A., LaFontaine, M.F.,...& Bauman, W.A. (September 2018). *Progressive sublesional bone loss occurs into the second decade after SCI*. Presented at the Academy of Spinal Cord Injury Professionals Annual Meeting, New Orleans, Louisiana.

### **4. Columbia University**

Funding supported development of two in-house pelvic support systems for training of human functions; a pelvic-assisted walker support system for over ground walking, and a stair-mill system with a Tethered Pelvic Assist Device (TPAD).

Funding also supported Sunil Agrawal, Ph.D., and three other personnel in the assembly, testing, and research of the two pelvic support systems. The researchers began preliminary evaluation of the pelvic-assisted walker with healthy individuals. Preliminary tests were performed to verify the capabilities of this system when the pelvic module is attached to a human user. Both motion tracking and force tracking capability of the pelvic module were evaluated. In the near future, researchers expect to perform preliminary studies with healthy subjects while walking with the device. The stair mill system was used to conduct an experiment which measured the kinematics and kinetics of climbing with the handrail and hands-free condition at different speeds. Using 12 non-SCI young adults the experiment provided insights into continuous stair-mill climbing at different speeds and when holding the handrail. As stepping rate increased, the handrail did not significantly influence the ratio of the phase time over the gait cycle. However, the handrail did affect both the amount of muscle activation and the joint angle change. The study also obtained muscle synergies of stair-mill climbing to help understand the

mechanism of stair-mill climbing as synergy modules. The advantages of muscle synergies are to lower the number of muscles to monitor during the motion and to help the researchers select representative muscles to control TPAD for gait training in the future. The results of this study will advance current rehabilitation methods for SCI patients and develop innovative solutions to reduce risk of falls for this patient group.

## **5. Cornell University**

This funding previously supported the purchase of a deformable mirror and high-end data processing computer for use with Dr. Schaffer's three-photon imaging system. The deformable mirror will be critical for imaging deep in the spinal cord of mice to study cellular changes after SCI. The spinal cord induces significant optical aberrations that degrade the laser focus and impact the signal strength from fluorescent markers. Correcting for these optical aberrations using the deformable mirror looks like it will yield 5-10 times greater signal strength. Currently this grant partially funds supplies necessary for SCI experiments with this imaging system. Preliminary data obtained with this imaging system was used in the successful application to the National Science Foundation for a large center grant funded through the BRAIN initiative. Dr. Schaffer is one of five Co-PI's on this award. Dr. Schaffer's lab's efforts will focus on spinal cord imaging and this will greatly enhance the knowledge base of normal neural circuit function in the spinal cord as well as the tool kit to examine changes in function after spinal cord injury.

Presentations: Cheng, Y.T., Denotta, S.L., Jones, J.S., Rivera, D.A., Hu, S., Raikin, J., ...& Schaffer, C.B. (2018). *In vivo three-photon excited fluorescence imaging in the spinal cord of awake, locomoting mice*. Presented at the Society for Neuroscience Meeting, San Diego, California.

## **6. Feinstein Institute for Medical Research**

Funding was used to provide partial salary support for the principal investigator, Dr. Ona Bloom, and a research coordinator, Ms. Ashley Chory. Dr. Bloom was responsible for overseeing all aspects of SCI-related projects, supervised all tasks, including training of all study personnel, screening of potential participants for enrollment, enrollment/inclusion of participants, performing biological assays and data analysis. The research coordinator maintained all regulatory agency related documents and correspondence, input basic screening data into a clinical database, screened potential participants for enrollment, performed sample processing and biological sample analysis, facilitated communication between study team members and trained study team members in some aspects of the study. The following three research projects continue:

- "Biomarkers of Spontaneous Recovery from Traumatic SCI," aims to build an easy-to-use, predictive model of recovery after SCI that uses inflammatory mediators. Researchers are studying white blood cells and other biological responses in the blood, as well as recovery of physical abilities, during the first year after SCI.
- "Biomarkers in Pediatric SCI/Abnormalities," aims to investigate biological responses in discarded blood samples from children with SCI or congenital abnormalities.

- “Strive for Wellness Research Outcomes,” aims to investigate changes in physical and mental wellness as a result of participating in a structured and safe physical activity and wellness program for persons with SCI.

Relatively little is known about the biological processes that influence physical recovery and wellness in people after SCI. The data that researchers will collect will enable them to fill gaps in knowledge and advance the ability to predict and promote physical recovery and overall health in persons with SCI. The data generated by these projects will be used to provide support of feasibility and scientific rationale for larger grant applications in SCI research.

Publications: Bank, M., Gibbs, K., Sison, C., Kutub, N., Papatheodorou, A., Lee, S., ...& Bloom, O. (2018). Age and Other Risk Factors Influencing Long-Term Mortality in Patients with Traumatic Cervical Spine Fracture. *Geriatr Orthop Surg Rehabil.* May 3:9:2151459318770882. doi: 10.1177/2151459318770882.

Herman, P.E., Bloom, O. (2018). Altered leukocyte gene expression after traumatic spinal cord injury: clinical implications. *Neural Regen Res*, 13(9):1524-1529. doi:10.4103/1673-5374.237112on.

## 7. Health Research, Incorporated

Funding continued to supported personnel in their work to develop and validate a new therapeutic method for improving recovery of useful motor function after SCI. This SCI research project specifically explores the therapeutic benefit of combining spinal reflex conditioning and locomotor training.

Funding also supported personnel in their study of the role of gap junctions between nerve cells and the improper control over the nerve cells that determine activity of the external urethral sphincter muscle (i.e. EUS motoneurons) during urinary dysfunction caused by SCI. The development of technology for the chronic study of EUS and bladder muscle activity will provide a valuable experimental platform that facilitates longitudinal studies of lower urinary tract function and may be used to develop a novel therapy for treatment of urinary dysfunction after incomplete SCI.

## 8. Icahn School of Medicine at Mount Sinai

Funding supported Principal Investigator, Hongyan Zou, MD, Ph.D., and other personnel studying the hypothesis that Plexin-B2 is an important modulator of the immune responses in SCI. Researchers have conducted detailed time course analysis at 3 days post injury (dpi), 7 dpi and 14 dpi, and have detected the upregulation/sustention of Plexin-B2 in activated microglial/macrophages after SCI. However, at 21 dpi researchers found that Plexin-B2 waned. Researchers enlarged their cohort size and conducted more comprehensive behavior studies, including but not limited to, Basso Mouse Scale for Locomotion (BMS), rotarod ladder walking and Von Frey hair study for sensory recovery. This research showed that the conditional deletion of Plexin-B2 hampers functional recovery after SCI. Researchers expanded their study to examine the underlying mechanisms of the poor recovery function and found that Plexin-B2 signaling plays a more crucial role in early state of microglial response after SCI. Researchers will build upon their current studies by linking how microglia integrate biochemical cues, cytoskeleton changes and physical forces control wound healing. Their study has the

potential of defining Plexin-B2 as a novel therapeutic target for immunomodulation after SCI. The study not only has a strong impact on advancing basic science, but also has translational value of exploring new signaling pathway regulating immune response after SCI.

Presentations: Zou, H. (September 2018). *Plexin-B2 regulates microglia coralling and wound compaction after SCI*. Presented at the Molecular Mechanisms of Neuronal Connectivity Meeting, Cold Spring Harbor, NY.

## **9. New York University**

Using the Eppendorf 633500020 Thermal Cycler, funded through this award, the laboratory has generated preliminary data suggest that cranial motor neurons (CMNs) are more resistant than spinal motor neurons (SMNs) to SOD1 A4V overexpression. From the three original cells lines express SOD1 G93A we made two lines from each; one with the inducible expression of Ngn2, Isl1 and Lhx3 (the NIL lines) and the other with Ngn2, Isl1 and Phox2a inducible expression. CMNs survive better than isogenic SMNs expressing the same levels of SOD1 A4V. Thus, NIP-programmed CMNs are more resistant to the intracellular stress induced by expressing mutant SOD1 A4V protein at 25 days in culture. The results obtained from this funding are the basis of a grant to be submitted to the NIH.

