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I. Background, including a brief literature review

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. In addition to motor paralysis, these injuries are also 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 the most severe injuries including those with clinically motor complete SCI have essentially no hope of neurologic recovery, even with therapeutic intervention.

Also, the mechanisms discovered from these early feasibility studies can be translated into new therapeutic approaches that may not need to use epidural stimulation for those who are treated early after intervention of have more incomplete injuries. This approach provides potential therapies for the most severely injured individuals but also knowledge about the nervous system following injury that can be used for all those who have paralysis.

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 stepping, standing 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.

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 overall general hypotheses. 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 and provide new knowledge that can be used for new therapy strategies that need not require epidural stimulation. 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 and autonomic 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.

A. Motor Control

Spinal cord epidural stimulation (scES) has been applied alone in a number of individuals with clinically complete spinal cord injury (SCI), showing that some alternating stepping-like actions can be induced [3]. scES has been used in combination with repetitive stepping on a treadmill for facilitation of walking-like movements after an incomplete lesion in two individuals showing stepping could be sustained for a longer period of time with less effort in the presence of scES [4]. This report provides promising results of a combination of locomotor training and scES. In contrast to our presently proposed studies on clinically complete SCI research participants, the previously reported studies included patients with incomplete injuries who already had some stepping ability before treatment [5]. Several recent findings suggest that a combination of scES and locomotor training in individuals with clinically complete SCI could be very successful for the recovery of standing and walking. Locomotor-like patterns can be facilitated by scES in complete spinally transected rats [6], cats [7], and humans with clinically complete SCI [8]. However, the long-term functional changes that occur in the spinal cord after clinically complete SCI have not been comprehensively examined, and it is likely to require more complex and adaptive stimulation protocols in order to achieve optimal results.

***Standing*:** Activity-dependent rehabilitation alone is normally not sufficient for promoting significant neurological and functional recovery after a SCI graded A or B [9, 10]. We have demonstrated that epidural stimulation of the lumbosacral spinal cord can promote an “enabling state” of the patient to generate EMG patterns sufficient for standing in response to weight-bearing related sensory information provided to the limbs [2]. It was postulated that the stimulation alters the physiological state of the lumbosacral spinal circuitry and proactively influences afferent information to enable standing rather than directly inducing a motor task. Although motor output can be modulated by relatively small changes in sensory information during standing, none of the research participants were able to balance themselves during standing without self-assistance with upper limbs. More recently, the combination of lumbosacral spinal cord epidural stimulation and stand training has been shown to gradually promote recovery of full weight-bearing and standing over-ground in two clinically motor complete and two clinically sensory and motor compete individuals with paraplegia [11]. A substantial

part of the success with each of these interventions appears to be related to the fact that the spinal cord can use ensembles of sensory information to generate appropriate motor responses even without inputs from the supraspinal centers, only in the presence of epidural stimulation. This reflects a unique automatic ability of the spinal cord, where its neural circuitry can interpret complex sensory information and make appropriate decisions to generate successful postural and locomotor tasks when the central state of excitability is optimized. Studies on complete SCI animal models highlighted the importance of sensory information in modulating postural motor output, and suggested that balance training with epidural stimulation can only partially restore postural control [12-14]. These findings suggest that synergic approaches to stand balance training with epidural stimulation should be attempted for enhancing the recovery of balance control. One of these approaches may be related to the use of targeted muscle vibration stimulation, which can augment sensory information and promote postural adjustments [15, 16]. In addition, improving the characteristics of stand balance training by providing a more challenging, and still safe, balance training environment (i.e., providing external assistance-as-needed for trunk and hips control while reducing the occurrence of research participant's upper limb self-assistance for balance) may also enhance the recovery of sensory-motor processes for standing balance control [310].

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.

