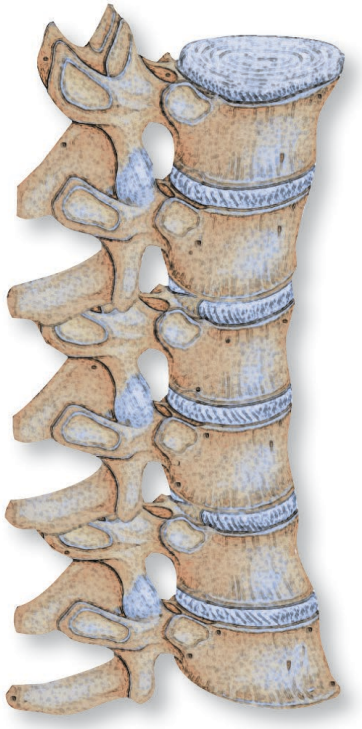
NEW YORK STATE OF OPPORTUNITY. Department of Health



SPINAL CORD INJURY RESEARCH BOARD

Annual Report

January 1, 2019 to December 31, 2019

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 1,300 New York residents suffer traumatic SCIs[1] joining approximately 291,000 people living in the United States who have SCI.[2] 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 SCI Statistical Center reported that first-year costs for an individual with SCI range from approximately $368,562 to more than $1,129,302, with annual costs thereafter ranging from approximately $44,766 to $196,107[2]. 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.[2] 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.

1 New York State Department of Health, Bureau of Occupational Health, and Injury Prevention, 2019 data

2 "Spinal Cord Injury Facts and Figures at a Glance." *National Spinal Cord Injury Statistical Center.* University of Alabama at Birmingham, 2019. Web. 7 August 2019. 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, Wadsworth Center, Extramural Grants Administration 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 are two (2) vacancies to be filled by the Temporary President of the Senate and the Governor. The current composition of the SCIRB includes seven (7) researchers, two (2) clinicians and two (2) spinal cord-injured persons. Members serve four-year terms.

III. SCIRB OPERATIONS

In fiscal year 2019-20, $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 2019 (see Section IV. below).

No changes were made to the SCIRB's bylaws in 2019. The bylaws can be found at https://www.wadsworth.org/extramural/spinalcord/advisory-board/statutes-bylaws.

IV. MAJOR ACTIVITIES OF THE SCIRB

Business Meetings

At its January 25, 2019 meeting, the SCIRB recommended funding for three (3) predoctoral and four (4) postdoctoral fellowship awards from the "Individual Predoctoral and Postdoctoral Fellowships in SCI (Round 4)" Request for Applications (RFA) for a total of $1.16 million. These are three-year awards and contracts began in August 2019. A tabular summary of this procurement can be found in Appendix 1.

The following two (2) RFAs were released in 2019:

- "Projects to Accelerate Research Translation (PART) and Innovative, Developmental or Exploratory Activities (IDEA) in Spinal Cord Injury (Round 4)"
- "Translational Research Projects in Spinal Cord Injury (Round 3)"

A January 2020 meeting will be planned so the SCIRB can recommend funding for the "PART and IDEA in Spinal Cord Injury (Round 4)" and "Translational Research Projects in Spinal Cord Injury (Round 3)". These multi-year contracts are anticipated to start in 2020. A summary of these procurements will be featured in the SCIRB's 2020 Annual Report.

At its September 13, 2019 meeting, the SCIRB approved the release of the following RFA for release in 2020/2021:

- "Individual Predoctoral and Postdoctoral Fellowships in Spinal Cord Injury (Round 5)"

A fall 2020 meeting will be planned so the SCIRB can recommend funding for the "Individual Predoctoral and Postdoctoral Fellowships in Spinal Cord Injury (Round 5)." These two-year contracts are anticipated to begin in 2021.

Previously Recommended SCI Research Contracts

By January 2019, four (4) PART contracts and seven (7) IDEA in SCI (Round 1) contracts completed their second year. The scientific progress resulting from these multiyear awards can be found in Appendix 2.

By March 2019, the Institutional Support for SCI Research in New York State (Round 6) contracts completed their second 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 2019, the five (5) remaining Individual Predoctoral and Postdoctoral Fellowships in SCI Research (Round 1) contracts completed their third year. The scientific progress resulting from these three-year awards can be found in Appendix 2.

In May 2019, three (3) PART and four (4) IDEA in SCI (Round 3) contracts began. The scientific progress resulting from these three- and two- year awards, respectively can be found in Appendix 2.

Also, in May 2019, two (2) contracts from the Translational Research Projects in SCI (Round 2) completed their first year. The scientific progress resulting from these multiyear awards can be found in Appendix 2.

In June 2019, three (3) PART and eleven (11) IDEA in SCI (Round 2) contracts completed their first year. The scientific progress resulting from these three- and two-year awards, respectively can be found in Appendix 2.

By August 2019, two (2) Translational Research Projects in SCI (Round 1) contracts completed their third year. The scientific progress resulting from these five-year awards can be found in Appendix 2.

By September 2019, five (5) of six (6) Individual Predoctoral and Postdoctoral Fellowships in SCI (Round 3) contracts completed their first year. The scientific progress resulting from these three-year awards can be found in Appendix 2.

Also, by September 2019, seven (7) Individual Predoctoral and Postdoctoral Fellowships in SCI (Round 2) contracts completed their second year. Two (2) awards concluded early. The scientific progress resulting from these three-year awards can be found in Appendix 2.

By the end of 2019, the two (2) remaining Collaborations to Accelerate Research Translation (CART) contracts which had received no-cost extensions completed their final year. The scientific progress resulting from these three-year awards can be found in Appendix 2.

NYS SCI Research Symposium

The SCIRB and the NYS DOH will plan to host a future NYS SCI Research Symposium in 2021. The 2021 symposium will highlight recent advances and developments in basic and translational SCI research, feature presentations of the research supported by the Program, and invite international/national speakers to present new discoveries. The last Symposium was held in New York City at the Rockefeller University in October 2018.

Appendix 1

2019 Individual Predoctoral and Postdoctoral Fellowships in SCI (Round 4) Recommendations for Award

Organization	Funding Mechanism/Research Category	Project Title	Sponsor Principal Investigator and Fellow	Recommended Funding
Burke Medical Research Institute[1]	Postdoctoral/ Rehabilitation	Paired Brain and Spinal Cord Stimulation to Augment Motor Responses in People with Cervical Myelopathy	Jason B. Carmel, M.D., Ph.D. and Bushra Yasin, M.D., Ph.D.	$192,366
Columbia University	Predoctoral/ Rehabilitation	Improving Upper Body Trunk Control in Spinal Cord Injury Patients Through Robotic Rehabilitation Training	Sunil K. Agrawal, Ph.D. and Tatiana D. Luna, M.S.	$135,600
Icahn School of Medicine at Mount Sinai	Postdoctoral/ Rehabilitation	The Effects of Incorporated Exoskeletal-Assisted Walking in SCI Acute Inpatient Rehabilitation	Ann M. Spungen, Ed.D. and Chung-Ying Tsai, Ph.D.	$189,476
New York University School of Medicine	Predoctoral/ Cellular Regeneration & Therapeutics	Enteric Glial Cells Cause Gastrointestinal Complications Post Spinal Cord Injury	Dan R. Littman, M.D., Ph.D. and Hyeon Kyu (Alice) Kwon, B.S.	$135,600
Regenerative Research Foundation	Postdoctoral/ Cellular Regeneration & Therapeutics	Modulation of Inflammation Following SCI Using Plasmid IL-10 Microbeads	Sally Temple, Ph.D. and Elizabeth Fisher, Ph.D.	$184,686
Rensselaer Polytechnic Institute[2]	Predoctoral/ Cellular Regeneration & Therapeutics	Neuroprotective Curcumin-Releasing Hydrogel for Treatment of Acute Spinal Injury	Ryan J. Gilbert, Ph.D. and Jessica Funnell, B.S.	$135,600

Organization	Funding Mechanism/Research Category	Project Title	Sponsor Principal Investigator and Fellow	Recommended Funding
University of Rochester Medical Center	Postdoctoral/ Cellular Regeneration & Therapeutics	The Role of Phosphodiesterase 10A in Inflammation After Spinal Cord Injury	Bradford C. Berk, M.D., Ph.D. and Chia Hsu, Ph.D.	$189,366
			Total (7 awards)	$1,162,694

1 Dr. Carmel accepted a position at Columbia University; a PI change request was denied, and this contract was terminated.
2 Dr. Gilbert declined this predoctoral award on behalf of Jessica Funnell who accepted another fellowship opportunity.

Appendix 2

Scientific Progress Resulting from SCI Research Board-Funded Projects

PART/IDEA in SCI (Round 3)
IDEA Contract Term 5/1/19-4/30/21; PART Contract Term 5/1/19-4/30/22

Progress Reporting Period
5/1/19-10/31/19

7 Awards, Procurement Total: $4,198,058

1. **Bronx Veterans Medical Research Foundation, Inc.,**
   William A. Bauman, M.D.
   PART: $826,939
   Treatment with Romosozumab versus Denosumab to Improve Bone Mineral Density and Architecture in Subacute SCI.

   Introduction/Background: Persons with SCI lose substantial amounts of bone below the level of injury, predisposing them to fracture. With the advent of robotic exoskeletal-assisted devices, direct stimulation of the spinal cord (epidural stimulation), and the future possibility of repairing the connections in the spinal cord to permit walking, it is of obvious clinical relevance to have bones of the leg that remain strong enough to bear the body's weight while walking without fracture. To date, there has been no literature on the successful prevention of bone loss at the knee in persons with SCI. The knee is the skeletal site where most persons immobilized by SCI will suffer a fracture, and the risk of fracture walking is considerably greater than that of sitting in a wheelchair.

   Progress towards specific aims: The experimental design was modified since contract initiation; hence, the study is awaiting approval from the medical center's Institutional Review Board before they can begin.

   Future Directions: Researchers will test the ability of romosozumab, to prevent osteoporosis at the knee soon after SCI. In one study group, romosozumab will be administered for one (1) year, followed by one (1) year of denosumab. This treatment will be compared to persons with recent SCI who receive denosumab for two (2) years and a control group who received no medication after SCI. Images of the regions of interest of the leg (the hip and knee) will be obtained by dual energy x-ray absorptiometry and compared.

   Impact: To prevent osteoporosis, which is important to health, wellbeing, and independence of persons with chronic SCI.

   Publications: Researchers submitted a manuscript for publication that shows the efficacy of a new class of medication, denosumab, to prevent bone loss at the knee in persons with recent SCI.

2. **Icahn School of Medicine at Mount Sinai**
   Ravi Iyengar, Ph.D.
   IDEA: $342,914
   Systems Therapeutics for Spinal Cord Injury

   Introduction/Background: Novel drugs, in particular drug combinations that elicit effects on both the neuronal cell bodies and the injury site are required to promote axonal regeneration and functional motor recovery after SCI. Taking a systems biology approach, the research team has predicted a novel 4-drug combination that has a robust effect in promoting axonal regeneration in the injured rat optic nerve. The team is now applying these drugs to the injured spinal cord to assess if they can promote not only regeneration but motor functional recovery as well.

