Title: Cortical functional connectivity as an early biomarker of recovery in spinal cord injury

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1. Abstract

a. Provide no more than a one page research abstract briefly stating the problem, the research hypothesis, and the importance of the research.

Early detection of response to therapeutic intervention in patients with chronic spinal cord injury (SCI) is crucial, as it will enable early adjustment or termination of intervention in non-responding patients, minimizing undue stress and financial burden. The goal of this study is to establish resting state fMRI (rsfMRI) outcome measures as novel non-invasive imaging biomarkers for early prediction of neurological improvements in response to intervention.

Cortical reorganization that occurs in SCI patients during therapeutic interventions underlies neurological and functional recovery. Thus, the ability to observe the degree of reorganization would allow investigation of therapeutically induced cortical changes in SCI patients that are predictive of recovery. Conventional fMRI allows observation of such changes using explicit tasks (task activated fMRI; tfMRI). However, SCI patients are often unable to complete the required tasks. tfMRI also has limited capacity to observe functional organization of the entire brain, making the method better suited to assess local regions. In contrast, rsfMRI doesn't require explicit tasks. This has strong clinical appeal, as it allows the use of an identical protocol for all, regardless of the degree of physical limitations. Moreover, rsfMRI allows localization of functionally homogeneous regions (parcels) of the entire brain with a single image acquisition – providing the means to efficiently estimate cortical functional organization in a data-driven manner. Two popular categories of such parcellation approaches include Independent component analysis (ICA; a method that uses a blind source separation algorithm to identify intrinsic brain functional networks) and temporal clustering, such as spectral clustering analysis (SCA; a method that uses spectral clustering to identify spatially coherent brain parcels). To date, these parcellation approaches have not been used to investigate functional reorganization associated with post-SCI intervention.

Functional electrical stimulation (FES) cycling is an intervention for SCI patients, in which patterned electrical pulses are delivered to lower extremity muscles to initiate cycling movement. Our group has shown that FES cycling, but not passive cycling (i.e., movement is driven only by the cycle's motor, without electric stimulation), leads to improvements in sensorimotor function, as measured using the International Standard of Neurological Classification for Spinal Cord Injury (ISNCSCI) scoring system. Unfortunately, early detection of response to intervention using ISNCSCI alone is challenging, as observation of clinically significant improvement (increase in ISNCSCI scores) requires at least 4 weeks of intervention. In contrast, previous tfMRI studies have shown that cortical reorganization can be observed after as little as one week of intervention. We hypothesize that reorganization of the sensorimotor cortex underlies neurological and functional recovery during intervention, and that such cortical reorganization will occur prior to observation of clinical improvement. We therefore propose that rsfMRI outcome measures, such as between-network connectivity (BNC; calculated as the degree of temporal dependency between functional networks), can be used to detect early changes in brain functional networks that are predictive of recovery.

If successful, these studies will: 1) provide a new and effective clinical tool to study plastic cortical changes that occur after SCI, 2) provide new imaging biomarkers that are predictive of progress towards recovery in response to therapy, and 3) extend our knowledge about the functional reorganization that takes place during and after therapeutic intervention. The developed methods may have broader applications in number of other diseases that affect motor functions, such as stroke and Parkinson's disease.

2. Objectives (include all primary and secondary objectives)

Aim 1. Characterize the baseline time profile of the rsfMRI outcome measures in chronic SCI patients, acquired over 4 weeks of passive (sham) cycling. In order to quantitatively assess the sensitivity of rsfMRI outcome measures to early functional reorganization that occurs in response to FES cycling, baseline time profile of the outcome measures in the absence of the active stimulation must be established. Accordingly, patients will participate in a 4-week passive cycling program. At weeks 0, 2, and 4, sensory and motor network BNC (calculated using ICA- and SCA-derived brain parcels) and ISNCSCI scores will be measured. *Hypothesis: we will observe stable baseline measures of sensory and motor cortex BNC and ISNCSCI scores of the patients during the 4-week passive cycling program, with minimal to no change in values.*

Aim 2. Characterize the time profile of the cortical reorganization in chronic SCI patients that occurs during a 4-week FES cycling program. *Hypothesis: We will observe early sensorimotor cortex BNC changes after 2 weeks of FES cycling, that are predictive of changes in ISNCSCI scores at week 4.* Specifically, we hypothesize that: a) Changes in sensory network BNC at week 2 will correlate with changes in sensory ISNCSCI scores at week 4; b) Changes in motor network BNC at week 2 will correlate with changes in motor ISNCSCI scores at week 4. To test these hypotheses, we will first measure the sensory and motor network BNC and ISNCSCI scores at weeks 0, 2, and 4, then correlate the changes in sensory and motor network BNC at week 2 with the changes in respective ISNCSCI scores at week 4. Finally, longitudinal (pre- and post- intervention) inter- and intra-subject reproducibility of the ICA- and SCA-derived parcels will be assessed.

3. Background (briefly describe pre-clinical and clinical data, current experience with procedures, drug or device, and any other relevant information to justify the research)

Spinal Cord Injury and Rehabilitation.

