Protocol Title: Recovery of cardiovascular function with epidural stimulation after human spinal cord injury

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Overall Objective: We propose to demonstrate that epidural stimulation (ES) can be used to recover significant levels of autonomic control of cardiovascular and respiratory function as well as the ability to voluntarily control leg movements below the injury level. This intervention would provide an immediate therapeutic alternative to individuals who now have no recourse for treatment. The reduction in the cost to the health care system, care givers and society would be dramatic.

Introductory Statement and Background: In the context of studies of human locomotion, we made the astonishing observation that three individuals who had been diagnosed as clinically motor complete (unable to voluntarily activate muscles below their level of lesion) developed the ability to voluntarily move their toes, ankles, knees and hips ***only*** in the presence of tonic ES of the lumbosacral spinal cord when also receiving intense locomotor training [1]. Even more surprising, over a period of months, they reported improved temperature regulation, bowel and bladder function, and normalized sexual activity. We also measured significant improvements in cardiovascular and respiratory function that persisted throughout the day even without stimulation.

Cardiovascular Function. Cardiovascular diseases are the leading cause of morbidity for individuals with SCI [2]. Reduction in peripheral blood flow increases the risk of pressure ulcers, parasympathetic imbalance increasing vasovagal syncope, fatigue and cognitive dysfunction, and symptomatic orthostasis which are manifestations of the reduced quality of life experienced by these patients [3-5]. With time, post injury episodes of orthostatic hypotension and autonomic dysreflexia become more prominent and persist in many individuals with cervical and high thoracic injury for the rest of their lives [3, 5, 6]. The causes of orthostatic hypotension in individuals with SCI are multifactorial and can be classified into either neurogenic (related to injury to the autonomic nervous system which controls cardiovascular function and has tracts through the spinal cord) or other physiologic consequences of SCI [3-5] . Other conditions that could contribute to orthostatic hypotension following SCI include altered baroreceptor sensitivity, lack of skeletal muscle pumps, cardiovascular deconditioning, altered salt and water balance and altered cerebral vascular autoregulation. A combination of sympathetic agonists and venous compression devices are the only measures available for treatment of these symptoms.

We propose that cardiovascular control is mediated by the spinal cord as the key integrator of complex signals from the periphery and from supraspinal centers in the brain stem (Figure 1). This spinal circuitry is moment by moment driven by peripheral input modulated plasticity to optimize the systemic blood pressure and heart rate. We have demonstrated in rats with chronic SCI that the sacral cutaneous or visceral afferents produced significant activation of the sympathetic activity within the renal nerve and correspondingly significant alteration in arterial blood pressure [6]. ES in the absence of descending input can modify the excitability of the relevant spinal interneuronal pools allowing them to respond to peripheral autonomic input and approximate normal cardiovascular control. It is also possible that ES restores conduction properties of residual damaged or non-functional, but anatomically intact axons across the spinal injured segment. If this is the case, then ES alone without the cardiovascular stress of stand training should restore near normal cardiovascular control. If, however, the spinal cord circuitry

is the key controller, then stand training with ES will be needed to optimize autonomic function. The ultimate goal is to develop appropriate parameters for ES that will activate spinal sympathetic circuits sufficient to maintain a more normal arterial blood pressure. If we can improve the management of this secondary complication of SCI, we would not only improve the health of this disadvantaged population but also dramatically reduce health care expenditures.

Respiratory function. Respiratory complications result in death and ongoing health problems in people with chronic SCI [2]. Following SCI, control of respiratory muscles innervated from within and below the injury zone is altered leading to paralysis [7, 8] and respiratory function deficits [9] which significantly impede recovery [10] and diminish quality of life [11]. Exercise programs involving limb muscles are known to increase fitness and improve ventilatory function in individuals with chronic SCI [12, 13]. However, none of the respiratory rehabilitative modalities have yet been proven to be clinically effective in patients with chronic SCI [14]. Preliminary results of locomotor training studies from our center indicate that the locomotor step training in individuals with motor complete SCI leads to increased spinal motor output to respiratory muscles, but does not improve the voluntary initiated respiratory muscle activating patterns. It has been shown that spinal networks for locomotion and respiration are closely related [15]. However, the three individuals who received ES increased their respiratory capacity. Therefore, based on our observations, we hypothesize that ES of the lumbosacral spinal cord will lead to re-activation of respiratory motor control and task specific training may enhance this effect in individuals with clinically motor complete SCI.

Restoration of voluntary control of movement. The critical role of the corticospinal tract in generating voluntary movements with the spinal cord simply being a conduit of these signals in primates has long been a widely held belief. This premise then limits the options for recovery to paralysis only occurring with repair or regeneration of these pathways. Our recent observations of three individuals with motor complete injury regaining the ability to voluntarily move their hips, knees and ankles upon command only in the presence of ES (Figure 2) challenges this theory and provides a novel treatment strategy for paralysis. The conceptual basis of our approach is that epidural stimulation combined with task-specific training re-engages existing spinal circuits and challenges novel post-injury circuitry to reorganize in functionally significant ways [1, 16, 17]. We provide a cellular environment that enables sensory-motor and autonomic circuits to recover significant levels of function. We are capitalizing on the inherent functional capacity that is built into these systemic circuits. Another point of significance in our approach is the high level of functional interdependence among the many physiological systems impacted by complete motor paralysis. The first motor complete subject studied, who has trained to stand and to voluntarily move his legs with ES for over 2 years, showed restoration of cardiovascular, respiratory, bladder, bowel, temperature regulation and sexual function in addition to recovery of movement and ability to stand.

Preliminary Data: Preliminary data in one motor complete quadriplegic patient with chronic hypotension and symptomatic orthostasis have shown that with optimized epidural stimulation parameters, we can maintain systolic blood pressure (SBP) 20% above his nadir and prevent symptomatic hypotension.

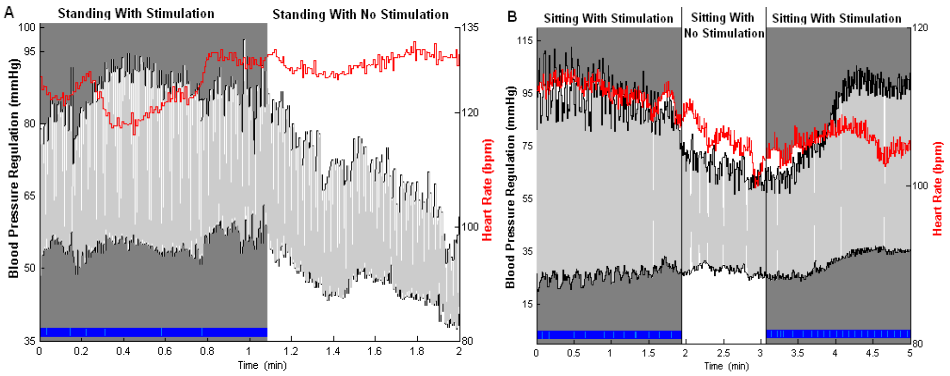

Figure 1. Continuous blood pressure and heart rate recordings from the individual with cervical motor complete SCI during standing **(A)** and sitting **(B)** with and without epidural stimulation.

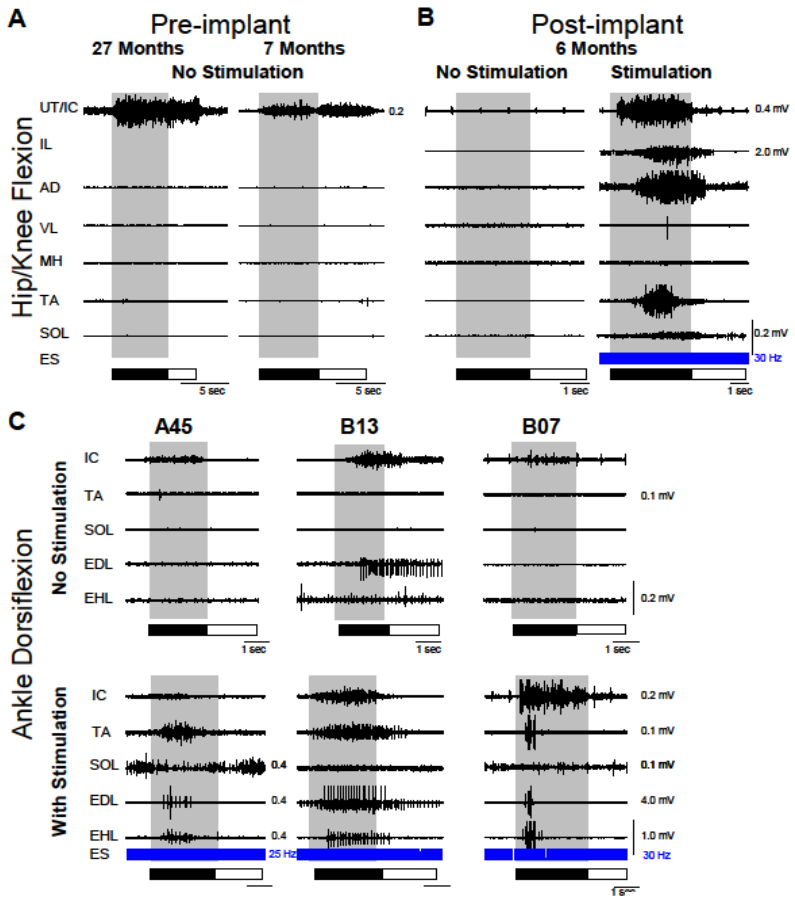

Figure 2. Lower extremity EMG activity during voluntary movement occurred only with epidural stimulation in three individuals with motor complete spinal cord injury. EMG activity from a subject B07 during voluntary attempts of left hip and knee flexion (A) without stimulation at 27 and 7 months prior to implantation; (B) without out stimulation (left panel) and with stimulation (right panel). EMG activity during attempts of ankle dorsiflexion without stimulation (top panels) and with stimulation (bottom panels) (C) from B07 (D) from subject A45 and (E) from subject B13. Muscles, surface EMG: Upper trapezius (UT), intercostal (IC), adductor magnus (AD), vastus lateralis (VL), medial hamstrings (MH), tibialis anterior (TA), soleus (SOL); fine wire EMG: iliopsoas (IL), extensor digitorum longus (EDL), extensor halluces longus (EHL). Black bars represent the command to flex and white bars represent the command to relax. Gray highlighted indicates verbal command to 'flex'. Blue indicates tonic stimulation.

<u>Rationale</u>: We propose to determine the functional gain that can be achieved in voluntary control of movements below the level of injury and autonomic nervous system function as a result of activation of spinal circuits with epidural stimulation (ES) in humans with paralysis. In addition to the scientific advances, the proposed experiments are essential to translating this therapeutic approach to a larger scale, which is needed to have a meaningful clinical impact. ES for recovery of neurological function in patients with severe SCI is not widely used because of uncertainty regarding the mechanisms of action and convincing evidence of efficacy in a larger numbers of subjects. Our approach will allow us to determine specific types of ES needed for

voluntary movement and autonomic nervous system dysfunction which lays the groundwork for expedient translation to larger numbers of individuals with SCI.

Current clinical methods of diagnosis of clinically complete SCI may not be sensitive enough to detect residual functional synapses across the lesion [18]. We propose to use a series of neurophysiological approaches that can detect different sources of supraspinal influence on spinal circuitry and identify specific pathways including vestibulospinal, reticulospinal, corticospinal and long propriospinal pathways that may remain viable or emerge with ES and task specific training after complete motor paralysis [19-32]. Such residual connectivity would be identified by the presence of voluntarily controlled movement or evoked motor potentials occurring only in the presence of epidural stimulation. Identifying the essential supraspinal-spinal pathways needed to recover these voluntary movements will advance our knowledge of human neural control of movement and provide critical information for developing repair and regeneration strategies and in determining the type and severity of patient that could benefit most readily in regaining voluntary control using epidural stimulation.

Research Plan:

1. Specific Aims
    a. **Specific Aim 1**. Demonstrate whether cardiovascular dysfunction improves in response to ES and stand training.

Hypothesis 1.1: ES and stand training will significantly increase resting systolic blood pressure (SBP) and diastolic blood pressure (DBP) and decrease heart rate (HR) to a greater extent than ES alone.

Hypothesis 1.2: Serum catecholamine levels will increase with ES and to a greater extent with ES and stand training.

Hypothesis 1.3 Cardiac structure and function will improve with ES and to a greater extent with ES and stand training.

- ***a. End systolic and end diastolic volumes***
- ***b. Systolic function and ejection fraction***
- ***c. Cardiac Output***

Hypothesis 1.4 Orthostatic hypotension and orthostatic tolerance will improve with ES and to a greater extent with ES and stand training.

Hypothesis 1.5 Arterial stiffness will decrease with ES and to a greater extent with ES and stand training.

Hypothesis 1.6 Metabolic indices will improve with ES and to a greater extent with ES and stand training.

The changes in cardiovascular function in individuals who received stand training and ES could have been induced by the ES alone or may have required the combination of ES and stand training. Since these individuals had received extensive stand and step training without these changes in cardiovascular parameters prior to the ES implant, the changes could not be attributed to the training alone. Thus ES in combination with stand training may be needed to regain vascular tone and reactivity. Peripheral autonomic control is lost with spinal cord damage above

the level of sympathetic output to the sympathetic ganglia. In individuals without neurologic injury who were subjected to weightlessness, experienced loss of cardiovascular control as observed with astronauts experiencing orthostatic hypotension, syncope on standing, and tachycardia [3]. Changes in blood distribution occur with postural changes. These blood distribution changes stimulate peripheral autonomic nerves that activate smooth muscles, reducing the capacitance of the vasculature and increasing cardiac return, preventing decreases in SBP and increases in heart rate.

b. **Specific Aim 2**. To evaluate whether task specific training combined with ES can improve pulmonary function and restore activation of respiratory muscles in individuals with SCI.

Hypothesis 2.1: Task specific stand training using ES parameters optimized for standing will result in more successful generation of voluntary respiratory muscle activation than ES without this task specific training.