   Progress towards specific aims: Dr. Mustafa Siddiq is taking lead of this project. He is working at the Bronx VA under the supervision of our co-PI, Dr. Christopher Cardozo. The Bronx VA this September just completed renovations on a brand-new surgical suite specifically for rat contusions using the Horizon impactor. Dr. Siddiq has already completed a preliminary set of eight (8) animals with contusions and he will have completed animal studies proposed in Aim 1 by spring 2021. He will also have completed a large part of the histologic analysis by then as well. He then will begin assessing the effects of the 4-drug combination on the glial population at the injury site and will see if this promoted any synaptogenesis in areas of robust regeneration.

   Future Directions: The team is preparing to initiate testing of their 4-drugs, by first applying them individually to either the injury site (for Taxol and adenomatous polyposis coli (APC)) or by injecting them directly to the cell bodies in the brain (for HU-210 and Interleukin 6 (IL-6)). Then they will begin testing different combinations of the 4 drugs and the complete 4 drugs application to assess for regenerating axons, modulating the glial scar, and motor recovery. They will also begin looking at synaptic markers for the drugs that show a robust effect.

   Impact: The project will determine if taking a systems approach to predicting novel drug combinations will be beneficial in promoting axonal regeneration in the injured spinal cord and for recovering some motor function.

3. **Icahn School of Medicine at Mount Sinai**
   David F. Putrino, P.T., Ph.D.
   IDEA: $357,639
   Virtual Reality to Reduce Pain in the Upper Extremities After Spinal Cord Injury

   Introduction/Background: After a SCI, there is damage to the spinal somatosensory circuit, leading to aberrant signals that are interpreted by the brain as neuropathic pain (NP). NP is a common and highly disabling condition that greatly decreases overall quality of life, whilst increasing risk of suicide and opioid addiction. Virtual Reality (VR) is an emerging technology that is currently being applied as a treatment option for many forms of pain, including NP. The research team believes that VR neurorehabilitation can help to activate downstream areas of the brain involved in movement and motor imagery decreasing the sensation of pain.

   Progress towards specific aims: Since the start date for the study, the team has submitted and received approval by their Institutional Review Board. They have

successfully enrolled 12 participants, which have been randomized and allocated to three different VR groups. For all the enrolled participants, they have successfully collected demographic data including SCI level and etiology, as well as pain quality and intensity and psychometric properties that may influence responses to VR.

Future Directions: The researchers will continue to enroll participants and their endeavors to guarantee the successful completion of the trial. After completion of the protocol, they will conduct data analysis to determine pain relief effect sizes across different VR groups, as well as the association between the psychometric properties and pain relief effects.

Impact: This study will help to further investigate the feasibility and efficacy of such treatment, bringing to light knowledge about the neurophysiology of NP. The team's hope is that this information will provide invaluable insight that can be used to guide accessible and affordable chronic-pain rehabilitation strategies, and ultimately, benefit a larger SCI population.

4. **University of Rochester**
   Gail V.W. Johnson, Ph.D.
   IDEA: $360,000
   Transglutaminase 2 as a Therapeutic Target to Facilitate Recovery After Spinal Cord Injury

   Introduction/Background: When the spinal cord is injured, astrocytes react by mounting a defensive reaction aimed at limiting tissue damage. However, this response may inhibit functional recovery or fail to support the survival of nerve cells. The response of astrocytes to injury is highly regulated and the protein transglutaminase 2 (TG2) likely plays a pivotal role. Deletion of TG2 from astrocytes significantly increases their ability to survive ischemic insults and protect neurons from ischemic-induced cell death. Dr. Johnson and her team will test specifically removing TG2 from astrocytes or using a highly selective, novel TG2 inhibitor and assess improvement outcomes after a SCI.

   Progress towards specific aims: For the team to test their hypothesis that astrocytic TG2 impedes the recovery process after SCI, they began breeding the necessary mice; with their control littermates to expand the colony so that they have the necessary number of mice needed for their studies. Further, they have been optimizing their contusion injury model and their immunostaining protocols, so that they can produce reproducible injuries and carry out functional analyses using both the Catwalk™ system and scoring by observers blinded to the genotype.

   Future Directions: In their preliminary studies, they only did functional analyses out to 28 days; however, over the next 6 months they will extend measures out to 46 days. Further, they will collect the mice at 46 days and carry out immunohistochemical analyses and plan on doing initial measures of gene expression. They also plan to establish the protocols to do axonal measures.

   Impact: Understanding how removing TG2 from astrocytes improves functional outcomes after a SCI is fundamental to developing pharmacological strategies that target TG2 as a therapeutic strategy for the treatment of central nervous system injuries.

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

Edmund R. Hollis, Ph.D.
PART: $963,000
Rehabilitation and Cortical Remodeling After Surgical Intervention for Spinal Cord Injury

Introduction/Background: Upper extremity nerve transfer surgery is a state-of-the-art intervention for individuals with mid to low cervical injury; however, functional outcomes after surgery have been highly variable, to date. The research team aims to evaluate the physiological and functional outcomes promoted by intensive rehabilitation in individuals that undergo nerve transfer surgery. Outcomes as well as underlying neurophysiological changes will be tracked in humans, with parallel experiments in an established pre-clinical mouse model.

Progress towards specific aims: The research team has obtained Institutional Review Board approval for the pilot clinical trial outlined in Aim 1. There has been significant interest in the research program, e.g., eight (8) individuals have undergone pre-screening and are being considered for enrollment. One (1) individual has undergone baseline testing and will undergo baseline electro diagnostics prior to referral to Dr. Justin Brown at the Massachusetts General Hospital for pre-surgical consult.

The team has tested several pre-clinical approaches for increasing the rate of regeneration following nerve transfer in Aim 2. Additionally, they are currently refining the methodology for mapping the motor representations in the brain with higher fidelity than previously possible.

Future Directions: In Aim 1, enrollment will begin for clinical studies. In Aim 2, chronically injured SCI mice will undergo nerve transfer surgery and rehabilitation.

Impact: They anticipate that mapping brain changes with post-surgical recovery and intensive motor training will show brain reorganization associated with re-innervation and functional recovery from training. Both the human and animal arms of the study will provide complementary and supporting evidence. Furthermore, the pre-clinical model will allow a more specific evaluation of physiological changes in the brain that underlie recovery; while the human study will determine potential physiological biomarkers for recovery and test the feasibility of intensive rehabilitation around the time of functional re-innervation.

6. **Winifred Masterson Burke Medical Research Institute**
Botir T. Sagdullaev, Ph.D.
IDEA: $357,566
Blood Flow Control and its Impairment in Spinal Cord Injury

Introduction/Background: Researchers will identify the cellular components that control blood flow to spinal cord circuitry and their role in SCI. Accumulating evidence, including their preliminary data, indicate that both pericytes and cholinergic neurons are structurally compromised in SCI. They will use optogenetic tools in combination with a vasomotor response assessment and advanced imaging approaches to define the neurovascular networks in rodent models of SCI injury to test the following hypotheses: 1) SCI damages pericytes, the vasomotor elements of capillaries, impairing blood flow control in the regions around and below the site of injury, and 2) reduced neurovascular interactions between cholinergic neurons and pericytes impair vasomotor activity in SCI.

Progress towards specific aims: During the initial reporting period Dr. Sagdullaev and his team have evaluated and optimized spinal cord clearing techniques that will enable a comprehensive and detailed analysis of spinal cord neuronal and vascular networks (they tested iDSCO, improved CUBIC and SHIELD protocols).

Future Directions: During the next period, they will begin evaluating neurovascular interactions between cholinergic motor neuron circuits and vascular components and how they are altered in SCI. This physiological analysis will then be followed by a structural characterization of the neurovascular unit using optimized clearing protocol.

Impact: Impaired blood flow leading to spinal cord ischemia is recognized as one of the most important factors determining the severity of SCI and clinical outcomes. Functional compromise to the networks responsible for regulating blood flow may account for this neurovascular failure after SCI. Understanding the mechanisms of vasomotor dysfunction will reveal novel therapeutic targets and provide additional approaches for treating paralysis.

7. **Winifred Masterson Burke Medical Research Institute**
   Jian Zhong, Ph.D.
   PART: $990,000
   Repetitive Transcranial Magnetic Stimulation (rTMS) as a means to Promote Corticospinal Tract (CST) Axon Regeneration

   Introduction/Background: Recently, evidence has accumulated that a relatively simple method of brain stimulation, rTMS, can slightly improve some symptoms of paralysis. The research team will use mouse models of SCI to investigate what mechanisms are activated by rTMS treatment that could lead to improved nerve function, and how much improvement may be possible using rTMS alone or in combination with other interventions. Their preliminary data suggest that rTMS can activate RAF-MEK signaling, an intracellular mechanism that can drive axon growth and regeneration. Finally, the team will treat a group of volunteer SCI patients with rTMS to see how well they tolerate the therapy while participating in a rehabilitation program.

   Progress towards specific aims: For Aim 1, the research team has generated all mouse lines that they plan to use and started to test the combined effects of rTMS with genetic modifications in promoting CST axon regeneration. They are now in the process of cross breeding the lines and producing substantial numbers of mice with defined genotypes needed to complete the experiments outlined in their grant proposal.

   For Aim 2, they have successfully carried out the transsynaptic tracing experiment and a manuscript is in preparation. In collaboration with Dr. Chris Schaffer's group, they have also recently been able to detect calcium transients in spinal interneurons in live mice.

   For Aim 3, they have set up a collaboration with Dr. Gail Forrest, Associate Professor, Director of Center for Spinal Stimulation, and Dr. Guang Yue, Professor and Director of the Center for Stroke Rehabilitation, both at the Kessler Institute for Rehabilitation in New Jersey, to examine the safety of long-term use of rTMS in SCI patients.

   Future Directions: The researchers' aims to develop a novel treatment strategy for SCI patients by translating the findings obtained from animal models to human clinical practice are expected to fill the current gap in knowledge as to whether non-invasive

brain stimulation via rTMS could be a promising route toward axon regeneration after SCI. If successful, the team will collaborate with rehabilitation hospitals to test to which extent rTMS can be used to improve axon regeneration and neurological outcomes in SCI patients.

Impact: If the researchers' aims are successful, they will have laid a firm basis for the development of new treatment strategies for SCI patients, in line with the goal of the RFA to foster the translation of results from basic (preclinical) research into the next research phase.