There is a perception in the field that significant neurological recovery in chronic SCI patients is unlikely. Recent studies, however, suggest that continued rehabilitation may enable recovery of sensation, function, and mobility in chronic SCI patients, months and even years after injury; leading to increased interest in interventions for chronic patients. In particular, active rehabilitation therapy, which aims to induce neurological improvements through continued physical activity, is receiving renewed attention, as it can increase muscle mass and strength. Functional electrical stimulation (FES) cycling is a widely-adapted component of the active rehabilitation therapy. This approach uses small electrical pulses that are applied to lower extremity muscles to initiate cycling movement. Previous clinical and preclinical studies have shown that FES increases the degree of physical integrity and functional recovery in SCI. Also, in a recent retrospective cross-sectional clinical study, our group showed that FES cycling, but not passive cycling (i.e., movement is driven only by the cycle's motor, without electric stimulation), leads to 80% composite motor-sensory score responder rate, higher quadriceps muscle mass (36%), higher muscle strength (35%), and higher quality of life and functional ability in SCI patients. As a widely-practiced component of the active rehabilitation therapy program with proven benefits for neurological recovery of chronic SCI patients, FES cycling is an excellent candidate for assessing the SCI patient response to an intervention program.

The International Standard of Neurological Classification for Spinal Cord Injury (ISNCSCI) scoring system is the most widely used clinical classification system of SCI. However, monitoring of response to intervention using only ISNCSCI is challenging. While ISNCSCI is excellent in describing the neurological level of SCI, its ability to describe the degree of functional loss, and therefore the sensitivity to changes in function, is limited. This is a significant issue, as it prevents early detection of response to intervention. While alternative neurological assessment measures are available, unfortunately, acquisition of such measures is often time-consuming. Given these limitations of the currently available outcome measures for SCI interventions, there is a compelling clinical need for new biomarkers that are: 1) measurable in heterogeneous SCI patient cohorts, and 2) sensitive to early responses to SCI intervention programs. *We hypothesize that resting state fMRI (rsfMRI) outcome measures will effectively fulfill these requirements*.

Resting State Functional MRI.

In addition to the degree of structural integrity at the injury epicenter that directly contributes to the capacity for neurological recovery in SCI patients, changes in brain functional networks are also thought to affect functional and neurological recovery. For example, plastic changes of somatosensory representation within cortex have been extensively reported both in previous preclinical and clinical studies, which are thought to be caused by the increased utilization of spared parts of the body. Two task activated fMRI (tfMRI) studies by Jurkiewicz et al are particularly relevant, as they showed that SCI patients who experienced recovery demonstrated a progressive enlargement in the volume of movement-related primary motor cortex (M1) activation and decreased activation in associated cortical sensorimotor areas. In contrast, those who experienced persistent paralysis demonstrated activation in M1 that was significantly reduced and progressively decreased in associated cortical sensorimotor areas. Authors conclude that together, the results suggest an association between motor task-related fMRI activation and degree of motor function post-injury. However, whether and how the observed cortical changes contribute to recovery remains largely unexplored. Having the ability to observe the pattern and degree of cortical functional reorganization would enable us to study changes that may be predictive of progress towards recovery.

In 1995, Biswal et al showed that blood oxygenation level dependent signals in functionally connected brain regions fluctuate in a highly correlated manner, even in the absence of explicit task performance (therefore the name “resting state” fMRI; rsfMRI). There are three major advantages of rsfMRI that are especially relevant to this project: 1) easy applicability to heterogeneous SCI cohorts: Unlike the conventional tfMRI, rsfMRI does not require explicit tasks. This allows the use of an identical protocol for patients in various stages of diseases and interventions, regardless of their degree of physical limitation. 2) capacity to observe functional organization within major brain networks within a clinically feasible timeframe: RsfMRI allows localization of functionally homogeneous regions (parcels) of the entire brain with a single image acquisition – providing the means to efficiently estimate cortical functional organization in a data-driven manner. Two popular categories of such parcellation approaches include Independent component analysis (ICA; a method that uses a blind source separation algorithm to identify intrinsic brain functional networks) and temporal clustering, such as spectral clustering analysis (SCA; a method that uses spectral clustering to identify spatially coherent brain parcels). To date, these parcellation approaches have not been used to investigate functional reorganization associated with post-SCI intervention. 3) high sensitivity to functional changes – studies have shown that functional connectivity (FC) measures are sensitive to changes in brain function, by showing that functional reorganization could be observed after as little as one week of intervention. Together, these advantages suggest that rsfMRI outcome measures, such as between-network connectivity (BNC; calculated as the degree of temporal dependency between functional networks) may be ideal for clinical observations and evaluation of the extent and pattern of cortical plasticity in SCI patients in response to therapeutic intervention.

Here, we propose to examine functional organization within major brain networks of SCI patients using rsfMRI, and to assess the relationship between rsfMRI and clinical measures. In addition to

development of a robust, clinically feasible rsfMRI data analysis pipeline, this study is expected to result in improved understanding of the functional reorganization that takes place during and after intervention.

4. Study Procedures

a. Study design, including the sequence and timing of study procedures (distinguish research procedures from those that are part of routine care).

This will be an early phase 1 (phase 0) clinical trial. Specifically, the study will be performed as a randomized, parallel group trial to determine if the amount of changes in brain functional connectivity outcome measures (i.e., between network connectivity) is significantly different between the group of patients that perform FES cycling and another group of patients that perform passive (sham) cycling.

Patients of the International Center for Spinal Cord Injury (ICSCI) at the Kennedy Krieger Institute (KKI) will be identified as meeting the inclusion criteria by reviewing the medical records held by the ICSCI. Once the patient consents by signing the consent form, official screening of subjects will be performed. Subjects will be randomized into 2 groups: FES cycling (Group 1; n=24) and passive cycling (Group 2; n=24). The patients will undergo either an FES cycling or a passive cycling sessions for 4 weeks, 3 times a week. **Acquisition of rsfMRI measures**: MRI will be performed on all participants at the beginning (prior to cycling sessions) and at the end of the 2nd and 4th weeks of the intervention program. **Acquisition of clinical measures**: ISNCSCI and SCIM (Spinal Cord Independence Measure) evaluations will be performed to coincide with the dates of MRI acquisitions, to determine the neurological level and the degree of sensory and motor impairments. Detailed study timeline can be found in Figure 1.

