

Task and physiological specific stimulation for recovery of autonomic function, voluntary movement and standing using epidural stimulation and training after severe spinal cord injury

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Task and physiological specific stimulation for recovery of autonomic function, voluntary movement and standing using epidural stimulation and training after severe spinal cord injury.

I. Overall and Specific Aims

We propose to understand the role of lumbosacral spinal cord epidural stimulation (scES) in recovery of autonomic nervous system function, voluntary movement, and standing in individuals with severe spinal cord injury (SCI). Thirty-six individuals with severe SCI who have cardiovascular and respiratory dysfunction and who are unable to voluntarily move their legs or stand will receive scES for cardiovascular function, voluntary movement, or standing with and/or without weight-bearing stand. Training will consist of practicing voluntary movements or standing in the presence of specific scES configurations designed for the voluntary movements of the legs and trunk (Vol-scES), or epidural stimulation configurations specific for standing (Stand-scES). Specific configurations epidural stimulation for cardiovascular function (CV-scES) will be provided during sitting and supine and during maneuvers of orthostatic or cardiovascular stress. Ability to move voluntarily, stand, as well as cardiovascular, respiratory, bladder, bowel and sexual function will be assessed in these individuals with chronic severe spinal cord injury. Quality of life and costs of health care also will be assessed.

An estimated 270,000 newly injured Americans are living with spinal cord injury (SCI) [1], and the prognosis for those with complete motor paralysis is particularly poor. Such injuries are often accompanied by cardiovascular, respiratory, bladder, bowel, and sexual dysfunction, and attendant medical, personal, and economic impacts can be devastating. We will test hypotheses related to neural control of human movement and cardiovascular function after human SCI, while also obtaining knowledge for optimizing spinal cord epidural stimulation as a therapeutic intervention. We will work to quickly translate scES to larger numbers of patients with SCI, many of whom currently lack treatment options for the secondary consequences of their injuries. We will also assess the effect of scES on quality of life and health care costs.

Specific Aim 1: Understand the effect of epidural stimulation alone using specific configurations on outcomes for voluntary movement, cardiovascular function, and independent standing.

Specific Aim 2: Understand the effect of epidural stimulation in combination with stand training on outcomes for voluntary movement, cardiovascular function, and independent standing.

Specific Aim 3: Understand the effects of scES alone and scES with stand training on respiratory, bladder, bowel, and sexual function.

Specific Aim 4: Assess the impact of scES and training on quality of life.

Specific Aim 5: Measure impact of scES and training on healthcare resource utilization.

We will recruit and enroll 36 participants who have non-progressive SCI, who are 18 years of age or older, and who have been injured for at least 2 years. They will not be able to voluntarily move all single joints of the legs, and will have cardiovascular, respiratory, bladder, bowel and sexual dysfunction. They will be randomized into one of four different treatment interventions among two groups.

Group A: Receive one of the following interventions:

- 1) Specific scES parameters optimized for successful voluntary movement (Vol-scES) during voluntary leg movement and trunk mobility training while sitting or lying supine.
- 2) Specific scES parameters optimized for maintaining normal systolic blood pressure during scES (CV-scES) during sitting or lying supine.

Group B: Receive the same scES as the three interventions as Group A with the addition of Stand-scES during weight-bearing stand training.

After at least 80 sessions of the interventions, Group A will then receive at least 80 sessions of the same scES as in the first 80 sessions with the addition of Stand-scES during stand training. Group B will repeat the same interventions that were done in the first 80 sessions. This trial will allow us to determine the degree of functional gain, below injury voluntary control, and recovery of autonomic function that can be gained as the result of activation of spinal circuits with scES with or without weight bearing stand training with Stand-scES in humans with severe paralysis. We will compare scES alone to scES with weight bearing stand training to both identify optimal parameters and to determine whether stand training is needed to achieve improvement in cardiovascular, respiratory, bladder, bowel, and sexual function as well as the ability to move the legs voluntarily and the ability to stand. In addition to securing scientific advances, our proposed experiments are critical to translating this novel therapeutic approach to a larger scale, and to expand its clinical impact.

II. Research Strategy

A. Challenge, Innovation and Impact Statement: An estimated 1,275,000 Americans are living with spinal cord injury (SCI) [1], and the prognosis for those with complete motor paralysis is particularly poor. Such injuries are often accompanied by cardiovascular, respiratory, bladder, bowel and sexual dysfunction, and attendant medical, personal, and economic impacts can be devastating. The current treatment paradigm is to provide compensatory therapeutic interventions which focus on improved function above the spinal cord lesion, with the singular hope that in the future some regenerative approach – perhaps using stem cell technologies – will reach clinical trials. The hope of stem cell-based interventions is a lingering one, precisely because it is thought that re-establishing 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 clinically motor complete SCI have essentially no hope of neurologic recovery, even with therapeutic intervention.

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. We propose to demonstrate that spinal cord epidural stimulation (scES) can be used to recover significant levels of autonomic control of cardiovascular, respiratory, bladder, bowel and sexual function, as well as the ability to stand and 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, and the expected reduction in SCI-related healthcare and caregiver costs would be dramatic. The U.S. could save an estimated \$400 billion on direct and indirect lifetime costs if we can develop therapies to treat SCI. Also, the cost in emotional stress and well-being to the individual and family is demanding.

From a scientific perspective, this novel intervention challenges the long-held belief that with the development of the primate's cortex, the spinal cord became solely a conduit to carry signals from the brain to execute movement.

B. Rationale: In the context of studies of human locomotion, we made the astonishing observation that four 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 scES of the lumbosacral spinal cord when also receiving intense locomotor training [2]. Even more surprising, over a period of months, they reported improvements in temperature regulation and bladder and bowel function and normalization of sexual function. We also measured significant improvements in cardiovascular and respiratory function that persisted throughout the day even without stimulation. These observations have led us to three assumptions. First, the observation that precise coordinated voluntary movement after complete paralysis can be executed only in the presence of epidural stimulation demonstrates a key role for the spinal circuitry. Second, the function of residual anatomical connections that are clinically undetectable can reach neural activation and functional significance via activity-dependent plasticity via scES and task specific training. Third, secondary complications of injury can be attributed not only to the direct loss of supraspinal input, but also to the lack of weight-bearing neuromuscular activity generated by proprioception. These novel hypotheses challenge current theoretical paradigms for the control of movement in humans and indicate possible therapeutic treatments that have not been considered previously for those with severe paralysis. A collaborative team of scientists and clinicians will comprehensively and systematically study a cohort of individuals who suffer chronically from severe SCI with paralysis and associated cardiovascular, respiratory, bladder, bowel and sexual dysfunction. We will test hypotheses of the neural control of human movement and autonomic function while also obtaining knowledge for optimizing therapeutic strategies that can be immediately translated to larger numbers of patients who now have no treatment options. We will obtain comprehensive, quantitative and sensitive neurophysiological outcomes that will improve our understanding of the physiology of SCI and recovery, and will help us to design therapeutic interventions that can treat those with paralysis and autonomic dysfunction, regardless of the cause.

1. Voluntary Leg and Trunk 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. If this premise is correct, options for recovery from paralysis would be limited to repair or regeneration of these pathways. Our recent observations of four individuals with motor complete injury regaining the ability to voluntarily move their hips, knees and ankles upon command only in the presence of scES (Figure 1) challenges this theory and provides a novel treatment strategy for paralysis.

2. Cardiovascular Function. Cardiovascular diseases are the leading cause of morbidity for individuals with SCI [3]. Individuals with high-lesion SCI often present with cardiac, vascular, and cognitive dysfunction and frequently exhibit orthostatic hypotension and autonomic dysreflexia, which contribute to the reduced quality of life often reported by these patients [4-6]. With time post injury, episodes of orthostatic hypotension and autonomic dysreflexia become more prominent in many individuals with cervical and high thoracic injuries. The causes of cardiovascular dysfunction in individuals with SCI are multifactorial, and can be classified into either neurogenic (related to autonomic nervous system injury which controls cardiovascular function and has tracts through the spinal cord) or other physiologic consequences of SCI [3-10]. A combination of sympathetic agonists and venous compression devices are the only measures currently available for treatment of these symptoms.

We have observed in preliminary studies that scES can increase blood pressure during hypotension (Figure 2). We propose that cardiovascular control is facilitated by the spinal cord as a key integrator of complex

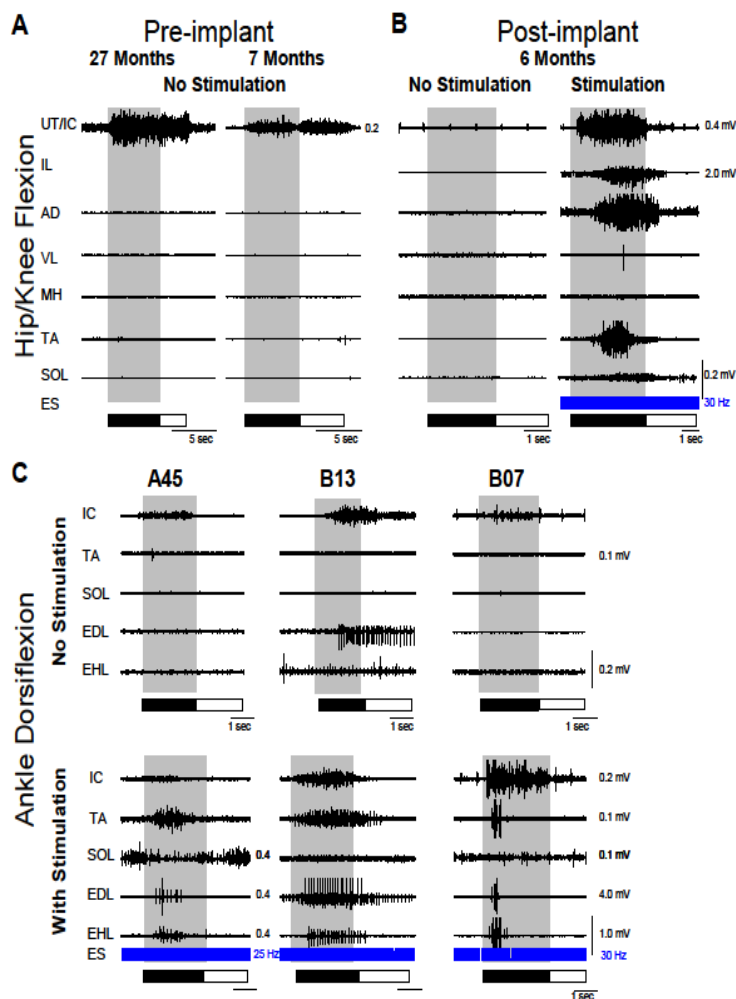


Figure 1. Lower extremity EMG activity during voluntary movement occurred only with epidural stimulation in three individuals with motor complete spinal cord injury. EMG activity from a subject B07 during voluntary attempts of left hip and knee flexion (**A**) without stimulation at 27 and 7 months prior to implantation; (**B**) without out stimulation (left panel) and with stimulation (right panel; 4V, 30 Hz, 210 μ s, 2-13-/7+). 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. The stimulation parameters needed were different for the successful execution of the left and right legs of an individual and also varied among the three subjects. 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 hallucis 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.

FIGURE 1

signals from the periphery and from supraspinal centers in the brain stem. This spinal circuitry is continuously driven by peripheral input to optimize the systemic blood pressure and heart rate. scES 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 possible that scES restores conduction properties of residual damaged or non-functional axons across the spinal injured segment. If this is the case, then scES alone without the cardiovascular stress of stand training should restore near normal cardiovascular control. If, however, the spinal cord circuitry is a key controller, the task specific training with scES will be needed to optimize autonomic function.

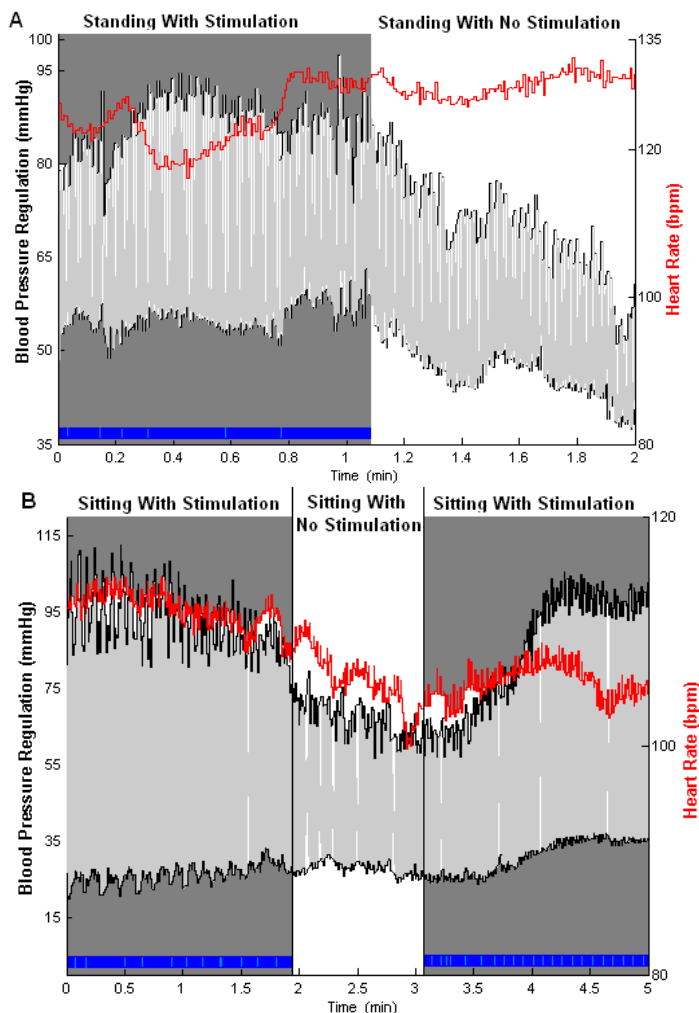


Figure 2. Continuous blood pressure and heart rate recordings from the individual with cervical motor complete SCI during standing (**A**) and sitting (**B**) with and without epidural stimulation. The individual was able to maintain his blood pressure in both sitting and standing positions with epidural stimulation. Blood pressure significantly decreased when stimulation was turned off and recovered again when the stimulation was turned on. Blue indicates tonic stimulation

We have demonstrated in rats with chronic SCI that activation of sacral cutaneous or visceral afferents increases sympathetic activity within the renal nerve and correspondingly alters arterial blood pressure [11]. The ultimate goal of the proposed project is to develop appropriate parameters for scES in humans with chronic SCI that will cause tonic activation of spinal sympathetic circuits and improve arterial blood pressure (but will not produce responses that result in development of life-threatening autonomic dysreflexia). scES-induced improvements in resting arterial blood pressure will likely lead to a cascade of secondary cardiovascular improvements including increased peripheral blood flow, reduced OH, increased venous return and cardiac pump function, and improved cerebral perfusion and associated improvements in cognition. Indeed, preliminary data in one motor complete quadriplegic patient with chronic hypotension and symptomatic OH have shown that with optimized epidural stimulation parameters, we can maintain systolic blood pressure 20% above his nadir and prevent symptomatic hypotension.

a. Central arterial stiffness. Individuals with high SCI experience dramatic fluctuations in blood pressure during events such as orthostatic challenges, which are a result of impaired sympathetic modulation [12] and likely contribute to this population's three to four-fold increase in cardiovascular disease [13]. Previous evidence demonstrates that impaired sympathetic drive to the central vasculature promotes arteriosclerotic development, leading to an accelerated aging process that includes elastin disruption and increased collagen deposition [14]. Our recent evidence supports this mechanism, which showed that the age related arteriosclerotic burden on the cardiovascular system (as measured by aortic pulse wave velocity) of those with high SCI is accelerated over 40 years [15]. These data are alarming, and highlight the need for novel treatments aimed at reversing and/or preventing the acceleration of central arterial stiffening post SCI. To serve this purpose, scES may serve as a viable treatment to mitigate the declining health of the central vasculature by activating dormant sympathetic axons within the spinal cord, re-establishing sympathetic drive,

and subsequently stabilizing blood pressure during daily living. Through epidural stimulation optimized with cardiovascular parameters, we hypothesize the number and severity of hypotensive episodes will be reduced (i.e. restored sympathetic drive), which will thereby inhibit the accelerated arteriosclerosis observed in the central vasculature of those with SCI and reduce cardiovascular disease risk [16, 17].

b. Cardiac structure and function. In the clinical SCI population, the few studies that have assessed cardiac function tend to agree that SCI is associated with a reduction in end-diastolic volume and attenuated systolic cardiac performance, as defined by reduced ejection fraction and stroke volume [18-21]. Recent evidence also suggests that diastolic function, inferred through the measurement of early-to-late filling velocity, is impaired in individuals with SCI [22]. In animal models of SCI, left-ventricular developed pressure and the rates of contraction and relaxation are impaired after high- or mid-thoracic SCI [23, 24]. There is also evidence of ventricular remodeling in the chronic stages of SCI as evidenced by increased myocardial collagen deposition [24, 25]. In both human and animal SCI, pump function of the left ventricle can be improved by exercise training [26] and/or mechanical compression of the legs and/or abdomen [27, 28], which are both thought to elicit cardiac improvements via increased venous return. We anticipate that scES optimized for cardiovascular function will activate spinal sympathetic circuitry and increase vascular tone and consequently blood pressure to 'load' the heart in a similar way to that of mechanical compression of the abdomen. It is also plausible that scES optimized for voluntary and/or stand training may activate lower limb musculature to a sufficient degree that the muscle pump can be re-engaged to facilitate improved venous return from the lower limbs.

c. Cerebrovascular function after SCI. We have recently shown that cardiovascular autonomic dysfunctions after SCI are primary factors that lead to declining health of the cerebral vasculature [19, 20]. Impaired cerebrovascular function and structure are associated with increased risk of stroke as well as cognitive dysfunction [29, 30]. As stroke risk is 200-300% times higher, and cognitive dysfunction occurs in up to 60% of those with SCI [3, 31], a critical appraisal of the association between autonomic dysfunction and cerebrovascular health is needed to improve our understanding and elucidate potential preventative and therapeutic targets. In two seminal clinical trials, we have shown that improving orthostatic hypotension using midodrine improves cerebrovascular function, orthostatic tolerance, and cognition [32, 33]. These studies provide strong evidence that mitigating orthostatic hypotension can improve cerebrovascular function and associated clinical conditions. Therefore, there is a dire need for a novel intervention aimed at reducing cardiovascular dysfunction in those with SCI, as this has the potential to reduce cardiovascular disease risk, improve cerebrovascular function and restore cognition in this population. scES, when optimized for improving cardiovascular function is hypothesized to increase blood pressure and reduce the severity of orthostatic hypotension, which will provide a similar benefit to pressor pharmacological agents after SCI, and partially restore normal cerebrovascular function and cognition in those living with SCI.

3. Bladder, Bowel and Sexual Function. Bladder, bowel and sexual dysfunctions rank among the top disorders affecting quality of life after SCI [34, 35]. We have exciting new preliminary data from several individuals with severe injuries to support recent intriguing case reports indicating improved bladder and bowel function as well as improved sexual function after undergoing an activity-based rehabilitation, locomotor training, and scES in combination with locomotor training. In addition, we have observed dramatic interference with stepping ability when the individual's bladder is full, suggesting interaction of locomotor and bladder circuitry.