Individual Predoctoral/Postdoctoral Fellowships (Round 3)
Contract Term 9/1/18-8/31/21

Progress Reporting Period
3/1/19-8/31/19

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 SCI, 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 to restore arm and hand function to people with cervical SCI. 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 SCI. 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: The researchers proposed to pair brain stimulation and spinal cord stimulation targeting the reticulospinal tract to strengthen neural connections for functional recovery. To find the best latency between two stimulations, they measured the latency of stimulating the reticular formation and responsive local field potential in the spinal cord, and the maximum latency to evoke forelimb muscle electromyography. To realize simultaneous paired cortex and spinal cord stimulation, local field potential recording and multi-muscle electromyography recording, they programmed in Tucker-Davis Technologies (TDT) synapse software and established an all-in-one physiology collection system and tested with simulated data.

   Future Directions: The researchers will become familiar with behavior tasks (Aim 2) and explore the optimal latency for paired brain and spinal cord stimulation (Aim 1).

   Impact: The researchers' experiments have the potential to improve their understanding of spared connections after SCI and whether those connections can be recruited using a promising therapeutic approach using the cortico-reticulospinal tract as the potential responsible pathway. The personal career development 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. 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: Using a virus to make CST neurons sensitive to light or to a special injected drug, Dr. Yang was able to stimulate CST neurons either physically with light, or tonically with a drug. In the previous reporting period, the research team demonstrated that phasic and tonic stimulation of CST axons produced morphological changes, e.g., axonal outgrowth, axon branching and outgrowth into the cervical motor pools. They explored whether stimulated axons increased synaptic connections onto a specific class of interneurons in the corticospinal circuit that is responsible for transmitting signals to motoneurons (termed Chx10 interneurons). They found that physically stimulated axons formed proportionately more contacts onto Chx10 interneurons than their non-stimulated neighbors in the same section. Increases in axonal sprouting and putative synaptic contacts imply strengthened CST connectivity, which should be detectable as changes in the motor map.

They also tested motor map responses by electrically stimulating the forelimb region in the primary motor cortex (M1) and recorded electromyograms (EMG)s from a forelimb muscle. They found that in physically stimulated animals, the stimulated region showed significantly lower current thresholds than a neighboring region of motor cortex that was not stimulated. Likewise, tonic stimulation also lowered the current threshold to evoke an EMG response at the stimulated site, but not in an analogous region in the opposite hemisphere. Thus, stimulating motor cortex physically or tonically for 10 days produces an increase in CST axonal outgrowth, an increase in contacts onto interneurons and a reduction in threshold of motor evoked responses.

Future Directions: In the next reporting period, the researchers will examine the targeting of CST axons with spinal cord neurons in tonically stimulated animals, add a drug only group, increase the numbers of animals to complete the initial phase of the study and compare the efficacy of phasic versus tonic activation of motor cortex using a neuronal activity-dependent marker, termed cFos.

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, 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 (8-+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 the third cervical vertebrae (C3) through the eighth cervical vertebrae (C8) or the sixth cervical vertebrae (C6) through the C8 segments of the spinal cord in their home-cage as well as during supervised skilled motor training.

   Progress towards specific aims: The researchers performed experiments in 10 rats and their findings indicated that cervical spinal cord structures stimulated with epidural stimulation are similar to lumbar spinal cord neural structures. Results also showed that rats trained with epidural stimulation (below the lesion) and motor training have greater training effects than motor training alone. Additionally, rats in the non-trained group do not show obvious signs of recovery in skilled function.

   Future Directions: The researchers will continue the above-mentioned experiment with more rats. They will also start their first set of implantation experiments in rats with stimulation electrodes across the lesion. They will also continue to collect electrophysiology data from their ongoing experiments and eliminate histology data from their experiments.

   Impact: This project will assist in developing a biofeedback 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: During the last 6 months, Dr. Ruven, has spent significant effort toward establishing a new, systematic approach to quantify protein distribution more accurately in axonal endings. She has applied this more in-depth quantitative analysis on growth cones and dystrophic endings of cultured dorsal root ganglia neurons.

First, she established a densitometric analysis method to assess the protein distribution in the whole axonal endings by using high-resolution confocal microscopy. This new analysis method, will be subsequently employed for investigating the distribution of additional proteins, labeled via immunocytochemistry, in the axonal endings.

Dr. Ruven also identified that there is no difference in the total concentration of ubiquitinated proteins in dystrophic endings formed on chondroitin sulfate proteoglycans (CSPGs), vs. both growth cones and bulbous endings that form on laminin. This is contrary to the research team's previous preliminary data and proves the point that the original results seen with single plane epifluorescence imaging likely arose from a shape/volume artifact. Her work additionally highlights that when analyzing axonal endings with different shapes and volumes, a more accurate, densitometric z-stack-based analysis is more appropriate than analysis based on epifluorescence imaging or even single-plane confocal images.

Future Directions: The research team will utilize additional markers in their analyses, extending beyond the UPS system, to investigate the protein degradation pathway more deeply as a potential therapeutic target. They will investigate whether the originally observed differences of these markers (especially markers of autophagy) reflect a true difference in protein concentration or are also reflective of different shape and total volumes of dystrophic endings vs. growth cones. In addition, Dr. Ruven is planning to also use cultured cortical neurons to investigate the effect of UPS manipulation on neurite outgrowth *in vitro*.

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: SCI 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: Progress towards determining functional and structural changes in the intracortical circuits and testing the role of intracortical circuits in skilled behavior after injury has been shown. The research team has performed preliminary experiments to establish calcium imaging of hindlimb intracortical neurons, used viral transduction during rehabilitation from SCI to label the hindlimb intracortical axons in reporter mice and two photon imaging of axons and boutons, and performed the optogenetic experiment to silence the forelimb cortex locally as well as the hindlimb intracortical circuits during recovery from SCI. The researchers have refined the infra-red LED-sensor system that controls their optical system in response to animal's reaching in their skilled forelimb behavioral task.

Future Directions: Ongoing experiments include structural imaging of hindlimb intracortical axons with labeling of forelimb intracortical neurons and optogenetic silencing of forelimb and hindlimb areas locally as well as intracortical circuits projecting from hindlimb to forelimb cortex.

Impact: The expected findings will inform combinatorial strategies that target cortical plasticity 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

   The contract has been terminated early because this RFA does not allow for substitutions of the named fellows. At this time, the SCIRB has remained steadfast in their desire to fund only Individual (non-transferrable) Pre- and Post-doctoral Fellowships; however, another alternative RFA for the funding of Fellowships may be agreed upon in the future that does not restrict the award to one specific individual.

PART/IDEA in SCI (Round 2)
IDEA Contract Term 6/1/18-5/31/20; PART Contract Term 6/1/18-5/31/21

Progress Reporting Period
6/1/19-11/30/19

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 Synaptojanin 1 in Functional Recovery After Spinal Cord Injury

   Introduction/Background: Apolipoprotiens 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 SCI 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: A comparison of functional recovery of time between mice carrying human ApoE3 and those carrying human ApoE4 was completed and showed reduced function in the ApoE4 mice. Studies of mechanisms responsible are under way. An investigation as to whether reducing levels of a protein called synaptojanin-1 (synj1) (which is increased in ApoE4 carriers) improves function after SCI will be performed over the next 6 months.

   Future Directions: Researchers have validated an animal model for studying the reasons for the poor outcomes in those with SCI who have the ApoE4 variant.

   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 fifth thoracic

vertebra (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 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: Potential participants have been screened for enrollment in the trial and eligible, consented 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 has shipped to the research pharmacist at the Bronx VA Medical Center.

Future Directions: Testing will begin on eligible participants in January 2020.

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 SCI. 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: The researchers recent cohort tested the efficacy of 4-AP on forepaw motor performance in rats with subacute SCI. A fourth cervical vertebra (C4) SCI was performed on the animals, they recovered for four (4) weeks, and then were trained to perform a skilled walking task and a food manipulation task over the following three (3) weeks. Once the animals showed signs of a stable and reproducible performance, they were given 4-AP doses (high and low doses) similar to therapeutic levels in people. The researchers' preliminary data suggest that the dose of 4-AP that achieves similar serum levels as human trials does not have an impact on motor performance in rats.
Future Directions: The researchers will plan to test the effect of multiple doses of 4-AP on motor learning after SCI. They also plan to inject a dye into the brains of animals

which will allow them to analyze which pathways might mediate recovery of behavior and verify that the injury in each of the animals are similar to one another by looking at the sections of tissue near the site of injury under the microscope. They plan to continue repeating these experiments in larger cohort of animals to confirm their findings.

Impact: If successful, the positive results will significantly impact the field of SCI because the results will provide a new therapeutic approach which is safe (4-AP is already FDA approved) and effective, and deeper insights about biology of recovery.

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 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 29 participants before and after 36 sessions of exoskeletal assisted walking (EAW).

   Researchers performed a pilot study of samples from six (6) participants (pre and post EAW). RNA-Seq libraries were created and 100 RNA-Seq reads were collected. Data was trimmed, and transcripts were normalized using the recommended default parameters. Following normalization, gene analysis was completed, and researchers found that some signaling pathway genes were significantly enriched. Of interest, with EAW, the pro-inflammatory toll-like receptor (TLR) signaling pathway was downregulated in all six (6) participants. Additional analysis of covariates and independent samples from more participants is needed to judge the significance and consistency of this data.

   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.

Publications: Bloom, O., Herman, P.E., & Spungen, A.M. (2019). Systemic Inflammation in Traumatic Spinal Cord Injury. *Experimental Neurology*, 325, 113143. doi: 10/1016/j.expneurol.2019.113143.

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 advanced bioinformatic analyses and identified that novel environmental sensors, including hypoxic inducible factors, are enriched in the genes displaying unique changes of DNA methylation during axon regeneration.

The research team has also optimized neuronal differentiation protocol to generate various types of neurons from human stem cells. The system will allow them how axon growth and regeneration is controlled.

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 and conduct functional analysis.

Impact: This research will advance the field of axon regeneration by providing new insights into molecular regulators of axon growth gene program. Their data will also have significant implication for stem cell-based regenerative therapy.

Publications: Wahane, S., Halawani, D., Zhou, X., & Zou, H. (2019). Epigenetic Regulation of Axon Regeneration and Glial Activation in Injury Responses. *Frontiers in Genetics,* (10), 640.

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: To avoid a potential immunogenic response, the researchers optimized a PEST (peptide sequence that is rich in proline, glutamic acid, serine, and threonine) degron on their bifunctional anti-synuclein-PEST intrabodies for human use. VH14-hPEST resulted in efficient degradation of endogenous alpha-synuclein in human induced pluripotent stem cell (iPSC) derived neurons. Additionally, a novel anti-synuclein bifunctional intrabody, N77K, can efficiently degrade both human and lamprey synuclein. Preliminary studies in the *in vivo* lamprey SCI model demonstrated that that N77K-mPEST improved behavior compared to empty vector controls.

   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.