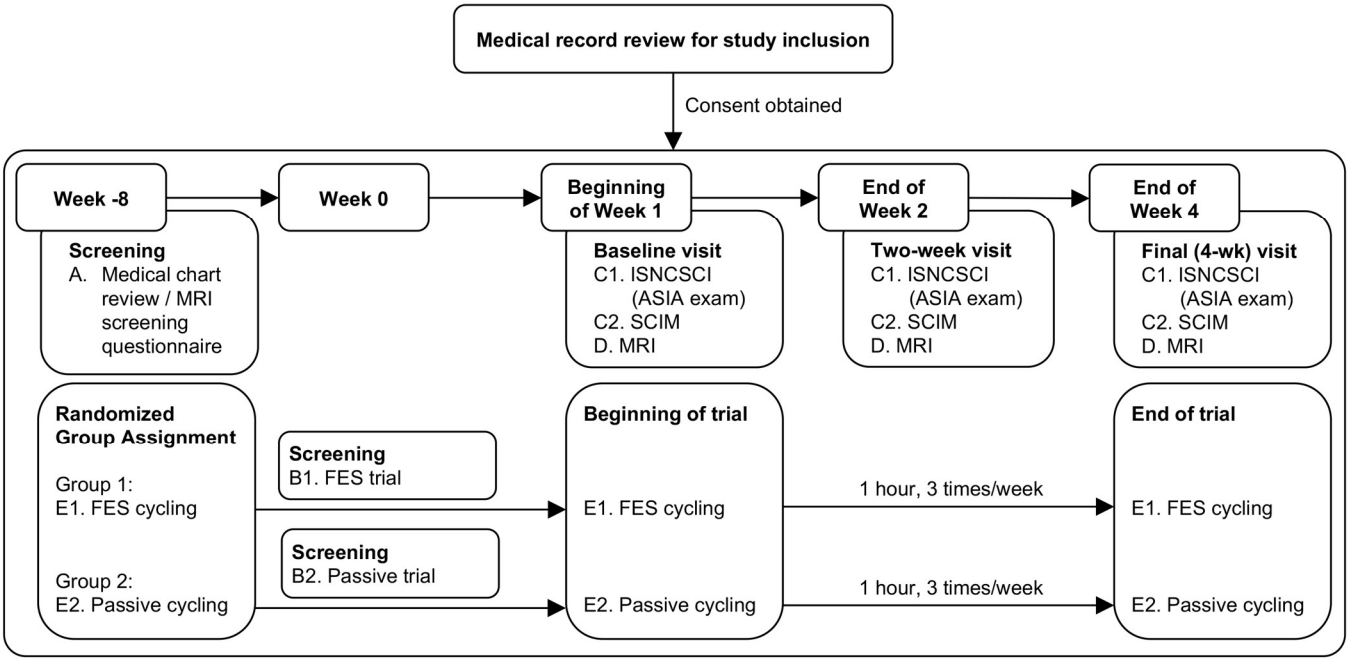

Figure 1. Study Timeline.

- SCI patients assigned to FES cycling group will take part in the following evaluations:
  - A: Medical chart review / MRI screening questionnaire
  - B1: FES cycling trial
  - C1: Neurological evaluation (ISNCSCI – more commonly known as ASIA exam)
  - C2: Functional evaluation (SCIM)
  - D: MRI

E1: FES cycling

- SCI patients assigned to passive cycling group will take part in the following evaluations:
  A: Medical chart review / MRI screening questionnaire
  B2: Passive cycling trial
  C1: Neurological evaluation (ISNCSCI – more commonly known as ASIA exam)
  C2: Functional evaluation (SCIM)
  D: MRI
  E2: passive cycling

A: Medical chart review / MRI screening questionnaire

For all subjects with spinal cord injury, their medical chart will be reviewed for the following information:

Demographics
Medical history (including labs)
Physical history
Neurological history
Medication history
Therapeutic plan
Physical/occupational/aquatic therapy history (including outcome measures)

This information is obtained as part of a routine care. The participant does not have to be physically present during this process. At this time, a standard MRI screening questionnaire will also be completed to be sure that it is safe for the participant to have an MRI exam.

B: Cycling trial

B1. FES cycling trial: This is a routine exam performed for people with spinal cord injury at the ICSCI. Once medically cleared to start an exercise program, subjects who are assigned to the FES cycling group will undergo a trial use of the FES cycle. This trial of FES will be overseen by a trained physical therapist experienced at setting up the FES cycle. The goal of this will be to ensure that the patient is willing and able to use the cycle. Participants who fail the FES trial will be excluded from the study. For additional details regarding the FES component, please see the ***E: Cycling*** section below. If the patient has successfully participated in an FES cycling program previously, this step will be omitted.

B2. Passive cycling trial: This is a routine exam performed for people with spinal cord injury at the ICSCI. Once medically cleared to start an exercise program, subjects who are assigned to the passive cycling group will undergo a trial use of the FES cycle (**but with the stimulation component OFF**). This trial of passive cycling will be overseen by a trained physical therapist experienced at setting up the FES cycle. The goal of this will be to ensure that the patient is willing and able to use the cycle. Participants who fail the passive cycling trial will be excluded from the study. For additional details, please see the ***E: Cycling*** section below. If the patient has successfully participated in an FES cycling program previously (with or without the stimulation component), this step will be omitted.

