OFFICE OF HIGHER EDUCATION

Spinal Cord Injury and Traumatic Brain Injury Research Grant Program, 2020 Report

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About the Minnesota Office of Higher Education

The Minnesota Office of Higher Education (OHE) is a cabinet-level state agency providing students with financial aid programs and information to help them gain access to postsecondary education. The agency also serves as the state's clearinghouse for data, research and analysis on postsecondary enrollment, financial aid, finance and trends.

The Minnesota State Grant Program is the largest financial aid program administered by the Office of Higher Education, awarding up to $207 million in need-based grants to Minnesota residents attending eligible colleges, universities and career schools in Minnesota. The agency oversees other state scholarship programs, tuition reciprocity programs, a student loan program, Minnesota's 529 College Savings Plan, licensing and early college awareness programs for youth.

About This Report

This is a legislative-mandated report. As requested by Minnesota Statutes, section 3.197, OHE estimates that the total cost of preparing this report was $1,662.03, including staff time.

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Contents

Introduction

The State of Minnesota established the Spinal Cord Injury and Traumatic Brain Injury (SCI-TBI) Research Grant Program effective July 1, 2015. Minnesota 2015 Session Law, Chapter 69 directed the Commissioner of the Minnesota Office of Higher Education (OHE) to establish a grant program for institutions in Minnesota to conduct research that would lead to new and innovative treatments and rehabilitative efforts for the functional improvement of people with spinal cord injuries and traumatic brain injuries. Research areas include, but are not limited to, pharmaceutical, medical devices, brain stimulus, and rehabilitative approaches and techniques. **Appendix A** provides a copy of the grant program's founding statute.

In July 2018, the Spinal Cord Injury and Traumatic Brain Injury Grant Program was given a Special Revenue Account by Minnesota Management and Budgt in order to extend project periods from 1-2 years to a 2-5 year timeline. Beginning with the FY 2020 competition, grantees are given 2-5 years to complete their research projects, with a possibility for an extension based on their progress and the complexity of the research. The timeline extension is crucial for the completion of projects based on lengthy Institutional Review Board (IRB) review processes and unexpected challenges that occur naturally with complex research and experimentation.

For the 2020-2021 biennium, $3,000,000 was made available for each year from the 2019 Omnibus Higher Education Bill (Minnesota 2019 Session Law, Chapter 69) to support the SCI-TBI Grant Program, with a 3 percent administrative fee. As directed by the program's statute, the Commissioner of the Office of Higher Education, in consultation with the program's Spinal Cord Injury and Traumatic Brain Injury Advisory Council (Advisory Council), will allocate 50 percent of the grant funds to research involving spinal cord injuries and 50 percent to research involving traumatic brain injuries throughout the biennium.

Spinal Cord Injury and Traumatic Brain Injury Advisory Council

The 2015 statute language that established the grant program also established the Spinal Cord and Traumatic Brain Injury Advisory Council. The Commissioner, in consultation with the Advisory Council, has the responsibility of awarding the SCI-TBI grants and developing the program. In 2015, an initial 12-member Advisory Council was set up using the Open Appointments process of the Minnesota Secretary of State's office. In 2017, the statute language was updated to include two new seats: 1) Veteran with a Traumatic Brain Injury, and 2) Physician Specializing in the Treatment of Spinal Cord Injury. Both seats were filled in 2018, although the Veteran with a Traumatic Brain Injury representative resigned at the end of 2018 due to personal reasons.

One persistent challenge in maintaining continuity within the Advisory Council is securing veterans with a spinal cord injury or traumatic brain injury to fill those corresponding roles. Many veterans who have joined the council do not persist through their first year for personal reasons, mainly related to their health and wellness. A future consideration is to reconfigure those council seats so that veterans with these injuries may send a representative from Veterans Affairs in their place.

In 2019, Mr. Robert Wudlick, advocate and community member with a spinal cord injury, was chosen to serve as the Advisory Council chair for the 2019-2020 biennium. Several of the 2018 appointments were also up for renewal. The Commissioner of the Office of Higher Education selected the Advisory Council through the Minnesota Secretary of State's Open Appointments process. The full membership of the Advisory Council is shown below; **new and reconfirmed members are bolded:**

Table 1: Advisory Council Roster

Member	Representing
Dr. Uzma Samadani	Physician specializing in the treatment of traumatic brain injury
Dr. Maxim C-J Cheeran	University of Minnesota Medical School
Dr. Peter J. Grahn	Mayo Clinic
Dr. Margaret M. Weightman	**Courage Kenny Rehabilitation Center (replacing Ms. Nancy Ann Flinn)**
Dr. Nova McNally	Hennepin County Medical Center
Dr. Andrew W. Grande	**Neurosurgeon**
Mr. Robert Wudlick, Chair	**Person with a spinal cord injury**
Mr. Matthew Rodreick	Family member of a person with a spinal cord injury
Ms. Carly Challender	**Person with a traumatic brain injury**
Mr. Brian Morrissey	**Veteran who has a spinal cord injury**
OPEN	Veteran who has a traumatic brain injury
Dr. Mark Gormley	Gillette Children's Specialty Healthcare
Mr. Bruce Richard Everling	**Family member of a person who has a traumatic brain injury**
Dr. Ann Parr	Physician specializing in the treatment of spinal cord injury

COVID-19 Disturbances and Actions Taken

Fiscal Year 2021 Annual Research Grant Proposal Solicitation Schedule

Initially, the FY 2021 grant cycle was set to take place over the late winter/spring of 2020, over the course of the following timeline:

December 2, 2019	Request for Proposals available to applicants
February 28, 2020	Deadline for receipt of intent to submit forms
4:30 p.m., April 3, 2020	Deadline for receipt of proposals
May 11, 2020	Proposal Review Meeting/Project Presentations
June 1, 2020	Notification of recommendation for grant award
July 1, 2020	Project funding begins with grant contract encumbrance

While OHE received nearly 50 intent to submit forms by February 28, the onset of the coronavirus disease 2019 (COVID-19) created some concern for proceeding with the timeline set for this grant cycle. On March 13, 2020, President Donald Trump announced a national State of Emergency due to the rapid spread of the disease. With so much uncertainty surrounding the state and immediate future of health and medicine, the Advisory Council decided to call an emergency meeting to discuss a plan of action. In the meantime, OHE sent an email to all applicants letting them know that proposal submission would be postponed due to the virus.

On April 3, instead of collecting and disseminating SCI-TBI proposals, the Advisory Council met virtually and decided to reschedule the FY 2021 grant cycle until winter 2021. Furthermore, it was brought to OHE's attention that research institutions such as the University of Minnesota and Mayo Clinic were diverting resources to respond to COVID-19 through testing, vaccination development and subsequent trials. Many current SCI-TBI grantees were asked to shut down their labs and active clinical trials until further notice, despite the threat of losing valuable research and materials during the closure. While each institution had different protocols around the use of resources and continuation of projects during COVID-19, all projects were halted. Most principal investigators were instructed to continue to pay staff salaries in order to maintain their projects. Because of the debilitating cost of shutting down labs/clinical trials, losing animals/cells/human subjects, and restarting projects once allowed, OHE and the Advisory Council decided to prioritize current grantees so that their progress would not be lost. Therefore, a one-third of FY 2021 funds would be set aside for a supplemental funding competition, open only to current grantees in order to keep them afloat during and after COVID-19. The remainder of funds would be awarded to new projects during the rescheduled FY 2021 grant cycle. On 7/30/2020, the following email was sent, notifying grantees of the supplemental grant opportunity:

> Hello current SCI-TBI grantees!
>
> I'm writing to notify you that the Minnesota Office of Higher Education, in collaboration with the Spinal Cord Injury and Traumatic Brain Injury Advisory Council, are releasing a one-

time supplemental grant RFA to assist in recovering losses due to the onset of COVID-19 and its ongoing impact on these projects. This competition is only open to our current grantees; this funding pertains only to projects that have needed to suspend their project or adjust their protocol due to COVID research, treatment, etc. and have experienced material or financial loss due to the project disruption.

Funding

$1M of our annual SCI-TBI budget (a total of $3M) has been set aside in order to accommodate loss recovery. The funding cap per project is $50,000.

Requested funds should encompass expenses incurred between March 13, 2020 and August 1, 2020.

Application

The application is attached to this email. This is a closed competition.

To apply, fill out each question in the attached application. Please make sure you are clear, concise, and thorough about the information you include. Avoid use of jargon if possible.

E-mail the application, budget, and budget narrative to **Alaina.DeSalvo@state.mn.us** by August 28, 2020, 4:30pm.

Criteria

The proposals will be reviewed by the SCI-TBI Advisory Council and evaluated on the need for project, potential impact, and safety precautions in addition to the budget.

Timeline

Supplemental RFP released: July 30, 2020
Applications due: August 28, 2020
Notification of award: September 21, 2020

Funding would be included into existing contracts as a grant amendment. If you have any questions, let me know!

Thank you.

Supplemental Grant Selection Process

On September 17, 2021, the Advisory Council convened virtually to evaluate and select supplemental grant proposals. A total of 26 proposals were submitted; 13 to supplement spinal cord injury research and 13 to supplement traumatic brain injury research.The council was split into two groups: one specializing in spinal cord injury proposal assessment and one specializing in traumatic brain injury proposal

assessment. The format and review process mirrored that of the annual research grant review; however, the criteria used to evaluate proposals was altered to fit the purpose of this unique, specific situation and addressing project disruptions caused by COVID-19. A copy of the proposal evaluation criteria can be found in **Appendix B.**

Each proposal was reviewed and scored by members of the specialty-area review panel reflective of the proposal's research focus. For the review, Advisory Council members with a scientific background gave particular attention to the scientific and technical merit of the proposals, while members with patient or community perspectives gave particular attention to the importance of the proposed research for patients. Proposals were scored individually and discussed during the meeting. Advisory Council members were required to disclose any conflict of interest with any submitted proposals. If direct conflict of interest was present, the Advisory Council member did not review the proposal and was excused from the room when the proposal was discussed.

Through this process, the Advisory Council completed their reviews of the 26 supplemental grant proposals submitted to the Office of Higher Education. All 26 proposals were recommended for funding, with many of the budgets requiring further modifications from the grantee to reflect strict losses over the course of four months, and not the cost of recouping those losses. A total of $787,673 was awarded, $310,337 to supplement spinal cord research and $477,356 to supplement traumatic brain injury research.

COVID-19 Emergency Supplemental Grant Project Summaries

A summary of projects selected for supplemental funding, and an explanation of what is being funded is listed below.

Spinal Cord Injury Research

iOptimize: Optimization of Epidural Stimulation for Spinal Cord Injury, HCMC/Hennepin Healthcare Research Institute, University of Minnesota, receives $46,904

This project is an exciting opportunity to develop a novel approach to treat chronic spinal cord injury. However, without access to the additional data and the appropriately skilled employee, the intended progress necessary for the completion of the study would be untenable. As such, additional funding and time is needed to complete the project. Supplemental funding will be used to retain study staff at Hennepin County Medical Center and at the University of Minnesota for this study. Research staff is building up to previous capacity to accommodate all study activities at a faster than planned rate, including: monthly follow ups, screening new study candidates, training for autonomic visits, analyzing data, and working on development for the iOptimize study. As such, the successful completion of the grant requires an additional five-six months of salary support to recoup for missed data collection time points (monthly follow up visits and patient preference data points) as well as the appropriate analysis of the procured data.

Principal Investigator(s): Dr. David Darrow, (214) 564-0623, **darro015@umn.edu**.

Intranasal Insulin to Improve Recovery Following Cervical Spinal Cord Injury, HealthPartners Institute, receives $19,363

Like several other projects, the COVID-19 pandemic has considerably slowed research and additional funds to retain paid research staff is necessary to complete this project. HealthPartners Institute, being a non-profit laboratory, relies heavily on academic volunteer internships (students) during both the academic semesters and during the summers to help with laboratory tasks. Two of these tasks include animal behavior tests with subsequent analyses and immunohistochemical analyses of brain slices. Tasks such as these are valuable learning tools for undergraduate students planning on attending both graduate and medical school, and are beneficial for the lab as they help perform data analyses. Indeed, the internship program allows the lab to keep costs low for studies such as these. Unfortunately, due to the pandemic, all volunteer internships across the HealthPartners organization were cancelled starting in March, and are still not allowed. As such, the principal investigator had to use staff in the lab to compensate – this impacts the budget, which accounted for volunteer time. Supplemental funding will be used to recover the costs to the budget to pay for extra time and extra personnel who were working on

this project unexpectedly. Without obtaining funding to support the time to replace the students, the aims of this study would not be completed.

Principal Investigator(s): Dr. Leah R. Hanson, (651) 495-6352, **Leah.R.Hanson@HealthPartners.com**.

iRehab: Discovering Outpatient Rehabilitative Measures for Epidural Stimulation Assisted Movement, Hennepin Healthcare Research Institute/Minneapolis VA, receives $15,369

The COVID-19 emergency has impacted this project in several ways. First, all human research activities were temporarily suspended at Hennepin Healthcare as well as the Minneapolis VA. This hindered and significantly delayed the patient screening process, as they recently received several referrals for potential veteran E-STAND candidates. Moreover, they have been unable to move forward with monthly follow ups at the Hennepin County Medical Center site. This has resulted in a loss of electromyography (EMG), bicycle, and evaluation of at home exercises with different device parameters. No materials were lost as a result of the COVID-19 pandemic. The primary cost associated with this disruption is staff salary support.

During the period when enrollment in human clinical trials was suspended, the principal investigator continued to compensate research staff. Research staff is vital to the success of the study and is essential for enrollment of future patients and completion of the study. Therefore, supplemental funding will be used to retain research staff so that they are available for patient enrollment to offset the funds that were spent during the period when enrollment of new patients was suspended.

Supplemental funds are necessary to maintain the scope of the project because the timelines have been escalated after COVID-related delays. Significant increases in staff effort will need to be instituted to effectively compensate for lost time while strategies are developed to overcome analysis hurdles to preserve data from disjoint participant enrollment. While the scope of this particular project has become high priority as a potential method of mitigating future study interruptions due to COVID using remote data collection platform, other aspects of the trial design have not allowed the flexibility to proactively compensate for such circumstances. As such, the funds will go toward compensating staff so that they may enroll and work with patients as planned.

Principal Investigator(s): Dr. David Darrow, (214) 564-0623, **darro015@umn.edu** and Dr. Uzma Samadani, (917) 388-5740, **Uzma@Samadani.com**.

ESTAND 2.0 – Bridge to clinical approval of eSCS for SCI, Hennepin Healthcare Research Institute/University of Minnesota, receives $44,908

The COVID-19 disruption to this project has been significant. All human subject research at Hennepin County Medical Center was immediately suspended on March 16th, to follow state and hospital regulations. While this suspension ended on June 10, investigators were unable to schedule follow up visits with study participants until July. As a result, this limited their ability to perform monthly follow-up visits for roughly five months, which led to a lapse in time and data collection for each of the E-STAND subjects. Moreover, some study participants and autonomic collaborators were unable to fly to the United

States as it conflicted with international travel restrictions. The study has also had to put a temporary hold on all screening and implantation surgeries as a result of the pandemic.

Supplemental funds are necessary to maintain the scope of the project as well as the retention of research staff that are essential for the viability of the study and completion of the original aims. Therefore, supplemental funding will be used to retain study staff at Hennepin County Medical Center and at the University of Minnesota for this study. The successful completion of the study requires an additional five-six months of salary support to recoup for missed data collection time points (monthly follow up visits) as well as the appropriate analysis of the procured data.

Principal Investigator(s): Dr. David Darrow, (214) 564-0623, **darro015@umn.edu.**

***Non-invasive Transcutaneous Spinal Cord Stimulation for Recovery of Hand Function After Spinal Cord Injury*, Hennepin Healthcare Research Institute/University of Minnesota receives $9,787**

All human subject research at Hennepin County Medical Center was suspended between the dates of March 16 and June 10. As such, it was difficult for investigators to work on preliminary screening for potential study candidates that had expressed interest in participating in SCI research prior to the pandemic. Given the significant delay and challenges during COVID, a new relationship was established and a plan for pivotal FDA trial was created. In order to compensate for the significant amount of additional work required to create and implement a nascent protocol with collaborators GTX and the University of Minnesota site, substantial coordinator and post-doctoral time will be necessary. Therefore, supplemental funding will go toward supporting those positions in order to complete the project.

Principal Investigator(s): Dr. David Darrow, (214) 564-0623, **darro015@umn.edu**.

Therapeutic targeting of cellular senescence to promote repair of the chronically injured spinal cord, Mayo Clinic, receives $17,316

In light of the COVID pandemic, Mayo Clinic instituted an essential research only policy in March. Research Laboratories were asked to specifically ramp down all but essential laboratory activities. Due to rotating furloughs of Animal Husbandry staff, Mayo Clinic also requested the principal investigators reduce animal colonies to all but the essential animals for colony maintenance. Although the investigative team had just received some mice from Jackson laboratories to continue with the research project, "Therapeutic targeting of cellular senescence to promote repair of the chronically injured spinal cord," these mice were ultimately euthanized as they became older than the approved experimental protocol would allow. These mice were not the only loss due to the pandemic, as certain reagents, kits, and surgical supplies had also expired during the period of shutdown. Mayo Clinic also mandated social distancing and work from home where possible, Dr. Scarisbrick and her team took rotating shifts to keep the laboratory processes and animal studies associated with this project running, albeit at highly reduced levels.

