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TITLE: Advanced Restoration Therapies in Spinal Cord Injury

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14. ABSTRACT Evidence suggests that functional electrical stimulation (FES) can improve the function of the central nervous system (CNS) after injury or disease. Using FES-based therapies to treat spinal cord injured patients in our clinic, we have observed neurological and physical improvements. Investigations in our basic science research laboratory address the mechanisms by which FES promotes the cellular and molecular CNS regeneration that forms the foundations of this recovery.. SPECIFIC AIM 1: Determine if functional electrical stimulation (FES) in a mouse model of chronic spinal cord injury (SCI) induces proliferation and differentiation of genetically labeled oligodendrocyte progenitor cells (OPCs) (Months 1-12). SPECIFIC AIM 2: Determine if FES induces remyelination by mature oligodendrocytes in a mouse model of chronic SCI (Months 1-24). SPECIFIC AIM 3: Determine if functional electrical stimulation in a mouse model of chronic SCI induces cortical plasticity as measured by resting state functional magnetic resonance imaging (rs-fMRI) (Months 1-24). Relevance: The studies proposed will continue to investigate the role of FES-based restorative therapies in promoting neurological and functional recovery in chronic SCI. We will use existing transgenic mouse lines that enable the genetically labeling of cellular populations to further our understanding of the mechanisms through which FES induces functional recovery. Additionally, we will use a newly developed transgenic mouse lines that enables us to examine the dynamics of myelin formation. We will also further our imaging work by developing methodology to use rs-fMRI for examination of cortical plasticity in response to FES.		

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Nothing listed				
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SECTION 1 – INTRODUCTION

The spinal cord injury (SCI) disrupts conduction of sensory and motor signals (Belegu et al., 2007). The severity of the injury, and the extent of the neurological impairment following SCI, limits the subsequent neurological recovery. Persons with severe SCI experience limited neurological recovery (Kirshblum et al., 2004; McDonald et al., 2002), and the chances of neurological recovery become even smaller in the chronic phase of SCI (McDonald et al., 2002). Previous studies, however, suggest that through continued rehabilitation efforts, recovery of sensation, function, mobility, and independence in individuals with chronic SCI is possible, months and even years after injury (Harkema et al., 2012; McDonald et al., 2002; Sadowsky et al., 2013). This information has led to increased focus in the development of rehabilitation programs appropriate for individuals with chronic SCI. In particular, active rehabilitation therapy, which aims to induce neurological improvements through continued physical movements, is receiving renewed attention, as studies show that it can effectively increase muscle mass and strength, as well as the independence in activities of daily living (ADL) in individuals with SCI (Harkema et al., 2012; Karimi, 2013; Lorenz et al., 2012; Sadowsky et al., 2013). One of the most widely adapted intervention components of active rehabilitation therapy includes functional electric stimulation (FES) (Harkema et al., 2012; Karimi, 2013; Lorenz et al., 2012). The mechanisms through which FES induces functional recovery remain poorly understood. Remyelination has been suggested as a possible mechanism considering it is an activity-dependent process, however, clinical studies are not sufficient to address this question. Therefore, we have undertaken a preclinical study where we have developed a mouse model of FES in chronically injured mice. In addition, we are using this methodology in transgenic mice that allow us to lineage trace neural progenitor cells that have shown to be induced to proliferate and differentiate upon FES. Furthermore, the current study is allowing us to generate a transgenic mouse that promises to be transformative not only for the study of FES in myelination but in all myelin related pathologies.

SECTION 2 – KEYWORDS

Spinal cord injury (SCI)

Neural progenitor cells (NPCs)

Functional electrical stimulation (FES)

Neurological recovery

Myelination

Remyelination

SECTION 3 – ACCOMPLISHMENTS

What were the major goals of the project?

SPECIFIC AIM 1: Determine if functional electrical stimulation (FES) in a mouse model of chronic spinal cord injury (SCI) induces proliferation and differentiation of genetically labeled oligodendrocyte progenitor cells (OPCs) (Months 1-12).

SPECIFIC AIM 2: Determine if FES induces remyelination by mature oligodendrocytes in a mouse model of chronic SCI.

