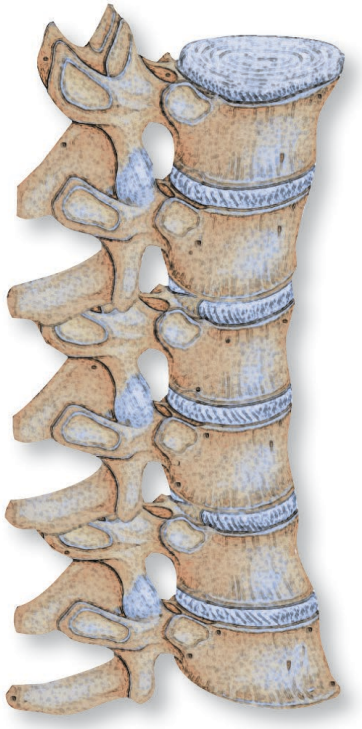
NEW YORK STATE OF OPPORTUNITY. Department of Health



SPINAL CORD INJURY RESEARCH BOARD

Report

January 1, 2020 to December 31, 2021

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 296,000 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 $379,698 to more than $1,163,425, with annual costs thereafter ranging from approximately $46,119 to $202,032[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. Due to the COVID-19 pandemic and the temporary closure of many research labs, this report includes progress made in 2020 and 2021.

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.

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, 2021. Web. 18 November 2021. https://www.nscisc.uab.edu/

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 the Wadsworth Center's website: 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). By the end of 2021, the current composition of the SCIRB includes six (6) researchers, two (2) clinicians and two (2) spinal cord-injured persons. Members serve four-year terms. No new appointments were made in 2020 or 2021 and the three (3) vacancies include one (1) to be filled by the Temporary President of the Senate, one (1) to be filled by the Governor, and one (1) to be filled by the Speaker of the Assembly.

III. SCIRB OPERATIONS

In fiscal year 2020-21, $8.5 million was programmed to support SCI research and in fiscal year 2021-22, $6.075 million was programmed to support SCI research.

Meetings are announced at least two weeks in advance whenever possible and are open to the public (in-person and/or remote) in accordance with Open Meetings Law (Article 7 of the Public Officers Law). 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.

The SCIRB held one meeting in 2020 (see Section IV. below). By the end of 2021, no meetings were held due to the delay in business from the COVID-19 pandemic; however, two (2) procurements were released by the New York State Department of Health, Wadsworth Center, Extramural Grants Administration on behalf of the SCIRB.

No changes were made to the SCIRB's bylaws in 2020 or 2021. 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 24, 2020 meeting, the SCIRB recommended funding from the "Projects to Accelerate Research Translation (PART) and Innovative, Developmental or Exploratory Activities (IDEA) in Spinal Cord Injury (Round 4)" Request for Applications (RFA). Eight (8) IDEA awards and one (1) PART award for a total of $3.84 million was recommended for funding. These are two and three-year awards, respectively, and contacts originally scheduled to begin in 2020 began in November 2021 due to the COVID-19 pandemic.

A tabular summary of this procurement can be found in Appendix 1.

Although the SCIRB did not recommend funding from the "Translational Research Projects in Spinal Cord Injury (Round 3)" RFA, the Board encouraged applicants to resubmit their projects with recommended revisions from the peer review critiques.

Funding Opportunities

The following RFAs were scheduled for release in 2020 but were delayed due to the COVID-19 pandemic; the first two (2) were eventually released in 2021:

- "Projects to Accelerate Research Translation (PART) and Innovative, Developmental or Exploratory Activities (IDEA) in Spinal Cord Injury (Round 5)"
- "Translational Research Projects (TRP) in Spinal Cord Injury (Round 4)"
- "Individual Predoctoral and Postdoctoral Fellowships in Spinal Cord Injury (Round 5)"

A May 2022 meeting will be planned so the SCIRB can recommend funding for the PART & IDEA (Round 5) and TRP (Round 4) opportunities. These multi-year contracts are anticipated to start in 2022. A summary of these procurements will be featured in the SCIRB's 2022 Annual Report.

Previously Recommended SCI Research Contracts

By January 2021, three (3) of eleven (11) PART and IDEA in SCI (Round 1) contracts completed their final year. The scientific progress resulting from these multiyear awards can be found in Appendix 2.

By March 2021, the Institutional Support for SCI Research in New York State (Round 6) contracts completed their fourth 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.

In May 2021, three (3) PART and four (4) IDEA in SCI (Round 3) contracts completed their second year. The scientific progress resulting from these three- and two- year awards, respectively can be found in Appendix 2.

Also, in May 2021, two (2) contracts from the Translational Research Projects in SCI (Round 2) completed their third year. The scientific progress resulting from these multiyear awards can be found in Appendix 2. In 2021, the Health Research, Inc. contract was transferred to Albany Research Institute, Inc. for the completion of the proposed work.

In June 2021, three (3) PART and eleven (11) IDEA in SCI (Round 2) contracts completed their third year. The scientific progress resulting from these three- and two-year awards, respectively can be found in Appendix 2.

By August 2021, two (2) Translational Research Projects in SCI (Round 1) contracts completed their final year. The scientific progress resulting from these five-year awards can be found in Appendix 2.

Also, by August 2021, five (5) Individual Predoctoral and Postdoctoral Fellowships in SCI (Round 4) contracts completed their first progress reports. The scientific progress resulting from these three-year contracts can be found in Appendix 2.

By September 2021, five (5) of six (6) Individual Predoctoral and Postdoctoral Fellowships in SCI (Round 3) contracts completed their third year. The scientific progress resulting from these three-year awards can be found in Appendix 2.

Also, by September 2021, three (3) of the nine (9) Individual Predoctoral and Postdoctoral Fellowships in SCI (Round 2) contracts 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 Department of Health discussed hosting the next NYS SCI Research Symposium in 2021 but planning in-person events was delayed by the COVID-19 pandemic. The SCIRB will discuss scheduling this at their next business meeting in 2022. A future symposium would 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.

Appendix 1

2020 PART and IDEA in SCI (Round 4) Recommendations for Award

Organization	Funding Mechanism/Research Category	Project Title	Principal Investigator	Recommended Funding
Cornell University	IDEA/Cellular Regeneration & Therapeutics	Investigating Regenerative Goldfish Extracellular Matrix in Mammalian SCI	Yadong Wang, Ph.D.	$ 360,000
Icahn School of Medicine at Mount Sinai	IDEA/Rehabilitation	Long Term Effects of rTMS in Chronic Neuropathic Pain in People with SCI	Mar Cortes, M.D.	$ 357,170
Research Foundation for SUNY Stony Brook	IDEA/Cellular Regeneration & Therapeutics	Targeting CaV3.2 Calcium Channel to Treat Chronic Neuropathic Pain Following SCI	Michelino Puopolo, Ph.D.	$ 360,000
Research Foundation for SUNY Stony Brook	IDEA/Rehabilitation	Neuroprotective Strategies to Minimize SCI Tissue Damage and Improve Bladder and Respiratory Function	Irene C. Solomon, Ph.D.	$ 357,782
Research Foundation for SUNY, University at Albany	IDEA/Cellular Regeneration & Therapeutics	Elemental Analysis of a Gene Network Implicated in CNS Axon Regeneration	Ben G. Szaro, Ph.D.	$ 351,031

Organization	Funding Mechanism/Research Category	Project Title	Principal Investigator	Recommended Funding
The Research Foundation of CUNY obo College of Staten Island	IDEA/Rehabilitation	Function of Spinal Locomotor Centers During Transspinal Stimulation After SCI	Maria Knikou, P.T., Ph.D.	$ 359,004
The Trustees of Columbia University in the City of New York	IDEA/Rehabilitation	Wheelchair Robot for Active Postural Support (WRAPS) of People with Spinal Cord Injury	Sunil K. Agrawal, Ph.D.	$ 346,220
Winifred Masterson Burke Medical Research Institute	IDEA/Cellular Regeneration & Therapeutics	Modulation of Presynaptic Partners of Corticospinal Neurons to Improve Motor Recovery After Spinal Cord Injury	Yutaka Yoshida, Ph.D.	$ 360,000
Winifred Masterson Burke Medical Research Institute	PART/Cellular Regeneration & Therapeutics	Novel Combinatorial Approach to Improve Motor Recovery After Spinal Cord Injury	Yutaka Yoshida, Ph.D.	$ 990,000
			Total (9 awards)	$3,841,207

Appendix 2

Scientific Progress Resulting from SCI Research Board-Funded Projects

Individual Predoctoral/Postdoctoral Fellowships (Round 4)
Contract Term 8/1/19-7/31/22

Progress Reporting Period
2/1/21-7/31/21

5 Awards, Procurement Total: $1,162,694

1. **Columbia University**
   Sunil K. Agrawal, Ph.D. and Tatiana D. Luna, M.S.
   Predoc: $135,600
   Improving Upper Body Trunk Control in Spinal Cord Injury Patients Through Robotic Rehabilitation Training

   Introduction/Background: The Robotics and Rehabilitation (ROAR) Lab has previously developed a robotic Trunk Support Trainer (TruST) and a Robotic Upright Stand Trainer, (RobUST). These cable driven devices can provide assistive and resistive forces to the participant at the level of the trunk. One of the main goals of this project is to explore the assistive contributions these robotic devices can make in training SCI participants to regain trunk control in standing, seated, and transitioning from sit-to-stand.

