# FIRST-STIM

*CNS Growth **F**actor Release and Changes in the **I**nflammatory Environment in **R**esponse to Electrical **St**imulation in **S**ubjec**t**s with **I**nflammatory **M**yelopathies.* A Phase II randomized, single blinded, 100 subject clinical trial of functional electrical stimulation in the treatment of inflammatory myelopathies

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# Johns Hopkins Medicine - eForm A

**Title: FIRST-STIM**
*CNS Growth **F**actor Release and Changes in the **I**nflammatory Environment in **R**esponse to Electrical **St**imulation in **S**ubjects with **I**nflammatory **M**yelopathies.*
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## 1. Abstract

Noninfectious inflammatory myelopathies were previously often categorized as idiopathic transverse myelitis, but advances in neuroimaging and neuroimmunology have allowed more specific diagnoses, such as multiple sclerosis (MS) and neuromyelitis optica (NMO). With the exception of relapsing remitting multiple sclerosis (RRMS) there are no existing therapies that alter the course of these diseases. People with these disorders inexorably accumulate disability. In virtually every person with secondary progressive MS (SPMS) ambulation will be significantly affected leading to use of canes, walkers, and then wheelchairs. Functional electrical stimulation (FES) cycling is a method of applying low level electrical currents to the leg and buttock muscles to cause the weakened or paralyzed muscles to contract and produce a cycling motion of the legs. It has been used most in rehabilitation of patients with traumatic spinal cord injuries (SCI). Over the recent years FES cycling has become an increasingly important modality in rehabilitation of patients with paralysis. It has been shown to have multiple primary medical benefits including: increased muscle mass [1;2], improvements in bone density [3], enhanced cardiovascular function [4], improved bowel function [5], decreased spasticity [6] and reductions in bladder infection rate [7]. More importantly it has been shown to improve recovery from SCI. In a recent pilot trial [IRB protocol NA_00015238] with MS patients at our center we showed improvements on a broad array of functional and neurologic outcome measures including gait, upper extremity dexterity, and quality of life. Further, analysis of cerebrospinal fluid before FES and 3 months after initiating FES cycling revealed an enhanced neural repair program and a reduced inflammatory environment within the CNS. **One of the most important questions unanswered in regards to this technology is: How much FES is required to result in the most optimal recovery?** We currently use an experience based approach (3 to 5 FES cycling sessions per week, 1 hour each). From extensive clinical experience in our center, we suspect that the more FES is applied the better recovery is. In order to find the optimal dose of FES we identified a biomarker that can be measured in the spinal fluid of individuals receiving FES. **Brain derived neurotrophic factor (BDNF) levels correlate closely with functional recovery** [8;9]. Voluntary exercise induces a BDNF-mediated mechanism and promotes neural plasticity in the CNS. **Currently, there are no controlled clinical trial data available on the effect of FES ergometry on neurotrophin release and functional recovery following neurological injury.** Our center specializes in chronic rehabilitation of patients with paralysis. In our clinical experience electrical stimulation leads to accelerated recovery of neurological function compared to traditional rehabilitation [10]. We speculate that this is in part due to local neurotrophin release resulting in neuroprotection and repair. It is unclear what the optimal "dose" of FES is, i.e., what dose is required for maximal recovery of function. **Here we propose to measure CSF BDNF concentrations in response to FES ergometry in patients with inflammatory myelopathies. In addition we will measure markers of inflammation in response to FES.** These data will be crucial for the design of a phase 3 clinical trial evaluating the efficacy of FES in patients with inflammatory myelopathies.

2. **Objectives** (include all primary and secondary objectives)

**Primary Objective: To determine which FES ergometry dosing regimen results in the most significant increase in CSF BDNF levels in subjects with inflammatory myelopathy.** We will test the hypothesis that 5 FES cycling sessions per week result in greater increase in CSF BDNF concentrations as compared to 1 or 3 sessions and passive cycling.

a. We will measure CSF concentrations of BDNF in response to FES ergometry (**primary outcome**).
b. We will investigate whether there is a correlation between plasma and CSF BDNF concentrations.

**Secondary Objective: To quantify changes in the CNS inflammatory environment in response to FES ergometry in subjects with inflammatory myelopathy.** We will test the hypothesis that concentrations of IL-6, IL17, TNFα, IL-1ß, IL-23, and IL-12 change in response to FES ergometry.

a. We will measure CSF concentrations of IL-6, IL17, TNFα, IL-1ß, IL-23, and IL-12 in response to FES ergometry.

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)

**Multiple sclerosis (MS)** is an inflammatory demyelinating disease of the central nervous system (CNS) of unknown etiology. It is the most common non-traumatic cause of neurologic disability in young adults and affects between 250,000 and 400,000 persons in the United States [11;12]. The disease usually manifests in the third to fourth decade of life and affects woman two to three times more commonly than men. In about 85% of patients, the course of disease starts with a period of unpredictable relapses and remissions (called relapsing remitting MS, or RRMS). After some number of years the majority of those patients will enter a phase of the disease where they develop slowly progressive disability (called secondary progressive MS, or SPMS). In another 15% of patients the disease onset is not marked by relapses, but rather by an insidiously progressive accumulation of disability. This subtype is called primary progressive MS (PPMS). Most treatments are only effective in RRMS.