SPECIFIC AIM 3: Determine if functional electrical stimulation in a mouse model of chronic SCI induces cortical plasticity as measured by resting state functional magnetic resonance imaging (rs-fMRI).

What was accomplished under these goals?

SPECIFIC AIM 1:

1) Major activities: Performed experiments in mice with C57BL6 genetic background to determine if FES enhances cell proliferation in mice that are in subacute and subchronic stages of contusive SCI. Performed experiments in mice with C57BL6 genetic background to determine their neurological recovery in response to FES in subacute and subchronic model of contusive SCI. Performed experiments in nestin-CreER:YFP mice to determine if FES enhances cell proliferation in mice that are in subchronic stages of contusive SCI. Performed experiments in nestin-CreER:YFP mice to determine their neurological recovery in response to FES in subacute and subchronic model of contusive SCI.

2) Specific objectives: determine the effect of FES on proliferation of cells in the subacute and subchronic stages of SCI.

3) Significant outcomes or key results:

a) Application of FES for one week in a subchronic model of SCI (n = 6 per group) does not affect cellular proliferation in the spinal cord.

b) Application of FES for four weeks in a subacute model of SCI (n = 6 per group) elicits a mitogenic response in the injured spinal cord. The peak of this mitogenic response is the injury epicenter however the effect extends up to 5-mm rostral and caudal from the injury epicenter (Figure 1).

- c) Application of FES for four weeks in a subchronic model of SCI improves motor functions (n = 11 for non-FES treated group; n = 13 for FES treated group) (Figure 2).
- d) We have completed an experiment where we have treated spinal cord injured non-transgenic mice in a subchronic stage of SCI with FES for four weeks. Currently, processing tissue from this experiment. We will assess if FES induces a mitogenic response in this stage of SCI.
- e) Application of FES for four weeks in a subchronic model of SCI improves motor functions (n = 10 for non-FES treated group; n = 12 for FES treated group) (Figure 3).
- f) We have completed an experiment where we have treated spinal cord injured nestin-CreER:YFP mice in a subchronic stage of SCI with FES for four weeks. Currently, processing tissue from this experiment. We will assess if FES induces a mitogenic response in this stage of FES.

SPECIFIC AIM 2:

- 1) Major activities: We generated homozygous and heterozygous MBP_MBP-CTRN_MBP-RFP mice. Performed experiments in mice with C57BL6 genetic background to determine if FES enhances number of oligodendrocytes and myelination in mice that are in subacute and subchronic stages of contusive SCI. Performed experiments in nestin-CreER:YFP mice to determine if FES enhances differentiation of NPCs in mice that are in subchronic stages of contusive SCI.
- 2) Specific objectives: determine the effect of FES on differentiation of neural progenitor cells in the subacute and subchronic stages of SCI. Determine the effect of FES on remyelination in the subacute and subchronic stages of SCI.
- 3) Significant results or key outcomes:
 - a) We have generated homozygous and heterozygous MBP_MBP-CTRN_MBP-RFP mice. The mice faithfully express MBP, however, the homozygous mice display a shivering phenotype. The mouse can be used as reporter for MBP that can distinguish changes in myelination versus oligodendrocyte differentiation.
 - b) Application of FES for four weeks in a subchronic model of SCI (n = 6 per group) increases the number of mature oligodendrocytes in the spinal cord. Unlike the mitogenic response, number of mature oligodendrocytes in the spinal cord is increased the most caudal from the injury epicenter; however, the difference extends 5-mm rostral and caudal from the injury epicenter (Figure 4).

c) We have completed an experiment where we have treated spinal cord injured non-transgenic mice in a subchronic stage of SCI with FES for four weeks. Currently, processing tissue from this experiment. We will assess if FES induces a differentiation and improves myelination in this stage of SCI.

d) We have completed an experiment where we have treated spinal cord injured nestin-CreER:YFP mice in a subchronic stage of SCI with FES for four weeks. Currently, processing tissue from this experiment. We will assess if FES induces a differentiation and myelination of nestin-labeled cell in response in this stage of SCI.