   Progress towards specific aims: To accomplish their first aim to characterize trunk control with TruST in SCI participants, the researchers brought in five (5) SCI participants with a neurological SCI level ranging from the fourth thoracic vertebra in the spine to the eleventh (T4-T11). Using a video motion capture system, they characterized the participants' seated trunk range of motion with and without the support of the robotic device TruST. They observed in one session, that with the use of TruST, participants showed a greater ability to increase their active seated trunk workspace.

   To get a better understanding of the effects the robotic force controller has on posture and balance, ten (10) able bodied participants were brought in for a postural standing experiment using RobUST. They compared their postural balance reactions with the assistance of the cable robotic device versus assistance from a traditional handrail support. They observed that participants with assistive forces from RobUST, enhances postural balance without significantly removing muscular control mechanisms that are of interest in re-training postural control strategies in standing, nor decreasing ground reaction force distribution. They also investigated how participants motion is altered with a load on the pelvis during squatting, a common intervention prescribed to improve sit-to-stand.

   Future Directions: Their results show cable driven robotic platforms potential as training devices to expand seated and standing workspace for individuals with SCI. A training pilot study would need to be implemented next. The cable robotic device has several functionalities and could be further tested to assist in creating a training paradigm.

Impact: Their results demonstrate that TruST can expand the seated workspace area and increase the active trunk's excursion of individuals with SCI.

Their results also showed pelvic assistive forces from RobUST allowed participants to have similar postural center of pressure outcomes as holding a handrail, but without inhibiting as significantly the electromyography activity of the postural muscles nor decreasing the ground reaction force distribution. The pelvic support via RobUST also decreased postural excursions for all perturbation directions.

Presentations: Luna, T.D., Santamaria, V., Omofuma, I., & Agrawal, S.K. *Control Mechanisms in Standing during Simultaneously Receiving Perturbations and Active Assistance from the Robotic Upright Stand Trainer (RobUST).* IEEE International Conference on Biorobotics and Biomechantronics, Virtual 2020.

Publications: Luna, T.D., Santamaria, V., Omofuma, I., Khan, M.I., & Agrawal, S.K. (2021). Postural Control Strategies in Standing with Handrail Support and Active Assistance from Robotic Upright Stand Trainer (RobUST). *IEEE Transactions on Neural Systems and Rehabilitation Engineering,* 29:1424-1431.

2. Icahn School of Medicine at Mount Sinai

Ann M. Spungen, Ed.D. and Chung-Ying Tsai, Ph.D.
Postdoc: $189,476
The Effects of Incorporated Exoskeletal-Assisted Walking in SCI Acute Inpatient Rehabilitation

Introduction/Background: The potential functional and health benefits of exoskeletal-assisted walking (EAW) have been demonstrated in persons with chronic SCI. However, few studies have been published that have an EAW intervention for persons with SCI during acute inpatient rehabilitation (AIR). Researchers will test the effect of early incorporated EAW training in AIR on accelerating functional recovery and reducing pain and inflammation.

Progress towards specific aims: The study received IRB approval on 06/29/2021 and 20 participants are enrolled in the study. Eleven participants were assigned to the EAW group, while the other nine (9) were in the control group. Seventeen of the participants have completed the study, one is currently active, and there were two study withdrawals (in the EAW group) due to temporary research suspension by the Mount Sinai Hospital in response to the COVID-19 pandemic in 2020 and severe shoulder and wrist pain during EAW. The COVID-19 pandemic delayed this study's enrollment progress. The preliminary results show that the AIR with EAW group compared to the AIR only group had significantly better improvement in the total motor score and the sub-score of respiration and sphincter management from Spinal Cord Independence Measure.

Future Directions: The research team will keep enrolling participants and try to achieve the enrollment goal. The fellow will continue to develop skills to establish a solid foundation for a career in SCI research. The preliminary data will be submitted as an abstract to SCI research conferences and used for future grant applications.

Impact: If the study results demonstrate that AIR with EAW training can improve functional independence better than AIR standard of care for people with SCI, this would serve clinicians and clinical practice guidelines as an important reference for including

EAW into the standard of care for inpatients during acute and subacute SCI rehabilitation.

3. **New York University School of Medicine**
   Dan R. Littman, M.D., Ph.D. and Hyeon Kyu (Alice) Kwon, B.S.
   Predoc: $135,600
   Enteric Glial Cells Cause Gastrointestinal Complications Post Spinal Cord Injury

   Introduction/Background: The project is to define the cellular pathways involved in gastrointestinal (GI) complications post spinal cord injury (SCI). Homeostasis is disrupted in the intestinal compartment of SCI patients; however, very little is known about the cause of such disruption and the resulting GI complications when the complications can drastically impact the patients' quality of life. Many recent studies show enteric nervous systems as crucial part of intestinal homeostasis, so the fellow aims to characterize and investigate the role enteric glial cells (EGCs) in keeping intestinal homeostasis as well as microbiome.

   Progress towards specific aims: With newly developed protocol for isolating high-quality EGC-specific RNA from colon, the fellow sequenced and revealed some interesting genes that are EGC specific. Ltf, Lactoferrin, is one of the highly enriched gene in the EGCs, which is also validated at the protein level. Further investigation revealed possible interaction of EGC's LTF with goblet cells, which are a kind of intestinal epithelial cells that produce mucus.

   Future Directions: They will further characterize the enteric glial cells by altering their environments to understand how EGC functions to keep intestinal homeostasis and microbiome.

   Impact: This is the first dataset characterizing the EGCs from colon during homeostasis along with validation on protein level. LTF is an antimicrobial peptide that sequesters free iron which is crucial to bacterial growth, so it's specificity to EGCs in the GI tract is intriguing. Also, possibility that glial cells may interact with intestinal epithelial cells to regulate mucosal health is a novel function of glial cells. Finally, as astrocytes of the brain do not express Ltf, this may be used as tool to manipulate the EGCs and study their functions without perturbing the astrocytes in the future.

4. **Regenerative Research Foundation**
   Sally Temple, Ph.D. and Elizabeth Fisher, Ph.D.
   Postdoc: $184,686
   Modulation of Inflammation Following SCI Using Plasmid IL-10 Microbeads

   Introduction/Background: Infiltration of immune cells into the spinal cord (SC) following injury exacerbates initial damage, resulting in neuronal death and poor patient outcomes. The inflammatory cascade post-SCI is well characterized, and now, single cell analysis enables a deeper understanding of how treatments can modify the inflammatory response. The researchers' goal is to treat injured SC with Interleukin-10 (IL10) plasmid DNA beads (IL10pDNA) to modulate immune cells to reduce damage and promote repair. IL10 is a known anti-inflammatory cytokine primarily targeting microglia and macrophages, key regulators of immune activation in the SC. They will compare the effect of treatment at the single cell level. These experiments will enable identification of novel pathways impacting injury recovery.

Progress towards specific aims: The researchers made significant progress towards analyzing cell populations. Tissue has been collected and sectioning begun for in vivo validation of the flow cytometry findings. They also have optimized methods for single nuclear isolation and RNA sequencing.

Future Directions: The fellow plans to complete sectioning of harvested spinal cords and analyzing tissue for the immune cell populations present at 3- and 7-days post injury, for control vs IL10pDNA beads. They also plan to perform the injury and collect the 30- and 60-day timepoint to perform flow cytometry, collecting the tissue for histological analysis. Aim 2: They will perform SCI surgeries for 3- and 7- days post injury to collect for single cell analysis.

Impact: After completing this study the researchers will have discovered the effects of IL10pDNA beads on immune cell subpopulations at key times after injury, and their gene expression at the single-cell level, providing insights into novel pathways for regulating immune cell infiltration following SCI.

5. University of Rochester Medical Center
Bradford C. Berk, M.D., Ph.D. and Chia Hsu, Ph.D.
Postdoc: $189,366
The Role of Phosphodiesterase 10A in Inflammation After Spinal Cord Injury

Introduction/Background: The objective of this project is to test TP-10 as a novel therapy that improves motor recovery and decreases muscle atrophy after SCI. The research team will investigate specific inflammatory pathways that link spinal cord injury-mediated macrophage activation and neuromuscular dysfunction. They hypothesize that PDE10A inhibition will improve motor function and reduce muscle atrophy after SCI by decreasing local tissue damage and inflammasome activation.

Progress towards specific aims: To determine the role of PDE10A in demyelination and motor impairment after SCI, the researchers performed T9 contusion injury in animals and treated them with vehicle or the PDE10A inhibitor and once daily after injury. They found PDE10A expression was significantly increased in the SCI group compared to the sham group. Furthermore, the TP-10-treated group had significantly decreased NLRP3 mRNA levels compared to the vehicle treated group. Notably, TP-10-treated mice demonstrated a significantly larger gastrocnemius mass than the vehicle treated group.

To define the mechanistic role of PDE10A-mediated inflammasome activation in macrophages and microglia, they evaluated the role of PDE10A in inflammasome activation by measuring ASC speck formation, caspase-1 activity, GSDMD cleavage, and IL-1β secretion after nigericin or ATP stimulation in lipopolysaccharide (LPS) primed macrophages. They found that PDE10A inhibition by TP-10 blocked ASC speck formation, LPS-nigericin-induced caspase-1 activation, GSDMD cleavage, and IL-1β secretion. These data suggest that PDE10A regulates inflammasome assembly and activation.

Future Directions: The team will determine the effect of the PDE10A inhibitor, TP-10, to decrease inflammation and improve functional recovery after in vivo injury. Mechanistically, they will determine the role of the PDE10A-PKA (protein kinase A) pathway on NLRP3 inflammasome assembly.