**Secondary Progressive MS (SPMS):** SPMS is a category of MS that refers to a patient who had an initial relapsing and remitting course of MS (RRMS) who then progressively worsens over months (at least six) to years. Most patients with RRMS eventually convert to SPMS, usually between 10 and 20 years after disease onset. A patient with SPMS may still experience relapses but does not stabilize between relapses. The predominant clinical pattern is one of continued clinical worsening. As time passes, relapses become less discrete, and the pattern becomes one of continued worsening without relapses. Conversion to SPMS is a poor prognostic sign, in that most patients continue to worsen despite medical intervention. Many patients with SPMS spontaneously stabilize for considerable periods of time, although they only rarely recover after deficits have persisted for 6 months. The pathogenic mechanisms underlying conversion from RRMS to SPMS are incompletely understood. Likely possibilities include failure of remyelination or progressive axonal injury. Most clinical trials have failed to show efficacy in SPMS. Mitoxantrone is the only FDA-approved treatment for SPMS. However, this chemotherapeutic agent has significant side effects that limit its use. Moreover, it is probably only useful in the group of patients that still are transitioning from RRMS to SPMS and still have relapses. SPMS patients without inflammatory relapses probably do not benefit from this relatively toxic treatment. Consequently, its use in SPMS is limited. There remains a lack of a good, safe therapy for SPMS patients.

**Transverse myelitis (TM):** TM is a focal inflammatory disorder of the spinal cord, resulting in motor, sensory, and autonomic dysfunction. It has an incidence of 1-4 new cases per million people per year, affecting individuals of all ages with bimodal peaks between the ages of 10 and 19 years and 30 and 39 years. There is no sex or familial predisposition to TM. It is characterized clinically by acutely or subacutely developing symptoms and signs of neurologic dysfunction in motor, sensory, and autonomic nerves and nerve tracts of the spinal cord. There is often a clearly defined rostral border of sensory dysfunction, and spinal MRI and lumbar puncture often show evidence of acute inflammation. When the maximal level of deficit is reached, approximately 50% of patients have lost all movements of their legs, virtually all patients have bladder dysfunction, and 80 to 94% of patients have numbness, paresthesias, or band-like dysesthesias. Autonomic symptoms consist variably of increased urinary urgency, bowel or bladder incontinence, difficulty or inability to void, incomplete evacuation, or bowel constipation. Longitudinal case series of TM reveal that approximately one third of patients recover with little to no sequelae, one third are left with moderate degree of permanent disability, and one third have severe disabilities [13].

**Neuromyelitis optica (NMO):** NMO is a distinct inflammatory demyelinating disease consisting of optic neuritis in combination with a longitudinally extensive TM in which the spinal cord lesion extends over three or more vertebral segments, but usually without significant brain involvement. It is characterized by the NMO-IgG autoantibody marker, which targets the water channel aquaporin-4. NMO-related myelitis attacks differ from the partial myelitis of MS in that they are longitudinally extensive, centrally based within the cord, and usually causes more severe bilateral impairment. CSF inflammation during active NMO relapses can be dramatic, revealing marked pleocytosis and high protein. Neurological recovery from NMO myelitis is very limited as compared to MS.

**Inflammatory Response:** MS is an inflammatory disorder of the CNS characterized by recurring episodes of inflammatory demyelinating lesions with prominent astrogliosis. IL-17 producing cells (Th17) play a critical role in disease induction in experimental autoimmune encephalomyelitis (EAE) [14]. IL-17 regulates cytokines (TNFα, IL-1β and IL-6) known to stimulate IL-6 production by astrocytes. CSF IL-6 is elevated in transverse myelitis (TM) and predicts acute and long term disability [15]. IL-17 and IL-6 production from peripheral blood mononuclear cells in TM and early MS (<2 years) has been shown to be increased and induce astrocyte IL-6 production through IL-6 [16]. Astrocytes have been shown to produce IL-6 in the CNS in response to TNFα and IL-1β, both of which are increased in the presence of IL-17. IL-17 is primarily made by a subset of activated memory CD4+ T cells distinct from Th1 or Th2 cells, called Th17. IL-23 produced by macrophages and dendritic cells appears to play a supporting role in the continued stimulation and survival of Th17 cells. IL-23 and IL-12 have antagonistic properties, as IL-23 supports IL-17 production, whereas IL-12 inhibits it [17]. IL-17 in turn stimulates macrophage production of IL-6, TNFα and IL-1β, all of which have been implicated in MS pathology and astrocyte IL-6 production [18].

**Functional Electrical Stimulation (FES):** Patients with complete or partial paralysis (as in spinal cord injury) often become severely de-conditioned due to the inability to exercise. One of the major goals of FES is to enable such patients to get the important benefits of exercise. Studies of exercise physiology have demonstrated that the benefits of exercise depend on duration, reciprocity of the movements (one limb to the other) and mechanical load of the movements. Also, to improve cardiovascular conditioning the heart rate must increase to a certain range. Passive movement, such as that provided by robotic systems or human-assisted movement predictably will not satisfy the requirements of load, duration and cardiovascular factors required to maximize the benefits of exercise. An FES ergometer allows coordinated stimulation of

the nerves of the patient's legs and induces contraction of the muscles leading to a cycling motion. These stimulations are controlled by a computerized, multi-channel stimulator with an electronic controllable motor cycling system capable of providing controlled resistance. Unlike passive movements, the active contraction of muscles provides load bearing on the limb which is important for the integrity of muscle and bone. Furthermore, it elevates the heart rate providing cardiovascular benefit.