SPECIFIC AIM 3:

1) Major activities: Evidence suggests that within minutes after SCI, decreases in spontaneous neuronal activity are observed in cortical areas that correspond to the injured as well as to the non-injured limbs. Moreover, these decreases were correlated with poor long-term recovery. Thus, we hypothesized that an intervention to attenuate the decreases in neuronal activity applied immediately post-SCI would accelerate neurorehabilitation. Transcranial magnetic stimulation (TMS) is a non-invasive brain stimulation method that has shown to induce cortical excitation and plasticity beyond the stimulation period. We have recently demonstrated that TMS therapy following traumatic brain injury in rats have rescued neuronal activity and led to improvement in behavioral tests (Lu et al., *Scientific Reports*, 2015). Therefore, we have determined if TMS can improve functional outcome post-SCI. SCI was induced at segment T9 in adult rats. The sensory and motor functions were evaluated in the weeks following the injury.

2) Specific objectives: We tested the outcome of TMS therapy on sensory and motor functions in three groups: SCI rats that received TMS 10 min after the procedure (acute-TMS); SCI rats that received the TMS starting two weeks after the procedure (chronic-TMS), and SCI rats that received sham TMS (no-TMS). Sensory responses to hind limb stimulation were assessed using functional MRI (fMRI). High-frequency (20 Hz) TMS was applied over the somatosensory cortex for 10 min, using a custom built rodent coil. TMS stimulation was applied three-times per week for a total of six weeks. Blood oxygenation level-dependent (BOLD) fMRI was carried out with an 11.7-T scanner which permitted high spatial and temporal resolution. The extent of the fMRI responses to hind limb tactile stimulation were calculated in the corresponding primary somatosensory areas.

3) Significant results or key outcomes: Preliminary results (Figure 5) show that the acute-TMS group demonstrated the greatest sensory responses with 50.89 ± 8.24 and 51.22 ± 4.92 activated pixels over the right (RHL) and left hind limb (LHL), respectively; the chronic-TMS group showed moderate sensory responses with 35 ± 2.61 and 32.8 ± 4.04 activated pixels in RHL and LHL; finally, the no-TMS group exhibited low sensory responses with 25 ± 4.73 and 13 ± 1.53 activated pixels in RHL and LHL, respectively. Motor behavior was assessed by weekly grid walk test, which indicated that the acute-TMS group had fewer number of footfall errors compared to the chronic-TMS and no-TMS groups. Together, these results reveal the efficacy of TMS in improving outcomes after SCI. We anticipate that application of TMS as a therapeutic strategy could be readily translated into the clinical setting as an alternative or adjuvant to traditional rehabilitation strategies.

4) Other achievements: N/A

What opportunities for training and professional development has the project provided?

Nothing to Report.

How were the results disseminated to communities of interest?

Nothing to Report.

What do you plan to do during the next reporting period to accomplish the goals?

SPECIFIC AIM 1:

- 1) Complete the data analysis on the neurological recovery in mice that were treated with FES in subacute and subchronic.
- 2) Complete data acquisition and analysis on the cellular proliferation in mice treated with FES in in subacute and subchronic stages of contusive SCI.

SPECIFIC AIM 2:

- 1) Complete evaluation of the structure of myelin sheaths in MBP_MBP-CTRN_MBP-RFP transgenic mice.
- 2) Complete data acquisition and analysis on remyelination in mice treated with FES in in subacute and subchronic stages of contusive SCI.

SECTION 4 - IMPACT

What was the impact on the development of the principal discipline(s) of the project?

We have developed a model for induction of FES in mice that allows us to study neurological recovery after SCI. Our methodology is similar to what is used in the clinic to treat SCI patients. The work completed here looked at the specific responses that were induced by FES.

What was the impact on other disciplines?

The methods and aims presented here are applicable to any disorder that can be altered through activity-based interventions.

What was the impact on technology transfer?

Nothing to report.

What was the impact on society beyond science and technology?

FES has shown to alter the life of SCI patients by enabling them to be involved in activities that promote neurological recovery and mental wellbeing. We hope that our work will continue to inspire SCI patients to maintain an active lifestyle.