Impact: These studies will contribute the foundational knowledge needed to develop a new pharmacological treatment that reduces inflammation in response to SCI, and thereby may improve recovery and enhance quality of life.

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
11/1/20-4/30/21

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: Six (6) participants were enrolled for screening and two (2) participants were identified as eligible for study enrollment. The researchers reported that participant recruitment was significantly impeded, and inpatient admissions were lower than expected due to the COVID-19 pandemic. They expect inpatient admissions and participants recruitment/enrollment to increase with the proliferation of immunizations and the public health outlook improving.

   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 co-PI, Dr. Christopher Cardozo. Dr. Siddiq has completed a cohort of rats to which the drugs were delivered to the injury site by gelfoam administration or by injections to the motor cortex of the brain. The researchers have completed a set of animals with contusions with or without treatments; however, they were never able to start work with the full 4-drug combination, in part due to issues with obtaining the compound due to unanticipated delays in getting local, State and Federal approval to use it for this application; delays in approval were also due to the COVID-19 pandemic. The animals with the incomplete combinations were labeled with green fluorescent protein (GFP)-expressing viruses by injection into the motor cortex.

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. All animals are labeled with GFP-viruses, and they are using the iDISCO technique (a technique to label the whole sample and chemically clear it for enhanced resolution) and visualizing their samples on the multiphoton microscope. 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.

Publications: Siddiq, M. M., Hannila, S. S., Zorina, Y., Nikulina, E., Rabinovich, V., Hou, J.,…& Filbin, M.T. (2021). Extracellular Histones, a New Class of Inhibitory Molecules of CNS Axonal Regeneration. *BioRxiv*, 365825.

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: The research team has successfully enrolled 26 participants, which have been evaluated, randomized, and allocated to three different VR groups. 17 participants have completed the protocol.

On March 16, 2020, recruitment of new participants and all in-person study visits were suspended due to COVID 19 determinations. As such, no new participants were enrolled between March and August 2020. In August 2020, the team received IRB approval to update their protocol to account for virtual sessions in light of the COVID-19 pandemic and on August 22, 2020, they restarted recruitment efforts.

Future Directions: Since the contract term has ended, the team will no longer be recruiting participants under this contract. 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. They will present the data in peer-reviewed publications and at scientific conferences.

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 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: The first aim of this proposal was to test the hypothesis that astrocytic TG2 impedes the recovery process after SCI, and the second aim was to test the hypothesis that pharmacological inhibition of TG2 results in significantly improved outcomes following SCI. The team has successfully achieved both aims; deletion of TG2 from astrocytes improves recovery after SCI, and TG2 inhibitors also significantly improved functional recovery after an SCI.

Future Directions: In the future the researchers plan on testing different treatment paradigms to determine optimal outcomes with the TG2 inhibitor following SCI. In addition, they plan on carrying out additional studies to fully delineate the mechanisms by which TG2 inhibition or deletion enhances recovery following SCI.

Impact: The findings from these studies solidify the conceptual framework that inhibiting TG2, is beneficial for SCI. Further, both deleting TG2 from just astrocytes or using a TG2 inhibitor that acts in all cells yield very similar outcomes; both significantly improve

functional recovery from SCI. These findings pave the way for the development of TG2 inhibitors for the treatment of SCI.

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 researchers have obtained Institutional Review Board approval for the pilot clinical trial outlined in Aim 1. Of eight (8) individuals pre-screened, six (6) were ultimately enrolled before they closed enrollment. This is half of the targeted enrollment and is largely due to the restrictions on clinical research during the COVID-19 pandemic. They anticipate that these individuals will provide sufficient power for their study as they are assessing changes within individuals.

   They also have demonstrated that their refined pre-clinical approach for enhancing the regenerative response in mice improves regeneration of axons required for movement. Aim 2 experiments including evaluating the recovery of forelimb function in mice following nerve transfer to treat chronic SCI are ongoing.

   Future Directions: In the next reporting period, the researchers anticipate that at least two (2) individuals will undergo nerve transfer surgery to treat chronic SCI. In Aim 2, they are also testing non-invasive transcutaneous stimulation to augment recovery following nerve transfer surgery in chronically injured SCI mice.

   Impact: The research team anticipates 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 their fourth and final reporting period, the research team has continued the investigation of neurovascular interactions and their contribution to the CNS pathophysiology. They have established for the first time the connectivity maps of all major elements of the neuro-glia-vascular system. To further characterize local cholinergic circuits and the structural relationship to pericytes, they have dissected activity-induced distribution of acetylcholine at capillary regions and their vasomotor effect. Using genetic tools, they have measured the distribution of acetylcholine upon targeted cholinergic cell stimulation. This was followed by an immunohistochemical analysis of the underlying neurovascular elements.

Future Directions: Our new data indicate that the levels of acetylcholine can be reliably measured using the novel genetically encoded biosensor. Furthermore, this allows for both spatially- and temporally accurate assessment of acetylcholine signaling in vivo, the goal of ongoing and future studies.

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.

Publications: Ivanova, E., Bianchimano, P., Corona, C., Eleftheriou, C.G., & Sagdullaev, B.T. (2020). Optogenetic Stimulation of Cholinergic Amacrine Cells Improves Capillary Blood Flow in Diabetic Retinopathy. *Investigative Ophthalmology & Visual Science*, 61(10):44.

Ivanova, E., Corona, C., Eleftheriou, C.G., Bianchimano, P., & Sagdullaev, B.T. (2020). Retina-specific Targeting of Pericytes Reveals Structural Diversity and Enables Control of Capillary Blood Flow. *Journal of Comparative Neurology*, 529(6):1121-1134.

Kovacs-Oller, T., Ivanova, E., Bianchimano, P., Sagdullaev, B.T. (2020). The Pericyte Connectome: Spatial Precision of Neurovascular Coupling is Driven by Selective Connectivity Maps of Pericytes and Endothelial Cells and is Disrupted in Diabetes. *Cell Discovery*, 16;6(1):39.

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 faced setbacks due to the COVID-19 pandemic causing a complete halt to their lab work from mid-March 2020. They spent much of the last six (6) months rebuilding their mouse colonies. They have regenerated enough transgenic mice to resume their SCI experiments.

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. Unfortunately, this work was interrupted since the animal transfers between the labs were suspended due to the COVID-19 pandemic, but they plan on resuming transfers as soon as they are permitted to do so. In the interim, they have been using a 2-photon microscope at Burke to compare the efficacy of several new GCaMP constructs.

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. They have obtained approval from the Kessler IRB to carry out the human study as well as permission to transfer the TMS stimulator and coil from Burke to Kessler Rehabilitation Center for the proposed experiments. Since they are not allowed to enter the building in Kessler due to the pandemic, they trained two postdoctoral fellows from Kessler to operate the machine at Burke.

Following the tests runs on able-bodied volunteers, they are now ready to begin working with subacute SCI volunteers as proposed. However, there are delays since the number of new SCIs dropped dramatically during 2020-21 due to the COVID-19 pandemic, such that the Kessler Institute for Rehabilitation has not admitted a single new spinal cord injury inpatient from September 2020 through May 2021.

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
3/1/20-8/31/20[1]
3/1/20-8/31/21[2]

6 Awards (5 current, 1 previously concluded), 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

   Per the University's website, Dr. Yang is listed as a former post-doctoral research scientist. At the time this report was written, no further progress was claimed or reported for year 3 of this project. The contract will be terminated early if Dr. Yang was in fact no longer working at the University on this project during year 3.

2. **Research Foundation of CUNY, The City College of New York[1]**
   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: The researchers studied the effects of stimulation on SCI animals, specifically, subjects received a contusion at the fourth cervical vertebra (C4) and phasic stimulation for 10 days following the injury. A map of the motor representation was then obtained, and the tissue analyzed for axonal outgrowth. Their research shows that stimulation after injury lowers the current threshold at the site of

stimulation but not in the surrounding regions in the same hemisphere. They were unable to observe a sufficiently large number of axons in the gray matter to determine changes in outgrowth because of the sparse numbers of spared corticospinal tract axons located caudal to the injury.

Future Directions: Going forward, and in accordance with University COVID-19 restrictions, the researchers will continue to add to their cohort of C4 contused animals. In the next cohort, they will use a milder injury to have more spared axons for morphological analyses. They need to add a control group of SCI animals that receive no additional stimulation, as well as add a group that receives tonic stimulation. They will also collect and analyze more electrophysiological data for changes in response latency which may indicate demyelination of axons after injury. By studying the morphological, synaptic and physiological changes that occur with SCI and stimulation, they can develop targeted therapies for restoring motor function in injured individuals.

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[2]
Prithvi K. Shah, Ph.D., Pawan Sharma
Predoc: $117,570
Activity-dependent Closed-loop Neuromodulation of the Cervical Spinal Cord for the Recovery of Skilled Upper Limb Function After a Spinal Cord Injury

Introduction/Background: Permanent impairments of hand function greatly deteriorates the quality of life for people with a cervical SCI (cSCI). Gains in recovery of upper limb motor function with epidural stimulation (ES) are modest. In this research 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 or 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 on 29 rats and their findings indicate that for the trained task, rats receiving ES below the injury site in acute or chronic phase along with motor rehabilitative training demonstrated modest recovery in the skilled upper limb function more than the rats receiving motor training or no training at all. In contrast, for the untrained tasks, the researchers did not observe significant difference in motor recovery between experimental groups.