Multiple FES cycling systems have been developed (Ergys, StimMaster, RT300) and are FDA-cleared for use in general rehabilitation. Functional Electrical Stimulation cycling allows the equivalent of 6000 steps in one hour (when cycling at 50 rpm), a little over half the normal number of steps a healthy adult takes on average per day. Electrical stimulation of hind limbs in rats and humans have shown the predicted benefits of exercise, when used with sufficient load and duration [19;20]. Since time limitations are a common barrier to treatment of individuals that are disabled, the ability to provide rehabilitative treatment benefits in three to five 1 hour sessions per week is important. These time intervals are suggested to produce the benefits of exercise (see the Surgeon Generals Healthy 2010 report).

A growing body of evidence suggests that electrical stimulation can promote peripheral and central nervous system repair following injury. Following complete spinal cord transection in rats, lower extremity FES induced an 82-86 % increase in cell birth in the lumbar spinal cord. FES doubled the proportion of the newly-born cells which expressed nestin and other markers suggestive of tripotential progenitors suggesting that controlled electrical activation of the CNS may enhance spontaneous regeneration after neurological injuries [21]. In a model of rat femoral nerve transection and repair, electrical stimulation promoted BDNF release from motor neurons and enhanced preferential motor reinnervation across the distal nerve stump. This stimulation paradigm also promoted functional recovery following femoral nerve repair [22]. In a similar model, electrical stimulation restored the specificity of sensory axon regeneration into the cutaneous branch of the femoral nerve. In addition, electrical stimulation promoted the expression of growth associated protein-43 and enhanced the number of regenerating sensory axons in the femoral nerve across the distal stump [23]. In a model of dorsal column transection (level T8), electrical stimulation promoted regeneration of CNS axons from dorsal root ganglia in a mechanism that likely involves cAMP signaling [24]. Finally, electrical stimulation applied to the cortical pyramids in rats enhanced synapse formation in the spinal cord during development and following corticalspinal tract injury [25;26].

Most of the cellular mechanisms of regeneration are activity-dependent. Elegant studies have clearly demonstrated that activation of the CNS is an important variable influencing the cellular mechanisms associated with regeneration, particularly axonal growth. The role of activity may be even more important in conditions where normal activity is reduced, as in spinal cord injury [27]. Examples where activity plays a critical role in development and plasticity include activity-dependent gene expression [28-32], modification of synaptic strength [e.g., long-term potentiation] [33;34], synapse elimination [33], myelination and maintenance of myelination [27;35-37], and axonal growth [38-40]. The widespread dependence of development and plasticity in the CNS on neural activity suggests that optimized neural activity might also be important for regeneration, given the common cellular mechanisms participating in both processes [35;41]. This is further supported by evidence that increased neural activity enhances multiple components of spontaneous regeneration while decreased activity inhibits it [42-48].

FES dosing:

**FES in Spinal Cord Injury:** There are numerous publications demonstrating practical benefits from FES in SCI patients including: increased muscle mass [1;2], improvements in bone density [3], enhanced

cardiovascular function [4], improved body composition[49], improved bowel function [5], decreased spasticity [6], improved glucose metabolism[49], and reductions in bladder infection rate [7]. In addition, electrical stimulation could someday be used in combination with other therapies to enhance functional recovery from SCI. One example is the use of electrical stimulation to facilitate partial-body-weight-supported walking in experimental animals following a spinal cord injury [50]. Solomonow et al demonstrated in 70 patients that 14 weeks of FES walking (3 h per week) can improve total cholesterol, LDL levels, and hydroxyproline/creatinine ratios and reduce spasticity in patients with paraplegia from SCI [51;52]. In another study after 10 weeks of FES cycling (2-3 session per week), 18 subjects with SCI (paraplegia and tetraplegia; complete and incomplete injuries), showed increased lean muscle mass, improved ASIA motor and sensory scores, and reductions in serum levels of IL-6, TNF-alpha, and C-reactive protein [49]. Based on the above noted studies we chose a study design of 1, 3, and 5 FES cycling sessions per week. Because of expected difficulty with compliance and practicality, especially in future Phase 3 clinical trials with long follow-up, we did not include the presumed maximum dose of 7 FES cycling sessions per week.

Circuitry for central pattern generation (CPG) is located in the lumbar region of the spinal cord (approximately L2-5) [53;54]. Limited input can activate the CPG and produce inter-limb CPG activation [53;55]. For example, focal sensory activation or stimulation as well as non-focal activation (e.g. epidural stimulation or intrathecal delivery of NT-3 or BDNF) can produce complex lower limb cycling like movements. Therefore, since lower limb movements have been associated with neurotrophins, it is conceivable that the elaboration of neurotrophins after FES underlies the biological basis for neural reorganization and functional improvement. It is for this reason that we wish to sample the CSF of patients before and after FES cycle therapy.

**FES in MS:** There is limited clinical data available on the use of FES in MS. In a double-blind, randomized controlled trial using whole-body vibration in MS patients Schufried et al demonstrated improved postural control and walking speed [56]. A pilot trial with 12 MS patients who underwent FES cycling (3 sessions/week for 2 weeks) demonstrated improved spasticity but failed to show improvement in strength and walking speed [57]. Long term FES bracing (3-12 months) for foot drop has been shown to increase strength and walking speed suggesting that it strengthens activation of motor cortical areas and their residual descending connections in patients with MS [58]. A randomized trial with 44 subjects with SPMS and foot drop who received FES bracing or exercise for 18 weeks demonstrated that exercise may provide a greater training effect on walking speed and endurance than FES, although the FES group performed to a significantly higher level with FES than without for the same outcome measures. It was therefore recommended to study the combined therapeutic effects of FES and exercise for this patient group [59]. In a recent pilot trial [IRB protocol NA_00015238] with MS patients at our center we showed improvements on a broad array of functional and neurologic outcome measures including gait, upper extremity dexterity, and quality of life. Further, analysis of cerebrospinal fluid before FES and 3 months after initiating FES cycling revealed an enhanced neural repair program (increased CSF TGF-β3) and a reduced inflammatory environment within the CNS (decreased Interferon-γ, IL-7, IL-8).