In the last six months, researchers made progress characterizing the dosing and time course of different zinc chelating drugs *in vivo*. They have also made progress in chelating zinc following dorsal hemi section injury in mice and analyzed the animals' health following treatments. They made technical progress on optimizing zinc autometallographic (AMG) staining of sections to detect free zinc in animals. Zinc detection uses sodium selenite to create zinc selenium crystals that are silver enhanced with AMG development. The new protocol avoids IP injections since IP injections can negatively affect animal health.

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: New *in vivo* data shows retained viability, anisotropic positioning at the SCI site and neurite extension and synaptogenesis achieved with dramatically reduced therapeutic cell numbers. Researchers have also advanced in vitro data on their stem cell derived spinal motor neurons that includes RNA seq, bioinformatics, electrophysiology and transplantable neural circuitry and increased reproducibility of *in vivo* data.

Future Directions: By using a modified injection platform for the rat hemicontusion on a fourth-fifth cervical vertebrae (C4-C5) injury model they have achieved reproducible neural ribbon delivery. This allows the research team to better compare conditions that include shipped neural ribbons versus onsite generated, with and without chondroitinase ABC, and density of cells, as well as neural stem cells versus spinal motor neurons.

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 who 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 the ClinicalTrials.gov. The research study now is deposited in the clinical trials website and can be found at NCT03669302. Furthermore, they completed independent

experiments for control subjects, published one paper on the effects of transspinal stimulation on corticospinal excitability during treadmill walking in healthy control subjects, and completed analysis of transspinal conditioning effects on flexion reflex. Furthermore, three (3) individuals with SCI in Group A (locomotor training only-control group) have completed training and assessments. Additionally, two (2) individuals with SCI in Group B (30 Hz transspinal stimulation) have been scheduled to participate in 2020, one (1) in Group C (0.3 Hz transspinal stimulation) completed training and assessments, and two (2) have been scheduled for 2020.

Future Directions: The PI's plans for the next six (6) months are to continue establishing eligibility and enrollment of interested participants along with the Co-PI at the James J. Peters VA Medical Center, to increase the number of enrolled participants 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.

Publications: Pulverenti, T.S., Islam, M.A., Alsalman, O., Murray, L.M., Harel, N.Y., & Knikou, M. Transspinal Stimulation Decreases Corticospinal Excitability and Alters the Function of Spinal Locomotor Networks. (2019). *Journal of Neurophysiology*, 122(6), 2331-2343.

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 improve motor outcomes substantially 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 have developed the pellet reaching

task that the rats will perform under robotic control. They have a working robot with effective force cancelling characteristics that operates in separate modes for assisting, resisting, and perturbing movements in a controlled manner. The results show that they can now obtain performance data for animals reaching to different locations in the workspace.

Future Directions: Since the researchers completed the 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 obtain quantitative baseline performance data and 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 (4AP), 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. Moreover, most other pharmacological therapies that have been investigated need to be administered within three hours post-injury, which creates enormous challenges in clinical trial design and implementation.

Progress Towards Specific Aims: The researchers confirmed that appropriate serum concentration levels of 4AP can be achieved with implantation of continuous release pumps. Secondly, researchers confirmed interesting changes at three (3) days post-injury (after treatment initiation but prior to behavioral improvements) in the patterns of gene expression associated with 4AP treatment.

Future Directions: The next stage of this work, in respect to analysis of serum levels of 4AP, will focus on completion of experiments on pump-mediated delivery. They will extend their analyses of gene expression and inflammation.

Impact: This research will provide a detailed investigation of the potential of their approach as a new treatment to decrease damage in acute SCI and bringing their discoveries forward to clinical trials.

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 completed histological analyses to define the spatio-temporal expression pattern of ZEB2 following SCI. They have also started completing 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 generated genetically engineered mice that will lack ZEB2 specifically in astrocytes to show that ZEB2 is a key regulator of astrogliosis and locomotor recovery.

Future Directions: They expect to complete their analysis of in vitro astrocyte-culture studies to probe Zeb2os regulation of ZEB2 protein levels following injury. They also expect to have complete studies testing whether glial scar formation is disrupted in mice lacking one or both genetic copies of the ZEB2 gene. They expect to submit a manuscript to describe their findings.

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 IB) 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 completed the experiments required to isolate this genetic material and once all samples are collected and the library preparation finished, the core facility will perform next generation sequencing on an Illumina HiSeq 4000. Researchers also used a nanostring technology to assess gene expression patterns in the injured nerve, which identified a strong activation of the complement system in the regenerating sciatic nerve. Their mechanistic studies are ongoing.

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. Researchers are currently breeding double B-RAF GOF PTEN LOF mice to assess axonal localization of EGFPL10 in CST axons after SCI. Since this requires several

rounds of crossing, mouse breeding and genotyping are still ongoing. Also, based on their pilot experiments to characterize the translatomes of regeneration-competent CST axons, they need to use 50 genetically modified mice for one mRNA TRAP for each group.

Future Directions: The research team will generate sufficient mice to carry out the planned TRAP experiment as planned. Dr. Fabricio Nicola participated in the animal breeding and will received training in carrying out the TRAP study as well as RNA seq data analysis. Dr. Nicola's work on this project has concluded; however, Dr. Hao Feng will take over these experiments and verify the results using in situ hybridization to analyze the expression of B-RAF regulated transcripts in corticospinal neurons and CST axons before and after SCI.

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 SCI (Round 2)
Contract Term 5/1/18-4/30/22

Progress Reporting Period
5/1/19-10/31/19

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 moved forward with usability testing, mapping tool comparison, hardware testing, and software development. They completed the first phase and started the second phase of usability testing, which uses a five-point Likert scale to assess clinicians' background knowledge, responses to training materials and methods, and specific competencies, it also gathers their feedback on the current state of the system.

   Researchers understand the importance of correct electrode placement throughout training and have compared several mapping techniques for prevision and accuracy. These include measuring boney landmarks, using ultraviolet medical markers, sketches, and photographs; and making templates with transparent sheeting. Once these methods have been vetted, the research team will introduce them to the clinicians for review and comparison.

   Researchers are exploring the use of multi-electrode arrays and automatic site selection algorithms to reduce the training and time requirements of selecting nerve stimulation and EMG recording sites.

   Researchers have bench-tested a Proportional Integral Derivative (PID) controller with four (4) subjects that's used for automatically controlling reflex elicitation. They are now comparing the PID controller to an automated Heuristic Controller to determine which achieves better control of M-wave size.

   Future Directions: Researchers will complete the second phase of usability testing, compare new algorithms for controlling M-wave size, continue to advance the reflex conditioning protocol, and develop and test multi-grid arrays appropriate for optimizing H-reflex elicitation and recording.

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: Thompson, A.K., Pouliot, B.A., Wolpaw, J.R., & Gill, C.R. (2019). *Can Combining Reflex Conditioning and Motor Practice Enhance Beneficial Plasticity in People with Chronic Incomplete SCI?* Society for Neuroscience, Neuroscience 2019, Chicago, Illinois.

Wolpaw J.R., Chen, L. Yang, X, Chen Y, & Chen X.Y. (2019). *Combining H-reflex Conditioning and Locomotor Training Enhances Locomotor Recovery in Rates with Incomplete Spinal Cord Injury.* Society for Neuroscience, Neuroscience 2019, Chicago, Illinois.

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. Researchers discovered that treatment with a repurposed drug in the acute/sub-acute injury period can 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: The general focus of their research is treatment with the potassium channel blocker 4-aminopyridine (4AP), starting 18-24 hours after injury, and their studies thus far indicate that effects of 4AP are sufficiently robust to jusitify planned progress to clinical trials.

The researchers analyzed the effects of age on the response to 4AP treatment of acute traumatic SCI because most experiments in this field are conducted on rats that are 8-10 weeks old (equivalent to young teenage humans in terms of spinal cord structure and function). They therefore have conducted contusion injuries on 1-year old rats (their response to injury is more severe than seen in 8–10-week-old rats), which are thought to be developmentally more equivalent to 30-year-old humans.

Secondly, the researchers combined physical therapy with 4AP treatment to test whether this would unexpectedly decrease the benefits of 4AP.

Future Directions: The researchers will complete analyses of the combination of 4AP treatment with treadmill training as well as experimental physical therapy. In addition, they will continue the analyses of effects of 4AP on the recovery of 1-year old rats.

Impact: The researchers implemented an analytical approach that indicates 4AP treatment is robust enough to be useful across a range of injury variability greater than most studies and allows early integration with physical therapy. They are starting treatment at a more clinically useful time point than is the case for most experimental SCI therapies. Moreover, this analytical approach offers a general strategy for examining the robustness of experimental therapies.

Individual Predoctoral/Postdoctoral Fellowships (Round 2)
Contract Term 9/1/17-8/31/20

Progress Reporting Period
3/1/19-8/31/19

9 Awards (2 concluded), 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 initiated subject enrollment in January 2018 and recruited eight (8) participants, which includes four (4) able-bodied participants and four (4) participants with SCI; however, two (2) SCI participants were excluded for not meeting the full inclusion criteria or passing the screen session. Five (5) participants completed the experiment and preliminary data showed different responses in able-bodied and SCI participants toward different stimulation intervention. 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: This is the final report due to the policy of the SCIRB fellowship award, Dr. Wu has accepted a NIH grant and is terminating the contract one year early. This fellowship award provided an excellent mentorship between the researchers on every aspect to build upon the ability of Dr. Wu to become an independent researcher. Therefore, Dr. Harel assisted Dr. Wu to have his first grant from NIH as a principal investigator.

   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.

Publications: Wu, YK., Saeed, S., Limonta, J., Bailey, E., Maher, M., & Harel, N. Proceedings #52: Development of Surface EMG- triggered Closed Loop Stimulation for Individuals with Spinal Cord Injury. (2019). *Brain Stimulation. Basic, Translational, and Clinical Research in Neuromodulation,* 12 (2), e125–e126.

Wu, YK., Saeed, S., Maher, M., Bailey, E., LiMonta, J., Hussain, …& Harel, N. Abstract #127: Non-Invasive Cervical Root Stimulation to Facilitate Corticospinal Transmission. (2019). *Brain Stimulation. Basic, Translational, and Clinical Research in Neuromodulation,* 12 (2), e44.

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 two (2) years 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 12 healthy participants to analyze the effects of different levels of BWS on gait stability. 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 three (3) patients with SCI and three (3) healthy matched controls. Results showed that the participants with SCI were able to adapt the recovery reaction for different amplitude of the perturbations but did not show aftereffects on gait stability.

Five (5) VR platforms have been developed: an infinite walker, a software to deliver pseudo-random oscillations of the visual field, a virtual floor maze test developer, a catch and throwing game for dual task training, and a VR environment that provides visual and audio biofeedback on foot placement to induce changes to spatial parameters of gait.

Future Directions: This is a final report because Dr. Martelli was hired full-time at another University. Scientific papers based on the results of the studies will be revised and submitted.

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 in patients with SCI aimed at reducing the risk of falling. This award has equipped Dr. Martelli with the scientific training and specific knowledge in SCI to pursue an independent career in this field. He has analyzed and gained 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.