Funding was also used to procure reagents and personnel that manage lab operations. Results of this research titled, Divergent Ascl1 and Neurog2 neuronal programming, were presented at the 2018 Neural Development Gordon Research Conference.

## **10. Regenerative Research Foundation**

Data analysis of single cell spinal cord injury data and manuscript preparation continues. Single-cell analysis tools continue to mature. Many are not fully adapted to handling complex multifactor or time-series data sets. To counter this shortcoming, several analysis tools have been utilized and integrated into our analysis pipeline as we try to identify the different cell types collected during single cell transcriptomic analysis. These tools include Monocle, Seurat, SC3, Scater, SCDE/Pagoda, S3. In addition to our previous results from clustering, cluster consensus and pseudotime analyses, this analysis is leading to the identification of specific genes that identify the cell types at play in the response to injury. Funding was used to support the personal services of Liz Fisher, Ph.D., who manages the animal care responsibilities associated with this project, injury modeling and histological analysis of samples; Nathan Boles, Ph.D., who provided data analysis and advisement; and Thomas Kiel, Ph.D., who performs primary analysis of the single cell data.

Training was conducted on the ICell8 machine with Takara trainers. Trainers verified the operating conditions of the ICell8 machine and trained Dr. Kiehl and Steve Lotz on the current operating procedures for the single-cell 3' differential expression workflows. Takara also provided training on their new full-length transcriptional analysis protocol. Both workflows were tested using a variety of cell sample types.



## 11. Rensselaer Polytechnic Institute

Funding supported Dr. Gilbert and two other personnel studying Electrical Stimulation of Human Schwann Cells: specifically, the changes to Schwann cell growth and production of cytokines for repair. Schwann cells can be neurosupportive in spinal cord injuries and have been shown to promote the regrowth of injured axons via the use of both transplanted Schwann cells and peripheral nerve grafts to bridge an injury to the spinal cord. The researchers used AC stimulation applied at low (20 Hz) and high (1 kHz) frequency via a pulse generator. The cells were fixed with paraformaldehyde and stained with 4', 6'-diamidino-2-phenylindole (DAPI) to identify all nuclei. Researchers observed a significant increase in cell number as measured by number of DAPI-labeled nuclei. Longer stimulations and daily stimulations are planned. The researchers concluded that primary Schwann cells demonstrate an increase in cell number related to frequency; which is a novel finding.

Publications: D'Amato, A.R., Bramson, M.T.K., Corr, D.T., Puhl, D.L., Gilbert, R.J. & Johnson, J. (2018). Solvent Retention in Electrospun Fibers Affects Scaffold Mechanical Properties. *Electrospinning*, 2(1). <https://doi.org/10.1515/esp-2018-0002>.

Johnson, C.D., D'Amato, A.R., Puhl, D.L., Wich, D.M., Vesperman, A., Gilbert, R.J. (2018). Electrospun fiber surface nanotopography influences astrocyte-mediated neurite outgrowth. *Biomedical Materials*, 13(5):054101. doi: 10.1088/1748-605X/aac4de

Ziemba, A.M., Lane, K.P., San Segundo, I.M., D'Amato, A.R., Mason, A.K., Sexton, R.J., ... & Gilbert, R.J. (2018). Poly-L-lactic acid-co-poly (pentadecalactone) Electrospun Fibers Result in Greater Neurite Outgrowth of Chick Dorsal Root Ganglia in Vitro Compared to Poly-L-lactic Acid Fibers. *American Chemical Society (ACS) Biomaterials Science & Engineering*, 4(5):1491-1497. doi: 10.1021/acsbomaterials.8b00013.

## 12. Research Corporation of Long Island, Inc. – Northport VA Medical Center

Funding was used to purchase the equipment listed below for SCI experiments:

- Biosafety cabinet
- CO<sub>2</sub> incubator
- Freezer
- Refrigerator

Funding continued to support personnel examining the effects of new gene therapy for improving transmission in damaged spinal cord and recovery after SCI in animals so that results can be translated into clinics. Their recent human studies revealed that administration of repetitive spinal electro-magnetic stimulation (sEMS) induced long-lasting modulation of M-wave and H-reflex responses, as well as frequency-dependent depression (FDD) of H-reflex in SCI participants.

Publications: Arvanian, V.L. and Petrosyan, H.A. (2018). Adeno-Associated Virus Vector Mediated Gene Delivery to Neurons and Glial Cells in Damaged Spinal Cord. In Mingjie Li and B. Joy Snider, *Gene Therapy in Neurological Disorders*. 15: 287-298.

Presentations: Arvanian, V.L., Petrosyan, H.A., Liang, L., Alessi, V., Ballas, N., Kondiles, B.R., Horner, P.J. (July 2018). *Electro-magnetic stimulation and exercise training*

*combined with implantation of neuralized-pluripotent stem cells (NiPSCs) immediately after spinal cord injury in rats.* Abstract presented at the Federation of European Neuroscience Societies, Forum of Neuroscience, Berlin, Germany.

Arvanian, V.L., Petrosyan, H.A., Zou, C., Hu, W., Leone, C., Tesfa, A., ...& Sisto, S.A. (November 2018). *Combined administration of electro-magnetic stimulation (EMS) at spinal level and at leg muscles to modulate neurophysiological properties at spino-muscular circuitry in healthy and SCI humans.* Abstract presented at the Society for Neuroscience, San Diego, California.

Petrosyan, H.A., Liang, L., Tesfa, A., Fahmy, M., Zou, C., Sisto, S., Arvanian, V. (November 2018). *Spinal electromagnetic stimulation induces modulation of M-wave and H-reflex responses and recovery of frequency-dependent depression of H-reflex in chronic spinal cord injured rats.* Abstract presented at the Society for Neuroscience, San Diego, California.

### **13. Research Foundation for SUNY – Downstate Medical Center**

Funding supported principal investigator, Dr. Salvador Dura-Bernal, and proposed objectives in terms of computation simulation of primary motor cortex (M1) neural circuits and analysis of M1 in vivo experimental data. Dr. Dura-Bernal's project is aimed at elucidating M1 neural coding mechanisms to help build autonomous bidirectional brain-machine interfaces for SCI patients. Funding also covered the corresponding Facilities and Administrative Costs.

Preprint: Gleeson, P., Cantarelli, M., Marin, B., Quintana, A., Earnshaw, M., Piasini, E., ...& Silver, R.A. (2018). Open Source Brain: a collaborative resource for visualizing, analyzing, simulating and developing standardized models of neurons and circuits. *bioRxiv*: 10.1101/229484.

Publications: Cantarelli, M., Marin, B., Quintana, A., Earnshaw, M., Court, R., Gleeson, P., Dura-Bernal, S., Silver, R.A., Idili, G. (2018). Geppetto: a reusable modular open platform for exploring neuroscience data and models, *Phil. Trans. R. Soc. Lond. B. Biol. Sci.*, 373, 1758. doi: 10.1098/rstb.2017.0380.

Zhao, Y., Hessburg, J.P., Asok Kumar, J.N., Francis, J.T. (2018). Paradigm Shift in Sensorimotor Control Research and Brain Machine Interface Control: The Influence of Context on Sensorimotor Representations, *Front. Neurosci.*, 12, 579. doi: 10.3389/fnins.2018.00579.

Presentations: Dura-Bernal, S., Suter, B.A., Quintana, A., Cantarelli, M., Gleeson, P., Rodriguez, F., ...& Lytton, W.W. (November 2018). *NetPyNE: a GUI-based tool to build, simulate and analyze large-scale, data-driven network models in parallel NEURON.* Abstract/Poster presented at the Society for Neuroscience, San Diego, California.

### **14. Research Foundation for SUNY – Stony Brook**

Staff continue to receive training to operate equipment previously purchased with this funding. The equipment will assist with expanding SCI surgical core facilities, the assessment of spontaneous locomotor and explorative motor behaviors, and performing high throughput biochemical assays.

This equipment will be used by SCI researchers that are examining the efficacy of various therapeutic interventions to yield changes in protein biomarkers that could subsequently serve as targets for enhancing therapeutic strategies to further facilitate functional recovery. In addition, data obtained using this equipment can be used in future grant applications.