**Brain-derived neurotrophic factor:** BDNF is an activity-dependent secreted protein that, along with its receptors, is expressed widely in the central nervous system and is critical to neuronal survival, growth and activity-dependent plasticity. BDNF plays a critical role in exercise-induced cell proliferation. Exercise leads to an increase in BDNF and its downstream effectors on synaptic transmission in the brain and spinal cord [60;61]. Exposure of adult rodents to environmental enrichment and exercise induces neurogenesis in the hippocampus and is correlated with an elevation in hippocampal BDNF levels [62]. Exercise activates cyclic AMP response element binding protein (CREB) and the mitogen-activated protein kinase (MAP-K)

pathway [63]. The MAP-K cascade facilitates the phosphorylation of CREB and synapsin-I [64;65]. Synapsin-I is involved in synaptic vesicle clustering and release while CREB plays a role in long term plasticity and memory [66-68]. Phosphorylation of CREB is involved in activation of its target genes including BDNF [69].

In paralyzed muscle groups, substitution for voluntary contractions with electrical stimulation may allow for activity and sensory feedback in which both have shown to enhance natural levels of BDNF. BDNF mRNA levels increase by greater than 10-fold within 3 h after stimulation with the non-NMDA receptor agonist, kainite [70]. Chronic exercise can increase the expression of genes that encode several brain neurotrophins such as BDNF, nerve growth factor, and galanin. Voluntary exercise induces a BDNF and NT-3-mediated mechanism and promotes plasticity. Exercise induced up-regulation of BDNF has been shown to enhance recovery after traumatic brain injury [9]. In a model of traumatic SCI in rats, Coumans and colleagues demonstrated that axonal growth back into the spinal cord below the lesion was seen only in the presence of BDNF (infusion rate: 0.001mg/h x 14 days) or NT-3 (infusion rate: 0.0005mg/h x 14 days). Furthermore, the restoration of anatomical connections across the injury site was associated with recovery of function with animals exhibiting plantar foot placement and weight-supported stepping [8].

There is experimental evidence that BDNF can cross the blood–brain barrier [71]. According to these results, BDNF changes within the CNS and CSF might be paralleled by changes of BDNF serum levels. In a trial comparing 64 patient's CSF and plasma BDNF levels Laske et al failed to show a correlation between BDNF levels in these compartments [72].

Reports about BDNF CSF concentrations vary widely. Reported concentrations are ranging from 6.16 ± 1.07 pg/ml [73] to 242 ± 37 pg/ml [74] to 21.3 ng/ml [72] in adults. In children concentrations were reported ~10 ± 1 pg/ml [75].
In neurological disease and injury, BDNF concentrations are generally higher. For example, there was an almost 4 fold increase reported in patients with Parkinson's disease as compared to controls [73]. Two hours following traumatic brain injury in children there was a 2.5 fold increase of CSF BDNF levels[75].
In the normal CNS, neurons have a major role in the synthesis of BDNF, while in the injured CNS, glial (microglial) cells produce BDNF [76].
The discrepancies of the different BDNF levels might be explained by the difference of assays used. BDNF assays are now commercially available (RayBiotech, Norcross GA, USA). With this assay the minimum detectable dose of BDNF is typically less than 0.3 ng/ml without cross reactivity to other cytokines (Reproducibility - Intra-Assay: CV<10%, Inter-Assay: CV<12%).

## 4. Study Procedures

**Study Design:**
Phase 2 trial.
This selection study will be performed as a randomized, parallel Group Trial to determine the amount of FES ergometry required to achieve maximal CSF BDNF levels in individuals with inflammatory myelopathy.

Subjects will be randomized into 4 groups (FES1: 1 session of FES ergometry per week, FES3:3 sessions of FES ergometry per week, FES5: 5 sessions of FES ergometry per week, and Passive: 3 sessions of passive cycling per week). Session duration is 60 minutes. After obtaining a detailed physical and spasticity testing, baseline serum and CSF BDNF levels will be measured. The subjects will then receive

FES ergometry or passive cycling according to their group assignment for 3 weeks. At that time the subjects will return for a physical exam, spasticity testing, and repeat serum and CSF BDNF level testing. The primary investigator will be blinded to the results. The Study Time Line is found in Figure 1.