Extra personnel expenses during the COVID shutdown were related to the costs of the shutdown requests made by Mayo Clinic and to implement social distancing as mandated by Mayo Clinic and the State of Minnesota. The personnel costs also covered activities to maintain the project, including maintaining

personnel with the necessary skill sets to drive to drive forward preparations for the expected ramp up in August. Supplemental funding will ensure that any losses incurred during the COVID shutdown will not impede on project completion.

Principal Investigator(s): Dr. Isobel A. Scarisbrick, (507) 284-0124, **scarisbrick.isobel@mayo.edu**.

Enhancing Rehabilitation with Neuromodulation for Veterans with Spinal Cord Injury, Center for Veterans Research and Education, receives $15,911

COVID-19 led to the suspension of enrollment for all human subject research at the Minneapolis VA Health Care System. In practice, the COVID-19 emergency limited the lab's ability to restart the project in order to ensure that they had a safe plan in place for both patient enrollment and protection of the research team. Since this study depends upon enrollment of patients with chronic spinal cord injury (SCI), the investigators were unable to enroll or screen new patients and collect further data during this time period.

The primary cost associated with this disruption is staff salary support. During the period when enrollment in human clinical trials was suspended, the lab continued to compensate research staff, as they are vital to the success of the study and essential for enrollment of future patients and completion of the study. Supplemental funding will retain research staff so that they are available for patient enrollment to offset the funds that were spent during the period when the enrollment of new patients was suspended.

Principal Investigator(s): Dr. Uzma Samadani, (917) 388-5740, **Uzma@Samadani.com** and Dr. David Darrow, (214) 564-0623, **darro015@umn.edu**.

Training Transplanted spinal Neuronal Progenitor Cells (sNPCs) to Function after Chronic Spinal Cord Injury, University of Minnesota, receives $9,431

All animal/cell work for this project was suspended during the pandemic, as collaborators were not considered essential employees. This project demanded physical implementation of probe adjustment within animals to move the project forward. In addition, the research team lost rats that had previously been injected with the cells and had to undergo tail nerve electrical stimulation within two weeks after cell injection. In order to continue, the investigators will need to reinject animals with the cells and conduct TANES on these rats, house them for 16 weeks to study impact of Cells +TANES on functional recovery and fund personnel to conduct TANES and functional studies. The supplemental funds are necessary to recoup the losses incurred due to the COVID-19 closure and fund personnel.

Principal Investigator(s): Dr. Ann M. Parr, (612) 625-4102, **amparr@umn.edu**.

Optogenetic for Corticospinal Tract Stimulation in Combination with Transplanted Spinal Neuronal Progenitor Cells after Spinal Cord Injury, University of Minnesota, receives $3,352

All animal/cell work for this project was suspended during the pandemic, as collaborators were not considered essential employees. In addition rats previously injected with optogenetic virus (retrograde-

AAV-ChR2) to investigate stimulation parameters were lost. This is significant because this project demanded physical implementation of probe adjustment within animals to move the project forward.

With supplemental funding, this research team plans to order new animals and continue testing the optimal stimulation parameters; the protocol has not changed. Additionally, they will have to move cell culture space due to it being a shared location, which will incur plumbing costs and the acquisition of a new cell culture microscope.

Principal Investigator(s): Dr. Ann M. Parr, (612) 625-4102, **amparr@umn.edu**.

Spinal Cord Regeneration by Cell Reprogramming in Chronic Spinal Cord Injury, University of Minnesota, receives $50,000

The COVID-19 outbreak forced the University of Minnesota to shut down all research that did not relate to the diagnosis and treatment of COVID-19. All employees were instructed initially to work from home, and only those labs working on COVID-19 projects were allowed on campus. As a consequence, this cell reprogramming project for spinal cord injury work in the lab was halted. The personnel that were working on this project worked at home doing reviews of the scientific literature on this topic. Because investigators were only able to partially complete Aim 1, many of the reagents needed to culture their astrocytes expired and needed to be replaced. Cells that had grown to be reprogrammed in tissue culture had to be discarded and now need to be replaced.

Supplemental funds will be used to replace the reagents for the project's tissue culture experiments that expired and the astrocytes that were lost when the lab shut down. These reagents will be required to maintain the scope of the project. In addition, funds will be needed to continue supporting personnel to continue working on the project beyond the initial targeted time period because of the delays caused by COVID-19.

Principal Investigator(s): Dr. Walter C. Low, (612) 626-9203, **lowwalt@umn.edu**.

3D Bioprinted Spinal Neural Progenitor Cell (sNPC) Scaffolds Accelerate Functional Neuronal Network Formation both in vitro and in vivo after Spinal Cord Injury, University of Minnesota, receives $39,151.08

All animal/cell work for this project was suspended due to the COVID-19 shut down. Investigators were able to maintain already printed scaffolds, but needed to completely suspend all animal/cell work for this project and lost rats that were purchased for transplantation of 3D bioprinted scaffolds. This project demands cell production, cell printing, and surgeries that all need to be done in person to move the project forward. The investigative team will need to reprint scaffolds for transplantation, order several new rats, and maintain 3D printed scaffolds for three-four weeks while they self-assemble into spinal organoids in order to transplant them. Supplemental funds are necessary to recoup the losses incurred due to the COVID-19 closure and fund personnel to complete Aim 2. They will need to purchase new animals, house them, reprint scaffolds for transplantation and allow them to mature for three-four weeks.

Principal Investigator(s): Dr. Ann M. Parr, (612) 625-4102, **amparr@umn.edu**.

Optimization of iPSC-derived oligodendrocyte progenitor cell (OPC) manufacture - A key step toward patient treatment, University of Minnesota, receives $38,845

On March 13, investigators on this project were ordered to shut down all non-essential research at the UMN Stem Cell Institute and had to cease all related operations. The project personnel were excluded from campus until late May, and no experimental work was possible during this time. Most of the long-term experiments under way at the time of shutdown had to be terminated prematurely, including a number of hybrid experiments that take place over the period of 50 days and some of the organoid myelination studies. The researchers were able to collect some intermediate time-points as they left the lab but had to discard some experiments, and lost media and reagents that had been partially used or would be out of date on projected return. As the University of Minnesota implemented their Sunrise return to work plans at the end of May, investigators were able to receive permission for a progressive return to the laboratory to restart experiments. However, in addition to enhanced cleaning and PPE use, both the Medical School guidelines and the Stem Cell Institute Sunrise plan have required social distancing and reduced occupancy, and this has severely restricted time back in the lab and reduced the number of experiments they have been able to conduct. Equipment and space is shared with other groups, and therefore time needed to be coordinated so that groups do not overlap. The SCI lab has also implemented a reduction in occupancy to 50% that impacts the time we can be in the laboratory. Also it has come to the researcher's attention that the company that manufactures the Cell-MateTM material they were using for the organoid system went out of business during the COVID-19 emergency, so they have had to source an alternative.

More lab time for cell culture is the most immediate and long-term need for the project to remain on track for the foreseeable future. Supplemental funds will be used to recoup some of the lost time either by increasing researcher effort or extending the project length to enable more experimental time. Under the constraints of the Sunrise plan, the investigators will have to maximize the laboratory time so they can get in the Stem Cell Institute without major infrastructure changes. They will also have to continue to closely manage and coordinate use of the cell culture equipment with the other SCI groups to maximize the number of experiments that they can conduct. The other parts of the project involving immunohistochemistry, microscopy, imaging and molecular biology are less constrained. Supplemental funding will allow this team to regain the lost experimental effort as well as replace media and reagents and cell culture plastic-ware lost as a result of having to prematurely terminate a series of long term experiments that were underway on lockdown.

Principal Investigator(s): Dr. James R. Dutton, (612) 626-2762, **dutto015@umn.edu**.

Traumatic Brain Injury Research

Improving Functional Outcomes Through Optimization of Surgical Subdural Hematoma Evacuation Technique, CentraCare Health/St. Cloud Hospital, receives $47,345.50

Q1 & Q2 of 2020 were slated for retrospective Neuroimaging data acquisition through the central VA database system as well as data curation. Due to impacts related to COVID-19, including administrative

delays in graduate student onboarding, IRB application review, IRB application revisions, and on-site access, these critical research steps have not been possible. With necessary focus on COVID initiatives on the part of the VA central IRB, no significant progress has been able to be made when it comes to garnering their approval or access to data.

Despite this significantly reduced capacity, salary charges for UMN BICB agreements funding collaborator and graduate student efforts ($17,042.43/Quarter) and lab research scientist 25% effort ($6,630.22/Quarter) have remained. While some progress has been made in preparation for receipt of data, including early models for processing DICOM data and partitioning anatomical features based on Hounsfield units. This work will be beneficial once we are able to access the necessary data, but alone cannot achieve the aims of the grant.

The efforts of the project's collaborator, graduate student, and research scientist were required during this time for contractual reasons despite the inability to make critical progress on the study. The successful completion of the study requires two additional quarters of support for data acquisition and curation that has not been possible.

Supplemental funds are necessary to maintain the scope of this project because the investigators have yet to obtain the necessary data set, due to administrative backlogs resulting from COVID, to complete any aim of the grant. The project is an exciting opportunity to evaluate a concept with the potential to drastically improve the care of TBI patients through machine learning. However, without access to the data set, the researchers cannot fully understand the complexities related to this novel and detailed analyses. As such, the project needs the current budgeted time for the project and an additional two quarters worth of effort to complete the project.

Principal Investigator(s): Dr. Uzma Samadani, (917) 388-5740, **Uzma@Samadani.com**.

Acute Biomarkers for Traumatic Brain Injury Classification Across the Severity Scale, CentraCare Health/St. Cloud Hospital receives $49,049

The COVID-19 emergency impacted the project in multiple ways. The first is access to pertinent clinical data. As sub-projects expanded with new findings and hypotheses, there was need for further clinical data analysis and neuroimaging review. Administrative mandates at all work sites resulted in decreased access to computers maintained behind firewalls for data confidentiality. Additionally, a hiring freeze at the University and suspension of IRB activity resulted in delayed ability to onboard necessary staff to support ongoing efforts in Q2 &Q3 of this year. This further slowed progress on the aims of the grant.

During Q2 & Q3, the investigators incurred two sets of extra personnel costs as a result of the COVID-19 emergency. These are necessary salary charges from UMN BICB agreements for collaborator and graduate student efforts ($23,924.50/Quarter), and the need to utilize outside contractors for scientific writing to complete manuscripts past the allotted budget ($1,200). Essentially all analytic projects were halted for the majority of Q2 and Q3 due to the aforementioned circumstances, and thus critical productivity from our UMN collaborators was lost. Additionally, since the investigators were not able to hire a new scientific

writer, they had to rely on outside contractors (which charge a higher rate) to complete necessary revisions for ongoing manuscripts.

The efforts of UMN collaborators were required during this time for contractual reasons despite the inability to make critical progress on the study. Additionally, extra costs had to be re-allocated to pay outside scientific writer contractors to finish work that was in progress and time sensitive. The successful completion of the study requires two additional quarters of support for advanced data analysis and dedicated scientific writer time that has not been possible.

Principal Investigator(s): Dr. Uzma Samadani, (917) 388-5740, **Uzma@Samadani.com**.

***Improving Communication about Sexual Health for Persons Undergoing Acute Inpatient Rehabilitation following Traumatic Brain Injury (TBI)*, Center for Veterans Research and Education/Minneapolis Veterans Affairs Health Care System, received $14,427.42**

All human subject research has been put on hold at Center for Veterans Research and Education facility, which has required halting of all patient participant recruitment. In addition, the investigators on this project experienced direct disruptions to the inpatient TBI rehabilitation program and staffing due to a reprioritization of resources and shifting of staffing and programming within the facility to meet the new clinical demands caused by the COVID-19 emergency. Additionally, a number of the team nurses also were deployed off site to serve at Veterans Homes and VA's in other states. This resulted in a less stable rehabilitation team composition due to members of the team being reassigned to other areas in the facility, e.g., COVID-19 rehabilitation, and outside of our facility. This continues to have a direct impact on the number of admissions they are receiving in our inpatient TBI rehabilitation program, which will negatively affect the number of participants enrolled in the originally allotted time for the project

This project lost six months of time and will need at least that much additional time (likely more) in order to recruit enough patient participants for our pre-rehabilitation team training comparison group.

Extra personnel expenses are the sole financial cost to this project inflicted by the COVID-19 emergency, estimated to be around $14,427.42 in total costs for the period from March 13, 2020 – August 1, 2020, though the actual cost is a bit higher than this due to the project still being on hold at the time of this supplemental application. These costs are directly related to funds applied toward the salaries of the research assistant and statistician.

The supplemental funds are necessary for funding the salaries of a research assistant and statistician for their support on the project for the timeframe for which their services will be needed to complete the project. Without the supplemental funds, the principal investigator will have to terminate these positions on the project before the project is completed.

Principal Investigator(s): Dr. Melanie Blahnik, 612-467-1792, **melanie.blahnik@va.gov**.

Acupuncture Treatment for Chronic Post-traumatic Headache in Individuals with Mild Traumatic Brain Injury, HealthPartners Institute, receives $19,849

At the beginning of the COVID-19 emergency, HealthPartners suspended all in-person visits for human subjects research trials. Although some studies eventually received approval to resume, the Principal Investigator and other study staff were out on furlough, thus they were unable to move forward with the study. Additionally, they planned to utilize the Rehabilitation Department at the Neuroscience Center (NSC) for Acupuncture treatment sessions. In order to maintain social distancing requirements, the space they were going to use for treatment sessions is now being occupied. Investigators now plan to conduct the acupuncture treatment sessions in the 3rd floor clinical research treatment rooms at NSC. However, the clinic treatment tables are not sufficient for acupuncture treatment. Therefore, the investigators would like to purchase one massage table to be used for acupuncture treatment in the new space.

The months of March and April 2020 were direct loses due to COVID-19, which disrupted the study timeline. Activities that were completed during these two months will need to be repeated. The investigators currently need to expand study personnel time due to study delays, and if possible, would like funding to support the cost of personal protective equipment (PPE) necessary to ensure the safety of study staff and participants.

The supplemental funding will be used to support extra personnel time that will be needed due to the study delay and furloughs. The investigators are asking to cover costs incurred in March and April 2020, as the activities completed during these months will need to be repeated. HealthPartners Institute furloughed staff; therefore, we did not charge to the account for the months of May-July 2020. While we were able to conserve these costs, this also disrupted the study timeline. The supplemental funds are necessary so that they can safely continue the study, while making up for lost time due to the suspension of research trials and personnel time lost due to furloughs.

Principal Investigator(s): Dr. Amanda A. Herrmann, 651-495-6356, Amanda.A.Herrmann@HealthPartners.com.

Cortical Spreading Depolarization after Severe Traumatic Brain Injury, Hennepin Healthcare Research Institute, receives $36,633

COVID-19 led to the suspension of enrollment for all human subject research at Hennepin County Medical Center between March 16, 2020 and June 10, 2020. In practice, the COVID-19 emergency limited the investigator's ability to restart the project until early August in order to ensure that we had a safe plan in place for both patient enrollment and protection of our research team. Since this study depends upon enrollment of patients with acute traumatic brain injury, they were unable to enroll new patients and collect further data during this time period.

Due to the suspension of patient enrollment, investigators lost and are at risk of losing subdural electrodes that were acquired for implantation in patients enrolled in the study. However, given the unanticipated duration of the hiatus from patient enrollment, the electrodes passed their expiration date for safe use in humans.

During the period when enrollment in human clinical trials was suspended, investigators continued to compensate our research staff, who are vital to the success of the study for enrollment of future patients and completion of the study. Therefore, they are requesting supplemental funding to retain research staff so that they are available for patient enrollment to offset the funds that were spent during the period when the enrollment of new patients was suspended.

Principal Investigator(s): Dr. Samuel Cramer, (Contact PI), (612) 624-6666, **cram0080@umn.edu**; Dr. David Darrow (Co-PI); Dr. Thomas Bergman (Co-PI).

Identification of Brainwide Network Activity Changes in Post-traumatic Epilepsy to Optimize the Therapeutic Effect of Vagus Nerve Stimulation on Post-traumatic Epilepsy, Mayo Clinic, receives $49,921

To develop a novel technology around the fUSimaging in *in vivo* behaving animal, several personnel with different expertise and activities need to work together, which the project investigators could not and cannot pursue in the current social distancing condition. In this technology development activity, they need to have at least three people in one rodent experiment suite; one for ultrasound imaging expert, second for animal care and training person, and the third for electrophysiology expert. Even though they spread the extra load (e.g., behavior monitoring and stimulation device controlling) to these people, it is still challenging. Thus, the investigators currently separate activities into the fUSimaging in the anesthetized rats and the electrophysiology monitoring with behavior monitoring. This way, they can limit the number of personnel performing the experiment and maintain social distancing requirements. However, using this method, they cannot monitor changes during the course of the PTE development despite being able to capture pre- and the post-TBI. For this reason, the investigators propose RNA analysis in plasma collected from the TBI rat over the course of the PTE development. The current emergent supplemental funding will help them adopt the genetic approach to fill the knowledge gap between before and after PTE development.

They will not request to add new personnel under this supplemental funding stream. Instead of hiring extra personnel, they will have the principal investigator take additional responsibility to direct animal care and surgery, since she has experience and knowledge in all proposed animal-related activities. They will continue support the graduate-student level personnel with full safety concern.

The investigators plan will keep our original research goals: 1) determine the underlying mechanisms of PTE; and 2) evaluate the therapeutic effect of the VNS on PTE. They will use the genetic approach to determine the underlying biological mechanisms of TBI-associated epilepsy over the course of the PTE development. To do so, they will collect plasma from the TBI rats and analyze the changes in the mRNA level. Since the Mayo provide extensive expertise in the genomic and genetic approaches, they will utilize the well-established Mayo facility to reach their goal.