#### **15. Research Foundation for SUNY – University at Albany**

Using the Model CM 1950 Cyrostat, which was previously funded through this award, the team has generated frozen histological sections of retina, hindbrain, and spinal cord after injury for analysis by histochemical procedures. The data generated using this instrument will be disseminated in publications and meetings.

The researchers have successfully been building ChIP-seq libraries from the three injured tissues at the peak period of regenerative axon outgrowth, and their uninjured controls, to identify empirically active promoters (H3K4me3; H3K27ac) and enhancers (H3K27ac) and their repressed counterparts (H3K9me3; H3K27me3). They have successfully built and sequenced ChIP seq libraries for the aforementioned histone modifications for the following tissues and conditions: H3K4 me3, H3K27ac, H3K27me3, H3K9me3 and input controls for the eye after optic nerve injury, the contralateral uninjured eye of the same animals, and naive, uninjured eyes. For each set, two independent biological replicates were made from pooling 6 eyes each. They aimed to obtain 30 million sequencing reads successfully aligned against the *X. laevis* genome of each replicate for the two active histone marks (H3K4me3 and H3K27ac), and 100 million such reads for the two repressive marks. They exceeded their targets for the first set and obtained about 70 million successfully aligned reads each for the second. They also obtained similar data for one of two intended biological replicates of tadpole SCI hindbrain and of frog SCI hindbrain (5 pooled brains each), with their respective uninjured controls. They will continue progress toward developing the precise conditions needed to carry out ChIP-Seq on *Xenopus* neural tissues. Researchers anticipate that new data will provide a foundation for building new understanding of the molecular genetic mechanisms that permit some CNS neurons to recover function and others to lose this ability after traumatic injury. They hope that this knowledge will enable the design of new therapeutic approaches to reverse this loss in human SCI.

Presentations: Belrose, J.L., Sammons, M.A., Gibbs, K.M., Szaro, B.G. (2018). *Using Xenopus laevis to identify genes involved in successful vs. unsuccessful central nervous system axon regeneration*. Presented at the 17th International Xenopus meeting, Genetics Society of America, Seattle, Washington.

#### **16. Research Foundation of CUNY – Staten Island**

Funding was previously used to purchase an Lokomat 6 Professional. In clinical trials led by Dr. Maria Knikou, they have delivered transspinal and transcortical paired associative stimulation at rest and during Lokomat gait training in people with SCI. The data generated from these studies supports future grant applications to the NIH and NYSDOH.

Zaghloul Ahmed, PT, Ph.D. will investigate the molecular mechanism of trans-spinal direct current stimulation (tsDCS) by testing its effect on the expression of vascular

growth factor (VGF). The investigation will be in animals with and without spinal cord injury. Funds have been used to pay for supplies and partially pay the salary for Sreyashi Samaddar, Ph.D., a post-doctorate fellow hired to work on this project. Data generated from this project will be used to support applications to NIH, Department of Defense, and NYSDOH.

Publications: Knikou, M., Murray, L.M. (2018). Neural Interactions between Transspinal Evoked Potentials and Muscle Spindle Afferents in Humans. *Journal of Electromyography and Kinesiology*.

Presentations: Islam, M.A., Li, J., Pulverenti, T.S., Murray, L.M., Knikou, M. (2018). *Regulation of human soleus H-reflex phase dependent modulation by transspinal stimulation during treadmill walking*. Poster presented at the NYC Neuromodulation 2018 in New York, New York.

Islam, M.A., Li, J., Pulverenti, T.S., Murray, L.M., Knikou, M. (2018). *Transspinal stimulation affects muscle activation patterns during treadmill walking in healthy humans*. Poster presented at the Society for Neuroscience, San Diego, California.

Murray, L.M., Li, J., Islam, M.A., Pulverenti, T.S., Knikou, M. (2018). *Transspinal stimulation during treadmill walking alters soleus H-reflex excitability in healthy humans*. Society for Neuroscience, San Diego, California.

## **17. Research Foundation of CUNY – The City College of New York**

The goal is to build institutional capacity for molecular-based approaches to SCI research at the City College of New York (CCNY). Upon execution, the outcomes are expected to lead to demonstration of organotypic and cellular experimental systems, regeneration-associated gene panel and microRNA panel focused on SCI that would be applied for subsequent studies of SCI pathological processes and prospective molecular therapies.

Progress on the genomic bioinformatics component of the work has continued briskly. While microarray and RNA sequencing studies may implicate hundreds of genes in SCI, researchers have focused on transcripts and probes that have been validated in specific experiments. Using functional or signaling pathway mapping and scoring tools such as Cytoscape, researchers have organized the transcripts into functional nodes so that key signaling cascades involved in SCI recovery may be identified. Their preliminary inference suggests that 2-4 key nodes account for most of the effects that have been reported in the literature.

Funding was used to purchase surgical supplies, antibodies, tissue cultureware, reagents and probes and general laboratory supplies. Funding also supported the animal procurement and husbandry.

## **18. Syracuse University**

Funding supported a technician/manager of the zebrafish facility. Essential reagents were purchased for the maintenance and characterization of zebrafish mutants and the maintenance of wild type (WT) lines that are essential for spinal cord research. These mutant lines are invaluable for elucidating how spinal cord neuronal functional properties

are specified. The materials, resources and data generated from the first year of this SCIRB funded contract generated essential reagents and preliminary data for a recently funded National Science Foundation (NSF) grant.

Preprint: Hartwell, R.D., England, S.J, Monk, N.A.M., Van Hateren, N.J., Baxendale, S., Marzo, M., ...& Whitfield, T.T. (2018). Anteroposterior patterning of the zebrafish ear through Fgf- and Hh-dependent regulation of *hmx3a* expression. bioRxiv: 10.1101/451963.

Publications: Wilk, R., Ali, N., England, S.J., Lewis, K.E. (2018). Using Zebrafish to Bring Hands-On Laboratory Experiences to Urban Classrooms. *Zebrafish*, 15(2):156-178. doi: 10.1089/zeb.2017.1503.

Presentations: Lewis, K. (June 2018). *International Specification of Zebrafish Interneurons*. Poster presented at the International Zebrafish Conference in Madison, Wisconsin.

## 19. University of Rochester

Funds are being used to support two researchers studying a specific population of human astrocytes, derived by human induced pluripotent stem cell technologies, in the treatment of contusion injuries of the spinal cord.

The goal of this study is to test the hypothesis that the current protocols for generating the desired astrocyte subpopulation are now sufficiently refined to yield cells that are effective in the treatment of traumatic SCI and can provide benefit even when transplantation is delayed.

Several studies are planned under this research area and the researchers have tested current astrocyte derivation protocols in contusion of SCI to confirm that the currently derived cells have therapeutic potential. Extensive characterization of the astrocyte populations was carried out prior to transplantation and confirmed that the cells have increased expression of a variety of factors of interest in promoting recovery from SCI and other types of damage to the CNS.

Thus far, researchers have seen significant improvements of astrocyte-transplanted animals 14 to 17 days after transplantation. The superiority of the astrocytes over NSCs also was seen in more fine-grained motor analyses and showed significant improvement in respect to maximum contact intensity, multiple footprint parameters related to planar stepping, and swing speed. The research team believes this to be a promising beginning to the project.

## 20. Winifred Masterson Burke Medical Research Institute

Funding supported the purchase of a Digitimer DS8R BiPhasic Constant Current Stimulator, which is now fully installed, training is complete, and it is in use. This equipment is being used in the 'Improving hand function in chronic SCI with combined robotic training and tDCS' study. The objective of this trial is to strengthen the spared connections between the brain and the muscle after spinal cord injury by pairing direct current electrical stimulation with robotic training. The other project is a collaborative project with Dr. Kathleen Friel (Director, Clinical Laboratory for Early Brain Injury

Recovery), 'Neurophysiological characterization of adults with cerebral palsy'. This equipment is being used to determine if a down-regulating conditioning H reflex protocol will help spasticity and gait parameters in adults with cerebral palsy. The data produced using this equipment include a manuscript describing the results of the "non-invasive paired-pulse stimulation to improve lower extremity motor recovery in chronic spinal cord Injury" is currently under review. The data of this project was submitted as an R21.