Future Directions: During the training period, Mr. Sharma published a research article and is working on three other manuscripts generated directly as a result of the pre-doctoral fellowship.

Impact: Overall, the research team's present findings provide encouraging evidence to further investigate aES as a therapeutic tool for the human population with cSCI, especially as a neural prosthetic device. If proven to be effective, aES can significantly improve the independence and quality of life of the population with cSCI.

Publications: Sharma, P. and Shah, P.K. (2021). In Vivo Electrophysiological Mechanisms Underlying Cervical Epidural Stimulation in Adult Rats. *Journal of Physiology*, 599: 3121-3150.

4. Winifred Masterson Burke Medical Research Institute[1]
Vibhu Sahni, Ph.D., (originally 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, which 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 this reporting period, Dr. Ruven has next established the age (postnatal day 4 or P4) and spinal segmental levels (at the second cervical vertebra (C2), second thoracic vertebra (T2), and eleventh thoracic vertebra (T11)) at which the injury should be done to produce dystrophic axonal endings. At P4 the CST is still growing into the spinal cord and the pioneer axons are beginning to reach the lumbar cord. To the researchers' surprise, they found the CST has fully lost the ability to regenerate when injured at the C2 level but was able to fully regenerate at the T11 level. The researchers also found that a T2 injury had an intermediate response where no axons were able to regenerate long-distances in the dorsal funiculus. These results are especially intriguing as the established thinking in the field would have predicted that while the CST is still developing, CST axons will maintain their regenerative ability at all segmental levels. However, it had not been shown before because conventionally used neonatal SCI models are quite severe and therefore not able to distinguish the contribution of intrinsic regenerative ability versus extrinsic factors in the spinal cord to the decline in axon regenerative ability. The researchers' new model allows the team to focus more clearly on the intrinsic regenerative ability of corticospinal neurons as the extrinsic spinal environment is left relatively intact after the injury.

The research team is now preparing a manuscript introducing this new neonatal SCI model, as well as their surprising results about an early and segmentally distinct loss of CST regenerative ability. In addition, these results have motivated many further ideas and projects in the lab that Dr. Ruven will be working toward next.

Future Directions: In the next reporting period, the researchers are planning to submit a manuscript describing their new neonatal SCI model, as well as the intriguing results about the segmentally distinct loss of CST regenerative ability. The paper is currently planned as a brief report to Nature Neuroscience. Also, they will continue working on novel ideas and new questions raised by these intriguing and exciting results. In the follow-up projects, they are further investigating the exact time-course of regenerative ability loss in different spinal segments, as well as possible reasons behind these segmental differences.

In addition, they will work on finalizing the methods to visualize single axonal endings (dystrophic endings versus growth cones) in the spinal cords obtained from mice that underwent their newly developed microsurgical SCI. Using an intersectional mouse line will enable sparser axon labeling, and this, in turn, will enable Dr. Ruven to resolve single axonal endings for imaging. Next, they will combine their approach with immunohistochemical staining for stress response protein synthesis markers. This should allow the team to deeply investigate the biology of these dystrophic endings compared to the growth cones in vivo.

Also, since they identified distinct regenerative responses at different spinal segments, they can further investigate whether protein degradation or synthesis pathways (especially stress response pathways) play any role in this segmentally distinct regeneration phenomenon. For that, they plan on using immunohistochemistry to compare protein levels in axonal endings after injuries at distinct spinal levels.

Impact: This project addresses an important barrier for SCI repair and has the potential to shift how researchers target dystrophic axonal endings. The researchers' 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 the 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[1]
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: During this period, the fellow made progress on determining structural changes in the intracortical circuits and testing the role of intracortical circuits in skilled behavior after injury.

Future Directions: The fellow's future directions initially included completing the structural imaging studies using viral expression of fluorescent reporter proteins to visualize intracortical circuits in vivo. Also, optogenetic silencing experiments were supposed to be continued with conditional expression of a light-activated inhibitory channel in intracortical connections; however, the fellow had to resign and is no longer an employee of the Institute.

Although a replacement fellow was identified and proposed for replacement by the Institute to continue the research, applications received in response to this procurement were evaluated and scored based on the fellow and the development plan as submitted. The purpose of these awards is to support promising fellows during their mentored training and research period under the guidance of outstanding faculty PI/sponsors. This award is not designed to fund the proposed research and the contract has been terminated early.

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
12/1/19-5/31/20[1]
6/1/20-11/30/20[2]
12/1/21-5/31/21[3]

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

1. **Bronx Veterans Medical Research Foundation, Inc.[1]**
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: Immunohistochemical (IHC) data comparing ApoE3 and ApoE4 spinal in cord segments, above and below the injury, revealed a greater staining for the active astrocytic marker GFAP, and microglial marker IBA-1 on ApoE4 sections surrounding the lesion site compared to sections from ApoE3 mice. Conversely, neurite outgrowth, detected with GAP-43, is decreased in ApoE4 sections compared to ApoE3.

Differences in spinal cord transcriptomic profiles by RNA-seq, in segments immediately above, below and at the lesion site were analyzed between ApoE3 and ApoE4 animals, at 7- and 21- days post injury. The number and variation of differentially expressed genes (DEGs) were significantly different between ApoE variants, and between spinal cord tissue from above versus below the injury site.

Surgeries and behavior testing were made for ApoE3 and ApoE4 animals normal (+/+) or reduced (+/-) levels of Synaptojanin-1 (Synj1). Functional recovery was compared between homozygous synj1 (+/+) and heterozygous synj1 (-/+) animals at 7- and 14-days post injury. Studies in male mice revealed a significant gain of function in ApoE4 synj1(+/-) mice compared to ApoE4 synj1(+/+). No significant differences were observed between ApoE3 synj1 (-/+) and synj1 (+/+) animals.

Future Directions: The researchers data validates the use of a novel animal model for studying the mechanisms responsible for the poor functional outcomes in those with SCI who have the ApoE4 variant. These results may open a new avenue for research on

functional recovery after SCI and help the development of novel pharmacologic interventions.

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.[1]
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 commonly prescribed medications to treat low BP is midodrine. However, in 2014 the FDA approved another medication, droxidopa (Northera). Researchers will test the effect of escalating dose of Droxidopa on seated systolic blood pressure in an open label trial. Researchers will determine the effect, compared to placebo, on supine systolic blood pressure and changes in cerebral blood flow and systolic blood pressure during a 70-degree head-up tilt maneuver.

Progress Towards Specific Aims: Since their last progress report (December 2019), they have identified and screened 11 participants. Of the 11 screened:

- eight participants are eligible,
- two have been enrolled,
- one completed study procedures,
- one is currently active,
- two participants signed consent but have not started the trial.

Since that time the study was placed on administrative hold in March 2020 and all research activities were stopped due to the COVID-19 pandemic. As of this report, they plan to resume some research activities in July.

Future Directions: Testing will begin on eligible participants in late August or September.

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: Progress has yet to be submitted at the time this report was published.

   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**[1]
   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: The research team has been measuring if exoskeletal-assisted walking (EAW) changes the immune system in persons with SCI, such as reducing inflammation or changing genes that are activated within white blood cells. To date, they have collected 29 blood samples from participants before and after 36 sessions of EAW training.

   Progress towards inflammatory proteome profiling is almost complete, with c-reactive protein and other inflammatory protein levels to be assayed in a pilot number of

participants. For inflammatory proteome profiling, RNA-sequencing is ongoing. As a first step, the researchers performed a pilot study of samples from the first six (6) participants who completed pre and post EAW sample collection.

Future Directions: The Centers for Medicare and Medicaid Services (CMS) recently issued a billing code (effective 10/1/20) for the ReWalk Exoskeleton and will seek coverage from national, state, and private payors. This greatly raises the potential impact of the research team's study.

The research team is now analyzing additional samples and examining potential correlations with other outcomes measured in the parent study, including walking ability, autonomic/cardiovascular function, body composition, bowel function, bladder complications, vagal tone, anabolic hormone levels, lipid profiles and quality of life.

Impact: As exercise modalities for persons with SCI are limited, identification of EAW 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 exercise/activity effects.

Together with the new CMS code for exoskeleton use by this population, the results of this study are likely to inform future clinical trials of health benefits of exoskeleton use by individuals with SCI.

Publications: Preliminary data and a description of this study was delivered as a selected oral abstract presentation by Dr. Bloom (PI) at the annual meeting of the International Spinal Cord Society (ISCOS): Bloom O, Arcese A, Spungen AM. The impact of walking on the immune system of persons with chronic spinal cord injury. November 7, 2019. Nice, FR.

5. Icahn School of Medicine at Mount Sinai[1]
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: Through advanced bioinformatics analyses, the research team has identified many transcription factors of the basic-helix-loop-helix Per-Arnt-Sim (bHLH-PAS) family that might be involved in the epigenetic regulation of regeneration-associated genes (RAGs). bHLH-PAS transcription factors are novel environmental and physiological sensors engaged in circadian rhythm, hypoxia response, and environmental/physiological sensors. They have conducted extensive studies over the past two (2) years to establish their potential importance in regulating axon regeneration gene pathway.

The researchers have also optimized neural differential protocols from human embryonic stem cells into various types of neurons. Using this system, they are exploring applicability of novel epigenetic mechanisms to enhance axon growth potential of induced neurons, which may have implications for stem cell-based regenerative medicine.