Of potential mechanistic importance is that task-specific training in animals influences the expression of neurotrophins, and we have data from male rats with severe SCI contusions showing reversal of injury-induced elevation of neurotrophin levels (nerve growth factor - NGF) with training. Our human and animal findings suggest that activity-dependent plasticity affecting the lumbosacral spinal circuitry may also have the potential for a beneficial effect on bladder, bowel and sexual function. Thus, the effects of epidural stimulation and task-specific training in humans with SCI on bladder, bowel and sexual function will be systematically studied, including the effect of different epidural stimulation parameters on the fill/void cycle during cystometry and the effects of training on bladder neurotrophin levels. Various combinations of epidural stimulation parameters (stand or voluntary or cardiovascular parameters) and task-specific training paradigms (stand training or voluntary leg movement training) that have been selected based on pilot studies in four subjects will be employed.

a. Urinary Bladder Dysfunction in SCI. Development of a neurogenic bladder is a cause of significant morbidity and mortality in the SCI population [36, 37]. Bladder complications following an upper

motor lesion or supra-sacral injury include over activity of the detrusor muscle leading to incontinence, sustained high pressure within the bladder wall, and sphincter-detrusor dyssynergia [38, 39]. SCI individuals often exhibit chronic vesico-ureteral reflux into the renal pelvis, leading to hydronephrosis and ultimately renal failure. Additionally, the backward flow of urine introduces bacteria into the kidneys and can lead to sepsis and hospitalization. Even though optimal sterile conditions are sought in bladder care management, most SCI patients develop urinary tract infections, which are the number one medical concern affecting overall health and medical costs [40]. With the common use of anticholinergic medications, side effects such as dry mouth and constipation further exacerbate the underlying urologic dysfunction, making compliance difficult [36]. Up regulation of neurotrophic factors post-SCI [41] is responsible for the re-emergence of the spinal voiding reflex within 2 weeks of injury [42], but chronic changes in NGF appear to be responsible for bladder afferent hypersensitivity, hypertrophy, and sprouting of axons [43], all of which lead to bladder over-activity. NGF and brain derived neurotrophic factor (BDNF) have been implicated as key factors in a variety of bladder dysfunctions [44]. NGF delivery (intrathecal infusion at L6-S1 spinal level in adult rats) causes bladder DRG afferents to become hyper-excited and results in detrusor hyperreflexia [45], while NGF removal, via antibody treatment (intrathecal infusion at L6-S1 spinal level in adult rats), has been shown to relieve detrusor hyperreflexia and detrusor-sphincter dyssynergia [46, 47]. Sequestering BDNF (daily tail vein administration of TrkB-Ig₂, which specifically binds BDNF and neutralizes it) has been shown to improve bladder function in a chronic cystitis model [48]. Exercise therapies also influence neurotrophin expression in visceral target organs, not just skeletal muscle [49, 50]. We therefore hypothesize that epidural stimulation and task-specific training will influence neurotrophin levels within the spinal cord and periphery, leading to enhanced visceral function.

b. Bowel Dysfunction in SCI. Gastrointestinal problems are also a cause of significant morbidity and mortality in the SCI population [51, 52]. Stool evacuation is a complex process involving relaxation of the pelvic floor, contraction of the recto-sigmoid, and the inhibition of the anal sphincters [53]. Many with chronic SCI lose conscious control of defecation (contraction of the external anal sphincter is the primary mechanism for deferring evacuation) and suffer from chronic constipation and fecal incontinence [51, 54-56]. Synergistic activity between colonic smooth muscle and the striated anal sphincter muscle is lost [57]. Dysmotility, decreased transit time and loss of tissue compliance may be associated with the loss of descending modulation of the sympathetic supply [57].

c. Sexual Dysfunction in SCI. In men with SCI, the degree of sexual dysfunction depends on level of injury and completeness of injury. For our research, only injuries cranial to T10 are considered, as the spinal reflex arcs for erection and ejaculation are considered to be left intact, with only the removal of supraspinal input [58]. Most individuals with SCIs above T10 demonstrate reflexogenic erections of varying degrees in response to very slight stimulation of the penis [59, 60]. Those with complete injuries above T10 are most likely to have reflex erections but not psychogenic erections. Though erections are easily initiated, they are not easily sustained (proposed to be a result of altered penile sensitivity) [61, 62] so that intercourse is difficult if not impossible without treatment such as intracavernous injections. In 95% of SCI men with lesions cranial to T10, normal ejaculation is severely impaired or impossible [60, 63] despite the intact spinal reflex arc, suggesting the ejaculatory reflex circuitry is more dependent on supraspinal control than is the erection circuitry. The ejaculatory dysfunction may also relate to the decrease in penile sensation. Many SCI individuals who do not respond to normal tactile stimulation of the penis may ejaculate to intense vibratory stimulation of the ventral penile midline [64], suggesting that massive recruitment of all low and high threshold penile mechanoreceptive afferent neurons can provide enough input to the spinal ejaculatory circuit. In addition to erectile dysfunction and ejaculatory failure, abnormal sperm motility and viability as early as two weeks post-SCI contribute to neurogenic reproductive dysfunction post-SCI [65]. In females with SCI, impairments in genital responses and sexual arousal have been documented [66-68]. Fertility in females is not an issue, although there are special issues to address during pregnancy [66, 67, 69].

III. Research Approach

A. General Approach: We propose to understand the role of scES activating spinal circuits and weight-bearing stand training on the recovery of below injury voluntary control of movements, standing, and the autonomic nervous system function in humans with severe paralysis. Training will consist of lying supine or sitting while (1) practicing voluntary movements with specific Vol-scES configurations designed for the voluntary movements of the legs and trunks; or (2) maintaining normal systolic blood pressure during maneuvers of orthostatic stress with specific CV-scES configurations. By comparing among the two Group A interventions we will assess whether scES with specific configurations for each function are needed to improve

the ability to move voluntarily and/or recovery of cardiovascular function. Group B will have the same training but will also include weight-bearing stand training. Comparing across Group A and B, we will assess the role of stand rehabilitation for improving the ability to move voluntarily, and/or recovery of cardiovascular function. In a secondary study we will evaluate the effect of these 4 interventions of respiratory, bladder, bowel and sexual function in these individuals with chronic severe spinal cord injury. Quality of life and costs of health care also will be measured.

Our approach differs from any current strategies in the following ways: 1) Our stimulation configurations (intensity, frequency, pulse width and electrode combination) are selected for the specific functional goals or physiological responses and the current physiological state of the spinal circuitry; 2) we stimulate with the minimal intensity to avoid direct motor responses, but maximize facilitation of function derived from proprioception and supraspinal influences; and 3) the facilitating mode of stimulation allows for existing sources of sensory feedback generated by functional events to neuromodulate; in essence, to control the excitability of the spinal circuitry so that the functional capacity intrinsic to these circuits can be realized. In the case of voluntary activation, it enhances the potential to engage novel supraspinal-spinal connectivity. In the case of autonomic control, the intervention re-engages circuits involved in cardiovascular control. In the case of standing, stimulation re-engages the circuitries associated with load-related proprioception and cutaneous input that may be critical for motor control in a gravity environment. After paralysis, individuals with severe SCI essentially exist without significantly opposing gravity because they cannot stand or walk.

None of these specific interventional strategies had ever been attempted after severe paralysis in humans until recently [2, 70, 71]. The conceptual basis of our approach is that lumbosacral scES combined with task-specific training re-engages existing spinal circuits and challenges novel post-injury circuitry to reorganize in functionally significant ways. We provide a cellular environment that enables sensory-motor and autonomic circuits to recover significant levels of function, and 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 four motor complete individuals studied, who trained to stand and to voluntarily move their legs with scES for over 2 years, have benefited from some restoration of cardiovascular, respiratory, bladder, bowel, and temperature regulation. They have also noted some normalization of sexual function in addition to the ability to move voluntarily and to stand without physical assistance. This study will further our understanding of the mechanisms underlying the restoration of voluntary movement and standing while also quantifying the changes in the secondary conditions and understanding underlying mechanisms in a larger cohort of SCI individuals. In addition to advancing our scientific knowledge, the proposed experiments are essential to translating this therapeutic approach to a larger scale, which is needed to have a meaningful clinical impact. This use of scES is not widely used for recovery of neurological function in patients with severe SCI due to uncertainty regarding the mechanisms of action and a lack of convincing evidence of efficacy in larger numbers of subjects. Our approach will allow us to determine specific types of scES needed for movement and autonomic nervous system dysfunction, and this will lay the groundwork for expedient translations to other neurologic disorders and diseases that cause paralysis, including stroke, traumatic brain injury, movement disorders and cerebral palsy.

B. Experimental Design: We will enroll, implant and complete the interventions in 36 research participants who have sustained a SCI in the proposed experiments. We anticipate we will need to screen 108 potential research participants to enroll 36 individuals who will complete the study. This sample size will provide sufficient replication per study group ($n=6$) from which variance and effect size estimates for each study hypothesis can be calculated, and hypothesis tests conducted. We will also select individuals to assure that there are a minimum of 25% ($n=9$) women to adequately represent the percentage in the SCI population. We will study each cohort of patients comprehensively, and each individual will be allocated to the group interventions based on the method of minimization. Research participants will be randomized into group interventions. 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, will advance both clinical and scientific knowledge in this highly complex population. 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, bladder, bowel and sexual function.

1. Recruitment, pre-screening, screening and enrollment

a. Recruitment. Potential research participants will be identified by our research database (UofL IRB 06.0647) that currently contains over 6,000 individuals. When individuals agree to be entered into the database they answer a series of IRB approved questions that allow us to identify the most likely candidates to approach. We anticipate that we will need to contact approximately 200 individuals in the initial stage of recruitment. Potential research participants will be selected in the order that they initially entered the database. Those who have already been screened for other studies from the Kentucky Spinal Cord Injury Research Center (KSCIRC) Screening Protocol (UofL IRB 07.0224) and/or have completed studies previously will be contacted first. The KSCIRC Screening protocol allows for general screening of individuals for all studies conducted at the Kentucky Spinal Cord Injury Research Center. These individuals would not need to go through the pre-screening process as all of the information normally collected during pre-screening (below) would have been collected during the KSCIRC screening protocol.

The clinical coordinator will contact the individual and provide them with general information regarding the research study. If the individual is interested in learning more about the study, the clinical coordinator will set up an info session via teleconference or a meeting at Kentucky Spinal Cord Injury Research Center with a project leader, Dr. Harkema, Dr. Angeli, and/or the Research Manager. During the info session the project leader will describe the time commitment, the requirement of surgery, the general assessments and interventions involved and that the interventions will be selected randomly as well as that the individual will need to live in Louisville during the research study. The potential risks will also be discussed. All questions from the potential research participant will be answered. The objective of this info session is to provide a first educational session of the research program and ascertain if given the time commitment, need to relocate, and the need for invasive surgery, the individual has an interest in being screened for the study. If they have an interest in potentially participating in the study, we will then send them a copy of the consent form for review and ask them to discuss the study with their family, friends, and physicians and to call the clinical coordinator if they decide to consent to participate in the screening process.

b. Pre-Screening (1st consent). If the potential research participant agrees to screening we will give them the choice to conduct some pre-screening assessments at their location. Pre-screening will be offered because the majority of the research participants will likely not be from our geographic region. Travel and housing costs will be the research participant's financial responsibility. Therefore, we would like to allow a pre-screening process to avoid those who would be ineligible after review of standard medical assessments having to travel to Louisville at their expense. They will also have the option to have all the pre-screening assessments conducted at the Kentucky Spinal Cord Injury Research Center in Louisville.

If they choose the pre-screening option, the clinical coordinator will request their medical history related to their spinal cord injury that may include surgical and medical records from their initial injury; spinal cord MRIs, DXAs, hospitalizations and/or surgeries they have had since their injury; and medical records from their physician visits that document their overall health. We will also have them fill out a self-report of their medical status. If they have not had a spinal cord MRI within 3 years, we may request they obtain one. We will request a drug screening test. If they are female, they will be asked to obtain a pregnancy test. All medical tests will be covered by the research study using a pre-paid credit card loaded with the costs of the tests. The clinical coordinator will obtain pricing and send a gift card pre-loaded with the funds for the testing to avoid any insurance billing or out of pocket expense. The clinical coordinator will work with the potential research participant to identify the medical location for the tests and the costs and will provide the pre-paid credit card for payment. The individual can decide if they would like to conduct all or some of these medical tests at their location.

These records will be reviewed by the study doctor and he/she may request consultation from Drs. Boakye, Neimat and/or Hirsch. The study physician(s) will provide a medical recommendation to Dr. Harkema as to whether they should be invited to the screening process. If the study physician(s) approve the screening and the potential research participant chooses to come to Louisville to complete the screening, the clinical coordinator will contact the individual and identify the dates for their screening. The research staff will then work with the research participant to schedule their screening assessments at Kentucky Spinal Cord Injury Research Center.

c. Screening (2nd consent). If the individual is potentially eligible for the study, as determined from the pre-screening assessments, from the KSCIRC Screening (UofL IRB 07.0224) or had chosen from the info session to have all screening assessments done onsite at the Neuroscience Collaborative Center, and is

interested in participation, he/she will be consented for screening. If the individual has had prior assessments these will be used by the investigators in the screening process to determine eligibility so all assessments listed may not be done to avoid unnecessary repetition. The investigators of this project, Dr. Maxwell Boakye, Dr. Neimat, Dr. Glen Hirsch, Dr. Alexander Ovechkin, Dr. Claudia Angeli, and Dr. Susan Harkema, will participate in the initial screening of the research participants. All eligible SCI research participants will be invited to the Neuroscience Collaborative Center at Frazier Rehab Institute to discuss the complete protocol and its risks and benefits with Dr. Harkema and/or Dr. Angeli. Dr. Harkema and/or Dr. Angeli will verbally review all other assessments that will occur and explain that the research participant can refuse any of the assessments and discontinue their participation at any time without penalty. Dr. Harkema and/or Dr. Angeli will answer any questions the potential research participant or family members may have in relation to the research. All potential risks will be discussed with the potential research participant.

The potential research participant will be assessed for medical eligibility by the research team's neurosurgeon, cardiologist and study physician. The study physician will conduct a medical history and physical examination and determine medical eligibility based on the clinical inclusion criteria (#1-4, see section IV.A below) and exclusion criteria (#1-6, see section IV.A below) and consideration of the risk assessment specific for the individual based on their medical history and examination. Following medical clearance for the assessments and participation in the screening portion of the study by the study physician, the potential research participant will undergo behavioral, neurophysiological, and cardiovascular assessments (Appendix #1). Pre-usual care assessments may also be done during the screening visit to reduce the number of study visits for participants who do not live locally. Potential study eligibility will be determined by the investigators based on the evaluation of these assessments to be consistent with the inclusion criteria (#5-9, see section IV.A below). The research participant will then be seen by the study cardiologist who will review the medical record and the cardiovascular assessments. The study neurosurgeon will then review their medical history and the MRI and provide a preliminary recommendation for surgical implantation. *Drug/Nicotine Testing:* The intent is to avoid nicotine users. If a potential research participant tests positive for nicotine during the screening period or the post-usual care/pre-implant assessments, they will be ineligible for the study. After implantation, we will conduct random nicotine testing using a urine sample and levels will be recorded. Nicotine testing will then be done daily until levels are negligible and then weekly for one month. If nicotine levels remain absent, testing will return to random testing. The individual will not be removed from the study after implantation but nicotine levels will be monitored and if detected, levels will be recorded.

Dr. Harkema and/or Dr. Angeli will meet with the potential research participant at the end of the screening and review the results of the assessments and discuss their eligibility. If they are ineligible, the reasons will be explained and they will be asked if they would like to remain in the database for recruitment in future studies. If they are eligible, then all the potential risks will be discussed with them. They will be informed that if they are still interested in participating in the study the next phase is for them to continue with their current daily activities for at least 80 days without any intervention (Usual Care). In some cases an additional pregnancy test may be done if the pre-screen and screen time points are more than 6 months apart.

2. Usual Care (3rd Consent). If the individual is determined to be eligible for the Usual Care period of the study, as determined by the screening assessments, he/she will be consented for Usual Care. The Usual Care period will last for at least 80 days without any intervention and the participant will continue with his/her current daily activities. Dr. Harkema will encourage the research participant to discuss the study with family, friends, and physicians during this period to facilitate their ability to reach the most informed decision regarding surgical implantation and full enrollment into the study. They will be informed that they are not consenting to surgical implantation – that consent would be signed when they return for the surgical implant and intervention phase of the study. They will also be informed that during the Usual Care phase of the study they can decide not to continue on with the study and let us know at any time. At the completion of the Usual Care phase of the study they will complete the post-usual care behavioral, cardiovascular, respiratory, bladder, bowel, and sexual function assessments (Appendix #1). If the individual meets all the inclusion and exclusion criteria, and is given medical clearance by the study from the cardiologist and the study physician and is recommended for possible implantation by the study neurosurgeon, then they will be enrolled in the study and randomized.

3. Surgical Implantation and Interventions (4th consent). If the research participant decides to enroll in the surgical implantation and intervention phases of the study, the research participant will return to the Kentucky Spinal Cord Injury Research Center. Dr. Harkema and/or Dr. Angeli will meet with the potential research participant to discuss the potential risks of the study and explain that the assessments they

conducted to determine eligibility for the Usual Care phase of the study will be repeated. Also, the medical eligibility and assessments will be reviewed by the investigators to determine eligibility for surgical implantation and the interventional phase of the study. The research participant will sign the consent form for surgical implantation and intervention and then complete the pre-implant assessments (Appendix #1). The research participant will be assessed by the research team's neurosurgeon, cardiologist and study physician. The neurosurgeon will review all aspects of the surgery including the potential risks specific to the surgery and the medical device with the individual. Dr. Maxwell Boakye (study neurosurgeon), Dr. Glen Hirsch (study cardiologist), or Dr. Joseph Neimat (back-up study neurosurgeon) will review the results and collaborate on whether the individual is medically eligible. Dr. Susan Harkema, Dr. Claudia Angeli, and/or Dr. Alexander Ovechkin, will review the results and collaborate on whether the individual is scientifically eligible for the study. The physicians will provide a medical recommendation for surgical implantation and participating in the interventional phase of the study. Dr. Harkema and/or Dr. Angeli will meet with the potential research participant at the end of the pre-implant assessments and review the results of the assessments and discuss their eligibility. If any new information is identified that would determine they are now ineligible, the reasons will be explained and they will be asked if they would like to remain in the database for recruitment in future studies. If they are eligible, then all the potential risks will be discussed again. Dr. Harkema and/or Dr. Angeli will answer any questions the potential research participant or family members may have in relation to the research. They then will be asked whether they would like to participate in the spinal cord implantation and intervention phase of the study. They will be told they can take more time to make their decision if needed. If they decide not to continue on with the study they can let us know and there will be no penalty. They can remain in our database, if desired, for recruitment in future studies.

a. Surgical Implantation. Spinal cord epidural stimulation is administered by a multi-electrode array implanted in the epidural space over the dorsum of the spinal 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, and 5 electrodes for the proposed experiments). 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 functional activities, such as standing or voluntary movement or physiological responses such as blood pressure regulation.