Principal Investigator(s): Dr. Su-Youne Chang, (507) 293-0511, **chang.suyoune@mayo.edu** and Dr. Azra Alizad.

Switching off the thrombin receptor to enhance recovery after traumatic brain injury, Mayo Clinic, receives $19,839

In light of the COVID-19 pandemic, Mayo Clinic instituted an essential research only policy in March. Research Laboratories were asked to specifically ramp down all but essential laboratory activities. Due to rotating furloughs of Animal Husbandry staff, Mayo Clinic also requested that these investigators reduce their animal colony to all but the essential animals for colony maintenance. The changes instituted were formally submitted to Mayo Clinic leadership for approval. The project "Switching off the thrombin receptor to enhance recovery after traumatic brain injury" involves several transgenic mouse lines, that the investigators have derived or which were received from other investigators. These lines, therefore, are not easily replaceable, and they put in considerable effort to maintain these genetic strains during the shutdown period.

As Mayo Clinic also mandated social distancing and work from home where possible, Dr. Scarisbrick and her team took rotating shifts to keep the laboratory processes and animal colony maintenance associated with this project running, albeit at highly reduced levels. The team did experience some loss of mice and expiring reagents/kits during the lockdown. Fortunately, they were able to maintain the genetic strains needed for our studies to continue, that is PAR1 knockout mice and mice with a PAR1 floxed allele. Extra personnel expenses during the Covid shutdown were related to the costs of the shutdown requests made by Mayo Clinic and to implement social distancing as mandated by Mayo Clinic and the State of Minnesota. Personnel costs were also incurred for animal genotyping and breeding necessary for the maintenance of the unquie genetic strains used in the research under consideration. Without supplemental funding, loss of Personnel time, animal and reagent costs will not be recovered, and this will limit the investigators' ability to complete the project as described.

Principal Investigator(s): Dr. Isobel A. Scarisbrick, PhD, (507) 284-0124, **scarisbrick.isobel@mayo.edu**.

Combined tDCS and Cognitive Training to Reduce Impulsivity in Patients with Traumatic Brain Injury, Center for Veterans Research and Education/Minneapolis Veterans Affairs Health Care System, receives $46,370

All in-person visits for this project were halted both at the Minneapolis VA and University of Minnesota. New enrollment visits were cancelled and postponed. The two patients who completed intervention completed partial follow up visits over the phone. Personnel were retained during the COVID-19 disruption because they were already trained on and experienced with study procedures. Experienced staff will be crucial for re-starting subject visits when research reopens. Supplemental funding will help support these experienced personnel for the duration of the study, which will likely extend longer than expected due to COVID-related slowdowns.

Principal Investigator(s): Dr. Casey S. Gilmore, (612) 467-2261, **casey.gilmore2@va.gov**.

Theta Burst Stimulation for Headaches After Traumatic Brain Injury, Center for Veterans Research and Education/ Minneapolis Veterans Affairs Health Care System, receives $24,755

All in-person research interactions were halted in March 2020 and the project was placed on an administrative hold by the Minneapolis VA's Office of Research and Development due to COVID-19. The employee hiring process at the Minneapolis VA was put on hold due to COVID-19. The project investigators have not been able to begin data collection due to COVID-19. Due to these disruptions, they will need to enroll more participants than previously anticipated. They also expect data collection will take longer than previously anticipated due to COVID-19; therefore, we need to extend the timeframe for data collection and the Research Assistant position. The goal of this study is to obtain 20 complete datasets. Due to COVID-19 precautions and expected future disruptions to data collection, these investigators predict the need to enroll up to 30 participants. Therefore, they are requesting additional participant payment costs, time, and salary support to complete the data collection.

Principal Investigator(s): Dr. C. Sophia Albott, (612) 787-5146, **albot002@umn.edu**.

Evolution of acute and chronic effects on neuronal activity and morphology following mild cerebral cortical traumatic brain injury using multi-scale optical imaging in behaving mice, University of Minnesota, receives $44,925

There were three categories of losses due to the COVID-19 emergency. The first, and major loss, is research staff time that severely impacted the project's experimental work. The second, is loss of animals due to premature cessation of optical imaging experiments and due to restrictions on breeding GCaMP6f transgenic animals during the shutdown. The third category is the lost surgical supplies and polymer windows and head-fixation implants with the ending a cohort of animals in March.

During the period when laboratory research was suspended, the University of Minnesota required that investigators fully compensate research staff. The research staff is vital to conducting the study and essential for completing the remaining experiments in the study. Therefore, a major component of our request for supplemental funding is salary support for the three key research staff needed to complete the remaining experiments and analyses. The principal investigators are requesting four months supplemental salary funding for the three staff as these individuals are necessary to complete the experimental studies.

Principal Investigator(s): Dr. Timothy J. Ebner (contact PI), (612) 624-1560, **ebner001@umn.edu**; Dr. Samuel W. Cramer (Co-PI); Dr. Clark C. Chen (Co-PI); Dr. Suhasa B. Kodandaramaiah (Co-PI).

Harnessing exosomes as a biomarker and therapeutic approach to traumatic brain injury, University of Minnesota, receives $50,000

The COVID-19 emergency resulted in a complete shutdown of the laboratory from March until July. Following regulations, all in person work was ceased and animal work was concluded. Planned surgical procedures and in vitro work was halted, and animal colonies were downsized. Animal work was slated to continue starting in April. Animals that were on hand were culled or moved onto COVID related projects

on campus. This means the animal colony will have to be replaced and the timeline will be greatly impacted. Exosomes currently in use in March were disposed of and could not be frozen, as were the reagents and antibodies in use for in vitro analysis. As the project is beginning to start again, the first aim is to begin isolating exosomes again in cord blood stem cells and then implement this in our TBI model to confirm projected results.

The project's current objective is the reestablish our animal groups. The need is to maintain animals in the correct age and group and then to isolate exosomes for use in the TBI model. There will be costs associated with reestablishing that colony. Additionally, the exosomes isolated and the reagents used for their culture and analysis must be acquired again. The exosomes could not survive another freezer/thaw cycle, and many of the media/reagents are no longer viable.

In order to complete this project, the funds must be made available to reestablish the investigator's animal colony and obtain stem cells and exosomes again. The costs associated with these steps, along with the funds already spent prior to the closure, would mean that the end goals of the project would need to be restructured. The supplemental funds will allow the project to maintain personnel. The post-doctoral fellow's work on this project is critical and is no longer covered by her fellowship, as it would have been from March-July.

Principal Investigator(s): Dr. Andrew Grande, (612) 624-6666, **grande@umn.edu**.

Reprogramming astrocytes into neurons to provide therapeutic benefit in TBI, University of Minnesota, receives $39,985

The COVID-19 emergency caused a complete shutdown of work on this project. Due to University of Minnesota regulations, all in-person work ceased and all animal experiments were concluded. While some aspects of this project were frozen and able to be started again, other work was lost. Animal colonies were downsized to assist with the limited animal research staff. Animal breeding was also halted. Specific to this project, investigators had to decrease the number of animals in their colony. Ongoing studies were assigned to be finished, but it was not possible to maintain animals until the long-term time points that they were originally set for. This means more animals will be needed to start this experiment again, as well as bring it to the full time point. Animal breeding will also need to begin again. The animals will need to be replaced, and the study must be started again to run its full course. Animals that were maintained during the lock down resulted in animal housing fees over the several months that they were made to wait.

Viral vectors and media are a few of the other items that need to be replaced. Media prepared before the lock down is no longer usable, and many antibodies also need to be replaced. Animals that were able to be maintained over the lock down period incurred daily per diem costs for housing and care.

A post-doctoral fellow that was assisting on this project had her salary covered through her fellowship. That fellowship expired over the summer, and now the work that was due to be covered on this project must be covered using grant funding. She is offering the same percentage of effort on the project, but the supplemental funds will be used to assist in covering her funding while working through the animal and

cellular work that was to be done over the summer. Additional funds for supplies/PPE will allow the investigators to continue to maintain that safety without having to reduce our budget for materials critical to the project.

Principal Investigator(s): Dr. Andrew Grande, (612) 624-6666, **grande@umn.edu**.

***Multilineage 3-dimensional brain organoids to model intracranial pressure linked to chronic traumatic encephalopathy*, University of Minnesota, receives $14,610**

The project came to a complete stop in March when the University announced the restrictions on non-COVID research. Due to the nature of the project, it was not possible to continue any cellular experiments or work outside of the laboratory. Experiments that were set to be run in April and May were pushed back and are the principal investigator is hopeful to restart them soon as the lab is reopening. Cell lines must be reestablished and then organoid generation can begin again. The shutdown did cause a major delay in the timeline, but strong results from prior work to the stoppage can be continued now that the lab has been approved to reopen on a limited capacity.

The supplemental funding will be used to cover the salaries of the individuals on this project throughout the lock down. No additional personnel need to be added to the project at this time, but the supplemental budget will allow the project team to meet all of their goals without having to re-budget to cover material funds. The supplemental funds will allow investigators on this project to recover the cost of the media, reagents, cells and kits that were lost when the lab was forced to lock down. It will also allow them to cover the salary of the members of this project without taking away from the funding that has been designated for materials and supplies. Without the supplemental funding, aims would have to be cut, or team members would have to be assigned to other projects, slowing the progress down.

Principal Investigator(s): Dr. Andrew T. Crane, (612) 626-9212, **atcrane@umn.edu**.

Characterizing the neuroinflammation associated with sequential TBI in a rodent model, University of Minnesota, receives $19,627

All projects (that were not related to COVID 19) were halted at the University of Minnesota until return to work protocols was initiated in late July/August. Animals in this project's breeding colonies (that could be purchased) and any remaining animals purchased for experiments when the State "stay at home" order was issued were humanely euthanized. Some antibody and enzyme (PCR) reagents that were purchased at the beginning of the project year had to be discarded as they reached their expiration dates. Time to restart the experiments include logistical considerations like ordering animals, scheduling surgery rooms, and analysis equipment. With COVID-19 the throughput capacity of shared laboratories have decreased considerably to maintain physical distancing requirements.

No additional personnel expenses were incurred, except lost time, which will be recouped by extending the grant period. We hope to use the supplemental funds to support staff salaries and fringe for the added time.

Laboratory re-entry/use protocols require managed access to allow increased physical distancing between employees. All laboratory staff are required to wear surgical grade face covering to mitigate potential spread of the coronavirus. Cost for added PPE is requested in the supplement application.

Principal Investigator(s): Dr. Maxim C. Cheeran, (612) 626-9930, **cheeran@umn.edu**.

FY 2021 Annual Research Grant Timeline and Anticipated Outcomes

Based on the results of the emergency supplemental funding competition, over $2M is remaining to fund FY 2021 research projects through the SCI-TBI Annual Research Grant funding mechanism. The timeline for the annual research grant opportunity is as follows:

October 1, 2020	Request for Proposals available to applicants
November 20, 2020	Deadline for receipt of intent to submit forms
4:30 p.m., January 11, 2021	Deadline for receipt of proposals
February 23, 2021	Proposal Review Meeting/Project Presentations
March 15, 2021	Notification of recommendation for grant award
April 1, 2021	Project funding begins with grant contract encumbrance

On November 20, 2020, OHE received a total of 49 intent to submit forms, indicating that there may be a high volume of submissions compared to prior years. Details, including project descriptions and award amounts will be included in the FY 2022 report.

Updated progress and/or outcomes of the projects listed in this report are typically disseminated to the public during the Minnesota Spinal Cord Injury and Traumatic Brain Injury Research Symposium. The date of the event was scheduled for February 2021; however, it has been postponed due to COVID-19 disruptions. Once rescheduled, an invitation will be extended to legislators so that the stories and experiences of patients can be heard. One such story from an SCI patient, Kathy Allen, can be found in **Appendix C**. Discoveries and innovations will also be shared with the scientific community through national presentations, journal articles and publications, and future collaborations. For a list of preliminary accomplishments from completed projects, see **Appendix C**.

Last year, OHE partnered with the Ohio Department of Education (ODE), which recently acquired funding for spinal cord injury research. This partnership shows much potential to share learnings, information, and discoveries through national or regional coordination between programs. OHE and ODE will continue to work together in hopes of furthering and deepening spinal cord injury research and getting closer to a cure for paralysis. OHE is willing to work with any state or entity that secures funding for SCI and/or TBI research in order to strengthen the likelihood of finding a cure.

The Spinal Cord Injury and Traumatic Brain Injury Advisory Council anticipates that through the innovations cited in the recommended research projects, and collaboration with other nationally-reknowned researchers, the novel outcomes from the funded projects should lead to advances in the fields of spinal cord injury and traumatic brain injury.

Highlighting Community Voices in the Spinal Cord Injury and Traumatic Brain Injury Grant Program

This winter, as an attempt to fully understand the impact of the SCI-TBI Grant Program, a survey was created and disseminated to all past and current SCI-TBI grantees. Rob Wudlick, Chair of the SCI-TBI Advisory Council, solicited updates, impact, and feedback from Principal Investigators. The results of the survey, in addition to patient testimony, are outlined below.

Survey Results

As of Februrary 22, 2021, a total of 19 principal investigators who are either current or past SCI-TBI Research Grant grantees responded to the participation survey. This constitutes as about ¼ of the total number of grantees who have participated in the program.

One of the first questions in the survey highlights how student involvement is crucial to this program, allowing for students of varying levels participate in real, professional medical/scientific research opportunities. The following table shows the number of students involved in these 19 projects, separated by education level.

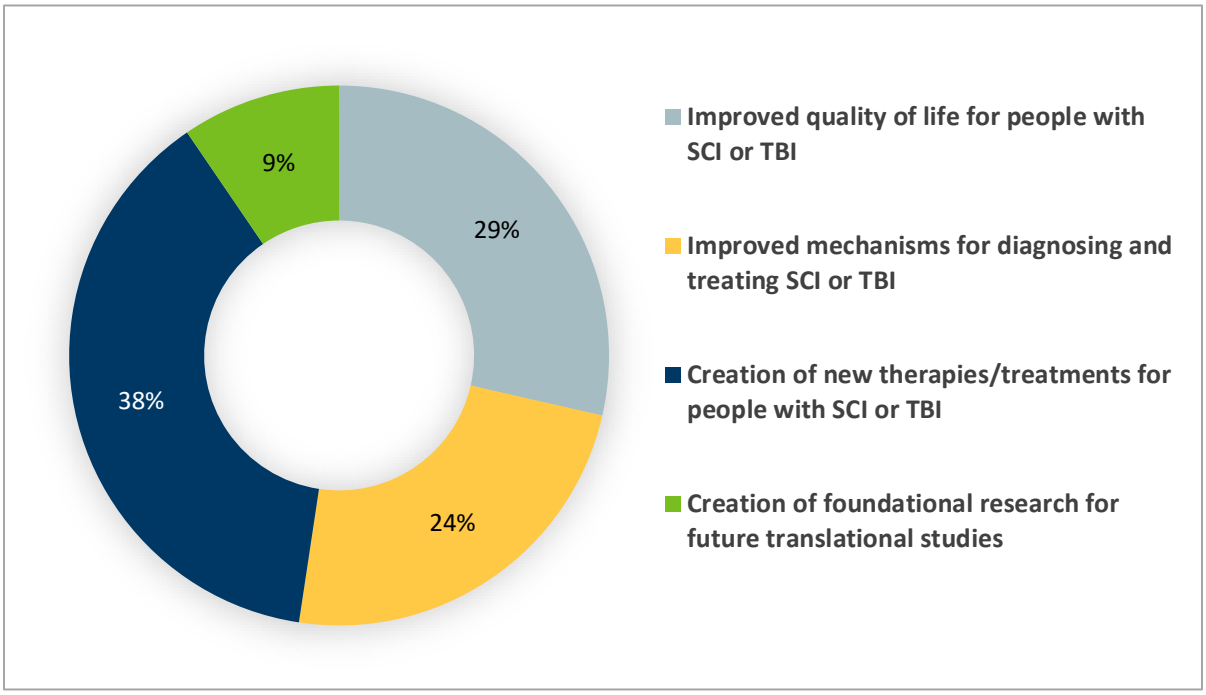
Table 2: Student Involvement in SCI-TBI Research

Student Type	Number of Students Involved
High School	3
Undergraduate	42
Graduate	30
Post-Doctoral	16
Medical	9

As indicated by the responses we received, students play an active role in the funded projects, allowing for both increased capacity related to project effort as well as unique educational experiences for the students.

While creating educational opporotunities for students is important, the program is focused on impacting everyday Minnesotans living with a spinal cord injury and/or a traumatic brain injury. The next graph illustrates the many different ways state-funded SCI-TBI research projects have an impact on the community.

Figure 1: Human Impact of SCI-TBI Projects, based on available survey responses



Other important survey data to note, based on the 19 responses we received:

- As of February 2021, a total of 717 participants with SCI and/or TBI have been recruited in clinical trials that were led the 19 survey respondants.
- Principal investigators were able to secure an additional $2,838,000 from various sources to further their projects beyond the SCI-TBI Research grant.
- These projects led to a total of 26 articles published in national journals and other publications, with more in progress.
- These projects have generated 7 patents for products that improve the quality of life for people living with SCI and/or TBI.
- Many of the projects funded through the SCI-TBI Research Grant program have inspired further investigations by researchers across the United States and internationally.