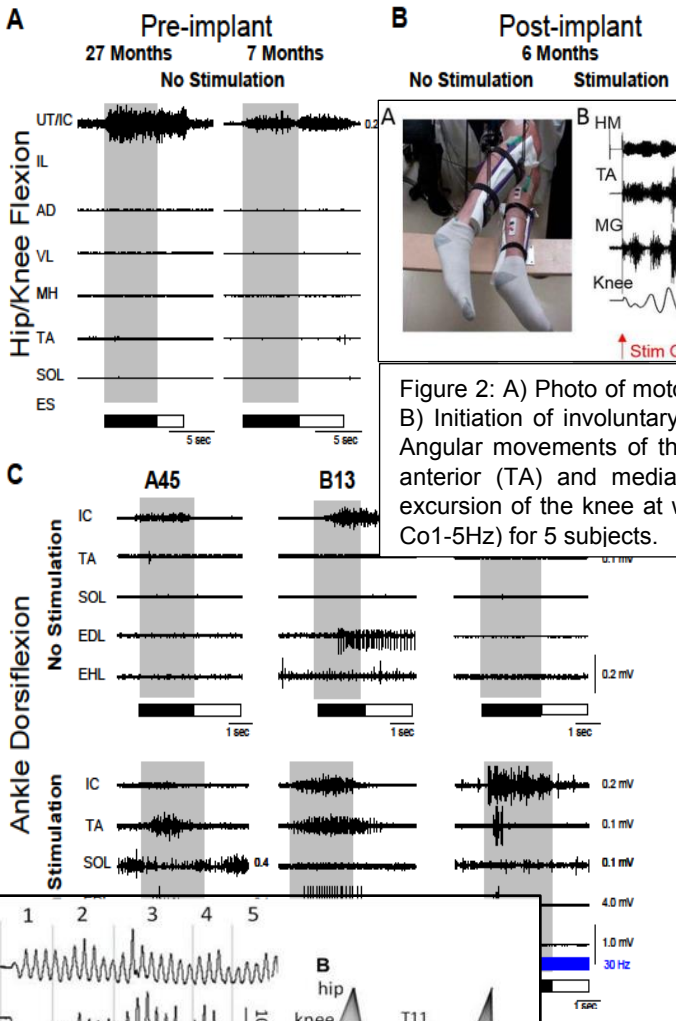


Figure 1. Lower extremity electromyography activity during voluntary movement occurred only

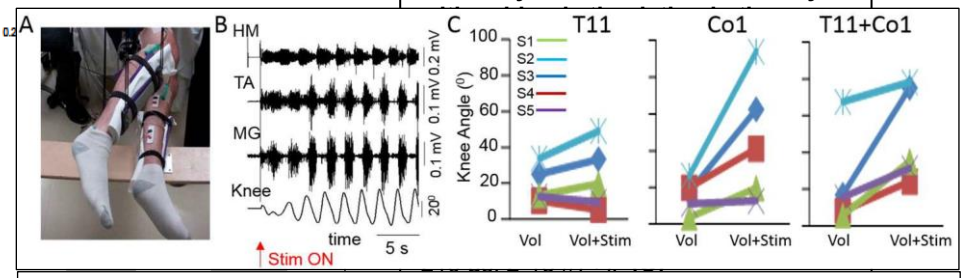


Figure 2: A) Photo of motor complete SCI individual placed in gravity neutral device. B) Initiation of involuntary stepping movements induced by pcEMC at T11 (30Hz). Angular movements of the knee joint and EMG activity in hamstring (HM), tibialis anterior (TA) and medial gastrocnemius (MG) muscles are shown. C) Angular excursion of the knee at with different stimulation sites and frequencies (T11-30Hz, Co1-5Hz) for 5 subjects.

the left and right legs of an individual and varied among the three subjects. Muscles, surface electromyography: Upper trapezius (UT), intercostal (IC), adductor magnus (AD), vastus lateralis (VL), medial hamstrings (MH), tibialis anterior (TA), soleus (SOL); fine wire electromyography: iliopsoas (IL), extensor digitorum longus (EDL), extensor hallucis longus (EHL). Black bars represent the examiner's command to flex and white bars represent the command to relax. Gray highlighted indicates subject's verbal command to 'flex'. Blue indicates tonic stimulation.