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

Martelli, D., Prado, A., Xia, B., Verghese J., & Agrawal, S.K. (2019). Development of a Virtual Floor Maze Test – Effects of Distal Visual Cues and Correlations with Executive Function in Healthy Adults. *IEEE Transactions on Neural Systems and Rehabilitation Engineering,* 27(10), 2229-2236.

3. Cornell University

Chris B. Schaffer, Ph.D., Yu-Ting Cheng, 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 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 can 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 (Df/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. They are continuing to work on optimizing their capability to visualize clean, unbiased calcium signals from the spinal cord cells in awake, moving animals by trying different transgenic line, virus preparation and signal gains from the microscope end. At the same time, they will carefully assess the stabilization of the treadmill and the spinal window clamping system to reduce motion artifact during locomotion as much as possible.
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.

Presentations: Cheng, Y.T., Denotta, S.L., Jones, J.S., Rivera, D.A., Hu, S., Raikin, J., …& Schaffer, C.B. (2019). *In Vivo Three-photon Excited Fluorescence Imaging in the Spinal Cord of Awake, Locomoting Mice*. Society for Neuroscience, Neuroscience 2019, Chicago, Illinois.

Publications: Cheng, Y.T., Lett, K. M, & Schaffer, C.B. (2019). Surgical Preparations, Labeling Strategies, and Optical Techniques for Cell-Resolved, *In Vivo* Imaging in the Mouse Spinal Cord. *Experimental Neurology,* 318: 192-204.

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 joined 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 enabled researchers to determine biomarkers of spontaneous recovery in adults with SCI. The experience provided 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 also in parallel, initiated 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 provided 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 was trained in immunology and lab techniques relevant to the project. He was introduced to the clinical/demographic data and clinical/functional outcome measures used in SCI. He received formal and practical instruction to analyze samples from this study and performed measurements of samples from this study. He served as a mentor to undergraduate interns on lab techniques and topics related to SCI research.

Future Directions: This is the final report because Dr. Deckert has accepted a faculty position at Gonzaga University in Spokane, Washington. The fellow's career goals, and training development plan has allowed Dr. Deckert to advance his skills and knowledge which supported his faculty appointment. The projects in which Dr. Deckert participated in are still ongoing in the lab.

Impact: This training award equipped Dr. Deckert with the scientific training and specific knowledge in SCI to pursue an independent career as a faculty member in biomedical

science. This training award created 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 SCI 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.

   In this reporting period, they observed that RGFP966 treatment improved microglial phagocytosis, suggesting it contributes to increased debris clearance and thus reduced inflammasome area, eventually leading to improved axon regeneration at the lesion site.

   Further, they examined a combinatorial treatment of RGFP966 and intrathecal injection of AAV-BMP4 to improve axon regeneration after SCI. However, perhaps due to the low infectivity rates, this combinatorial treatment did not result in improved motor recovery, neither did it enhance repair. Taking this into account, the research team decided not to pursue this line of investigation any further and decided to focus their efforts on the HDAC3 inhibition alone.

   Dr. Wahane is currently analyzing the transcriptomes of 24 clusters of cells from the preliminary analysis of single-cell RNA sequencing data of CNS cell types with either vehicle or RGFP966 treatment.

   Future Directions: It’s clear to the research team that HDAC3 inhibition improves motor recovery and injury resolution. Identifying the underlying molecular basis of microglial transcript changes will help uncover target genes downstream of HDAC3, making RGFP966 a potential therapeutic drug for SCI repair.

   Impact: 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 unaffecting normal physiological

functions of microglia and macrophages. Further tasks that Dr. Wahane has outlined for this project will shed light on molecular mechanisms that are affected by loss of HDAC3 and take researchers closer in defining changing gene signatures under HDAC3 regulation.

Publications: Wahane, S., Halawani, D., Zhou, X., & Zou, H. (2019). Epigenetic regulation of axon regeneration and glial activation in injury responses. *Frontiers in Genetics,* 10.

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: 17β-estradiol (commonly known as estrogen and referred to as E2) is a steroidal hormone that differentially affects all cells in the CNS. Estrogen is potently 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: Over the duration of this fellowship, Dr. D'Amato 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 for unprecedented timescales on the order of 1-10 years. Further, he has demonstrated that the biomaterial provides contact guidance for neurite extension *in vitro*. Lastly, he demonstrated that E2 released from the biomaterial is neurotrophic and neuroprotective *in vitro,* and significantly rescues neuronal death compared to biomaterial free controls.

Future Directions: The contract ended in December 2018, since Dr. D'Amato graduated from RPI's Ph.D. program. In future experiments, the biomaterial will be employed in animal models of SCI to demonstrate translational efficacy.

Impact: This study led to the development of a new biomaterial guidance scaffold for nervous tissue regeneration that delivers a neuroprotective and potentially regeneration-promoting poly(pro-drug) of E2 on the timescale of multiple years. The various neural culture assays demonstrated the impact of the biomaterial as an entirely new approach for biomaterial drug delivery for neural engineering applications.

Publications: D'Amato, A.R., Puhl, D.L., Ziemba, A.M., Johnson, C.D.L, Doedee, J., Bao, J, & Gilbert, R.J. (2019). Exploring the Effects of Electrospun Fiber Surface Nanotopography on Neurite Outgrowth and Branching in Sensory Neuron Cultures. *PLoS One*, *14* (2).

7. 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 and they recorded muscle responses in awake and anesthetized animals with chronic EMG electrode implantation. They performed troubleshooting on basal forebrain cholinergic neurons with a targeted toxin (saporin) and measured the effect on motor learning, overall health, and various kinds of behavior. 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, dHβE and Atropine). Additionally, researchers are breeding transgenic mice (Thy1-ChR2, α7 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 α7 conditional knockout mice, use saporin to investigate the effect of basal forebrain cholinergic neuron deletion on rehabilitation after SCI, record the changes in neuronal morphology using advanced *in vivo* imaging, 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. These findings will provide new opportunities for pharmacological modulation and for combinatorial treatments that support the recovery of corticospinal circuit function after injury.

PART/IDEA in SCI (Round 1)
IDEA Contract Term 1/1/17-12/31/18*; PART Contract Term 1/1/17-12/31/19

Progress Reporting Periods
7/1/18-12/31/18[1]
1/1/19-6/31/19[2]
7/1/19-12/31/19[3]

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

1. **Bronx Veterans Medical Research Foundation, James J. Peters VA Medical Center[1]**
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. The findings of this study suggest a positive role for T cells in bone loss associated with SCI. This new knowledge could help develop new drugs that would protect against bone loss, thus reducing the risk of bone fracture in SCI patients.

Progress Towards Specific Aims: In the final reporting period, researchers completed the proposed experiments of Aims 1 and 2. They carefully assessed changes in bone structure and histology as well as in bone turnover markers during different stages of SCI. This included experiments that were performed on T cell deficient and T cell reconstituted animals and underwent control or SCI surgery for one, two, and five weeks. Researchers confirmed their findings by comparing bone structure of normal (WT) and T cell deficient animals after control and SCI surgery. Researchers also evaluated the changes in T cell subsets, secretion of cytokines, and metabolic changes in osteoblast/osteocyte cells. Their results strongly suggest a favorable role for T cells in modulating bone loss during SCI.

Future Directions: This is the final report, the findings that emerged from this project lay the foundation for future investigations focusing on the interaction between the immune system and the skeleton during SCI. Experiments evaluating the effect of modulators of the immune function on SCI skeleton will be of high interest. The PI, Dr. Hesham Tawfeek, and his team are currently reviewing all data form years one and two and manuscripts are expected to be submitted and published in the next months. Research results from this project will be disseminated and made easily accessible to the research community and public at large. Dr. Tawfeek and his team plans to continue and expand this investigation to further improve their understanding of bone loss in SCI individuals.

Impact: The need for alternative therapeutic interventions to reduce bone loss after SCI has been urgent because of the uncertainty surrounding the use of anti-resorptive drugs

and other rehabilitation and intervention measures to prevent severe osteoporosis after SCI. The results of this project provide new knowledge and evidence that supports a positive role for T cells in SCI-induced bone loss. The research team's data highlight the importance of promoting the immune function as a new strategy to reduce bone loss with SCI patients especially in those with impaired immune function such as high-level SCI injury and immunocompromised patients. Thus, Dr. Hesham and his team anticipate that their studies will lead to the discovery of new therapeutic strategies for patients with SCI.

2. **Cornell University[2]**
Chris B. Schaffer, Ph.D.
IDEA: $350,876* No Cost Extension Approved Through 12/31/19
Imaging Neural Activity in the Spinal Cord of Awake Mice After Spinal Cord Injury

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

Progress Towards Specific Aims: Dr. Schaffer continues 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 upstream SCI. Technical improvements were made and they measured calcium transients from neural activity with excellent signal to noise from spinal cord neurons at depths of more than 300 μm.

Future Directions: Now that Dr. Schaeffer and his team have achieved good signal to noise imaging from deep-lying spinal cord neurons, they will start training cohorts of animals for awake imaging and begin experiments to follow the activity of the deep-lying neurons that play a role in coordinating limb motion.

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 SCI and correlate those changes with changes in hindlimb motion.

Publications: Cheng, Y.T., Lett, K.M., & Schaffer, C.B. (2019). Surgical Preparations, Labeling Strategies, and Optical Techniques for Cell-Resolved, *In Vivo* Imaging in the Mouse Spinal Cord. *Experimental Neurology*, 318, 192-204.

3. **Research Corporation of Long Island, Inc., Northport VA Medical Center[3]**
Victor L. Arvanian, Ph.D., D.Sci
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 Neuralized Pluripotent Stem Cells (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. Therefore, they have begun managing, maintaining, and using similar stem cells at their facility through consultation with Dr. Nurit Ballas, who is an expert in stem cell Research and Stony Brook University.

Based on results of recent experiments, conducted in the laboratories of Victor L. Arvanian, Ph.D., D.Sci and Dr. Horner, they hypothesize that spinal electro-magnetic stimulation (sEMS) and exercise combined with transplantation of NiPSCs will induce improvement of function in chronic SCI.

Progress Towards Specific Aims: During reporting period, researchers have optimized the procedure of NPCs transplantation to ensure the best survival rate following contusion SCI and began the experiment in which adult rats received NPC treatment combined with spinal electro-magnetic stimulation and exercise. They expect that transplantation of NPCs will partially compensate for the loss of neurons after SCI. While administration of sEMS will guide the growing projections to establish synaptic contacts with host motoneurons, and exercise is applied to improve plasticity, they anticipate that combining these three treatments will improve function after chronic SCI.

Future Directions: Researchers have begun an experiment to examine effects of combinatorial treatment combined with sEMS and exercise with expectation to see recovery of function They will continue their pilot study and examine effects of sEMS in non-injured human subjects. Researchers will examine cellular and molecular mechanisms underlying modulatory effects of sEMS on H-reflex in naïve and SCI animals and begin to examine whether sEMS will induce modulation of H-reflex in SCI humans.