Patient Testimonies

> *I am writing you this email in support of research funding and would like my story to be included in the Minnesota SCI-TBI Legislative Report.*
>
> *My name is Geoff Jessup. On June 22 2014 I suffered a spinal cord injury while out recreationally riding off road dirt bikes with my 2 younger sons. At the time I didn't believe I was participating in any sort of high risk activities but in a blink of an eye I laid on the ground without the ability to move my legs. My official injury status is a T4 complete spinal cord injury. To describe what the feeling of not being able to move my lower body to people who take it for granted is very difficult as I could imagine someone doing the same to me before I was injured and not even remotely understanding what they were describing. The uncontrollable muscle spasms, the neuropathic pain, the bladder/bowel issues to name only a few were things that would never cross my mind if and when I saw someone in a wheelchair or were told they had a spinal cord injury.*
>
> *I was asked what being included in to the Estand study meant to me once. I think the first time asked my eyes welled up as I recall hearing I was accepted the very first time. Beyond some ability to move my legs for the first time in 4 years, almost completely eliminate my muscle spasms and give me some better control over my bladder and bowel issues It also gave me hope. Hope that there were still opportunities to improve my quality of life and also hope for the many young guys that I had met in my original rehabilitation after surgery. You see I was 54 at time of injury and even though I was very active playing all types of sports that I very much miss I had 30 years on some of the young guys that I met in rehab and could only imagine that they had no idea of what great things in life they were going to miss because of their injury.*
>
> *Alaina If you thought it was beneficial I would be more elaborate with some details and I could forward a short video of my leg movement after 4 years of not being able to.*
>
> *Really in the end those of us with a spinal cord injury just want to feel that our numbers and not too small that they don't deserve the funding to work towards a cure.*

-Geoff Jessup

> *I am a quadriplegic on a ventilator for 13 plus years now. I am messaging to say Thanks for keeping the funding for spinal cord research! I know it is expensive but the impact of a successful treatment will result in more than anything can pay for for many individuals!*

-Nick Doriott

> *I'd like to thank you for your support in continuing funding for spinal cord injury and traumatic brain injury cure research! I'm personally affected by this because when I was barely 13 in 1994 I was crossing the street on my bike, I had the right-of-way, when a*

negligent driver who wasn't watching the road, his turn signal, or the upcoming next turn that corresponded with his turn signal, (where I was crossing from), drove his El Camino into me doing over 55 mph.

Among the MANY other physical injuries & trauma I sustained from that, (shattered femur & shin, head trauma which nearly knocked my eye out of my skull & the doctors had to push it back in, brain swelling, hematomas, & countless surgeries to save my life, going into my head several times, needing a shunt put in my brain to drain fluid to my stomach for life, hardware put in my entire left leg, exploratory surgery to check for internal bleeding, having piece of my skull removed to accommodate my brain swelling and having it put back in weeks later, having a rib removed and relocated into my neck with hardware to fuse it, a tracheostomy, a feeding tube in my stomach that was later able to be removed, needing a pacemaker put in because I was literally dying/my heart stopping/the crash cart called in to my ICU room every 5 minutes for several weeks, having the pacemaker removed later on, & countless other surgeries after all of that but because of it), my neck was broken at the 2 highest levels possible, C1 & C 2, (same levels that Christopher Reeve was), leaving me quadriplegic & 100% ventilator dependent 24/7. Since then I've undergone more physical & emotional trauma than most people would have in 10 lifetimes. I'm literally on life support 24/7 & completely dependent on others for all of my needs from below my jaw down. I have been this way for 26+ years.

Spinal cord injuries can happen to anyone at any time. It doesn't matter how old or young you are, (there was another kid at Gillette Children's hospital for his physical rehab at the same time I was, same level of injury as me, & he was only 7), it doesn't matter what your race is, it doesn't matter what your gender is, it doesn't matter what your beliefs are, it doesn't matter how it happens. 10,000 Americans alone, (& probably more by now, I got this statistic years ago when writing a letter similar to this, I'm a very big advocate about SCI cure research & funding for it), suffer a spinal cord injury every year leaving them paralyzed to some degree, including the highest degree that I am. Continued funding for researching SCI & TBI is INCREDIBLY IMPORTANT & could even lead to finding cures for other nervous system or neurological injuries or ailments. Medical science is mysterious that way, just look at penicillin. Who would've thought mold would be the cure for so many things that used to kill people all the time.

I, my family, everyone who has suffered a SCI & has been living with the aftermath for years-decades, & peoples who are going to suffer a SCI leaving them paralyzed to any degree, (could be someone you know, could be someone you love, could be you), needs this funding& thanks you for supporting it!!! TBI research funding that is included with this is important as well. Head injuries can happen at any time to anyone & can severely mess the person up. I've seen the aftermath of head injuries personally.

-Angelique Novak

I have been a quadriplegic for over 33 years and have been a cure advocate since day one. Since 1987, everything has changed, cars, houses, landscapes, even your breakfast cereal, not paralysis. With the help of the Research Grant Program, this is no longer true. The State of MN is now partnering with and helping drive some of the most innovative science toward a common goal to eradicate this most ugly human affliction known to man. Additionally, because the State is paying for the cares for these people, ANY improvements in bodily function will most certainly pay off huge dividends in reducing cares costs.and not to mention, what a health IMPROVEMENT would do to someone's life and mental well-being…put a price on that!

-Jeffrey Toby

To Whom it may Concern:

In October of 2006, I suffered a T8, ASIA A spinal cord injury from a fall during sleepwalking. At the 2016 W2W (Working 2 Walk) Symposium Minneapolis, I listened to a presentation about an epidural stimulation clinical trial starting soon at HCMC (Hennepin County Medical Center) called ESTAND (Epidural Stimulation After Neurologic Damage). I met with Dr. David Darrow to discuss what the trial would entail and about the surgery itself.

After looking through my medical records and discussing with the clinical trial team, Dr. Darrow told me that they were not sure this would work for me, given the severity of my injury, the length of time that had passed since my injury and my age-52 at the time of surgery. I went into this clinical trial with no preconceived notions of success. If it didn't work, then we knew what the limitations were.

On September 27, 2017, I had the epidural stimulator implanted! 2 weeks later, on the 11th anniversary of my spinal cord injury, we turned the stimulator on. It worked! There was quite a bit of excitement in that room and yes, I did shed a few tears of joy!

As the trial progressed, I noticed that my shoulders didn't hurt anymore. My left shoulder was so painful before the trial. I was worried that I may need surgery. I am able to sit up straighter in my wheelchair and that has allowed me to push more efficiently. My gray area of nerve sensitivity has disappeared and the pins and needles nerve pain has gotten much better! My legs don't bounce around anymore. I can ride in a car without becoming stiffer than a board and being thrown back in my chair. I can maintain a more even body temperature. It is nice not to take 2 hours to warm up. My bowel program is shorter. My bladder is still a work in progress. I didn't expect all of these changes. In the past few years, our community has made it clear that while walking again would be great, it is all of the other stuff the affects our quality of life.

Without the Minnesota Spinal Cord Injury/Traumatic Brain Injury Research Grant program, the ESTAND clinical trial would not have happened. Why deny people a good quality of life after injury? Let's prevent shoulder injuries due to overuse, drug use for spasms, nerve pain,

> *bladder leakage, long bowel programs, poor temperature control. The list is long. Please continue to fully fund the MN SCI/TBI Research Grant Program! Thank you for reading about my experience!*

-Kathy Allen-SCI survivor, Crosslake, MN

Appendix A: Copy of Statute

LAWS OF MINNESOTA 2019

136A.901 SPINAL CORD INJURY AND TRAUMATIC BRAIN INJURY RESEARCH GRANT PROGRAM.

Subd 1. Grant program

The commissioner shall establish a grant program to award grants to institutions in Minnesota for research into spinal cord injuries and traumatic brain injuries. Grants shall be awarded to conduct research into new and innovative treatments and rehabilitative efforts for the functional improvement of people with spinal cord and traumatic brain injuries. Research topics may include, but are not limited to, pharmaceutical, medical device, brain stimulus, and rehabilitative approaches and techniques. The commissioner, in consultation with the advisory council established under section 136A.902, shall award 50 percent of the grant funds for research involving spinal cord injuries and 50 percent to research involving traumatic brain injuries. In addition to the amounts appropriated by law, the commissioner may accept additional funds from private and public sources. Amounts received from these sources are appropriated to the commissioner for the purposes of issuing grants under this section.

Subd. 2. Report

By January 15, 2016, and each January 15 thereafter, the commissioner shall submit a report to the chairs and ranking minority members of the senate and house of representatives committees having jurisdiction over the Office of Higher Education, specifying the institutions receiving grants under this section and the purposes for which the grant funds were used.

136A.902 SPINAL CORD AND TRAUMATIC BRAIN INJURY ADVISORY COUNCIL.

Subd 1. Membership

The commissioner shall appoint a 14-member advisory council consisting of:

(1) one member representing the University of Minnesota Medical School;
(2) one member representing the Mayo Medical School;
(3) one member representing the Courage Kenny Rehabilitation Center;
(4) one member representing Hennepin County Medical Center;
(5) one member who is a neurosurgeon;
(6) one member who has a spinal cord injury;
(7) one member who is a family member of a person with a spinal cord injury;
(8) one member who has a traumatic brain injury;
(9) one member who is a veteran who has a spinal cord injury;
(10) one member who is a veteran who has a traumatic brain injury;
(11) one member who is a family member of a person with a traumatic brain injury;
(12) one member who is a physician specializing in the treatment of spinal cord injury;
(13) one member who is a physician specializing in the treatment of traumatic brain injury; and
(14) one member representing Gillette Children's Specialty Healthcare.

Subd. 2. Organization

The advisory council shall be organized and administered under section 15.059, except that subdivision 2 shall not apply. Except as provided in subdivision 4, the commissioner shall appoint council members to two-year terms and appoint one member as chair. The advisory council does not expire.

Subd. 3. First appointments and first meeting

The commissioner shall appoint the first members of the council by September 1, 2015. The chair shall convene the first meeting by November 1, 2015.

Subd. 4. Terms of initial council members

The commissioner shall designate six of the initial council members to serve one-year terms and six to serve two-year terms.

Subd. 5. Conflict of interest

Council members must disclose in a written statement any financial interest in any organization that the council recommends to receive a grant. The written statement must accompany the grant recommendations and must explain the nature of the conflict. The council is not subject to policies developed by the commissioner of administration under section **16B.98**[1].

Subd. 6. Duties.

The advisory council shall:

(1) develop criteria for evaluating and awarding the research grants under section **136A.901**[2];
(2) review research proposals and make recommendations by January 15 of each year to the commissioner for purposes of awarding grants under section **136A.901**[3]; and
(3) perform other duties as authorized by the commissioner.

[1] https://www.revisor.mn.gov/statutes/cite/16B.98
[2] https://www.revisor.mn.gov/statutes/?id=136A.901
[3] https://www.revisor.mn.gov/statutes/?id=136A.901

Appendix B: Covid-19 Emergency Supplemental Research Grant Proposal Evalution Criteria

Minnesota Office of Higher Education

Spinal Cord Injury and Traumatic Brain Injury Research Grant Program

Emergency Supplemental Funding for Research Impacted by COVID-19 Grant Evaluation Criteria

Applicant Organization:

Amount Requested:

Project Title:

Reviewer ID Number:

Total Score:

As you read through the proposal, rate each section on its ability to demonstrate the following criteria for funding, when applicable.

1. **Need for funding** **Section Score: ____/40**

Estimated funds remaining do not seem to satisfy the completion of the original aims of project
COVID-19 has prevented the grantee from lab use or entering patient sites/has limited contact with patients
COVID-19 has caused grantee to not only halt, but lose, progress made on project
COVID-19 has caused the grantee to lose project materials (i.e. rodents, cells, etc.)
COVID-19 has caused the grantee to lose staffing on project
Grantee has supported salaries for project team during COVID-19 closures
Grantee has had to spend grant dollars during COVID-19 on expenses not directly related to the progression of their project
Project team was not able to create a remote protocol for continuing project at full capacity during COVID-19 closures
If scope/project aims were to narrow due to capacity or resource issues, the project may lose integrity or decrease the value of new-knowledge gained through research

2. Potential Impact of funding **Section Score: /40**

Additional funding would maintain or increase progress on the project
All expenses incurred throughout COVID-19 closures are directly related to lab closures/project suspension
Supplemental funding would maintain project integrity despite loss of progress on the project
Supplemental funding would prevent loss of staff due to the need to find other work
The PI has a clear and feasible plan for restarting their project
The PI has support from their institution in completing their project
The PI has a clear understanding about future lab closures and how to adjust protocols to accommodate future COVID-19 outbreaks

3. Safety **Section Score: /10**

The PI has funds necessary to return to their project safely, with PPE and social distancing protocol for their staff
The PI will continue their project by going through the necessary avenues laid out by their institution's COVID-19 response plan
The PI will immediately suspend their project if instructed to by their institution, or if they believe continuing their project would put staff at risk in any way

4. Budget **Section Score: /10**

The budget is clearly articulated and makes sense based on the current status of the project
The budget only includes reimbursement for losses incurred from March 13, 2020 – August 1, 2020 due to COVID-19 closures
The budget is justifiable and represents a feasible plan of action
The budget only includes staff, materials, etc. that were essential to the original project protocol or are essential to a revised protocol

General Comments (be specific!):

Appendix C: Status/Accomplishments of Funded Projects and the Dissemination of New Knowledge

Institution: Hennepin Healthcare Research Institute
Principal Investigator: Dr. David Darrow
Project Title: *Optimizing Epidural Spinal Cord Stimulation to Restore Cardiovascular Function after Spinal Cord Injury*
Grant Cycle: FY2018

Accomplishments: Using established methodologies in combination with our optimization algorithm, we have been able to determine SCS parameters that restore cardiovascular function in those with autonomic dysreflexia. Our tablet application has allowed us to collect data on autonomic parameters, remotely, through questionnaires. In addition, using the tilt-table, we are able to collect autonomic data, with and without stimulation, to assess changes in cardiovascular function. Through this, we are able to determine parameters that aid in restoring cardiovascular function. This finding has shown to improve cognitive function and increase energy levels.

Moreover, the development and implementation of our application has allowed us to collect data remotely through the home-exercise in conjunction with accelerometers and questionnaires. Such data allows us to assess the efficacy of each setting with regards to improvements in volitional movement, autonomic function, and bowel/bladder control while the participant is in the comfort of their own home. Through this, we are able to continuously refine setting parameters based on participant preference. In addition, this home-exercise also serves as a form of therapy that can either enhance the participants' current regimen or provide a form of activity for those that do not participate in rehabilitation programs for various reasons.

Dissemination: The media has been our primary vehicle for disseminating our results to communities of interest. We have used our updated website to create blog posts that include links to our publication, news articles and presentations given by our investigators. Through Dr. Darrow's social media, study updates and information have been provided as well. Moreover, patient progress videos have been posted on social media accounts, such as YouTube, where they have been shared by others in the community. Additionally, a study guide was also created for our participants which entails information on our study and links to our publications and news articles. We also send monthly updates to each subject via email, which includes current findings, presentations, and articles.

Institution: University of Minnesota
Principal Investigator: Dr. Andrew Grande
Project Title: *Therapeutic Application for Non-hematopoietic Umbilical Cord Blood Stem Cells (nh-UCBSCs) in Traumatic Brain Injury: Immune Modulation with Acute and Long Term Benefits*
Grant Cycle: FY2018

Accomplishments:
Major Goal #1:

1. Activities included creating a controlled cortical impact in rats and treating injured rats with nh-UCBSCs at 48 hours post-injury. Animals were tested using a battery of behavioral tasks quantifying lateral

asymmetry in injured animals. Live animals were also imaged using Ferumoxytol enhanced MRI, in an effort to identify macrophage activity in the brain of injured animals. At the conclusion of the study, animals were euthanized and brains isolated for flow-cytometric analysis of immune cells within the brain.

2. Specific objectives of this study were to reduce neuroinflammation associated with TBI through intravenous infusions of nh-UCBSCs, as measured by MRI and flow cytometry. Reductions in neuroinflammation would be correlated with reductions in behavior deficits.
3. Results from this study are variable and inconclusive. The behavioral data indicate the presence of lateral asymmetry in injured animals, with a preference towards using the forepaw of the uninjured hemisphere, although treatment with nh-UCBSCs did not ameliorate this deficit (Figure 1A). Live imaging through MRI proved highly variable and no conclusions could be made. Inflammation measured through flow cytometry noted a trend towards reduction in the number of neutrophils present in the injured (ipsi) hemisphere of treated rats, relative to injured rats injected with vehicle (Figure 1B). No other alterations in immune cells were observed in injured animals. The results from this study led us to re-examine the premise on which this study was designed. Our initial assumptions were that the neuroinflammation following TBI should be similar to stroke, on which we have well established data indicating a robust immune response at 9 days post-stroke. Given a minor difference was observed in the diversity and number of immune cells present in the brain post-TBI we decided to establish a timeline of neuroinflammation in a mouse model (Major Goal #2).

Major Goal #2:

1. Activities include developing a unilateral controlled cortical impact model in mice with time and injury severity as variables. A mild-injury was developed with the goal to create minimal gross pathology to the brain. A moderate-injury was developed with the goal to create a cavitation in the brain. Animals were tested using a battery of behavioral tasks quantifying lateral asymmetry and memory deficits. Animals were euthanized at 6-hours, 24-hours, 3-days, 7-days, 14-days, and 28-days post-injury and brains isolated for flow-cytometric analysis of immune cells within the brain.
2. Specific objectives were to quantify the diversity and total numbers of a wide-range of immune cells within the brain and whether these changes can be correlated with behavioral deficits.
3. Results from this study confirms our hypothesis following analysis of the results from Major Goal #1. The infiltration of immune cells within the brain following injury is very dynamic, particularly in the total number of macrophages and the unique macrophage phenotypes (Figure 2A-D). We were also able to identify behavioral deficits associated with the mild- and moderate-TBI mouse (Figure 2E-F).