SECTION 5 - CHANGES/PROBLEMS

Changes in approach and reasons for change

We have changed the scope of Specific Aim 3. In the new Specific Aim, we want to determine if non-invasive transcranial magnetic stimulation (TMS) can improve functional outcome post-injury.

Actual or anticipated problems or delays and actions or plans to resolve them

Nothing to report

Changes that had a significant impact on expenditures

As approved in our no cost extension we have purchased a high-speed camera for calcium imaging, and added the camera to an inverted Olympus fluorescent microscope. We have hired a postdoc to complete work on Specific Aim 3.

Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents

Nothing to report

Significant changes in use or care of human subjects

Nothing to report

Significant changes in use or care of vertebrate animals.

Nothing to report

Significant changes in use of biohazards and/or select agents

Nothing to report

SECTION 6 – PRODUCTS

Publications, conference papers, and presentations

Nothing to report.

Journal publications

Nothing to report.

Books or other non-periodical, one-time publications.

Nothing to report.

Other publications, conference papers, and presentations.

Nothing to report.

Website(s) or other Internet site(s)

Nothing to report.

Technologies or techniques

Nothing to report.

Inventions, patent applications, and/or licenses

Nothing to report.

Other Products

To date we have generated several transgenic mouse lines that promise to be transformative in the study of oligodendrocytes and myelination during development and in various pathologies that are pertinent to the healthcare of military personnel.

SECTION 7 - PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?

Name:	Visar Belegu, PhD
Project Role:	PI
Researcher Identifier (e.g. ORCID ID):	NA
Nearest person month worked:	9.6
Contribution to Project:	Dr. Belegu has supervised all the work performed in this project. In addition, Dr. Belegu has performed SCI surgeries, electrode implantations, FES stimulation, and neurological assays.
Name:	Ali Fatemi, MD
Project Role:	Co-PI
Researcher Identifier (e.g. ORCID ID):	NA

Nearest person month worked: 4.8
Contribution to Project: Dr. Fatemi has overseen and analyzed the data obtained in Specific Aim 1 and 2.

Name: Michael Johnston, MD
Project Role: Key Personnel
Researcher Identifier (e.g. ORCID ID): NA
Nearest person month worked: 3.6
Contribution to Project: Dr. Johnston has overseen and analyzed the data obtained in Specific Aim 1 and 2.

Name: Gallit Pelled, PhD
Project Role: Key Personnel
Researcher Identifier (e.g. ORCID ID): NA
Nearest person month worked: 1.9
Contribution to Project: Dr Pelled has supervised experiments performed in Specific Aim 3.

Name: Jineta Banerjee, PhD
Project Role: Postdoctoral Fellow
Researcher Identifier (e.g. ORCID ID): NA
Nearest person month worked: 6.5
Contribution to Project: Dr. Banerjee has performed the fMRI assessment in Specific Aim 3.

Name: Su Liu, PhD
Project Role: Key Personnel
Researcher Identifier (e.g. ORCID ID): NA
Nearest person month worked: 10.2
Contribution to Project: Dr. Liu has assisted Dr. Belegu in performing SCI surgeries, electrode implantation. In addition, she has performed the electrical stimulations, perfusions, staining and imaging for animals in Specific Aim 1 and 2.

Name: Sade Rodriguez, BS
Project Role: Laboratory Technician
Researcher Identifier (e.g. ORCID ID): NA

Nearest person month worked: 12
Contribution to Project: Mrs. Rodrigues has assisted in all experiments for Specific Aim 1 and 2.

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?

Nothing to report

What other organizations were involved as partners?