Funds have been used to recruit, train, and support a Research Assistant, Jeremy Fidock, whose primary role has been to run the ongoing "Improving hand function in chronic SCI with combined robotic training and tDCS" project. Mr. Fidock worked on the data analysis and manuscript preparation for the "non-invasive paired-pulse stimulation to improve lower extremity motor recovery in chronic spinal cord Injury" study, which is currently under review. A primary task of this funding is to support the development of human studies and clinical trials in spinal cord injury patients. A DOD CDMRP Spinal Cord Injury Research Program Clinical Trial Award application was submitted for the October 15, 2018 funding cycle. This is a multisite clinical trial, in collaboration with Kessler Rehabilitation, designed to test the hypothesis that brain-computer interface-targeted electromagnetic field treatment will improve upper extremity function in sub-acute SCI patients.

Publications: Cortes, M., Alias, G.G., & Tansey, K.E. 2018, July. A "Snapshot" of the Advances in SCI Therapeutics. *Neurotherapeutics*, (3):527-528. doi: 10.1007/s13311-018-0654-z.

## **Translational Research Projects in Spinal Cord Injury (Round 1)**

**Contract Term 8/15/16-8/14/21**

**Progress Reporting Period  
2/1/17-8/14/17**

**2 Awards, Procurement Total: \$8,771,302**

### **1. Columbia University**

Sunil K. Agrawal, Ph.D.

Sub-applicant: University of Louisville Research Foundation

\$5,033,354

Tethered Pelvic Assist Device (TPAD) and Epidural Stimulation for Recovery of Standing in SCI

Introduction/Background: The goal of this reporting period was to extend the design of the TPAD for stand training of patients with SCI. This robotic system mimics the manual training of standing for patients with SCI. The Robotics and Rehabilitation (ROAR) Laboratory at Columbia University is collaborating with the Department of Neurological Surgery at the University of Louisville (UOL).

Progress Towards Specific Aims: During the first half of the year the researcher focused on the final assembly of the TPAD system. This involved integration of electrical components, programming of the computer interface, and preliminary testing and validation of the different modules. The validation studies composed of applying desired forces using cables on an object while measuring the forces applied to it via a force-torque sensor and applying active trunk assistance using the stand trainer and passive pelvic resistance with a group of healthy subjects. Slight modifications to the stand trainer were made based on these studies and were reevaluated. After becoming satisfied with the performance of the stand trainer at Columbia's ROAR lab, components for a second trainer were sent to the University of Louisville. Researchers from Columbia travelled to Louisville and assembled the second stand trainer. Once fully assembled the University of Louisville stand trainer was tested on two healthy individuals, and one individual with spinal cord injury.

Future Directions: With both machines functioning and initial human safety tests completed, the researchers will focus on human subject studies. The studies will first target healthy subjects to determine feasibility of the technology, characterize human behavioral responses to different perturbations and force fields, and establish training results with healthy participants. The results of these studies will provide baseline results which will be used to compare those obtained with patients with spinal cord injury. Both teams will simultaneously perform pilot testing on patients with spinal cord injury.

Impact: The goal of this robotic system is to allow effective assistance, positioning, and support for the SCI patient during training. It will significantly help in the stand training of SCI patients by supporting them at the trunk, pelvis, and the knees.

Publications: Khan, M.I., Santamaria, V., & Agrawal, S.K. (2018). Improving Trunk-Pelvis Stability Using Active Force Control at the Trunk and Passive Resistance at the Pelvis.

*IEEE Robotics and Automation Letters*, 3(3):2569-2576. doi: 10.1109/LRA.2018.2809919.

Martelli, D., Luo, L., Kang, J., Kang, U.J., Fahn, S., & Agrawal, S.K. (2017) Adaptation of Stability during Perturbed Walking in Parkinson's Disease. *Scientific Reports*. 7(1): 17875. doi: 10.1038/s41598-017-18075-6.

Presentations: Martelli, D., Kang, J., & Agrawal, S.K. (2018). *A Perturbation-based Gait Training with Multidirectional Waist-Pulls Generalizes to Split-Belt Treadmill Slips*. Abstract published in the 7<sup>th</sup> IEEE International Conference on Biomedical Robotics and Biomechatronics, Enschede, Netherlands. doi:10.1109/BIOROB.2018.8487618.

## **2. Research Foundation of CUNY, The City College of New York/CUNY School of Medicine**

John Martin, Ph.D.

Sub-applicants: Bronx Veterans Medical Research Foundation, Inc. and Columbia University

\$3,737,948

Combined Motor Cortex and Spinal Cord Stimulation to Promote Arm and Hand Function After Chronic Cervical Spinal Cord Injury

Introduction/Background: The overall goal of this project is to translate a promising therapy for improving arm and hand function after cervical SCI from animal models to humans. Regaining hand function is the highest priority for people with cervical SCI. Researchers use combined brain and spinal cord electrical stimulation to promote recovery, strengthen connections and improve arm and hand function after SCI.

Progress Towards Specific Aims: Major progress continues to be made. For rat replication experiments, behavioral data replicated the original behavioral findings in an independent laboratory. The Carmel lab has shown efficacy of dual spinal-motor cortex electrical stimulation in improving forepaw manipulation skills and skilled locomotion after C4 bilateral contusion. For the large animal translational study, they continue to add animals to the study and provide further support for improvement in the reach-to-grasp task and strengthening of motor responses evoked by motor cortex electrical stimulation. The cervical contusion injury has been further refined. Preparation for the human phase of the study is ongoing through consultation with other facets of the program and related neurostimulation experiments performed at the Bronx VA Medical Center.

Future Directions: Data will be presented at scientific meetings, after feedback is received the rat experiments will be written. The large animal model is complete. The researchers will add subjects to assess efficacy of therapeutic neuromodulation in C4-injured animals. In addition, they will continue to consult on developing the human translational studies, which are scheduled for the latter half of the project.

Impact: Using the therapeutic neuromodulatory strategy key rat behavioral findings have been replicated. This is an important milestone in SCI research. The researchers are on the path to successfully translate this approach to their large-animal cat model. As the three phases, and component aims, progress, they move closer to the final goal of being in the position to initiate a trial in humans with cervical SCI.



Presentations: Truong, D.Q., Maglione, C., Valter, Y., Zannou, A.L., Shereen, A.D., Williams, P.T.J.A, . . . & Bikson, M. (2018). *Scaling spinal cord injury models for non-invasive stimulation*. 21<sup>st</sup> Annual North America Neuromodulation Society Meeting, Las Vegas, Nevada.

Williams, P.T.J.A, Brandenburg, J.R., Truong, D.Q., De Paolis, A., Cardoso, L., Bikson, M., & Martin, J.H. (2018). *Enhanced neuromodulation with paired brain and spinal cord stimulation therapy in a large animal model of cervical contusion injury*. Presented at the Society for Neuroscience, San Diego, California.

Williams, P.T.J.A., Brandenburg, J.R., Truong, D.Q., Seifert, A., Sarkar, A., Xu, J., ...& Martin, J.H. (2018). *Translational neuromodulation of motor-output using trans-spinal direct current stimulation (tsDCS) in a large animal model*. 21<sup>st</sup> Annual North America Neuromodulation Society Meeting, Las Vegas, Nevada.

Yang, Q., Ramamurthy, A., Lall, S., Santos, J., Zareen, N., Alexander, H., ...& Carmel, J.B. (2018). *Motor cortex and cervical spinal cord electrical stimulation promotes forelimb motor function after spinal cord injury in rats*. Society for Neuroscience, San Diego, California.

## Individual Predoctoral/Postdoctoral Fellowships (Round 1)

Contract Term 3/1/16-2/28/19

### Progress Reporting Periods

9/1/17-2/28/18<sup>1</sup>

3/1/18-8/31/18<sup>2</sup>

**5 Awards, Procurement Total: \$695,041**

#### 1. **Columbia University**<sup>2</sup>

Jason Carmel, M.D., Ph.D., Hongguen Park, Ph.D.