Major Goal #3:

1. Objective: To determine the source and impact of migrating brain macrophages induced in response to TBI.
2. Activities:
    (a) Evaluation of BM Chimeras and Macrophage depletion. Two experiments were performed to standardize the BM transplant and macrophage depletion characteristics. Blood was collected from animals at 4 weeks post transplantation and chimerism was assessed using the congenic markers on myeloid cells (CD45.1-recepient and CD45.2-donor).
    (b) Evaluation of macrophage migration into the brain (from the periphery) post TBI. Bone marrow chimeras were used to determine the number of cells that migrated from the bone marrow through peripheral circulation (compared to a resident tissue origin).
3. Results: In the first experiment standardizing the transplant procedure, we lost 60% of our transplant recipient animal and less than 25% of animals had >80% blood chimerism with donor leukocytes

(CD45.2^{+}). After changing the irradiation protocol for myeloablation and transplant bone marrow cell preparation, we achieved 75% survival with >80% donor chimerism observed in 2/3 of the animals and > 50% chimerism in all transplanted animals.

Three months after transplantation, 8 chimeric animals with 80 to 90% leukocyte chimerism were selected and treated with diphtheria toxin (DT, 100 ng/mouse) to deplete DTR tagged donor cells in the chimera. At 24 h after DT treatment, bone marrow, brain, blood, spleen, cervical lymph node, inguinal lymph node, and peritoneal cavity cells were analyzed for macrophages and lymphocytes. No depletion of macrophages was observed in DT-treated mice. Escalating DT dose was titrated in F1-DTR-mCherry transgenic mice to determine the threshold for DT effect by analyzing brain, blood, bone marrow, spleen and CLN at 24 h. There was reduction in number of Ly6C(hi) macrophages in the blood at 100 and 200 ng doses, but these doses did not affect total macrophages in all tissues analyzed, including the brain. This result indicates that the macrophage depletion with this system was effective only for certain subpopulations of macrophages and did not impact the tissue compartment. We hypothesized that poor drug penetration into tissues, rapid repopulation of depleted populations or insufficient expression of DTR in certain populations of macrophages (i.e. tissue macrophages). We tested mCherry expression in tissue macrophages as a surrogate for DTR expression and found low expression levels in tissue macrophages, suggesting a low DTR expression in these cells. mCherry was detected predominantly in the ly6C population in blood. Other methods for macrophage depletion are being explored.

We then used chimeric mice with congenic markers (CD45.1/CD45.2) to ask if peripheral (bone marrow derived) macrophages migrated into the brain post TBI. At 7 d post TBI, chimeric mice (>50% donor cells in blood, bone marrow, and CLN) were assessed for donor and recipient contribution to the neuroinflammatory response to TBI. As expected, all the microglial cells in the brain were of host (recipient) origin, i.e. not repopulated from the BM. Since these experiments were done >20 weeks post-transplant we also confirm that replacement of microglia from peripheral sources is a slow process, if at all. This finding also alludes to a brain source for replenishment of these resident brain

macrophages. Post TBI, CD45(hi) total infiltrating macrophages numbers were high (as previously shown) and were of both recipient and donor origin. However, CD45(hi) Ly6C(hi) inflammatory macrophages were mostly (>75%) derived from recipient, even in animals with 80% donor derived cells in the blood. This finding suggests either (1) there is a local tissue source for macrophages or (2) there is selective recruitment of host derived cells into sites of inflammation (even when blood chimerism is as high as >80%). This also means that our therapeutic modulation of macrophage responses in the brain would need to be directed to a tissue source (than circulating cells), giving more credence to the hypothesis that umbilical cord blood stem cells use a distal tissue directed mechanism to modulate brain inflammation.

Dissemination: Results from these studies have been presented at the 2019 Minnesota Neurosurgery Society Annual Meeting as an oral presentation, the 2019 NexGen Stem Cell Conference as an oral presentation, 2019 National Neurotrauma Society Annual Symposium, and the Society for Neuroscience conference 2019.

Two manuscripts describing the kinetics of neuroinflammation in (1) mild- and (2) moderate-injured mice are currently being written with the intent to submit before the end of 2020.

Institution: Hennepin Healthcare Research Institute
Principal Investigator: Dr. David Darrow
Project Title: *Epidural Stimulation for Spinal Cord Injury*
Grant Cycle: FY2018

Accomplishments: The development and implementation of our application has allowed us to collect data remotely through the home-exercise in conjunction with accelerometers and questionnaires. Such data allows us to assess the efficacy of each setting with regards to improvements in volitional movement, autonomic function, and bowel/bladder control while the participant is in the comfort of their own home. Through this, we are able to continuously refine setting parameters based on participant preference. In addition, this home-exercise also serves as a form of therapy that can either enhance the participants' current regimen or provide a form of activity for those that do not participate in rehabilitation programs for various reasons.

Using the MOTOmed bike, a motorized therapy device, we now have the capability of capturing various elements of interest regarding volitional movement. Specifically, we are able to collect data related to the duration of active pedaling, power generated, spasticity, muscle tone, and sidedness among others. This data is collected with and without stimulation to assess the aforementioned variables. Such data allows us to assess changes, either improvements or declines, in subjects' volitional movement throughout the duration of the study. In addition, it has provided the ability to reinforce the relationship between our developed tablet application and optimized setting parameters. Current analysis is preliminary but holds promise.

Dissemination: The media has been our primary vehicle for disseminating our results to communities of interest. We have used our updated website to create blog posts that include links to our publication, news articles and presentations given by our investigators. Through Dr. Darrow's social media, study updates and information have been provided as well. Moreover, patient progress videos have been posted on social media accounts, such as YouTube, where they have been shared by others in the community. Additionally, a study guide was also created for our participants which entails information on our study and a link to our publication. We also send monthly updates to each subject via email, which includes current findings, presentations and articles.

Institution: Center for Veterans Research and Education
Principal Investigator: Dr. Casey S. Gilmore
Project Title: *Combined tDCS and Cognitive Training to Reduce Impulsivity in Patients with Traumatic Brain Injury*
Grant Cycle: FY2019

Accomplishments: All necessary study approvals, including IRB approvals from both the VA and the University of Minnesota (UMN), and the subcontract with UMN to allow us to collect MRIs at the Center for Magnetic Resonance Research there, have been obtained. We have enrolled the first subject in the study. Through coordination with the Rehabilitation and the Addiction Recovery Services care teams at the VA, we have a list of potential participants whom we have begun to contact.

Dissemination: None.

Institution: Center for Veterans Research and Education
Principal Investigator: Dr. Gary Goldish
Project Title: *Head-Mounted Display Virtual Reality in the Treatment of Upper Extremity Function in Acute TBI Rehabilitation: A Comparison Study to Conventional OT Treatment Alone*

Grant Cycle: FY2019

Accomplishments: We have worked on optimizing the flow of our study. We have developed a standardized study definition of a 'repetition'. A rep is defined as a volitional movement of the more-affected UE that describes any of the following:

- UE leaves a starting point and returns to a resting position (Score as 1 rep given the return to rest is a more passive, non-purposeful movement)
- UE leaves a starting point and has a purposeful, active, controlled return (Score as 2 reps given the purposeful, active return movement)
- UE starts and has a clear stop before changing directions (Score as 1 rep)

If simultaneous movement is occurring at multiple joints, the joint most distal is counted. Based on this definition, two research staff were trained on how to count reps. Counters independently counted reps during 17 instances of VR and table top activities. Our biostatistician (Amy Gravely) computed intra-rater reliability (ICC > .9). Due to the high ICC we feel confident using one counter going forward.

All study personnel were trained on how to implement the VR intervention, how to conduct the assessments used in study, and best practices when conducting research. Our clinician collaborators continue to be excited about the potential use of VR in the clinic for rehabilitation.

The study has been actively recruiting subjects since October 2019. The study team meets weekly to discuss recruitment and problem solve barriers to participation. We have screened 14 potential subjects and enrolled none (see consort below). The primary reasons for excluding subjects have been no UE impairments, other medical conditions that prevent participation (e.g., wearing a c-collar on the neck), and dual TBI/stroke diagnoses. We had originally excluded cases of dual TBI/stroke diagnoses due to the differences in recovery trajectories, but after discussion with the study team have decided to include these subjects in the future as clinicians state that dual diagnoses can be common in the Veteran population served at the Minneapolis VA. Therefore, to have a representative sample, we will include Veterans with dual TBI/stroke diagnoses moving forward.

In December, 2019 we met with Jack Avery, the Minneapolis VA's CARF Administrator, to get insight into the Polytrauma census over time. In our meeting we discovered that there has recently been a lower number of TBI admits than is typical in prior years.

We have submitted an amendment to include active duty service members along with Veterans in this study. The Minneapolis VA polytrauma center has a partnership with the Department of Defense (DoD) in which the polytrauma program serves active duty military members in addition to Veterans. The DoD and VA have a strong commitment to the identification and patient-centered treatment of active duty service members and Veterans who are in recovery from a TBI. Including active duty service members in this study helps underline this commitment and may aid in recruitment.

Dissemination: None.

Institution: HealthPartners Institute
Principal Investigator: Dr. Leah R. Hanson
Project Title: *Intranasal Insulin to Improve Recovery following Cervical Spinal Cord Injury*
Grant Cycle: FY2019

Accomplishments: The start-up activities of this project continue to take longer than anticipated including delivery of tools/devices and successful implementation of the model in the lab. We anticipate that we will need the one year cost extension. We continue to meet bi-monthly with the research team.

We have the necessary equipment and training to conduct the behavioral testing and the biochemical analysis. While we received the FEJOTA clip last summer, it did not work properly and we needed to return it for modification and re-calibration. Customs also delayed that process. Upon its return, we still were not able to successful cause a reliable cord injury. So, we have amended our IACUC application to include an alternate model of inducing injury with the impactor. The amendment was approved. The machine was ordered, but some of its parts are currently on back order from China. We are hopeful that we will receive the impactor before March, 2020.

Dissemination: None.

Institution: Mayo Clinic
Principal Investigator: Dr. Igor Lavrov
Project Title: *Spinal Cord Tissue Regeneration through Schwann Cell Seeded Hydrogel Scaffolds with Spatial-Selective Electrical Stimulation*
Grant Cycle: FY2019

Accomplishments: For the purpose of the project we further developed a comprehensive combinatorial approach that combined an hydrogel (OPF+) scaffold that contained drug eluting microspheres and loaded with genetically modified Schwann cells with electrical stimulation and motor training in spinal cord injured rats. Immediately following transection, a scaffold embedded with glial-cell derived neurotrophic factor (GDNF) expressing SCs and drug-eluting microspheres will be combined with EES for neuromodulation of spinal cord circuitry with locomotor training.

The treatment tested in this study is a combination of many individual therapies investigated by our group in the past. We combined genetically modified cells, with small molecules, scaffolds, electrical stimulation, and motor training on a treadmill in a rat model of spinal cord injury. Electrodes were placed in the muscles as well as above and below SCI. The spinal cord was transected at T9 and the GDNF/SC-RAPA-OPF+ was implanted.

Adult, female Sprague Dawley rats were trained to step on a treadmill system for one week after which they were implanted with an epidural stimulation electrode (L2) and intramuscular EMG electrodes into the tibialis anterior (TA) and medial gastrocnemius (MG) muscles. Following one week after the first surgery, a complete spinal cord transection will be performed at the T9 thoracic level followed by implantation of a GDNF/SC-RAPA-OPF+. Custom-built electrode for EES was implanted on top of the scaffold covering the segments T8-T10. Following implantation and recovery, EES with locomotor training will be performed for 8 weeks. Implanted rats were followed for 7 weeks following thoracic spinal cord transection. We analyzed gait recovery using open field testing, kinematics, and electromyography from the hind-limbs. At week 6, a group of rats were re-transected through the scaffold to determine the extent of recovery.

The functional effect of this treatment was determined by treadmill assisted kinematic analysis, electrophysiological assessment, and open field testing. Rats implanted with scaffolds recovered better EES-enabled stepping than rats with no scaffold (Figure 2). Specific improvements in gait parameters such as step height, step length, toe fluctuation, and drag phase were demonstrated. In addition, there were significant improvements in the knee, ankle, and MTP angle displacements.

As early as 2 week following SCI we found some improvement in stepping in animals that were implanted with the scaffolds compared to no scaffolds when they were electrically stimulated (40 Hz, 1-2.5V) below injury.

Rats that received our combinatorial therapy (scaffold group) had better stepping on the treadmill when induced by epidural electrical stimulation than those that did not get the regenerative therapy (no scaffold group). (A) Representative stick diagrams of the gait from kinematic analysis. Although there was a trending difference in (B) BBB motor scores without EES assistance, there were significant improvements in (C) step height, (D) step length, (E) toe fluctuation, (F) drag phase, (G) hip angle displacement, (H) Knee angle displacement,(I) Ankle angle displacement, and (J) MTP angle displacement. No-Scaffold EES VS Scaffold EES*, Scaffold No-EES VS Scaffold EES#, No-Scaffold EES VS Scaffold No-EES$.

In particular we saw improvements in angular displacement of the knee, ankle, and MTP as well as step length, step height, toe fluctuation, and drag phase (Figure 2 and 3). There were no significant differences in stepping when there was no electrical stimulation. After re-transection of the scaffold we found some reduction in motor function, still, the motor performance was better compare to rats with no scaffold that could indicate on the influence of regenerated fibers on sub-lesional network spasticity.

Average (8 responses) spinal cord motor evoked potential (SCMEP) for rats with scaffolds and EES assisted motor training 1 week, 6 weeks, and post re-transection. Two representative rats that were treated with scaffolds and EES are shown (A and B). Red dotted lines indicate time of EES pulse and the middle response (MR and later response (LR) are indicated in the blue box. (C) The peak to peak amplitude of the middle response is shown for rats 6 weeks after injury and following retransection with stimulation at S1.

Overall, our results indicate that there is sub-functional reconnection through the scaffold and that this connection influences organization of spinal circuitry and stepping induced by EES.

We were also recording the BBB open field test with and without electrical stimulation. Kinematic analysis is very sensitive in picking up changes in gait and we wanted to see if any gait benefits of EES could be observed in open field testing. Novel modifications were made to our behavioral testing for this study by record the BBB locomotor open field test with and without ongoing electrical stimulation (Figure 5). Hind-limb BBB motor scores improved in rats with scaffolds and EES during stimulation at 6 weeks when compared to those without scaffolds (Figure 5B). Rats with scaffolds and EES before re-transection (Figure 5C) demonstrated no differences on BBB motor scale with stimulation on or off. After re-transection (Figure 5D) the rats with scaffolds declined in motor function when the EES was off. With EES on, their function was similar to before the re-transection. These findings support the hypothesis that the sublesional circuitry is augmented through stimulation and regeneration of axons prior to retransection. After re-transection of the regenerated fibers, EES assisted gait functions measures through BBB scoring and kinematics persisted (Figure 5). Combinatorial treatment may therefore aid functional recovery though the influence of regenerated connections on reorganization of the sub-lesional circuitry.

Basso, Beattie, and Bresnahan (BBB) motor scores for rats implanted with scaffolds or only transected. (A) BBB score was record by 3 observers when then EES was off. Rats with scaffolds that received EES (blue) or no EES (green) trended to do better than the rats with no scaffold (red). (B) When the BBB scores were recorded with subthreshold EES on, the rats with scaffolds did significantly better than the rats without scaffolds 6 weeks post injury. (C) Rats with scaffolds and EES had similar BBB scores with EES on or off before the rats were retransected. (D) After retransection, there was an expected drop in BBB score when the EES was off, however, the BBB score was better with EES on, similar to before retransection.

The rats that were regularly stimulated during motor training and kinematics were compared to the rats that did not receive any stimulation. These rats were implanted with scaffolds loaded with GDNF Schwann cells and Rapamycin microspheres. We demonstrated that stimulated rats have better angular displacement of their hip, knee, ankle, and MTP.

Angular displacements of joints in rats implanted with scaffolds that received EES during motor training and testing compared to those that did not. Stimulated rats had greater hip, knee, ankle, and MTP angle compared to non-stimulated rats. After re-transection (week 7, past red dotted lines), stimulated rats had similar angle displacement to non-stimulated rats without re-transection.

The stimulated rats also had greater stride length, step height, toe fluctuation, stance phase, and swing phase duration with reduced drag phase compared to non-stimulated rats (Figure 7). Improvements in step height, stance phase, and drag phase persisted in re-transected stimulated rats compared to unstimulated rats with no second injury. This re-emphasizes our thought that there may be changes facilitated at the circuitry below injury that is strengthened with our regenerative therapy and repeated stimulation.

Improvements in motor function on treadmill testing. Stimulated rats had greater stride length, step height, toe fluctuation, stance phase and swing phase duration. Drag phase decreased in stimulated rats. The stimulated rats that were re-transected had improvements persist over the unstimulated rats that did not receive a re-transection (week 7, past red dotted lines).

Immunohistochemical analysis was performed to determine the number of axons passing the lesion site through the scaffold, as well as characterize the synaptic connections in the lumbar spinal cord segment. Staining with neurofilament showed evidence of axons traversing through the scaffold (Figure 8). Staining on scaffold sections, along with myelin basic protein, helped to identify the number of myelinated and unmyelinated axons are passing through the length of the scaffold. Axons were observed to regenerate across the scaffold length. At the same time, there was no differences in axon numbers between rats with scaffolds regardless of EES.