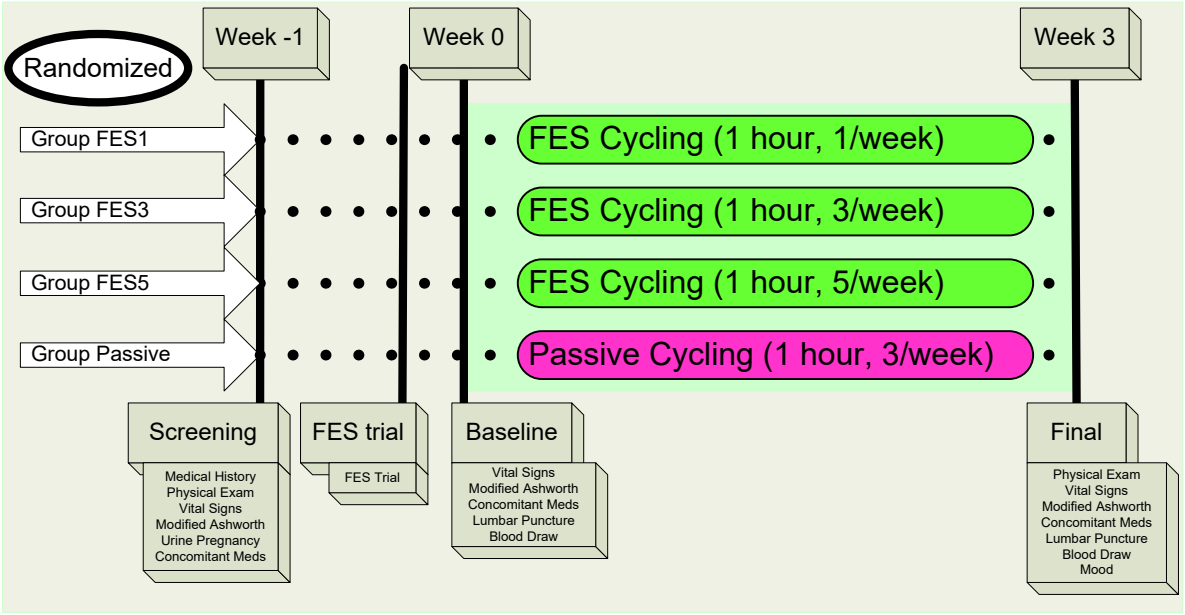


Figure 1: Study Time Line

## Study Treatments

All activities will be conducted at INI and are supervised by a licensed physical therapist. This includes all cycling activities; all study procedures, the screening and final visits. Urine (for rapid urine pregnancy testing) will be sent to the Johns Hopkins Medicine (JHM) Pathology lab.

### *Functional Electrical Stimulation Ergometry*

RT300 ergometer (Restorative Therapies, Inc., Baltimore, Maryland) are currently installed at the International Neurorehabilitation Institute (INI) according to INI 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 with a frequency according to the assigned study group (1 to 5 session of FES ergometry per week).

### *Passive Ergometry*

The passive ergometry treatment group will use the same RTI 300 ergometer however during this period of treatment assignment 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 ergometry will be required to have one hour of passive therapy 3 times a week for the entire duration of treatment assignment.

## Study Assessments

The tests listed below will be performed for all subjects. The data collection schedule in Table 1.

*a) Medical History*

This is a routine exam. A detailed medical history will be obtained by the study physician including history of present illness, review of systems, current medications, family medical history, social history, and allergies.

*b) Physical Exam*

This is a routine exam. A study physician will obtain a detailed clinical exam including a standard general medical exam (head, ears-nose-throat, neck, heart, lungs, abdomen, extremities), and neurological exam (cranial nerves, reflexes, American Spinal Injury Association exam).

*c) Vital Signs*

This is a routine exam. Vital signs will be obtained including blood pressure, heart rate, respiratory rate, temperature, height, and weight.

*d) Modified Ashworth Scale (MAS)*

This is a routine exam performed for people with spinal cord injury. Muscle tone will be assessed using the Modified Ashworth Scale (MAS). The MAS is a widely used neurological rating scale, ranging from 0 (normal) to 5 (rigidity). It measures neurological impairment and disability based on the ratings of an observer or neurologist through structured definitions. The MAS will be conducted by the study neurologist at baseline and at the final study visit.

*e) Fast Urine Pregnancy Test:*

This is a routine exam performed for women of child bearing age who do not take any birth control prophylaxis. Urine samples will be obtained at KKI. The urine (for rapid urine pregnancy testing) will be sent to the Johns Hopkins Medicine (JHM) Pathology lab.

*f) Concomitant Medications*

This is a routine exam performed for people with spinal cord injury. Concomitant medications will be recorded.

*g) FES Trial*

This is a routine exam performed for people with spinal cord injury at the ICSCI. Once medically screened to continue participation in the study, subject 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.

*h) CSF Analyses via Lumbar Puncture*

This is a routine procedure with an experimental analysis. Cerebrospinal fluid (CSF) will be collected by one of the study investigators at KKI. Approximately 12-15ml of CSF will be collected during each procedure.

Neural activity triggered by FES may induce an increase in nerve growth factors, or neurotrophins as described in the background section of this document. Such factors, including brain derived neurotrophic

factor (BDNF), nerve growth factor (NGF), ciliary neurotrophic factor (CNTF), glial cell line-derived neurotrophic factor (GDNF), neurotrophin-4 (NT4), and neurotrophin-3 (NT3) have been shown to decrease after cessation of neural activity. BDNF has been linked to improved functional outcomes following CNS injury. We therefore chose BDNF as a surrogate marker to determine FES efficacy. In addition to BDNF we will also look at several other neurotrophins and inflammatory markers as noted above.
CSF will be processed as previously described [77]. Briefly, CSF will be processed with immediate decellularization by centrifugation and freezing at -80°C. CSF analyses will be done on paired samples using cytokine arrays (purchased from RayBiotech) according to manufacturer instructions.