Impact: The triple combination treatment of NPCs, 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., Horner, P.J, Krencik, R.C., Liang L., Ballas, N., Lasek, K.., …& Petrosyan, H. (2019). *Delayed implantation of neural stem cells (NSCs) combined with spinal electro-magnetic stimulation (SEMS) and exercise training in chronic spinal cord injured rats*. Society for Neuroscience, Neuroscience 2019, Chicago, Illinois.

Liang, L., Petrosyan, H., Sisto, S.A., & Arvanian, V.L. (2019). *Differential effects of Low Frequency (0.2 Hz) and High Frequency (20Hz) spinal electromagnetic stimulation in modulating parameters of H-reflex responses in chronic spinal cord injured rats.* Society for Neuroscience, Neuroscience 2019, Chicago, Illinois.

Petrosyan, H., Fahmy, M., Tesfa, A., Liang, L., Sisto S.A., & Arvanian V.L. (2019). *Spinal electromagnetic stimulation results in immediate pain reduction and induces long-lasting functional improvements in patients with chronic low back pain (LBP). A pilot study.* Society for Neuroscience, Neuroscience 2019, Chicago, Illinois.

4. **Research Foundation for SUNY Stony Brook[3]**
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 used sEMS with eight (8) non-injured adults. The researchers have completed collecting data using the same sEMS protocol of 3 sessions to compare data between SCI adults and non-injured adults. The gathered data will allow for comparison as they investigate outcomes in SCI after five (5) weeks of sEMS only versus a combination of sEMS and LT.

Future Directions: One trainer was hired for the LT component and seven (7) additional trainers will be hired and attend a national training in 2020. Recruiting material were sent to two (2) local facilities and three (3) support groups with SCI patients for additional participants. After completion, statistical analysis will be done within and between the SCI group and SCI+LT group.

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

5. **Research Foundation for SUNY Stony Brook[2]**
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 and the therapeutic potential of rAIH exposure 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 been completed. 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. Hence, the research team identified optimal O_2 fractional concentration for use in evaluating the therapeutic potential of repeated bouts of AIH on LUT function and respiratory motor activities following SCI.
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Future Directions: The researchers will continue to 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 SC and finalize a draft manuscript for publication.

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.

Presentations: Collins, W.F. III & Solomon, I.C. (2019). *Acute intermittent hypoxia-induced amelioration of bladder hyperactivity: a non-invasive therapeutic intervention to improve bladder function in SCI.* Gordon Research Conference, Central Nervous System Injury and Repair, Spinal Cord Injury and Repair: From Molecules to Function, Waterville Valley, New Hampshire.

Solomon, I.C. & Collins, W.F. III (2019). *Characteristics of hypoxia-induced reflex micturition events (rME) in urethane anesthetized adult female rats with SCI*. Gordon Research Conference, Central Nervous System Injury and Repair, Spinal Cord Injury and Repair: From Molecules to Function, Waterville Valley, New Hampshire.

Solomon, I.C. & Collins W.F. III. (2019). *Impact of severity and duration of acute hypoxic exposure on lower urinary tract (LUT) function in urethane-anesthetized adult female rats.* Society for Neuroscience, Neuroscience 2019, Chicago, Illinois.

6. **Research Foundation for SUNY, University at Albany**[3]
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 protein coding 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. One inflammation related gene, SOCS3, was found to be upregulated in axons, but not cell bodies of injured neurons, suggesting it has pro-regenerative effects in frogs (unlike in mammals where it inhibits axon regeneration). Also, expression of an enzyme that

alters hnRNP K was upregulated in successful CNS axon regeneration, suggesting it serves as a point of convergence for transcriptional and post-transcriptional changes in gene expression during CNS injury.

Future Directions: This is the final report, and a manuscript is under review. Future project efforts will be aimed at further exploring SOCS3, and the role of genes involved in the epigenetic control of gene expression during successful CNS axon regeneration.

Impact: Successful completion of these objectives provided new information which could ultimately lead to new therapies for treating SCI in humans. Such information is key to developing rational approaches to treating SCI and other forms of CNS trauma in humans.

Presentations: Szaro, B.G. (2019). *Identifying Core Genes Involved in Successful Vertebrate CNS Axon Regeneration.* Invited lecture at the Animal Biotechnology & Biomedical Sciences Seminar Series, University of Massachusetts, Amherst, Massachusetts.

Publications: Priscilla, R. & Szaro, B.G. (2019). Comparisons of SOCS mRNA and Protein Levels in Xenopus Provide Insights into Optic Nerve Regenerative Success. *Brain Research*, 1704: 150-160.

7. Research Foundation of CUNY, College of Staten Island[3]
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 (PAS) 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 continue transspinal-transcortical PAS and Lokomat training sessions and experiments in people with motor incomplete SCI, motor complete SCI, and at rest in healthy control subjects. The team has delivered close to 400 sessions and completed at least 85 experiments in a total of 21 individuals with SCI thus far.

Future Directions: The principal investigator will complete transspinal-transcortical PAS and Lokomat training in additional participants with SCI, train staff/personnel for neurophysiological data analysis, submit research for publication, develop manuscripts, continue reduction and statistical analysis of acquired data, and present preliminary findings at National and/or International conferences.

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.

8. **The Trustees of Columbia University in the City of New York**[1]
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 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 their results suggest that Pum2 prevents the axonal synthesis of proteins that inhibit axon growth and regeneration.

Future Directions: This is the final progress report, and the researchers are working on experiments for a resubmission of a manuscript to report their findings. The project has generated the experimental basis for another ongoing project in the laboratory, in which they focus on the impact of Pum1/Pum2 on synapse formation and maintenance.

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.

9. **Weill Medical College of Cornell University**[3]
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: 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^+, and a variant of NR called dihydro-NR (NRH) in treatment of SCI, using a rat thoracic contusion model of SCI.

Progress Towards Specific Aims: The research in Aim 1 was largely focused on development of synthetic methods, biodistribution and pharmacologic characterization of NR and NRH in unlabeled as well as isotopically labeled forms. It was determined that NR and NRH could provide dose-dependent increases in NAD concentrations in uninjured as well as injured spinal cords. Their quantitative assessment of overground locomotion showed improvement in NR-treated animals. NR-treated animals also showed increased hindlimb contact intensity and print area indicating improved hindlimb locomotor strength. The more potent NRH was found to have unexpected toxicity attributed to rapid metabolism and possible rapid energy depletion.

Future Directions: With improvements in NRH administration, such as IV dosing, toxicity of NRH could be overcome and NRH, due to enhanced potency versus NR could be a viable compound for post-injury studies in SCI.

Impact: The likelihood of development of NR or NRH as a possible treatment for SCI is improved by the described studies, with modification in delivery of NRH likely to remove toxicity.

Publications: Yang, Y., Mohammed, F.S., Zhang, Z., & Sauve, A.A. (2019). Dihydronicotinamide Riboside is a Potent NAD^+ Concentration Enhancer *In Vitro* and *In Vivo*. *The Journal of Biological Chemistry,* 294(23), 9295-9307.

10. Winifred Masterson Burke Medical Research Institute[1]
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. Chronic SCI 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. 27 participants were enrolled to participate in the study. There are only 13 complete data sets, which are comprised of complete baseline, post, and follow-up evaluations.

Future Directions: This is the final report for this study, and a larger grant exploring the optimal dose of the training, the optimal training timing, and exploring what patients benefit the most, will be submitted in the future.

Impact: The combination of anodal tDCS with hand robotic training in people with chronic SCI has shown promising results in improving functional outcomes when compared with the sham stimulation group. This opens the door to use this approach in other neurological disorders that have an impairment in the corticospinal tract.

11. Winifred Masterson Burke Medical Research Institute[1]

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. The research team's objective is to develop methods to detect newly formed synapses in the post-injury cord. They hypothesize that synaptic connections in the cord can be directly visualized using a novel fusion protein as a tracer to identify newly formed synapses between sprouting or regenerating corticospinal tract (CST) axons and propriospinal interneurons in the injured spinal cord. 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: This is the final report for this contract. 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 after an experimental SCI. 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 are collaborating with Dr. Chris Schaffer's laboratory at Cornell University to establish a protocol for 3-photon excitatory fluorescent *in vivo* live imaging of axons and interneurons in the spinal cord. The spinal gray matter that lies beneath a sheath of dense white matter makes live imaging far more challenging than imaging at similar depths in the brain. Thus, the inclusion of Dr. Schaffer's research team is essential since they are one of the premier labs in the field of multiphoton imaging of the CNS.

Future Directions: Experiments are ongoing to demonstrate *de novo* synaptogenesis following an eighth thoracic vertebra hemisection injury. The imaging experiments are also ongoing. A manuscript reporting CST regeneration enabled by B-RAF signaling will be submitted for publication.

Impact: At the conclusion of this study, the research team will have obtained, for the first time, an overview of the rewiring of CST connectivity in the injured mouse spinal cord. This knowledge will be essential for the development of novel therapeutic approaches to facilitate the axon and circuit regeneration, and may be especially useful for the evaluation of training/rehabilitation efforts designed to modulate circuit formation in the spinal cord after SCI.

Institutional Support for SCI Research (Round 6)
Contract Term 3/1/17-2/28/22

Progress Reporting Period
3/1/19-8/31/19

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

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

   Funding was used for the development and validation of protocols to test the hypothesis that H-reflex operant conditioning can improve locomotion in people with chronic incomplete SCI and can also improve other skills that use the same spinal cord circuitry. This non-invasive therapy can complement other therapies and enhance functional recovery for people with SCI and other neuromuscular disorders.

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

   Funding was used to support personnel to perform preliminary bone histological and histomorphometric analyses to expand on studies to include additional readouts for organ function recovery in SCI rats following treatment with FL2-siRNA to maintain bone health and prevent osteoporosis.

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

   Funding supported personnel on current proof-of-concept studies such as determining the precision of dual energy x-ray absorptiometry scans and bioimpedance spectroscopy in SCI and able-bodied individuals. The research team feels strongly that the preliminary work will lead to several grant applications.

4. **Columbia University**

   Funding supported Sunil Agrawal, Ph.D. in the assembly, testing, and research of the two pelvic support systems to advance current rehabilitation methods and develop innovative solutions to reduce risk of falls for SCI patients.

5. **Cornell University**

   Currently this grant partially funds supplies necessary for SCI experiments with mice, specifically for imaging aimed at understanding the normal function of spinal cord circuits in coordinating limb motion.

6. **Feinstein Institute for Medical Research**

   Funding was used to partially support the research team's personnel to increase their knowledge of biological processes that influence neurological recovery and overall health in persons with SCI. The data that they will collect with this support is enabling them to fill gaps in knowledge and advance the ability to predict and promote physical recovery and overall health in persons with SCI. Also, data collected within these

projects has been used to provide support of feasibility and scientific rationale for larger grant applications in SCI research.