Scaffold implanted at thoracic level 9 after spinal cord transection can be seen containing axons (white) 7 weeks after injury. In rats implanted with scaffolds there was no difference in the amount of axons through the scaffold in those that received EES or those that did not. Therefore, our scaffold supports axon regeneration which is not affected by EES in the lumbroscaral cord.

As there was an improvement in gait in rats with scaffolds during treadmill walking with the assistance of EES, we investigated synapse formation within the sublesional circuitry at the level of the lumbar cord, corresponding to the anatomic location of the central pattern generators Immunostaining co-localized synaptophysin, a marker of synaptic vesicles, with either choline acetyltransferase for motor neurons and calbindin for interneuron populations (Figure 9A-D). The number of synaptic vesicles was measured by QuPath image analysis software and was normalized to the cell area for each of the neuronal subtypes (Figure 9E-F). The median number of synaptophysin on ChAT and calbindin positive neurons was greater in rats that had scaffold implantation and stimulation than in rats with scaffolds alone (no EES) or those injured with stimulation (no scaffold control). The distribution was intermediate for rats with scaffolds but without EES stimulation This result parallels the observed in motor recovery seen in the kinematics analysis where the recovery of rats with scaffolds was intermediate to those with no scaffold and to those with scaffold with stimulation. Regenerating fibers through the scaffolds influenced the reorganization of the sublesional circuitry at the motor and interneuron synaptic level. The combination of epidural electrical stimulation of the sublesional circuitry with regenerating fibers through the scaffold further enhanced synaptic modification, which may correspond to improved kinematic, electrophysiologic and BBB functional improvements.

Behavioral data showed that rats with scaffolds even after re-transection performed better than rats with no scaffolds. ChAT (cyan; B) labelled the motor neuron populations, calbindin (green; C) labelled a subpopulation of interneurons, synpatophysin (red; C) was used to label synaptic vesicles. This data demonstrates that rats with scaffolds and retransection had greater synpatophysin on ChAT (E) and Calbindin (F) labelled neurons. The

number of synaptophysin points on neurons from rats with scaffold without EES was intermediate to that of no scaffold and scaffold with EES. Therefore, there were greater synaptic connections in the lumbroscaral spinal cord of scaffold rats after retransection than rats with no scaffolds. Median +/- 95% confidence interval. Kruskal-Wallis test with Dunn's multiple comparisons. * compared to no scaffold; # compared to scaffold with no EES.

Dissemination: The results of this study were presented at several international and local meetings and were disseminated to communities of interest.

Institution: Mayo Clinic|
Principal Investigator: Dr. Kristin Zhao
Project Title: *Direct Comparison of Transcutaneous and Epidural Spinal Stimulation to Enable Motor Function in Humans with Motor Complete Paraplegia*
Grant Cycle: FY2019

Accomplishments: Upon receiving approval of our IDE protocol from the FDA, as well as protocol approval from Mayo Clinic's IRB, we conducted a preliminary screening of a list of potential study participants that were eligible based on the inclusion and exclusion criteria of this clinical trial. We identified the first potential participant and, after obtaining informed consent, performed screening tests. During the screening phase, a baseline MRI identified a possible unhealed fracture in the medial femoral condyle of the right leg. The subject passed all other screening criteria; however, the presence of a possible unhealed fracture resulted in screen failure. This potential participant expressed interest in repeating the screening once adequate time had passed to allow the fracture to heal, and therefore, re-screening was conducted at a later date. Imaging showed that the fracture lines had resolved, however, the subject then exhibited bilateral gastrocnemius tendon tears, and thus failed screening again.

We identified and obtained informed consent to screen a second potential participant. This candidate passed the screening phase and was enrolled in September 2019 to participate in 6 months of TESS, followed by one month of rest and another 6 months of TESS. To date, this participant has completed 46 sessions of TESS with rehabilitation and has achieved bouts, each lasting approximately one minute, of TESS-enabled independent standing.

A third participant recently consented to the study, passed screening, and was enrolled in January, 2020 to participate in 6 months of TESS, followed by implantation of an EES system, one month of rest, and then 6 months of EES. To date, this participant has completed 5 sessions of TESS with rehabilitation. To date, no serious adverse events have occurred.

The first two clinical trials participants are currently completing the initial TESS phase of this study. We have observed positive outcomes for both subjects during TESS, such as improvements in standing and seated posture. To date, we have not reached the study phase of EES system implantation. Therefore, we are unable to compare TESS-enabled outcomes to EES-enabled outcomes at this time.

Dissemination: None.

Institution: Mayo Clinic
Principal Investigator: Dr. Isobel Scarisbrick
Project Title: *New Combinatorial Strategies for Regenerative Repair of the Injured Spinal Cord*
Grant Cycle: FY2019

Accomplishments:

Specific Aim 1: To determine if genetic inhibition of PAR1 function initiated at an acute time point after SCI improves axon regeneration and recovery of function and any additive effects of BDNF gene therapy. These studies are in progress. We have demonstrated successful expression of recombinant BDNF in the spinal cord of adult mice following microinjection of AAV-BDNFGFP.

Specific Aim 2: To determine if pharmacologic inhibition of PAR1 initiated at an acute time point after SCI improves axon regeneration and recovery of function and additive effects of BDNF gene therapy. We have completed animal studies delivery a PAR1 small molecule inhibitor daily, 5d per week for 60d alone or in combination with AAV-mediated delivery of the powerful growth factor BDNF. The final analyses of behavioral outcomes and histopathological assessment of changes in the spinal cord, including signs of neural repair are currently underway.

We are excited to report that early outcomes assessments point to improvements in function in mice treated with a PAR1 inhibitor. Histological analyses and quantification of changes in axons and synapses are currently underway.

Specific Aim 3: To identify small molecule inhibitors of PAR1 with potent pro-regenerative effects toward human neurons and synergistic effects in combination with recombinant BDNF.

We have established the growth conditions, staining and quantitative PCR protocols for the human iPSC derived ventral spinal cord neurons in our laboratory at the Mayo Clinic. We have also implemented a robotic screening approach to monitor changes in neurite outgrowth with PAR1 inhibition in a high throughput manner in conjunction with Dr. Dutton's laboratory at the University of Minnesota. Results to date suggest that inhibiting PAR1 improves neurite growth in human neurons as our parallel studies suggest occurs in murine model systems.

Early results suggest that administration of a PAR1 small molecule inhibitor improves clinical and histopathological outcomes in a murine compression model of traumatic spinal cord injury, as was suggested by our preliminary studies at the time of grant submission. We are currently determining if combining this approach with delivery of recombinant BDNF further enhances the regenerative potential of the injured spinal cord. Early results from cell culture studies support the hypothesis that blocking PAR1 will improve neuron survival and neurite outgrowth in response to growth factor therapy.

Dr. Scarisbrick along with the Co-I Dr. Dutton have submitted several grant applications using new preliminary data generated as a result of this project. This includes an NIH R01 grant application and a Minnesota Partnership Grant application. While these new applications were not funded, they form the basis for additional future studies.

Dissemination: None.

Institution: Hennepin Healthcare Research Institute
Principal Investigator: Dr. David Darrow
Project Title: *iRehab: Discovering Outpatient Rehabilitative Measures for Epidural Stimulation Assisted Movement*
Grant Cycle: FY2019

Accomplishments:
Hennepin Site:
Thus far, 1,012 people with spinal cord injury have completed our prescreening survey and 30 of these people have completed in-person formal screening. Of these potential participants, 13 subjects have been enrolled.

These participants have successfully undergone stimulation implantation surgery. Five participants have completed the study, while the other participants are undergoing the study protocol.
Currently, seven subjects are participating in at home therapy exercises, which are tracked through an app made for the study. Additionally, these patients are undergoing bicycle therapy with and without spinal cord stimulation, during their routine follow ups.
Minneapolis VA Site:

Of the 1,014 people screened, 65 indicated that they were veterans. Veterans were also contacted through information obtained from the Minneapolis VA and referred by Minneapolis VA physicians. Of these candidates, 4 have been screened in person and 1 subject was enrolled and has successfully undergone stimulation implantation surgery. This subject has completed the study as of July 2019.
To date, six subjects have demonstrated ability to bike with the stimulator on. One of our subjects is enrolled in the ABLE program through Courage Kenny while participating in our study. She has seen major improvements in strength with and without the stimulator on documented in the rehab notes. Her experience will be useful in developing a rehab plan that can be used by cSCI patients using eSCS.

Dissemination: None.

Institution: Hennepin Healthcare Research Institute
Principal Investigator: Dr. David Darrow
Project Title: *iOptimize: Optimization of Epidural Stimulation for Spinal Cord Injury*
Grant Cycle: FY2019

Accomplishments:

Hennepin Site:

Thus far, 1,014 people with spinal cord injury have completed our prescreening survey and 30 of these people have completed in-person formal screening. Of these potential participants, 14 subjects have been enrolled. These participants have successfully undergone stimulation implantation surgery. Five participants implanted had 13 follow-up visits and completed the study. Additionally, the most recent participants (thirteen and fourteen) have had their one month follow-up visit. Each of the fourteen subjects has undergone spatial mapping (specific aim 1) for volitional movement. This data has been processed by our UMN engineering team to help determine patient specific spatial settings to pair with the temporal optimization protocol, which was previously developed in our study. This multifaceted approach has improved our rate of setting optimization and will be used to guide our intensive spatial setting testing at the UMN when that portion of the study begins later this year.

Ten of our patients were found to have cardiovascular dysfunction with enrollment in the autonomic arm. Autonomic subjects are also given a set of device parameters for autonomic dysreflexia and are optimized using application guided survey responses about effectiveness. In-person testing with the Finapres has been used to guide determination of what autonomic settings are strongest. Our Canadian collaborators have come to HCMC for autonomic testing with these individuals per the protocol. We have successfully purchased the finapres MIDI as outlined in the grant budget.

University of Minnesota Site:

A postdoctoral student was hired as described in the grant proposal and has been instrumental in temporal and spatial setting optimization. She works with subject survey and accelerometer data from the subjects' home

training from the study created tablet application. Her work is guided by Dr. Netoff and his lab is instrumental in guiding setting optimization.

Aim 2 and 3 of the grant application laid out a plan for 2 subjects to undergo intensive in patient testing after implantation with a boston scientific epidural stimulator. The devices have been donated and the site IRB is being pursued to begin the enrollment and surgeries. The process of procuring IRB approval has been slower than anticipated in the grant timeline. This will likely drive back our anticipated timeline for the surgeries and follow ups. However, progress is being made in the meantime. A protocol and consent have been developed, training on study assessments have been administered, and other collaborators have been added to the study. Additionally, we've been working closely with Boston Scientific to get the documents necessary to prepare and FDA submission, so the study may be started at the University of Minnesota. We anticipate that the study will start very soon and have organized all aspects in order to be as prepared as possible.

The spatial mapping protocol implemented as part of this grant (Aim 1) has been incredibly successful. A majority of device programming before protocol implementation relied on temporal setting optimization (stimulation frequency and pulse width). Now that setting optimization has been expanded to consider the thousands of spatial settings, we have found that spatial settings may be very pivotal for ensuring control of both lower extremities independently and for recruiting new sets of muscles previously not innervated by the stimulation. These spatial setting findings can help create a map of each subject's spinal cord to ensure that stimulation is directed to the correct nerve roots and muscles to allow for controlled volitional movement in more muscles.

Dissemination: None.

Institution: Hennepin Healthcare Research Institute
Principal Investigator: Dr. Samuel Cramer
Project Title: *Cortical Spreading Depolarization after Severe Traumatic Brain Injury*
Grant Cycle: FY2019

Accomplishments: We have enrolled a single subject in the study. We are actively developing the analytic tools to stream line analysis of the ECoG recordings we have obtained from the single subject. We are actively recruiting subjects. We have also submitted a manuscript describing the study protocol which is currently under review.

We are happy to have finally enrolled a subject after failing to enroll several subjects during the screening phase.

Dissemination: None.

Institution: University of Minnesota
Principal Investigator: Dr. Brendan J. Dougherty
Project Title: *Targeting Estrogen Receptors to Restore Spinal Plasticity in Acute Spinal Cord Injury*
Grant Cycle: FY2019

Accomplishments: Overall, funding from this Tier 1 Pilot Project has been efficiently utilized to advance the overall research goals of our laboratory, address initial goals of our proposed research Aims, and establish baseline preliminary data critical for future funding. Specific to the Aims of our initially SCI/TBI proposal, we have made progress towards both proposed Aims. Graduate student Rebecca (Feczer) Barok is independently completing all SCI surgeries and post-operative care. Findings include preliminary evidence supporting our Aim 1 hypothesis that activation of spinal, membrane associated estrogen receptors using E2-BSA is sufficient to

restore plasticity of respiratory motor output. In addition, we completed trials of immunohistochemistry for the three known estrogen receptors in the cervical spinal cord as proposed for Aim 2 in support of on-going studies.

These preliminary data have been used as the basis for multiple additional grant submissions including an NIH R01, A Craig H. Neilsen Foundation Pilot SCI study and multiple internal UMN Research proposals. Critiques from those submissions have significantly shaped the emphasis of our research in year 2 of this SCI/TBI funding period to include more supportive evidence of the role of estrogen in respiratory neuroplasticity. We are currently collecting data for a study exploring whether one-week of hormone supplementation (estrogen or DHT, a testosterone derivative) will improve respiratory function and the expression of plasticity in rats with sub-acute SCI (2-wks post injury). This study is planned for completion prior to the conclusion of our funding and will be submitted for publication by the end of the year. Additionally, we have completed preliminary neurophysiological studies identifying estrogen receptor beta as the critical receptor permitting plasticity in female rats, while both estrogen receptor beta and estrogen receptor alpha are needed to permit plasticity in males. Further, it appears that estrogen may act as a critical homeostatic regulator of spinal cord microglia, and that reductions in estrogen availability in both females and males, as seen following SCI, may alter microglial phenotype and impair the ability to express spinal neuroplasticity. This is a research direction that we are actively pursuing.

Dissemination: None.

Institution: University of Minnesota
Principal Investigator: Dr. Ann M. Parr
Project Title: *Training Transplanted Spinal Neuronal Progenitor Cells (sNPCs) to Function after Spinal Cord Injury*
Grant Cycle: FY2019
Accomplishments: The project has two Specific Aims:

1. To determine whether the application of rose Bengal phototoxic glial scar ablation will improve the survival, differentiation, and integration of sNPCs in the chronically injured rat spinal cord; and,
2. To determine whether the transplantation of sNPCs (the addition of glial scar ablation will be determined by Specific Aim 1) can produce functional synapses and locomotor recovery in our rat model of chronic moderate contusion SCI and whether the addition of TANES would enhance this recovery.

<u>Progress so Far</u>

• Chronically contused injured rats were either glial scar ablated or not ablated and injected with human iPSC derived sNPCs.
• Rats were sacrificed 4 weeks, 8 weeks and 16 weeks and spinal cords were harvested.
• The harvested cords were cryosectioned.
• The cords are currently being processed for histological and immunohistochemical assay.

Dissemination: None.

Institution: University of Minnesota
Principal Investigator: Dr. Ann M. Parr
Project Title: *Optogenetics for Corticospinal Tract Stimulation in Combination with Transplanted Spinal Neuronal Progenitor Cells after Spinal Cord Injury*
Grant Cycle: FY2019

Accomplishments: We are currently testing optimal hindlimb stimulation strategies. We have optimized transduction of virus in our human spinal neuronal progenitor cells, in lumbar segments of the spinal cord and in the cortex. Currently, we have developed robust protocols for transducing cells in motor cortex from hindlimb motor pools within the spinal cord. We have also developed a technique to map viral transduction within the

entire brain and spinal cord by tissue clearing the brain and spinal cord and measuring the normalized fluorescent intensity within the brain, then registering the fluorescent intensity to an atlas. Finally, we have optimized coordinates for stimulation of hindlimb motor-evoked potentials but we are still in the process of optimizing stimulation parameters.

Dissemination: None.

Institution: University of Minnesota

Principal Investigator: Dr. Timothy J. Ebner

Project Title: *Evolution of Acute and Chronic Effects on Neuronal Activity and Morphology following Mild Cerebral Cortical Traumatic Brain Injury using Multi-Scale Optical Imaging in Behaving Mice*

Grant Cycle: FY2019

Accomplishments: In the first 18 months of funding, we have: 1) developed a modified See-Shell (fenestrated See-Shell) to allow single or multiple controlled cortical impact as a mTBI model; 2) developed and implemented sophisticated analyses including novel ways to dissect out temporal-spatial patterns of activity, functional network connectivity and automated single cell identification; 3) assembled a team of investigators and identified additional recourses for the project; 4) studied two cohorts of mice (impact and sham) with a single controlled cortical impact; and 5) studied two cohorts of mice (impact and sham) with repeated controlled cortical impacts. The two cohorts of mice were studied with wide field and two-photon (2P) Ca2+ imaging, behavioral assays (rotarod and open field), and immunohistochemistry.

We reported results from the single mTBI group in the 12 month report. The Ca^{2+} imaging data suggested modest decreases in cortical functional connectivity following mTBI. Given the clinical observation that multiple mild TBIs are more likely to result in long-term deficits, we decided to study multiple cortical impacts. Therefore, in this report we focus on the experimental results in which the controlled cortical impact was repeated 3 times. Using the fenestrated See-Shells that allow opening of the window and access to the cortical surface, a single cortical impact of 1 mm depth at 0.4 m/sec was delivered over 3 days in GCaMP6f or wild type C57Bl/6 mice. The later were used for histological and/or behavioral assessments and the former for wide field and 2P Ca^{2+} imaging. Importantly, the fenestrated See-Shell allows for excellent optical access with repeated impacts, and therefore, we can perform imaging of neuronal activity before and following mTBI.