Future Directions: The researchers are finalizing additional experiments to validate the novel roles of bHLH-PAS family of transcription factors in regulating gene expression to reactivate axon growth program. They are in the process of preparing manuscripts to report these exciting findings.

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: Zhou, X., Wahane, S., Friedl, M.S., Kluge, M., Friedel, C.C., Avrampou, K.,…& Zou, H. (2020). Microglia and Macrophages Promote Corralling, Wound Compaction and Recovery after Spinal Cord Injury via Plexin-B2. *Nature Neuroscience,* (3):337-350.

6. Regenerative Research Foundation[1]
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: Following SCI alpha-synuclein (α-synuclein) undergoes an intracellular cascade of pathogenic misfolding, abnormal accumulation, and trans-cellular propagation. SCI induces synuclein aggregation and neurotoxicity, as observed in vertebrate animal models ranging from lampreys to rodents, implicating this process as a novel therapeutic target. However, none of these events proceed in the absence of the primary intracellular α-synuclein misfolding event. They address this issue by utilizing cutting edge recombinant antibody technologies to develop novel bifunctional intrabodies with the potential to eliminate synuclein accumulation following SCI using the cell's normal protein clearing process.

Progress Towards Specific Aims: The researchers identified anti- α-synuclein intrabodies targeting synuclein to the proteasome for degradation. To avoid a potential immunogenic response, the proteasomal targeting signal was optimized for human use by substitution of the mouse PEST degron with the human PEST (hPEST) degron from ornithine decarboxylase. VH14-hPEST resulted in efficient degradation of endogenous α-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. Initial studies in the in vivo lamprey SCI model demonstrated that N77K-hPEST was potentially neuroprotective on a cell-by-cell basis.

Future Directions: Additional studies are warranted to determine the extent to which bifunctional anti-synuclein-PEST intrabodies improve anatomical and functional recovery in the established in vivo lamprey SCI model. Additionally, targeted degradation of endogenous synuclein by bifunctional anti-synuclein human-PEST intrabodies should be evaluated in higher vertebrate models of SCI.

Impact: SCI induced synuclein aggregation is a novel therapeutic target. These studies utilize highly selective intrabodies for synuclein engineered to direct synuclein to the proteasome for degradation using the cell's normal clearing process. The researchers hypothesize that targeted degradation of α-synuclein will preserve spinal cord tissue and expand residual neural circuitry, thereby reducing neurological damage and attenuating disability.

7. Regenerative Research Foundation[1]
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: The last six (6) months impacted the progress made due to the COVID-19 pandemic, limiting access to lab space in both experimental locations from March through the end of the reporting period. Planned experiments were scaled back, including culling animal colonies, which will take time to reestablish. Progress was made on optimizing biotinylated dextran amine (BDA) injections as well as repeating optic nerve crush studies and optimization of systemic clioquinol administration.

Future Directions: Future studies will characterize animals which have received zinc chelation following dorsal hemisection injury, as well as further characterization of zinc chelation agents used following SCI.

Impact: The researchers predict zinc chelation following SCI will enhance nerve regrowth, which could lead to new therapeutics for clinical SCI. They demonstrated multiple drugs chelate zinc *in vivo* in brain and spinal cord both before and following SCI when administered systemically, which will be used to test effects on nerve regrowth in injured animals.

8. **Research Foundation for SUNY, SUNY Polytechnic Institute**[3]
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: Neural damage to the spinal cord is life altering with substantial impact on the quality of life for the individual and caregivers and has significant economic impact. Stem cell-based therapies may alleviate partially or fully neural damage, but how best to achieve consistency remains unclear. The research team evaluated and advanced state of the art strategies with human neural stem cells (NSCs) or mature spinal motor neurons (SMNs) in a rat hemicontusion model of SCI. To retain neuroprotection of transplanted cells in a harsh SCI microenvironment the researcher's evaluated encapsulation in novel alginate-based neural ribbons and modulation of inhibitory glycosaminoglycans using chondroitinase ABC (an enzyme, which promotes neurite outgrowth and regeneration).

Progress Towards Specific Aims: The researchers evaluated multiple challenges in SCI that are: NSCs versus SMNs; neural protection and survival of transplants; host integration; and modified encapsulation technology for shippable, transplantable alginate-based neural ribbons. The neural ribbon technique required technical modifications to injection and verified placement by MRI. Optimal SMNs were achieved by substantial improvements to spinal neural differentiation protocols, evaluation of neuron 'maturity', and encapsulation as preformed validated circuits.

Future Directions: The researchers will expand animal testing numbers and behavioral studies, including methods to evaluate transplanted neural circuitry integration with host and neuron regeneration. An intravital window may be beneficial for early real time in vivo analysis during circuit (re)formation.

Impact: This study is game changing for transplantation of neurons and neural circuits, bypassing the need to differentiate from NSCs in vivo. Novel protocols for anatomically matched human SMNs and support cells that achieve survival and enhanced integration by preformation of neural circuitry in alginate ribbons.

Publications: Olmsted, Z.T. & Paluh, J.L. (2021). Stem Cell Neurodevelopmental Solutions for Restorative Treatments of the Human Trunk and Spine*. Frontiers in Cellular Neuroscience,* 15.

Olmsted, Z.T. & Paluh, J.L. (2021). Co-development of Central and Peripheral Neurons with Endoderm in Elongating Multi-lineage Organized (EMLO) Gastruloids. *Nature Communications,* 12, 3020.

Olmsted, Z.T., Stigliano, C., Badri, A., Zhang, F., Williams, A., Koffas, M.A.G…& Paluh, J.L. (2020). Fabrication of Homotypic Neural Ribbons as a Multiplex Platform Optimized for Spinal Cord Delivery. *Scientific Reports*, 10(1): 12939.

Olmsted, Z.T., Stigliano, C., Scimemi, A., Wolfe, T., Cibelli, J., Horner, P.J., & Paluh, J.L. (2021). Transplantable Human Motor Networks as a Neuron-directed Strategy for Spinal Cord Injury. *iScience.* 24(8): 102827.

9. Research Foundation of CUNY, College of Staten Island[1]

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 could 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: The research lab of the PI closed on March 17, 2020 through September 1, 2021 because of the COVID-19 pandemic. However, the lab operation is still not as before COVID-19 and tremendous safety measures are under effect for visitors to enter campus.

The research lab of the Co-PI also closed in mid-March 2020 through August 2020. However, multisession protocols such as the one in this funded project are not allowed until vaccinations are well underway. They are now preparing this site for full study activities.

Future Directions: The research team will continue to work on transspinal stimulation and locomotor training to develop innovative non-invasive methods to improve motor function and quality of life in people with spinal cord injury.

Impact: Transspinal stimulation may be used before or during locomotor training to enhance the benefits of exercise-induced neuroplasticity and neurorecovery. Based on the researchers recent published findings, transspinal stimulation paired with brain stimulation during assisted step training reorganizes the function of both flexor and

extensor reflexes, promoting a more physiological gait pattern and locomotor EMG activity in people with Spinal Cord Injury similar to the step training only protocols.

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 4^{th} cervical segment contusion injury.

Progress Towards Specific Aims: Final progress has yet to be submitted at the time this report was published.

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[2]

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 have continued their analyses of 4AP serum concentrations and analysis of 4AP levels achieved with implantation of slow-release pumps and have confirmed that they can use continuous release pumps to achieve appropriate serum levels of 4AP. Secondly, they have extended their analyses of gene expression at three (3) days post-injury and have identified multiple ways in which 4AP treatment favorably alters gene expression after SCI.

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[1]

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 investigated ZEB2 protein and Zeb2os RNA expression levels after SCI. They have shown ZEB2 and Zeb2os expression levels are up-regulated selectively in astrocytes after SCI. These changes in expression levels are part of a larger set of changes in gene expression that share several important similarities to wound healing responses in non-neural tissue.

The researchers used genetically engineered mice that lacked Zeb2 specifically in astrocytes and have shown that after SCI the loss of Zeb2 reduces the astroglial response, increases lesion size, and delays the spontaneous recovery of motor function.

Future Directions: The researchers have completed the proposed studies and have published the main findings in *Cell Reports*. They are drafting two additional manuscripts that further discuss the implications of their findings. They are also conducting new studies to manipulate the molecular mechanisms identified by studies to address whether they can modulate the astroglial response to SCI and improve the recovery of locomotor function. The post-doctoral fellow support by this grant, Dr. Ana Vivinetto, has received additional support from the Craig Neilsen Foundation to pursue the new studies.

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.

Publications: Vivinetto, A.L., Kim, I.D., Goldberg, D., Tarabykin, V.S., Hill, C.E., Cho, S., & Cave, J.W. (2020). Zeb2 is a Regulator of Astrogliosis and Functional Recovery after CNS Injury. *Cell Reports,* 31:107834.

13. Winifred Masterson Burke Medical Research Institute[1]
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: The researchers have established sensitive flow cytometry and fluorescence-activated cell (FAC) sorting protocols for injured nervous tissue to assess the distribution of immune profiles at different post-injury time points in the sciatic nerve, de-afferented dorsal root ganglia and the lumbar spinal cord. Detailed analysis of these data revealed a highly dynamic and rapidly changing inflammatory milieu. They have isolated half of the genetic samples from a defined population of sensory neurons using the Translating Ribosome Affinity Purification (TRAP) methodology. The low yield of the TRAP technique required double the number of animals initially planned. The high-quality translating mRNA samples already isolated are in storage at the Genomic and Epigenomic Core facility at Weill Cornell Medicine, awaiting library preparation for next-generation sequencing (NGS). Once all samples are collected and the library preparation finished, the core facility will perform NGS on an Ilumina HiSeq 4000.