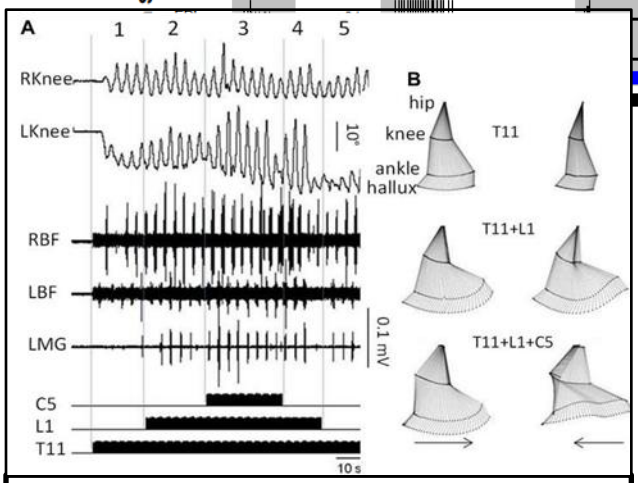


Figure 3. A: angular excursions of the right (R) and left (L) knee joints and corresponding EMG activity in the RBF, LBF, and LMG muscles with pcEMC at T11 with and without stimulation at L1 and/or C5 in non-injured subject in a GND are shown. B: stick diagram decompositions (40 ms between sticks) of the movements of the right leg during 1 step cycle during stimulation at T11, T11+L1, and T11+L1+C5 are shown. Arrows under the stick diagrams indicate the direction of movement.

Transcutaneous Stimulation: While multiple combinations of stimulation parameters can be effective in enabling motor functions, our data from SCI subjects[2 , 1 7] have significantly narrowed the windows for a given set of parameters to achieve a given motor response. Importantly the most effective combination of parameters varies from subject to subject and even from time to time within a given subject during progressively improving performance (Figure. 2). We have developed a model using a GND to test the ability to generate rhythmic stepping patterns in response to changes in stimulation frequency, intensity and sites (Figure. 2A). This has proven to be a highly sensitive and easily manageable device for detecting the efficacy of a given set of stimulation parameters in generating bilateral locomotor-like movements, a task that has been absent for more than a year in AIS A/B subjects. This paradigm, being free of weight-bearing, allows the locomotor spinal circuits to experience and relearn

rhythmicity without the "burden" of generating high levels of force[18]. The GND serves as a surrogate to understand the interactive elements of the stimulation parameters with the objective to formalize fundamental principles in regulation of the spinal networks to coordinate motor pools for postural and locomotor behaviors.
We have studied five completely paralyzed subjects in response to spinal cord Transcutaneous Stimulation (scTS) applied once a week for approximately 18 weeks. We observed that 1) the combination of scTS stimulation at different sites are subject-specific (Figure. 2) and 2) the functional state of the spinal circuitry changes with continued treatment (Figure. 3). Initially none of the SCI subjects could voluntarily initiate rhythmic stepping- like movements with any detectable rhythmic EMG activity of the leg muscles. After four weekly training sessions with pcEmc in the GND, the subjects had recovered a significant level of voluntarily facilitated knee and hip movements. The subjects were capable of generating voluntary flexion of specific joints on command during pcEmc with appropriate EMG bursting patterns. We concluded: 1) the magnitude of the range in knee oscillations was increased with voluntary effort plus pcEmc even within the first test session, 2) there was a further increase in the range of these oscillations with just four weeks of training (one session per week) with pcEmc (t2 vs. t1 in Figure. 2) by 18 weeks the voluntary movement was as great without as with stimulation and 4) there was notable subject-specificity for the stimulation site that evoked the greatest responses to the interventions at all phases [17]. Also we have demonstrated that pcEmc applied to T11 results in step-like movements in non-injured subjects when their legs were placed in a GND. The addition of stimulation at L1 and then C5, progressively increased the amplitude of these movements and the EMG activity in most subjects (Figure. 3). An important finding of the study is that in the majority of cases multi-site stimulation produces a more robust response compared to single site stimulation. The synergistic and interactive effects of scTS suggest a multi-segmental convergence of descending and ascending, and most likely propriospinal, influences on the spinal neuronal circuitries associated with locomotor activity[18].