The procedure will be performed by Dr. Maxwell Boakye or Dr. Joseph Neimat at University of Louisville Hospital to implant the epidural stimulating electrodes and stimulator in a single surgical procedure under general anesthesia following standard medical procedures. As per standard practice, a Medtronic representative will bring the stimulator to the operating room and provide technical support throughout the implantation procedure. The MRI compatible 5-6-5 Specify electrode, and Intellis Adaptive Neurostimulator, (MEDTRONIC, Minneapolis, MN, USA) will be implanted at the T11-L1 vertebral level which corresponds with L1-S1 spinal cord levels (lumbosacral spinal cord) guided by fluoroscopy. Neurophysiological mapping will then occur. The lead wires will be tunneled subcutaneously to exit 5 centimeters from the incision site. The implantable neurostimulator will be internalized and the connecting wires for the implanted electrodes will be tunneled under the skin and connected with the battery generator that will be placed either in the abdominal area or the lower back.

Initially, the individual 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 of the spine, with dissection carried deeply to the laminae. A partial laminectomy may be performed at the spinal interspace providing a site for electrode insertion. The incision will be approximately 2.0 – 2.5 inches. The electrode will be passed along the dorsal aspect of the epidural space in a cephalic direction to the T11-L1 vertebral levels over the group of spinal cord nuclei where activation of the muscles occurs. Fluoroscopy and neurophysiological parameters will be used to determine the desired lead placement. The neurophysiological parameters will be utilized to determine the optimal lead placement by monitoring the motor system using electrical stimulation of the spinal cord at the T11-L1 spinal segments. Surface EMG electrodes from the quadriceps, hamstrings, adductor, gluteus maximus, tibialis anterior, and triceps surae, bilaterally and fine-wire EMG electrodes from the iliopsoas will also be used to record the multi-segmental motor responses induced by 2 Hz stimulation. Dr. Harkema and/or Dr. Angeli will monitor these responses and evaluate the spatial orientation of the electrode. The electrode may then be repositioned to achieve the

desired placement. The appropriate placement of the electrode is critical in order to achieve motor and autonomic responses.

Following the location of optimal lead placement, the participant will be rolled to the left lateral decubitus position (or right lateral depending on the side of the abdomen the stimulator will be placed) and the surgeon will proceed with the internalization of the implantable neurostimulator into the subcutaneous area of the abdomen. The wires of the implanted epidural electrodes will be tunneled under the skin and connected with the battery generator that will be placed in the abdominal area. 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 electrode. The battery pack 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 of Louisville Hospital for monitoring. 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 monitored for blood pressure, pulse, and temperature changes. Following discharge from inpatient the individual will recover at home for 2-3 weeks and be monitored by the research nurse with daily communication and periodic visits to inspect the wound healing and recovery. The research nurse will continuously update Drs. Boakye, Neimat and/or the study doctor. If any complications arise, the study physicians will be informed immediately and the research participant will be provided with medical care. We do not anticipate any increased risks other than the well-recognized accepted risks of surgery (for details see Surgical Risks section IV.C below).

b. Interventions. After recovery from surgery, *post-implant, pre-intervention* behavioral and cardiovascular assessments will be conducted (Appendix #1). Mapping of the motor evoked responses related to spatial electrode selection, amplitude and/or frequency [72] will be conducted and the specific configurations and parameters optimal for voluntary movement, standing and cardiovascular function will be identified. The pattern of electrically active electrodes, as well as electrode amplitude, stimulating frequency, and stimulating pulse width can be remotely programmed. Since different spatial activation patterns and frequency parameters affect different spinal circuits, the array can be reconfigured, within limits, to bias its facilitating effects toward different functional activities, such as standing or voluntary movement or physiological responses such as blood pressure regulation. After determining the configurations for voluntary, standing, and blood pressure regulation, the individual will then begin his or her assigned group intervention for approximately 80 sessions for 6 hours each session. After intervention #1 is completed, the post-intervention #1 assessments will be conducted (Appendix #1, post-intervention #1) and the individual will continue to complete his or her second assigned group intervention. Post-intervention #2 assessments will be conducted after the completion of the second intervention. After about 40 sessions in each intervention, mid-training assessments will be conducted.

4. Experimental Groups/Interventions (n=36 research participants).

Two factors are varied in this study, the stimulation frequency (voluntary, cardiovascular, or standing) and the presence or absence of weight bearing stand training. Crossing these yields 4 possible interventions: A1, A2, B1, and B2, where A=no weight bearing stand training, B=with weight bearing stand training, 1=voluntary parameters Vol-scES and 2=cardiovascular parameters CV-scES. A description of each of the four different interventions is provided below.

Group A:

- Vol-scES during voluntary leg movement training while sitting or lying supine (A1).
- CV-scES during sitting or lying supine (A2).

Group B:

- Stand-ES during stand training + Vol-scES during voluntary leg movement training (B1).
- Stand-ES during stand training + CV-ES during sitting or lying supine (B2).

Each participant begins 80 sessions of Usual Care to obtain baseline levels, followed by two intervention periods. In the first intervention period (intervention 1, at least 80 sessions), participants will be randomized to one of the four different interventions (A1, A2, B1, or B2). Each research participant will complete at least 80 sessions of the intervention to which they are randomized. At the completion of at least 80 sessions of intervention 1, clinical and neurophysiological assessments will be conducted (Appendix #1). Some assessments may be completed more often.

At the conclusion of the first intervention period, a second intervention period of at least 80 sessions will be commenced. During this second intervention period, participants that had not been receiving weight bearing stand training will be crossed over to receive it. Participants who had been receiving weight bearing training will repeat their current intervention. Thus, participants who had A1 or A2 in intervention 1 would cross over to B1 or B2, respectively, while participants who had B1 or B2 would repeat their intervention.

In what follows it is often convenient to refer to groups (the specific cohort of 9 participants) by their initial intervention in period 1, thus we will refer to groups as A1, A2, B1, or B2, as appropriate.

After at least 80 sessions of the second intervention are completed, assessments are conducted. If an individual does not complete at least 80 sessions of their assigned group intervention, their data will still be evaluated.

Table 1. Interventions for research participants (n=36)			
(n=18) Group A		(n=18) Group B	
Intervention #1			
N=9 A1 Vol-scES 6 hours	N=9 A2 CV-scES 6 hours	N=9 B1 Vol-scES 6 hours <u>and</u> Stand-scES + weight bearing training 2 hours	N=9 B2 CV-scES 6 hours <u>and</u> Stand-scES + weight bearing training 2 hours
Intervention #2			
B1 Vol-scES 6 hours <u>and</u> Stand-scES + weight bearing training 2 hours	B2 CV-scES 6 hours <u>and</u> Stand-scES + weight bearing training 2 hours	B1 Vol-scES 6 hours <u>and</u> Stand-scES + weight bearing training 2 hours	B2 CV-scES 6 hours <u>and</u> Stand-scES + weight bearing training 2 hours
Home and Community Integration			

Appendix #1 gives a detailed outline for the timeline and procedures for an individual research participant.

The research participant will be given the choice to have the implant removed by the study neurosurgeon. The neurosurgeon will remove the stimulator if requested by the research participant.

If they choose to continue using the implant they will be given the option to participate in the Home and Community Integration phase. They may then receive configurations for the behaviors and/or physiological

responses that were not in their assigned group interventions. They will participate in daily sessions identifying configurations for each of the other interventions that they did not receive during the study. Configurations will only be released to the research participant after being conducted safely in the laboratory and demonstrated by the research participant that they can safely implement the scES independently from the research staff. If the research participant would like to continue scES, they will be provided with stimulation configurations that were not part of their group interventions. This will require that they will stay in Louisville for approximately 8 additional weeks to undergo home and community integration of the stimulation parameters. The duration of stay will be variable and depend on the time it takes to identify effective configurations for each behavior and/or physiological response.

Follow up studies will be conducted at 6 months and 1 year. If an individual has removed the epidural stimulator, then the assessments will be done without stimulation only.

Follow up assessments will occur at 6 months and one year after post-interventions completion (Appendix #1).

5. Blood Draws for Laboratory Testing

Blood will be drawn at Screening, Pre-Usual Care, Pre-Implant, Post-Intervention #1, Post-Intervention #2, and Follow-Up time points to test for the following levels in the blood: lipid panel, fasting insulin, fasting glucose, complete metabolic panel, angiotensin, renin, aldosterone, leptin, interleukin 6, tumor necrosis factor alpha (TNF- α), plasminogen activator inhibitor 1 (PAI-I), complete blood count, GGT, c-reactive protein (CRP), erythrocyte sedimentation rate (ESR), albumin, procaltitonin, prealbumin, vitamin D, and trimethylamine-N-oxide (TMAO). Blood may be drawn prior to surgery to test for the following: complete blood count (CBC), basic metabolic panel (BMP), PT/PTT, and blood type and screen. Blood that will be drawn prior to surgery to test for infection is described below.

C. Specific Aims.

Specific Aim 1.

Understand the effect of epidural stimulation alone using specific configurations on outcomes for voluntary movement, cardiovascular function and independent standing.

Primary Analyses:

Hypothesis 1a: Primary Efficacy Hypothesis: CV-scES will result in greater cardiovascular functional improvements than those achieved during Usual Care Intervention.

Hypothesis 1b: Secondary Efficacy Hypothesis: Task specific voluntary movement training using Vol-scES will result in greater improvements in voluntary movement than those achieved during Usual Care Intervention.

Hypothesis 1c: Secondary Efficacy Hypothesis: Stand Stand-scES (parameters optimized for standing) will result in greater improvements in the ability to stand than those achieved during the Usual Care Intervention.

Secondary Analyses:

Hypothesis 1d: Task specific voluntary movement training using Vol-scES (parameters optimized for voluntary movement) will result in more successful voluntary movements than CV-scES.

Rationale: 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. Other possibilities are that the scES retrogradely stimulates residual axons that anatomically cross the lesion but are not functional, or, that scES restores the conduction properties that are lost with demyelination or other consequences of damage [2, 70, 73-76]. If only the restoration of the conduction of the residual supraspinal connections are needed, then the scES **alone** without voluntary 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 scES would also be required to optimally generate voluntary movement.

Methods: Linear statistical models will be fit with coefficients relating to the three stimulation methods. This hypothesis will be tested by if the Vol-scES estimates are significantly higher than those for CV-scES or Stand-scES stimulation parameters. See Section G for details.

Hypothesis 1e: CV-scES (parameters optimized for cardiovascular function) will result in greater cardiovascular functional improvements than Vol-scES.

Rationale: We propose that scES, when optimized for cardiovascular function to normalize blood pressure and reduce the severity of orthostatic hypotension will result in improvements of cardiovascular function. We hypothesize the number and severity of hypotensive episodes will be reduced (i.e. restored sympathetic drive), which will thereby inhibit the accelerated arteriosclerosis observed in the central vasculature of those with SCI and reduce cardiovascular disease risk [16, 17]. We anticipate that scES optimized for cardiovascular function will activate spinal sympathetic circuitry and increase vascular tone and consequently blood pressure to 'load' the heart in a similar way to that of mechanical compression of the abdomen.

Methods: Linear statistical models will be fit with coefficients relating to the three stimulation methods. This hypothesis will be tested by if the CV-scES estimates are significantly higher than those for Vol-scES or Stand-scES stimulation parameters. See Section G for details.

Hypothesis 1f: Stand-scES (parameters optimized for standing) will result in improvements in the ability to stand without physical assistance than Vol-scES or CV-scES.

Rationale: It is also plausible that scES optimized for stand training may activate lower limb musculature to a sufficient degree that standing without physical assistance will occur even without weight-bearing training. The scES for cardiovascular would not activate the lower limb musculature. The scES for voluntary trunk and leg movements would not activate the lower limb musculature in a manner that would promote standing if task-specificity is important for the recovery.

Methods: Linear statistical models will be fit with coefficients relating to the three stimulation methods. This hypothesis will be tested by if the Stand-scES estimates are significantly higher than those for Vol-scES or CV-scES stimulation parameters. See Section G for details.

Specific Aim 2.

Understand the effect of epidural stimulation in combination with stand training on outcomes for voluntary movement, cardiovascular function and independent standing.

Secondary Analyses:

Hypothesis 2a: Task specific voluntary training using Vol-scES combined with task specific stand training using Stand-scES will result in more successful voluntary movement than voluntary training using Vol-scES alone.

Rationale: Stand training (weight-bearing training with dynamic assistance) with simultaneous Stand-scES 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 [77-79]. 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 [80], indicating that loss of load related sensory information drives neural plasticity that results in deleterious effects. Due to these insights and others, we believe that the most efficacious approach to restoration of voluntary movement is to combine scES with voluntary training and scES with stand training.

Given that each of our subjects to date has received stand training with Stand-scES as well as training for voluntary movement of the limbs with Voluntary-scES, it may require the combination of both 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, so there may be overlap of circuitries controlling standing and voluntary movement. In testing this hypothesis we will determine whether there can be complementary effects of stand and voluntary leg movement training, or whether stand or voluntary training is necessary.

Methods: Linear Statistical Models will be fit containing parameters for the main effect of each stimulation setting (Vol, CV, and Stand) and additive effects of weight bearing stand training for each stimulation setting. This hypothesis will be tested using the stand training coefficient corresponding to Vol-scES. See section G for more details.

Hypothesis 2b: CV-scES (parameters optimized for cardiovascular control) combined with task specific stand training using ES parameters selected for stand training will significantly improve blood pressure control along with cardiac and cerebral function to a greater extent than ES optimized for CV alone.

Rationale: We propose that muscle activation with Stand training (weight-bearing training with dynamic assistance) with Stand-scES will further activate spinal sympathetic circuitry and increase vascular tone and thus blood pressure in addition to these mechanisms occurring with CV-scES.

Methods: Linear Statistical Models will be fit containing parameters for the main effect of each stimulation setting (Vol, CV, and Stand) and additive effects of weight bearing stand training for each stimulation setting. This hypothesis will be tested using the stand training coefficient corresponding to CV-scES. See section G for more details.

Specific Aim 3:

Understand the effects of scES alone and scES with stand training on respiratory, bladder, bowel and sexual function.

Secondary Analyses:

Hypothesis 3a: CV-scES selected for cardiovascular function will result in greater improvements in respiratory, bladder, bowel and sexual function than Vol-scES selected for voluntary movement

Rationale: We propose that CV-scES will further activate spinal autonomic circuitry and thus influence similar systems, respiratory, bladder, bowel and sexual function in addition to these mechanisms occurring with improvements in the cardiovascular system.

Methods: Linear Statistical Models will be fit containing parameters for the main effect of each stimulation setting (Vol, CV, and Stand) and additive effects of weight bearing stand training for each stimulation setting. This hypothesis will be tested using the contrasts between the stimulation settings both with and without weight bearing stand training. See Section G for more details.

Hypothesis 3b. CV-scES selected for cardiovascular function combined with task specific stand training using Stand-scES will result in greater improvement in respiratory, bladder, bowel and sexual function than CV-scES alone.

Rationale: Exercise programs involving limb muscles are known to increase fitness and improve ventilatory function in individuals with chronic SCI [81, 82]. However, none of the respiratory rehabilitative modalities have yet been proven to be clinically effective in patients with chronic SCI [83]. Preliminary results of locomotor training studies from our center indicate that the weight bearing 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 [84]. We have also found that weight-bearing exercise improves bladder function. Thus the combination of CV-scES with scES stand training will provide the greatest effect.

Methods: Linear Statistical Models will be fit containing parameters for the main effect of each stimulation setting (Vol, CV, and Stand) and additive effects of weight bearing stand training for each stimulation setting. This hypothesis will be tested using the contrasts between the stimulation settings both with and without weight bearing stand training. See Section G for more details.

Specific Aim 4.

Measure impact of scES and training on quality of life.

Secondary Analyses:

Hypothesis 4a: Quality of Life will significantly improve with scES.

Rationale: Loss of movement results in reduced mobility and less access to the community and employment, significantly affecting individuals with SCI. They may also have low systolic blood pressure compared to uninjured or paraplegic patients [6]. These cardiovascular changes may contribute to poorer reporting of measures of general health, increased fatigue, cognitive deficits, impaired social well-being, and depression [85-92], leading to a decreased quality of life. We will administer quality of life measures to assess the impact of scES and training on these individuals' quality of life. We will use Spinal Cord Functional Index (version 1), selected scales from the Spinal Cord Injury-Quality of Life (SCI-QOL) measurement system and the EuroQoL 5-Dimensional 3 Level Scale (EQ-5D-3L). The SCI-QOL measurement system employs a computerized adaptive testing (CAT) approach to provide a reliable, valid and practical means for multi-dimensional quality of life assessment. The SCI-FI and SCI-QOL build on the work of the Patient Reported Outcomes Measurement Information System (PROMIS) and the Neurology Quality of Life Initiative (Neuro-QOL). Classical and contemporary test development methodologies were employed to develop the SCI-QOL.

Qualitative input was obtained from individuals with SCI and clinicians through interviews, focus groups, and cognitive debriefing. Item pools were field tested in a multisite sample (n=877) and calibrated using 2-parameter item response theory methods. Initial reliability and validity testing was performed in a new sample of individuals with traumatic spinal cord injury [93-95]. The SCI-QOL is a validated, reliable measurement

system consisting of psychometrically sound measures for individuals with SCI. The SCI-QOL also links to other measures designed for a general medical population.

The EQ-5D-3L captures an individual's self-reported health status at the time they complete the questionnaire. The EQ-5D-3L consists of 2 pages – the EQ-5D descriptive system and the EQ visual analogue scale (EQ-VAS). The EQ-5D-3L descriptive system comprises the following 5 dimensions: mobility, self-care, usual activities, pain/discomfort and anxiety/depression. Each dimension has 3 levels: no problems, some problems, extreme problems. The respondent is asked to indicate his/her health by ticking (or placing a cross) in the box against the most appropriate statement in each of the 5 dimensions. The EQ VAS records the respondent's self-related health on a vertical, visual analogue scale where the endpoints are labeled 'Best imaginable health state' and 'Worst imaginable health state.' This information can be used as a quantitative measure of health outcome as judged by the individual respondents [96]. Furthermore, we will conduct qualitative interviews.

Methods: In this study, we will employ SCI-FI scales to examine intervention impacts on the following quality of life domains: Basic mobility, Self-care, Fine Motor Function, Wheel Chair Mobility, and Ambulation. The SCI-QOL measures include: Bladder Management, Bowel Management, Pain Interference, Depression, Anxiety, Resilience and Ability to Participate in Social Roles/Activities. We will also administer the PROMIS global satisfaction with sexual function along with the Pure Pain Scale and the Revised Life Events Survey. We may assess these pre-usual care, post-interventions and during follow-ups.

Additionally, we will employ the EQ-5D-3L scales to examine the intervention impacts on the following quality of life domains: mobility, self-care, usual activities (e.g. work, study, housework, family or leisure activities), pain/discomfort, and anxiety/depression. Finally, we will conduct qualitative interviews using an interview guide developed specifically for this study that has been pilot tested.