In a subgroup of animals, immunostaining for inflammatory markers was performed at 24, 48, and 72 hours. These included GFAP as a marker for gliosis and Iba-1 for microglia activation. Repetitive mTBI results in a transient elevation of GFAP and Iba-1 at 24 hrs which does not persist at 48 or 72 hrs (a total of 20 mTBI and sham animals have been evaluated across time points). The transient increase in inflammatory markers is consistent with other models of mTBI and confirm that our model does not generate major structural damage in the cerebral cortex.

We have performed open field (data not yet fully analyzed) and rotarod testing. The rotarod data shows no difference between the sham and impact groups. Therefore, the multi-impact mTBI model does not appear to impart a significant motor deficit. This observation is consistent with clinical findings that focal motor behavior remains intact following mTBI.

Data suggests that repetitive mTBI has effects on the functional organization of the cerebral cortex for up to 4 weeks.

Although the changes did not result in focal motor behavior impairment, our hypothesis is that disruptions of cortical organization may effect more cognitive and executive functions, as observed in humans after mTBI.

Dissemination: None.

Institution: University of Minnesota

Principal Investigator: Dr. Dezhi Liao

Project Title: *The Roles of Tau in Chronic Traumatic Encephalopathy*

Grant Cycle: FY2019

Accomplishments:

1. At this very moment, we are focusing on experiments to test whether tau is required for mechanical injury-induced tau mislocalization. We have recently submitted our experimental results to Proceedings of National Academy of Science. The reviewers require that we perform our TBI experiments in cultures that had been made from tau knock-out mice. Accordingly, we have ordered tau knock-out mice from Jackson Lab and are collecting data for the revision of our article. We believe that it is of critical importance to publish our results in this prestigious journal.
2. The in vivo TBI device has been built and we are performing surgeries to test the device.
3. We are refining our hypothetical signaling framework that is responsible for neural deficits caused by tau phosphorylation, which is the key mediator for CTE:

   When we proposed the initial hypothesis, the specific residues in tau that are responsible for neural deficits associated with CTE were not known at that time. We have now narrowed down two distinct domains that are responsible for the pathways: the C sites of Ser396 and SerS404 as well as the B sites of Ser202, Thr205, Thr212, Thr217, and Thr23. The results have now been published in the Journal of Physiology.

4. We have now synthesized a novel tau peptide that may potentially be used to treat CTE. The University of Minnesota has filed a patent for the usage of this peptide.

Submitted Manuscript: Nicholas J. Braun, Patrick W. Alford, and Dezhi Liao (2019). Mechanical injuries of neurons induce tau mislocalization to dendritic spines and tau dependent synaptic dysfunction (requesting revision in PNAS)

Status: We have submitted our manuscript to Proc Natl Acad Sci U S A. It has passed the initial screening of editorial board. We will receive very positive feedbacks from the journal. However, *the reviewers request that we perform experiments in tau knock-out mice.* We are currently doing the experiment now.

Significance: Athletes and soldiers exposed to repeated traumatic brain injuries have increased risk of developing chronic traumatic encephalopathy (CTE), which is a neurodegenerative disease characterized by tangled deposits of the protein tau in the brain and loss of cognitive function. This study provides the first experimental evidence showing that mechanical stretching of neurons induces tau to be mislocalized to dendritic spines where it causes synaptic dysfunction. This cross-disciplinary study utilizes computational modeling, imaging, and electrophysiology to unravel a new cellular framework for how tau phosphorylation mediates trauma-induced synaptic dysfunction, highlighting the essential role of tau in functional deficits caused by traumatic brain injury.

Publication: Teravskis PJ, Oxnard BR, Miller EC, Kemper L, Ashe KH, Liao D. **Phosphorylation in two discrete tau domains regulates a stepwise process leading to postsynaptic dysfunction**[4]. J Physiol. 2019 Jun 13. doi: 10.1113/JP277459.

Significance: The published experimental results have refined our initial hypothesis in this proposal because of the in depth characterization of tau phosphorylation sites.

[4] https://www.ncbi.nlm.nih.gov/pubmed/31194886

Publication: Teravskis PJ, Covelo A, Miller EC, Singh B, Martell-Martínez HA, Benneyworth MA, Gallardo C, Oxnard BR, Araque A, Lee MK, Liao D. **A53T Mutant Alpha-Synuclein Induces Tau-Dependent Postsynaptic Impairment Independently of Neurodegenerative Changes**.[5] J Neurosci. 2018 Nov 7;38(45):9754-9767. doi: 10.1523/JNEUROSCI.0344-18.2018.

Significance: Some CTE patients also suffer from motor deficits. The results reported in this manuscript will provide new insights on the neurobiological mechanism underlying these deficits.

Patent:

Institution: The University of Minnesota
Title: TAU PEPTIDES, METHODS OF MAKING, AND METHODS OF USING
OTC #: 20170226
Date: February 29, 2019
Docket No.: 0110.000572US01
Previous Provisional Patent Number: 62/636,523 (filed on February 28, 2018)
Inventor: Dezhi Liao
Unit: Department of Neuroscience
Objective: Using tau peptides to treat neurodegenerative diseases (AD, FTD, PD and CTE) by blocking tau mislocalization to dendritic spines.
Funded federal grants:
Application ID: 1 R61 NS115089-01 [Ebner-PI, Liao-PI, Koob-PI (CONTACT)]
Title: Full human gene-replacement mouse models of ADRDs
Source of Support: NIH
Award Period Covered: 09/01/2019 – 08/31/2024
Months/year: 1.8 calendar
Annual Direct Cost: $492,669

Total Cost: $3,824,524

"Targeting tau phosphorylation and missorting to treat Alzheimer's Disease"

Major Goals: To the present, most animal models were made by random insertion of cDNA. In the proposed project, we will replace the whole mouse tau gene with human tau genes with mutations linked to tauopathy.

Role: multiple PI

Application ID: BMMB 1935834 (Alford-PI, Liao-co-PI)

Title: Mechanics of trauma-induced tauopathy

Source of Support: NSF

Award Period Covered: 09/01/2019-08/31/2021

Total direct cost: $400,000

Major Goals: To test how mechanical parameters affect tau mislicalization and associated synaptic deficits in chronic traumatic encephalopathy models.

Dissemination: None.

[5] https://www.ncbi.nlm.nih.gov/pubmed/30249789

Institution: University of Minnesota

Principal Investigator: Dr. Andrew W. Grande
Project Title: *Harnessing Exosomes as a Biomarker and Therapeutic Approach to Traumatic Brain Injury*
Grant Cycle: FY2019

Accomplishments: The effort of the past year was to focus on validation of the rat model of TBI in our lab. We needed to purchase a new impactor device in order to improve standardization of injuries across animals and have been able to observe significant deficits associated with the injury and replicable lesion volumes.

We are also optimizing protocols to isolate exosomes from minute quantities of blood from rats as this is necessary for long-term tracking of biomarkers from individual animals.

Finally, we have successfully isolated exosomes from our non-hematopoietic umbilical cord blood stem cell line, which will be used to treat rats with TBI.

We have established a collaboration with a bio-tech company which will isolate exosomes from our non-hematopoietic umbilical cord blood stem cell line using their isolation platform and will provide exosomes from this line for therapeutic applications in TBI to be tested alongside exosomes isolated at the University of Minnesota.

Dissemination: None.

Institution: University of Minnesota
Principal Investigator: Dr. Andrew W. Grande
Project Title: *Reprogramming Astrocytes into Neurons to Provide Therapeutic Benefit in TBI*
Grant Cycle: FY2019

Accomplishments: To identify all cells infected with the adeno-associated virus (AAV), we developed a strategy using an AAV expressing Cre on a GFAP promotor with the Ai9 transgenic mouse following TBI. Ai9 mice have a Lox-Stop-Lox tdTomato insert on the Rosa26 locus. In this approach, all infected cells will expressed Cre, excising the stop sequence, permanently expressing tdTom, regardless of reprogramming status. Intracranial injection of AAV6 resulted in few tdTom+ cells, while many astrocytes and neurons were tdTom+ following AAV9 injection. AAVrh10 injection into the injured brain resulted in many tdTom+ cells, more of which appeared to have an astrocyte morphology.

Given many $tdTom^+$ cells had a neuronal morphology at 7-DPI, we wanted to interrogate the phenotype of the cell soon after AAV administration. To test this, Ai9 mice were injected with AAVrh10 one week after TBI and euthanized 1-DPI for immunohistochemistry. Intracranial injection of AAVrh10-hGfABC1D::hASCL1:Cre resulted in tdTom+/Cre+ neurons. tdTom+ fiber tracts are also observed in the striosomes of the striatum. This suggests that in our system the hGfABC1AD promoter is not tightly regulated and results in leaky expression, or that GFAP is expressed in neurons not detectable through immunohistochemistry.

While many cells can be initially infected with the AAV construct, the number of astrocytes exiting the cell cycle during reprogramming is likely limited. We therefore were interested in the effects of AAV infection on the whole population of astrocytes. Immunohistochemistry of tdTom+ cells at three time points following intracranial injection of AAVs demonstrates an increase in the number of tdTom+ cells, particularly along the corpus callosum of the ipsilateral hemisphere. This suggests that a population of infected cells carrying the excised stop sequence have proliferated throughout the brain.

These data have been presented as a poster at the International Society for Stem Cell Research as well as the

Society for Neuroscience annual conferences. The topic of astrocyte reprogramming following cerebral injury has led to a platform session at the American Society for Neural Therapy and Repair annual conference, building strong collaborations with faculty from multiple universities.

Dissemination: None.

Institution: Center for Veterans Research and Education
Principal Investigator: Dr. Uzma Samadani
Project Title: *Enhancing Rehabilitation with Neuromodulation for Veterans with Spinal Cord Injury*
Grant Cycle: FY2019

Accomplishments: Only one subject has been recruited thus far in the study. The VA has many restrictions on how veterans will be recruited and finding candidates that fit the eligibility requirements has proved challenging. Six devices have been donated from Abbott Labs, so five devices remain for implantation. The team is currently primarily focused on obtaining additional recruits and developing outreach strategies that may increase our chances of recruitment. Over 50 veterans have reached out on our website, but have been excluded for various reasons. A total of 4 in person screenings have been completed. Unfortunately the most recently screened participant did not progress to enrollment. We are currently looking for other outreach opportunities.
The first subject at the VA has completed the study, and we are currently working on finding other veterans to screen. Through a combined effort through HCMC and the VA, we have been able to implant a total of 14 subjects and have seen incredible results with regards to partial restoration of volitional movement and autonomic function. Our hope is to find additional veterans who fit the criteria for the study.
Dissemination: None.

Institution: Hennepin Healthcare Research Institute
Principal Investigators: Dr. David Darrow, Dr. Ann Parr and Dr. Thomas Bergman
Project Title: *ESTAND 2.0 – Bridge to Clinical Approval of eSCS for SCI*
Grant Cycle: FY2020

Accomplishments: Since the grant has been awarded, the E-STAND study has made enormous strides towards accomplishing the aims of the grant. Thus far, 1,014 people with spinal cord injury have completed our prescreening survey and 30 of these individuals have completed in-person formal screening. Of these potential participants, 14 subjects have been enrolled (5 of which since this grant has been awarded).

These participants have successfully undergone stimulation implantation surgery and have marked benefits from the stimulator. Five participants have completed 13 follow-up visits and finished the study. Additionally, the most recent participants (thirteen and fourteen) have completed their one month follow-up visit. Each of the fourteen subjects has undergone spatial mapping for volitional movement and submit daily surveys that help access the functionality of each setting, with regards to their volitional and autonomic function. This data is processed by our UMN engineering team to help further optimize and categorize each patient's settings. This has been particularly effective when looking at specific functionalities, such as core-balance, transferring, digestion, etc. This multifaceted approach has improved our rate of setting optimization.

Additionally, ten of our study participants have been found to have autonomic dysreflexia and were enrolled in the autonomic part of the study. Currently, they are undergoing tilt-table testing to gain more insight on the effects of eSCS and blood pressure regulation, as well as cognition. Moreover, we have been able to implement

EEG during these assessments to further categorize the type of dysfunction these individuals are experiencing, and how eSCS helps improve these conditions.

Lastly, a long-term protocol and consent have been developed. We are hoping to move forward and submit this study to the FDA/IRB soon.

Equipment/Grant Purchases

1. All EEG headcaps and accessory equipment has been purchased.
2. An additional headbox for our Neuralynx machine has been obtained, which allows us to conduct EMG and EEG simultaneously on SCI patients.

Since the grant has been awarded we have successfully enrolled and implanted 5 additional study participants, which has brought us closer to our goal of studying eSCS in a more diverse and generalizable population. We anticipate to enroll several more subjects this spring. Additionally, we've been able to implement EEG and preliminary results suggest that eSCS helps cognitive function in AIS A/B paraplegia patients with cardiovascular dysautonmia.

Dissemination: None.

Institution: Hennepin Healthcare Research Institute
Principal Investigator: Dr. David Darrow and Dr. Thomas Bergman
Project Title: *Non-invasive Transcutaneous Spinal Cord Stimulation for Recovery of Hand Function After Spinal Cord Injury*
Grant Cycle: FY2020

Accomplishments: The protocol and consent form have been written to assess the outcomes of transcutaneous stimulation on hand and arm function in individuals with chronic cervical injuries. Once we have IRB approval for the study, Neurorecovery Technologies, a subsidiary of GTX medical, will provide us with the devices to be used for the study. We will also begin the hiring process for an occupational therapist, who will be responsible for providing each subject with a tailored hand/arm exercise program and for administering the stimulation therapy. The components of each exercise program will include warm-up and cool down periods, active and assisted range of motion exercise, strength training, re-education of motor skills through task specific progressive functional training for performing ADL (pinching, grasping, reaching, pulling). The study will be published on clinicaltrials.gov and we will begin screening interested individuals. Currently, we have identified 221 individuals that may fit the screening criteria for the study.

A study protocol detailing the methodology for this clinical trial has been written and we are currently seeking approval from the IRB for the study.

Dissemination: None.

Institution: Mayo Clinic
Principal Investigator: Dr. Mohamad Bydon
Project Title: *A Multi-Dose Safety and Feasibility Study of Autologous Culture Expanded Adipose Derived Mesenchymal Stem Cells (AD-MSCs) in the Treatment of Traumatic Spinal Cord Injury*

Grant Cycle: FY2020

Accomplishments: In the current clinical trial that this grant is funding, we are investigating the role of fat-cell derived mesenchymal stem cells (a type of stem cell found all over the body in multiple different tissue types) injected intrathecally (i.e., into the spinal canal) among patients who have suffered a spinal cord injury. This research study is being conducted at the Mayo Clinic in Rochester, MN where nearly one-third of MSC clinical trials across the nation are conducted and approximately 200 patients with spinal cord injury are treated on an annual basis.

In the first phase of the clinical trial, our team enrolled and treated a total of 10 patients with spinal cord injury. All treated subjects have successfully moved through the acute phase of treatment with no serious adverse events and demonstrating promising preliminary results. This research study has created three positions vital to the project and future studies that will spin-off this clinical research trial. At each follow-up visit, we learn more and more about spinal cord injury, which patients may benefit from this study, and how best to shape the future of clinical care and practice for our patients.

We are planning to open phase II of our clinical trial in the next several months, which will be a randomized controlled trial consisting of two arms of which one will receive stem cell treatment and rehabilitation therapy, while the second arm will receive only rehabilitation therapy. To date, all appropriate stakeholders have met to discuss how best to proceed with the study protocol. Our next steps include finalizing the appropriate study conduct and awaiting IRB approval for our modified phase II protocol.

Dissemination: None.

Institution: Mayo Clinic
Principal Investigator: Dr. Isobel Scarisbrick
Project Title: *Therapeutic Targeting of Cellular Senescence to Promote Repair of the Chronically Injured Spinal Cord*
Grant Cycle: FY2020

Accomplishments: The first experiments administering senolytic agents to animals with chronic spinal cord injury have been completed. Quantification of neurobehavioral and histopathological outcomes is underway. No deleterious effects of treatment were noted, indicating safety of this approach even in the context of spinal cord injury.

We have used preliminary data from this proposal to submit two other grant proposals for additional future funding.

Dissemination: None.

Institution: University of Minnesota
Principal Investigator: Dr. Walter Low
Project Title: *Spinal Cord Regeneration by Cell Reprogramming in Chronic Spinal Cord Injury*
Grant Cycle: FY2020

Accomplishments: Following the award of the above referenced project on cell reprogramming we performed the time lapse imaging in vitro, to better understand the temporal kinetics of when the reprogramming begins to occur. This is important to better elucidate the cellular mechanisms of reprogramming. Through these experiments, we have determined the following.

- Transduction was a rapid process, as we saw a fluorescent signal (indicating that mRuby was transduced and expressed in cells) after only a few hours.

- After ~2.5 to three days, we found that the astrocytes began to undergo morphological rearrangements, suggesting that they were in the reprogramming process. This is something that we saw with several biological replicates in two separate experiments. To note the timeframe we determined in these reprogramming experiments aligns with the timeframe in which Gong Chen has reported differential changes in transcripts in qPCR experiments.
- We compared the efficiency of reprogramming in mouse astrocytes and humans. Interestingly the human astrocytes showed a different behavior and appeared less responsive to the reprogramming, which is something that we will be following up with.

We also verified in vitro and in vivo that NeuroD1 was ectopically expressed in cells that express the reporter. In both applications, we saw the faithful expression of NeuroD1 expressed in transduced cells, which indicates the functionality of our system.