Pathways:

Following human spinal cord injury (SCI) there is a marked interruption in descending inputs to the spinal cord which results in reduced motor activity and impaired motor function. However, the spinal cord can recover and exhibits considerable plasticity at various levels of the motor pathway [20, 21]. Because most human SCI are not anatomically complete, recovery of function after injury most likely also involves plasticity in circuitry above the lesion [22]. Therefore, strengthening the remaining descending pathways can aid in the transmission of descending cortical and brainstem signals that have been identified as being important for motor function. In incomplete SCI participants, it has repeatedly been shown that strengthening of the corticospinal tract is associated with motor recovery [22-25]. To date, most intervention studies following SCI have focused on plastic changes occurring within the corticospinal tract alone, neglecting the potential impact of other extrapyramidal pathways on the recovery of motor function. Such pathways include the reticulospinal tract which is important for the generation of automatic and synergistic motor functions [26-28], the vestibulospinal tract which plays an important role in the control of balance [27, 29-31] and the long intersegmental propriospinal pathway which allows intersegmental integration of networks along the spinal cord [32-34]. Strengthening of these extrapyramidal pathways could potentially compensate for the loss of corticospinal input and positively affect recovery of motor function after SCI. Interestingly, following human SCI, and in the absence of training interventions, these non-pyramidal pathways already exhibit plastic changes, with non-pyramidal pathways strengthening after injury as potential compensation for the loss of corticospinal input [35-38]. However, studies are required to determine whether motor recovery following training coincides with strengthening of these non-pyramidal pathways in addition to strengthening of the corticospinal tract.

- ***Corticospinal Pathway***

The corticospinal pathway is perhaps the most-studied descending tract in the study of human movement control, and specifically volitional movement. This tract innervates all levels of the spinal cord and terminates within all regions of the spinal grey matter, including direct connections to the motoneurons [39, 40]. Corticospinal neurons originate in a variety of cortical areas, however, for the purpose of this study we are focusing on those originating within the primary motor cortex. Numerous animal and human studies have examined the role of corticospinal

input in the generation of voluntary movements, therefore we will not go into the details of those studies here. However, it is important to note that the corticospinal tract does not only have a role in generating isolated voluntary movements, or fine movement control but can also modulate the central pattern generator circuitry responsible for the generation of locomotion. In humans, transcranial magnetic stimulation has been utilized to tease out the role of the corticospinal tract in gait in non-injured individuals and after SCI. TMS activates the corticospinal neurons trans-synaptically resulting in the production of multiple descending volleys that evoke a motor potential [41, 42]. During gait, the use of TMS has demonstrated that corticospinal tract neurons are active and greatly modulate muscle responses even when gait is uncomplicated [43-46]. Additionally, EMG-EMG and EEG-EMG coherence studies also support the view that the motor cortex plays a role in walking [43, 47].

After SCI, the integrity of the corticospinal tract is significantly reduced and this is evident through the absence or decreased size of EMG responses below the lesion in response to i) voluntary effort and ii) TMS stimulation as well as iii) decreased EMG coherence within the 25-40 Hz band (for review see Field-Fote et al 2017). It is clear however that the corticospinal tract reorganizes after injury and exhibits considerable plasticity. This is evidenced by impaired cross-facilitation to muscles below the lesion [48-50], and increases in coherence and MEP amplitudes following interventions such as locomotor training [23, 24, 47]. In fact, the strength of the corticospinal tract may predict locomotor recovery following SCI [51]. Lastly, it is also likely that following injury the initiation of steps and the role of the motor cortex becomes more significant [52]. Although the above studies have provided strong evidence of the role of the corticospinal tract in the recovery of function after SCI there are some limitations. The tibialis anterior is the frequently-studied muscle and therefore it remains unclear if these findings translate to other muscles of the lower limbs. Additionally, several studies have failed to successfully measure TMS MEPs below complete lesions due to mechanistic limitations. Methodological concerns such as the type of coils used in these studies (figure-8 versus double cone coil) and whether participants were instructed to attempt a voluntary contraction while being stimulated may explain these discrepancies. Indeed, MEPs were successfully evoked in a recent study where a double cone coil was used and MEPs were recorded in muscles below the level of a complete lesion while participants attempted voluntary contractions [53]. Therefore, it is highly likely that even in the motor complete SCI individuals in this study, the use of ES and training facilitated transmission along the corticospinal pathway to allow individuals to gain the ability to voluntary move muscles below their lesion and to display independent overground walking which is commonly thought to engage the entire neuraxis including the corticospinal tract and not just the spinal CPGs [22].