Specific Aim 5.

Measure impact of scES and training on healthcare resource utilization.

Secondary Analyses:

Hypothesis 5a: Healthcare resource utilization will decrease with scES.

Rationale: SCI injuries are often accompanied by respiratory, cardiovascular, bladder, bowel and sexual dysfunction requiring greater healthcare utilization. The economic impact on the increased use of healthcare can be devastating. It is estimated that the total cost of care in the years after the first year of injury is \$165,554. The total cost of care includes the cost of hospitalization each year, nursing home care, outpatient services, physician fees, equipment, environmental modifications, supplies, attendant care, and vocational services [97].

Methods: We will collect healthcare resource utilization information from each research participant's insurance provider from two years prior to the intervention to two years after the intervention. We will also enroll a control group to more precisely estimate changes in resource utilization. The control group will be selected from the Neurorecovery Network (NRN) and will be enrolled under a separate protocol. Patients with complete or incomplete cervical or thoracic SCI are referred to the NRN and there receive intense locomotor training for three to four months. Controls will be matched on age, level of severity, duration of injury, and insurance type. Resource utilization data will be assigned to one of the cost categories above and a cost estimate will be assigned to the resources.

D. Outcomes

1. Functional Movement Assessments (Voluntary movement of legs and trunk)

a. Multi-Segmental Motor Responses (MMR).

Procedure: Motor responses in different leg muscles can be evoked by non-invasive stimulation of the dorsal lumbosacral spinal cord and recorded [98-100]. Such Multisegmental Monosynaptic Reflexes (MMR) [101, 102] are the basic components of the lower-limb muscle responses that are elicited by epidural stimulation of posterior lumbar cord structures. MMRs will be evoked by stimulating the spinal cord percutaneously between the T9 and L4 spinous processes. The AgCl cathode (pre-gelled, soft surface electrode) will be placed over the skin between T9 and T10, T10 and T11, T11 and T12, T12 and L1, L1 and L2, L2 and L3, and L3 and L4 spinous processes and two 50 x 100 mm large anodes will be placed bilaterally over the anterior spine of the iliac crest. The optimum site of stimulation will first be located by a hand-held electrode. The site of stimulation will be selected based on where the motor responses can be elicited in all the recorded muscles as

symmetrically as possible. A secured piece of foam rubber will be placed over the cathode with a strong elastic band wrapped tightly around the body.

Analysis: The MMR amplitude will be quantified as the peak-to-peak amplitude and/or area under the rectified curve using custom MATLAB R011A® software and/or Labchart 8.7® scripts. Recruitment curves will be constructed by plotting the MMR amplitude against stimulation intensity and threshold intensity, rate of recruitment, and plateau intensity will be identified per muscle using custom MATLAB R011A® software.

b. Functional Neurophysiological Assessment (FNPA).

Procedure: The FNPA assesses the motor capacity and control of the upper and lower extremities and trunk. Bilateral low-noise pre-amplified surface EMG electrodes with fixed space of 1.7 cm between the contacts are placed on skin using electrode gel over multiple muscles of upper and lower extremity and trunk including, but not limited to sternocleidomastoid (SCM), upper trapezius (UT), biceps brachii (BB), triceps brachii (TB), extensor carpi radialis (ECR), flexor digitorum profundus (FDP), abductor digiti quinti (ADQ), external intercostal (IC6 - 6th intercostal space), rectus abdominus (RA), erector spinae (EST10 - lateral to T10 spinous process), rectus femoris (RF), vastus lateralis (VL), medial hamstrings (MH-semitendinosus), tibialis anterior (TA), extensor digitorum longus (EDL) and lateral soleus (SOL), in individuals with injuries above T2. In individuals with injuries below the T2 neurological level, and thus with no impairment of upper limb musculature, BB, TB, ECR, FDP, and ADQ, were replaced with external oblique (EO), erector spinae at L2 (ESL2), peroneus longus (PL), medial gastrocnemius (MG), and flexor hallucis brevis (FHB) for greater resolution in the motor segments below the lesion. We may also use fine-wire EMG to acquire activity from the ilio-psoas, extensor hallucis longus, extensor digitorum longus or other deep muscles [103].

The study protocol contains a variety of tasks that the research participant will be asked to perform in the supine position. These tasks include, but are not limited to relaxation, reinforcement tasks, and intentional active movement of each joint. Vibration, assessment of reflexes, and plantar withdrawal will also be a component of this protocol. Protocol presentation rate will be adjusted to a comfortable pace for the research participant who may be reinstructed as needed for any of the motor tasks presented. The volunteer will be asked to relax for a minimum of 5 minutes at the beginning of the study to acquire a baseline of the electrical noise in his/her muscles and in the room. Reinforcement maneuvers, including deep breath, shoulder shrug, neck flexion with and without resistance, or an alternate Jendrassik maneuver will be performed at the beginning of volitional testing. Corresponding volitional motor tasks will then be performed to match the muscles being recorded at that time. Reflexive testing, including deep tendon reflexes, clonus, Babinski, and reaction to vibration may be performed after volitional testing. This may be done with Voluntary Spinal Cord Epidural Stimulation (Vol-scES).

Analysis: To quantify responses, data will be rectified mean, integrated and burst duration will be calculated for each attempt per muscle. Averages and standard deviations of average values per muscle will be calculated from three attempts in all events except relaxation. Relaxation will be divided into 30 second intervals. Average and standard deviation of integrated and mean values will be determined from 10 intervals in 5 minute of relaxation per muscle per person.

c. Neuromuscular Recovery Scale (NRS).

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 [104, 105], 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.

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 quinti (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), peroneus longus (PL), medial gastrocnemius (MG), soleus (SOL), flexor hallucis brevis (FHB) or longus (FHL), extensor hallucis 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 ilio-psoas (IL), or other deep muscles, including the hand and feet muscles listed above. Standard needle insertion sites for each muscle will be used [106].

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, an 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.

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%.

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.

d. Neuromuscular Voluntary Movement Assessments.

Procedures: Assess specific leg and trunk muscle electromyography (EMG) and force generation during targeted intentional movements. EMG, kinematic and kinetic analysis may be performed on the lower extremities and/or trunk during voluntary movement attempts with and without Voluntary-sCES. Lower extremity and trunk muscle activation patterns will be evaluated using EMG soleus (SOL), medial gastrocnemius (MG), tibialis anterior (TA), medial hamstrings (MH), quadriceps (VL and RF), adductor (AD) and/or of related muscles using the MA300 System (Motion Lab Systems, Baton Rouge, LA). 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 bilaterally. We may also use fine-wire EMG to acquire activity from the ilio-psoas, extensor hallucis longus, extensor digitorum longus or other deep muscles muscle. Standard needle insertion sites for each muscle will be used [106]. Limb kinematics may include hip, knee and ankle angles that will be acquired using high speed passive marker motion capture (Motion Analysis, Santa Rosa, CA). Force generation will be measured with force transducer via a non-elastic cable throughout all supine movements (flexion and extension of the toes, ankles, knees and hips). 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.

Participants will be in a supine or sitting position. Participants will be asked to perform voluntary movements in response to verbal commands, visual cues or auditory cues. We will ask participants to perform movements requiring different levels of precision and endurance. 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). This may be done with scES.

Analysis: Efficacy in voluntary movement will be measured by calculating peak force relative to stimulation amplitude. Task specific leg muscle electromyography outcomes (amplitude, duration, and onset and offset) appropriate for the movements will also be used to assess quality of movement. 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. Other comparisons used in the analysis will include amplitude and duration of force generation; rate of movement; and accuracy of movement. Accuracy of movement relative to visual and auditory cues will be analyzed by slope and shape comparison between the computer generated signal and those produced by the movement [107].

e. Lower Extremity Torque Assessment with Electromyography (EMG)

Procedure: Participant will be sitting, lying supine or sideways on a Biodex Dynamometer. This dynamometer is commonly used in rehabilitation facilities to measure skeletal muscle force production. Participant may be strapped at the waist and/or chest into the chair attachment near the hip, knee or ankle. Isometric or isokinetic contraction of different lower limb muscles (i.e. triceps surae, tibialis anterior, quadriceps femoris and hamstrings) will be performed by the research participant voluntarily and/or combined with epidural stimulation. Voluntary-scES will be applied at optimal voltage. Lower extremity and trunk muscle activation patterns may be evaluated using electromyography soleus (SOL), medial gastrocnemius, tibialis anterior, medial hamstrings, quadriceps, adductor and/or related muscles using the MA300 System (Motion Lab Systems, Baton Rouge, LA). Electromyography 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 bilaterally. We may also use fine-wire electromyography to acquire activity from the ilio-psoas, extensor hallucis longus, extensor digitorum longus or other deep muscles muscle. Standard needle insertion sites for each muscle will be used [115]. We may ask the participant to practice moving their legs with Voluntary-scES in the Biodex Dynamometer during their training intervention. When they are practicing, these sessions will be done without EMG.

Analyses: Torque characteristics including mean, peak, and integrated torque, torque duration, and torque derivation of voluntary and electrical stimulation attempts will be calculated using customized Labview (National Instruments, Austin, TX) software. Efficacy in voluntary movement will be measured by calculating peak force relative to stimulation amplitude. Task specific leg muscle electromyography outcomes (amplitude, duration, and onset and offset) appropriate for the movements will also be used to assess quality of movement. Electromyography data will be full wave rectified and filtered using a 4th order bandpass Butterworth filter (40Hz - 500 Hz) representing the relative number and frequencies of the motor units recruited per burst. Integrated electromyography will assess the total electromyography activity generated during specific phases of the motor tasks.

2. Cardiovascular.

a. Ambulatory BP and Heart Rate monitoring.

Procedure: Twenty-four hour ECG will be performed using Edan 3-channel Holter with continuous heart rate monitoring. Twenty-four-hour ABPM will be performed using the ABPM-05 (Meditech) monitor. The device will be fixed with appropriate cuff sizes to the non-dominant arm and pre-programmed such that systolic blood pressure (SBP), diastolic blood pressure (DBP), and heart rate (HR) will be recorded automatically every 15 minutes during their daytime active period, then hourly overnight. For participants who have a scheduled bowel routine within the 24-hour monitoring period, recordings at 5-minute intervals for 30 minutes before, during, and for 30 minutes after the routine will be programmed. Additionally, participants will be instructed to initiate a self-measurement any time they experience symptoms of or completed an activity that may trigger a hypo- or

hypertensive episode. Testers will provide examples of such symptoms/episodes. Additionally, the subject diary will include examples of activities that may result in hypo- or hypertensive events and instructions on how to take a self-measurement in this instance. Participants will be asked to write down notes regarding blood pressure related symptoms, activities and their time-points, and the time they wake-up and fall asleep in a participant diary. In the case of tetraplegic individuals with impaired hand function, caregivers/nurses will be asked to provide notes in the diary. Participants will be given both verbal and written instructions for wearing the device to ensure blood pressure measurements are taken accurately.

Analysis: Twenty-four-hour ambulatory blood pressure monitoring data will be stored and analyzed upon completion of the assessment [108, 109]. The EasyABPM 1.1.1.2 software package (Meditech) provides several report files once the data is loaded onto the computer from the device monitor. Baseline SBP and DBP will be established by calculating the average of 3 consecutive resting blood pressures while seated in the morning. Baseline blood pressure will be used to assess the number of hypertensive and hypotensive events during the daytime [6, 12, 110-113]. The blood pressure and heart rate data will be divided into four time period groups: Total 24 hour Period, Active Period (awake), Passive Period (asleep), and Special Event Period (morning routine or bowel program). Each time period group will be further separated into three data parameters: systolic blood pressure, diastolic blood pressure, and heart rate. Histograms will be created demonstrating the mean percentage of time each data parameter falls within a specific data range during the allotted time period. Blood pressure will be separated into data ranges of 10 mmHg. Heart rate will be separated into data ranges of 10 beats per minute.

b. Orthostatic Stress Test

Procedure: The orthostatic stress test is utilized to assess orthostatic tolerance, to diagnose orthostatic hypotension, evaluate baroreflex responses, and assess beat-to-beat blood pressure and heart rate variability in individuals with SCI [114, 115]. Each participant will be assessed in the morning in a quiet, temperature-controlled (~72°F/22°C) laboratory at the Kentucky Spinal Cord Injury Research Center. Their diet will be restricted to exclude caffeine, alcohol, and foods that are high in fat the evening prior and the morning before the study. Participants will be asked to void their bladder right before arriving and to have completed their bowel program in advance if possible.

Participants will be placed in a cardiac chair (Chair Hydraulics, Steris Corp., Mentor, OH) or on a tilt-table (Hausmann Wheelchair Accessible Hi-Lo Tilt-Table, Patterson Medical, IL). Before the recording begins, each participant will be acquainted with the equipment and study setup. Continuous beat-to-beat arterial blood pressure will be recorded from a cuff placed around the finger using a Finapres Medical Systems unit. Brachial arterial blood pressure measurements will be taken periodically with a Dinamap V100 (GE Medical Systems) device for calibration and as a reference. A 3-lead or 12-lead ECG (ML132, AD Instruments) will be placed for electrocardiographic monitoring. Respiratory kinematics (chest and abdominal wall movements) will be acquired using two belt transducers (AD Instruments). Participants will rest in the supine position to allow for relaxation after the preparatory period. Next, baseline values will be recorded in the supine position. Then participants will be passively moved into the upright seated position in the cardiac chair or into the head up position on the tilt table (20-90 degrees) (Wecht et al. 2009, Wecht et al. 2003). While the participant is seated or tilted, we will record cerebral blood flow by transcranial Doppler, ultrasound of the heart and vessels, and pulse wave velocity (all of which are described in this protocol and previously approved) in addition to the standard beat-to-beat blood pressure and heart rate. The assessment will be stopped if: the participants experience symptoms of Orthostatic Intolerance, if high blood pressure persists without normalizing, or if the participant requests to be returned to the supine position.

While the participant is supine, seated, or head up tilted the Orthostatic Intolerance Questionnaire will be administered periodically to determine if the participant is experiencing symptoms of orthostatic intolerance. The Orthostatic Intolerance Questionnaire is a 0-10 scale (0= no symptom and 10= intolerable) questionnaire that ranks the following symptoms: headaches, dizziness, blurred vision, nausea, weakness, confusion, fatigue, ringing of ears, and passing out. Symptom severity, or lack thereof, while upright will be compared to the participant's symptoms in the supine position.

During the orthostatic stress test, there may also be blood drawn to measure plasma catecholamines. In this case, prior to beginning the study, a butterfly catheter will be inserted into an antecubital vein to allow for the collection of blood samples at the different time points. Eight milliliters of venous blood will be drawn from an antecubital vein at the end of the supine phase to assess baseline epinephrine and norepinephrine levels. In the seated or head up tilted position, to assess the catecholamine responses to orthostatic stress, blood will

be drawn at minutes 3, 10, and at the end of the upright or tilted period. If the participant experiences symptoms of orthostatic intolerance or requests to end the seated/tilted position early, blood will be drawn immediately upon return to the supine position.

In individuals with the implanted stimulator, cardiovascular parameters at rest and in response to orthostatic challenge (sit up or tilt table positioning) may also be examined during stimulation, with optimal stimulation parameters determined previously using an established protocol.

Analysis: Beat-to-beat systolic blood pressure, diastolic blood pressure, heart rate, and R-R intervals will be calculated from continuous blood pressure recordings and electrocardiography throughout the supine and upright/tilted periods. During the sit-up and head-up tilt, mean blood pressure and heart rate will be analyzed in 1-minute intervals during the supine and upright positions. During head-up tilt, continuous blood pressure will be analyzed to determine the percentage of systolic blood pressure beats that fall below 110 mmHg, as well as the percentage of beats that are decreased from average supine systolic blood pressure by 10 mmHg or more. Spectral power values and baroreflex variables will be calculated during the supine rest phase (see below). For sit-up and head-up tilt positions, spectral power / baroreflex variables will be analyzed during the 5-minute window that includes around the lowest 1-min systolic blood pressure interval; these outcomes may also be analyzed during the position change on the tilt table. We will also track the time duration of the head up tilt position as an outcome measure of orthostatic tolerance.

Autonomic balance will be assessed by spectral power density, based on Welch's modified periodogram technique. Beat-to-beat variables will be interpolated with a 5 Hz sampling rate by using a cubic spline, and then detrended with a straight line fitted to the data series. Power spectral density estimates will be made from 1-minute windows with 50% overlap. 512 points will be used in FFT. Spectral power will be calculated for low (LF, 0.04-0.15 Hz) and high (HF, 0.15-0.4 Hz) frequency regions by integrating the power spectral density curve by using trapezoidal integration.

The baroreflex sequence technique will be applied to evaluate baroreflex function. Beat-to-beat time series of systolic blood pressure and RR-interval will be scanned for consecutive beats that contain increasing and decreasing pressures and increasing and decreasing RR intervals. Three consecutive increasing or decreasing beats with an interbeat-difference of at least 1 mmHg and 4 ms will be identified as a "sequence." Systolic blood pressure sequences with an RR-interval sequence that follows, delayed by one beat, and a coefficient of determination $R^2 > 0.85$ will be coupled. The mean slope of all coupled sequences will be used to estimate baroreflex sensitivity (ms/mmHg). Baroreflex effectiveness index will be estimated by the ratio of coupled sequences compared to the number of systolic blood pressure sequences overall.

Analysis of blood samples will be conducted by Jewish Hospital, LabCorp, Quest Diagnostics, Dr. David Goldstein's analysis laboratory at the National Institute of Health, or other licensed biochemical laboratories.

c. Echocardiography.

Procedure: Research participants will be positioned on an echocardiography table in the left-lateral decubitus position. There will be a 3 lead ECG during procedure connected to participant as well as a blood pressure cuff. The participant's BP will be taken 5-6 times throughout procedure. Images will be recorded with state of the art echocardiography equipment (e.g. Philips X5-1 MHz xMATRIX array transducer on a Philips EPIQ 7 ultrasound system). A variety of standard, non-invasive measurements will be performed. Views will be taken in the parasternal long axis (PLAX), apical 4 chamber (A4C), apical 2 chamber (A2C), apical 3 chamber (A3C), and from the subcostal view (SubC). Using M-mode we may measure the Tricuspid Annular Plane Systolic Excursion (TAPSE). Five consecutive cardiac cycles will be recorded at the end of a tidal expiration and the mean value will be recorded for each parameter. Frame rate and imaging depth will be kept constant during within-subject acquisition. Four consecutive cardiac cycles will be recorded for off-line analysis [116]. To adjust for inter- and intra-individual variability of heart rate, raw data will be normalized to the percentage of systolic and diastolic duration using cubic spline interpolation of systolic and diastolic data points (Strain Analysis Tool, custom built software). The research participants will then lay on their back and Subcostal views may be taken of the Inferior Vena Cava (IVC) and the Hepatic Vein. The research participant is then repositioned to an 80 degree angle for much of the same measurements. This may be done with CV-scES.