The combination of stand training, specific for functional spinal proprioceptive activation, with ES, needed to increase the locomotor and respiratory spinal network responsiveness, might be required for successful respiratory reactivation. If voluntary movement training and stand training with ES will lead to the bigger respiratory effect, it will indicate that ES might facilitate both the strengthening of supraspinal and spinal pathways specific for locomotion and respiration.

c. **Specific Aim 3**. Identify mechanisms of restoration of voluntary movement with ES after paralysis.

Hypothesis 3.1: Task specific voluntary movement training using ES parameters optimized for voluntary movement will result in more successful voluntary movements than ES for cardiovascular function.

Hypothesis 3.2: Task specific stand training using ES parameters optimized for standing will result in more successful generation of voluntary movements than ES without task specific training.

We propose that the spinal circuitry is the key integrator of complex signals from the periphery and supraspinal centers that drive the final motor pool output. We predict that the circuitry is continuously adapting to activity-dependent events that functionally configure the spinal networks to optimize generation of a specific motor task. Another possibility is that the ES retrogradely stimulates residual axons that anatomically crossed the lesion but were not functional. Also, the ES could restore the conduction properties that were lost with demyelination or other consequences of damage [1, 16, 33-36]. If only the restoration of the conduction of the residual supraspinal connections is needed, then the ES alone without voluntary or stand training should be sufficient to restore voluntary movement of the legs. However, if the spinal cord circuitry is a key controller, then task specific training of voluntary movement in the presence of ES will also be required to optimally generate voluntary movement.

Stand training with ES may also be required for the recovery of voluntary movement given that the nervous system evolved in an anti-gravity environment and information related to load bearing has been shown to be a critical factor in modulation of neural activity [1, 37, 38]. Astronauts and research animals who are neurally intact return to earth with neural disorders such as spasticity and clonus that are common to individuals with spinal cord injury [39],

indicating that loss of load related sensory information drives neural plasticity that results in deleterious effects. Then weight-bearing with ES would also result in successful voluntary movement. The most efficacious approach may be the combination of ES with voluntary training and ES with stand training.

Given that each of our subjects to date has received stand training as well as training for voluntary movement of the limbs it may require the combination of stand training and ES to recover and improve voluntary movement. To some degree, even when standing, there is undoubtedly some critical element of voluntary control of posture and balance, and it seems almost inevitable that there will be some overlap of the circuitries of voluntarily moving the lower limbs when bearing weight as well as when one is not load bearing.

d. **Specific Aim 4**. Identify supraspinal descending pathways that could contribute to the restoration of voluntary movement with ES after paralysis.

Hypothesis 4.1: Stand training using ES parameters optimized for stand and voluntary training using ES parameters optimized for voluntary movement will significantly increase the amplitudes and decrease the latencies of evoked potentials of the leg muscles using transcortical electromagnetic stimulation (corticospinal) as well as facilitate the monosynaptic excitability of motoneuron pool of m.Soleus (H- reflex) after conditioning galvanic vestibular stimulation vestibular stimulation (vestibulospinal), an audio startle reaction (reticulospinal), and stimulation of the ulnar nerve (propriospinal).

Hypotheses 4.2: Voluntary movement training using ES parameters optimized for voluntary training will significantly increase the amplitudes and decrease the latencies of evoked potentials of the leg muscles induced by stimulation of the cortex (transcortical electromagnetic stimulation).

Hypotheses 4.3: Stand training using ES parameters optimized for stand training will significantly facilitate H-reflex and/or increase the amplitudes and decrease the latencies of evoked potentials of the leg muscles after conditioning with galvanic vestibular stimulation (vestibulospinal), induction of an audio startle reaction (reticulospinal), stimulation of the ulnar nerve (propriospinal) or by applying transcortical electromagnetic stimulation (corticospinal), demonstrating an activity-dependent effect on all supra-spinal pathways connectivity.

Hypotheses 4.4: The anatomical and axonal integrity of the neural tissue in the spinal cord (according to MRI) will have a positive relationship to the degree of restoration of voluntary movement after complete motor paralysis.

Experimental activation of descending motor tracts on leg muscles and the soleus H-reflex have been studied extensively in humans using cortical stimuli to activate the corticospinal tract [27, 30, 32], galvanic stimulation to activate the vestibulospinal tract [19, 21-24], auditory stimulation to activate the reticulospinal tract [29], and sensory stimuli to activate long propriospinal tracts [20, 25, 26, 28, 31]. The unique aspect of this proposal is that we will customize these experimental paradigms by conducting the neurophysiological assessments of all three prominent

descending pathways and the long propriospinal pathways together to comprehensively assess the extent of residual descending and long propriospinal pathways' viability in the spinal cord injury population [19-21, 23-25, 28, 40-65]. A soleus H-reflex recruitment curve and the conditioning stimulation paradigms (i.e. transmagnetic, galvanic, auditory startle, or ulnar nerve) specific to each pathway will be conducted. The amplitudes and latencies of the soleus H-reflex will be calculated.

2. Methods and Procedures

a. **General Experimental Design**. We will enroll 5 research participants who have sustained a SCI to participate in the proposed experiments. Our novel approach of conducting repeated experiments with comprehensive assessments in a smaller cohort of patients rather than a more traditional approach of including a large number of patients and focusing on a single outcome allows advancing both clinical and scientific knowledge. We have found success with the smaller cohort approach because we can employ more rigorous, quantitative and sensitive outcomes that not only inform us about the potential clinical efficacy but also provide further knowledge of the mechanisms of neural control of movement and other physiological mechanisms related to cardiovascular, respiratory and function and voluntary control of movement.

b. **Research participant enrollment**. Each research participant will be screened for medical eligibility by the neurosurgeon and physiatrist and for scientific eligibility by the site principal investigator (see Human Subjects section below). After eligibility is determined and consent procedures are implemented, the individual will undergo all clinical and neurophysiological assessments for voluntary movement, cardiovascular and respiratory function. Magnetic resonance imaging (MRI) will be conducted at the time of enrollment to establish structural integrity of the nerve tissue and to establish the severity of injury and diffuse tensor imaging to establish the axonal integrity. A standard MRI of the area of injury will be conducted and it will be read by a radiologist and the following measures will be made: 1) the number of sagittal cuts in which the signal change is present, 2) maximum height of signal change, 3) maximum area of signal change, 4) maximal canal compromise will calculated (MCC) and 5) maximal spinal cord compression (MSCC). All of these measurements will be made on the mid sagittal view.

Participants will continue with their current daily activities for 80 consecutive days without any intervention (usual care) followed by all assessments. Surgical implantation of the 5-6-5 Specify or 5-6-5 Specify SureScan MRI electrode, and Restore Advanced or RestoreAdvanced SureScan MRI Pulse generator, (MEDTRONIC, Minneapolis, MN, USA) encompassing the lumbosacral spinal cord guided by neurophysiological mapping will then occur (implant).

Surgical Procedure (Implantation of Medtronic Specify™ 5 -6-5 or Medtronic Specify™ 5 -6-5 SureScan MRI): Thoracic epidural stimulation is an operation that is commonly performed by neurosurgeons or pain management physicians for the treatment of intractable pain and spasticity involving the lumbar spine and legs. In this study, we will be inserting electrodes into the thoracic epidural space below the level of a spinal cord injury.

The Medtronic Specify 5-6-5 or Specify 5-6-5 SureScan MRI (Minneapolis, MN) 16-electrode epidural arrays and battery pack will be used and the operation will be performed at University Hospital in Louisville, KY. Two large bore IV cannulas, a urinary catheter, and endotracheal tube will be inserted. Muscle relaxant medications will be avoided which will allow motor evoked potentials to be performed intraoperatively. Prophylactic antibiotics will be administered intravenously with maintenance of adequate blood pressure. The patient will be placed in the

prone position on the operating table with all pressure points being well-padded, particularly over the pelvis, knees, abdomen, and eyes. A midline incision will be made in the thoracolumbar area, with dissection carried deeply to the laminae. A cross-table lumbar spine X-ray will be performed to confirm the correct level. An L2-L3 laminectomy will be performed to provide a site for insertion of the epidural electrodes. The Specify 5-6-5 or Specify 5-6-5 SureScan MRI electrode will be passed along the dorsal aspect of the epidural space in a cephalic direction to the T11-L1 level. Fluoroscopy using the O-arm will be used to confirm the position of the electrode. Neurophysiological parameters will be utilized to determine optimal lead placement by monitoring the motor system using electrical stimulation of the spinal cord at the T11-L1 spinal segmental levels. Electrophysiological tests will be performed under the direction of Dr. Harkema. If appropriate motor responses cannot be obtained initially, the electrode will be repositioned as many times as required in order to obtain optimal motor responses from the legs. Optimal position of the electrode will cover L1-S1spinal levels. The wire lead connected to the Specify 5-6-5 or Specify 5-6-5 SureScan MRI electrode will be sutured tightly to muscle to prevent its being dislodged. The surgical incision will be closed in layers, and a wire connected to the epidural electrode will be passed through the subcutaneous layer and tunneled subcutaneously to exit through the skin about 4 inches to the right of the midline. A sterile dressing will cover the external wire, and the midline incision will be closed.

The next part of the operation will be performed while the patient is in the supine or side position. The patient will be turned from the prone to the supine/side position keeping the area of the external wire exit sterile. The abdomen, lower thoracic area, and right flank will be prepped using betadine soap. A lower abdominal incision will be made approximately 4 inches in length and will be carried to the subcutaneous area directly external to the abdominal muscle layer. A wire passer will be threaded circumferentially to the lateral flank incision to the site of exit of the electrode wire to allow the distal portion of the electrode wire to be threaded through the wire passer. The distal (abdominal) aspect of the electrode wire will be attached to the battery pack of the Specify 5-6-5 or Specify 5-6-5SureScan MRI electrode. The battery back will be buried in the subcutaneous tissue directly external to the abdominal muscle. The battery pack will be sutured to muscle in order to prevent its migration. The surgical field will be irrigated using antibiotic solution to minimize infection. The abdominal incision will be closed in layers. The patient will be taken from the operating room and transferred to the recovery room.

The patient will be kept in the recovery room for 4-6 hours. He or she will stay overnight at University Hospital for monitoring and then will be transferred to the Frazier Rehab Institute or home. The patient will be monitored for blood pressure, pulse, and temperature changes. Fluid output will be recorded hourly to maintain appropriate homeostasis. The dressing over the incision will be changed 24 hours postoperatively. The patient will be discharged home and a research nurse will make daily visits to change dressings and check on the incision. The patient will recover at home for 2-3 weeks prior to any testing or stimulation is started.

ES is administered by a multi-electrode array implanted in the epidural space over the dorsum of cord. An implanted package containing stimulating circuits, rechargeable battery, and wireless communication activates the electrodes (16 platinum electrodes arranged in three columns of [5-6-5]). The pattern of electrically active electrodes, as well as electrode voltage, stimulating frequency, and stimulating pulse width can be remotely programmed. Since different spatial activation patterns and different frequency parameters affect different spinal circuits, the array can be reconfigured, within limits, to bias its facilitating effects toward different activities, such as cardiovascular control or voluntary movement.

Clinical and neurophysiological assessments will be repeated post implantation. Mapping of the motor evoked responses in response to spatial and amplitude/frequency responses [66] will be conducted and the specific configurations and parameters optimal for voluntary movement, standing and cardiovascular function will be identified.

The individual will then undergo ES stimulation optimizing cardiovascular function for 80 days for 2-6 hours per day (Appendix A). In our pilot data from the first three individuals, we observed in two individuals that as they continued stimulation they were able to maintain normal blood pressure levels without stimulation for some periods of time. In one individual, the stimulation was always needed to maintain systolic blood pressure within normal ranges, however if allowed to stimulate for longer periods of time they may have reached normal ranges without needing ES. Thus, the protocol now reflects ES time periods customized to the actual modulation of the blood pressure for each individual. The individual will monitor their blood pressure and use the stimulator during those periods when their systolic blood pressure is outside the targeted range. The individual chooses the time periods during the day they will use the stimulator with a minimum of 2 hours each day. In situations that 2 hours of continuous stimulation occurs, a break of at least 15 minutes of no stimulation should occur before resuming stimulation again. Cardiovascular ES is limited to 6 hours each day. Each session of ES can be less than 2 hours if the blood pressure remains within the targeted range without stimulation. Stimulation periods will be conducted and observed in the laboratory before being approved to be done in the home.

The same clinical and neurophysiological assessments again will be repeated (Post CV Training). The research participant will then undergo ES for voluntary movement (VM) sessions for 80 days for 2 sessions per day, in addition to continuation of ES for CV function. The CV sessions can be conducted at home. The voluntary sessions will be conducted both in the laboratory and at home. Each voluntary session will be focused on the leg and/or trunk. The remote device records the minutes of stimulation and parameters used so these will be collected on those days the research participants are not in the laboratory. For approximately the first 5 sessions of each intervention (CV or VM), the sessions may be conducted in the laboratory under the supervision of the investigators. The optimal parameters will be identified and programmed into the remote device. For the following sessions, the research participants will come to the laboratory at least once every 2 weeks and CV and VM parameters will be evaluated. The final 5 sessions may also be conducted in the laboratory (monitoring purposes, if necessary). The research participant will then complete the same clinical and neurophysiological assessments (Post VM Training).

In our previous early feasibility studies we have found that the intensity (daily session number) is a critical factor in the recovery of voluntary and independent standing recovery. The first three research participants in this pilot study did not regain sufficient independent standing to be able to take the Stand-ES to their home environment unlike those in our other study who had training twice a day. Thus, now in the final intervention we will include daily stand training sessions once or twice a day (1-hour each) with ES stimulation parameters optimized for standing for 80 sessions. Research participants will participate in 2-6 hours of cardiovascular and 2-4 hours of voluntary parameters outside of the stand training session. Total daily stimulation time will be 6-10 hours. All stand training and ES sessions will be conducted in the laboratory. Approximately every 10 sessions the cardiovascular and voluntary sessions may be conducted in the laboratory (monitoring purposes, if necessary). Then the final clinical and neurophysiological assessments will be conducted (Post Stand Training).