Organization Name:

Location of Organization:

Partner's contribution to the project

Financial support

In-kind support

Facilities

Collaboration

Personnel exchanges

Other

SECTION 8: SPECIAL REPORTING REQUIREMENTS

COLLABORATIVE AWARDS:

QUAD CHARTS:

SECTION 9: APPENDICES

Figures:

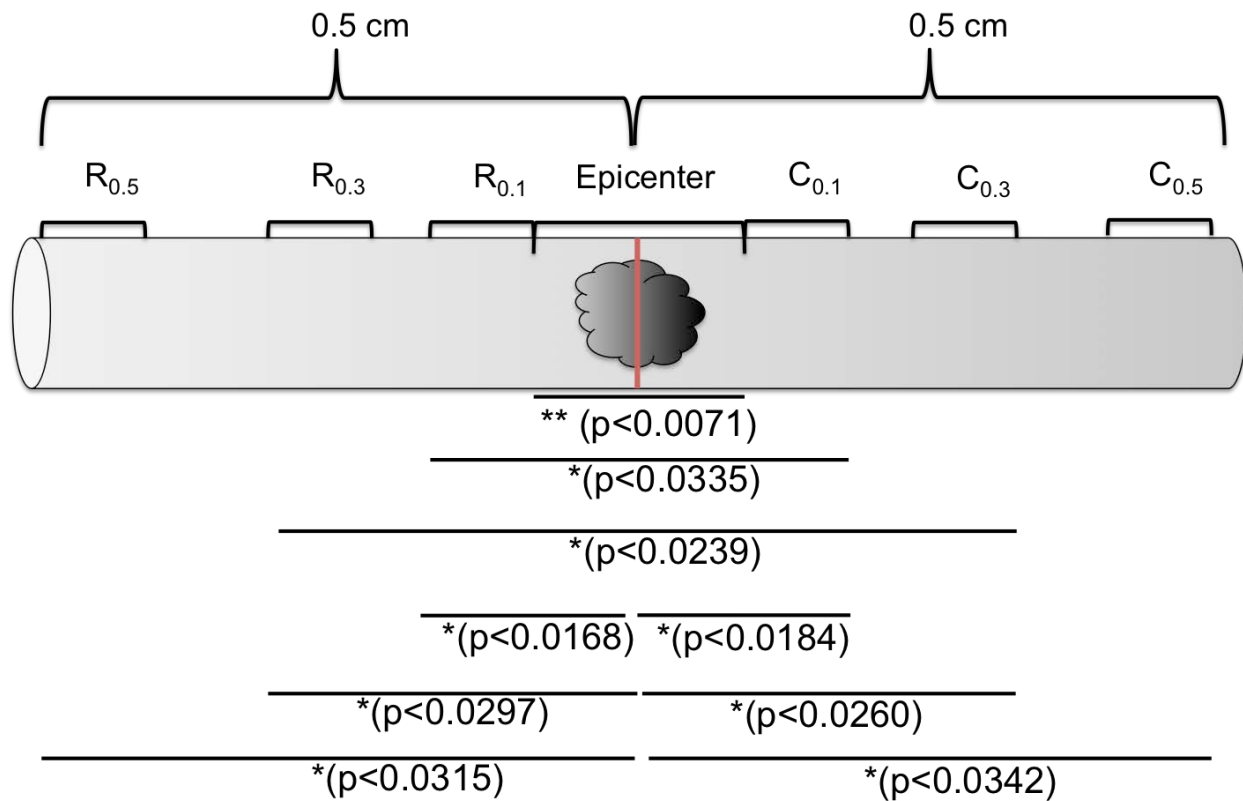


Figure 1. Cell proliferation in response to FES. R is rostral to the SCI epicenter; C is caudal to the SCI epicenter; the subscript numbers indicate the distance in centimeters from the injury epicenter. * signifies statistical difference by t-test between the FES and non-FES groups with a p-value of <0.05; ** signifies statistical difference by t-test between the FES and non-FES groups with a p-value of <0.01.