*i) Blood Draw (BDNF, PT/INR)*

This is a routine procedure with an experimental analysis. Approximately 5ml of venous blood will be drawn from the upper or lower extremities during each scheduled procedure for a maximum total of 20ml in a 3 week period. Blood samples will be processed as previously described [78]. Blood serum analyses will be done on paired samples using cytokine arrays for BDNF (purchased from RayBiotech) according to manufacturer instructions.

j) Mood assessment (using daily SMS text based Likert scale subjective measurement)

This is a routine assessment that is generally not part of the standard clinical exam at our center. We will assess mood on a Likert scale from 1 (low) to 10 (high) using "Mood24/7" (http://www.mood247.com). The use of this kind of mood assessment has been validated extensively and shown to correlate with a vast array of psychiatric mood rating scales [79]. Subjects will sign up with "Mood24/7" online via the HealthCentral mood tracking tool that is licensed from Johns Hopkins at the beginning of the study. The subjects will receive an automated daily message from "Mood24/7" requesting input in regards to their mood. The study team will only check on the data at the end of the collection period and not during the collection. Dr. Adam Kaplin serves as the mental health consultant in this study. If a subject were to express depressive symptoms at the end of the study, we will refer the subject to Dr. Kaplin. HealthCentral secures the confidentiality and privacy of patient data using industry best practices including documented standards and procedures for protecting sensitive data, encryption of data within the Mood24/7 database and tool interface, locking down all servers and network infrastructure supporting Mood24/7, limiting access to patient data to only employees that have a need, and special training for employees covering their responsibilities as far as protecting the confidentiality of sensitive data.

**Study Duration and number of study visits required of research participants:**

The study will last up to 4 weeks. The Study Time Line is found in Figure 1, and the Data Collection Schedule in Table 1.

**Study Assessments:**

The following post-consent screening will be performed at the Screening Visit (Week -1):

a. Medical History (about 30 minutes)
b. Physical Exam (about 30 minutes)
c.
d.
e. Fast Urine Pregnancy Test (about 15 minutes)
f.

The subject eligibility criteria will be reviewed after the Screening Visit.

The following assessments will be performed at the FES Trial Visits:

a. FES trial (about 30 minutes)

The subject eligibility criteria will be reviewed after the FES Trial Visit. Participants who fail the FES trial will be excluded from the study. If the subject is willing and able to proceed with the study the subject will be signed up with "Mood24/7", and scheduled for the Baseline visit.

The following assessments will be performed at the Baseline (Week 0) visit:

a. Vital Signs (about 5 minutes)
b. Modified Ashworth Scale (about 15 minutes)
c. Concomitant Medication (about 10 minutes)
d. Lumbar Puncture (about 30 minutes)
e. Blood Draw (about 5 minutes)

The following assessments will be performed at the Final (Week 3) visit:

a. Physical Exam (about 30 minutes)
b. Vital Signs (about 5 minutes)
c. Modified Ashworth Scale (about 15 minutes)
d. Concomitant Medication (about 10 minutes)
e. Lumbar Puncture (about 30 minutes)
f. Blood Draw (about 5 minutes)
g. Mood assessment review (about 5 minutes)

| | Screening | FES Trial | Baseline | Final |
|---|---|---|---|---|
| | 1 ¾ hours | ½ hours | 1 ¾ hours | 1 ¾ hours |
| Medical History | √ | | | |
| Physical Exam | √ | | | √ |
| Vital Signs | | | √ | √ |
| Modified Ashworth | | | √ | √ |
| Fast Urine Pregnancy | √ | | | |
| Concomitant Medications | | | √ | √ |
| FES Trial | | √ | | |
| Lumbar Puncture | | | √ | √ |
| Blood Draw | | | √ | √ |
| Mood | | | | √ |

Table 1: Data Collection Schedule

**Study Treatment:**

The total number of treatment visits depends on the study group:

- Group FES 1: a total of 3 FES ergometry visits scheduled 1 hour, 1 time a week for 3 weeks.
- Group FES 3: a total of 9 FES ergometry visits scheduled 1 hour, 3 times a week for 3 weeks.
- Group FES 5: a total of 15 FES ergometry visits scheduled 1 hour, 5 times a week for 3 weeks.
- Group Passive: a total of 9 passive ergometry visits scheduled 1 hour, 3 times a week for 3 weeks.

**Blinding:**

This study is a rater-blinded randomized trial. Study physicians and research staff who perform study measurements on participants will be blinded from the intervention the study participants receive. Because of the nature of the interventions, study participant cannot be blinded to the treatment they will receive.

**Justification why participants will not receive routine care or will have current therapy stopped:**

All participants will receive routine care for treatment of paralysis. 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.

**Justification for inclusion of a placebo or non-treatment group:**

Passive ergometry is chosen as one control to determine whether it is the active neuromuscular conduction triggered by FES that leads to alteration in CSF neurotrophin levels or whether any range of motion is sufficient. Physiological effects of FES ergometry require treatment for several months [10]. Our proposed

study is short (3 weeks). Although we expect a short term increase in neurotrophin levels, we do not expect any functional differences between the active and passive ergometry groups.

**Definition of treatment failure or participant removal criteria:**

Subjects may withdraw from the study at any time for any reason.

The subject may be removed from the study for any of the following reasons:
a) The subject experiences a medical emergency that necessitates discontinuation of therapy
b) 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.
c) For any medical reason at the discretion of the investigator.
d) Subject is not compliant: participants who miss more than 3 sessions of cycling for the duration of the study.
e) Subject becomes pregnant.

**Description of what happens to participants receiving therapy when study ends or if a participant's participation if 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 until resolution or stabilization of their adverse condition and then referred back to their treating SCI specialist.

## 5. Inclusion/Exclusion Criteria

**Inclusion criteria**

1. History of inflammatory myelopathy with onset at least 3 months prior.
2. Males and females between the ages of 18 and 65 years inclusive.
3. No FES ergometer (i.e. RT300 or equivalent) use within 4 weeks.
4. Ability to comply with procedures and follow-up.
5. Participants must be medically stable with no recent (1 month or less) inpatient admission for acute medical or surgical issues.
6. Access to a telephone with texting capabilities (SMS).
7. English language proficiency.