After the training and experiment period has ended, research participants will be released with a list of approved programs based on investigator review. When the individual returns for a follow-up visit, the time the research participant used each program will be downloaded from the stimulator.

Cardiovascular Function (Specific Aim 1):

Orthostatic Stress Test (with and without catecholamine blood draw) will be assessed in the morning in a quiet, temperature-controlled (~22 C) room. After arriving, participants will be asked to empty their bladder before beginning the study. A butterfly catheter will be inserted into an antecubital vein during instrumentation to allow the collection of blood without additional stress to the participant by appropriate clinical staff. Continuous arterial BP will be acquired from a finger cuff placed around the ring, middle, index finger or thumb (Finapres Medical Systems). The hand will be placed in an arm sling and kept at the level of the heart throughout the study. Manual arterial blood pressure measurements will be taken throughout the study with a digital blood pressure measurement device or via the auscultation method. A three or twelve-lead ECG (ML132, AD Instruments) will be placed for ECG monitoring. Rib cage and abdomen kinematics (respiratory kinematics) will be acquired using an inductive plethysmograph. Baseline recording for 15 minutes will begin after a 3-5 minute rest period that will follow subject preparation. At the end of 15-minute recording in the supine position, participants will be passively moved into the upright seated position (tilt chair) or upright position (tilt table). This position will be maintained for 15-18 minutes. Then the participants will be passively moved to the supine position for 10-15 minutes. Eight milliliters of venous blood will be drawn from an antecubital vein at the end of 15-minute supine to assess baseline catecholamine levels. Blood draw will be repeated at 3 min and at the end of 15 minutes of upright position. The test will be aborted if subjects become lightheaded or symptomatic of syncope. The Hemodynamic Test during standing, upright, sitting and supine positions with and without ES will be recorded using a Finapres system as described above. This assessment may be performed with and without epidural stimulation.

Blood pressure lability: 24-hour blood pressure monitoring (Specific Aim 1) and Heart Rate Variability: ECG Holter Monitor

Continuous blood pressure monitoring will be recorded over a period of 24 hours outside the lab (Meditech ABPM, Budapest, Hungary). In regards to the severity of AD, the participant's signs of OH (e.g. yawning, pallor) and subjective symptoms (e.g. light-headedness, dizziness) will also be assessed using validated autonomic questionnaires. Continuous ECG will be recorded over a period of at least 24 hours outside the lab with a 3,7 or 12 lead Holter monitor (EDAN Instruments, Inc). 24-hour measurements will be repeated throughout the training interventions.

Ultrasound: Measurements will be taken with and without epidural stimulation.

Arterial Stiffness (Specific Aim 1)

Arterial Pulse Wave Velocity (aPWV m/s) will be measured non-invasively and calculated by dividing the distance between measurement sites by the pulse transit time. Distance between the arterial measurement points will be measured using measuring tape along the surface of the body, held parallel to the testing table. The pulse transit is determined from the arterial blood pressure waves, which are collected at each arterial site. A pen-like device (model SPT-301; Millar Instruments Inc., Houston, TX) or Complior

Pulse Wave and Central Pressure Analyzer (Alam Medical, Vincennes, France) sensors will be applied to the carotid, femoral, brachial and other arterial sites using a light pressure to obtain arterial pressure waves. Heart rate will be recorded using a single-lead (lead I) electrocardiogram (ECG) (model ML 123, AD Instruments Inc., Colorado Springs, CO).

Arterial structure: Wall thickness and lumen diameter (Specific Aim 1)

Brachial and femoral arterial images will be collected using B-mode ultrasound for 10 cardiac cycles. Images will be analyzed using internal ultrasound software to determine lumen diameter and intima-media thickness.

Cardiac structure and function (Specific Aim 1)

Cardiac images will be collected non-invasively using Doppler ultrasound (GE Healthcare or Philips Healthcare) in a supine and seated position. Briefly, apical four and two-chamber views, and parasternal short and long-axis views will be collected and stored on the ultrasound for offline analysis. Indices of interest will include but not limited to: volumes (end systolic (ESV), end diastolic (EDV), diameters (intraventricular septum systole (IVSs) and diastole (IVSd), left ventricular internal diameter systole (LVIDs)), systolic function (left ventricular posterior wall systole (LVIDd) and diastole (LVPWd), ejection fraction (EF), cardiac output (CO), fractional shortening, mitral regurgitation (dP/dT)) and diastolic function (E/A, E/e' ratio, IVRT, DT).

International Autonomic Standards Evaluation (Specific Aim 1)

Until recently individuals with SCI were only examined with use of motor and sensory neurological standards in order to establish the level and the severity of the neurological impairment or AIS (American Spinal Injury Association Impairment Scale) resulting from the SCI. During the last decade, International Autonomic standards for evaluation of individuals with SCI were developed and implemented around the world (76). These short standardized forms collect data on cardiovascular (AD and OH) as well as other autonomic dysfunctions including bladder bowel and sexual dysfunctions.

Body Composition (Specific Aim 1)

DXA: Weight, height, and total body fat will be determined from a dual energy x-ray absorptiometry (DXA) scan (Hologic QDR 4500W, APEX System Software Version 2.3) at Jewish Hospital, performed in the supine position. Waist circumference will be measured in the supine position following a normal expiration, to the nearest 1cm midway between the lowest lateral border of the ribs and the uppermost lateral iliac crest. Waist circumference is considered the most practical bedside measurement of visceral adipose tissue. Hip circumference will be measured supine over the widest part of the femoral great trochanter. Waist/hip ratio and body mass index (measured weight in kilograms divided by the measured height [meters2]) will be calculated. Total body fat will be reported as total body fat in kilograms, and as a percent of total body weight determined by DXA scan. Height (length) will be measured using the electronic ruler function and weight from the DXA scan table scale feature. This is the preferred measure for assessing total body fat and has strong agreement with cadaver and chemical composition studies. Recent studies comparing DXA to CT and MRI have confirmed the validity and reliability of DXA to assess abdominal adiposity [26]. In addition, its ease of use makes it ideal for studying large populations. All scans will be performed with a (Hologic Discovery QDR 4500W (Hologic Inc., Bedford, MA), which has an error of less than 1% for body fat scans. An experienced technician will conduct scans according established protocol. All participants will be scanned with this methodology to ensure high internal validity (Intervention Protocol Version 2.0).

BOD POD Body Composition:

Procedure: The BOD POD is an air displacement plethysmograph that measures body composition, fat mass, fat-free mass, thoracic gas volume, and estimates resting metabolic rate. Participants will be asked to sit in the scanner, remaining still during the test. The full test requires only about 5 minutes and includes multiple short (about 40 seconds) volume calculations. The last scan for thoracic gas volume will only be conducted with individuals capable of holding the breathing tube to their mouth. Men will be asked to wear a form-fitting Lycra/spandex-type swim suit or single-layer compression shorts with no padding. Women will be asked to wear a form-fitting Lycra/spandex-type swim suit or single-layer compression shorts without padding and a single layer (not padded) sports bra. Participants may be asked to repeat the procedure.

Analysis: Body composition, fat mass and fat-free mass will be calculated by the Cosmed BOD POD software package based upon the measured or predicted thoracic gas volume and the equation of the chosen model. Resting Metabolic Rate is estimated from the thoracic gas volume and the activity level that the research participant self-reports. The Brozek and Siri models (based on sex and ethnicity) are standard with the software package. Additional models can be built into the software.

Metabolic Parameters (Specific Aim 1)

Blood draws: A trained technician will draw a venous blood sample. Participants will undergo a 12-hour fast the night before, including no food or drink including alcohol or caffeine (water is permitted). A complete blood count (white blood cells and differentials, erythrocytes, packed cell volume, hematocrit, platelets, hemoglobin and red cell indices) will be performed. Blood glucose control (HbA1c), fasting glucose, fasting insulin, atherogenic dyslipidemia (triglycerides, TC, LDL-c, HDL-c, TC/HDL-c), a pro-thrombotic state (PAI-1 and TAFI), a pro- inflammatory state (IL-6, and TNF-α), leptin, adrenal control (angiotensin, aldosterone and renin) will also be measured. Blood samples for PAI-1, TAFI, IL-6, and TNF-α will be analyzed using enzyme-linked immunosorbent assays (ELISA). Plasma levels of lipid and hemoglobin A1c will be analyzed through laboratory services using a Dade Behring RxL Max analyzer. This system has demonstrated very good intra and interassay reliability for lipid and glucose measures. A comprehensive drug screen (urinalysis or blood draw) will be done to assess the use of combustible drugs (nicotine and other drugs that can be smoked)

Aerobic Fitness Evaluation: Peak oxygen uptake test (VO2peak) (Specific Aim 1)

Participants will perform an exercise regimen. Participants will be asked to not perform any moderate or heavy exercise 12 hours prior to the test. Resting ECG, blood pressure (DinamapCarescape V100; GE Healthcare, Buckinghamshire, UK) and respiratory measures (ParvomedicsTruemax 2400, Sandy, UT, USA) will be collected two minutes prior to exercise. Heart rate will also be monitored with a chest strap heart rate monitor (Polar T31 heart rate monitor, Polar Electro Inc., Woodbury, NY, USA). Continuous blood pressure and heart rate will also be measured using the Finapres acquisition described in the orthostatic stress test. Each participant will perform a graded exercise test (GXT) on a total body recumbent stepper (NuStep T4 ergometer, Ann Arbor, MI) to measure VO2max. For participants with tetraplegia who have limited handgrip function, tensor bandages will be used to secure hands to the ergometer handles. For those participants unable to perform the test on the NuStep, an arm ergometer test protocol will be administered [29, 53]. Participants will be instructed to maintain a cycling rate of 60 rev/min for the duration of the test. After an initial warm-up at 0W, power output will be increased at a rate of 5 W/min for

participants with tetraplegia, or 10 W/min for participants with paraplegia, until volitional exhaustion (i.e. dropping below 30 rev/min). Participants will be asked to identify their ratings of perceived exertion (RPE) on the Borg scale every minute until the completion of exercise. Heart rate and oxygen uptake will be recorded on a breath-by-breathe basis for the duration of the test. The highest 15-second average of oxygen uptake during the test will be recorded as VO2peak.

Basal Metabolic Rate (BMR) (also called Resting Metabolic Rate – RMR) (Specific Aim 1)

BMR is the rate of energy expenditure by humans at rest (reported as kilocalories). The release, and using, of energy in this state is sufficient only for the functioning of the vital organs: the heart, lungs, nervous system, kidneys, liver, intestine, sex organs, muscles, brain and skin. BMR is measured under very restrictive circumstances when a person is awake, without moving or talking while the person is lying down in a best for about 45 minutes after fasting for 10-12 hours. A canopy hood will be placed to cover the participant head and shoulder, and BMR will be measured by expired gases through indirect calorimetry and will be analyzed with a Parvo Medic TrueOne 2400 (Sandy, UT). Analysis of the oxygen and carbon dioxide composition of the expired air will occur every 10 seconds. The cart will be calibrated with a 3-liter syringe for flowmeter calibration and the ambient air for gas calibration at least 30 minutes before testing as recommended by the manufacturer's guidelines. An accurate BMR measurement requires that the person's sympathetic nervous system not be stimulated, thus, it requires complete rest. BMR generally decreases with age and with the decrease in lean body mass. Increases in muscle mass and mitochondrial proliferation (for example: as a result of endurance training) result in increases in BMR.

Pulmonary Function Test, PFT (Specific Aim 2): PFT's will be performed in the participant's wheelchair using BreezeSuite System (MedGraphics, St. Paul, MN). Forced vital capacity (FVC) and forced expiratory volume in 1 second (FEV1) will be obtained. Three acceptable spirograms will be obtained and the result of their best attempt will be used [67]. MP45-36-871-350 Differential Pressure Transducer (Validyne Engineering, Northridge, CA) will be used to measure the maximum inspiratory pressure (PImax) and the maximum expiratory pressure (PEmax). The PImax will be measured during maximal inspiratory effort beginning at near residual volume and PEmax will be measured during maximal expiratory effort starting from near total lung capacity. The assessment will require a sharp, forceful effort be maintained for a minimum of 2 seconds. The maximum pressure will be taken as the highest value that can be sustained for one second. The maximum value from three maneuvers that varied by less than 20% will be averaged.