Analysis: Left Ventricular dimensions will be taken from the parasternal long axis (PLAX) view. This will include left ventricular internal dimension in diastole (LVIDd) and systole (LVIDs), the interventricular septal dimension (IVSDd) in diastole and the left ventricular posterior wall dimension in diastole (LVPWDd). The cross sectional area of the left ventricular outflow tract (LVOT) will be measured here also. Volumetric measurements may be determined from the A4C & A2C view using the modified Simpson's method in 2D and

3D. The LV Mass will be measured in 3D. Left ventricle inflow velocities during early and late diastole will be assessed using pulsed-wave and continuous wave Doppler at the mitral valve leaflet tips. Left ventricle outflow velocities will be assessed using pulsed-wave and continuous wave Doppler in the A4C or A3C views. Myocardial tissue velocities during systole, early diastole, and late diastole will be assessed using pulsed-wave tissue Doppler imaging of the septal wall at the level of the mitral annulus and at the right ventricular free wall. If we can get the full right ventricle in view we may measure the right ventricular area change and RV strain. From the A4C and A2C view the LA Volume will be measured along with the right upper pulmonary vein via pulse wave doppler. The RA Volume will be measured in the A4C view. LV strain may be assessed using parasternal short-axis at the papillary level, along with the A4C, A2C and A3C view using the program Echo Insight. Frame-by-frame twist and twist velocity values will be obtained by subtracting the apical rotation/rotation velocity from the basal rotation/rotation velocity. Myocardial dyssynchrony will also be examined by segmental analysis of LV mechanics. Standard dimensions, wall thickness, chamber volumes, systolic and diastolic function parameters will be calculated using commercially available software (e.g. Phillips and Echo Insight). Baseline comparisons to future measurements throughout training will be performed to assess for trends. Overall comparisons of measures from baseline to end of training will be performed using one-sided student's t-tests as the participants are their own controls with a $p < 0.05$ being considered statistically significant. For non-normally distributed variables the appropriate non-parametric test will be used for analysis (e.g. Kruskal-Wallis).

d. Arterial Pulse Wave Velocity (aPWV,m/s).

Procedure: This assessment will be measured non-invasively and calculated by dividing the distance between measurement (meters) sites by the pulse transit time (seconds). The subject will be in the supine position during the assessment. The distance between the arterial measurement points will be assessed using measuring tape along the surface of the body, held parallel to the testing table. The pulse transit time will be determined from the arterial blood pressure waves, which are collected at each artery, recorded from special sensors. A Complior Pulse Wave and Central Pulse Analyzer (Aim Medical, Vincennes, France) sensors will be applied to the carotid, femoral, brachial, or other arterial sites. A minimum of 10 consecutive pulses are required for analysis. This procedure has been previously described by Phillips et al 2014. Heart rate will be recorded using a single lead electrocardiogram (ECG) (ModalML 123, AD Instruments Inc, Colorado Springs, CO). This may be done with CV-scES.

Analysis: The Complior software package provides experimental acquisition and data report functions. Upon completion of the test, the software generates a document containing the Pulse Wave Velocity outcome measures and percentile for each participant.

e. 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. This may be done with CV-scES.

Analysis: The SSR recordings are among the frequently utilized techniques for the evaluation of integrity and functional capacity of the autonomic circuits after SCI [101, 102, 114, 117]. The outcomes will be calculated as a number of recognizable responses out of 10 stimulations, and averaged latency and averaged amplitude [118].

f. Vascular Ultrasound.

Procedure: Doppler ultrasound (Philips EPIQ 7 ultrasound system) will record blood flow, vessel structure, and vessel diameter of arteries and veins while the participants are supine, sitting, and/or tilted. Continuous beat-to-beat arterial blood pressure will be recorded from a cuff placed around the finger using a Finapres Medical Systems unit. Periodic brachial arterial blood pressure measurements will calibrate and confirm the finger waveform. A 3-lead or 12-lead ECG (AD Instruments) will monitor electrocardiographic activity. A Phillips L12-3 ultrasound transducer will be placed superficially and laterally over the blood vessel to be recorded at a fixed angle of 60 degrees. Arterial wall segments will be recorded longitudinally. Anatomic landmarks (i.e., bifurcation of the femoral and deep femoral arteries, bulb of the common carotid arteries) will ensure measurements are obtained from the same region for within and without participant reproducibility. Vascular ultrasound data will be saved as three-beat, five-beat, seven-beat, or 60-second clips and stored for offline analysis. Participants may be asked to alter their

breathing (i.e., inspire fully, expire slowly, suspend breathing, etc.) to enhance the ultrasound images. Frame rate and image depth used will be consistent within subjects. Testing procedures should take one hour to complete.

Analyses: Doppler ultrasound of arteries will measure flow and velocity of blood within the arteries, diameter of the arteries, and thickness of the intimal and medial layers. Peak and minimum arterial flow and velocity from three to seven consecutive cardiac cycles will be measured from the Doppler spectra and respective means and indices will be reported for each individual artery. Diameter of the arteries (cm) will be measured at systole (maximum diameter of the vessel) and diastole (minimum diameter of the vessel). Diameter will be measured as the distance from the inner near-wall intimal layer to the inner far-wall intimal layer, perpendicular to the vessel. Systolic and diastolic diameter and related indices will be reported each as the mean of three to seven consecutive cardiac cycles. Combined thickness of the intimal- and medial-layers of the common carotid artery will be measured from a 10-mm-long straight segment, free from atherosclerotic plaques. Thickness (mm) will be measured as the distance between the lumen-intima interface and the media-adventitia interface of the far wall. The mean of three to seven measurements obtained during diastole will be reported. To measure distensibility of the common carotid artery, continuous finger blood pressure waveform will be synchronized to the diameter of the common carotid artery recorded by ultrasound. Pulse pressure (ΔP), carotid artery diameter during diastole (D_d) and the maximum change in diameter during the cardiac cycle (ΔD) will be measured for each beat. Distensibility (mmHg^{-1}) will be calculated as $\Delta D / \Delta P / D_d$.

Doppler ultrasound of veins will measure velocity of blood during systole and diastole. The mean of three to seven successive cardiac cycles will be reported as systolic velocity (cm/s) and diastolic velocity (cm/s) within each respective vein. The ratio of systolic to diastolic velocity (systolic/diastolic) will be calculated from the means of each vessel and reported.

In individuals implanted with an epidural stimulator unit, Doppler ultrasound of arteries and veins may also be performed with stimulation using previously determined optimal stimulation parameters.

g. Transcranial Doppler.

Procedure: The Transcranial Doppler assessment is used to measure cerebral blood flow velocity in order to assess neurovascular coupling, cerebral autoregulation, and cerebrovascular reactivity (Willie, C.K. et al 2011). Participants will be assessed in a quiet, temperature-controlled laboratory at the Kentucky Spinal Cord Injury Research Center. Participants will be asked to abstain from caffeine, alcohol, and high-calorie meals in the morning before the study. Upon arrival, participants will be asked to empty their bladder before recording starts.

These assessments will utilize an ST3 Transcranial Doppler (Spencer Technologies) to measure Middle Cerebral Artery (MCA) and Posterior Cerebral Artery (PCA) blood flow velocity. The recordings will occur via two – 2MHz probes mounted bilaterally on the temporal bones using a fitted head-set. Ultrasound gel will be applied to the probes and the skin for better reading accuracy. During these experiments, we may record blood pressure, ECG, and respiratory kinematics, as detailed above using equipment and methods used during the *Orthostatic Stress Test*. We may also record end tidal CO_2 (ETCO_2) using a nasal cannula and gas analyzer (Gemini Respiratory Monitor, CWE Inc or 17515 CO_2 Analyzer Gold Edition, VacuMed). The PCA will be insonated at depths of 60-75 mm, while the M1 segment of the MCA will be insonated at 45-60 mm depth. Insonation of the desired artery will be confirmed using the angle of the probe, depth of insonation, the blood flow velocity of the artery, and the characteristics of the velocity waveform.

Once the desired arteries are insonated and confirmed, we will record up to 15 minutes of baseline values. We will ask the participant to perform a visual task to measure neurovascular coupling. This task involves up to 10 iterations of 30 seconds of “eyes closed”, followed by 30 seconds of “eyes open” according to the examiner’s instruction. During the “eyes open” component, the participant will be asked to follow a visual stimulus with their eyes. The visual stimulus may be the examiner’s hand or a moving image on a computer screen.

We may record the Transcranial Doppler during the Orthostatic Stress Test, scES, or portions of the Respiratory Motor Control Assessment which are all described in this protocol.

Analysis: Raw cerebral blood flow velocity (CBFv) waveforms will be inspected for noise and large spikes (<100 ms) will be removed using linear interpolation. Smaller non-physiological spikes will be removed by low-pass filtering all data at 20 Hz with a zero-phase Butterworth filter. ECG will be used to identify R peaks and calculate R-R intervals. Mean cerebral blood velocity and blood pressure values, calibrated periodically against brachial-cuff pressure recordings, will be calculated for each cardiac cycle. Mean values from baseline recordings will be compared to recordings from the experimental conditions described above.

R-R intervals, ETCO_2 , blood pressure, and CBFv data will be upsampled at 5 Hz with cubic spline interpolation and spectral analysis will be performed to identify a physiological mechanism for CBF responses.

3. Standing.

a. Standing Assessments (Independence and EMG).

Procedure: Assess the level of external assistance and leg and/or trunk muscle electromyography (EMG) during standing. EMG, kinematic and kinetic analysis may be performed on the lower extremities and/or trunk during standing with and/or without Stand-scES. Lower extremity and trunk muscle activation patterns will be evaluated using EMG that may include soleus (SOL), medial gastrocnemius (MG), tibialis anterior (TA), medial hamstrings (MH), quadriceps (VL and RF), adductor (AD) muscles and/or related muscles using the MA300 System (Motion Lab Systems, Baton Rouge, LA). 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 bilaterally. We may also use fine-wire EMG to acquire activity from the ilio-psoas, extensor hallucis longus, extensor digitorum longus and/or other deep muscles muscle. Standard needle insertion sites for each muscle will be used [106]. Limb kinematics may include arm, trunk, hip, knee and ankle 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 HRMat (TEKSCAN, Boston, MA) or forces during movement with a force transducer (Kistler, Amherst, NY).

Participants will be placed either in a body-weight supported harness over a treadmill (i.e. BWST) or on a customized over ground standing apparatus comprised of horizontal bars anterior and lateral to the individual. These bars will be used for upper extremity support and balance assistance as needed. Bungee cords will be placed across the upper tibias and hips for dynamic support. The participant will begin the sitting-to-standing transition by using the horizontal bars of the standing apparatus for assistance and support; trainers positioned at the trunk, pelvis and knees will manually assist as needed during this transition. If the participant's upper limbs and trunk control is insufficient to safely use the standing apparatus, he/she will be placed on the treadmill, and a body weight support system with a harness will be used to avoid trunk collapse and knee buckling. If, during standing, the knees, hips or trunk flex beyond the normal posture, assistance at the knees distal to the patella, at the hips below the iliac crest, and at the upper trunk will be provided manually by trainers to promote extension. Trainers will also promote slight knee flexion and extension to facilitate dynamic weight bearing to enhance neuromuscular activation. Research participants will take a break and rest at any time they feel the need to during the session. Research participants may take a break at any time during the session. 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. These assessments may be done with scES.

Analyses: 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. Other comparisons used in the analysis will include amplitude and duration of force generation; rate of movement; and accuracy of movement. [107]..

b. Dual-energy X-ray absorptiometry (DXA).

Procedure: Bone mineral density and body composition analysis will be performed by a Dual-Energy X-Ray Absorptiometry (DXA, Model Prodigy, GE Lunar, enCore version 10.5) bone densitometer. DXA is a method utilized to measure bone density by using high, 140kVp and low, 100kVp X-rays and multiple detectors, dual energy X-ray fan-beam, and a rotating C-arm. The beam sweeps across a region of interest on the scan area in a fan-shaped pattern, and is detected by a high-resolution multi-detector array to form a high quality image. The basic principle of the DXA data acquisition is based on the different bone and soft tissue attenuation characteristics at two pulsed X-ray levels. The system is calibrated using a drum comprised of known amounts of bone and soft tissue equivalent materials that is placed in the beam. As the beam passes through the participant, lower energy X-rays than higher energy X-rays are absorbed by the anatomical structures in the participant. The detectors in the C-arm then register the beam. The raw scan data, containing the attenuation values of tissue, bone, and the calibration value are relayed to a computer.

Analysis: Points and regions of interest are placed based on set protocols defined by Lunar Prodigy enCore software and according to research protocols for modified scans of the knees and ankles. The software algorithm interprets each pixel and creates an image and quantitative measurement of the bone and body

tissues. Bone mineral density and content values are analyzed for the total body, lumbar spine, lower thoracic anterior to posterior spine, hips, knees, ankles, and forearm of the dominant hand. Analysis of fat mass, lean mass, and percent fat mass can be reported for the entire body and head, arms, trunk, pelvis, and legs.

4. Respiratory.

a. Spirometry and Airway Pressure Measurements (Pulmonary Function Test).

Procedure: Standard Spirometry [119] will be performed in the seated and/or supine position by using the preVent™ pneumotach BreezeSuite Spirometer and Software (MedGraphics, St. Paul, MN). Forced Vital Capacity (FVC) and Forced Expiratory Volume in 1 Second (FEV₁) will be obtained and expressed as the percent of the predicted value for each subject based on a database of neurologically intact individuals, with no known pulmonary deficits, based on gender, age, height, weight, and race [120].

For measurements of airway pressure, the MP45-36-871-350 Differential Pressure Transducer with a UPC 2100 PC card and EasySense Software (Validyne Engineering, Northridge, CA) will be used to measure Maximum Expiratory Pressure (PE_{max}) and Maximum Inspiratory Pressure (PI_{max}). The PE_{max} will be measured during a maximal expiratory effort starting from total lung capacity and the PI_{max} will be measured during a maximal inspiratory effort beginning at residual volume [121, 122]. The mouthpiece incorporates a three way valve system together with plastic tubing from a Ventilatory Monitoring Adapter Circuit Kit (Airlife 001504). The mouthpiece includes a 1.5mm diameter leak hole to prevent glottis closure and to reduce buccal muscle contribution during the test [123, 124]. The assessment will require a sharp forceful effort to be maintained for 2 seconds. This assessment may be done with scES.

Analysis: For the standard spirometry assessment, three acceptable spirograms will be obtained and the results (FVC and FEV₁) from the best attempt will be reported. For airway pressure measurements, the maximum pressure will be taken as the highest value sustained over a 1 second interval [125]. The three best attempts varying by less than 20% will be averaged.

b. Respiratory Motor Control Assessment (RMCA).

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 (Inductotrace, Ambulatory Monitoring), and surface Electromyography (sEMG) (Motion Lab Systems, Inc, Baton Rouge, LA) of the muscles of respiration.

Respiratory muscle activation patterns will be evaluated using sEMG of respiratory-related muscles using the MA300 System (Motion Lab Systems, Baton Rouge, LA) [121]. 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 [126, 127]. The protocol begins in the seated position and consists of the following maneuvers followed by 5 minutes of relaxation in supine position: Spirometry, PE_{max}, PI_{max}, PE_{max} and PI_{max} 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). This assessment may be done with scES.

Analysis: The envelope of EMG activity for each muscle will be calculated using a root mean square (RMS) algorithm [127]. 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 [126, 127]. 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.

c. Resting Metabolic Rate (RMR).

Procedure: The resting or basal metabolic rate is the rate of energy expenditure by humans at rest (reported as kilocalories). The release and consumption of energy in this state is sufficient only for the functioning of the vital organs: the heart, lungs, nervous system, kidneys, liver, intestines, sex organs, muscles, and skin. The resting metabolic rate is measured under very strict circumstances when a person is awake, without moving or talking in the supine position. The test will take approximately 30-45 minutes and the participant will be required to fast for 10-12 hours beforehand. Once the participant is lying down on the hospital bed, a canopy hood will be placed over their head and shoulders to capture the expired air. The resting metabolic rate will be measured through indirect calorimetry using the Parvo Medic TrueOne 2400 system (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 the flowmeter calibration and the ambient air for the gas calibration at least 30 minutes before testing, as requested by the manufacturer. Resting metabolic rate generally decreases with age and with a decrease in lean body mass. Increases in muscle mass and mitochondrial proliferation result in increased values. We have based our procedure on the methods as previously described [128] (Buchholz & Pencharz 2004).

Analysis: Depending on the stability of the readings, the last 3-5 Resting Energy Expenditure (REE) values are averaged and recorded as the Resting Metabolic Rate. Instability in the readings may be due to voluntary movement, spasm, falling asleep or awakening, anxiety, talking, or distraction. The experimenter will judge the accuracy of the REE values based the data trends and upon observation of the participant.

5. Bladder.

a. Urodynamics.

Procedure: For the urodynamic study (necessary to determine bladder function/voiding pressures/degree of detrusor sphincter dyssynergia; [130, 131], a complex cystometrogram (to evaluate the filling phase of the bladder) with a pressure flow study and simultaneous abdominal pressures and flow rate (to evaluate the voiding phase of the bladder) will be performed. The cystometry evaluation is accomplished using standard procedures [132-134] (measuring bladder pressure during filling, possible uninhibited bladder contractions and maximum cystometric capacity), with determination of the leak point pressure and post-void residual volume when voiding is possible at the end of the study when a second uroflow can be obtained. A 12 French straight catheter will be used to empty the bladder completely for a urine sample to be assessed for the presence of blood, urobilinogen, glucose, ketones, bilirubin, protein, nitrites, leukocytes, pH, and specific gravity using DiaScreen reagent strips for urinalysis. Participants suspected of having a urinary tract infection will be triaged with the clinical research nurse as appropriate. Then a 7 French three way urodynamic catheter will be placed in the bladder to fill the bladder as well as to measure and record intra-vesical pressure. Another catheter with a balloon will be placed in the rectum to record the intra-abdominal pressure. Detrusor pressures will be calculated by subtracting the intra-abdominal pressure from the intra-vesical pressure.

The bladder will be filled at a slow rate with body temperature water. Each participant will be asked to cough to verify intra-abdominal catheter position, and will be instructed to communicate when s/he first feels a full bladder (first sensation); when s/he first feels the desire to urinate (first urge to void); and when s/he can no longer wait to void (maximum capacity). The volume of water and bladder pressure will be recorded. Uninhibited bladder contractions will be identified. Since the majority of SCI individuals have abnormal or no sensation, filling will be stopped when the participant has an involuntary contraction, increasing blood pressure (autonomic dysreflexia) or high intravesical pressures (greater than 60 cm of water). After stopping the fill, the bladder will be completely emptied and a residual volume obtained. A second fill/void cycle will then be done (starting again with an empty bladder) following the same procedure. If the participant's bladder emptied reflexively, a third assessment cycle will be done. For the third cycle, filling will cease prior to the volume that triggered the reflex void. The participant will then be asked to attempt to empty his/her bladder voluntarily; voiding bladder pressures will be recorded. In participants receiving spinal cord epidural stimulation (scES), following the initial fill/void cycle. Fill/void cycles will also be assessed using continuous scES and configurations optimized for bladder storage and voiding. Additional post-scES fill/void cycle may be performed.