For further characterization of immune cells accumulating in the injured nerve under regenerative and non-regenerative conditions, they used nanostring technology to assess gene expression patterns. This work identified strong activation of the complement system, an important branch of innate immunity, in the regenerating sciatic nerve. 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.

Publications: Jara, J.S., Agger, S., & Hollis, E. (2020). Functional Electrical Stimulation and the Modulation of the Axon Regeneration Program. *Frontiers in Cell and Developmental Biology*, 8.

14. Winifred Masterson Burke Medical Research Institute[1]

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: The researchers have confirmed that in B-RAF GOF mice, axonal translocation is absent prior to tamoxifen treatment and present post tamoxifen adminstration. The COVID-19 pandemic caused an almost complete halt to the researcher's lab work just as they had assembled the necessary greater animal numbers and begun tamoxifen treatment.

Future Directions: Assuming the positive trend continues with quick resumption of normal level operations, it will take them about nine (9) months to re-breed the mice and complete RNA-seq and data analyses.

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
11/1/19-4/30/20[1]
11/1/20-10/31/21[2]

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

1. **Health Research, Inc.[1]**
   Johnathan Wolpaw, M.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: The researchers substantially advanced their work aimed at streamlining and hardening the operant-conditioning system to make it robust and convenient for clinical use. During this grant period, they focused on simplifying the setup and modifying the EPOCs hardware and software to minimize the variability of the H-reflex across sessions and across participants. To accomplish this, they first compared a proportional integral derivative (PID) controller to the existing operator-dependent manual control of the stimulus. Second, they tested a multi-channel electrode array that will, once incorporated into the system, significantly simplify set-up procedures for clinicians. Third, they began to develop automated parameterization of the system by describing the impact of specific settings (e.g., pulse width) on the Hmax-to-Mmax ratio. In addition, they examined the sources of artifacts in the EMG.

   To facilitate the clinical use of the reflex conditioning protocol, they have drafted the first manual that includes instructions for system setup, operation, and troubleshooting. In the upcoming year, they will revise this manual based on feedback from users and further system development.

   From feedback to date, they have learned that clinicians want to understand the basic physiological mechanisms and principles of reflex conditioning. Thus, to increase their interest, engagement, and success in reflex operant conditioning, the researchers are creating a small reflex conditioning resource that provides basic background introductory material to clinicians. The researchers will continue to obtain input from clinicians to optimize the reflex conditioning guide and the user manual so that they are maximally meaningful and useful to clinicians.

To create the operant-conditioning system that is usable for clinicians the researchers have developed procedures to test the system's usability and robustness for patient screening/preliminary sessions, and reflex conditioning sessions. The user-test protocol consists of one introductory session (didactic presentation and demonstration) and four experimental sessions (i.e., two systems times two different types of sessions). The clinician test-users compared the existing system with the new system.

Each phase of the user test includes returning clinician users and novice clinician users. This enables the researchers to understand: the users' cumulative skill learning and knowledge of the system; and the usability of the system, both separately and in combination.

Future Directions: The researchers will prepare for and conduct the second phase of clinician user-tests including a further improved version of the developing software system, complete data collection to compare new algorithms for controlling M-wave size accurately and consistently throughout the study, continue to advance in automation and optimization of specific components of the reflex conditioning protocol (e.g., updating of the reward criterion), develop and test multi-grid arrays.

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.

2. University of Rochester[2]

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 our research is treatment with the potassium channel blocker 4-aminopyridine (4AP), starting 18-24 hrs after injury, and the researchers studies thus far indicate that effects of 4AP are sufficiently robust to jusitify planned progress to clinical trials.

During the past year the researchers have conducted experiments on two questions relevant to the clinical translation of their discoveries. The first of these is an analysis of the effects of age on the response to 4AP treatment of acute traumatic SCI. They have

conducted contusion injuries on 1 year old rats, which are thought to be developmentally more equivalent to 30-year old humans. Secondly, they have combined physical therapy with 4AP treatment to test whether the physical therapy would unexpectedly decrease the benefits of 4AP. This is important to know as one benefit of 4AP treatment is earlier improvement in motor function. In humans, such an outcome would lead to earlier initiation of physical therapy.

Future Directions: The researchers are continuing the analyses of effects of 4AP on the recovery of 1 year old rats, with an expanded number of animals and a longer treatment period.

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
3/1/20-8/31/20

9 Awards (3 active, 6 previously concluded), Procurement Total: $1,341,954

1. **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 are able to long-term depict the changes of firing patterns in CPGs along with functional recovery after SCI.

   Progress Towards Specific Aims: To image neuronal activities at a greater depth, the researchers incorporated deformable mirror into their 3P imaging system to compensate tissue-induced aberration and enhance signal strength in deep labeled structures. The researchers have established a robust experiment platform to image deep neuronal functions using 3P while the animals were spine-fixed and awake, locomoting. This novel imaging method enables a whole new class of experiments that aimed to investigate the neuronal basis of behavior in the spinal cord.

   Future Directions: Training in the Schaffer Lab has been preparing Dr. Cheng for a career in translational academic research. A postdoctoral fellow in the team will continue to finish up the final experiment, repeating more awake calcium imaging experiment.

   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.

2. **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: Upon activation, microglia undergo rapid morphological, immunological, and physiological transformations. The researchers' RNA-seq unveiled the underlying progressive transcriptional changes, while integrated single-cell transcriptomic analysis comparing steady-state, SCI, and HDAC3 inhibition revealed refined microglial subtype classification. They also documented diversification of other glial cells and a wide influence of HDAC3 in these cells, arising either directly, or indirectly from an altered inflammatory milieu.

The researchers identified four (4) transcriptional subtypes in microglia: an immunity focused MG1, a reactive MG2, an immediate response MG3, and a proliferative MG4. The current study provided a snapshot of microglial heterogeneity at 5 dpi; it is not clear whether individual microglial cells can switch from one subtype to another, or if proliferation largely accounted for subtype expansion. Future longitudinal studies with tracking different microglial subclusters will address whether functional assignment is fluid over time.

In summary, the researchers discovered a wide range of activation states in microglia both at baseline and after SCI. Activated microglia and influxed macrophages progressively acquired an overall reparative but highly diversified transcriptional profiles while maintaining their distinct transcriptional identifies. The injury responses and functional diversifications of immune and glial cells are influenced by chromatin regulator HDAC3, which can be explored to enhance neural repair.

Future Directions: Dr. Wahane will continue to improve skills for independent thinking, project planning, and running successful collaborations, with a long-term goal to become an independent researcher in the field of immunomodulation of neural injury 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.

3. 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: The researchers ablated basal forebrain cholinergic neurons with a targeted toxin (saporin), and measured the effects on motor coordination, learning and retention. They also studied the contribution of cholinergic inputs to medial prefrontal cortex and motor cortex to coordinated motor behavior. They are developing fiber photometry for recording cholinergic neuron activity. They also performed Lynx1 overexpression experiment and updated chemogenetic data.

Future Directions: Researchers will record neuronal activity during rotarod training by using fiber photometry and submit manuscript.

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
1/1/20-6/31/20[1]
7/1/20-12/31/20[2]

11 Awards (3 Active, 9 previously concluded), Procurement Total: $6,264,035

1. **Research Corporation of Long Island, Inc., Northport VA Medical Center[2]**
   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: The researchers have conducted an experiment using a new population of stem cells specific to spinal cord. They have created a structure of pluripotent stem cells expressing green fluorescent protein (GFP) to locate the presence of these cells in the spinal cord tissue. Rats received contusion SCI and two (2) weeks later were implanted with these NiPSCs followed with sEMS and exercise treatment.

   They have used one component of proposed combination treatment (i.e. sEMS) in human study and examined effect of sEMS on neurophysiological properties of spino-muscular circuitry in healthy and SCI humans.

   Future Directions: They have examined effects of proposing combination treatment in SCI rats and completed examination of the combinational treatment and each individual component of this treatment. Results demonstrate successful survival of the NiPSCs after transplantation, associated with improved function (evaluated using open field locomotion scoring), as well as extensive presence of 5-HT labeled fibers (based on results of 5-HT immunochemical staining).

   They also have translated one component (SEMS) to humans and accomplished a pilot study examining effects of SEMS in healthy and SCI humans.

Future Directions: Use current results of their animal experiment to translate proposing triple combination treatment to clinics.

Impact: Results of their experiments revealed beneficial effects of proposing triple combination treatment (NPCs, sEMS and exercise). These results provide a strong base for developing a novel, feasible and effective translational set of treatments for acute and chronic contusion SCI.

Their pilot human studies revealed that administration of sEMS (one component of combination treatment) may modulate H-reflex amplitude and threshold intensity of nerve stimulating current in both healthy and SCI humans, thus suggesting that sEMS may excitability at nerve-to-muscle circuitry. Thus, administration of sEMS may induce partial recovery of excitability at nerve-to-muscle circuitry in SCI human subjects.

2. **Research Foundation for SUNY Stony Brook**[2]
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 have completed four (4) participants with sEMS on higher spinal levels by using circular coils as a supplement to evaluate the effectiveness of electrodemagnetic stimulation. They will compare outcomes in SCI before and after 5 weeks of sEMS only versus sEMS and LT.