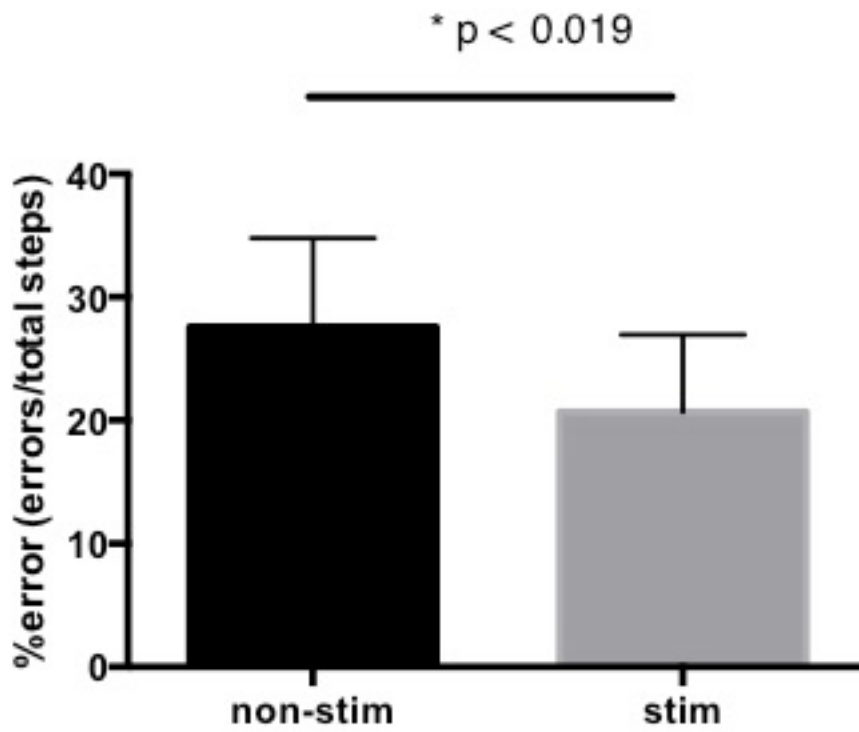


Figure 2. FES treatment of SCI in the subacute stage induced motor function recovery.