During the time when cystometric evaluation is being done, either EMG needle wire electrodes will be inserted through the skin at 3 and 9 o'clock on either side of the urethra in order to record the urethral sphincter activity or EMG surface patch electrodes will be placed near the urethral sphincter (ground electrode placed on the hip) to capture muscle activity during the cystometrogram. Topical anesthetic cream will be used prior to insertion of the needle electrodes. The EMG activity will evaluate coordination of the urethral and anal sphincters during the voiding phase and during possible uninhibited contraction episodes. Detrusor-sphincter dyssynergia will be evaluated and classified according to the Blaivas classification into: *type 1 DSD* characterized by a crescendo increase of the sphincter activity that reaches its maximum at the peak of the detrusor contraction (as the detrusor pressure begins to decline, sudden complete external sphincter relaxation occurs); *type 2 DSD* characterized by clonic contractions of the external urethral sphincter interspersed throughout the detrusor contraction (these Participants usually void with an interrupted spurting stream); or *type 3 DSD*, characterized by external urethral sphincter contraction persists throughout the entire detrusor contraction (these Participants void with an obstructive stream or cannot void at all) [135, 136].

A standard human non-invasive blood pressure (NIBP) monitoring system from AD instruments (ML282B1-X) will be used to measure any physiological changes during urodynamic assessment corresponding to autonomic reflexive responses to bladder filling. The NIBP system will record systolic, diastolic, and mean arterial pressure continuously during urodynamic assessment using a finger probe that is worn by the Participant. Heart rate and inter-beat interval will also be measured using the non-invasive finger probe. After the examination, participants may continue activities of daily living.

Analysis: Detrusor pressures will be calculated by subtracting the intra-abdominal pressure from the intra-vesical pressure. The volume of water (ml) and bladder pressure (cmH₂O) will be recorded at various stages of filling as well as at capacity. Uninhibited bladder contractions will also be recorded and noted if incontinence results. Leak point and maximum detrusor pressures (cmH₂O) will be recorded on the cystometrogram (CMG). Voiding efficiency (VE) will be calculated by the following equation: $VE = (\text{amount leaked/voided}) / (\text{amount leaked/voided} + \text{post-void residual volume}) * 100$. Bladder compliance will be calculated by dividing the change in detrusor pressure during that change in bladder volume and expressed as (ml/cmH₂O)[137]. Filling sensations will be noted and are defined as: First sensation of fullness (FSF) – the first sense that there is fluid in the bladder; First desire (FD) – the feeling that you would void at the next convenient moment; Strong desire (SD) – a compelling need to void that is less comfortable to postpone; Capacity (C) – the feeling that voiding cannot be delayed any longer. Uroflow parameters will be recorded if the participant is able to void voluntarily [138]. Documentation of the flow rate (ml/sec), voided volume, residual urine and the pattern will be noted (Normal, bell-shaped curve; Obstructed, intermittent). EMG patterns of detrusor dyssynergia will be assessed as Type I, II, or III (if applicable). Values reported will be based off the first CMG recorded during urodynamics and compared to previous time points.

b. Bladder Questionnaire.

Procedure: The International Lower Urinary Tract Function and Urodynamic Basic Spinal Cord Injury Data Sets [139, 140] will be used to assess bladder function. The items on the data set include awareness of need to urinate, bladder emptying method/s and frequency, any urinary incontinence, and medications/supplements used. Subjects will be asked to complete the questionnaire on the same day they present for Urodynamics as

time is generally allotted for discussion post-assessment. A private room will be used to complete the questionnaires.

Analysis: Information recorded on the lower urinary tract function data set will be combined with results from the Urodynamics procedure performed that day in order to provide a comprehensive assessment of the participant's bladder management at that particular intervention/time point.

c. Urinalysis/Urinary Biomarkers.

Procedure: Urine samples will be obtained using a sterile 12 French straight catheter that is inserted through the urethra into the bladder for cystometry. The bladder is always emptied prior to filling for cystometry recordings, so once the volume is measured, urine samples will be placed in sterile storage vials and stored at 4C for less than 3 hours. Procedures will follow similar methods established and used by multiple groups of investigators [141-143]. Briefly, samples will be centrifuged (3,000 rpm for 10 minutes) and 1 ml supernatant aliquoted into 1.5 ml tubes, with some used to determine creatinine concentration. Creatinine levels will be measured using a Siemens Clinitek Status⁺ analyzer and Siemens Multistix Pro 10LS testing strips for urinalysis. In addition to creatinine, urine samples will be tested for the presence of blood, urobilinogen, glucose, ketones, bilirubin, protein, nitrites, leukocytes, pH, and specific gravity. The supernatant aliquots will be frozen at -80 C for later processing. Urinary NGF and BDNF concentrations will then be determined on the thawed samples using the Emax ImmunoAssay System with specific ELISA kit per manufacturer instructions [141-143].

Analysis: The concentration of NGF and BDNF in each urine sample will be extracted from a standard curve and normalized to the concentration of urinary creatinine (NGF/Cr, BDNF/Cr). All samples will be run in triplicate and the values averaged.

d. Ultrasound (Bladder and Kidney).

Procedure: An ultrasound (US) exam is a noninvasive, painless diagnostic technique that makes use of how sound waves travel through the body. When sound waves pass through the body, they bounce off tissues and organs and the reflected waves can be used to make images of the organs inside. The sound waves do not damage body tissue and there is no radiation [144]. The participant will report to the Urogenital and Bowel Lab at Frazier Rehab Institute on the 11th floor to have US imaging performed using a Phillips EPIQ 7 US scanner. Generally, no prior preparation, such as fasting or medication cessation is required. However, for a bladder US, participants may be asked not to empty the bladder prior to the procedure. The procedure should last about 45-60 minutes and is performed by a certified sonographer. Participants will be able to resume a usual diet and activities following the procedure.

The participant will be assisted on the examination table in the appropriate assessments position (see kidney and bladder evaluations). A clear, water-based gel will be placed on the skin over the analyzed area in order to allow for smooth movement of a hand-held probe (transducer) over the skin and to eliminate air between the skin and the transducer for the best sound conduction. The organs of interest will be scanned in real-time through all tissue planes in at least two orthogonal directions.

Images of both kidneys will be obtained in the longitudinal and transverse planes for purposes of comparison and to exclude absence of either kidney. The right kidney may be visualized with an anterior subcostal approach using the liver as a sonographic window. With the participant in the left lateral decubitus position he or she may be asked to take and hold a deep breath in order to extend the liver window so that it includes the inferior pole of the kidney. If parts of or the entire kidney may not be seen in this view due to interposed loops of bowel, the kidney can be imaged using an intercostal approach in the right flank between the anterior axillary line and mid axillary line. For this approach, the participant can be placed in the decubitus position with a bolster under the lower side with the arm of the upper side fully abducted, thus spreading the intercostal spaces. To obtain transverse images, the transducer is rotated 90 degrees counter-clockwise from the longitudinal plane. Once in the transverse plane, the transducer can be moved superiorly and medially, or inferiorly and laterally to locate the renal hilum. Images cephalad to the hilum represent the superior pole and those caudad represent the inferior pole. The left kidney lacks the hepatic window, necessitating an intercostal approach similar to the one described for the right flank. The kidneys will be assessed for abnormalities of the renal sinus and parenchyma such as calculi, blood flow and degrees of hydronephrosis: Mild or Grade I (any hydronephrosis up to Grade II), Moderate or Grade II (the calices are confluent resulting in a "bear's paw" appearance), or Severe or Grade III (the hydronephrosis is sufficiently extensive to cause effacement of the renal parenchyma). Other abnormalities identified including cysts and masses may require additional

diagnostic evaluation. Measurements may be made of the dimensions of abnormal findings and the length and width of the kidneys [145].

The bladder will be imaged to assess for volume, thickness, and blood flow, evidence of distal ureteral obstruction, diverticula and for calculi. The bladder will be imaged from top to bottom and from side to side, in transverse and sagittal planes, respectively. Note that while a full bladder facilitates bladder scanning, distension may be a cause of artifactual hydronephrosis and is therefore to be avoided in scanning the kidneys.

Analysis: Analysis of kidney and bladder morphology, grades of hydronephrosis, the presence of masses, cysts and/or obstructions will be recorded by the radiologist and compared to prior evaluations. All exams will be reviewed with the study doctor.

6. Bowel

a. Bowel Questionnaire.

Procedure: The International Bowel Function Basic Spinal Cord Injury Data Set [146] will be used to assess bowel function. The items on the data set include awareness of need to defecate, defecation method and bowel care procedures, average time for defecation, frequency of defecation, frequency of fecal incontinence, and medications/oral laxatives used. The average time to defecation has been modified from the original data set to include a larger choice of times (smaller ranges). Participants will be asked to complete the questionnaire on the same day they present for either Urodynamics (when bladder and sexual function questionnaires are completed) or following Anorectal Manometry. The daily voiding diary will include a place to record the length of time for defecation, so that accurate data is collected and a time-line for any changes can be detected.

Analysis: Information recorded on the bowel function data set will be combined with results from the Anorectal Manometry procedure performed that day in order to provide a comprehensive assessment of the participant's bowel management at that particular intervention/time point. The average time required for defecation will be averaged across time points for each participant.

b. Anorectal Manometry (ARM).

Procedure: This is an assessment that evaluates bowel function in individuals with constipation or stool leakage. The assessment measures the strength of the anal sphincter muscles, the sensation of fullness of the rectum, reflexes that control bowel movements and activation of the rectal and anal muscles. The participant will report to the Urogenital and Bowel Lab at Frazier Rehab Institute on the 11th floor to have ARM assessment using the Aquarius® LT system by Laborie Medical Technologies (Williston, VT, USA). Participants will be asked to perform 2 Fleet® enemas 2 hours prior to the study. Participants will be asked to refrain from eating anything during the two hours prior to the procedure. Participants may continue taking regular medications with small sips of water at least 2 hours prior to the study. However, if UDS is performed in conjunction with ARM, participants should not take bladder medication. The assessment takes approximately 60 minutes and is performed by a registered nurse.

While the participant is in the left lateral decubitus position, a small, flexible tube, about the size of a thermometer, with a balloon at the end is inserted into the rectum. The catheter is connected to the Aquarius® LT that measures the pressure. During the assessment, the small balloon attached to the catheter may be inflated in the rectum to assess the normal reflex pathways. The nurse or technician may also ask the participant to squeeze, relax, and push at various times. The anal sphincter muscle pressures are measured during each of these maneuvers. To squeeze, the participant tightens the sphincter muscles as if trying to prevent anything from coming out. To push or bear down, the participant strains down as if trying to have a bowel movement. Two measurements are obtained: first, an anal sphincter electromyography (EMG), evaluates the nerve supply to the anal muscle. Anal sphincter electromyography (EMG) is recorded with a small plug electrode placed in the anal canal. The participant then is asked to relax, squeeze and push at different times. The anal sphincter muscle electrical activity is recorded and displayed on a computer screen. Anal sphincter EMG confirms the proper muscle contractions during squeezing and muscle relaxation during pushing. The second measurement is the time it takes to expel a balloon from the rectum. For this procedure, a small balloon is inserted into the rectum and then inflated with water. The participant tries to defecate (expel) the small balloon from the rectum. The amount of time it takes to expel the balloon is recorded. Prolonged balloon expulsion suggests a dysfunction in the anorectal area. During the ARM assessment period, subjects'

blood pressure, heart rate and oxygen saturation will be monitored using the Dinamap Carescape V100 (GE Healthcare). After the examination, participants may continue activities of daily living.

Analysis: Analysis will be based on published protocol for assessing Anorectal Manometry in both healthy and spinal cord injured adults [147-149]. Pressure values during rest and squeeze will be obtained. Maximum resting anal sphincter pressure is defined as the difference between the baseline pressure (atmospheric pressure) and the maximum anal sphincter pressure at rest (At each level, i.e., 1 cm, 2 cm, and 3 cm from the anal verge). Maximum squeeze pressure is defined as the difference between the baseline pressure and the highest pressure that was recorded at any level within the anal canal during the squeeze. Maximum sustained squeeze pressure is defined as the difference between the baseline pressure and the mean of three highest values for anal sphincter pressure that was sustained for >15 s at any level within the anal canal. Squeeze duration is defined as the longest time interval, in seconds, between the onset of increase in anal sphincter pressure and the return of this pressure curve to baseline values. Pressure changes during balloon inflation will be the difference between the baseline pressure and highest intrarectal pressure, and the difference between the baseline and the highest intra-anal pressure at any level within the anal canal. The mean of the three highest rectal and anal pressures will be used to assess this reflex response. Rectoanal pressure changes when bearing down will be assessed with three attempts to bear down in order to identify the participant's defecation pattern. This recording can be used to measure the intrarectal pressure, the residual anal pressure and the percent anal relaxation. Residual anal pressure is defined as the difference between the baseline pressure and the lowest (residual) pressure within the anal canal, when the participant was bearing down. The percent anal relaxation is calculated using the following formula: percent anal relaxation = anal relaxation pressure/anal resting pressure times 100. To provide an overall index of the changes in the rectal and anal pressures during simulated defecation, a defecation index may be calculated: defecation index = maximum rectal pressure when straining ÷ minimal anal residual pressure when straining. Rectal sensation is also monitored and defined as: the lowest volumes of air that evoke a first sensation, a constant sensation of fullness/bloating (constant sensation was defined as a feeling that persisted for >15 s), a desire to defecate, and an urgent desire to defecate, and the maximum tolerable volume were recorded. Rectal compliance will be calculated from the slope describing the relationship between the balloon volume (dV) and the intrarectal pressure (dP) at steady state: compliance = dV/dP cc mm Hg.

7. Sexual Function.

a. Sexual Function in Male Participants.

Procedure: Measures of erectile function will be assessed using the full International Index of Erectile Function (IIEF) questionnaire which is readily available in many publications [150]. It has been validated in 32 languages and can be used cross-culturally [151]. Briefly, there are 15 questions that can be divided into 5 unique domains: erectile function, intercourse satisfaction, orgasmic function, sexual desire, and overall satisfaction [150]. The Erection Hardness Grading Scale (Grades 1-4) is defined as follows: increase in size of penis but no hardness (rigidity) as Grade 1; increase in size and slight increase in hardness (rigidity), but insufficient for sexual intercourse as Grade 2; increase in hardness (rigidity) sufficient for sexual intercourse but not fully hard (rigid) as Grade 3; fully hard (rigid) as Grade 4 [152, 153]. Participants will be asked to complete the questionnaires on the same day they present for Urodynamics (when bladder and bowel questionnaires are completed). A private room will be used to complete the questionnaires.

Analysis: A score of 0-5 is awarded to each of the 15 questions that examine the 5 main domains of male sexual function: erectile function (maximum score of 30), orgasmic function (maximum score of 10), sexual desire (maximum score of 10), intercourse satisfaction (maximum score of 15) and overall desire (maximum score of 10). Analysis of the questionnaire is an adjunct to the participant's detailed sexual history and examination. The following guidelines have been suggested:

1. Participants with low IIEF scores (<14 out of 30) in Domain A (Erectile Function) may be considered for a trial course of therapy with Sildenafil unless contraindicated. Specialist referral is indicated if this is unsuccessful.
2. Participants demonstrating primary orgasmic or ejaculatory dysfunction (Domain B) should be referred for specialist investigation.
3. Participants with reduced sexual desire (Domain C) require assessment of blood levels of androgen and prolactin.
4. Psychosexual counseling should be considered if low scores are recorded in Domains D and E but there is only a moderately lowered score (14 to 25) in Domain A.

b. Sexual Function in Female Participants.

Procedure: The Female Sexual Function Index (FSFI) along with the International SCI Female Sexual and Reproductive Function Basic data set will be used [58, 154, 155]. These are recommended tools for assessing female sexual function for clinical trials. The 19 question FSFI yields an overall score and 6 index scores in the following categories: desire, arousal, lubrication, orgasm, satisfaction and pain. Participants will be asked to complete the questionnaires on the same day they present for Urodynamics (when bladder and bowel questionnaires are completed). A private room will be used to complete the questionnaires.

Analysis: The questionnaire has 19 questions that assess six domains of sexual function including desire, arousal, lubrication, orgasm, satisfaction and pain. Score ranges for items 3–14 and 1–19 are 0–5 and for items 1, 2, 15, 16 are 1–5. The composite score is determined by the sum of domains multiplied by the domain factor. Add the six domain scores to obtain the full scale score (see below). The full-scale score range is from 2 to 36 with higher scores associated with a lesser degree of sexual dysfunction. It should be noted that within the individual domains, a domain score of zero indicates that the participant reported having no sexual activity during the past month. Participant scores can be entered in the right-hand column.

Domain	Questions	Score Range	Factor	Minimum Score	Maximum Score	Score
Desire	1, 2	1 – 5	0.6	1.2	6.0	
Arousal	3, 4, 5, 6	0 – 5	0.3	0	6.0	
Lubrication	7, 8, 9, 10	0 – 5	0.3	0	6.0	
Orgasm	11, 12, 13	0 – 5	0.4	0	6.0	
Satisfaction	14, 15, 16	0 (or 1) – 5	0.4	0.8	6.0	
Pain	17, 18, 19	0 – 5	0.4	0	6.0	
Full Scale Score Range				2.0	36.0	

E. Electrode Configuration Selection and Interventions.

1. Mapping Studies.

Procedure: Initially research participants will participate in mapping studies to identify the neurophysiological response to various anode and cathode selections, amplitude, frequency and pulse width combinations to provide a basis for identifying the specific configurations for each intervention. These may include ramped changes in voltage at a given frequency, ramped frequencies at a given voltage, or variations of pulse widths with different anode and cathode electrode combinations. These studies will be done as the first stimulation studies after surgery and continue throughout the study as needed.

2. Voluntary Intervention.

Procedure: Participants will participate in laboratory sessions until all optimal configurations for joint movement and trunk control are found. Participant will demonstrate safe use of the patient programmer and ability to perform the leg voluntary movement independently or in the presence of a care giver. This will take approximately 3-5 days. Once the participant has demonstrated safe use of programs they will be allowed to conduct the sessions at home. Participants will be asked to train daily (7 days a week) for a total of 80 sessions.

Participants in the Voluntary-scES group (A1) will receive Core voluntary training focusing on trunk exercises, sitting balance, and lower extremity training for 6 hours daily. Voluntary training will be performed in the laboratory under supervision of trained research staff until they can perform each task safely and independently. Then they will be able to conduct their training at home. Approximately every two weeks, lower extremity voluntary configurations will be assessed by research staff to make sure they are still optimized for the desired movement. They may train in the laboratory more often.

3. Cardiovascular Intervention.

Participants will undergo testing in the laboratory to determine the optimal stimulator configuration(s) for blood pressure regulation. The first 5 training sessions will be performed in the laboratory. Once 3 consecutive stable days are achieved with the same stimulation parameters and the participant has demonstrated safe use

of the programmer, they will be sent home for one day to perform the stimulation at home. The participant will return to the laboratory the following day for stimulation with blood pressure monitoring. Provided cardiovascular parameters remain stable, the participant will be allowed to train at home for 3 days prior to returning to the laboratory for stimulation with monitoring. If cardiovascular parameters remain stable, the participant will be able to begin the home training program of 6 days a week and 1 day in lab training. Participants will be asked to train with CV-scES for CV for approximately 6 hours daily (7 days a week) for a total of 80 CV training sessions. Participants are instructed to monitor blood pressure for at least 15 minutes prior to starting CV-scES each day in order to insure a stable baseline prior to starting stimulation.