Presentations: Stein, A., Schwab, J., Bloom, O., Popovich, P., & Krisa, L. (2019). *Translational Studies of Immune System Dysfunction after SCI.* 2019 SCI Summit, Annual Scientific Meeting, American Spinal Injury Association. Waikiki, Hawaii.

Bloom, O. (2019). *Biomarkers of Spontaneous Recovery from Traumatic SCI*, Gordon Research Conference, Central Nervous System Injury and Repair, Spinal Cord Injury and Repair: From Molecules to Function, Waterville Valley, New Hampshire.

Publications: Pekmezaris, R., Kozikowski, A., Pascarelli, B., Handrakis, J., Chory, A., Griffin, D., & Bloom, O. (2019). Participant-Reported Priorities and Preferences for Developing a Home-Based Physical Activity Telemonitoring Program for Persons with Tetraplegia: A Qualitative Analysis. *Spinal Cord Series and Cases*, 48, 2-7.

7. Health Research, Incorporated

Funding continued to support Dr. Yu Wang in his work to validate H-reflex conditioning with locomotor training as a therapeutic method for improving recovery of useful motor function after SCI. The results from this study should lead to future applications for funding of animal and human studies exploring this combined therapy to enhance functional recovery after SCI.

Presentations: Wang, Y., Chen, L., Wolpaw, J.R., Chen X.Y. (2019). *Impact of Globus Pallidus or Sensorimotor Cortex Ablation on Spinal Motoneuron GAD67 and KCC2 and on Ventral Horn Interneuron GLUR2/3: Initial results.* Society for Neuroscience, Neuroscience 2019, Chicago, Illinois.

Wolpaw, J.R, Chen, L., Yang, X.X., Wang, Y., Chen, Y., Chen, X.Y. (2019). Combining H-reflex Conditioning and Locomotor Training Increases the Rate and Magnitude of H-Reflex Change and Enhances Locomotor Recovery in Rats with Incomplete Spinal Cord Injury. Society for Neuroscience, Neuroscience 2019, Chicago, Illinois.

8. Icahn School of Medicine at Mount Sinai

Funding supported the following investigators to conduct SCI-focused research, Principal Investigator, Hongyan Zou, MD, Ph.D., Co-Principal Investigator, Roland Friedel, Ph.D., and Associate Scientist, Yong Huang, M.D., Ph.D. The research team's laboratories generated new pilot data on novel function Plexin-B2 signaling in mediating innate immune response after SCI. These data will form the foundation for future grant applications. A manuscript is currently under review in *Nature Neuroscience* made possible with the support of this funding.

9. New York University

Funding supported the extension of human pluripotent stem cell system in the lab by Research Assistant, Asha Babu.

The results obtained from this funding were used as preliminary data to develop better human motor neuron differentiation strategies.

Publications: An, D., Fujiki, R., Iannitelli, D., Smerdon, J.W., Maity, S., Rose, M.F.,…& Mazzoni, E.O. (2019). Stem Cell-Derived Cranial and Spinal Motor Neurons Reveal Proteostatic Differences Between ALS Resistant and Sensitive Motor Neurons. *Elife*, 8: e44423.

10. Regenerative Research Foundation

Funding was used to support Liz Fisher, Ph.D., for manual annotation of data and cell types, sample tracking and manuscript preparation; Nathan Boles, Ph.D., for data analysis and advisement on cell markers; and Thomas Kiel, Ph.D., for data analysis and equipment management/maintenance.

During this period, the research team's efforts have been focused on manual annotation of the clustered data, assigning clusters and subgroups to specific known cell-type classifications and annotations, and cleaning the data. The gene lists identified after refining the data have been much more amenable to the identification of cell-type as well as network analysis. The data has served as a platform for building collaborations and continues to contribute to other grant applications related to SCI and neurodegeneration.

11. Rensselaer Polytechnic Institute

Funding was used to support student stipends and develop pilot studies of: interleukin-4 (IL-4) releasing biomaterials; poly-estrogen biomaterials; poly (pro-drug) coatings to improve intracortical electrode biocompatibility; and a biomaterial educational program.

Publications: Ziemba, A.M., Lane, K.P., Balouch, B., D'Amato, A.R., Totsingan, F., Gross, R.A., & Gilbert, R.J. (2019). Lactonic Sophorolipid Increases Surface Wettability of poly-L-lactic Acid Electrospun Fibers. *American Chemical Society (ACS) Applied BioMaterials,* 2 (8): 3153-3158.

D'Amato, A.R., Puhl, D.L., Ellman, S.A.T., Balouch, B., Gilbert R.J., & Palermo E.F. (2019). Vastly Extended Drug Release from Poly(pro-17β-Estradiol) Materials Facilitates *In Vitro* Neurotrophism and Neuroprotection. *Nature Communications,*10(1): 4830.

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

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

- Isoflurane systems with accessories
- Pulse stimulator
- Battery backup systems for confocal microscope
- Wireless EMG system for electrophysiology recordings

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. Specifically, results of electrophysiological recordings revealed that combined treatment with AAV-NG2Ab and AAV-NT3 improve excitability of surviving axons in damaged spinal cord. This research provides a strong foundation for translation results of their animal experiments into humans.

The research team has begun using electro-magnetic stimulation (EMS) in human studies at Northport VA Medical Center (collaboration with Dr. Fahmy) and Stony Brook University (Collaboration with Dr. Sisto) and results are being analyzed.

Presentations: Arvanian, V.L., Horner, P.J., Liang, L., Ballas, N., Lasek, K., Gumudaveli S., & Alessi, V. *Effects of Delayed Implantation of Neuralized-Pluripotent Stem Cells (NPSCs) Combined with Electro-Magnetic Stimulation and Exercise Training in Chronic Spinal Cord Injured Rats*. Society for Neuroscience, Neuroscience 2019, Chicago, Illinois.

Liang, L., Petrosyan, H., Sisto, S.A., & Arvanian, V.L. *Differential Effects of Low Frequency (0.2 Hz) and High Frequency (20 Hz) Spinal Electromagnetic Stimulation in Modulating Parameters of H-Reflex Responses in Chronic Spinal Cord Injured Rats.* Society for Neuroscience, Neuroscience 2019, Chicago, Illinois.

Petrosyan, H., Fahmy, M., Tesfa, A., Liang, L., Sisto S.A., & Arvanian V.L. *Spinal Electromagnetic Stimulation Results in Immediate Pain Reduction and Induces Long-Lasting Functional Improvements in Patients with Chronic Low Back Pain (LBP). A Pilot Study.* Society for Neuroscience, Neuroscience 2019, Chicago, Illinois.

13. Research Foundation for SUNY – Downstate Medical Center

Funding supported principal investigator, Salvador Dura-Bernal, Ph.D., 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. The research supported through this funding resulted in new funding, to develop the software tool that facilitates simulation of cortical microcircuits

Publications: Gleeson, P., Cantarelli, M., Marin, B., Quintana, A., Earnsah, M., Sadeh, S., ...& Silver, R.A. (2019). Open Source Brain: A Collaborative Resource for Visualizing, Analyzing, Simulating and Developing Standardized Models of Neurons and Circuits**.** *Neuron,*103 (3), 395-411.

Presentations: Doherty, D.W., Dura-Bernal, S., Neymotin, S.A., & Lytton, W.W. (2019). *Identifying avalanches in simulated mouse primary motor cortex (M1).* 28th Annual Computational Neuroscience Meeting (CNS'19), Barcelona, Spain.

Dura-Bernal, S., Suter, B.A., Neymotin, S.A., Shepherd, G.M.G., & Lytton, W.W. (2019). Phase-Amplitude Coupled Oscillations and Information Flow in a Multiscale Model of M1 Microcircuits. 28th Annual Computational Neuroscience Meeting (CNS'19), Barcelona, Spain.

14. Research Foundation for SUNY – Stony Brook

Funding was used to purchase equipment for expanding respiratory measurement core facilities for SCI experiments. Specifically, the plethysmography equipment can be used by SCI researchers at Stony Brook that are examining the efficacy of various therapeutic interventions to yield improvements in respiratory function following SCI.

15. Research Foundation for SUNY – University at Albany

The researchers continued analysis of the ChIP-seq data during this reporting period and is still ongoing with no new conclusions to report at this time.

The researchers also began *in situ* hybridization and immunohistochemistry analyses of retina after optic nerve crush to provide more direct histochemical evidence to support the RNA-seq, whole genome bisulfite methylation sequencing (WGMS) database and ChIP-seq analyses.

For the next reporting period, once experiments on retina are complete, the researchers plan to extend the analysis to frog and tadpole hindbrain after SCI. Collectively, they anticipate these data will provide a foundation for building new understanding of the molecular genetic mechanisms permitting some CNS neurons to recover function after traumatic injury to the CNS while others lose this ability.

16. Research Foundation of CUNY – Staten Island

Funding was previously used to purchase a Lokomat 6 Professional and is currently used daily. 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.

Zaghloul Ahmed, PT, Ph.D. continues to 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 is in animals with and without SCI Funding provides partial support 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.

Publications: Knikou, M. & Murray, L.M. (2019). Repeated Transspinal Stimulation Decreases Soleus H-reflex Excitability and Restores Spinal Inhibition in Human Spinal Cord Injury. *Public Library of Science (PLOS) One,* 14 (9): e0223135.

Richards, T.M., Sharma, P., Kuang, A., Whitty, D., Ahmed, Z., & Shah, P.K. (2019). Novel Speed-Controlled Automated Ladder Walking Device Reveals Walking Speed as a Critical Determinant of Skilled Locomotion after a Spinal Cord Injury in Adult Rats. *Journal of Neurotrauma,* 36 (18), 2698-2721.

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).

Funding was used to purchase surgical supplies, antibodies, tissue culture ware, reagents and probes and general laboratory supplies. Funding also supported the animal procurement and husbandry. Overall, these purchases contribute to the goal of the project, which is to demonstrate that the experimental systems the researchers are characterizing can be suitable for probing structural and functional correlates of spinal tissue degeneration and regeneration in an accessible *in vitro* system.

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 this SCIRB funded contract generated preliminary data for a recently funded National Science Foundation (NSF) grant and future grant applications.

Presentations: Lewis, K E. (2019). *Interneuron Specification in the Zebrafish Spinal Cord*. 14th International Zebrafish Conference, Suzhou, China.

Lewis, K.E. (2019). *Interneuron Specification in the Zebrafish Spinal Cord*. 8th Annual Strategic Conference for Zebrafish Investigators, Pacific Grove, California.

19. University of Rochester

Funding supported a pilot study of the utility of 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 these experiments is to test the hypothesis that a regeneration-promoting population of astocytes can be reproducibly generated *in vitro* from distinct sources of iPSCs, and that these astrocyte subpopulations are effective in the treatment of traumatic SCI and can provide benefit even when transplantation is delayed in a rat model of thoracic contusion injury.