Respiratory Motor Control Assessment (RMCA) (Specific Aim 2):

Procedure: This assessment combines standard spirometry and airway pressure measurements (as described in the Pulmonary Function Test /PFT/ section), 3-lead Electrocardiography (ECG) (ML132, AD Instruments), beat-by-beat arterial Blood Pressure (BP) recordings from a finger cuff (Finapres Medical Systems), respiratory kinematics using inductive plethysmography (lnductotrace, Ambulatory Monitoring), and surface Electromyography (sEMG) (Motion Lab Systems, Inc, Baton Rouge, LA) of the muscles of respiration. This assessment may be performed with and without epidural stimulation.
Respiratory muscle activation patterns will be evaluated using sEMG of respiratory-related muscles using the MA300 System (Motion Lab Systems, Baton Rouge, LA) [68]. sEMG input will be amplified with a gain of 2000, filtered at 4-1000 Hz and sampled at 2000-10000 Hz. The

electrodes will be centered over the muscle belly parallel to the muscle fibers. The skin will first be prepped with a sterile alcohol swab before electrode placement and held in place with either Tegaderm Film (3M Req#1624W) or Cover-Roll Stretch Tape (BSN Medical, Hamburg, Germany). The ground electrode(s) will be placed over the acromion process bilaterally.
The RMCA will utilize a multi-muscle sEMG-based measure of motor output from the central nervous system recorded during voluntary tasks attempted in the supine and sitting position [69, 70]. The protocol begins in the seated position and consists of the following maneuvers followed by 5 minutes of relaxation in supine position: Spirometry, PEmax, PImax, PEmax and PImax sustained for 5 seconds, deep breath, coughing, and a Valsalva maneuver. Each maneuver will be cued by an audible tone and repeated three times. After the 5 minute relaxation period, the aforementioned maneuvers will be repeated in the supine position. The supine position protocol will exclude the Valsalva maneuver, while adding a neck flexion against resistance, shoulder shrug, hip and knee flexion, and a sit-up task. sEMG of left and right neck, trunk, limb muscles including but not limited to submental, sternocleidomastoid, scalene, upper trapezius, lower trapezius, upper portion of pectoralis major, intercostals, the diaphragm, rectus abdominus, obliques, and the paraspinals will be recorded using a multi-channel EMG system MA300 with pre-amplified electrodes (MotionLab Systems Inc., Baton Rouge, LA) or an Eclipse Neurological Workstation (Axon Systems Inc., Hauppauge, NY) with pairs of recessed, FE9 silver-silver chloride cap surface electrodes (Grass Instruments, W Warwick, RI).
Analysis: The envelope of EMG activity for each muscle will be calculated using a root mean square (RMS) algorithm [69]. Analysis windows will be determined from the event marker recorded with the cuing tone that signaled the subject to begin the task. The overall amount of EMG Magnitude (μV, Mag) and the Similarity Index (SI), that quantitate the multi-muscle distribution of activation during Maximum Expiratory Pressure Task (MEPT) in research participants compared to that of healthy subjects will be calculated using a vector-based analysis as previously described [69, 70]. In brief: multi-muscle activity parameters will be calculated using averaged RMS amplitudes from each SCI subject for comparison to group values from non-injured (NI) subjects. The resulting Mag parameter is the amount of combined sEMG activity during MEPT calculated as a length of the resultant vector. The SI provides a value between 0.0 and 1.0 (most similar) equal to the cosine of the angle between the resultant multi-muscle distribution vectors in SCI subject to that of NI subjects. To perform the maximum airway pressure tasks, subjects will produce maximum respiratory efforts for 5 seconds blowing into the Airlife 001504 circuit (Allegiance Healthcare Corp., McGaw Park, IL). Airway pressure; sEMG; breathing rate and chest wall kinematics will be monitored simultaneously by using Powerlab acquisition system (ADInstruments, Colorado Springs, CO).
For the bursts analysis, EMG data will be full wave rectified and filtered using a 4th order bandpass Butterworth filter (40 Hz - 500 Hz) representing the relative number and frequencies of the motor units recruited per burst. Integrated EMG will assess the total EMG activity generated during specific phases of the motor tasks. Co-activation values of inspiratory and expiratory muscles and the degree of coordination in the breathing-related oscillations will be evaluated through principal component analysis.

EMG, kinematics and kinetics experiments (Specific Aim 3): During the experiments, the research participants will be placed on the treadmill in an upright position and suspended by a cable in a harness (i.e. BWST) or on an overground standing device. Voluntary leg movements will be performed on a mat or seated in a chair. Following standard skin preparation techniques, bipolar surface EMG electrodes may be placed bilaterally on the following muscles: soleus (SOL), medial gastrocnemious (MG), tibialis anterior (TA), medial hamstrings (MH), quadriceps

(VL and RF) and adductor (AD) muscles. Fine-wire EMG electrodes may be used for deep muscles of the hip and foot. Limb and trunk kinematics including hip, knee and ankle angles will be acquired using high speed passive marker motion capture (Motion Analysis, Santa Rosa, CA). When appropriate we will measure individual ground reaction forces (GRF) using HRMat (TEKSCAN, Boston, MA) or forces during movement with a force transducer (Kistler, Amherst, NY). These experiments will be performed with and without epidural stimulation.

NeuroRecovery Assessment using EMG activity: Standing, Stepping, and Overground motor tasks: (Specific Aim 3)

***Procedure**:* Assess the level of muscle activation and amount of external assistance required during standing and stepping in a body-weight supported treadmill environment, as well as overground motor tasks. Efficacy of arm, trunk and leg movement recovery and incorporation of independent motor tasks will be measured by the NRS [71, 72], comprised of fourteen motor tasks, and combined with EMG, kinematic, and kinetic analyses. This population has ongoing medical issues related to their spinal cord injury and so in some cases we may not complete the assessments with EMG depending on the physical status of the research participant. This will not affect the overall integrity of the data set. The research participant will be asked to perform these tasks as independently as possible with and without epidural stimulation

Materials: EMG, kinematic, and kinetic analysis will be performed on the upper and lower extremities and/or trunk during stepping, standing, and overground motor tasks. Muscle activation patterns will be evaluated using EMG that may include but is not limited to the following combinations of muscles: sternocleidomastoid (SCM), upper trapezius (UT), biceps brachii (BB), triceps brachii (TB), extensor carpi radialis (ECR), flexor digitorum profundus (FDP), abductor digiti quniti (ADQ), external intercostal (IC6 - 6th intercostal space), rectus abdominus (RA), external oblique (EO), erector spinae (ES), rectus femoris (RF), vastus lateralis (VL), medial hamstrings (MH), adductor (AD), tibialis anterior (TA), peroneous longus (PL), medial gastrocnemius (MG), soleus (SOL), flexor hallucis brevis (FHB) or longus (FHL), extensor hallicus longus (EHL), and extensor digitorum longus (EDL) using the MA300 System (Motion Lab Systems, Baton Rouge, LA). We may also use fine-wire EMG to acquire activity from the illio-psoas (IL), or other deep muscles, including the hand and feet muscles listed above. Standard needle insertion sites for each muscle will be used [73].

EMG input will be amplified with a gain of 2000, filtered at 4-1000 Hz and sampled at 2000-10000 Hz. The bipolar surface electrodes will be placed over the muscle belly parallel to the muscle fibers. The skin will first be prepared by shaving and cleaning the area with a sterile alcohol swab before electrode placement. The ground electrode(s) will be placed over a bony surface of the lower leg(s). Limb kinematics will include trunk, and upper and lower extremity angles that will be acquired using high speed passive marker motion capture (Motion Analysis, Santa Rosa, CA). When appropriate, we will measure individual ground reaction forces (GRF) using a zebris FDM-T System (zebris Medical GmbH, Isny, Germany) or forces during movement with a force transducer (Kistler, Amherst, NY). Blood pressure and heart rate may be measured using either a manual blood pressure monitor (Dinamap V100, GE Medical) or by a finger cuff (Finapres Medical Systems). Temperature may be monitored using a customized sensor system. A NIRS optode may be placed on a muscle of the leg to measure muscle oxygenation and hemodynamics.

The research participant will be asked to perform motor tasks as independently as possible. Research staff will score each of these tasks based on the algorithm. The following tasks may be performed in separate sessions (e.g. overground tasks, standing, or stepping on different days).

Sit: The research participant will be sitting unsupported at the edge of the therapy mat with both feet touching the ground with hips and knees in 90-degree angles. The research staff will then ask the participant to sit without upper extremity support to attain or maintain best posture. Depending on participant abilities, participant may be asked to maximally reach forward and to each side while maintaining appropriate posture and balance.

Reverse Sit Up: From an unsupported sitting position at the edge of the mat with feet on the ground with hips and knees in 90-degree angles, the research participant will be asked to slowly lower his/her trunk down to the mat without assisting the movement with his/her arms. Depending on participant abilities, participant may be asked to maintain trunk position, rotate trunk, and return to upright sitting.

Sit Up: From a supine position on the mat with feet on the ground with hips and knees in 90-degree angles, the research participant will be asked to return to a sitting position without the use of his/her arms.

Trunk Extension in Sitting: The research participant will be sitting with feet flat on the floor with hips and knees in 90-degree angles and chest resting on his/her lap. The research participant will be asked to return to an upright sitting position without the use of arms. Depending on participant abilities, participant may be asked to slowly lower trunk down to chest and return to upright sitting without use of arms.

Overhead Press: The participant will begin the task by sitting with their best posture at the edge of the therapy mat with both feet touching the ground with hips and knees in 90-degree angles and hands down by their sides. The participant will be asked to curl their hand towards the shoulder then press their hand towards the ceiling while straightening the elbow. As the participant progresses through the task with appropriate kinematics, they may be asked to perform the task while holding a one, three or five pound dumbbell. Each upper extremity will be performed and scored separately. Stability assistance can be given to the participant at the trunk, up to the level of the inferior borders of the scapulae while they are performing this task.

Forward Reach and Grasp: The participant will begin the task by sitting with their best posture at the edge of the therapy mat with both feet touching the ground with hips and knees in 90-degree angles and hands down by their sides. A table will be placed in front of the participant with the height adjusted to be one inch below the wrist crease with their elbow flexed to 90 degrees. An empty 12 oz. can, will be placed on the table, arm's length away. The participant will be asked to reach forward, grab the can, bring it to their mouth, and set it back down on the table. As the patient progresses through the task with appropriate kinematics, they will be asked to perform the task with a full 12 oz. can. Each upper extremity will be performed and scored separately. Stability assistance can be given to the participant at the trunk, up to the level of the inferior borders of the scapulae while they are performing this task.

Door Pull and Open: The participant will begin the task by sitting with their best posture at the edge of the therapy mat with both feet touching the ground with hips and knees in 90-degree angles and their arm resting on the table. The table height will be adjusted to be one inch below the wrist crease with their elbow flexed to 90 degrees. The participant will be instructed to pull their hand back to the side of their body as if opening a door. As the participant progresses through the task, they will be instructed to perform additional movements, including pronation and supination. Once the participant performs the movements with appropriate kinematics, they will be asked to perform the task with a 3 pound dumbbell and to pick up a key, insert it in a lock and turn the key 90 degrees. Each upper extremity will be performed and scored separately. Stability assistance can be given to the participant at the trunk, up to the level of the inferior borders of the scapulae while they are performing this task.

Can Open and Manipulation: The participant will begin the task by sitting with their best posture, at the edge of the therapy mat with both feet touching the ground with hips and knees in 90-degree angles and hands down by their sides. A table will be placed in front of them with the height adjusted to be one inch below the wrist crease. The participant will be asked to simultaneously reach and place both hands around a container with a lid. As the participant progresses through the task with appropriate kinematics, they will be asked to perform more advanced skills, including stabilizing the can with one hand while using a lateral pinch to remove the lid with the other. There will be items in the can that the participant will be asked to remove and translate with the tips of their fingers. Each upper extremity will be performed and scored separately. Stability assistance can be given to the participant at the trunk, up to the level of the inferior borders of the scapulae while they are performing this task.

Sit to Stand: The research participant will be asked to stand up from a seated position at the edge of the mat, with hips and knees in 90-degree angles, without the assistance of his/her arms. If the participant is able to raise his body 50% off the mat, the research staff will assist as needed during the latter 50% of standing. Depending on participant abilities, participant may be asked to stand up while holding 20 pounds and to stand up from a seated position with hips at 100-degree flexion angles.

Stand: The research participant will be asked to stand overground with proper posture. Assistance will be provided by research staff only as needed. Depending on participant abilities, participant may be asked to reach maximally reach forward and laterally, achieve and maintain tandem stance, and achieve and maintain single-limb stance while maintaining proper posture and balance.

Walking: From a standing position, the research participant will be asked to shift weight laterally and forward while in a stride position. Assistance will be provided by research staff only as needed. Depending on participant abilities, participant may be asked to take repetitive steps, step over an object, descend an incline, complete a pivot turn all while walking as well as run 10 meters while maintaining proper posture and balance.

Stand Adaptability: From a standing position over a treadmill with overhead body weight support, the research participant will be asked to maintain best posture without use of upper extremity support. The research staff will assist only as needed at body segments not being assessed. Body weight support will then be lowered until the research participant can no longer maintain proper posture without assist. Depending on participant abilities, participant may be asked to resist perturbations at the trunk with body weight support less than 20%, perform squats, and maintain single-limb stance all while maintaining proper posture and balance with body weight support less than 10%.

Step Retraining: From a standing position over a treadmill with overhead body weight support, the research participant will be asked to maintain proper posture and natural arm swing while the research staff assists him/her to walk at speeds of 2.0 mph or greater. The research staff will provide assist to maintain proper stepping kinematics. Body weight support will then be lowered until the research participate is unable to maintain proper posture and/or the research staff are unable to maintain proper stepping kinematics. Depending on participant abilities, participant may be asked to step over objects, adjust to varying random treadmill speeds, and achieve a running pattern all while maintaining proper posture, with support from research staff to maintain stepping kinematics, at various levels of body weight support. We may step continuously for up to 10 minutes.

Step Adaptability: From a standing position over a treadmill with overhead body weight support, the research participant will be asked to maintain proper posture and natural arm swing while walking at speeds of 0.6-1.2 mph. The research staff will provide assist to provide proper

posture and stepping kinematics only as needed. Body weight support will then be lowered until the research participate is unable to maintain proper posture or stepping kinematics. Depending on participant abilities, participant may be asked to step over objects, adjust to varying random treadmill speeds, and achieve a running pattern all while maintaining proper posture and stepping kinematics at various levels of body weight support.
*Analysis**:*** Based on the performance across categories, 4 phase scores can be assigned. Phase 1 represents the greatest impairment relative to normal movement patterns with most people being non-ambulatory. In Phase 2, people begin to stand and weight support independently. Phase 3 denotes walking with varying skill levels. Phase 4 reflects normal locomotor and transfer performance with marked adaptability to varying conditions. EMG data will be full wave rectified and filtered using a 4th order bandpass Butterworth filter (40 Hz - 500 Hz) representing the relative number and frequencies of the motor units recruited per burst. Integrated EMG will assess the total EMG activity generated during specific phases of the motor tasks. Co-activation values of agonists and antagonist muscles and the degree of coordination in the movements will be evaluated through principal component analysis.