4. Stand Intervention.

During the stand training sessions, participants will use the custom designed standing apparatus described in the 'Standing Assessments' section above. In case of upper limbs and trunk control insufficient for safely using the standing apparatus, participants will be placed on the treadmill, and a body weight support system with a harness will be used to avoid trunk collapse and knee buckling. In this case, the level of body weight support will be continuously reduced over the course of the training sessions as the individuals increase their ability to bear weight on the lower limbs. 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 during 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. Participants will undergo 80 sessions of stand training (two 1 hour sessions, 5 days per week), always with lumbosacral epidural stimulation. Participants will be encouraged to stand for as long as possible throughout the training session, with the goal to stand for 120 minutes with the least amount of assistance. Seated resting periods will occur when requested by the individuals. This will be done with Stand-scES.

F. Home and Community Integration.

Participants that choose to keep the stimulator at the conclusion of the laboratory training phase and would like to receive stimulation configurations that were not part of their group interventions will receive home and community integration for approximately 2-6 weeks depending on the intervention(s) needed. For voluntary activity, configurations will be optimized for trunk and lower extremity movement. Participants will be asked to demonstrate safe use of the patient programmer while practicing lower extremity movements from the supine or seated position.

For those participants not achieving independent standing during the stand training phase of the protocol, the home and community integration will include training in a customized standing device. Standing devices will be designed to match the level of assistance required to safely translate the training to the home environment. This phase can also include training of care givers or personal trainers to assist the participants in achieving safe and independent standing.

G. Statistical approaches.

For the primary aims (comparing to baseline), paired sample t-tests will be used the primary endpoints (hypotheses 1a, 1b, 1c) comparing the results at the end of the second intervention period to the baseline period.

For the secondary aims, each endpoint will be analyzed separately with a common linear model framework. We fit a linear model with effects for each stimulation setting (voluntary, cardiovascular, and standing) and an effect of weight bearing stand training within each stimulation setting. Thus, for example we have a coefficient which measures the additional benefit of weight bearing stand training and a stimulation setting over and above that stimulation setting alone. There are separate coefficients for the benefit of stand training within Vol-scES, CV-scES, and Stand-scES. Formally, we fit a linear model of the form

$$\begin{aligned} \text{Response} = & \beta_{\text{Vol-scES}} I_{\text{Vol-scES}} + \beta_{\text{CV-scES}} I_{\text{CV-scES}} + \beta_{\text{Stand-scES}} I_{\text{Stand-scES}} + \\ & \beta_{\text{VT}} I_{\text{Vol-scES and Training}} + \beta_{\text{CVT}} I_{\text{CV-scES and training}} + \\ & \beta_{\text{ST}} I_{\text{Stand-scES and training}} + \text{error} \end{aligned}$$

where the β terms give the regression coefficients and I_x is an indicator variable equal to 1 if X occurs and 0 otherwise. The indicator variables function to only include the coefficients of the model for treatments the participant actually receives. Thus, for a participant receiving CV-scES and stand training, the model would reduce to

$$\text{Response} = \beta_{\text{CV-scES}} + \beta_{\text{CVT}} + \text{error}$$

Due to the partial crossover, no carryover effects are included in the model (sensitivity analyses using only the first period data will also be summarized). Random effects for each subject may be included when appropriate.

For hypotheses related to differences between the stimulation settings (hypotheses 1d, 1e, 1f, 3a, 3b), contrasts between the stimulation parameters will be used to test the hypothesis. For example, to test the superiority of CV-scES to the other two settings, the contrasts $\beta_{\text{CV-scES}} - \beta_{\text{Vol-scES}}$ and $\beta_{\text{CV-scES}} - \beta_{\text{Stand-scES}}$ will be used (testing if the difference is greater than 0) if interest centers on stimulation alone, and the contrasts $\beta_{\text{CV-scES}} + \beta_{\text{CVT}} - \beta_{\text{Vol-scES}} - \beta_{\text{VT}}$ and $\beta_{\text{CV-scES}} + \beta_{\text{CVT}} - \beta_{\text{Stand-scES}} - \beta_{\text{ST}}$ if interest centers on stimulation with stand training.

For hypotheses related to the effect of stand training above and beyond stimulation only, the parameters, β_{VT} , β_{CVT} , and β_{ST} may be tested directly (for hypotheses 2a, 2b, 2c)

Comparisons between stimulation frequencies have 90% power to detect effect sizes of 1.7 or higher with size 0.05 (one-sided) tests.

H. Uniqueness of initiative. The present proposal is designed to determine the effectiveness of a novel intervention on the recovery of multiple physiological systems after complete paralysis. There is no current intervention with demonstrated effectiveness in the recovery of motor or autonomic function after complete paralysis except via epidural spinal cord stimulation. The intervention of epidural stimulation applied with our newly developed stimulation parameters is based on a fundamentally sound neurophysiological concept: that the spinal circuitry can be neuromodulated to a physiological state with modest levels of epidural stimulation whereby proprioception itself can contribute to the recovery of the ability to stand and voluntarily control movement below the level of injury. Our experimental strategy embraces a completely new philosophy where the objective is to understand how multiple systems that are normally highly integrated and interdependent respond as such after a severe spinal cord injury, and to establish the efficacy of ES in regaining the ability to:

1. improve cardiovascular function,
2. volitionally control movement,
3. stand independently
4. improve bowel and bladder function,
5. improve sexual function
6. improve respiratory function,
7. improve quality of life, and
8. reduce health care costs.

In addition to securing scientific advances, our proposed experiments are essential to translating this novel therapeutic approach to a larger scale, and to expand its clinical impact. Therapy using scES 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 larger numbers of subjects. Our approach will allow us to determine the specific types of scES needed for recovery of voluntary movement and autonomic nervous system dysfunction, and this will lay the groundwork for expedient translations to treatment of other neurologic disorders and disease that cause paralysis, including stroke, traumatic brain injury, movement disorders and cerebral palsy.

IV. Human Subjects

A. 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. 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 recruit and screen approximately 108 individuals to reach an enrollment in the proposed study of 36 research participants who have sustained a severe SCI. We anticipate we will need to pre-screen approximately 200 individuals to identify 108 for screening. Frazier Rehab Institute evaluates approximately 300 chronic SCI outpatients each year. We also have a database of over 5,000 people with SCI who have expressed interest in participating in our research programs. We will select individuals to assure that there are a minimum of 25% (n=9) women to adequately represent the percentage in the SCI population.

Inclusion/Exclusion Criteria.

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

Inclusion criteria:

- 1) At least 18 years of age
- 2) non-progressive SCI
- 3) at least 2 years post injury
- 4) stable medical condition
- 5) unable to voluntarily move all individual joints of the legs
- 6) unable to stand independently
- 7) cardiovascular dysfunction including presence of persistent resting low blood pressures and/or symptoms of autonomic dysreflexia and/or orthostatic hypotension and/or dysregulation in response to postural changes and/or highly variable blood pressures in a 24 hour period
- 8) urodynamic dysfunction including dyssynergia and/or inability to void voluntarily and/or low voiding capacity
- 9) respiratory dysfunction including at least 15% deficit in predicted pulmonary function outcomes;

Exclusion criteria:

- 1) ventilator dependent
- 2) untreated painful musculoskeletal dysfunction, fracture or pressure sore
- 3) untreated psychiatric disorder or ongoing drug abuse
- 4) cardiovascular, respiratory, bladder, or renal disease unrelated to SCI
- 5) bladder botox injections less than 12 months prior to implant
- 6) colostomy bag, urostomy
- 7) any implanted pump (i.e., baclofen pump, pain pump, etc)
- 8) ongoing nicotine use
- 9) pregnant at the time of enrollment or planning to become pregnant during the time course of the study.

B. Potential Risks

The frequency is an estimated range of the likelihood that the risk will occur. These are general ranges: Rare (0-10%), Less likely (11-30%), Likely (more than 30%) chance that these risks may occur.

The study may involve the following risks and/or discomforts:

Surgical Risks

Surgical procedures are associated with numerous risks, including death. Risks associated with general surgery include, but are not limited to:

Likely

- Mild Discomfort
- Bruising
- Development of scar tissue around the electrode
- Bleeding
- Constipation

Less Likely

- Cellulitis at the incision site
- Infection at the incision site with washout
- Ileus

Rare

- Wound dehiscence
- Infection resulting in explant
- Seroma
- Complications from anesthesia
- Pneumonia (cervical injury, non-ambulatory
- Pneumonia (others)
- Epidural hemorrhage without compression
- Epidural hemorrhage requiring surgery
- Hematoma without compression
- Hematoma requiring surgery
- Cerebrospinal fluid leak resulting from a hole or tear in the dura
- Blindness
- Excessive blood loss
- Heart Attack
- Death

Electrode/Device Risks

Less Likely

- Undesirable change in stimulation
- Jolting or shocking

Rare

- Allergic response
- Hardware malfunctions
- Migration
- Erosion
- Breakage or failure resulting in further injury to the spinal cord

Risks of Assessments and Interventions

Likely

- Skin irritation from hand placements of trainers
- Skin irritation from adhesive tape, sensors, wires, and/or pads
- Tingling feeling from the stimulation
- Dizziness by breathing in and out hard
- Dizziness during sitting, standing or stepping
- Skin irritation from vein needle and/or fine wire insertion
- Slight discomfort from the pressure of the ultrasound probe
- Skin irritation from ECG sensor placement

Less Likely

- Bleeding and/or bruising from fine wire insertion and/or blood draw
- Pain and/or infection from blood draw
- Skin abrasion from hand placement of trainers

- Feelings of claustrophobia
- Shortness of breath
- Significant changes in heart rate and/or blood pressure
- Muscle and joint soreness

Rare

- Chest pain
- Joint sprain
- Nausea
- Fall
- Broken bones requiring medical treatment
- Broken bones requiring surgical treatment and long-term medical follow-up

Risks of Bladder Assessments

Likely

- Feeling of shyness
- Significant changes in heart rate or blood pressure
- Autonomic dysreflexia symptoms that resolves when the cause is removed

Less Likely

- Mild discomfort, especially during urination after Urodynamics
- Urinary tract infection requiring oral antibiotics
- Discomfort from lying still for ultrasound

Rare

- Autonomic dysreflexia symptoms but the cause cannot be identified and the high blood pressure does not resolve and medical intervention is required
- Urinary tract infection requiring intravenous antibiotics
- Excessive pain, fever, chills

Risks of Bowel Assessments

Likely

- Feeling of shyness
- Significant changes in heart rate or blood pressure
- Autonomic dysreflexia that resolves when the cause is removed

Rare

- Autonomic dysreflexia symptoms but the cause cannot be identified and the high blood pressure does not resolve and medical intervention is required
- Bleeding of the rectum
- Bowel infection requiring oral antibiotics
- Bowel perforation in those with previous rectal surgery, bowel inflammation, or bowel obstruction

Risks of Sexual Function Assessments (Questionnaires)

Likely

- Feeling of shyness

Risks of Stand and Voluntary Training Interventions

Likely

- Skin irritation from hand placements of trainers
- Skin irritation from adhesive tape, sensors, wires, and/or pads
- Dizziness during sitting, standing, or stepping
- Skin irritation from fine wire insertion

Less Likely

- Bleeding and/or bruising from fine wire insertion
- Skin abrasion from hand placements of trainers
- Shortness of breath
- Significant changes in heart rate and/or blood pressure
- Muscle and joint soreness

Rare

- Chest pain
- Joint sprain or muscle strain
- Fall
- Broken bones requiring medical treatment
- Broken bones requiring surgical treatment and long-term medical follow-up

Risks of Cardiovascular Training Intervention

Less Likely

- Shortness of breath
- Significant changes in heart rate and/or blood pressure

C. Adequacy of Protection against Risk

1. Recruitment and Informed Consent: Recruitment of patients will be performed through our secure research database that includes over 5,000 individuals registered with SCI. All potentially eligible research participants will be invited to Frazier Rehab Institute to discuss the complete protocol, including risks and benefits with Dr. Harkema and/or designated research staff. The informed consents 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. All potential research participants will be encouraged to read the pre-screening, screening, and surgical interventions informed consent(s) and discuss the study with their physician, family and friends, before signing each of the three IRB approved informed consents. The original signed informed consents will be kept in a locked cabinet in a locked room within an area of controlled access. A scanned copy will be stored on our server with password protection. Eligibility checklists will be signed by Dr. Harkema with all source documentation and stored in a locked cabinet in a locked room within an area of controlled access. A scanned copy will be stored on our server with password protection.

After the research participant signs each consent document, an Eligibility Checklist is completed as medical and scientific eligibility is determined. This is placed in research medical record along with supporting source documentation. These will be reviewed by the research manager and the Principal Investigator. The Eligibility Checklist will be signed by the person verifying eligibility and by the Principal Investigator who is responsible for final determination of eligibility. All source documentation (medical and scientific) for eligibility will be placed in the research medical report. The research manager will do periodic internal audits of all enrolled research participants research medical report to ensure compliance.

If a participant is employed by the University of Louisville, we will review and discuss a risk management plan. The plan will be signed by the investigator and the 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/her employee file.

To protect confidentiality, each research participant will be assigned a coded identifier with no association to their identity. This identifier will distinguish all evaluations and analyses. Data will be stored on computer media

and video and will be secured in a locked storage area. Only members of the research team including research staff, post-doctoral students and graduate students will have access to the data for analyses. Dr. Harkema will have access to the coding of the coded identifier to the research participants.

No individual will be allowed to participate in the study without being examined by the study physicians. All eligible research participants will be encouraged to discuss the study with their primary physician, in order to minimize physical risks. Participants will be continuously monitored for any signs of discomfort or risks.

2. Surgery, Assessments, and Interventions

a. General protection against risk

No individual will be allowed to participate in the study without being examined by the study physicians. All eligible research participants will be encouraged to discuss the study with their primary physician, in order to minimize physical risks. Participants will be continuously monitored for any signs of discomfort or risks by a designated research staff member during every assessment and intervention. The skin integrity will be checked and the joints will be examined for swelling or redness after every assessment and training session. Blood pressure and heart rate will be routinely measured. If discomfort or any risks persist, the recording or training session will be immediately discontinued and the research nurse will be contacted immediately to assess the participant. If needed the study physician or physician assistant will be notified and will also examine the individual. Immediate medical care will be provided when necessary. Their primary care provider will be informed when necessary. If there are no medical events during the intervention or assessment this will be noted and verified with a signature by a research team member.

Before and after every experiment and training session, a physical therapist or activity based technician will examine the subject's skin for irritations and abrasions. If skin irritations or abrasions are caused by the recording or stimulating electrodes, or hand placements of trainers, electrode and hand placement will be modified appropriately. Further, the physical therapist or activity based technician will constantly monitor the subject's skin and muscle for signs of muscle strain, joint sprain and skin irritation (e.g. temperature and redness).

Each participant will be assigned an observer to accompany the research participant to experiments and training sessions. The responsibility of the observer is to communicate with the research participant and monitor their well-being. The observer, the activity based technicians, the research nurse and/or the research physical therapist will monitor the research participant daily for skin redness, swelling of joints or spasticity as well as other issues. Adverse events will be reported as required by the institutional review board. The assigned observer may be either a University of Louisville Employee or a KentuckyOne Health Employee.

b. Epidural Stimulation: Electrode Configuration, Assessments and Interventions

Drs. Harkema, Angeli, Rejc and/or research team members will continually assess the appropriate stimulation parameters including configurations, voltage and frequency. Stimulation parameters used during mapping, assessments and interventions will be closely monitored by the research team. Every research participant will be slowly acclimated to stimulation. This may help them 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. Blood pressure and heart rate will be closely monitored 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, the stimulation will be immediately discontinued and the research nurse will be contacted immediately to assess the research participant. If needed the study physician or physician assistant will be notified and will also examine the individual. Immediate medical care will be provided when necessary. Their primary care provider will be informed when necessary.

Research participants will undergo training about stimulator use for each configuration and intervention. Testing of optimal stimulation parameters and ranges will be performed in the laboratory to make sure stimulation is safe for the research participant. Stimulation programs given to each participant will be restricted to those used and tested in the laboratory. Participants will be instructed to call the research physical therapist and/or assigned research team member immediately if complication from stimulation develops during home training programs. 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.

c. Imaging Assessments

Participants undergoing a DXA scan will be exposed to small amounts of radiation. During one scan a participant may be exposed to less than 200 millirem of radiation, a dose that is less than what is obtained from natural (sun, earth, etc.) and man-made sources each year. The radiation dose that a participant will receive from this study is well below the levels that are thought to result in a significant risk of harmful effect. The known risks of MRI are the possibility that loose metallic objects might be attracted into the magnet, or that metallic prostheses or pacemakers might be affected by the magnet. All individuals will be screened for any metallic implants and loose metal objects and they will be removed from subjects as per the standard clinical practice. Although the only known risks of MRI exams are from metallic objects or implants, the possibility that there might be unknown risks cannot be ruled out. If a research participant experiences feelings of claustrophobia while in the MRI machine, we will stop the testing. The research participant will be informed that there are no known animal or human data on the potential for birth defects, so they should not participate in the test if they are pregnant, nursing, or anticipate becoming pregnant.

d. Cardiovascular Assessments

Blood pressure and heart rate will be continuously monitored throughout the assessment. If the individual displays symptoms of syncope (dizziness, light headedness, darkening of vision) during the sit up procedure, they will be immediately returned to the supine position and their legs raised above the heart. The assessment will then be discontinued. Trained, experienced, and certified nurses or technicians will use approved procedures during all blood draw experiments to reduce the risk of skin irritation, bleeding, and bruising. Participants will also be asked if they have a preferred blood draw location before catheter insertion. Lidocaine spray may be used to prepare the skin and reduce pain during needle insertion.

e. Respiratory Assessments

In the event of dizziness, shortness of breath, lightheadedness, or significant changes in blood pressure and/or heart rate, time will be allowed for the participant to recover between trials. Research participants will be asked about any skin allergies prior to the assessment, and in the instance of a skin sensitivity or allergy, tests with various hypoallergenic tapes for electrode adherence will be conducted. In the event that a participant is uncomfortable, we will allow time to do routine pressure relief and postural adjustments to maintain comfort.

f. Bladder, Bowel and Sexual Function Assessments

Assessments will be performed under the supervision of the study physician(s) with University of Louisville Gastroenterology and Urology clinics. Both during and in the days following the procedure, participants will be monitored for excessive discomfort, pain, and other adverse reactions. A nurse or specialist trained in the procedures will perform the assessment. The participant's blood pressure will be continuously monitored during the assessment and the procedures will be stopped and catheters removed with either a sustained high systolic blood pressure recording and/or intolerable autonomic dysreflexia symptoms. Symptoms associated with infection will be addressed immediately and the participant's primary care provider will be notified if needed. Participants experiencing excessive pain, chills, or fever will be triaged accordingly. If needed, the study physician or physician assistant will be notified and will also examine the individual. Immediate medical care will be provided when necessary. Their primary care provider will be informed when necessary. Viscous Lidocaine will be applied through the urethra in an attempt to dampen a burning sensation. Participants experiencing excessive pain, chills, fever, or other external symptoms of an infection will be treated accordingly. Both during the assessment and in the days following the procedure, participants will be monitored for excessive discomfort, pain, bleeding, and other adverse reactions.

g. Surgical Implantation

The 5-6-5 Specify electrode, (MEDTRONIC, Minneapolis, MN, USA) and Intellis Adaptive Neurostimulator, (MEDTRONIC, Minneapolis, MN, USA) will be surgically implanted. The devices are FDA approved and meet US safety regulations. The patient will be apprised of the surgical risks by Dr. Boakye and/or Dr. Neimat. To minimize risks of infection each patient will be administered intravenous antibiotics throughout the operation and for 24 hours postoperatively. Individuals will be induced by general anesthesia and will be closely

monitored by the anesthesiologist for changes in blood pressure, pulse, and temperature. In the unlikely event of an infection, the patient may require prolonged intravenous antibiotics, reopening of the incision to irrigate and drain an abscess, or even removal of the epidural electrodes. Scrupulous attention for hemostasis should prevent a postoperative hematoma from occurring. However, if a hematoma develops and is clinically significant, timely surgical evacuation of the clot will be performed. The research participant will stay overnight at University of Louisville Hospital for observation. There may be discomfort from the operation which will be treated with pain medications as required.