We have also performed experiments where we administered the reprogramming constructs to astrocytes 3 days prior to transplantation into the mouse nervous system. We have done this in both uninjured and noninjured mouse nervous system. Once we know the result of these experiments it may suggest the possibility of using the transplantation of reprogrammed astrocytes into spinal cord-injured mice, which may be helpful for severe injuries that have a large cavitation and cell loss.

We also designed and ordered additional reprogramming constructs using the human GFAP Cre in order to compare the efficiency of reprogramming. While the plasmid arrived, we are waiting for the gene engineering core facility to clone the hGFAP promotor into our Cre constructs. Based on conversations with investigators at the Society for Neuroscience annual meeting, we believe that this promotor will be more efficient. Once we receive the construct, it will be tested in vitro and in vivo in the spinal cord to optimize the titer and determine the efficiency.

Dissemination: None.

Institution: University of Minnesota
Principal Investigator: Dr. Ann M. Parr
Project Title: *3D Bioprinted spinal Neural Progenitor Cell (sNPC) Scaffolds Accelerate Functional Neuronal Network Formation both In Vitro and In Vivo after Spinal Cord Injury*
Grant Cycle: FY2020

Accomplishments: We have discovered that our bioprinted spinal organoids are able to survive *in vitro* for at least six months and maintain a neural identity throughout that time. We have also validated the orthotopic layering of cell types within our spinal organoids through next generation sequencing techniques such as RNA-sequencing. We have found that our organoids are capable of producing all six layers within the ventral spinal cord and that they maintain a spinal cord positional identity 21 days post-printing. Further, we have optimized tissue clearing techniques with our organoids that allow us to visualize the organoids in their entirety and map their projections. Finally, we have been able to optogenetically stimulate neurons within the organoids even after six months post-printing. We are now preparing to transplant our spinal organoids into a rat spinal cord injury model.

Dissemination: None.

Institution: University of Minnesota
Principal Investigator: Dr. James Dutton
Project Title: *Optimization of iPSC-Derived Oligodendrocyte Progenitor Cell (OPC) Manufacture – A Key Step*

Toward Patient Treatment
Grant Cycle: FY2020

Accomplishments: Since the receipt of the funding we have started to implement the research plan as set out in the proposal and laboratory experiments are ongoing. For Aim 1 we have initiated the cell aggregate experiments, introducing our accelerated hiPSC-derived ventral spinal neural progenitor cells in undirected aggregate differentiation protocols and are currently assessing the timing of phenotype transitions and comparing the results with the protracted "industry standard" protocols. For the work of Aim2 we are continuing to optimize the directed differentiation protocol and have made an interesting and potentially significant discovery regarding the differentiation signaling required in our approach to maintain the desired pre-oligodendrocyte progenitor cell phenotype. We are now examining this finding in greater detail. We have also started the 3D hydrogel co-culture experiments designed to provide an in vitro model to demonstrate myelination by oligodendrocytes generated from iPSC-derived OPCs. Our initial testing of the proposed method indicated no practical issues with incorporating iPSC derived cells in the hydrogel and initiating the prolonged culture periods and we are now testing methods for analyzing cell phenotypes and function. We are excited by the potential of this approach and will have more to report in the future.
Dissemination: None.

Institution: Center for Veterans Research and Education
Principal Investigator: Dr. Melanie Blahnik
Project Title: *Improving Communication About Sexual Health for Persons Undergoing Acute Inpatient Rehabilitation for Traumatic Brain Injury (TBI)*
Grant Cycle: FY2020

Accomplishments: The TBI research grant activities to date have been centered around the start up of the project. An application for the study was submitted for review by the Minneapolis VA Health Care System's (MVARCS) Institutional Review Board (IRB) and Research and Development Committee (RDC), from whom we have received approval to commence the study. A research assistant was hired and trained to support the research project, as well as the MVARCS Neurorehabilitation (TBI) Sexual Health and Intimacy Work Group that is involved in developing components of the research project focused around the clinical teams, such as the Sexual Health TBI Inpatient Rehab Template for use in the computerized patient record system and the development of the stafftraining curriculum. The Sexual Health TBI Inpatient Rehab Template was developed and trialed and is now fully implemented into the computerized patient record system (CPRS) at the MVARCS. The principal investigator and research assistant for the project have completed in person trainings on use of the clinical template with Inpatient Neurorehabilitation (TBI) Team members by rehabilitation discipline. A manual with photos of the template and corresponding instructions also was created for staff as a reference. This template

also serves as a research data collection method. Patient measures also were finalized and participant files were assembled for the team psychologists who are administering the measures as an additional date collection method. A staff survey was developed and trialed for use with REDCap, a digital survey tool, which also will serve as research data collection and will be administered to staff serving as participants in the research in the next couple of weeks. The research assistant has compiled four study protocol binders. The binder has a step-by-step guide for the research project, copies of the patient measures, scoring information for the patient measures, and clinical templates. The research assistant developed scoring and clinical documentation templates to assist the team psychologists with streamlining the process of entering clinical information obtained from the patient measures into CPRS at the MVAHCS. The research assistant has compiled over 115 articles pertaining to the project in a spreadsheet as a resource for the

Work Group. This spreadsheet has acted as an annotated digital library allowing members of the Work Group to quickly access the information they are looking for. This spreadsheet also contains links for accessing the actual articles. Databases for both patient and staff data have been setup. The codebook to help identify variables has also been written to accompany the databases. Our Work Group has maintained biweekly meetings to address challenges and questions as they come up. Within these biweekly meetings we are continuing to plan for the upcoming interdisciplinary staff training. The Work Group has developed training materials, including 20 written case studies to provide examples and guidance for putting the training into place. We are now transitioning to the "pre-staff training" data collection phase of our project.
Dissemination: None.

Institution: Center for Veterans Research and Education
Principal Investigator: Dr. C. Sophia Albott
Project Title: *Theta Burst Stimulation for Headaches after Traumatic Brain Injury*
Grant Cycle: FY2020
Accomplishments: TBD.
Dissemination: None.

Institution: HealthPartners Institute
Principal Investigator: Dr. Amanda A. Herrmann
Project Title: *Acupuncture Treatment for Chronic Post-Traumatic Headache in Individuals with Mild Traumatic Brain Injury*
Grant Cycle: FY2020
Accomplishments: We received Institutional Review Board approval for this study on January 10th, 2020. We plan to begin volunteer acupuncture training sessions at the beginning of February and recruitment and enrollment of participants by the end of February/early March.
Dissemination: None.

Institution: Mayo Clinic
Principal Investigator: Dr. Su-Youne Chang and Dr. Azra Alizad
Project Title: *Identification of Brainwide Network Activity Changes in Post-Traumatic Epilepsy to Optimize the Therapeutic Effect of Vagus Nerve Stimulation on Post-Traumatic Epilepsy*
Grant Cycle: FY2020

Accomplishments: To reach our goal, we proposed two aims. In Aim 1, we plan to optimize fUSimaging protocol for *in vivo* behaving rats (Aim 1-1) and will detennine changes in brain-wide network activities associated with PTE development and seizure on-set area in the brain (Aim 1-2). In Aim 2, we will perfonn VNS and validate therapeutic effects of VNS on PTE rats. Since the grant started in July 2019, we fully optimized the surgical procedures for fUSimaging and TBI as well as fUSimaging protocol in anesthetized rats. We also designed, fabricated, and evaluated the cuff-style stimulation electrode for the VNS. Furthermore, we recently got the approval for the IACUC protocol for the chronic fUSimaging and completed chronic surgeries onto two rats for fUSimaging. After the *surgery,* the rats were survived longer than 1 month and there was little sign of inflammation and infection in the *surgery* animals. The rats were euthanized after 1 month of the surgery. We are currently trying to determine the brain areas which are activated by the VNS in normal anesthetized rats and completed the first batch of PTE model *surgery.* We will soon start video-EEG to identify PTE animals and then will perform fUSimaging on to the PTE rats. Since there were some staffing changes, we plan to increase Dr. Su-youne Chang's effort and she will perform animal *surgery* and electrophysiology recording to successfully

complete the project within the timeline. Since we optimized fUSimaging protocol for anesthetized rats, we plan to submit a poster abstract to the 8th Annual Minnesota Neurotnodulation Symposium, which will be held in April 19-20, 2020.
Dissemination: None.

Institution: Mayo Clinic
Principal Investigator: Dr. Isobel Scarisbrick
Project Title: *Switching off the Thrombin Receptor to Enhance Recovery after Traumatic Brain Injury*
Grant Cycle: FY2020

Accomplishments:

Aim 1 - To determine if turning off PAR1 pharmacologically after repetitive mild TBI improves outcomes.

We have successfully established the repetitive mild TBI (r-mTBI) model at Mayo Clinic. Our efforts in this regard are in close collaboration with Dr. Regina Armstrong at the Uniformed Services University. In our first studies, we were successful in establishing a model of reduced social interaction in mice with r-mTBI. We also documented that this is underpinned by microgliosis. In Year 2 we will complete studies to demonstrate the neurobehavioral impact of pharmacological inhibition of PAR1 initiated after r-mTBI.
Aim 2 - To determine if turning off PAR1 genetically improves outcomes after repetitive mild TBI.
We have completed breeding of PAR1 knockout mice to obtain sufficient numbers of males and females to complete experiments to determine the impact of switching OFF PAR1 genetically on recovery after TBI. We will directly compare PAR1 knockouts to wild type mice and determine any differences between males and females. Studies, including histological analyses are still underway.
Dissemination: None.

Institution: University of Minnesota
Principal Investigator: Dr. Andrew T. Crane
Project Title: *Multilineage 3-Dimensional Brain Organoids to Model Intracranial Pressure Linked to Chronic Traumatic Encephalopathy*
Grant Cycle: FY2020
Accomplishments: We have established two-lines of human induced pluripotent stem cells in the lab which have been cultured in commercially available differentiation media to generate microglia which are currently being characterized. We have also packaged a plasmid linking green fluorescent protein to Tau into a lentivirus, which has been used to infect both lines of human induced pluripotent stem cells with variable success. We are currently working on optimizing the infection and sorting protocols to maximize yield of cells that can be utilized downstream for brain organoid generation. A pressure chamber that will be used for repeated stress of the brain organoids has been designed and is currently being fabricated.
Dissemination: None.

Institution: University of Minnesota
Principal Investigator: Dr. Maxim C. Cheeran
Project Title: *Characterizing the Neuroinflammation Associated with Sequential TBI in a Rodent Model*
Grant Cycle: FY2020
Accomplishments: The central hypothesis of this present study is that a second injury after a mild traumatic brain injury (TBI) will result in an enhanced neuroinflammatory response that persist longer and leads to behavioral deficits in the affected animal. To study inflammatory response in the brain consequent to TBI, we

characterized immune cell phenotypes that infiltrate the brain in 9-week-old C57BL/6 mice after single moderate TBI.

Moderate TBI was achieved by delivering an impact over the dura mater with velocity of 6m/s, a dwell time of 100ms reaching a cortical impact depth of 1 mm using an impactor device mounted on a stereotactic frame. Control animals for this experiment received a sham injury, where animals received anesthesia and a craniotomy, but did not receive an impact injury. Immune cell phenotypes in the brain and cervical lymph node were characterized by flow cytometry at various times starting at 6 h to 30 d following injury.
The results showed that migration of neutrophils and macrophages into ipsilateral hemisphere of the brain, occur early after injury with peak neutrophil infiltration at 24 h post injury (dpi), and macrophages at 3 dpi. The CD45(hi) macrophages in the brain showed a higher expression of proinflammatory activation markers like CD86, Ly6c, and MHCII. Notably, a temporally biphasic macrophage activation profile was observed in the brain, where proinflammatory CD86(+) macrophage numbers increased at 3 dpi then reduced to control levels by 7dpi and increased again at 30 dpi.

In addition, we observed significant increase in CD4(+) T lymphocytes at 14 dpi and increased microglial activation with higher MHCII expression at 30 dpi. These results suggest that inflammation persists in the brain after a moderate TBI, even up to 30 dpi. This increase in CD86(+) macrophages at 30 dpi was also observed in a mild TBI model (impact velocity of 4m/s) long after the minor motor deficits were resolved (7 dpi). Apart from transient increase in neutrophils at 24 hpi, there was no significant change in immune cell numbers on contralateral hemispheres. To characterize inflammation in the brain after repetitive TBI (double hit), first mild injury (impact velocity of 4m/s) was delivered on the right hemisphere and the animal was allowed to recover for 7 days. At 7 days after the initial injury, a moderate second injury was delivered on the left (opposite) hemisphere. Immune cell phenotypes were characterized at 7 d after second injury on both hemispheres of the brain by flow cytometry. Although immune cell infiltration and activated phenotype of immune cells were observed predominantly in the left (ipsilateral) hemisphere after moderate injury, an increase in infiltrating macrophages and MHC II(+) activated microglia was seen in both hemispheres 7 d after second injury.
This data suggests an exaggerated inflammatory response in the brain after second injury on the contralateral (right) brain hemisphere that received a mild injury 7 d prior. We are currently repeating this experiment to increase the numbers for analysis and plan to perform gene expression analysis to evaluate inflammatory cytokine genes by RT-PCR in addition to characterizing immune cell phenotypes. Additionally, behavioral analysis to assess the impact of the "second hit" on sensory motor function will be evaluated. In the next funding year, experiments are planned to study the impact of altering the macrophage activation profile with nhUCBSC treatment on the neuroinflammatory response to second TBI. The observation of an exaggerated inflammation on the previously affected side of the brain after a second trauma given 7 days after the first hit supports our initial hypothesis. We are working on assessing the impact of nHUCBSC treatment on this phenomenon.

Dissemination: None.

Institution: ExercisAbilities
Principal Investigator: Melanie Brennan
Project Title: *Validation of Cardiovascular Exercise Tests for Individuals with Traumatic Brain Injury*
Grant Cycle: FY2020

Accomplishments: To date, ExercisAbilities has acquired the Madonna ICARE and the SciFit arm crank ergometer, which has enabled the researchers to recruit participants and begin the intake process. IRB approval was finalized in November of 2019. At this time four participants have been recruited to begin the study. In

January of 2020 we started our first participant. We have the next 3 scheduled to begin incrementally every 4 weeks through March. Recruitment for the remainder of participants is ongoing.

We are thrilled to be in initial stages of participant recruitment and data collection and have many interested participants that we are working to enroll currently.

Dissemination: None.

Institution: CentraCare Health – St. Cloud Hospital
Principal Investigator: Dr. Uzma Samadani
Project Title: *Effectiveness of a Neck-Strengthening Program for the Prevention or Mitigation of Sports Concussion Injuries in Student Athletes*
Grant Cycle: FY2019

Accomplishments: 513 subjects were enrolled in this study between 8/2017 - 4/2018. Enrollment has now ceased. Data analysis and manuscript writing has now commenced.

1. **https://pubmed.ncbi.nlm.nih.gov/29998204-traumatic-brain-injury-reduction-in-athletes-by-neck-strengthening-train/**
2. **https://pubmed.ncbi.nlm.nih.gov/31637268-injury-rate-in-tacklebar-football/?from_sort=date&from_term=Toninato+J&from_cauthor_id=29998204&from_pos=2**

Dissemination: None.

Institution: CentraCare Health – St. Cloud Hospital
Principal Investigator: Dr. Uzma Samadani
Project Title: *Improving Functional Outcomes Through Optimization of Surgical Subdural Hematoma Evacuation Technique*
Grant Cycle: FY2019

Accomplishments: We have formed an official collaboration with Dr. Yuk Sham from the Computer Sciences department at the University, who will be overseeing and mentoring Atishya Ghosh who is a PhD student. She will be focusing her PhD on this specific problem. Data acquisition has begun.

Dissemination: None.

Institution: CentraCare Health – St. Cloud Hospital
Principal Investigator: Dr. Uzma Samadani
Project Title: *Traumatic Brain Injury Classification and Outcome Assessment*
Grant Cycle: FY2019

Accomplishments: Over 700 subjects have been enrolled in the study and enrollment has now concluded. One manuscript detailing the predictive power of certain biomarkers to predict positive head CT's has been published in the journal – World Neurosurgery, and multiple others are in the process of being finalized. **https://pubmed.ncbi.nlm.nih.gov/31051301-glial-fibrillary-acidic-protein-gfap-outperforms- s100-calcium-binding-protein-b-s100b-and-ubiquitin-c-terminal-hydrolase-l1-uch-l1-as-predictor-for-positive-computed-tomography-of-the-head-in-trauma-subjects/**

Dissemination: None.

Institution: CentraCare Health – St. Cloud Hospital
Principal Investigator: Dr. Uzma Samadani
Project Title: *Vagus Nerve Stimulation to Augment Recovery from Traumatic Brain Injury: Evaluation of Patients with Moderate Injury*
Grant Cycle: FY2019

Accomplishments: 14 subjects were enrolled in this study and enrollment has now concluded. Data analysis and manuscript writing has commenced.

A medical textbook chapter using the pilot data from this study has been submitted for review. Additionally, the data was presented at the International Brain Injury Alliance meeting in Toronto.

Dissemination: None.

Institution: CentraCare Health – St. Cloud Hospital
Principal Investigator: Dr. Uzma Samadani
Project Title: *Acute Biomarkers for Traumatic Brain Injury Classification Across the Severity Scale*
Grant Cycle: FY2020

Accomplishments: Over 700 subjects were enrolled in the previous study. With Dr. Uzma Samadani transferring hospitals, the amount of data already collected, and the advanced statistical and machine learning required to continue analysis – this grant funding has been reallocated to analyzing and publishing the data from the pilot study.
Dissemination: None

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