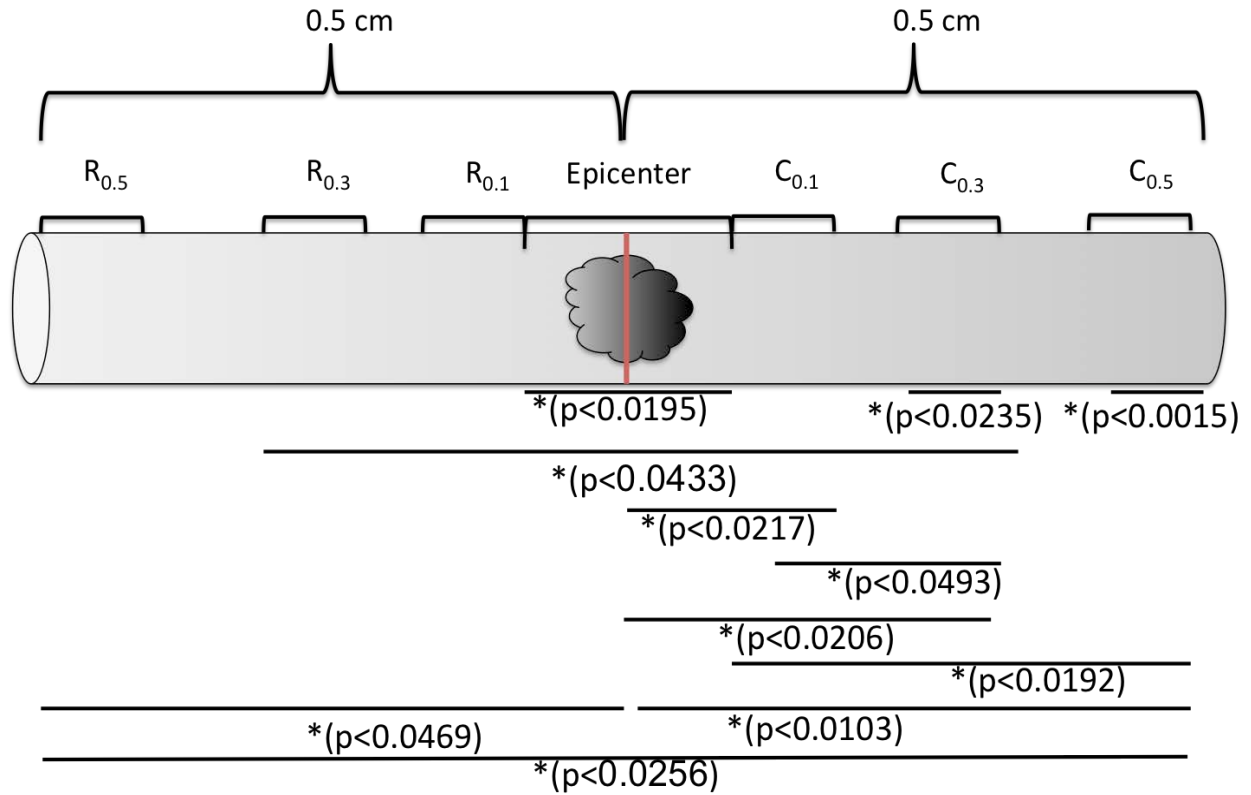


Figure 3. Number of oligodendrocytes increases in response to FES. R is rostral to the SCI epicenter; C is caudal to the SCI epicenter; the subscript numbers indicate the distance in centimeters from the injury epicenter. * signifies statistical difference by t-test between the FES and non-FES groups with a p-value of <0.05; ** signifies statistical difference by t-test between the FES and non-FES groups with a p-value of <0.01.

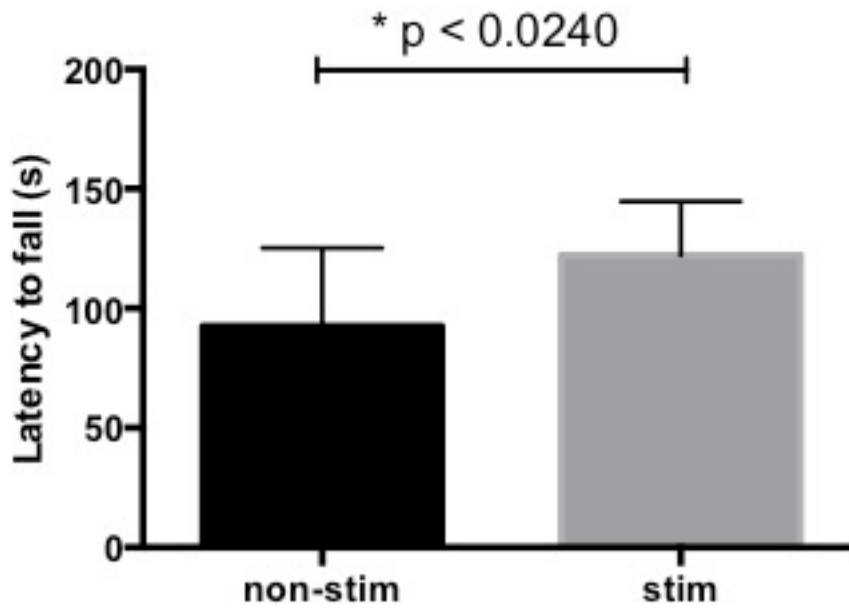


Figure 4. FES treatment of SCI in the subchronic stage induced motor function recovery.

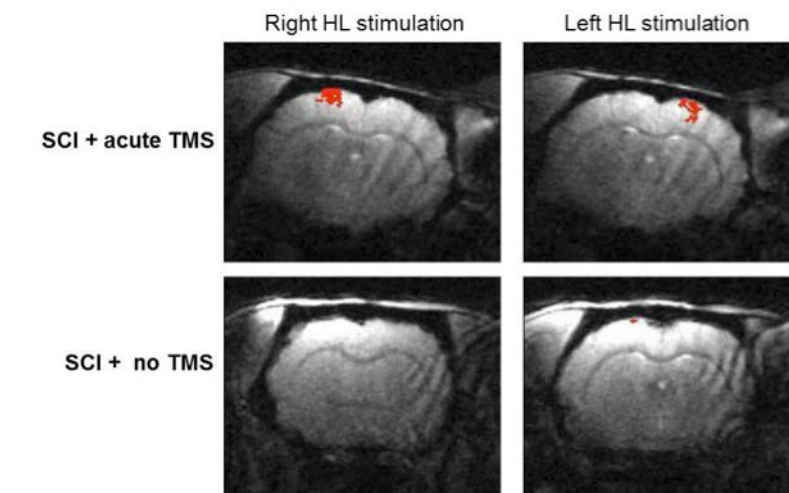


Figure 5. fMRI activation maps in response to tactile hind limb (HL) stimulation. SCI rats that received TMS therapy starting 10 min following injury procedure (acute- TMS) have shown greater sensory responses in primary somatosensory cortex of HL representation compared to rats that did not receive any TMS.