C: Neurological evaluation

C1. American Spinal Injury Association (ASIA) Impairment Scale (ISNCSCI) evaluation: American Spinal Injury Association (ASIA) Impairment Scale (ISNCSCI) evaluation is part of a routine care

evaluation performed for SCI patients at the International Center for Spinal Cord Injury (ICSCI) at Kennedy Krieger Institute. While its formal name is ISNCSCI, it is more commonly referred to as the ASIA exam. The evaluation consists of: 1) a motor component, where strength of ten key muscles (elbow flextors, wrist extensors, elbow extensors, finger flexors, finger abductors, hip flexors, knee extensors, ankle dorsiflexors, long toe extensors, and ankle plantar flexors) on each side of the body is evaluated, and 2) a sensory component, where touch and pinprick sensations are tested on 28 dermatomes on each side of the body. The total evaluation time will not exceed 60 minutes.

C2. Spinal Cord Independence Measure Version III (SCIM-III): The Spinal Cord Independence Measure Version III (SCIM-III) is a part of a routine care evaluation performed for individuals with SCI that assesses daily living and mobility activity performances. Specifically, it assesses the degree of self-care (feeding, grooming, bathing, and dressing), respiration and sphincter management, and mobility abilities (bed and transfers and indoors/outdoors). SCIM-III is frequently used in both clinical practice and clinical trial settings and is used to help guide clinicians in determining treatment goals and objectives for patients with an SCI. The SCIM takes approximately 30-45 mins to administer and score. However, the assessment will concurrently during the study participants' FES biking session, and will not contribute any additional time to the total study duration.

D: Magnetic Resonance Imaging (MRI)

This is a research procedure and not part of a routine care. All MR imaging will be performed at the F.M. Kirby Research Center using 3T scanners and consist of two main components: structural imaging of brain and spine, and functional imaging of brain and spine. Prior to imaging, an MRI technician will always perform an in-person safety screening, during which time any contraindications to MRI (e.g., aneurysm clips, pacemaker, etc.) will be identified. The total MR scan time will not exceed 90 minutes.

Structural imaging of brain and spine: A structural MRI study of the spine and brain will be performed. The subject will be instructed to lie still during structural MRI sequences, and the subject will be permitted to sleep or to watch television projected onto a screen.

Functional imaging of brain and spine: A functional MRI study of the spine and brain will be performed. The participants will be instructed to stay as still as possible with their eyes fixated on a cross hair on a screen.

The MRI will be performed in a single visit, according to the participants' convenience, tolerance, and availability. The neurological evaluation can be scheduled the same day as the MRI Imaging according to the subjects' convenience, tolerance, and availability.

E: Cycling

By default, cycling activities will be conducted at KKI and supervised by a licensed physical therapist experienced at setting up the FES cycle. However, in response to the ongoing coronavirus pandemic and the recent surge of the omicron variant, and with an ultimate goal of minimizing risks to the study participants, if a study participant has their own FES cycle at home, the participant will be able to choose to conduct the FES cycling at home instead of at KKI. If the participant decides to pursue the at-home FES cycling, a licensed physical therapist will conduct a televisit, during which time the therapist will supervise and monitor the FES sessions via Zoom or Microsoft Teams.

Once this protocol is approved, we will continue to offer online FES cycling sessions for study participants who prefer not to come for in-person visits. In order to maintain the consistency of FES cycling throughout the 4-week program, participants will not be able to switch between in-person and at-home FES cycling programs. The screening and MRI visits will still be conducted at KKI in person. All procedures listed below are routine practices in ICSCI.

E1. Functional Electrical Stimulation cycling: The FES cycling group (Group 1) will use RT300 ergometer (Restorative Therapies, Inc., Baltimore, Maryland). The RT300 ergometers are currently installed at ICSCI according to Kennedy Krieger Institute and Johns Hopkins Hospital safety guidelines. The muscles chosen for stimulation will be the bilateral glutei, quadriceps and hamstrings. The FES stimulation parameters will be set as follows: waveform biphasic, charged balanced; phase duration typically of 250 microseconds; pulse rate 33 to 45 pulses per sec (pps). The stimulus intensity of each channel will be adjusted for individual patients and muscle group so that a tolerable stimulation is provided that will generate a cycling action (0-140 mA). Target cycling speed is 50 RPM. Resistance will be automatically adjusted by the FES bike according to the subject's performance. When fatigue occurs, participants will continue cycling with electrical stimulation and motor support. FES therapy will be administered for one hour per session 3 times a week.

E2. Passive cycling: The passive cycling group (Group 2) will use the same RTI 300 ergometer however during this period stimulation will not be turned on. Instead, continuous motor support will be activated resulting in passive cycling. Target cycling speed is 50 RPM. Participants assigned to passive cycling will be required to have one hour of passive therapy 3 times a week for the entire duration of treatment assignment.

b. Study duration and number of study visits required of research participants.