- ***Reticulospinal Pathway***

The reticulospinal pathway may also play a significant role in the recovery of motor function after SCI. The reticulospinal pathway originates in the reticular formation, and more specifically the nuclei reticularis pontis oralis and caudalis in the pons and the nucleis reticularis gigantocellularis and magnocellularis in the medulla. A portion of this tract descends ipsilaterally, while some descend almost evenly bilaterally [26, 54]. These two pathways descend throughout the ventral, ventrolateral funiculi and in the case of medullary axons also through the dorsolateral funiculus with terminations either on spinal interneurons and axial motoneurons at all levels of the spinal cord (pontine pathways) or terminations in laminae VII-VIII and axial and limb motoneuron nuclei [54]. Additionally, a single reticulospinal neuron can project to multiple motoneuron pools indicating that this pathway may play a role in the activitation of gross muscle synergies associated with motor function [26]. In animals, the role of the reticulospinal pathway has been implicated in the intersegmental integration of locomotor activities generated by spinal interneuronal circuitry (e.g. central pattern generators) distributed throughout the spinal cord [26]. It appears that the reticulospinal pathway is necessary for the production of automatic and synergistic locomotor functions. These pathways are of interest following spinal cord injury as more recently an increasing number of animal studies have shown that these brainstem pathways are important for recovery of function. It has been demonstrated that reticulospinal axons have a higher propensity for regeneration than other descending axons (e.g. corticospinal tract) and sprout extensively into grey matter structures contributing to improvements in gross motor behaviours following injury [28, 55, 56].

In autopsy findings in the human spinal cord, it appears that a high density of reticulospinal axons are present within the cervical cord, with most axons terminating in the cervical enlargement [27]. It has been suggested that beyond the cervical enlargement, these axons are replaced by propriospinal axons, indicating a reticulo-propriospinal relay that may play a role in recovery of function after human SCI [27]. After human SCI, it has

been demonstrated that the reticulospinal pathway appears to be strengthened [36, 57]. Additionally, this has also been shown in individuals with deficits in the corticospinal tract without motor weakness, indicating that this tract may allow for motor compensation in the presence of reduced descending corticospinal input [58]. Lastly, only very recently has it been demonstrated that the reticulospinal pathway is responsible for gross finger movements following human SCI [59], however, it remains to be determined whether the integrity of this pathway below the level of a complete lesion can account for other types of motor recovery following human SCI, and whether the excitability of this pathway can be altered with interventions.

- ***Vestibulospinal Pathway***

Vestibular information is pertinent in balance control during a variety of movements. The vestibular apparatus consists of the semicircular canals and otolith organs within the ears. These irregularly firing afferents provide information about angular velocity, linear acceleration and velocity of the head [31]. The role of the vestibulospinal pathway in human movement control has best been elucidated through the use of galvanic stimulation (GVS). GVS directly modulates the firing of vestibular afferents and produces a signal of head movement that has a whole-body effect on motor responses. Specifically, it has been demonstrated that only muscles engaged in a balance task are modulated by GVS and that the response is distributed in response to the load placed on the limb [31]. Additionally, small changes in posture such as shifts in body weight and distance between feet can drastically alter the amplitude of the responses. When comparing the effects of GVS during a variety of different tasks such as overground walking, treadmill walking, standing and bicycling it becomes apparent that the vestibulospinal pathway likely plays a greater role in tasks where changes in the centre of mass and base of support are dynamic [60-62]. Additionally, the lack of effect of GVS during cycling again reiterates its role in balance control, despite similar muscle activations in ankle muscles during walking and cycling [61]. Lastly, when comparing walking to standing, although there are similarities in the responses to GVS there are also some notable differences. Namely, there appears to be different patterns of muscle activity depending on the task (coactivation and increased involvement of muscles around the ankle joint during walking) [60-62]. Finally, studies examining changes in the efficacy of transmission along the vestibulospinal pathway after SCI are very few. In one study it was demonstrated that GVS-evoked responses in the trunk muscles correlated well with the level of impairment of the individual [63]. Additionally, when examining GVS-evoked responses in the leg muscles the magnitude of responses was similar to those of non-injured participants, however the latency of the responses was significantly delayed indicating compromised transmission of the vestibulospinal signals [29]. However, it is important to note that in all these studies only individuals with incomplete SCI were examined. In a more recent study examining motor complete SCI individuals, vestibular-evoked myogenic potentials were only elicited in the legs of 2 individuals, however, the authors highlight the need to refine the experimental protocol which utilizes a different method to elicit vestibulospinal responses [53]. Nevertheless, this study highlights that even after a severe SCI (AIS A/B) there may still be residual vestibulospinal pathways below the level of the lesion. Therefore, the likelihood of strengthening these residual pathways with ES and training exists and perhaps underlies the recovery of such tasks like independent standing and walking which require greater balance control.