MG and Soleus H-reflex (Specific Aim 4): All EMG data will be collected at 2000 Hz with custom-written acquisition software (National Instruments, Austin, TX, USA). We will record bilateral EMG (Motion Lab Systems, Baton Rouge, LA, USA) from same muscles as above. The soleus H-reflex will be evoked by monopolar electrical stimulation of the posterior tibial nerve at the popliteal fossa using a 1-ms pulse, generated by a constant current stimulator (DS7A, Digitimer, UK) and will be recorded by surface monopolar differential electrodes placed over the soleus muscle. A minimum 10 control and conditioned reflexes will be recorded in every trial. The indifferent electrode will be placed above the patella for selective stimulation of the nerve trunk. The EMG signal will be amplified and band-pass filtered (10 Hz-500 Hz) before being sampled at 2 kHz (1401 plus running Spike 2 software). The digitized EMG signals will be rectified and the size of M-wave and H-reflex responses will be measured as the area under the full-rectified waveforms. Soleus H-reflexes will be recorded as designated by each specific supraspinal pathway protocol (described in detail below). For all conditioning experiments, amplitude and latency changes of the soleus H-reflex will be used to quantify the effects of the TMS, galvanic, auditory or ulnar nerve stimulation. Control H-reflexes will be evoked interleaved with those conditioned by the respective stimulation.

Corticospinal pathways (Specific Aim 4): We will administer single pulse transmagnetic stimulation using a Magstim 200 single-pulse stimulator with a double cone coil for activating lower extremity musculature while the research participants are in the supine position. We will position the coil approximately 0-2 cm anterior to the vertex to locate the hotspot left and right tibialis anterior and quadriceps muscles. We will position the coils tangentially to the scalp with intersection of both wings at 45 degrees to midline for optimal motor cortex stimulation. We will use Signal software (Cambridge electronic design, UK) to trigger motor evoked potential (MEP) data acquisition. We will perform MEP data analysis using Signal software (Cambridge electronic design, UK). Mean peak-to-peak MEP amplitudes (average of 8-10 trials) at intensities 10%, 20%, 30%, 40%, 50%, 60% and 70% above rMT will be used to generate stimulus response curves. Using SigmaPlot curve-fitting software, stimulus response curves will be fitted with the Boltzmann function: MEPa= P/1+exp ((I50-I)/k), where P is the Plateau amplitude, I is the intensity, I50 is the amplitude at 50% of plateau and k is slope parameter of the steepest portion of the curve.

Research participants will be in a supine position with a fixed hip, knee and joint angle. For those individuals who can maintain a voluntary contraction, an additional series of tests will be conducted with background EMG activity. For soleus H-reflex modulation single pulses will be used to condition the H-reflex induced by posterior tibial nerve stimulation at interstimulus intervals ranging between 0 and 100 s (see 8,33,61,68). We will measure the MEP's in response to incrementing levels of TMS over the leg area of the primary motor cortex and generate recruitment curves. We will measure changes in threshold, slope and the maximum amplitude of the recruitment curve to determine if severity of injury, time since injury, or locomotor training influences the excitability or functional connectivity of the corticospinal pathways. We will also compare the reproducibility of these parameters in non-disabled research participants to verify these changes are not attributed to inherent variability of the measurements. We will calculate peak-to-peak values for the MEP response and those responses at a given stimulation frequency will be averaged and plotted versus the stimulation intensity. If background EMG is elicited we will average the amplitude from a 25 ms window prior to stimulation.

Vestibulospinal pathways (Specific Aim 4): We will administer galvanic stimulation (rectangular pulses, 300 ms, 2-4.5 mA) with Digitimer DS5 Isolated Bipolar Constant Current Stimulator using 2.5 cm diameter electrodes placed over the mastoid processes for the assessment of the vestibulospinal pathways. The digitimer will be externally triggered by our Labview program and used to condition the soleus H-Reflex. The research participants will be lying with the head of the mat fixed (30 degrees) because posture influences the responses. Control H-reflexes will be evoked interleaved with those conditioned by auditory stimulation with the time randomly between 10 and 20 seconds to allow adequate recovery of the motoneuron pool. A minimum of 5 responses of control and condition will be measured and averaged with conditioned responses expressed as percentage of control values. Peak-to-peak amplitude will be calculated and the mean amplitude and standard deviation for each of the conditioned and control reflexes. The conditioned reflexes will be expressed as a percentage of the control reflexes.

Reticulospinal pathway (Specific Aim 4): The reticulospinal pathway will be evaluated using soleus H-reflex amplitude under conditioning stimulation via auditory stimulus (30 ms tone of 90 dB at 700 Hz) that will be delivered using binaural earphones. EMG will be recorded from the sternocleidomastoid muscle to confirm the startle response (10). The soleus H-reflex will be elicited 50 ms after the sound to peak after 75-125 ms and return to baseline values after 250 ms (24). Amplitude changes of the soleus H-reflex will be used to quantify the effects of the auditory stimulation. Control H-reflexes will be evoked interleaved with those conditioned by auditory stimulation with a time separation of at least 2 minutes. A minimum of 5 responses of control and condition will be measured peak-to-peak and averaged with conditioned responses expressed as percentage of control values.

Long propriospinal pathway (Specific Aim 4): The long propriospinal system will be evaluated using soleus H-reflex amplitude under conditioning stimulation of the ipsilateral ulnaris nerve at the wrist joint via surface electrodes with trains of 3 rectangular pulses (pulse duration: 0.5 ms, pulse interval: 3 ms) (20,47). The soleus H-reflex will be elicited 100 ms after the ulnaris nerve stimulation. The intensities of the stimuli will be expressed as multiples of the threshold for the direct M response of the abductor pollicis brevis muscle. The stimulus will be applied every 3 s in a randomized, interleaved conditioned and unconditioned stimuli sequences.

Somatosensory Evoked Potential:

Procedure: Somatosensory evoked potentials (SEPs) - Somatosensory evoked potentials are recorded through surface electrodes to measure conduction in the peripheral nerves, cervical and lumbosacral spinal cord, deep brain structures, and sensory cortex (Asanuma 1981. Skin is cleaned with alcohol and prepared using mild abrasive conductive paste for the placement of surface electrodes on the scalp over the Fz, CZ, C3 and C4 locations of the International 10-20 System for EEG electrode placement. Electrodes are also placed over the 7th cervical, 12th thoracic, right or left side of iliac crest and on popliteal fossa medially and distally. Stimulation electrodes are placed over the median nerve at the wrist and tibial nerve of the right and left upper and lower limbs. Ground electrodes are placed on the forearms and thighs. Repeated single-pulse electrical stimulation is delivered at intensities of 1 and 1.5 times motor threshold for the recorded muscles for each of the four nerves evaluated. Averaged responses for up to 256 stimuli will be recorded for each of these two intensities for the right and left median and tibial nerves. Simultaneous bilateral stimulation will be applied if no recognizable responses are recorded from the scalp leads. Stimulation delivery rates will not exceed 2 per second.
Analyses: Average latencies will be calculated for N20 & P23 peaks for the Median nerve and N45 & P37 peaks for the Tibial nerve using Natus (Viking).The peak to peak amplitude can also be calculated using Natus (Viking).

Sympathetic Skin Responses (SSR):
Procedure: Sympathetic skin responses (SSR): The SSR will be elicited by stimulation of supra-orbital, median and/or tibial nerves, and recorded bilaterally and simultaneously from both hands and feet to assess the extent of disruption to spinal autonomic pathways. Five to ten electrical stimuli (duration 0.2 ms; intensity 3-60mA) will be applied Supra orbital nerve, the median nerve and posterior tibial nerve, in random order and with variable and long-time delays to minimize habituation.
Analysis: The SSR recordings are among the frequently utilized techniques for the evaluation of integrity and functional capacity of the autonomic circuits after SCI [74-77]. The outcomes will be calculated as a number of recognizable responses out of 10 stimulations, and averaged latency and averaged amplitude [78].

Interventions:

1) ES Cardiovascular Parameters during sitting or lying supine
2) ES Voluntary Parameters during voluntary leg movement training
3) ES Stand Parameters during stand training overground or on BWST

c. **Significance**. The prognosis for those who suffer a devastating spinal cord injury (SCI) that results in complete paralysis, cardiovascular, respiratory, and bladder dysfunction is extremely poor. The combined medical and personal economic impact of these complications of the average spinal cord injured patient over the average life span are devastating, with an estimated 1,275,000 Americans with a SCI (1). The current paradigm is to provide compensatory therapeutic interventions which focuses on improved function above the lesion, with the singular hope that in the future some regenerative approach, perhaps using stem cell technologies, will reach clinical trials; because, it is thought that reestablishing anatomical connectivity of supraspinal input to the spinal cord is essential for recovery of movement and autonomic function. In this proposal, we challenge 1) the concept that movement and autonomic function can only be restored by re-establishing anatomical connections from supraspinal to spinal

neurons; and 2) that those with the diagnosis of clinically motor complete SCI indicates a prognosis of essentially no recovery. Individuals with complete motor paralysis suffer from a myriad of complications that result in mortality, morbidity, hospitalization, high burden of care and health care costs and a drastically lowered quality of life.

d. Human Subjects

Human Subjects Involvement and Characteristics: There are approximately 1,275,000 Americans with a SCI. Fifty-six percent of the injuries occur in people aged 16 to 30, with an average age of 31, and 82% of the total population are male. Minorities make up 38% of SCI cases and while every effort will be made to recruit minorities, based on the incidence rates, their participation may be limited. Every effort will be made to recruit women, though only about 18% of SCI patients are female. Although many people have secondary health issues after SCI, such as spasticity and frequent urinary tract infections, they are otherwise healthy individuals. Standing and activity dependent training is considered beneficial for people with SCI, who are otherwise confined to a wheelchair, as immobilization can contribute to secondary pathologies such as osteoporosis, leg muscle contractures, decreased cardiovascular health, pressure sores and muscle atrophy. Pregnant women with SCI will not be studied because the risks to the fetus are unknown. No other vulnerable subjects will be included.

We will enroll five research participants who have sustained a SCI (AIS A, B or C) to participate in the proposed experiments. Frazier Rehab Institute evaluates approximately 300 chronic SCI outpatients each year. We also have a database of over 200 people with SCI who have expressed interest in participating in our research programs.

All research participants, irrespective of gender, will be selected based on the following

Inclusion criteria.

1) non-progressive SCI with motor paralysis above T1; American Spinal Injury Association Impairment Scale (AIS) A, B or C; 2) 21 – 70 years of age; 3) greater than 2 years post injury; 4) stable medical condition; 5) unable to voluntarily move all single joints of the legs; 6) cardiovascular dysfunction including presence of persistent resting low blood pressures and/or symptoms of autonomic dysreflexia and/or orthostatic hypotension; 7) respiratory dysfunction including at least 15% deficit in predicted pulmonary function outcomes; and 8) presence of functional segmental reflexes.

Exclusion criteria:

1) ventilator dependent; 2) painful musculoskeletal dysfunction, unhealed fracture, severe contracture, or untreated pressure sore/wound that might interfere with training; 3) untreated psychiatric disorders; 4) ongoing drug abuse; 5) cardiovascular, respiratory, bladder, or renal disease unrelated to SCI; 6) severe anemia (Hgb<8 g/dl) or hypovelemia; 7) HIV or AIDS related illness (self-reported or based on chart review); 8) ongoing use of combustible drugs (e.g. Nicotine and illicit: methamphetamine, marijuana, cocaine, etc); and 9) unable to wean off from anti-spasticity medications.

Each research participant will be screened for medical eligibility by a neurosurgeon and physiatrist and for scientific eligibility by the principal investigator. After eligibility is determined and consent procedures are implemented, the individual will undergo clinical and neurophysiological assessments for voluntary movement, cardiovascular and respiratory function. Random drug testing will be conducted throughout the study to verify the participant is abstaining from the use of nicotine and other combustible drug products.

During the experimental stand session, participants will use an overground standing device or will be placed on the treadmill in an upright position and suspended in a harness by an overhead pulley at the maximum load at which knee buckling and trunk collapse can be avoided. A trainer positioned behind the participant will aid in pelvis and trunk stabilization. The trainer will ensure that the trunk and pelvis are not flexed or hyper-extended during standing. Trainer(s) positioned at the lower limb will provide manual facilitation using a customized technique developed by this research team that facilitates knee extension standing. Trainer(s) promote knee extension by applying gentle pressure at the tibial tuberosity and stimulation of the patellar tendon. Manual facilitation at the trunk-pelvis and at the legs will be used only when needed. For those participants trained on the body weight support system, the body weight support (BWS) will be continuously reduced over the course of the training sessions as the subjects increase their ability to bear weight on the lower limbs. Manual facilitation will be reduced with independence of standing. Assessment of standing, EMG activity, kinematics and kinetics during standing, pulmonary and voluntary function will be conducted prior to the onset of training and after approximately 4 months (80 sessions) of each intervention

Experimental testing, electrode implantation and subsequent training interventions will be initiated after the subject has been evaluated and the research team has determined compliance with the selection criteria. Maxwell Boakye, MD, Joseph Neimat, MD or Camilo Castillo, MD will complete a medical history and neurological examination to determine medical eligibility for each SCI subject. Susan Harkema, PhD will determine study eligibility based on the inclusion and exclusion criteria and by the medical recommendation of Dr. Boayke and Dr. Castillo. Experimental testing and training interventions will be initiated after the subject has been evaluated and determined in compliance with the selection criteria. The subjects will not be concurrently enrolled in any other experimental studies unless approved by the investigators. All subjects will sign an informed consent that has been approved by the University Institutional Review Board (IRB) prior to entering the study. Each subject will be assigned a subject identification number to designate all evaluations.

Sources of Materials: Data obtained will be utilized specifically for research and teaching purposes only. Members of the research team including research assistants, post-doctoral students and graduate students will have access to the materials for analyses. Only Dr. Harkema will have access to the coding of the identification number to the research participants.