Patients will be evaluated for appropriateness of enrollment and informed consent will be obtained. During the next 8 weeks, the screening process will be completed. A trial of FES will be performed at Kennedy Krieger Institute ICSCI to ensure that the participant is able to tolerate the stimulations of this exercise modality. If he or she is willing and able to proceed with the study then baseline data will be obtained at the beginning of week 1. The detailed study timeline can be found in Figure 1. The study duration for each assessment is as follows:

A: Medical chart review: N/A
B: FES/passive cycling trial (30 min)
C1: Neurological evaluation (up to 1 hour, part of routine care in ICSCI; total of 3 times over 4 weeks)
C2: Functional evaluation (up to 45 min, part of routine care in ICSCI; total of 3 times over 4 weeks; to be performed simultaneously during one of E1 or E2 sessions)
D: MRI (up to 90 minutes; total of 3 times over 4 weeks)
E1: FES cycling (1 hour, 3 times a week)
E2: Passive cycling (1 hour, 3 times a week)

We expect to enroll an average of 4 subjects each month into the trial with a total number of subjects of 48 (24 per group). Since participants will be enrolled in the study for 4 weeks, then the entire duration of the study will be around 1.5 years. After completing 24 subjects enrollment will be paused for interim analysis when it will be decided whether enrollment will continue.

c. Blinding, including justification for blinding or not blinding the trial, if applicable.

Because of the nature of the interventions (i.e., electrical stimulation), study participants cannot be completely blinded to the treatment they will receive. This is because many participants will have a residual motor and sensory functions and 'feel' which intervention they are receiving. Therefore, this study will be

performed as a single-blinded randomized trial. Study physicians and research staff who perform study measurements on participants will be blinded from the intervention the study participants receive.

d. Justification of why participants will not receive routine care or will have current therapy stopped.

All participants will receive routine care for treatment of spinal cord injury. Participants will not be asked to stop their current medications or therapy. All subjects will continue to consistently perform their usual ("baseline") amount of physical activity throughout the study, including home exercise stretching program, cardiovascular conditioning, etc. "Baseline" activity is defined as amount of physical activity performed for at least 1 month prior to enrollment. Routine care will not be interrupted.

e. Justification for inclusion of a placebo or non-treatment group.

Patients who are assigned to the passive cycling group will act as a non-treatment group. By comparing the outcome measures of the FES- and Passive-cycling groups, we will be able to determine whether it is the active neuromuscular conduction triggered by FES that leads to alteration in cortical functional connectivity, as opposed to a simple movement of muscles. Physiological effects of FES cycling require treatment for several months. Our proposed study is short (4 weeks). And as such, we expect minimal neurological differences between the FES and passive cycling groups. In contrast, we expect early, observable, and detectable differences in cortical functional connectivity between the FES and passive cycling groups.

f. Definition of treatment failure or participant removal criteria.

Subjects may withdraw from the study at any time for any reason. Additionally, a subject may be removed from the study for any of the following reasons:

- The subject experiences a medical emergency that necessitates discontinuation of therapy
- The subject experiences a serious adverse event that is judged to be likely related to the assigned treatment group or is of severity that warrants discontinuation of assigned treatment.
- For any medical reason at the discretion of the investigator.
- Subject is not compliant: participants who miss more than 3 sessions of cycling for the duration of the study.
- Subject becomes pregnant.

g. Description of what happens to participants receiving therapy when study ends or if a participant's participation in the study ends prematurely.

Upon completion of the study, participants will be referred back to their treating SCI specialist who will be made aware of the treatment and follow-up. The same protocol will be followed for patients who terminate the study early. In the event of discontinuation due to adverse event, participants will be followed by the PI and a medical supervisor of the study (Dr. Sadowsky) until resolution or stabilization of their adverse condition and then referred back to their treating SCI specialist.

5. Inclusion/Exclusion Criteria

Only subjects who agree voluntarily to participate in these procedures after they have been fully informed will be included, and who can comprehend the nature of these procedures can participate. Also, their actual involvement will be discussed with them repeatedly to assure adequate knowledge. Subjects will be

included only if one can understand the nature of the experiment as presented in English, and can cooperate with symptom assessment in English. 48 subjects will be recruited in total over a period of two years.

Inclusion criteria:

- Adult (18-99 years) men and women of all ethnic groups
- SCI, traumatic
- Thoracic (T1-T12) and lumbar (L1, L2), without the involvement of lower motor neurons.
- ASIA classification A-D
- Chronic injury: > 6 months from the injury
- Satisfactory general health
- No FES ergometer (i.e. RT300 or equivalent) use within 4 weeks.
- Ability to comply with procedures and follow-up

Exclusion criteria:

- Contra-indication to MR study (*e.g.,* cardiac pacemaker, claustrophobia, aneurysm clip, etc.)
- History or clinical evidence of moderate or severe brain injury
- Major spine deformity (*e.g.* scoliosis, kyphosis, subluxation)
- Movement disorder or severe spasticity preventing ability to lay still for extended periods required for imaging.
- Women who are pregnant
- Concurrent lower motor neuron disease such as peripheral neuropathy that would exclude lower extremity electrical excitability
- Unstable long bone fractures of the lower extremities.
- Subjects with history of inability to tolerate electrical stimulation.

Pregnancy is associated with significant physiological deviations with respect to cerebral spinal fluid dynamics and blood flow in the middle cerebral artery that are different than what is seen in women who are not pregnant. Gonadal steroids associated with pregnancy are also implicated in marked changes in cognitive function. Because of the significant changes from the normal, non-pregnant condition it is important to exclude pregnant women from many studies. If they were not excluded their data would significantly add to the variance of the physiological and behavioral measures**.** Furthermore, that variance is not readily interpreted at this time.

6. Drugs/ Substances/ Devices

a. The rationale for choosing the drug and dose or for choosing the device to be used.