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Advanced Restoration Therapies in Spinal Cord Injury

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PI: Visar Belegu, PhD

Org: Hugo W. Moser Research Institute at Kennedy Krieger, Inc.

Award Amount: \$1,032,569.11
(+ \$965,926 – optional year 2)

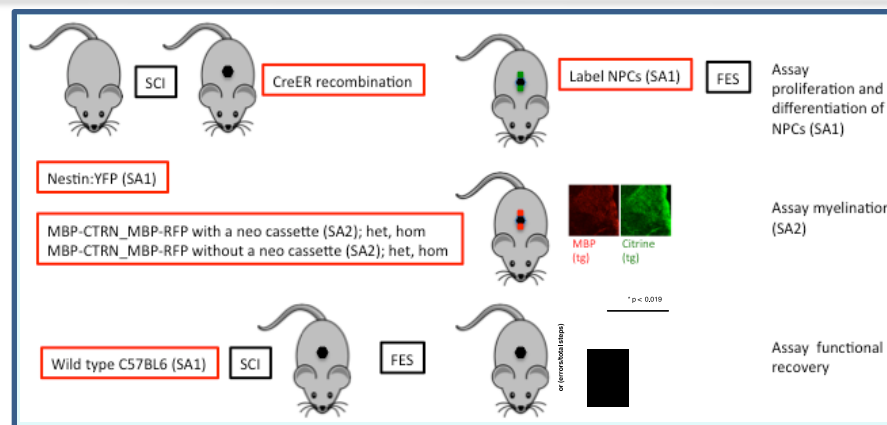


Study/Product Aim(s)

- SPECIFIC AIM 1: Determine if functional electrical stimulation (FES) in a mouse model of chronic spinal cord injury (SCI) induces proliferation and differentiation of genetically labeled oligodendrocyte progenitor cells (OPCs)
- SPECIFIC AIM 2: Determine if FES induces remyelination by mature oligodendrocytes in a mouse model of chronic SCI
- SPECIFIC AIM 3: Determine if FES in a mouse model of chronic SCI induces cortical plasticity as measured by resting state functional magnetic resonance imaging (rs-fMRI)

Approach

We aim to investigate the role of functional electrical stimulation in generating neurological recovery following spinal cord injury. Specifically, we will use transgenic mice to investigate if such an effect is mediated through cells of the oligodendrocyte lineage.



Accomplishments: (1) FES elicits a mitogenic response in subacute stages of SCI. (2) FES induces motor function recovery in subacute and subchronic stages of SCI. 3) FES increases number of oligodendrocytes in subacute stages of SCI. 4) Treatment with transcranial magnetic stimulation in the acute stages of SCI demonstrates the greatest sensory response.

Timeline and Cost

Activities	Year	1	2
Determine if FES induces proliferation & differentiation of OPCs			
Determine if FES induces remyelination by mature oligodendrocytes			
Determine if FES induces cortical plasticity			
Estimated Budget (\$K)		\$1,033	\$966

Goals/Milestones (Example)

Y1 Goal – System demonstration

- ☒ Obtain regulatory approval for animal work from Johns Hopkins IACUC and USAMRMC ACURO.
- ☒ Initiate generation of transgenic animals.
- ☒ Initiate and completed SCI, and FES implantation, and electrical stimulation for two sets of experiments.
- ☒ Develop task-activated fMRI sequences and data processing pipeline.

Y2 Goals – System validation

- ☐ Complete the analysis on the effects of FES in proliferation and differentiation of spinal cord progenitor stem cells.
- ☐ Complete the analysis on the effects of FES in myelination.
- ☒ Complete the analysis on the effects of FES in neurological recovery.

Comments/Challenges/Issues/Concerns

- No cost extension.

Budget Expenditure to Date

Projected Expenditure: \$ 1,998,495

Actual Expenditure: \$1,421,385