- ***Propriospinal Pathways***

As one of several components that form part of the motor pathway, the propriospinal pathway includes interneurons that link several segments of the spinal cord and consists of both short and long projections within the cervical, thoracic and lumbar spinal cord. The short propriospinal neurons span only a couple segments, while the long ascending and descending pathways allow coupling between the cervical and lumbar enlargements with cell bodies originating in C3-4 and terminating in L2-5 and vice versa. After injury, and subsequently during recovery, marked plasticity within the propriospinal system can contribute to functional reorganization following SCI in animal models [64-67]. More specifically, after an incomplete injury in the rat, corticospinal tract axons were shown to exhibit sprouting to both short and long propriospinal neurons [65]. Of interest, only contacts with longer propriospinal neurons that bridged the lesion were maintained [65]. These long propriospinal neurons arborized onto lumbar motoneurons facilitating recovery of locomotion in these animals [65]. Additionally, it has been demonstrated that propriospinal circuitry undergoes significant remodeling after injury. In a delayed, staggered hemisection model in mice, it has been demonstrated that a

relay of short propriospinal neurons in the thoracic spinal cord can provide an alternative route to bypass the lesions and allow transmission of neural signals that can facilitate locomotor recovery [67]. The pharmacological ablation of thoracic propriospinal neurons abolishes the recovery of stepping in these same animals [IEEE Trans Neural Syst Rehabil Eng. 2019 Sep;27(9):1855-1864. doi: 10.1109/TNSRE.2019.2933381. Epub 2019 Aug 5.67]. Additionally, this pathway can be shaped by training interventions, with increased labelling of short propriospinal neurons in quadrupedal step-trained rats where cervicolumbar coupling would be targeted [68].

In humans, propriospinal pathways account for a large proportion of the fibres within the spinal cord, with a greater percentage of fibres in the lumbar spinal cord attributed to this pathway [27]. While most research has focused on the cervical (C3-4) propriospinal pathways [69, 70], some have demonstrated interlimb reflexes in humans as well [32, 37, 71-74]. Specifically after human SCI, interlimb reflexes thought to be mediated by propriospinal neurons appear to undergo plastic changes following injury [37, 38, 74-76]. The link between the excitability of these likely propriospinally-mediated interlimb reflexes and functional neurophysiological assessment (FNPA) or ASIA scores has not been examined, despite the interesting finding that the more rostral the lesion, the greater the gain in locomotor function following SCI [77]. This finding indicates that the circuitry within the spinal cord that enables locomotor activity is not restricted to one specific segment of the spinal cord, but rather spans a network of intersegmental pathways. In humans, it is well established that CPG mechanisms contribute to locomotion and that afferent feedback modulates this activity [78-84]. It has also been suggested that the phase and task-dependent modulation of various reflexes in the upper limbs during arm cycling indicate a similar CPG organization to that describing locomotion of the legs [34, 85-87]. In addition, strong evidence has shown the existence of task-dependent coupling between arm and leg muscle activity during walking, crawling and swimming [33]. During treadmill walking, interlimb reflexes in both arms and legs evoked by cutaneous nerve stimulation were phase-modulated during the walking cycle and task-dependent [88]. Additionally, reciprocal organization of reflex responses from hand to foot and from. foot to hand were observed. Taken together, these findings have suggested the existence of coupled neural oscillators, with two controlling arm movements, and another two controlling leg movements [33]. This coupling may explain why following SCI, gait is significantly improved when patients are able to utilize arm swings in combination with body-weight supported locomotor training [89, 90]. Additionally, following incomplete cervical SCI, rhythmic arm motions were able to elicit locomotor muscle activity in leg muscles [91]. Therefore, it is apparent from numerous findings that innate circuitry within the spinal cord can generate rhythmic, patterned muscle activity at various levels of the spinal cord that involve both the arms and the legs. Additionally, strengthening the cervicolumbar coupling within the cord may translate to greater recovery of locomotor function following SCI [25, 92] because it incorporates this circuitry along the spinal cord rather than utilizing a discrete network at a specific level of the spinal cord. Therefore, it is highly likely that the recovery of stepping following ES and locomotor training in individuals with complete SCI could arise through strengthening of these long propriospinal pathways.