**Exclusion criteria**

1. Cardiovascular disease as defined by previous myocardial infarction, unstable angina, requirement for anti platelet agents, congestive heart failure, or stroke NYHA Class III or IV, history of arrhythmia with hemodynamic instability.
2. Uncontrolled hypertension (resting systolic BP>160mmHg or diastolic BP >100mmHg consistently).
3. History of epileptic seizures.

4. Subjects who have a pacemaker, an implanted defibrillator or certain other implanted electronic or metallic devices other than baclofen pumps.
5. Subjects who are unwilling to agree to two CSF examinations (lumbar punctures).
6. Unstable long bone fractures of the lower extremities.
7. Pregnancy.
8. Subjects having Stage 2 or greater sacral decubitus ulcer.
9. Subjects with history of inability to tolerate electrical stimulation.
10. Malignancy.
11. Active drug or alcohol use or dependence that, in the opinion of the site investigator, would interfere with adherence to study requirements.

**6. Drugs/ Substances/ Devices**

a. The rationale for choosing the drug and dose or for choosing the device to be used.
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.
c. Justification and safety information if non-FDA approved drugs without an IND will be administered.

Most individuals with inflammatory myelopathy have lost normal voluntary control of lower extremity function. Functional Electrical Stimulation cycling with the RT-300 allows the equivalent of 6000 step cycles in one hour (when cycling at 50 rpm), a little over half the normal number of steps a healthy adult takes on average per day. Six lower extremity muscle groups are simultaneously stimulated with this device. It is therefore the most efficient device in delivering electrical stimulation to subjects with complete spinal cord injury and was therefore chosen for this study.

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.

**7. Study Statistics**

a. Primary outcome variable.

**BDNF CSF level testing**

BDNF levels in the CSF of the study subjects will be measured in response to FES ergometry. BDNF has been linked to improved functional outcomes following CNS injury. We therefore chose BDNF as a surrogate marker to determine FES efficacy.

b. Secondary outcome variables.

**CSF analysis**

Exploratory analysis to further understand the CSF response to FES cycle ergometry. It will be aimed at examining neurotrophins and define the molecular signature of repair in the CSF of patients with spinal cord injury.

**Spasticity level:**

FES cycle ergometer therapy is expected to improve spasticity. This will be assessed by Modified Ashworth testing.

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

**Sample size and power:**

This is a dose finding clinical trial. Because there is limited data on BDNF CSF levels, the sample size cannot be computed at this time (power analysis cannot be performed). Instead we will use an adaptive sample size re-estimation strategy. Based on *in vivo* BDNF animal data we decided an effect size of 20% to be significant [80].

After enrolling 48 subjects (12 per group) we will estimate 2 quantities: the mean BDNF CSF level per group and the within group standard deviation of BDNF levels (averaged across the 4 groups). The sample size may then be adjusted based on the estimated effect size (estimated mean per treatment group - 80% of that estimated mean) and the estimated standard deviation. If the recalculated total sample size is equal or less than 100 then the study will proceed. Otherwise the study will be terminated at that point.

The required sample size will be computed such that it provides 80% probability of correctly selecting the amount of FES that yields the highest mean BDNF given a true 20% mean difference between the highest mean and the next highest mean.

**Analysis plan:**

Dependent Variables: CSF BDNF concentration, Serum BDNF concentration, CSF GDNF concentration, CSF NT-3 concentration, CSF NT-4 concentration, CSF CNTF concentration, CSF NGF concentration, CSF IL-6 concentration, CSF IL17 concentration, CSF TNFα concentration, CSF IL-1ß concentration, CSF IL-23 concentration, CSF IL-12 concentration, modified Ashworth score, mood.

Independent Variables: Intervention (FES ergometry); Timing

Approach: The distributions of all outcome variables (CSF BDNF concentration, Serum BDNF concentration, CSF GDNF concentration, CSF NT-3 concentration, CSF NT-4 concentration, CSF CNTF concentration, CSF NGF concentration, CSF IL-6 concentration, CSF IL17 concentration, CSF TNFα concentration, CSF IL-1ß concentration, CSF IL-23 concentration, CSF IL-12 concentration, modified Ashworth score, mood) will be described by treatment group (mean, median, standard deviation). The group that has the highest mean change in BDNF concentration will be used for further studies (future Phase 3 clinical trial).

Exploratory analysis will be performed to compare mean values of GDNF, NT-3, NT-4, CNTF, NGF, IL-6, IL17, TNFα, IL-1ß, IL-23, IL-12 concentration among the 4 groups using analysis of variance or suitable non-parametric methods (Kruskal-Wallis Test) if the outcome appears to be non-normally distributed.

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 #80.

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

a) The subject experiences a medical emergency that necessitates discontinuation of therapy

b) 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.

c) 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.

* Subject not compliant:
- 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. This allows combining the risk and risk management in the same section for each risk.

b. Steps taken to minimize the risks.

All assessments and intervention sessions will be supervised by the Principal Investigator and/or a licensed research Physical Therapist trained in the use of FES.

For all the study procedures, the risks and risk management are defined in the sections below.

*Intervention Risks:*

**FES**

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.

*Assessment Risks:*

**Medical History**

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.

**Physical Exam**

There are no known risks associated with this assessment.

**Vital Signs**
There are no known risks associated with this measurement.

**Modified Ashworth Scale**
There is a risk of eliciting spasms during the test. Subjects will feel a quick pull of the muscle, which might be painful at times. **To minimize the risk,** one of the co-investigator trained in the procedure will perform the test.