Future Directions: One trainer was hired for the LT component, and four (4) additional trainers are under the process of hiring which was on hold due to the COVID-19 pandemic. After completion of the last two phases, statistical analysis will be done within the SCI group and SCI+LT group; cross group comparison will also be analyzed.

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

3. **Research Foundation of CUNY, College of Staten Island**[1]
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: Recruitment, experiments and/or administration of rehabilitation intervention in people with SCI has been completed. The researchers registered the study (ClinicalTrials.gov, NCT04624607) to be able to publish the results of the project as it is required from many clinical scientific journals.

Future Directions: Data analysis, development of manuscripts and submission of original research articles for publication are the next steps.

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

Publications: Islam, M.A., Zaaya, M., Comiskey, E., Demetrio, J., O'Keefe, A., Palazzo,…& Knikou, M. (2020). Modulation of Soleus H-reflex Excitability Following Cervical Transspinal Conditioning Stimulation in Humans. *Neuroscience Letters,* 732: 135052.

Zaaya, M., Pulverenti, T.S., Islam, M.A., & Knikou, M. (2020). Transspinal Stimulation Downregulates Activity of Flexor Locomotor Networks during Walking in Humans. *Journal of Electromyography and Kinesiology*, 52: 102420.

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

Progress Reporting Period
9/1/19-2/29/20[1]
3/1/20-2/28/21[2]
3/1/21-8/31/21[3]

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

1. **Albany Research Institute, Inc. – Albany Stratton VA Medical Center[3]**

   Funding was to support staff who direct aspects of SCI work; their work will enable new applications focused on developing new therapeutic methods that enhance recovery for people with SCI.

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

   Research supported by this funding is aimed at elucidating the therapeutic potential of targeting the novel microtubule regulatory protein FL2 to promote axonal regeneration after SCI. The data generated with this funding will further the research team's understanding of how the microtubule cytoskeleton regulates axonal growth, and may potentially provide the groundwork for a novel therapeutic target for promoting functional recovery after SCI. Their data has been used to apply for several SCI and nerve regeneration related grants. A manuscript is under review.

3. **Bronx Veterans Medical Research Foundation – James J. Peters VA MedicalCenter[3]**

   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. Genetic analyses will serve as one of the primary study outcomes for which a future clinical trial may be designed.

4. **Columbia University[1]**

   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.

   Right now, the engineering feasibility of the two systems are being verified. In the coming years, the systems will be extended for pilot studies with SCI participants.

   Presentations: Stramel, D. & Agrawal, S. K. *Validation of a Forward Kinematics Based Controller for a Mobile Tethered Pelvic Assist Device to Augment Pelvic Forces during Walking.* IEEE Virtual Conference on Robotics and Automation, 2020.

   Publications: Chang, B. C. & Agrawal, S. K., Biomechanical Differences during Ascent on Regular Stairs and on a Stairmill. (2020). *Journal of Biomechanics*, 104.

5. Cornell University[3]

Currently this grant continues to partially fund 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[3]

This grant provides partial salary support and funding for three ongoing projects that share the goal of improving the understanding of how to best promote neurological recovery and wellness in people with SCI.

Publications: Morrison, D., Parrish, J., Arcese, A., Herman, P., Gibbs, K., Stein, A., & Bloom, O. (2021). Systemic Gene Expression Profiles According to Pain Types in Individuals with Chronic Spinal Cord Injury *Molecular Pain*, 17:17448069211007289.

Presentations: Bloom, O., Botticello, A., Delgado, A., Trevor Dyson-Hudson, T., Galea, M., Kirshblum, S.,…& Bryce, T.N. *Effects of the COVID-19 Pandemic on People with SCI: Understanding Acute and Long-term Physical, Psychosocial, and Functional Outcomes*. Annual Scientific Meeting of the American Spinal Injury, Virtual Meeting 2021.

Morrison, D., Bank, B., Stein, A., Herman, P., Lee, A., Boakye, M.,…& Bloom, O. *Biomarkers of Spontaneous Recovery from Traumatic SCI*. Annual Scientific Meeting of the American Spinal Injury, Virtual Meeting 2021.

7. Health Research, Incorporated[3]

Funding supported ongoing work to develop molecular methods to determine how transcriptional changes in spinal cord activity are correlated in rats with spinal cord injury responses to the various forms of therapy designed to recover motor function.

Presentations: Electro-cortical stimulation increases *Gad1* transcription of ventral spinal cord GABAergic interneurons but does not change *Gabbr1* expression of soleus motoneuron. Poster presented at Society for Neuroscience (SFN) global connectome January 11-13, 2021.

Electrocortical stimulation (ECS) of sensorimotor cortex (SMC) in rats decreases expression of G-protein-coupled inwardly rectifying potassium channel 2 (GirK2) in spinal cord motoneurons. To be presented at 2021 Neuroscience meeting.

Electrocortical stimulation (ECS) of rat sensorimotor cortex (SMC) may increase glutamate ionotropic AMPA receptors and their associated mRNA in spinal cord motoneurons. To be presented at 2021 Neuroscience meeting.

8. Icahn School of Medicine at Mount Sinai[3]

With the support of this funding, Principal Investigator, Hongyan Zou, M.D., Ph.D. and Co-Principal Investigator, Roland Friedel, Ph.D., are directing their laboratories to generate data on novel function of Plexin-B2 signaling in mediating innate immune

response after SCI and after peripheral nerve injury. These data also formed the basis of an NIH R01 grant application centered on Plexin-B2 and nerve repair.

Presentation: Zou, H. *Epigenetic and Glial Biology in Axon Regeneration.* Gordon Research Conference on Glial Biology, Maine, 2021.

9. New York University[3]

The research team reported development of a robust human motor neuron differentiation protocol producing high-quality cranial motor neurons (CMNs) and spinal motor neurons (SMNs). They have established a stem cell-based system to investigate the mechanisms that protect human CMNs from neurodegeneration.

Results obtained from this funding supported two new grant applications: one is pending, and one is funded by the Chan Zuckerberg Initiative Neurodegeneration Challenge Network (NDCN) to produce human motor neurons to analyze the effect of C9orf72 (a gene providing instructions for making a protein that is found in various tissues) mutations.

10. Regenerative Research Foundation[3]

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.

Single cell data has indicated multiple stages of B cell engagement, post-injury. During this period, significant effort has been dedicated to teasing out the distinctions of the various B cell subtypes as well as any time-dependent behaviors. The research team's single cell data collected contains clues about T cell interactions concomitant with the previously discussed dynamics. They expect this data to suffice as preliminary data for grant submissions to the NIH and to the NYS SCIRB.

11. Rensselaer Polytechnic Institute[2]

Support was requested to support student stipends and laboratory supplies. At the time this report was written, progress on their studies has yet to be submitted.

12. Research Corporation of Long Island, Inc. – Northport VA Medical Center[3]

The research team has several new insights regarding the possible processes and mechanisms of PSEMS (pulse at 20 Hz frequency for 5 sec with 25 sec break between pulses, total 40 trains for 20 minutes) They hypothesize that Buprenorphine and PSEMS might share common sites of action and suggest that PSEMS might carry potential to recover, at least partially, some deficits, including pain, after SCI. Preliminary results of their on-going pilot study indicate that PSEMS may induce reduction of chronic low back pain in humans and thus can serve as an effective, non-invasive treatment approach for chronic low back pain.

This research provides a strong foundation for translation results of their animal experiments into humans. Administration of electro-magnetic stimulation (EMS) at spinal

levels to stimulate spinal roots is FDA approved. They have begun using EMS in human studies at Northport VA (collaboration with Dr. Fahmy) and Stony Brook University (Collaboration with Dr. Sisto).

13. Research Foundation for SUNY – Downstate Medical Center[3]

Support was requested to support student stipends and laboratory supplies. At the time this report was written, progress on their studies has yet to be submitted.

14. Research Foundation for SUNY – Stony Brook[3]

The supplies purchased can be used by SCI researchers that are generating rodent contusion SCI models and/or examining the efficacy of various therapeutic interventions on tissue-level biomarkers as part of their evaluation. This information could aid in the identification of potential cellular targets in the development of future mechanistic studies and grant applications.

15. Research Foundation for SUNY – University at Albany[3]

The researchers anticipate that the ChIP-seq data, WGBS data, and 5hmC meDIP data will become a valuable resource for studying the epigenetics of SCI and other forms of CNS injury. By comparing differences in epigenetic states between situations where CNS axons successfully regenerate against those that do not, they have gained fundamentally new insights into molecular mechanisms underlying the success or failure of regenerative responses to CNS injury in a vertebrate animal model.

The raw sequencing data and the BigWig files derived from these have now been deposited at the National Center for Biotechnology Information (NCBI) in their Gene Expression Omnibus (GEO database.

They plan to use these data as preliminary data for additional awards from NYS SCIRB, the National Science Foundation, and the National Institutes of Health. Applications to the NSF and NYS SCIRB were submitted previously to build on this project, but reviewers advised us to strengthen the applications with publications from this work. In a previous reporting period, we completed the first stage of our study, which was published in BMC Genomics.

The long-term goal is to gain critical insights into how thyroid hormone, which is now established as directly responsible for the loss of CNS regenerative capacity in development, represses the ability of CNS neurons in adult vertebrates to express the genes crucial for this repair. Their hypothesis is that exposure to thyroid hormone during development introduces epigenetic modifications to chromatin structure (DNA methylation state/histone methylation-acetylation state) that determine whether the response to injury is regenerative or not.

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. They hope this knowledge will facilitate the design of new therapeutic approaches to attenuate or even reverse this loss in human SCI.