Potential Risk:

Risk from Surgery and Implantation of Neurostimulator: The surgical procedure performed to implant the 5-6-5 Specify or Specify 5-6-5 SureScan MRI electrode, (MEDTRONIC, Minneapolis, MN, USA) and RestoreAdvanced or Restore Advanced SureScan MRI Pulse generator, (MEDTRONIC, Minneapolis, MN, USA) involves minimal risk. Both devices are FDA approved and meet US safety regulations. The patient will be apprised of the surgical risks by Dr. Boakye or Dr. Neimat. As with any routine surgical intervention there is a risk of bleeding and infection. Other potential risks of this procedure involve: 1) development of scar tissue around the electrode; 2) epidural hemorrhage; 3) seroma; 4) hematoma; 5) CSF leak; and 6) skin erosion. Some complications such as lead migration and breakage of electrode or hardware could require follow up surgery. The subjects will be monitored postoperatively and will stay overnight at University of Louisville Hospital. The patient will be followed during that period by Drs. Boakye, Neimat or Castillo to monitor any complications of surgery.

Risk from Interventions and Experimental Procedures:

Stand and/or Voluntary Interventions: Because subjects must meet the criteria listed above, we expect that all subjects will be in good health. The studies described may involve the following physical risks and/or discomforts: 1) increased respiration or shortness of breath; 2) increased heart rate; 3) muscle and joint soreness; 4) lowering or elevation of blood pressure; 5) dizziness; 6) skin irritation from recording electrodes or hand placements of trainers; 7) skin abrasion from hand placements of trainers; 8) chest pain; 9) muscle strain or joint sprain from weight-bearing due to standing or stepping, or the force exerted by the trainers; and 10) fracture from weight-bearing due to standing or the force exerted by the trainers.

Most subjects will have increased respiration and heart rate due to an increase in activity. However, we do not expect the increase in respiration and heart rate to be greater than what is normally experienced during regular exercise. Many SCI subjects will likely sustain skin irritation from the recording electrodes or hand placements of the trainers. These conditions are considered to be minimal risks and are reversible.

There is some chance that subjects may sustain muscle and joint soreness, lowering or elevation of blood pressure, dizziness or skin abrasion from hand placements of the trainers. If these events occur, stand training and the experiment would cease immediately. Dr. Castillo will be alerted if the condition persists. These conditions are considered to be minimal risks and are reversible.

It is highly unlikely that a subject would feel chest pain or high blood pressure would occur that did not resolve within several minutes. These events have not occurred in our past experience. However, if this did occur the individual would be immediately transported to the respective hospital's Emergency Unit and Dr. Castillo will be notified. It is also highly unlikely that a subject would suffer a muscle strain, joint sprain or fracture from standing. These conditions are considered to be moderate risks but rarely occur. However if these events should occur, the subject would immediately stop stand training and would be evaluated by Dr. Castillo. Standard medical procedures will be provided. The subject's primary physician would be notified as needed. These conditions are considered to be moderate risks and are reversible.

Stimulation parameters used during training will be closely monitored by the research team. Subjects will undergo training about stimulator use at each site. Testing of optimal stimulation parameters and ranges will be performed in the laboratory to make sure stimulation is safe for the subject. Stimulation programs given to each participant will be restricted to those used and tested in the laboratory. Subjects will be instructed to call the therapist, nurse or assigned research team member immediately if complications from stimulation develop during home training programs. Dr. Castillo will be notified as needed.

If serious adverse effects such as autonomic dysreflexia, sustained elevation or reduction in blood pressure, or bradycardia or tachycardia have recurring onset on an individual or become present across the tested sample population the research team will evaluate the stimulation protocol. Stimulation parameters will be assessed initially limiting the voltage and frequency as well as selecting more localized configuration patterns that could reduce such effects. If a serious adverse event occurs as a result of stimulation, the research participant will be required to train in the laboratory for at least a week where they can be monitored by research staff. They will not be allowed to return to a home based stimulation program until they show stable responses to the stimulation and have been cleared by a physician. If stimulation parameters cannot be found to eliminate the onset of adverse effects the study will be stopped.

Orthostatic Stress Test: While undergoing this test, participants may experience dizziness, changes in blood pressure and heart rate or shortness of breath. Each participant will be closely

monitored for any drastic changes in the recordings of their blood pressure, heart rate or breathing or show visible flushing of their cheeks or face.

Blood Catecholamines: Though venipuncture is routinely done and relatively safe, participants may experience a feeling of light-headedness, bruising, Hematoma (blood accumulating under the skin), pain and/or infection. Participants will be monitored for any adverse effects or acute cases of the specified risks.

Pulmonary function tests: During testing, every research participant will be slowly acclimated to the equipment to make him/her feel comfortable. This will help the research participants avoid experiencing a lowered blood pressure or dizziness. Each participant will be closely monitored for blood pressure and heart rate throughout each sEMG recording. Testing will immediately cease if these values become abnormal or if the research participant feels tired, winded or has chest pain. If these conditions persist, Dr. Castillo will be immediately contacted to assess the participant and will notify the primary care provider when necessary. Before and after every sEMG recording, the participant's skin will be examined for irritations and abrasions. If skin irritations or abrasions are caused by the recording electrodes, placement will be modified appropriately.

Transcranial Magnetic Stimulation and Peripheral Nerve Stimulation: There are no known significant risks with this procedure at this time, because the magnetic fields at the strengths used are thought to be without harm. The exception is if you have a cardiac pacemaker, or a certain type of metallic clip in your body (i.e., an aneurysm clip in your brain). There are no known long-term adverse effects reported with the use of this device; however, this test is still under development. There may be unforeseen risks in the long-term that are currently unknown. The TMS device produces a loud clicking sound. Although studies have found no hearing impairments as a result of this sound, some subjects experience a mild temporary effect on their hearing. To minimize this possibility, participants will be given protective earplugs. In patients with epilepsy, activation of the brain could also activate a seizure. Patients with stroke can also develop seizures due to the brain scar. Therefore, magnetic stimulation of the brain could conceivably activate a seizure in a stroke survivor with such a scar. There may be slight discomfort associated with the electrical stimulation. If this becomes uncomfortable, stimulation will be reduced or stopped.

There are no identifiable psychological, sociological, economical or legal risks to the participants.

Adequacy of Protection against Risk:

Recruitment and Informed Consent: The research team will utilize the Human Locomotion Research Center's Potential Volunteer Database (UofL Study #06.0647) to search for potential research volunteers for any of our current research studies based on eligibility criteria (e.g. level of injury, cause of injury, etc.). Participants will be screened through IRB #07.0024 and through the screening consent for this protocol. All eligible subjects will be invited to the recruiting site to discuss the complete protocol, including its risks and benefits with Dr. Harkema (Louisville) and/or designated research staff. Afterwards, all potential subjects will be encouraged to read the informed consent and discuss the study with their physician, family and friends, before signing the IRB approved informed consent. The informed consent will be written in language that an eighth-grade student would be able to understand and will contain information on all studies to be performed as well as contact information if the subject and his/her associates should have any

questions. The original signed informed consent and two copies will be kept in Dr. Harkema's office. If a participant is employed by the University, we will review and discuss a risk management plan. The plan will be signed by the investigator, participant and witnessed by an individual outside of the study and department to ensure there is no coercion. A copy of the plan will be provided to the participant and placed in his employee file.

Protection against Risk: To protect confidentiality, each research participant will be assigned a coded identification number with no association to their identity. This number will distinguish all evaluations and analyses. Data will be stored on computer media and video and will be secured in a locked storage area of the laboratory. Only members of the research team including research assistants, post-doctoral students and graduate students will have access to the data for analyses. Dr. Harkema will have access to the coding of the identification number to the research participants. No individual will be allowed to participate in the study without being examined by Dr. Castillo. All eligible research participants will be encouraged to discuss the study with their primary physician, in order to minimize physical risks. To further minimize risks, the following precautions will be taken:

Stimulation: Every research participant will be slowly acclimated to stimulation. This may help the research participants avoid experiencing significant blood pressure fluctuations or dizziness. However, if these conditions should occur, stimulation will be modified or stopped, depending on the need to regulate the blood pressure. Each research participant will be closely monitored (blood pressure, oxygen saturation, heart rate) throughout stimulation sessions in the lab. Stimulation will immediately cease if these values become abnormal or if the research participant feels tired, winded or has chest pain. If these conditions persist, Dr. Castillo will be contacted immediately to assess the participant and will notify the primary care provider when necessary. Dr. Harkema and the research team will continually assess the appropriate stimulation parameters including configurations, voltage and frequency.

Orthostatic Stress Test, Pulmonary function tests, EMG Experiments: Participants will be continuously monitored for any signs of risks or discomfort. If these occur the recording or training session will be immediately discontinued. If any complications arise, the training will immediately stop and Dr. Castillo will immediately be informed. He or a designated associate will be available on campus during all data collections. In addition, the participant's primary care provider will be notified as necessary. Reports of symptoms associated with infection will be addressed immediately and the participant's primary care provider will be notified as necessary.

Transcranial Magnetic Stimulation and Peripheral Nerve Stimulation: Both during and in the days following the procedure, participants will be monitored for excessive discomfort, headaches and other adverse reactions. Reports of symptoms associated with seizures will be addressed immediately and the participant's primary care provider will be notified as necessary.

Data Safety and Monitoring Plan

An independent Data Safety Monitoring Board (DSMB) has been chartered to monitor the safety of research participants as well as the validity and data integrity of studies conducted at the Kentucky Spinal Cord Injury Research Center, University of Louisville and the Frazier Rehab Institute. Members of the DSMB serve in an individual capacity and will convene yearly to provide their expertise including recommendations regarding the continuation, modification, or termination of any or all phases of a study. The DSMB will review cumulative study data to evaluate safety, study conduct, scientific validity and data integrity. DSMB members may review current versions of the protocol and Informed Consent Form, and any subsequent amendments to ensure an understanding of a study's objectives and design. Day-to-day oversight of the study will be provided by the Principal Investigators. They will review all study data and any adverse events, and report all adverse events to the University of Louisville, IRB, DSMB chairperson and

sponsor as appropriate. Medical events that occur while the research participants are engaged in the research protocols also will be logged by a research nurse, physical therapists, physicians and/or staff. Any adverse events (AE) are collected on an Adverse Event Form and will be reported at the yearly DSMB meeting. An AE report will be generated for each event and will include a description of the event, when and how it was reported, as well as any official chart records or documentation to corroborate the event and a determination of attribution. Any AE that the PI determines to be definitely, probably, or possibly related to the research intervention, or serious in nature, and unexpected will be reported to the IRB, within 5 business days of the PI gaining knowledge of the event. Any unanticipated problems involving risks to research participants or others will include a corrective plan and measures to prevent reoccurrence. Such events will be reported to appropriate regulatory agencies as required within 5 business days of the PI gaining knowledge of the event.

e. Animals - NOT APPLICABLE

f. Biohazardous Materials - NOT APPLICABLE

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STUDY TITLE: RECOVERY OF CARDIOVASCULAR FUNCTION WITH EPIDURAL STIMULATION AFTER HUMAN SPINAL CORD INJURY

INFORMED CONSENT AND RESEARCH AUTHORIZATION for SURGERY AND TRAINING

RECOVERY OF CARDIOVASCULAR FUNCTION WITH EPIDURAL STIMULATION AFTER HUMAN SPINAL CORD INJURY

Industry Contracts number: OICB140483

Sponsor(s) name & address: Craig H. Neilsen Foundation
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Sponsor assigned number: ES2-CHN-2013(SH) (CDRF)

Investigator(s) name, Degree, University Department, & address:

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Frazier Rehab Institute
220 Abraham Flexner Way
Louisville, KY40202

Site(s) where study is to be conducted: University of Louisville Hospital, Louisville, KY;
Neuroscience Collaborative Center, Department of Neurological Surgery, University of Louisville, Louisville, KY

Phone number for subjects to call for questions: (502) 581-8675

Introduction and Background Information

You are invited to participate in a research study because disease or injury to the spinal cord has impaired your ability to walk. This study is being conducted by Susan Harkema, PhD, Maxwell Boakye, MD, Joseph Neimat, MD, Camilo Castillo, MD, Claudia Angeli, PhD, Enrico Rejc, PhD, and Glenn Hirsch, MD. The study is sponsored by the Craig H. Neilsen Foundation and will take place at University of Louisville Hospital and the Neuroscience Collaborative Center, University of Louisville. Approximately 5 individuals with spinal cord injury will be invited to participate in this study. Your participation in this study will last for approximately 20 months. If you are pregnant, anticipate pregnancy or are nursing you are not eligible to participate in this study.

Purpose

The purpose of this study is to evaluate the combined effects of electrical stimulation of the spinal cord and stand training on cardiovascular and respiratory function as well as the ability to voluntarily control leg movements below the injury level in patients with spinal cord injury.

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Procedures

Your participation in this study will last up to 20 months. After 20 months a decision will be made as to whether the stimulator will be left in place or removed. If the stimulator is beneficial, it will be left in place. If ineffective, a decision will be made to keep the stimulator in place or remove it. If you consent to participate, you will have the following procedures while you are in this study.

I. MD Evaluation:

- You will undergo a physical examination at each specified time point by the study doctor or neurosurgeon.
- There will be an examination (ASIA) of the sensation and strength in arms, hands and legs: Sensory and motor examination of upper and lower limbs according to the *International Standards for Neurological Classification of Spinal Cord Injury* are the main instruments used to document changes in neurologic function during recovery post-injury and in clinical trials.

II. Surgery:

- You will undergo spine surgery at University Hospital by Dr. Boakye. You will also be asked to sign an additional surgical consent form required by University Hospital. This requires an operation on your lower back performed under sedation by intravenous medications injected into your body.
- A 4 to 6" cut will be made in your lower back and one or two electrodes (devices to produce electrical stimulation) measuring approximately ½ x 3" will be inserted in your spinal canal.
- During this 4-5 hour operation several electrical stimulations will be given to your spinal cord in order to determine the best place for the sensor.
- One or two 3" x 1/2" battery (generators) will be put under your skin through a cut placed in the lower abdomen (one on each side). The research team will be able to adjust the electrical stimulator by an external device after surgery.
- Approximately half a cup of blood will be used during this operation, either from the surgical cuts or by the anesthesiologists who will draw blood to perform routine laboratory tests.
- The electrode(s) and battery generator(s) system will remain in place, but can be removed at your request.
- You will be monitored following your operation in the recovery room at University Hospital for approximately 4-24 hours and then may be transferred to the Frazier Rehab Institute or your residence.
- A second surgery will only be necessary in the rare case that the electrode wires become disconnected from the stimulator or the need to remove the electrode.