**Pregnancy testing**
There is the possibility of psychological stress occurring if testing shows that the subject may be pregnant. In the event that pregnancy test results are positive, this will be disclosed to the subject. **To minimize the risks,** if the subject is uncomfortable with this arrangement, her consent to participate in this study is not recommended.

**Mood assessment**
Aside from the risk associated with disclosing personnel or protected health information outside the research study, there are no known risks associated with this assessment. HealthCentral, that runs the Mood24/7 website tool that has been licensed from Johns Hopkins, secures the confidentiality and privacy of patient data using industry best practices including documented standards and procedures for protecting sensitive data, encryption of data within the Mood24/7 database and tool interface, locking down all servers and network infrastructure supporting Mood24/7, limiting access to patient data to only employees that have a need, and special training for employees covering their responsibilities as far as protecting the confidentiality of sensitive data. The study team will only check on the data at the end of the collection period and not during the collection. Dr. Adam Kaplin serves as the mental health consultant in this study. If a subject were to express depressive symptoms during the study, we will refer the subject to Dr. Kaplin.

**Concomitant Medications**
Aside from the risk associated with disclosing personnel or protected health information outside the research study, there are no known risks associated with this measurement.

**FES Trial**
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.

**Lumbar Puncture**
The risks of a lumbar puncture are rare and include back pain, leg pain, headache, bleeding, infection, paralysis, brainstem herniation and death. About 10% of people develop a headache after a lumbar puncture. This headache may require prolonged bed rest and in some cases a blood patch in the area where the lumbar puncture was done. **To minimize the risk,** lumbar punctures will be done only in approved outpatient facilities by a certified neurologist with experience at performing this procedure.

**Blood draw**

There risks of drawing blood include discomfort, bleeding or bruising where the needle enters the body, and in rare cases, fainting or infection. **To minimize the risks,** a trained nurse will draw the blood.

*Other Risks:*

**Time Commitment**
The time commitment for the treatment over the 3 weeks may be inconvenient. **To minimize the risks**: 1] The subject should contact the study coordinator if having problems scheduling the visits; 2] If we feel that the subject can not commit the time and effort, he/she will be excluded from the study; 3] The visits may cause moderate social, school and work disruption. The start of the study will be scheduled to lessen the disruption.

c. Plan for reporting unanticipated problems or study deviations.

**Data and Safety Monitoring Plan:**
The data and safety monitoring plan has two components: internal monitoring by the PI, and periodic monitoring by the Johns Hopkins Medical Institutional Review Board.

**Internal monitoring:** This will be conducted under the supervision of the PI. The PI will work very closely with the study team evaluating every step of the study milestones. The PI will also oversee the review of the patient history as also the thoroughness of the completed case report forms in conjunction with all study measures. The PI will also supervise the quality of the data entry and data quality. This will ensure continuous close monitoring for safety assessments and study measurement outcomes. The PI will report unanticipated problems or study deviations in writing to the KKI Office of Research Administration and JHM-IRB.

**Institutional Review Board:** In the event of any serious adverse event, the IRB will be notified and all further enrollment will be temporarily halted. Any medical questions should be directed to the PI. In the event of any injury, the services at any of the Johns Hopkins Hospital will be available to the subject. However, the subject will be responsible for the payment of any treatment or hospitalization required as a result of being injured while in the study. By signing the consent form, the subject does not waive any of the legal rights which he/she otherwise would have as a subject in a research study.

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 departmental locked cabinet and secure database program.

e. Financial risks to the participants.

There will be no financial risks to the subject. Any complications that arise from the treatment will be billed to the patients insurance. If the study participant has health insurance, the costs for any treatment or hospital care received as the result of a study-related injury will be billed to their health insurer. Any costs

that are not paid for by the health insurer will be billed to the patient. If the study participant does not have health insurance, he or she will be billed for the costs of any treatment or hospital care received as the result of a study-related injury.

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.

**Potential benefits of the proposed research to the subjects and others:**

Functional electrical stimulation has been shown to increase muscle mass [1;2], improve bone density [3], enhance cardiovascular function [4], improve bowel function [5], decrease spasticity [6] and reduce bladder infection rate [7]. There has been no formal evaluation of its efficacy in restoring function following traumatic and non-traumatic spinal cord injury. A surrogate marker for FES ergometry efficacy is the CSF level of BDNF.

In addition this study will help answer the question whether the inflammatory environment changes in response to FES and whether plasma and CSF BDNF levels correlate. If that were the case then future studies might be carried out using blood instead of CSF analysis which would be a less invasive approach then proposed in this trial.

The information from this trial will benefit society as whole and future patients with inflammatory myelopathies. This study is likely to increase scientific knowledge about the use of FES ergometry, inflammatory myelopathies, and in rehabilitation medicine in general. We will also understand more clearly from a molecular level the changes that take place regarding growth factors in the CSF following FES cycle therapy. If this therapy is able to improve functional recovery from inflammatory myelopathies it will not only be of clinical importance but also provide economical benefits in terms of better quality of life and function and lower societal costs.

**Importance of the knowledge to be gained:**

The efficacy of FES ergometry in restoration of function following inflammatory myelopathy has not been formally studied. If FES ergometry is found to be effective in inflammatory myelopathy, it may open a new window of hope for patients with other CNS injuries and propose a mechanism for recovery. There are risks associated with this trial however, the potential of the knowledge to be gained from the study, outweigh the risks.

## 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.

There will be no penalties for not completing the protocol. No monetary compensation will be provided.

## 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 INI, as well as any food/meals purchased throughout the day while engaged in study procedures.

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