Postdoc: \$172,902

Dissecting and Strengthening Corticospinal Connections After Spinal Cord Injury Using Advanced Neuroscience Methods

Introduction/Background: Spinal cord injury is a devastating disease that causes paralysis by disconnecting the brain and spinal cord. While motor function is impaired, some connections are spared and provide a potential substrate for therapeutic treatment. In this study, researchers aimed to identify the connections responsible for spontaneous recovery and to strengthen them to improve recovery.

Progress Towards Specific Aims: For Aim 1, researchers have made progress on the inactivation system, and to prove that the circuits they have identified are responsible for spontaneous function recovery, they will selectively inactivate the circuits.

To do this, they will use a viral inactivation tool, called Cre-dependent DREADD (Designer Receptor Exclusively Activated by Designer Drug). Cre-Dependent DREADD is comprised of two viruses injected in separate areas of the spinal cord. Using four injection sites, and EMG electrodes animals were trained before and after surgeries and tested for forelimb activity. These studies have confirmed successful inactivation of selected connection with behavioral test in awake animal and muscle responses in anesthetized animals in the previous year. In the last reporting period, researchers tried to inactivate selected connection in awake animal with muscle responses.

Future Directions: In completing Aim 1, researchers will keep trying to optimize inactivation to successfully silence selected connection in awake animal with testing muscle responses. In the final six-months of this Fellowship, the fellow will begin work on Aim 2: strengthening CST motor responses and promoting recovery of skilled forelimb function after chronic cervical SCI with motor cortex stimulation.

Impact: Once this project is completed successfully, the results will provide information regarding where in the brain and spinal cord stimulation with electrical current should occur to improve motor recovery.

#### 2. **Rensselaer Polytechnic Institute**<sup>1</sup>

Ryan Gilbert, Ph.D., Christopher Johnson, D.L., B.S.

Predoc: \$135,600

Magnetic Alignment of Electrospun Fibers for Treatment of Acute Spinal Cord Contusive Injury

Introduction/Background: Traumatic SCI usually results in an irregularly shaped contusion in the spinal cord that regenerating neurons cannot bridge. Electrospun fiber scaffolds have been successful as bridges that aid in regeneration by guiding neurons and glial cells across the injury. But these scaffolds must be implanted using invasive surgical techniques that risk infection and cause further damage to the tissue from the implantation. Instead, researchers propose using a composite material that consists of magnetic electrospun fibers suspended in an injectable hydrogel. The scaffold would be injected into the injury site, then the fibers would be magnetically realigned in the injury cavity using a magnetic field. This scaffold would be an injectable scaffold that provides aligned topography to guide cells across the injury site. Researchers optimized the magnetic characteristics of the fibers. They also determined how well the neurons interact with the magnetic fibers.

Progress Towards Specific Aims: Expansion of the magnetic nanoparticle (SPION) content in the fibers from 2% up to 8% will provide more options when determining the optimal fibers for realigning in a hydrogel and guiding cells across the injury site. It has been determined that magnetic properties of the fibers were directly related to the SPION content in the fibers. Testing has been conducted to prove whether the length of the fibers affected the movement and there appeared to be no clear trend. Further testing will correlate the hydrogel viscosity, another variable that affects the alignment of the fibers, to the rate of realignment of the fibers. A method to insert the scaffold into a small gauge needle has been developed, delivery of the fibers and hydrogel to a model injury site using the needle and realigning the fibers with two permanent magnets has been demonstrated. In their final report, they showed that the magnetic fibers increase neurite outgrowth compared to similar fibers that did not have the magnetic nanoparticles. This was an interesting finding because there were no clear physical characteristics of the fibers that explained the differences. This suggests that the longer growth has something to do with the nanoparticles embedded in the fibers. Researchers used dissociated neurons to determine that the fibers were not toxic to the cells and in fact, the SPION containing fibers increased the neurite growth by approximately 1.3x compared to the SPION free control.

Final Report: Researchers submitted their findings to the high impact journal, *ACS Applied Materials and Interfaces* in 2018. Christopher Johnson graduated with his Ph.D. degree in biomedical engineering from RPI in May 2018.

Impact: This work would create injectable electrospun fibers that can be reoriented using a magnetic field. Success of this approach would allow electrospun fibers to be delivered using minimally invasive surgical techniques, and to be combined with advanced injectable hydrogel approaches.

Publications: Johnson, C.D.L., Ganguly, D., Zuidema, J.M., Cardinal, T.J., Ziemba, A.M., Kearns, K.R., ... & Gilbert, R.L. (December 2018). Injectable, Magnetically-Orienting Electrospun Fiber Conduits for Neuron Guidance. *ACS Applied Materials & Interfaces*. doi:10.1021/acsami.8b18344.

Presentations: Johnson, C.D.L. & Gilbert, R.L. (October 2018). *Injectable, Magnetically-Aligned Electrospun Fiber Conduits for Neural Guidance*. Abstract presented at the Biomedical Engineering Society Annual Meeting, Atlanta, Georgia.

### **3. Research Foundation for SUNY, University at Albany<sup>2</sup>**

Ben Szaro, Ph.D., Rupa Priscilla, M.S.

Predoc: \$85,585

Intracellular Modulations of Cytokine Signaling Leading to Successful CNS Axon Regeneration in a Vertebrate Model

Introduction/Background: Studying animals such as frogs, which successfully recover from traumatic injury to the CNS can guide design of combinatorial therapies for treating human SCI. In mammals, expression of the SOCS3 gene directly inhibits CNS axon regeneration, but frogs, which also express SOCS3, somehow overcome this inhibition. Another SOCS gene expressed in mammals and frogs, SOCS2, functionally antagonizes SOCS3 in mammalian neural development and tumorigenesis. The hypothesis is that increased SOCS2 expression after injury in amphibians promotes CNS axon regeneration by counterbalancing SOCS3's inhibitory actions. The researchers tested this hypothesis by comparing SOCS2 (Aim 1) and SOCS3 (Aim 2) expression when animals recover from CNS injury (e.g., frog optic nerve and tadpole SCI) with when they do not (frog SCI) and by testing effects of manipulating expression of these genes on recovery (Aim 1, SOCS2; Aim 2, SOCS3).

Progress Towards Specific Aims: In previous reporting periods, the trainee developed reagents and refined techniques for measuring expression of SOCS2 and SOCS3 proteins and mRNAs and made reagents for inhibiting their expression in regenerating and developing CNS neurons. Perhaps most importantly, the differences she identified between frog and mammalian SOCS3 metabolism after CNS injury could have implications for therapeutic approaches toward treating SCI. For example, her results suggest that inhibiting SOCS3 degradation by calpain in mammalian axons might enhance pro-regenerative effects of SOCS3 after CNS injury and improve regeneration.

Final Report: The trainee's project helped resolve this paradox by comparing SOCS3 RNA and protein expression during optic nerve injury with those SOCS2, another SOCS protein that functionally antagonizes SOCS3.

Impact: This research has yielded new insights into the functions of SOCS proteins in CNS axon regeneration and suggests that reagents that reduce SOCS3 protein levels in neuronal cell bodies (e.g., activators of elongin B/C) and raise them in axons (e.g., calpain inhibitors) may promote recovery from CNS in mammals.

Rupa Priscilla received experience conducting research, presenting results at meetings, and mentoring undergraduate researchers. She also broadened her knowledge through seminar courses. She successfully defended her Ph.D. thesis and has submitted her research for publication. Rupa Priscilla graduated with his Ph.D. degree in Philosophy from University at Albany in June 2018.

Publications: Priscilla, R., Szaro, B.G. (October 2018). Comparisons of SOCS mRNA and Protein Levels in Xenopus Provide Insights into Optic Nerve Regenerative Success. Brain Research, S0006-8993(18)30522-5. doi: 10.1016/j.brainres.2018.10.012.

### **4. Research Foundation of CUNY, The City College of New York<sup>2</sup>**

John Martin, Ph.D., Alzahraa Amer, M.S.