The research participants will be monitored postoperatively and will stay overnight at University of Louisville Hospital. The patient will be followed during that period by the research nurse and the study physicians to monitor any complications of surgery. If the participant has any of these difficulties from the surgeries, the study physician will be contacted immediately.

h. Protection Against Infectious Disease From Surgery

An expanded set of screening laboratory tests will be done prior to surgery including screening for MRSA and multidrug-resistant Gram-negative bacteria in order to direct surgical prophylactic antibiotics (see Table below).

To reduce the risk of infectious disease from surgery, the following precautions will be implemented: 1) The number of the people in the operating room will be limited. 2) Access to the operating room will be limited (i.e., doors will be taped) during the entire procedure. Sterile core access will be utilized if entrance/exit is required. 3) New sterile instruments will be utilized in Stage II of the procedure and will be in the operating room prior to Stage I. 4) Electromyography equipment will be cleaned with ULH approved PDI® Sani-cloth AF-3 germicidal disposable wipes (PDI, Orangeburg, NY) and screens with PDI Easy Screen cleaning wipes prior to taking equipment into the operating room. 5) Surgical antibiotic prophylaxis will be prolonged to 48 hours. 6) Patients will be pre-screened with the laboratory tests in the table below. 7) MRSA patients will be decolonized, retested and repeated if necessary (see Box below). 8) Each patient will be instructed to clean their skin (neckline to toes) with a 2% chlorhexidine bath wipe pre-operatively per protocol. 9) Vancomycin powder will not be used. 10) A TYRX® Neuro Absorbable Antibacterial Envelope in the abdominal pouch site will be used.

Test	Purpose	When
Nasal Swab for MRSA	To determine if colonized to de-colonize and use vancomycin as pre-operative antibiotics	4 weeks prior
Peri-rectal swab for MDRO Gram-negatives	To determine if colonized, and, based on organism, consider decolonization and adjustment or pre-operative antibiotics	
Urinalysis	To identify infection for clearance prior to procedure	
Urine Culture		
C-reactive Protein	To obtain for baseline measure	1 week prior
Erythrocyte Sedimentation Rate		
Procalcitonin		
Prealbumin	To verify appropriate status for procedure	
Albumin		
Transthyretin		
25-Hydroxy Vitamin D		

chlorhexidine gluconate (2 or 4% solution) with daily washes or a disposable impregnated cloth

mupirocin ointment (2%) applied to the nares with a cotton-tipped applicator two to three times daily.

Administer for seven days. Retest. If positive then decolonize again with the two medications above and the following:

rifampin (600 mg PO daily)

plus either

doxycycline (100 mg twice daily)

or

trimethoprim/sulfamethoxazole (one double strength twice daily).

D. Medical Events Unrelated to the Study.

A medical event is defined within our program as “any untoward or unfavorable medical occurrence in a human research participant that occurs during the same time period the research participant is enrolled in the study and is not study related and is not serious.” It is an anticipated medical issue that is unfortunately common to individuals with chronic spinal cord injury and can occur daily, weekly monthly, or several times a year. These medical events can include urinary tract infections, kidney stones, hypotension, autonomic dysreflexia, skin sores and pressure sores with prolonged healing, joint swelling, joint soreness, joint sprain, ligament sprain, fracture, infections (non-UTI), spasticity and falls. Our standard operating procedure is that if any of these medical issues arise while directly participating in our research, if not immediately resolved, the study physician is notified and the individual is referred to the appropriate medical care. This medical care can be provided by our study physician or their colleagues or by the medical specialist of the research participant's choice. The study physician can medically treat the issue to resolution, if appropriate, and the research participant chooses the study physician as their clinical choice for medical treatment. In this case the study physician would notify the principal investigator only if the medical issue affects their medical eligibility in the study or any of the research assessments or training paradigms. If another physician treats the medical issue the study physician will follow the individual to only assess whether the medical condition affects their medical eligibility in the study or any of the research assessments or training paradigms and will notify the principal investigator. All non-study related medical events are followed by the research team and will be reported to the IRB at the time of the continuing annual review. A weekly report of all medical events is generated by the research nurse and sent to the study physician (and physician assistant), the principal investigator, and the study physical therapists.

E. Data and Safety Monitoring Board

James Guest, MD, PhD (Chair of DSMB)

Neurosurgeon, Clinical Professor

Department of Neurological Surgery

University of Miami and The Miami Project to Cure Paralysis, Miami, FL

Steven Schulman, MD

Cardiologist, Professor of Medicine

Johns Hopkins Hospital, Baltimore, MD

Thomas Kessler, MD, PhD

Urologist

University of Zurich Spinal Cord Injury Center, Research

Balgrist University Hospital, Zurich, Switzerland

Steven Kirshblum, MD
Medical Director, Spinal Cord Injury Rehabilitation
Kessler Institute for Rehabilitation, West Orange, NJ

An independent Data Safety Monitoring Board (DSMB) has been chartered to monitor the safety of research participants as well as the validity and 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 first DSMB meeting will occur in the first quarter of the first year of the funding period and will then meet yearly after that. 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 IRB and DSMB chairperson as appropriate. Medical events that occur while the research participants are engaged in the research protocols also will be logged by a research nurse, research physical therapists, study physicians and/or research staff. Any adverse events are collected on an Adverse Event Form and will be reported at the yearly DSMB meeting. An Adverse Event 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 Adverse Event that the principal investigator 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 principal investigator 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 principal investigator gaining knowledge of the event.

Research participants may be withdrawn from the study due to noncompliance. In the instance that a research participant is withdrawn, a request will be submitted to the FDA and the University of Louisville IRB to increase enrollment in order to obtain 36 data sets.

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Appendix #1

Appendix 1. Frequency of Outcomes for Aims 1 – 3.

Assessment Category #=with and without stim	Assessments	Pre-screen	Screen	Pre-Usual Care	Pre-implant	Post-Implant/ Pre-Intervention	Weekly	Periodic Throughout Intervention	Monthly	Post-Intervention #1	Post-Intervention #2	Follow Up(s)
Medical	SC MRI	X	or X									
	ASIA		X	X	X					X	X	
	Physician and PT Medical		X	X	X					X	X	X
	Pregnancy	X	X									
	Blood Work		X	X	X					X	X	X
	Drug Testing	X	X	X	X	X	X	X		X	X	X
Voluntary	MMR		X									
	FNPA		X	X	X	X#				X#	X#	X#
	NRS trunk and leg score		X	X		X#		X#		X#	X#	X#
	Lower Extremity Torque with EMG					X#		X#		X#	X#	X#
	Neuromuscular Voluntary Movement Assessments					X#		X#		X#	X#	X#
Cardiovascular	Ambulatory BP monitoring		X	X				X	X	X	X	X
	Ambulatory HR monitoring		X	X				X	X	X	X	X
	12 lead ECG				X					X	X	
	Orthostatic Stress Test		X	X	X	X#		X#		X#	X#	X#
	Vascular Ultrasound			X	X	X#		X#		X#	X#	X#
	Echo-cardiography		X	X	X	X#		X#		X#	X#	X#
	Arterial pulse wave velocity		X	X	X	X#		X#		X#	X#	X#
	Sympathetic skin responses				X					X	X	
Stand	Dexa		X		X					X	X	X
	Independence levels during stand -			X	X	X#	X#	X#		X#	X#	X#
	Standing Assessments				X	X#		X#		X#	X#	X#
Respiratory	Pulmonary Function Test		X		X	X#				X#	X#	X#
	Respiratory Motor Control Assessment		X		X	X#				X#	X#	X#
	Resting metabolic rate		X	X	X					X	X	X

Assessment Category #-with and without stim	Assessments	Pre-screen	Screen	Pre-Usual Care	Pre-implant	Post-Implant/ Pre-Intervention	Weekly	Periodic Throughout Intervention	Monthly	Post-Intervention #1	Post-Intervention #2	Follow Up(s)
Bladder	Urodynamics		X	X	X					X	X	X
	Bladder Questionnaire			X	X					X	X	X
	Urinalysis			X	X					X	X	X
	Ultrasound			X	X					X	X	X
Bowel	Bowel Questionnaire			X	X					X	X	X
	Anorectal Manometry			X	X					X	X	X
Sexual Function	International Dataset (Males)			X	X					X	X	X
	International Dataset (Females)			X	X					X	X	X
Quality of Life	SCI-QOL		X		X					X	X	X
	SCI-FI		X		X					X	X	X
	PROMIS		X		X					X	X	X
	EQ-5D-3L		X		X					X	X	X

Appendix #2

Medical Event Reporting Unrelated to the Research Protocol Version 2, 12/12/2016

A medical event is defined within our program as any untoward or unfavorable medical occurrence in a human research participant that occurs during the enrollment period of a study.

As the team is made aware of study participants' medical events, they will be recorded in a log for tracking and reporting.

Medical events that occur during a research visit will be reported to the study nurse and then elevated to the study physician if not immediately resolved. The physician will evaluate these medical events, to determine causality with relation to the research and referral for medical care.

A weekly report of all medical events is generated by the research nurse and sent to the study physician (and physician assistant), the principal investigator, the regulatory core, and the study therapists for review of causality and follow-up.

We have developed a table outlining the most common expected events in the spinal cord injury (SCI) population. These events occur with this population on a frequent basis. This table will allow the IRB and our staff to understand how expected and unrelated events will be documented and reported.

If a physician outside the study team treats the medical event, the study physician will follow the event to assess the following:

- The medical condition affecting their medical eligibility for research
- Determination on causality of the event
- The event's effect on research assessments and/or training paradigms

All conclusions reached by the study physician will be reported to the PI.

Characteristics of Medical Events in the Spinal Cord Injury Population (Unrelated to Study)					
Medical Event	Probability	Medical Event Severity	Medical Intervention	Serious Event	Reporting
Urinary Tract Infections	Likely	Mild	Antibiotics	No	Research Medical Log
	Likely	Moderate	Antibiotics	No	Research Medical Log
	Rare	Moderate	Hospitalization	No	IRB Continuation Review
	Rare	Severe	Hospitalization	Yes	IRB Continuation Review
Urinary Tract Infections that significantly interfere with research conduct	Rare	Moderate	Depends on specific diagnosis	No	IRB Continuation Review
	Rare	Moderate	Hospitalization	No	IRB Continuation Review
	Rare	Moderate	Hospitalization	Yes	IRB Continuation Review
Urinary Tract Infection/Kidney	Less Likely	Moderate	Depends on specific diagnosis	No	Research Medical Log
	Rare	Moderate	Hospitalization	No	IRB Continuation Review
	Rare	Severe	Hospitalization	Yes	IRB Continuation Review
Urinary Tract Infections/Kidney that significantly interfere with research conduct	Rare	Moderate	Depends on specific diagnosis	No	IRB Continuation Review
	Rare	Moderate	Hospitalization	No	IRB Continuation Review
	Rare	Severe	Hospitalization	Yes	IRB Continuation Review

Characteristics of Medical Events in the Spinal Cord Injury Population (Unrelated to Study)					
Medical Event	Probability	Medical Event Severity	Medical Intervention	Serious Event	Reporting
Kidney Stones	Less Likely	Moderate	Depends on specific diagnosis	No	Research Medical Log
	Rare	Moderate	Hospitalization	No	IRB Continuation Review
	Rare	Severe	Hospitalization	Yes	IRB Continuation Review
Kidney Stones – significantly interferes with research conduct	Rare	Moderate	Depends on specific diagnosis	No	IRB Continuation Review
	Rare	Moderate	Hospitalization	No	IRB Continuation Review
	Rare	Severe	Hospitalization	Yes	IRB Continuation Review

Characteristics of Medical Events in the Spinal Cord Injury Population (Unrelated to Study)					
Medical Event	Probability	Medical Event Severity	Medical Intervention	Serious Event	Reporting
Hypotension	Likely	Mild	None	No	None
	Rare	Moderate	Physician Referral	No	Research Medical Log
	Rare	Moderate	Depends on specific diagnosis; includes medication and/or hospitalization	No	IRB Continuation Review
	Rare	Severe	Depends on specific diagnosis; includes medication and/or hospitalization	Yes	IRB Continuation Review
Hypotension that interferes with research conduct	Less Likely	Moderate	Cease stand or step; Elevate legs above heart	No	None
	Rare	Moderate	Physician Referral	No	Research Medical Log
Hypotension that significantly interferes with research conduct	Rare	Moderate	Depends on specific diagnosis; includes medication and/or hospitalization	No	IRB Continuation Review
	Rare	Severe	Depends on specific diagnosis; includes medication and/or hospitalization	Yes	IRB Continuation Review

Characteristics of Medical Events in the Spinal Cord Injury Population (Unrelated to Study)					
Medical Event	Probability	Medical Event Severity	Medical Intervention	Serious Event	Reporting
Autonomic Dysreflexia	Likely	Mild	None	No	None
	Less Likely	Moderate	Identify and eliminate source	No	None
	Rare	Moderate	Depends on specific diagnosis; includes medication and/or hospitalization	No	IRB Continuation Review
	Rare	Severe	Depends on specific diagnosis; includes medication and/or hospitalization	Yes	IRB Continuation Review
Autonomic Dysreflexia that significantly interferes with research conduct	Rare	Moderate	Physician Referral	No	IRB Continuation Review
	Rare	Moderate	Depends on specific diagnosis; includes medication and/or hospitalization	No	IRB Continuation Review
	Rare	Severe	Depends on specific diagnosis; includes medication and/or hospitalization	Yes	IRB Continuation Review

Characteristics of Medical Events in the Spinal Cord Injury Population (Unrelated to Study)					
Medical Event	Probability	Medical Event Severity	Medical Intervention	Serious Event	Reporting
Skin sore	Likely	Mild	None	No	None
Skin sore that requires prolonged healing	Likely	Mild	None	No	None
	Likely	Moderate	None	No	Research Medical Log
	Less Likely	Moderate	Wound Care referral	No	Research Medical Log
	Rare	Moderate	Hospitalization	No	IRB Continuation Review
	Rare	Severe	Hospitalization	Yes	IRB Continuation Review
Skin sore that requires prolonged healing and that interferes with research conduct	Rare	Moderate	Depends on specific diagnosis	No	IRB Continuation Review
	Rare	Severe	Depends on specific diagnosis	Yes	IRB Continuation Review
Skin sore that requires prolonged healing and results in infection	Less Likely	Mild	Depends on specific diagnosis	No	Research Medical Log
	Less Likely	Moderate	Depends on specific Diagnosis	No	Research Medical Log
	Rare	Moderate	Hospitalization	No	IRB Continuation Review
	Rare	Severe	Hospitalization	Yes	IRB Continuation Review
Skin sore that requires prolonged healing and results in infection and interferes with research conduct	Rare	Moderate	Depends on specific diagnosis	No	IRB Continuation Review
	Rare	Severe	Depends on specific diagnosis	Yes	IRB Continuation Review

Characteristics of Medical Events in the Spinal Cord Injury Population (Unrelated to Study)					
Medical Event	Probability	Medical Event Severity	Medical Intervention	Serious Event	Reporting
Joint soreness with swelling	Likely	Mild	None	No	None
	Less Likely	Moderate - Reoccurs	None	No	Research Medical Log
	Less Likely	Moderate - Reoccurs	Physician referral for X-ray	No	Research Medical Log
Joint soreness with swelling that interferes with research conduct	Less Likely	Moderate - Reoccurs	Depends on diagnosis	No	IRB Continuation Review
Joint soreness with swelling – fracture or other orthopedic injury	Less Likely	Moderate	Depends on diagnosis	No	IRB Continuation Review
	Rare	Serious	Depends on Diagnosis	Yes	IRB Continuation Review
Joint soreness with swelling – fracture or other orthopedic injury that interferes with research conduct	Less Likely	Moderate	Depends on diagnosis	No	IRB Continuation Review
	Rare	Serious	Depends on diagnosis	Yes	IRB Continuation Review
Infections (non-UTI)	Likely	Mild	None	No	None
	Less Likely	Moderate	None	No	Research Medical Log
	Less Likely	Moderate - Reoccurs	Physician referral	No	Research Medical Log
	Less Likely	Moderate - Reoccurs	Hospitalization	No	IRB Continuation Review
	Rare	Severe	Hospitalization	Yes	IRB Continuation Review
Infections (non-UTI) that interfere with research conduct	Rare	Moderate	Physician referral	No	IRB Continuation Review
	Rare	Moderate - Reoccurs	Hospitalization	No	IRB Continuation Review
	Rare	Severe	Hospitalization	Yes	IRB Continuation Review

Characteristics of Medical Events in the Spinal Cord Injury Population (Unrelated to Study)					
Medical Event	Probability	Medical Event Severity	Medical Intervention	Serious Event	Reporting
Falls	Likely	Mild	None	No	None
	Likely	Moderate	None	No	Research Medical Log
	Less Likely	Moderate	Physician Referral	No	Research Medical Log
	Less Likely	Moderate	Hospitalization	No	IRB Continuation Review
	Rare	Severe	Hospitalization	Yes	IRB Continuation Review
Falls that interfere with research conduct	Rare	Moderate	Physician Referral	No	IRB Continuation Review
	Rare	Moderate	Hospitalization	No	IRB Continuation Review
	Rare	Severe	Hospitalization	Yes	IRB Continuation Review
Spasticity	Likely	Mild	None	No	None
	Likely	Moderate	None	No	None
	Less Likely	Moderate	Physician Referral	No	Research Medical Log
	Rare	Moderate	Hospitalization	No	IRB Continuation Review
	Rare	Severe	Hospitalization	Yes	IRB Continuation Review
Spasticity that significantly interferes with research conduct	Rare	Moderate	Physician Referral	No	IRB Continuation Review
	Rare	Moderate	Hospitalization	No	IRB Continuation Review
	Rare	Severe	Hospitalization	Yes	IRB Continuation Review