III. Post Surgical Procedures:

After your surgery, you will have a two week rest period. After your rest period, you will go through the experimental measures (as described on pgs 3-6), then Epidural stimulation (ES) to optimize the settings for cardiovascular control, standing and voluntary movement. This is followed by experimental measures (as described on pgs 3-6). The first intervention will be to optimize the ES setting for cardiovascular (CV) control. The second intervention is to optimize the settings for voluntary movement and those for cardiovascular control. Both the ES sessions for CV and voluntary movement will be conducted in the lab or at home (they are described on page 7). The final intervention will be standing with ES, and ES sessions for voluntary movement and for cardiovascular function.

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A. Experimental measures

1. FNPA (Functional NeuroPhysiological Assessment)
   - In this test, you will be lying down and asked to perform several movements.
   - We will paste sensors to your skin to record the electrical activity of your arm and leg muscles. Muscles recorded will be in your arms, trunk, legs, neck or a combination. Lower limb reflexes will also be performed.
   - This test will last approximately 2 hours.
2. Bladder Function Test
   - You will be asked to rest on an exam table on your back.
   - A small tube will be inserted in your bladder. Your bladder will then be filled with water. The doctor or technician will measure the pressure in your bladder.
   - We may measure how your leg muscles respond to your bladder being filled and emptied.
   - We may record continuous blood pressure and heart rate during the test.
   - We may repeat this test with stimulation following your surgery.
   - This test will last approximately 30-45 minutes.
3. Sympathetic Skin Responses (SSR)
   - We will apply a low electrical current over the skin of your hand and feet while you are lying down.
   - Sensors will be pasted to your skin to record the electrical activity of your muscles during the stimulation.
   - Warm towels or a moist heat pack may be applied at the top of the hands and feet if skin temperature is low.
   - This assessment will take approximately ninety (90) minutes to perform.
4. Reflexes (Pathways)
   - A low level of electrical stimulation will be used over the nerves of your neck, arms, legs, and/or back to measure how your leg muscles respond to the stimulation during standing and also during resting conditions.
   - A pad will be pasted over the skin of your neck, arms, legs, and/or back and a small current will pass through it.
   - For some tests, you will hear a loud sound, it might take you by surprise and we will measure how your leg muscles respond to your reaction to the sound.
   - For some tests, we will magnetically stimulate your brain (transcranial magnetic stimulation) and we will measure how your leg muscles respond to the stimulation of your brain.
   - We may use ES in combination with electrical stimulation on your neck, arms and/or legs.
   - We may use stimulation of your brain in combination with electrical stimulation on your legs.
   - Each test will take approximately 2-3 hours to perform.
5. Cardiovascular Function
   - We will measure your blood pressure, heart rate and breathing rate while you are lying down and also when you are moved to a seated position (tilt chair) or upright position (tilt table).
   - You will be asked to follow a diet excluding caffeine, alcohol, smoking, and foods that are high in fat on the evening prior to and the morning of study.
   - Devices will be pasted to your skin to record heart rate activity.
   - We may take a small blood sample (approximately 4 tablespoons) from your arm
   - We may repeat this test with stimulation following your surgery and measure how your muscles respond to stimulation during this test.

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- This test may be performed at the same time as the SSR and Ultrasound.
- This test may be performed with and without epidural stimulation.
- This test will take approximately 2 hours to perform.

6. Ultrasound

- We will be measuring your heart and your blood vessel structure, and muscle properties using ultrasound and light technology. Continuous heart rate and blood pressure may be monitored during the test.
- The ultrasound will be performed while you are lying down on your side, and again while you are in a seated position.
- We may repeat this test with stimulation following your surgery.
- This test will last about 1-2 hours.

7. Respiratory Motor Control Assessment (RMCA)

- We will record your lung volume, airflow, and airway pressure by using a mouth piece while you are sitting and/or lying down.
- You will be asked to inhale and exhale into the mouth piece when your nose is closed with a plastic clip.
- We will also record the muscle activity from your neck, chest, arms, legs, abdomen and back by placing adhesive sensors over your muscles and heart.
- We may ask your permission to shave skin at the sites of sensor placement.
- We will record how you are breathing by placing elastic belts around your chest and abdomen.
- We will record your blood pressure and heart rate using a finger cuff.
- This assessment may be performed with and without epidural stimulation.
- This assessment will last about three hours.

8. Body composition measurement

Skinfolds:

- We will measure how thick your skin is while you are lying on your back, sitting in a mat with back support, or standing (only if you able to stand independently). The measurements (distance around) your arms, legs, and trunk, and your body mass index (BMI) may also be measured and calculated.
- This test will take approximately 15 minutes to perform.

DXA:

- You will be asked to have dual energy x-ray absorptiometry (DXA) scan that measures body composition (fat, bone and lean tissue).
- This procedure usually takes an hour and is performed when your entire body is being scanned in a large machine while you are lying still on your back.

BOD POD Body Composition:

- If you are a man, you will be asked to wear a form-fitting Lycra/spandex-type swim suit or single-layer compression shorts with no padding. If you are a woman, you will be asked to wear a form-fitting Lycra/spandex-type swim suit or single-layer compression shorts without padding and a single layer (not padded) sports bra.
- You will be asked to remove all jewelry and to wear a swim cap to compress the hair on your head.
- You will be weighed on a scale.
- You will be asked to sit quietly for approximately 5 minutes in the BOD POD chamber.
- You may be asked to breathe through a tube at a set rate displayed on a computer monitor while in the BOD POD chamber.

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Body fat percentage:

- You will also have your body fat measured with a non-invasive technique called bioelectrical impedance spectroscopy (BIA).
- You will lie down for 5 minutes while 2 electrodes will be placed on your hands and feet.

9. Peak Oxygen Consumption Evaluation (VO2 max)

- This test is performed to determine the amount of oxygen your body uses during exercise.
- You will not be allowed to perform any moderate or heavy exercise for 12 hours before you have the test.
- We will ask you to breathe through a face mask that covers your nose and mouth when performing aerobic exercises on special equipment adapted for people with spinal cord injury.
    - Depending on your spinal cord injury, you will be asked to perform on either:
        - Arm crank ergometer (while remaining in your wheelchair)
        - Treadmill with body weight support for standing/stepping.
        - Seated stepper with push-pull arm movements (you will be transferred to the equipment).
        - Bench step (for SCI participants that can move their legs independently).
- We will monitor your heart and blood pressure before, during and after exercise.
- This test will last approximately one hour.

10. Basal Metabolic Rate (BMR)

- We will assess the amount of energy your body uses at rest.
- In preparation for the study, you will be asked to keep a log of the food you eat for a 3-day period and you will need to fast from food, alcohol and caffeine (you may only drink water) for 10-12 hours the night before the test.
- During the test, you will be asked to lie very still on your back without moving or talking.
- We will cover your head with a canopy hood and we will measure the gases in the air you exhale.
- This test will take approximately 45 min.

11. Motor activity (EMG):

- We will test the activity of your muscles while you are stepping; standing; supine or sitting.
- We may ask you to stand and/or step on a treadmill, while wearing a harness that is attached to an overhead suspension device that will support the weight of your body, or overground while placed in a standing frame or with an assistive walking device. Trainers will provide assistance as needed.
- Devices pasted to your skin will record the electrical activity of your muscles and the position of your limbs. We will have to shave to remove hair in the spots we place the electrodes.
- Devices pasted to your skin and surrounding your fingers and chest might be used to record temperature, blood pressure and respiration.
- We may repeat this test with stimulation following your surgery.
- These experiments will last from 2-3 hours. You will have the opportunity to rest at any time during the experiment.

12. Neuromuscular Recovery Scale

- We will ask you to complete the Neuromuscular Recovery Scale (NRS) assessment. In some cases we will also record your muscle activity.
- This assessment consists of 11 items performed in sitting and standing and 3 items performed while standing and stepping over a treadmill equipped with a harness and an overhead lift system that supports some of your body weight.

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- When we use EMG, sensors pasted to your skin will record the electrical activity of your muscles and the position of your limbs. We may have to shave areas of your skin in the spots where we place the sensors.
- We may use a needle to insert a fine wire into some muscles to record the electrical activity.
- We may measure continuous blood pressure, breathing rate, and temperature with different sensors.
- Research staff will provide assistance as needed throughout the assessment for safety.
- This assessment will take approximately 2 hours to perform. You will have the opportunity to rest at any time during the experiment.

13. Somatosensory evoked potentials test (SEP)

- We will apply an electrical current over the skin around your ankles and wrist.
- We will place sensors on your scalp, upper and lower back, and behind your knee that record the electrical activity of your brain during the stimulation.
- This test will take approximately ninety (90) minutes to perform.

14. Automatic blood pressure monitoring (ABPM)

- Blood pressure will be recorded over a period of 24 hours outside of the lab using a small device.
- You will be asked to wear this device on your arm throughout the day and while performing your normal daily activities.
- A questionnaire will be completed to validate the severity of autonomic dysreflexia.
- This test is 24 hours.

15. Blood tests:

- You will be asked to have a 12-hour fast the night before, including no food or drink (no alcohol or caffeine; water is allowed).
- A blood sample will be drawn. Approximately 2 tablespoons of blood is needed.
- This testing will take approximately 10 minutes.

16. Heart monitor (Holter monitor)

- Your heart rate and the electrical activity of your heart (electrocardiogram, ECG) will be recorded outside of the lab using a small device called a Holter monitor.
- Small pads will be pasted to your chest and abdomen area. Wires will be connected to these pads and plugged into the Holter monitor. You will be asked to wear this device throughout the day and while performing normal daily activities.
- This test lasts at least 1 day and up to 1 week.

17. Random Comprehensive Drug Screen

- You will be asked to provide a urine sample for drug screening at random times during the study.
- You will be asked to participate in this random drug screening throughout your study participation.
- Your results may cause you to be withdrawn from this study by the investigator.

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B. Intervention 1: Electrical Stimulation (ES) for Cardiovascular (CV) function:

- The electrical stimulation of the implanted device is activated by a remote wireless device.
- After your device is implanted, you will have ES conducted in the lab to adjust the settings necessary to affect cardiovascular control.
- You will be asked to take a "Patient Programmer" remote device home to turn the stimulation on and off and up and down within limits decided by the research team.
- You will have 80 consecutive days of stimulation to perform at home or in the lab for 2-6 hours per day. For monitoring purposes you may be requested to perform all sessions in the lab.
- The remote device records the minutes of stimulation and parameters used so these will be collected on those days you are not in the laboratory. The first five sessions of each set of ES will be performed in the lab under supervision of the research team. The setting will be adjusted by the research team as needed.
- For the remaining sessions, you will come to the lab once per week to have CV settings collected. Other CV and EMG parameters may be obtained at this time.
- The final series of sessions will be conducted in the lab.
- You will be asked to repeat the experiments listed above, prior to continuing on to the next intervention.

C. Intervention 2: Electrical Stimulation (ES) for Voluntary movement and CV function:

- You will have ES conducted in the lab to adjust the settings necessary to affect voluntary movement.
- You will be asked to take a "Patient Programmer" remote device home to turn the stimulation on and off and up and down within limits decided by the research team.
- You will have 80 consecutive days of stimulation to perform at home for 4-10 hours per day.
- Stimulation settings will be for 2-6 hours of CV function and 2-4 hours of voluntary movement. For monitoring purposes you may be requested to perform all sessions in the lab.
- The remote device records the minutes of stimulation and parameters used so these will be collected on those days you are not in the laboratory. The first five sessions of each set of ES will be performed in the lab under supervision of the research team. The setting will be adjusted by the research team.
- For the remaining, you will come to the lab once per week to have CV settings, voluntary movement settings collected and voluntary movement observed. Other CV and EMG parameters may be obtained at this time.
- The final five sessions will be conducted in the lab.
- You will be asked to repeat the experiments listed above, prior to continuing on to the next intervention.

D. Intervention 3: Electrical Stimulation (ES) for Standing, voluntary movement and CV function:

- The final ES will be conducted in the lab and will include daily stand training sessions with parameters for standing 1-2 sessions for 1 hour, and 2-6 hours of CV (Intervention 1), and 2-4 hours of voluntary movement (Intervention 2) settings. Total daily stimulation time will be approximately 6-10 hours.
- You will be helped to stand on a treadmill while wearing a harness that is attached to an overhead suspension device which will support the weight of your body, or stand overground in a standing device. Trainers will assist you as needed.
- You will have 80 sessions of ES and stand training.
- The standing testing will last from 2 to 4 hours.
- You will be asked to repeat the experiments listed above.