Predoc: \$135,600

Modulating Spinal Cord Neural Activity to Promote Recovery of Motor Function After SCI

Introduction/Background: SCI interrupts the corticospinal tract (CST), which connects the motor cortex, where movements are initiated with the spinal cord and where movements are more directly controlled by the actions of spinal cord neurons on muscle. The overall aim of this project is to strengthen the connections of the CST using spinal cathodal direct current electrical stimulation to promote motor function after injury. Direct current electrical stimulation is a non-invasive way to modulate spinal cord neuronal activity.

Progress Towards Specific Aims: For Aim 1, the researchers showed that both direct current electrical stimulation, and transspinal direct current (DC) electrical stimulation significantly enhanced spinal cord excitability. Aim 2, studies have been completed using these methods along with chronic motor cortex stimulation enhance corticospinal tract sprouting above and below contusion injuries. The studies in Aim 2, showed improved forelimb locomotor function and manipulation. The predoctoral student used Western blotting during the current period to assay the effects of neuromodulatory interventions on signaling pathways in corticospinal motor system neurons. She also continued the electrical stimulation studies to further examine the benefits in improving motor functions.

Future Directions: The researchers will focus on completing the remaining experiments and the predoctoral student will prepare her thesis. In addition, the trainee will attend courses on SCI and neural circuit study.

Impact: Spinal cord DC stimulation has the potential to become an important non-invasive neuromodulatory tool to promote spinal motor function after injury and to enhance the therapeutic effects of brain stimulation.

Presentations: Amer, Z. & Martin, J.H. (November 2018). *Theta burst electrical stimulation of the motor cortex produces functional reorganization of the corticospinal system in rats*. Abstract presented at the Society for Neuroscience, San Diego, California.

## 5. Winifred Masterson Burke Medical Research Institute<sup>2</sup>

Jian Zhong, Ph.D., Mariel Voutounou, Ph.D.

Postdoc: \$165,354

Promoting Intrinsic Growth Competency of Injured Neurons Using Genetic and Small Molecule Approaches

Introduction/Background: Neural repair after SCI remains challenging due to the limited intrinsic regenerative capacity of mature corticospinal motor neurons (CSMNs) and the inhibitory environment. Recently, researchers found that re-activation of specific intraneuronal growth signaling B-RAF is sufficient to promote axon growth and regeneration in the optic nerve after lesion. Their objective is to combine genetic manipulation of B-RAF, and the elimination of growth inhibitory molecules, to overcome these limitations to achieve regeneration, ultimately leading to functional recovery after experimental SCI. They are developing a multi-photon live imaging protocol to access the changes in the activity of CSMNs to monitor the ongoing recovery.

Progress Towards Specific Aims: A *Fezf2CreER<sup>T2</sup>* line to activate B-RAF in CSMNs has been generated. The B-RAF gain of function (GOF) mice lacking myelin-based growth inhibitor molecules (*LSL-kaBraf: Nogo(-/-) : MAG(-/-) : OMgp(-/-) : Fezf2CreER<sup>T2</sup>*) has

been generated to investigate the combinatory effects of B-RAF activation and the absence of inhibitory cues.

Viral constructs have been designed and generated to up- and down- regulate downstream effectors of B-RAF signaling pathways identified via RNAseq. Moreover, using a genetically modified mouse model in which both red and green fluorescent proteins are expressed in CSMNs, their study indicates that the excitation/emission spectra of red fluorescent protein are well suited for *in vivo* multi-photon imaging of the deeper layers in the adult mouse CNS.

Future Directions: Investigate the effects B-RAF activation in the presence or absence of myelin-based inhibitor on axon regrowth in SCI models. A three-photon excitation fluorescent imaging protocol will be set up for live imaging of the  $Ca^{2+}$  transients in CSMNs and spinal cord, first in intact animals. Analysis will then proceed to mice subjected to spinal cord injury surgeries, either unilateral pyramidotomy or dorsal hemisection, to determine changes in CSMNs activities associated with CNS axon sprouting or regeneration *in vivo*.

Impact: Researchers aim to boost the regenerative effect of B-RAF activation on injured mature corticospinal axons by combining it with the elimination of environmental growth-inhibitory cues. They are also testing the ability of specific miRNAs to promote axon regrowth after mouse SCI. The findings will contribute towards a better understanding of the intrinsic signaling mechanisms of SCI regeneration, but also novel therapeutic strategies. In addition, the multi-photon imaging protocol will provide a crucial tool for investigating the association between CSMNs activities and motor behavior recovery.

## CART/IDEA

IDEA Contract Term 11/1/15-10/31/17; CART Contract Term 11/1/15-10/31/18

### Progress Reporting Periods

5/1/2017-10/31/2017<sup>1</sup>

11/1/17-4/30/18<sup>2</sup>

5/1/2018-10/31/2018<sup>3</sup>

Procurement Total: \$5,719,548

#### 1. Albert Einstein College of Medicine<sup>2</sup>

David Sharp, Ph.D.

CART: \$1,197,182

Harnessing Microtubules to Enhance Urological Function after Spinal Cord Injury

Introduction/Background: SCI-related research both in animals and humans has traditionally focused on repairing, protecting or regenerating motor tracts, while changes in the sensory and autonomic nervous system after SCI remain understudied.

Progress Towards Specific Aims: This research evaluated the recovery of function in a rat model of SCI following Fidgetin-like 2 (FL2) knockdown. For Aim 2, studies have shown a robust improvement in axon growth in adult rat dorsal root ganglion neurons with FL2 depletion and have further shown that FL2 depletion attenuates the impact of inhibitory environmental cues on axon growth in these sensory neuronal cultures. While dorsal root ganglion neurons send an axon into the spinal cord and are a widely used as an *in vitro* model for SCI regeneration studies, they are technically part of the Peripheral Nervous System (PNS). PNS neurons have significantly more regenerative capacity than neurons of the CNS. Therefore, to further validate the efficacy of targeting FL2 to promote axon regeneration in the CNS, researchers established a fetal cortical neuronal culture system, taking advantage of their newly acquired transgenic mouse. The FL2 conditional knockout mouse has been genetically engineered so that the FL2 gene can be excised from select tissues or cells with high efficiency at any time during development. Researchers have optimized a procedure for the culture and efficient knockout of FL2 from embryonic cortical neurons and are now in the process of verifying the impact of FL2 on axon growth and the microtubule array in a CNS neuronal culture system. Secondly, their studies show that FL2 activity and/or expression in neurons is upregulated to response to select environmental cues *in vitro* and have shown that FL2 expression is upregulated very rapidly in response to injury in the spinal cord. Preliminary studies indicate that 1) FL2 activity is upregulated in response to select cellular stressors, and 2) FL2 activity is also dynamically regulated at the translational level in response to certain environmental cues.

Final Report: The main goal moving forward will be to further improve the delivery and efficacy of the therapeutics by bringing their *in vitro* approach using viral delivery of FL2-shRNA to the *in vivo* context. In theory, viral FL2-shRNA delivery would enable researchers to maintain FL2 at lower levels at later timepoints and for a more extended period of time.

In addition, researchers will continue to utilize the transgenic mouse for *in vitro* studies aimed at elucidating the role of FL2 in regulating regeneration after SCI. The ease and

high efficiency of generating FL2 knockout cells with adeno-virus-delivered cre recombinase make this system ideal for studies assessing the role of FL2 in regulating the response of both neurons and nonneuronal cells at injury sites. Researchers will continue studies aimed at understanding the dynamic regulation of FL2 expression in response to injury.

Impact: This study identifies a novel therapeutic target for promoting axon regeneration and recovery of SCI-related loss of motor and urogenital functions; will advance research in underexplored areas of autonomic and sensory dysfunction following SCI; and will advance our understanding of how the microtubule cytoskeleton can be targeted to promote neural regeneration in the CNS.

Presentations: Sharp, D. (2018). *Neuronal Cytoskeleton Subgroup X*. Presented for the American Society of Cell Biology Annual Meeting, Philadelphia, P.A.

## **2. Burke Medical Research Institute<sup>1</sup>**

Jason Carmel, M.D., Ph.D.