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. Funds have been used to support this trial to strengthen the spared connections between the brain and the muscle after SCI by pairing direct current electrical stimulation with robotic training.

The Burke Neurological Institute has established a research subject registry to facilitate successful completion of ongoing and future studies and enable more SCI patients to participate in clinical trials. The database will allow scientists within the Institute to recruit participants for upcoming and current clinical trials.

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’. The stimulator 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 will be presented at an appropriate national meeting and a manuscript prepared.

Presentations: Wong, V., Picci, C., Swift, M., Levinson, M., Sauve, A., Langley, B., & Willis, D. (2019). *Targeting A-Tubulin Acetylation to Promote Neurite Outgrowth and*

Functional Recovery After Injury. Mind Brain Behavior Symposium, Zuckerman Institute, New York, New York.

Translational Research Projects in SCI (Round 1)
Contract Term 8/15/16-8/14/21

Progress Reporting Period
2/15/19-8/14/19

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 three years, researchers focused on developing and testing the TPAD system. The two teams designed RobuST, to apply forces at three levels in the human body (at the trunk, pelvis, and the knees) when a subject is standing. The salient feature of RobuST is real-time control of 14 motors while being able to generate appropriate forces at the three levels on the human body. Pilot human studies (healthy subjects and SCI patients) have been performed at both laboratories.

   Future Directions: With both machines functioning and initial human safety tests and studies completed, the researchers will focus on subjects with SCI during the fourth year to understand the system capabilities and how the device could impact stand training of SCI patients. An FDA request to utilize RobuST for SCI patients implanted with a spinal cord epidural stimulator will be submitted for approval to begin training in this population prior to the end of the fourth year.

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

   Publications: Khan, M., Luna, T., Santamaria, V., Omofuma, I., Martelli, D., Rejc, E.,…& Agrawal, S.K. (2019). Stand Trainer with Applied Forces at the Pelvis and the Trunk: Response to Perturbations and Assist-As-Needed Support, *Institute of Electrical and Electronics Engineers, Transactions on Neural Systems and Rehabilitation Engineering,* 27 (9) 1855-1864.

   Santamaria, V., Luna, T., Khan, M., & Agrawal, S.K. (2020). The Robotic Trunk-Support Trainer (TruST) To Measure and Increase Postural Workspace During Sitting in People with Spinal Cord Injury, *Nature, Spinal Cord Series and Cases*, 6 (1).

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. The Carmel lab has shown efficacy of dual spinal-motor cortex electrical stimulation in improving forepaw manipulation skills and skilled locomotion after fourth cervical vertebra (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 results across animal subjects with cervical SCI is that dual modulation therapy strengthens corticospinal tract (CST) connections after injury. 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: The research team will pursue follow-up analyses on the role of brain stem pathways contributing to recovery after dual neuromodulation therapy; analyze viral tracing of CST axons in injured animals; and continue to consult on developing the human translational studies, which are scheduled for the latter half of the project.

Impact: The researchers are on the path to successfully translating components of their work into their large-animal cat model using the therapeutic neuromodulatory strategy key rat behavioral findings. 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.

Publications: Yang, Q., Ramamurthy, A., Lall, S., Santos, J., Ratnadurai-Giridharan, S., Lopane, M., …& Carmel, J.B. (2019). Independent Replication of Motor Cortex and Cervical Spinal Cord Electrical Stimulation to Promote Forelimb Motor Function After Spinal Cord Injury in Rats. *Experimental Neurology*; 320:112962.

Individual Predoctoral/Postdoctoral Fellowships (Round 1)
Contract Term 3/1/16-2/28/19

Progress Reporting Period
10/1/18-2/28/19

5 Awards (2 concluded in 2018), Procurement Total: $695,041

1. **Columbia University**
   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: SCI 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: The researchers' goal was to identify and strengthen connections between brain and spinal cord that are responsible for spontaneous recovery after SCI to improve functional recovery of hand movement. For identification, they analyzed anatomical changes in the candidate connections after labeling them with fluorescent proteins delivered by virus injection. To prove the necessity of these connections, they inactivated them with a technique that blocks electrical signals in specific connections. They made progress in identification of the connections that are responsible for treatment effect of our therapeutic intervention. They found that integration of the motor brain-spinal cord connection and sensory feedback in the spinal cord is key.

   Future Directions: This is the final progress report; however, because all the experiments were completed in healthy animals, they will continue future studies in animals with SCI to see whether the connections are responsible for the functional recovery of hands.

   Impact: The project results have provided the researchers further information regarding where in the brain and spinal cord stimulation with electrical current should occur to improve motor recovery. Optimization of the therapeutic paired brain and spinal cord stimulation continues.

2. **Research Foundation of CUNY, The City College of New York**
   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: During this three-year project, the trainee, Ms. Alzahraa Amer, has completed the major goals of the fellowship and has helped to expand how transspinal direct current electrical stimulation (tsDCS), alone and in combination with motor cortex epidural stimulation, to promote motor function after injury. Ms. Amer has mentored several medical students and biology students and has had the opportunity to present her findings, including internationally.

Future Directions: This is the final report, and the trainee has significantly advanced the City College of New York's (CCNY) understanding of the biochemical and physiological changes in the motor cortex and spinal cord produced by electrical neuromodulation.

Impact: The research team's studies suggest that spinal cord DCS 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.

3. Winifred Masterson Burke Medical Research Institute

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 neurons (CSNs) and the inhibitory environment. The researchers' 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: The researchers found that selective activation of B-RAF in adult CSNs promoted strong axon growth in the spinal cord after a dorsal hemi section or complete crush. Combination of B-RAF activation and absence of myelin-associated inhibitors promoted axon regeneration in an optic nerve crush model.

Loss of function of endogenous B-RAF antagonist, DUSP6, promoted robust axon growth in the spinal cord after a dorsal hemi section and resulted in the functional recovery. Their multi-photon imaging system detected the Ca^{2+} transients in CSNs and spinal cord interneurons of a free-walking adult mouse.

Future Directions: Although is the final report, the researchers are designing and generating clinical translational AAVs to activate B-RAF and target its downstream effectors. They are assessing the activity changes of CSNs and SINs in mice subjected to SCI surgeries to determine their association with axon regeneration *in vivo.*

Impact: Their study indicated that activation of the B-RAF signaling pathway elevates the growth competency of adult CSNs. The RAF-MEK pathway is therefore a promising target for spinal cord axon regeneration therapy. In addition, the three-photon *in vivo*

imaging protocol will provide a crucial tool for the community to investigate the CSNs and SINs' activities following injury and during 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 Period
5/1/2019-10/31/2019

8 Awards (6 concluded in 2017/2018), Procurement Total: $5,719,548

1. **Regenerative Research Foundation[3]**
   Sally Temple, Ph.D.
   CART: $1,097,684
   Sustained Delivery of IL10 and SHH to Promote Spinal Cord Regeneration After Injury

   Introduction/Background: Current SCI 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 microbeads to the injury site will counteract inflammatory processes, promote a regenerative environment, and improve recovery after SCI.

   Progress Towards Specific Aims: All work associated with Aim 1 (study of the impact of IL10 and SHH microbeads on the inflammatory response *in* vitro and their effects on cultured cells) 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. Histological analyses of spinal cord tissues of animals that received combined treatments of IL-10pDNA and SHH microbeads demonstrated a synergistic response to the therapuetics (relating to lesion volume, scar formation and myelination).

   Future Directions: Although this is the final reporting period, future directions may entail looking at cervical models of SCI or moving into higher verebrate models of SCI. Their promising results will be submitted for publication.

   Impact: Sustained delivery of IL-10pDNA and SHH microbeads (singly and together) can alter the post-injury inflammatory processes in the spinal cord. Combiation treatment significantly enhances behavioral recovery after SCI. Manuscripts are being prepared for publication.

2. **Research Foundation of CUNY, The City College of New York[2]**
   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 developed a pre-clinical cervical contusion injury model in rats that shares features with human cervical SCI. Their approach to therapy is to stimulate the spinal cord using transspinal direct current stimulation (tsDCS), where movements are executed, and to promote connections of the corticospinal motor system after injury.

Progress Towards Specific Aims: Researchers combined motor cortex and spinal cord stimulation, and successfully promoted voluntary forelimb function in rats after fourth cervical vertebra (C4) contusion injury. The researchers were able to identify that spinal cord stimulation was able to rescue cervical interneurons from degeneration after injury.

The researchers also discovered that CST fibers and muscle sensory nerve fibers compete for spinal synaptic connections (e.g., as one set of connections strengthens, the other weakens) and has important implications for implementing muscle training exercises in rehabilitation.

The researchers also focused on developing direct current stimulation targeting as a therapeutic strategy in rats.

Future Directions: Although this is the final report, using targeted tsDCS as a therapeutic strategy is a new non-invasive therapeutic intervention for SCI and has the potential to be implemented rapidly.

Impact: The researchers' studies are the first to show that electrical neuromodulation produces durable plasticity of the CST after cervical SCI. John Martin, Ph.D., has been presented findings both nationally and internationally, including at Institute of Neurosciences of Timone, Marseille, France, in 2019. The researchers hope the results open the possibility of using tsDCS to treat two major effects of cervical injury, arm weakness and spasticity. They have prepared a manuscript (in review) and their will continue their research to further study ways to implement brain and spinal cord stimulation to promote function after cervical SCI.

Presentations: Martin, J. H. (2019). *CNS Neuroregeneration Strategies: Discovery and Implementation.* Neuroregeneration Symposium, Houston Methodist Research Institute, Houston, Texas.

Martin, J.H. (2019). *Neuromodulation Symposium: Keynote, The Neurobiological Effects of Stimulating the Nervous System After Injury.* American Spinal Injury Association (ASIA), SCI Summit, Annual Scientific Meeting, Honolulu, Hawaii.

Martin, J.H. (2019). *Motor Cortex and Spinal Cord Injury Neuromodulation to Promote Motor Function After Injury.* UCLA Neurology Grand Rounds, Los Angeles, California.

Martin, J.H. (2019). *Modulating Neural Activity to Repair the Corticospinal System After Injury.* NeuroFrance 2019 Meeting, Marseille, France.

Martin, J.H. (2019). *Cortical Stimulation*. Gordon Research Conference, Central Nervous System Injury and Repair, Spinal Cord Injury and Repair: From Molecules to Function, Waterville Valley, New Hampshire.

Publications: Jiang, Y., Armada, K., & Martin, J.H. (2019). Neuronal Activity and Microglial Activation Support Corticospinal Tract and Proprioceptive Afferent Sprouting in Spinal Circuits After a Corticospinal System Lesion. *Experimental Neurology,* 321, 1113015.

Appendix 3

NEW YORK STATE SPINAL CORD INJURY RESEARCH BOARD

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1 Service commenced during 2019

2 Service concluded during 2019