16. Research Foundation of CUNY – Staten Island[3]

Funding provides partial support for Sreyashi Samaddar, Ph.D., a post-doctorate fellow hired to work on biochemical and PCR experiments. A Ph.D. student also worked on aspects of the (tracing) experiments. Data generated from these experiments will be used to support future IDEA applications.

17. Research Foundation of CUNY – The City College of New York[3]

Funding was used to purchase research supplies and general laboratory supplies. Funding also supported animal procurement and husbandry. Overall, these purchases contribute to the goal of the project, to build capacity for mechanism-based pharmacological approaches to SCI research at the City College of New York (CCNY). Successful mastery of the research team's experimental techniques as well as data generated from experiments would then facilitate additional resources to support a larger program of mechanism-based drug discovery for SCI. Their efforts over the last four (4) years have now begun to yield next-step results. Data generated from this project was used for a National Institutes of Health grant proposal. The research team has also begun planning an IDEA proposal.

18. Syracuse University[3]

The materials, resources and data generated through this funding generated essential components and preliminary data for a full proposal that was submitted to the National Science Foundation (NSF) to analyze the created mutants in more detail and establish a fully characterized Gene Regulatory Network for the specification of V0v neurotransmitter properties. This grant has now been funded. The funding is now helping the research team to create and maintain essential mutant lines, which are important for future grant applications.

Publications: Juárez-Morales,J.L., Weierud, F., England, S.J., Demby, C., Santos, N., Mazan, S., & Lewis, K.E. (2021). Evolution of *Ibx* Spinal Cord Expression and Function. *Evolution and Development*, 23: 404-422.

19. University of Rochester[3]

This funding is used to further develop the abilities of the University's Spinal Cord Injury Operational Network (SCION) to serve the needs of the SCI research community and has helped the princiapl investigators recruit new investigators to the field of SCI research.

SCION provides a central hub that enables high quality SCI research in multple laboratories, without requiring each laboratory to create its own unique resource and equipment base. SCION currently is equipped for traumatic spinal cord injury models (Infinite Horizons spinal cord contusion impactor), behavioral and sensory analysis (Catwalk gait analysis system, Gridwalk motor test, staircase forepaw motility test, Hargraves thermal hyperalgesia testing), electrophysiological analysis (motor evoked potential and somatosensory evoked potential measurements, intra-spinal evoked field potential measurements, in vitro multi-electrode array testing), treadmill-based physical therapy and advanced microscopy using design-based stereology (MicroBrightField Stereo Investigator and Neurolucida systems).

Publications: Elahi, A., Emerson, J., Rudlong, J., Keillor, J.W., Salois, G., Visca, A.,…& Proschel, C. (2021). Deletion or Inhibition of Astrocytic Transglutaminase 2 Promotes Functional Recovery after Spinal Cord Injury. *Cells*, 10(11): 2942.

20. Winifred Masterson Burke Medical Research Institute[3]

The research team is currently working on acquiring additional preliminary data for revised submissions to the Department of Defense – Congressionally Directed Medical Research Programs SCI Research Program Clinical Trial Award and the New Jersey Commission of Spinal Cord Research. Based on the work in developing these grant proposals, they held Institutional-level discussion between Burke Neurological Institute and Kessler Rehabilitation Institute about ways to facilitate and grow SCI clinical trials. This relationship has led to further collaborations between Burke researchers and Kessler researchers to facilitate recruitment and completion of an ongoing repetitive transcranial magnetic stimulation feasibility and safety study. This establishment of this relationship, as well as our new patient recruitment efforts, have been integral to the ability of Dr. Zhong to accomplish his ongoing NYS SCIRB funded trial. During the prior reporting period while on Covid-19 shut down, Dr. Zhong finalized plans with Kessler to complete his PART-funded studies and now that clinical research is reopened this study has continued. In addition, Dr. Zhong presented a webinar for interested members of the community hosted by the Burke Neurological Institute, entitled “Repairing Lost Connections – New Concepts to Treat Spinal Cord Injury,” which resulted in significant interest in this new trial.

This award has been used, in part, to develop the infrastructure to support an expanding spinal cord research focus. This has allowed the research team to recruit some of the best and brightest spinal cord injury researchers. The ongoing NYS SCIRB funding has been instrumental in this recruitment. One of their relatively recent recruits has just been awarded two (2) new grants from this funding. Dr. Yutaka Yoshida has been granted the Innovative, Developmental, Exploratory Activities (IDEA) and Projects to Accelerate Research Translation (PART) awards in this last cycle.

Publications: Vivinetto, A.L. & Cave, J.W. (2021). *Zeb2* Directs EMT-like Processes that Underlies the Glial Response to Injury. *Neural Regeneration Research*,16(9):1788-1790.

2/1/20-8/14/20[1]
8/15/20-8/14/21[2]

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

1. **Columbia University[2]**
   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: The goal during the first three (3) years of the project was to develop and test a Robotic Upright Stand Trainer for patients with SCI that mimics the manual training provided by physical therapists but requires far less physical effort from them. Detailed discussions and visits between the two teams at Columbia University and University of Louisville led to the design of RobUST. Based on the cable-based architecture of the Tethered Pelvic Assist Device (TPAD), RobUST was designed to apply forces at three levels in the human body when a subject is standing (at the trunk, pelvis, and the knees). This system is controlled by forces applied by four (4) wires at the trunk, eight (8) wires at the pelvis, and two (2) wires at the knees. Each wire is controlled in tension by a servomotor and the controller uses real-time data of the body segment from a motion capture system for closed-loop control. These motors are placed on a fixed frame around the standing area.

   A computer aided design (CAD) model was first created to visualize the design, its dimensions, and potential use of the system. Once the system design was critiqued and finalized, component mechanical parts were ordered, machined, and assembled as per the design of the system. Computers, data acquisition systems, sensors, and electronics were integrated within the overall system. With 14 motors driving 14 cables, associated drive electronics, in-line tension sensors, the overall electronics and cable management presented many challenges, which were overcome with critical thinking and collective experience of the team. The entire system was then programmed and tested at the ROAR lab at Columbia University.

   The salient feature of RobUST is real-time control of 14 motors while being able to generate appropriate forces on the human body, at the trunk and the pelvis. The design of such a system has not been attempted by researchers in the SCI community and as expected, presented several unique challenges. Once the system was tested with appropriate tension control of wires at Columbia ROAR lab, a copy of the stand-trainer was designed and installed at University of Louisville.

   RobuST is a novel robotic device and unique in the field of stand training of spinal cord injured patients. It uses a unique cable-driven design, state-of-the-art motors, real-time controller, and tension sensors. In addition, the system is integrated with a motion

capture system, force-torque sensors to measure ground reaction forces during standing, body-suspension harness, and other safety modules. The full system was designed and assembled at both Columbia ROAR laboratory and University of Louisville.

Future Directions: Pilot human studies are being performed at both places to characterize the human performance for both able bodied and those with SCI.

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. This is the first robotic device designed for stand training and the platform may significantly enhance the capability of clinical personnel to test different rehabilitation interventions of subjects with spinal cord injury.

Presentations: Luna, T.D., Santamaria, V., Omofuma, I., & Agrawal, S.K. *Control Mechanisms in Standing during Simultaneously Receiving Perturbations and Active Assistance from the Robotic Upright Stand Trainer (RobUST).* IEEE International Conference on Biorobotics and Biomechantronics, Virtual 2020.

Publications: Luna, T.D., Santamaria, V., Omofuma, I., Khan, M.I., & Agrawal, S.K. (2021). Postural Control Strategies in Standing with Handrail Support and Active Assistance from Robotic Upright Stand Trainer (RobUST). *IEEE Transactions on Neural Systems and Rehabilitation Engineering,* 29:1424-1431.

2. Research Foundation of CUNY, The City College of New York/CUNY School of Medicine[1]
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: Progress was significantly limited by the COVID-19 pandemic with the mandated shutdown. Nevertheless, during the lockdown, they were able to conduct a limited set of computer-based data analyses off-site. Upon partial reopening and resumption of experiments, they reestablished behavioral performance levels and have since performed the two (2) contusion injuries. They have also begun therapy for the chronic SCI subject and received IRB approval to amend the protocol for initial human phase to test safety and tolerability of cervical direct current stimulation (DCS).

Future Directions: The research team will determine the effects of contusion injury on reaching and step-cycle kinematics; integrity of the corticospinal axons at and around

the cervical contusion site; finalize experiments to establish a role for spinal motor neurons in mediating trans-spinal (ts) DCS efficacy; continue to add subjects to assess the therapeutic efficacy of dual motor cortex-spinal cord stimulation; finalize experiments on connection strength; and add new investigations on spinal motor neuron excitability by studying the physiological analogue of the mechanical stretch reflex; and implement spinal DCS in human studies.

Impact: Replication of the research team's therapeutic neuromodulatory strategy in the rat underpins development of and translation to the large-animal cat SCI model. They are on the path to translate this approach successfully to their large-animal cat model. The feline studies help move them towards establishing tsDCS as a non-invasive and well tolerated therapy for promoting spinal motor functions and motor behavior after SCI. Their findings provide evidence that a particular spinal neuron class, the motor neuron, is an important target of tsDCS. These results inform implementation in the human studies. 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.

Appendix 3

NEW YORK STATE SPINAL CORD INJURY RESEARCH BOARD

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1 Service commenced during 2020
2 Service concluded during 2020
3 Service commenced during 2021
4 Service concluded during 2021