IDEA: \$450,419

Delayed Versus Immediate Motor Training Following Brain Stimulation to Enhance Recovery in Rats with Chronic Corticospinal Tract Injury

Introduction/Background: For people with cervical SCI, restoring arm and hand function is the top priority. Researchers want to understand how to combine hand therapy (exercise) and electrical brain stimulation in chronic SCI. They hypothesized that training will be most effective delivered two weeks after electrical stimulation.

Progress Towards Specific Aims: Thus far, researchers have demonstrated that supination task can detect severe forelimb deficits and moderate improvements with training and stimulation. Regarding Aim 1, they have found that both electrical stimulation and rehabilitation training appear to be effective in improving functional outcomes regardless of relative timing between two therapies. Stronger effects were observed in animals receiving rehabilitation training compared with stimulation alone. Preliminary results for the Aim 2 indicate that anatomical results mirror our findings in the behavioral studies in that there is increased sprouting following both therapies but effects of behavior training is stronger.

Final Report: Researchers will complete quantifying the anatomical results and continue quantifying axon densities and distribution of the animals that have concluded the behavioral studies.

Impact: With a better understanding of how these two promising therapies should be combined, researchers expect that these results will help to design more effective therapies for people with SCI.

## **3. Burke Medical Research Institute<sup>1</sup>**

Principal Investigator: Dianna Willis, Ph.D.

IDEA: \$448,978

Alterations in Extracellular Vesicle Communication as a Cause of NMJ Dysfunction after SCI

Introduction/Background: Following SCI, changes occur that have been implicated in driving secondary events after the initial, primary injury. Among these changes are alterations in the



neuromuscular junctions (NMJs) at sites distant from the injury. Researchers worked on the hypothesis that changes in signals from the muscle cells to the neuron at the NMJ are a cause of NMJ dysfunction.

**Progress Towards Specific Aims:** The goal of Aim 1 is to identify the muscle-secreted microRNA changes following SCI. Researchers have generated the SCI animals, collected tissue from these animals, and isolated exosome preparations which were tested for the presence of microRNAs, and performed RNA deep sequencing on these preparations. Samples have been collected for electron microscopy, and the antibodies required for the immuno-EM have been optimized. These experiments are ongoing. Aim 2 is designed to elucidate the role of mir206 in the maintenance of NMJs. Tagged-mir206 constructs have been used to confirm the transfer from muscle cells to axons, and NMJ morphological analyses with knockdown and overexpression have been performed. The goal of Aim 3 is to determine whether muscle-secreted microRNAs regulate local protein synthesis to facilitate NMJ maintenance. Researchers have completed the bioinformatics-based target identification and have confirmed the veracity of these target RNAs using microRNA Trap system. *In vitro* co-cultures and NMJ formation have been optimized, and they have finished the knockdown experiments to complete this aim. They have identified several target RNAs in motor neuron axons whose local translation is required to maintain motor neurons.

**Final Report:** They are completing the final analyses of the collected data and are in the process of preparing a manuscript for publication. In addition, the data collected from this award has served as the preliminary data for a recently submitted National Science Foundation grant application aimed at better understanding the mechanisms of transfer of the microRNAs from muscles to motor neurons.

**Impact:** Researchers believe that a better understanding of the fundamental means of communicating between cells, and how this communication is disrupted following SCI, will point to potential therapeutic strategies for maintaining the NMJ following injury. Strategies that limit the propagation of secondary damage following injury would greatly impact long term recovery and quality of life.

#### **4. Health Research, Incorporated<sup>3</sup>**

Jonathan Carp, Ph.D.

IDEA: \$442,373

Role of Abnormal Urethral Sphincter Motoneuron Properties in Urinary Tract Dysfunction after Spinal Cord Injury

**Introduction/Background:** This project addresses how SCI affects the nerve cells that operate the external urethral sphincter (EUS), a muscle crucial for controlling urinary function. The central hypotheses are that: SCI produces long-lasting changes in these nerve cells; and these changes cause inappropriate EUS muscle activation, thereby impairing urinary control. First, researchers will identify SCI-induced abnormalities in these nerve cells by directly measuring EUS nerve cell properties with microelectrodes using SCI or intact rats. After collecting control data, the drug sensitivity of these nerve cells will be assessed to determine the mechanism of these effects. They will also determine whether SCI-induced changes in EUS nerve cell properties identified will contribute to SCI-induced urinary dysfunction by assessing voiding capabilities of rats with or without SCI before and during spinal administration of the same drugs.

**Progress Towards Specific Aims:** Researchers achieved developing decerebration-anesthetized rat preparation for performing urinary assessment without chemical

anesthesia; performing assessment of urinary function in decerebrate rats with or without SCI; performing pharmacological experiments in decerebrate rats with or without SCI in which a serotonin inverse agonist (eplivanserin) improved voiding efficiency in SCI rats, while the serotonin neutral antagonist did not; development of bladder port for chronic cystometry in freely-moving rats; and design and prototyping a 3D-printer-fabricated port for chronic cystometry in freely-moving rats.

Final Report: Researchers plan to conduct further experiments to identify neurons that are targets of eplivanserin's beneficial effects and determine if its systemic administration can improve SCI-induced urinary dysfunction.

Impact: Abnormal EUS activity after SCI can make voiding difficult or impossible without catheterization. The demonstration that eplivanserin improved urinary function after SCI in rats suggests the potential benefit of this class of drugs for treating this problem in people with SCI.

Publications: LaPallo, B.K., Wolpaw, J.R., Yang Chen, X., & Carp, J.S. (November 2017). Spinal Transection Alters External Urethral Sphincter Activity during Spontaneous Voiding in Freely Moving Rats. *Journal of Neurotrauma*, 34:3012–3026. doi: 10.1089/neu.2016.4844

## **5. Icahn School of Medicine at Mount Sinai<sup>2</sup>**

Noam Harel, M.D., Ph.D.

IDEA: \$391,353

Augmenting Hand Muscle Control in Cervical SCI through Paired Cortical and Cervical Stimulation

Introduction/Background: Researchers aim to improve function of spared nerve circuits after SCI through the use of electrical and magnetic stimulation.

They have developed a form of non-invasive electrical stimulation over the spinal cord that activates muscles in both hands simultaneously and comfortably. This technique, called cervical electrical stimulation (CES), works at the skin surface – no surgery is required. In this proposal, they are investigating the basic mechanisms, safety, and short-term efficacy of this new technique.

Progress Towards Specific Aims: 12 subjects without SCI and 10 subjects with SCI completed multiple sessions of repetitive stimulation via different combinations of CES, transcranial magnetic stimulation (TMS), and peripheral median nerve stimulation.

Preliminary results indicate that a potentially non-specific facilitatory effect is seen after repetitive paired stimulation across both SCI and non-SCI subjects. They also reported facilitatory effects using other interventions such as TMS either by itself or paired with peripheral median nerve stimulation.

When pairing CES with magnetic stimulation of the brain, they have observed an increase in nerve transmission between the brain and hand muscles either immediately after single pairs of stimulation, or for up to 30 minutes after a 20-minute period of stimulation.

When pairing CES with magnetic stimulation of the brain on a single-pulse basis, they have observed that CES can facilitate response to magnetic stimulation in a timing-dependent

fashion. This suggests that CES may also be able to facilitate response to voluntary hand movement, which comes from the same area of the brain that they are magnetically stimulating.

Clinical tests of hand strength (pinch dynamometry) and dexterity (grooved pegboard) after repetitive stimulation did not show significant changes.

Final Report: Data analysis of the many outcome measures is ongoing and they expect to publish at least two primary research papers reporting these results. These results have been presented at the American Society of Neurorehabilitation Annual Meeting, as well as at Grand Rounds for Mount Sinai Neurology and Rehabilitation Medicine.

Impact: This approach to stimulation has the potential to strengthen the brain's control over the spinal cord after SCI. It could also synergize with other types of treatment, such as physical rehabilitation and future drug treatments.

## **6. Icahn School of Medicine at Mount Sinai<sup>1</sup>**

Hongyan Zou, M.D., Ph.D.