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Potential Risks

This study may involve risks that are currently unforeseeable. The study may involve the following physical risks and/or discomforts:

Surgical Risks

- Mild Discomfort (likely)
- Bruising (likely)
- Development of scar tissue around the electrode (likely)
- Pneumonia (rare)
- Excessive blood loss (rare)
- Complications from anesthesia (rare)
- epidural hemorrhage (rare)
- Bleeding (likely)
- Infection at the incision site (less likely)
- Heart attack (rare)
- Blindness (rare)
- Death (rare)
- skin erosion (rare)
- hematoma (swelling of clotted blood) (rare)
- seroma (pocket of clear fluid that can develop in body after surgery) (rare)
- CSF leak (cerebrospinal fluid leak results from a hole or tear in the dura—outermost tissue that encloses the spinal cord and brain) (rare)

Electrode/Device Risks

- Hardware malfunctions (less likely)
- Undesirable change in stimulation (less likely)
- Jolting or Shocking (rare)
- Allergic response (rare)
- Migration (rare)
- Erosion (wearing down of the device, wires and/or electrodes) (rare)
- Breakage or failure resulting in further injury to the spinal cord (rare)

Assessment Risks

- Skin irritation from hand placements of trainers (likely)
- Skin abrasion from hand placements of trainers (less likely)
- Tingling feeling from the stimulation (likely)
- Dizziness during sitting, standing or stepping (likely)
- Shortness of breath (less likely)
- Significant changes in heart rate and/or blood pressure (less likely)
- Chest pain (rare)
- Muscle and joint soreness (rare)
- Joint sprain or muscle strain (rare)
- Fall (rare)
- Broken bone (rare)
- Skin irritation from vein needle and/or fine wire insertion (likely)
- Bleeding and/or bruising from fine wire insertion and/or blood draw (less likely)
- Pain and/or infection from blood draw (rare)
- Feelings of claustrophobia (less likely)
- Dizziness by breathing in and out hard (likely)

Intervention Risks

- Skin irritation from hand placements of trainers (likely)
- Skin abrasion from hand placements of trainers (less likely)
- Tingling feeling from the stimulation (likely)
- Dizziness during sitting, standing or stepping (likely)
- Shortness of breath (less likely)
- Significant changes in heart rate and/or blood pressure (less likely)
- Chest pain (rare)
- Muscle and joint soreness (rare)
- Joint sprain or muscle strain (rare)
- Fall (rare)
- Broken bone (rare)

Bladder Assessments

- Feelings of shyness (likely)
- Blood pressure changes (less likely)
- Mild discomfort, especially during urination after Urodynamics (less likely)
- Urinary tract infection (less likely)
- Discomfort from lying still for ultrasound (less likely)
- Excessive pain, fever, chills (are)

Transcranial Magnetic Stimulation:

You will be excluded from any testing using magnetic brain stimulation if you have any of the following:

- metal in head, except mouth (i.e. cochlear implant, implanted brain stimulators, aneurysm clips)
- increased intracranial pressure
- cardiac pacemakers
- implanted medication pump
- intracardiac lines
- significant heart disease
- history of stroke or other brain lesions
- personal or family history of epilepsy
- you are receiving tricyclic antidepressants or neuroleptics
- have had heavy alcohol consumption less than 48 hours prior to the test.

TMS Risks:

- Seizures from the magnetic brain stimulation (rare)
- Temporary or permanent hearing loss from magnetic brain stimulation (rare)

DXA Risks: You will be exposed to minimal amounts of radiation during the DXA scan. We are exposed to radiation on a daily basis both from natural (sun, earth, etc.) and man-made sources. The average radiation dose from these sources for those living in the United States is about 300 millirem per year. By comparison, your dose will be less than 200 millirem from the DXA scan. The radiation dose that you will receive from this study is well below the levels that are thought to result in a significant risk of harmful effect.

In addition, you may suffer harms that we have not seen before. If you should have any of these difficulties during assessments, we will stop. There are no reasonably foreseeable psychological risks, social risks, and/or legal risks. This study may involve risks that are currently unforeseeable.

Possible Pregnancy Risks

Pregnant women are excluded from this study, as the risk to the fetus is unknown. Women of child bearing age will be given a pregnancy test. You should discuss these risks with your doctor before signing this consent form. Talk to your doctor about the best method of birth control to use while you are in this study. If you are pregnant or become pregnant, your unborn child may suffer harms that we have not seen before. It is important that you tell someone on the research team at 502-581-8675 right away if you become pregnant during the course of this study. If you become pregnant, you will be terminated from the study by the study doctor.

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Benefits

We do not know the benefits of this study. Although there are no guarantees of benefits occurring during this experimental study, the information obtained from your participation in this study may help you and/or other patients who have/or will sustain spinal cord injuries in the future.

Alternatives

Instead of taking part in this study, you could choose to not participate. There are no alternatives to this study.

Research Related Injury

If you are injured by being in this research study, the study doctor will arrange for you to get medical treatment. Your study doctor has not set aside money to pay for treatment of any injury. You and your insurance will be billed for the treatment of these injuries. Before you agree to take part in this research study you should find out whether your insurance will cover an injury in this kind of research. You should talk to the study doctor or staff about this. If you are injured, there is no money set aside for lost wages, discomfort, disability, etc. You do not give up your legal rights by signing this form. If you think you have a research related injury, please call your study doctor, Camilo Castillo, MD at (502) 899-3623 (24 hour number).

Compensation

You will not be paid for your time or inconvenience while you are in this study.
You will be paid by prepaid card for travel based on the federal mileage rate and parking fees up to $75 dollars per day while you are in the study. Because you will be paid to be in this study the University of Louisville may collect your name, address, social security number, and keep records of how much you are paid. You may or may not be sent a Form 1099 by the University. This will only happen if you are paid $600 or more in one year by the University. This will not include payments you may receive as reimbursement for actual expenses based on receipts or actual miles traveled. We are required by the Internal Revenue Service to collect this information and you may need to report the payment as income on your taxes.

You can still be in the study even if you don't want to be paid.

Costs

There will be no additional costs to you for participating. However, you or your insurance company will be billed for all office visits and procedures that are part of routine medical care. It is your responsibility to find out what costs, if any, your insurance company will cover before taking part in the study. If you are injured by the research, there may be additional cost to you for participating. Otherwise there will be no additional cost to you.

HIPAA Research Authorization

The Health Insurance Portability and Accountability Act of 1996 (HIPAA) provides federal safeguards for your protected health information (PHI). Examples of PHI are your name, address, and birth date together with your health information. PHI may also include your medical history, results of health exams and lab tests, drugs taken and results of this research study. Your PHI may not be used or shared without your agreement, unless it meets one of the HIPAA exceptions.

State and federal privacy laws protect your health information. In most cases, health information that identifies you can be used or shared by the research team only if you give your permission by signing this form.

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If you sign this form your health information will be used and shared to answer the research questions described above and to make sure that the research was done correctly. The time period when information can be used or shared ends when all activities related to this study are completed.

Your access to your health information will not be limited during this study.

You do not have to sign this form. If you do not sign this form you may not participate in the study and health information that identifies you will not be shared with the research team.

Site(s) where health information about you will be used or shared for this research:

In our research, the research team will look at and may share information about you and your health. Federal law requires that health care providers and researchers protect the privacy and security of health information that identifies you. We may ask for your health information from the following:

Affiliated Sites:

University of Louisville
University of Louisville Hospital
Frazier Rehab Institute

Unaffiliated Sites: Any physician offices, clinics or medical facilities where you may seek treatment during this study.

Protected health information (PHI) that will be used or shared for research

Diaries and questionnaires
Discharge summaries
Healthcare provider orders
History and physical exams
Laboratory, x-ray and other tests
Records of your operation(s)
Medical Progress notes
Photos, videotapes or digital or other images
Records about the study device

Revocation of Research Authorization

You may cancel the permission you have given to use and share your protected health information at any time. This means you can tell us to stop using and sharing your protected health information. If you cancel your permission:

- We will stop collecting information about you.
- You may not withdraw information that we had before you told us to stop.
  - We may already have used it or shared it.
  - We may need it to complete the research.
- Staff may ask your permission to follow-up with you if there is a medical reason to do so.

To cancel your permission, you must complete a written "Revocation of Research Authorization" form located at the end of this document. You may also obtain a copy from your study doctor, designated personnel or from the Human Subjects Protections Program Office website (http://louisville.edu/research/humansubjects/subject-information).

Information Available on ClinicalTrials.gov

A description of this clinical trial will be available on http://www.ClinicalTrials.gov, as required by U.S. Law. This website will not include information that can identify you. At most, the website will include a summary

of the results. You can search this website at any time.

Confidentiality

Total privacy cannot be guaranteed. We will protect your privacy to the extent permitted by law. If the results from this study are published, your name will not be made public. Once your information leaves our institution, we cannot promise that others will keep it private.

Your information may be shared with the following:

- The sponsors, Craig H. Neilsen Foundation along with Christopher and Dana Reeve Foundation, and others hired by the sponsor to oversee the research
- The University of Louisville Institutional Review Board, Human Subjects Protection Program Office, Privacy Office and others involved in research administration at the University, and others contracted by the University for ensuring human subjects safety or research compliance
- The local research team
- People who are responsible for research and HIPAA oversight at the institutions where the research is conducted
- People responsible for billing, sending and receiving payments related to your participation in the study
- Government agencies, such as:
    - Office for Human Research Protections
    - Office of Civil Rights
    - Food and Drug Administration
- Data Safety Monitoring Board related to the study
- Others: Medtronic, maker of the device used in this Study.

Medtronic will keep your health information confidential in accordance with all applicable laws and regulations. Medtronic may use your health information for its business purposes, such as overseeing and improving the performance of its device, new medical research and proposals for developing new medical products or procedures, and another business purposes. Any reports or publications about the study or any other research will not include your name or a description of you. Information received during the study will not be used to market to you; your name will not be placed on any mailing lists or sold to anyone for marketing purposes.

Security

Your data will be kept private by being stored in a locked cabinet behind closed door with limited access. Electronic data is stored on a password protected computer with limited access in a locked area.

Conflict of Interest

This study does not involve a conflict of interest because the institution and investigators will not be compensated for your participation in it.

Voluntary Participation

Taking part in this study is completely voluntary. You may choose not to take part at all. If you decide not to be in this study, you won't be penalized or lose any benefits for which you qualify. If you decide to be in this study, you may change your mind and stop taking part at any time. If you decide to stop taking part, you won't be

penalized or lose any benefits for which you qualify. You will be told about any new information learned during the study that could affect your decision to continue in the study.

Termination

Your study doctor has the right to stop this study at any point. Your study doctor may take you out of this study with or without your okay. Reasons why this may occur include circumstances that arise which warrant doing so. The decision may be made either to protect your health and safety, or because it is part of the research plan that people who develop certain conditions may not continue to participate. If the study doctor believes that the pain or discomfort might pose a risk to you, you will be terminated from the study. If you become pregnant you will be terminated from this study.

Participation in Other Research Studies

You may not take part in this study if you are currently in another research study. It is important to let your doctor know if you are in another research study.

Contact Persons

If you have any questions, concerns, or complaints about the research study, please contact Susan Harkema, Ph.D. at (502) 581-8675. Once you are enrolled in this study, you will be given additional contact numbers that are answered 24/7 to reach a designated research staff member if you need immediate assistance.

Research Subject's Rights

If you have any questions about your rights as a research subject, you may call the Human Subjects Protection Program Office at (502) 852-5188. You may discuss any questions about your rights as a research subject, in private, with a member of the Institutional Review Board (IRB). You may also call this number if you have other questions about the research, and you cannot reach the study doctor, or want to talk to someone else. The IRB is an independent committee made up of people from the University community, staff of the institutions, as well as people from the community not connected with these institutions. The IRB has approved the participation of human subjects in this research study.

Concerns and Complaints

If you have concerns or complaints about the research or research staff and you do not wish to give your name, you may call the toll free number 1-877-852-1167. This is a 24 hour hot line answered by people who do not work at the University of Louisville.

STUDY TITLE: RECOVERY OF CARDIOVASCULAR FUNCTION WITH EPIDURAL STIMULATION AFTER HUMAN SPINAL CORD INJURY

Acknowledgment and Signatures

This informed consent document is not a contract. This document tells you what will happen during the study if you choose to take part. Your signature indicates that this study has been explained to you, that your questions have been answered, and that you agree to take part in the study. You are not giving up any legal rights to which you are entitled by signing this informed consent document. You will be given a copy of this consent form to keep for your records.

Subject Name (Please Print)	Signature of Subject	Date Signed
Printed Name of Legally Authorized Representative (if applicable)	Signature of Legally Authorized Representative	Date Signed

Authority of Legally Authorized Representative to act on behalf of Subject

*Authority to act on behalf of another includes, but is not limited to parent, guardian, or durable power of attorney for health care.

Printed Name of Person Explaining Consent Form	Signature of Person Explaining Consent Form (if other than the Investigator)	Date Signed
Printed Name of Investigator	Signature of Investigator	Date Signed

List of Investigators:	Phone Numbers:
Susan Harkema, PhD	(502) 581-8675
Maxwell Boakye, MD	(502) 540-3694
Joseph Neimat, MD	(502) 540-1430
Camilo Castillo, MD	(502) 333-8155 (office)
Glenn Hirsch, MD	(502) 852-7959
Claudia Angeli, PhD	(502) 581-8675
Enrico Rejc, PhD	(502) 581-8675

STUDY TITLE: RECOVERY OF CARDIOVASCULAR FUNCTION WITH EPIDURAL STIMULATION AFTER HUMAN SPINAL CORD INJURY

REVOCATION OF AUTHORIZATION FOR USE AND DISCLOSURE OF YOUR HEALTH INFORMATION FOR RESEARCH

PI Address: 220 Abraham Flexner Way
Louisville, KY 40202
PI Phone: 502-581-8675

Return To:

OR

Institutional Review Board
MedCenter One, Suite 200
501 E. Broadway
Louisville, KY 40202

Do not sign this letter unless you are withdrawing from this research. You will be sent confirmation that this notice was received.

To Whom It May Concern:

I would like to discontinue my participation in the research study noted above. I understand that health information already collected will continue to be used as discussed in the Authorization I signed when joining the study.

Your options are (***choose one***):

☐ **Withdraw from Study & Discontinue Authorization:**
Discontinue my authorization for the future use and disclosure of protected health information. In some instances, the research team may need to use your information even after you discontinue your authorization, for example, to notify you or government agencies of any health or safety concerns that were identified as part of your study participation.

☐ **Withdraw from Study, but Continue Authorization:**
Allow the research team to continue collecting information from my personal health information. This would be done only as needed to support the goals of the study and would not be used for purposes other than those already described in the research authorization.

_____ _____
Printed Name and Signature of Subject Date Signed

_____ _____
Signature of Subject's Legal Representative (if subject is unable to sign) Date Signed

_____ _____
Printed Name of Subject's Legal Representative Birthdate of Subject

_____
Relationship of Legal Representative to Subject

_____ _____
Subject's Address Subject's Phone Number

Optional: I am ending my participation in this study because:

_____