The RT-300 FES bike has been FDA-cleared for use in general rehabilitation (the device clearance number is K072398). It has been a standard rehabilitation treatment modality at our center for several years.

b. Justification and safety information if FDA approved drugs will be administered for non-FDA approved indications or if doses or routes of administration or participant populations are changed.

N/A

c. Justification and safety information if non-FDA approved drugs without an IND will be administered.

N/A

7. Study Statistics

a. Primary outcome variable.

Type	Name	Time Frame	Brief Description
Primary	International Standard of Neurological Classification for Spinal Cord Injury (ISNCSCI)	baseline, mid-intervention, post-intervention	Developed by the American Spinal Injury Association (ASIA) as a universal classification tool for spinal cord injury (SCI). The classification tool involves a sensory and motor examination to determine the neurological level of the injury and whether the injury is complete or incomplete. The tool also provides sensory and motor scores of upper and lower body, as well as right and left of the body. The ISNCSCI defines neurological level as the most caudal level at which sensory and motor function are intact. The completeness of the injury is graded according to the ASIA Impairment Scale (ISNCSCI).
Primary	Resting state fMRI functional connectivity	Baseline, mid-intervention, post-intervention	RsfMRI functional connectivity is defined as the temporal dependency of neuronal activation patterns (represented by the blood oxygenation level dependent (BOLD) signal time courses as measured using rsfMRI) of anatomically separated brain regions. There are number of methodologies one can use to characterize the degree and type of rsfMRI functional connectivity. One example is between-network-connectivity (BNC), which is defined as the degree of correlation between two time courses obtained from a pair of brain regions. Summary statistics of BNC (e.g., mean, variance), as well as the dynamic properties of BNC (e.g., dynamic functional connectivity) can be used to further summarize the characteristics of the functional connectivity in SCI population.
Primary	RsfMRI brain parcels	Baseline, mid-intervention, post-intervention	RsfMRI functional connectivity can also be used to identify functionally homogeneous brain regions, or parcels of the brain. The parcels' center of mass, spatial maps, average and variance of the BOLD signal time courses within the parcels can be used to characterize brain functional organization.

b. Secondary outcome variables.

Type	Name	Time Frame	Brief Description

Secondary	FES cycling parameters	During intervention	The degree of electrical stimulation is adjusted specifically for each patients, depending on the patients level of sensory and motor impairment, as well as the patient's level of tolerance for sensation and fatigue. Accordingly, the strength and frequency of the electrical stimulation applied during each FES cycling session will be recorded. The duration of the cycling session performed will also be recorded. These parameters may be used as higher-order confounds during statistical data analysis
Secondary	Passive cycling parameters	During intervention	The amount of time and the degree of resistance used by the patients during the passive cycling sessions will be recorded, and may be used as higher-order confounds during statistical data analysis.
Secondary	Personal information	Baseline	Patient's age, sex, handedness, and time since injury will be recorded, and may be used as higher-order confounds during statistical data analysis.
Secondary	SCIM	baseline, mid-intervention, post-intervention	The Spinal Cord Independence Measure Version III (SCIM-III) assesses daily living and mobility activity performances. Specifically, it assesses the degree of self-care (feeding, grooming, bathing, and dressing), respiration and sphincter management, and mobility abilities (bed and transfers and indoors/outdoors) and each category are scored using the SCIM questionnaire. SCIM-III is frequently used in both clinical practice and clinical trial settings and is used to help guide clinicians in determining treatment goals and objectives for patients with an SCI.

c. Statistical plan including sample size justification and interim data analysis.

Preliminary study: In this preliminary study, we hypothesized that BNC of sensorimotor and visual networks will correlate with the total ISNCSCI score, and performed the study to determine the feasibility of a larger population study. Twelve SCI patients were recruited, and the relationship between the ISNCSCI score and BNC measurements was examined. ISNCSCI sub-scores were first obtained, which were: total upper and lower body motor scores, and total sensory score. The sum of the three scores provided the total ISNCSCI scores. Analysis of the data consisted of preprocessing, PCA, and GICA, and total of 14 intrinsic functional components were identified. BNC between the sensorimotor and visual networks were computed, and plotted against the total ISNCSCI scores. Linear regression analysis was performed to find the best linear fit for the plots. Stronger BNC between the sensorimotor and visual networks was associated with lower total ISNCSCI scores (R2=0.35, p = 0.04; Figure 2~~Figure 2~~). In other words, patients with greater degree of physical impairment tended to have stronger functional connectivity between the sensorimotor and visual networks, suggesting compensatory plastic cortical changes that are related to clinical presentation of the patients. Overall, BNC measures showed good correlations with the total ISNCSCI scores, supporting our hypothesis.

Between network connectivity (BNC) measurements from the above preliminary study provided means, standard deviations, and correlation coefficients for 12 SCI patients. A power analysis1 revealed that a sample size of 24 subjects will be required to achieve 80% power at an alpha of 0.018; the alpha level was lowered with the correction for multiple comparisons in mind. We will therefore recruit a total of 48 patients. At this point, we do not have enough information about the variability of the BNC in response to the FES to perform a more stringent power analysis. We will use the data in the first 24 SCI subjects recruited to the study to assess the degree of variability in the metric. Based upon these estimates, we will then perform a power analysis to determine how many more subjects will need to be enrolled to assess the significance of our findings.