IDEA: \$360,000

The Role of HDAC3 in the Epigenetic Regulation of Functional Polarization of Microglia and Macrophages after Spinal Cord Injury

Introduction/Background: SCI results in neurological deficits that seldom recover. SCI triggers a multiphasic immune response. The innate immunity consists of microglia, resident immune cells in the CNS, and blood-born monocytes that differentiate into macrophages at the injury site. The innate immune response plays a dual role for tissue repair after SCI. Developing new strategies that can maximize the pro-repair while minimizing the detrimental aspect of the immune response represents a promising new direction for SCI therapy. In order to realize the promise of immunomodulatory therapy, a deeper understanding of the regulatory mechanisms of the diverse functions of microglia and macrophages in SCI is imperative. This proposal studies the novel function of HDAC3 in mediating the innate immune response after SCI. HDAC3 is an epigenetic enzyme that modifies the histone acetylation status of target genes. Researchers focused on studying the potential therapeutic effect of a specific HDAC3 inhibitor in promoting functional recovery after SCI.

Progress Towards Specific Aims: Researchers have demonstrated a robust upregulation of HDAC3 in the innate immune cells after SCI. Remarkably, blocking HDAC3 activity using a selective small molecule inhibitor can suppress inflammatory responses of microglia and macrophages, resulting in better neuroprotection and improved functional recovery in SCI model. They have also confirmed that HDAC3 mediates histone deacetylation in microglia and their inflammatory responses to classic inflammatory stimuli. Based on these results, their future research endeavors will optimize HDAC3-based immunomodulatory strategy and evaluate its efficacy in alleviating neuropathic pain and improving bladder function after SCI.

Final Report: Researchers are preparing a manuscript on the bioinformatics analysis of RNA-Seq data. This will provide important reference map for in vivo gene signature of the innate immune cells in their multiphasic responses to SCI. They will demonstrate at early stage, they upregulate cell cycle genes; at intermediate stage, ion channel genes are induced; and at later stage, extracellular matrix genes are involved. They will also carry out studies to assess histone acetylation status on target genes in microglia/macrophages, thus linking the enzymatic function of HDAC3 and inflammatory gene regulation

Impact: Their results reveal a novel function of HDAC3 inhibitor in promoting functional recovery after SCI by dampening inflammatory cytokines, thus pointing towards a new direction of immunomodulation for SCI repair

Publications: Kuboyama, T., Wahane, S., Huang, Y., Zhou, X., Wong, J.K., Koemeter-Cox, A.,...& Zou, H. (August 2017). HDAC3 Inhibition Ameliorates Spinal Cord Injury by Immunomodulation. *Science Reports*, 7(1):8641. doi: 10.1038/s41598-017-08535-4.

Loh, Y.E., Koemeter-Cox, A., Finelli, M.J., Shen, L., Friedel, R.H. & Zou, H. (February 2017). Comprehensive Mapping of 5-hydroxymethylcytosine Epigenetic Dynamics in Axon Regeneration. *Epigenetics*, 12(2), 77-92. doi: 10.1080/15592294.2016.1264560.

## 7. Regenerative Research Foundation<sup>3</sup>

Sally Temple, Ph.D.

CART: \$1,097,684

Sustained Delivery of IL10 and SHH to Promote Spinal Cord Regeneration After Injury

Introduction/Background: Spinal cord injury affects more than a million individuals in the US. Most were injured at a young age and suffer life-long consequences of paralysis and numerous medical complications. Current treatments are symptomatic, and do not result in recovery. Research into novel treatments that will improve regeneration and repair after SCI are imperative, as there is great unmet medical need. Researchers have developed bioengineered micro-sized beads made of a biodegradable, biocompatible and FDA approved material. They propose to test whether a combination of sustained IL10 plasmid (IL10 pDNA) and sustained sonic hedgehog growth factor (SHH) delivered via biodegradable biocompatible microbeads to the injury site will counteract inflammatory processes, promote a regenerative environment and improve recovery after spinal cord injury.

Progress Towards Specific Aims: All work associated with Aim 1 (study of the impact of IL10 and SHH microbeads on the inflammatory response in the spinal cord) has been completed.

For Aim 2, researchers studied the effects of separate SHH and IL10 pDNA microbead delivery on functional locomotor and histological recovery in acute and chronic SCI. There were no specific differences in spinal cord lesion volumes in SHH and IL-10pDNA microbead treated animals.

For Aim 3, research was conducted to test the combinatorial effect of IL-10pDNA and SHH microbead delivery on functional locomotor and histological recovery in rat acute and chronic SCI models. Data of 12 weeks post-SCI and post-injections demonstrated no difference to cumulative errors between IL-10pDNA and SHH microbead-injected versus empty microbead-injected animals groups.

Future Directions: Histological analyses of spinal cord tissues of animals that received IL-10pDNA or SHH microbeads alone or combined treatment is underway to investigate how these treatments affect lesion volume, scar formation and myelination.

Impact: Researchers will assess whether sustained delivery of IL-10pDNA and SHH microbeads can alter the post-injury inflammatory processes in the spinal cord and their potential therapeutic effectiveness for the treatment of SCI. Data will be obtained on the

effectiveness of combinatorial treatment with IL-10pDNA and SHH microbeads for recovery from SCI.

#### **8. Research Foundation of CUNY, The City College of New York<sup>2</sup>**

John Martin, Ph.D.

CART: \$990,000

Repairing the Damaged Corticospinal Tract after Cervical Spinal Cord Injury

**Introduction/Background:** The scope of the project is to develop electrical-stimulation based therapies for SCI. Researchers use a rat model of contusion injury of the upper part of the spinal cord, termed the cervical spinal cord. Their approach to therapy is to electrically stimulate the motor cortex, where movements are initiated, and stimulate the spinal cord, where movements are executed, and to promote connections of the corticospinal motor system after injury. In the prior reporting period, they completed development of their SCI model in the subject animals. They also combined motor cortex stimulation with transspinal direct current stimulation (tsDCS) to promote recovery in their SCI model. In the present reporting period, they expanded their experiments to study how stimulation effects movement performance errors after SCI.

**Progress Towards Specific Aims:** Researchers continued to examine effects of tsDCS in reducing movement errors after SCI. Using animal models and conducting reach to grasp tasks the researchers continued experiments with tsDCS stimulation, and the combined effects of moto cortex and spinal cord neuromodulation. These experiments continued to show corticospinal tract (CST) axon sprouting can be driven by combined motor cortex stimulation and tsDCS. They also continue to expand experiments determining if the effect of cathodal tsDCS on CST sprouting is due to a compensatory response to reducing afferent fiber projections. To prove their hypothesis a sufficiently large number of animals are needed draw a conclusion.

**Future Directions:** The researchers plan to continue to examine the effects of tsDCS in reducing movement errors after cervical contusion injury during the stimulation period, with a focus on changes in the performance of a reach-to-grasp task. They have also begun experiments to compare the effects of neuromodulation alone with neuromodulation plus behavioral rehabilitation. The research team will continue to examine the effect of tsDCS on afferent fiber sprouting. They also plan to continue to conduct combined modeling and physiological experiments to determine the effect of localizing tsDCS on spinal functions. Recently, a 12-month No-Cost-Time-Extension was approved for this award to allow the researchers to complete their work.

**Impact:** The researchers studies are the first to show that electrical neuromodulation produces durable plasticity of the CST after cervical SCI. They hope the results open up the possibility of using tsDCS to treat two major effects of cervical injury, arm weakness and spasticity. They also hope this research leads to development of tsDCS as a non-invasive therapy for CST and motor system repair after cervical SCI in humans.

**Publications:** Zareen, N., Dodson, S., Armada, K., Awad, R., Sultana, N., Hara, E., ...& Martin, J.H. (2018). Stimulation-dependent remodeling of the growth-promoting developmental signaling pathways. *Experimental Neurology*, 307, 133-144. doi: <https://doi.org/10.1016/j.expneurol.2018.05.004>.

### *Appendix 3*

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<sup>1</sup> Service commenced during 2018

<sup>2</sup> Service concluded during 2018