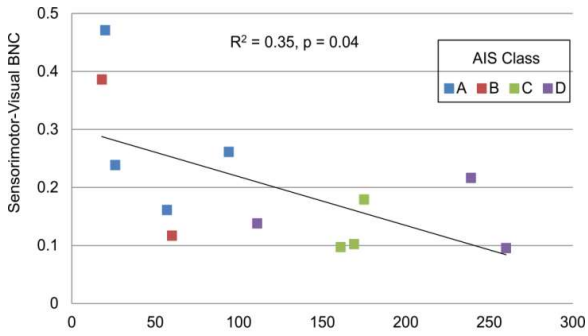

Figure 2. Correlations between sensorimotor-visual BNC measurements and total ISNCSCI scores in SCI patients suggest a compensatory plastic cortical changes that are related to clinical presentation of the patients. Patients with lower ISNCSCI score showed larger degree of functional connectivity between the sensorimotor and visual networks.

The spatial maps of ICA- and SCA-derived brain parcels will be compared across groups. First, the participant-specific sensory and motor brain parcels will be converted to z values, so image intensities reflect the degree to which the parcel is present in each participant's data. The brain parcels will be combined in a second-level random effects analysis using a two sample t-test in SPM8. Voxels that contributed unequally to the parcels across groups will be identified using a liberal voxel-wise p value of 0.01 and a cluster-level p value of 0.05 corrected for multiple comparisons2. The between network connectivity (BNC) between sensory and motor parcels will be calculated using Pearson's correlation coefficient between pairs of participant specific motor and sensory brain parcels3. Pairwise-correlations will be converted to Z-scores using Fisher's transformation, and the BNC across groups will be compared using two-sample t tests and adjusted p-values using a false discovery rate correction. Next, we will investigate whether sensory and motor BNC measured at week 2 of cycling program are related to motor and sensory International Standards for Neurological Classification of Spinal Cord Injury (ISNCSCI) scores measured at week 4, using a multivariate linear regression model analysis. Finally, the longitudinal inter- and intra-subject reproducibility of the ICA- and SCA-derived brain parcels will be asses using intra-class correlation coefficient (ICC).

d. Early stopping rules.

The principal investigator may discontinue the study if the interim statistical analysis as described above indicates that the number of subjects required for this trial exceeds 50.

Patients may withdraw from the study at any time for any reason. Any investigator may discontinue a patient for any of the following reasons:

- The subject experiences a medical emergency that necessitates discontinuation of therapy
- The subject experiences a serious adverse event (WHO Grade IV-V adverse events) that is judged to be likely related to the assigned treatment group or is of severity that warrants discontinuation of assigned treatment.
- For any medical reason at the discretion of the investigator.

Individual participants will be removed from the study if they experience toxicity or complications as described above.

- Fails to follow directions.
- Are not compliant: participants who miss more than 3 sessions of cycling for the duration of the study.
- Unwilling to perform the second lumbar puncture prior at the end of the study.
- Become pregnant.

8. Risks

a. Medical risks, listing all procedures, their major and minor risks and expected frequency.

The medical risks are defined in the section below ('Steps taken to minimize the risks'). This allows combining the risk and risk management in the same section for each risk.

b. Steps taken to minimize the risks.

A: Medical chart review / MRI screening questionnaire

Aside from the risk associated with disclosing personnel or protected health information outside the research study and possible discomfort with being asked personal questions, there are no known risks associated with this measurement.

B: FES trial & E: Cycling

The risk associated with FES is pain at the stimulation site. **To minimize the risk**, stimulation will only be done to the participant's tolerance level. There is a minimal risk of skin burn. To further minimize the risk of skin burn, only appropriate size electrodes will be used and they will replaced as per the manufacturer's recommendations.

The safety of FES for use during pregnancy has not been established. Therefore, pregnant women will not be enrolled in the study. **To minimize the risk**, any females who are of child bearing age will be asked to undergo pregnancy testing prior to the start of the study as well as agree to use an effective method of contraception during the study.

C: Neurological and Functional evaluation

There is a risk of discomfort at the site of the pin prick during the ISNCSCI evaluation. **To minimize the risk**, only the professionally trained personnel at the ICSCI will perform the ISNCSCI evaluation.

The risks associated with functional evaluation (SCIM) are extremely low. The tasks could cause boredom or maybe distracting for some participants. **To minimize the risk**, study participants will be informed that they are free to take breaks while answering the questionnaire as often as necessary.

D: MRI

The risks associated with magnetic resonance imaging are extremely low. They include the possibility that undetected metal in the subject's body could be displaced by the magnetic field of the MRI scanner. Participants could become claustrophobic while in the scanner. Participants could experience discomfort in attempting to remain still for up to 90 minutes in the scanner. The tasks could cause boredom or anxiety in some participants. Credit cards, watches, or other items could be damaged by the magnetic field if they are inadvertently brought into the scan room. Some volunteers may experience dizziness when being in the scanner. Head phones and ear-plugs are provided to the subjects to protect subjects from scanner gradient noise. **To minimize risk**, following measures will be taken:

- All subjects are screened for contra-indications for magnetic resonance, namely severe claustrophobia, pregnancy, cardiac pacemaker, orthodontics (braces) or other non-MR compatible ferromagnetic implant. In addition, access to the scan room is strictly controlled to ensure that no ferromagnetic materials are introduced. The scanners to be used in these studies are FDA approved and operates under radiofrequency power monitoring software at all times. All scan procedures at magnetic field strengths of 3T are FDA approved and are not classified as investigational devices.
- The subjects have the option of terminating the testing at any time without penalty if they so choose. The risks of psychological discomfort will be minimized by encouraging subjects to let the examiner know if they are feeling any discomfort, and by continuous monitoring by experienced operators for subject fatigue, inattention, or annoyance. Scanning will be halted if the subject so requests, or if the examiner feels that the subject is becoming uncomfortable. The subject will be encouraged to report any adverse symptoms during the MRI. The MRI can be stopped by the subject either by verbalizing the request to stop (which can be heard through the microphone in the MRI) or by pushing a "panic button" provided.
- A potential concern with MRI studies is so-called radiofrequency heating due to radiofrequency power deposition in the subject. The FDA has set strict and very conservative guidelines to guard against this risk. Such power deposition increases with the square of the magnetic field strength. However, it is possible to stay within these guidelines. To be 100% sure of compliance with the FDA guidelines, power deposition is monitored continuously by the software of the manufacturers and scans are not possible if the power deposition would exceed guidelines.
- This study includes operator-controlled changes to the pattern and timing of an MRI signal or pulse sequence that varies from the standard sequences used by the MRI manufacturer at lower field strengths. These changes will operate within FDA guidelines.

Other risks: Time commitment

The time commitment required to complete all study over 4 weeks may be inconvenient. **To minimize this risk**, the participant: 1) will be instructed to contact the study coordinator if the participant is having problems scheduling study visits, or adhering to scheduled study visits ahead of time; 2) will be excluded from the study if the participant cannot commit the time and effort to the study; 3) will be told that the start of the study will be arranged in such a way that it reduces any disruption to the participant's social, work or school commitments.

c. Plan for reporting unanticipated problems or study deviations.

During this study, medical history and physical examination will be performed at baseline and at regular intervals for measurement of residual sensorimotor functions in patients with spinal cord injury. All of the materials collected are for research purposes only, and data will be kept in strict confidence. No information will be given to anyone without permission from the subject. The consent form includes the informed consent statement required by Johns Hopkins University School of Medicine for studies involving human subjects. This statement guarantees confidentiality, which will be ensured by use of identification codes. All data, whether generated in the laboratory or at the bedside, will be identified with a randomly generated identification code unique to the subject.

The Principal Investigator (PI) will be responsible for ensuring participants' safety on a daily basis. The PI will also monitor participant safety, evaluate the progress of the study, to review procedures for maintaining the confidentiality of data, the quality of data collection, management, and analyses. Dr. Cristina Sadowsky, M.D., is the Clinical Director of the International Center for Spinal Cord Injury (ICSCI), and she will aid the PI in her capacity as a clinical mentor of the study in monitoring the study's participants' safety.

Adverse events (AE), unanticipated problems and/or study deviations will be reported in writing by the PI to the Johns Hopkins Medical Institute-IRB and Kennedy Krieger Institute Office of Research Compliance within 24 hours of notification. AE reports and annual summaries will not include subject- or group-identifiable material. Each report will only include the identification code. Twice annually, the PI will review study progress, data quality, and participants safety and share the reports with other study members of the group.

All research material will be kept confidential and any means of subject identification (name and history number) will be removed from all material for analysis or presentation. No identifying information will be made publicly available. In some cases, individual scans will be included in published papers or meeting papers or posters, but the identity of the subject in question will not be revealed. There are minimal legal risks associated with breach of confidentiality for this study. To minimize the risk of breach of confidentiality, access to participant/study data will be limited to study team members only. All study data will be stored in a department al locked cabinet and secure database program.

d. Legal risks such as the risks that would be associated with breach of confidentiality.

There are minimal legal risks associated with breach of confidentiality for this study. To minimize the risk of breach of confidentiality, access to participant/study data will be limited to study team members only. All study data will be stored in a department al locked cabinet and secure database program. Additionally, all research material will be kept confidential and any means of subject identification (name and history number) will be removed from all material for analysis or presentation. No identifying information will be made publicly available. In some cases, individual scans will be included in published papers or meeting papers or posters, but the identity of the subject in question will not be revealed.

e. Financial risks to the participants.

There are no financial risks to participants for any of the study tests. There will be no cost to the participant or the participant's insurance company for the MRI scans done specifically for this study. The scans will be paid by funds from this grant application. Subjects will be responsible for the cost of travel to and from the Institute, as well as any food/meals purchased throughout the day while engaged in study procedures. Valet parking is available at no cost.

9. Benefits

a. Description of the probable benefits for the participant and for society.

Individual subject: There is no direct benefit to the individual subject, except that the MRI report they receive may contain information useful to their physicians.

Society: Once developed and validated, the proposed MRI measures may yield surrogate biomarkers of treatment and/or rehabilitation therapy responses in SCI patients for use in clinical trials and drug discovery. Additionally, the developed methods may have broader implications in a number of other movement disorders (e.g., stroke and Parkinson's disease).

10. Payment and Remuneration

a. Detail compensation for participants including possible total compensation, proposed bonus, and any proposed reductions or penalties for not completing the protocol.

After completing the study, participants will receive a total of $525 compensation for their contribution. The costs for each visit are as follows:

- MRI visits: 3 sessions at $25 per session, totaling $75.
- ASIA visits: 3 sessions at $50 per session, totaling $150.
- FES visits: 12 sessions at $25 per session, totaling $300.

Compensation will be provided via a check, and participants will receive a structural images of their brain as a souvenir.

11. Costs

a. Detail costs of study procedure(s) or drug (s) or substance(s) to participants and identify who will pay for them.

There is no cost to study participants for any of the study tests. Subjects will be responsible for the cost of travel to and from the Kennedy Krieger Institute (Valet parking is available at no cost to study participant), as well as any food/meals purchased throughout the day while engaged in study procedures.