B. Cardiovascular and Respiratory Function

Cardiovascular diseases are the leading cause of morbidity for individuals with SCI [93]. 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 [94-96]. 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 [93-100]. 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 4**). We propose that cardiovascular control is facilitated by the spinal cord as a key integrator of complex 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

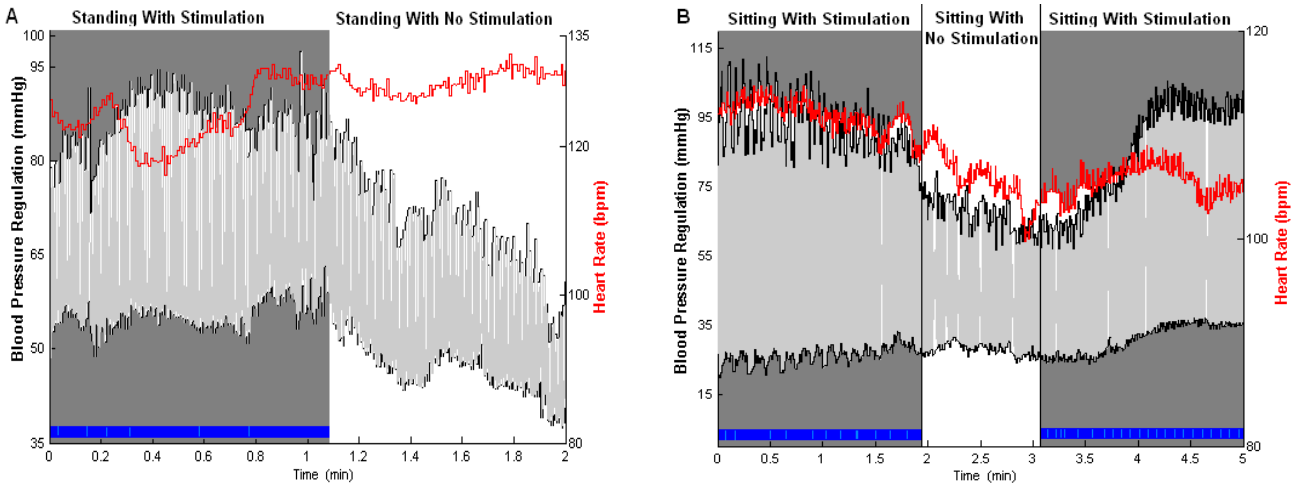


Figure 4. Continuous blood pressure and heart rate recordings from the individual with cervical motor complete SCI during standing **(A)** and sitting **(B)** with and without epidural stimulation. The individual was able to maintain his blood pressure in both sitting and standing positions with epidural stimulation. Blood pressure significantly decreased when stimulation was turned off and recovered again when the stimulation was turned on. Blue indicates tonic stimulation

scES will be needed to optimize autonomic function.

Krassioukov et. al has 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 [101]. 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 orthostatic hypotension, 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 orthostatic hypotension have shown that with optimized epidural stimulation parameters, we can maintain systolic blood pressure 20% above his nadir and prevent symptomatic hypotension.

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 [102] and likely contribute to this population's three to four-fold increase in cardiovascular disease [103]. 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 [104]. 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 [105]. 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 [106, 107].

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 [108-111]. Recent evidence also suggests that diastolic function, inferred through the measurement of early-to-late filing velocity, is impaired in individuals with SCI [112]. In animal models of SCI, left-ventricular developed pressure and the rates of contraction and relaxation are impaired after high- or mid-thoracic SCI [113, 114]. There is also evidence of ventricular remodeling in the chronic stages of SCI as evidenced by increased myocardial collagen deposition [114, 115]. In both human and animal SCI, pump function of the left ventricle can be improved by exercise training [116] and/or mechanical compression of the legs and/or abdomen [117, 118], 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.

Cerebrovascular function after SCI: Cardiovascular autonomic dysfunctions after SCI are primary factors that lead to declining health of the cerebral vasculature [109, 110]. Impaired cerebrovascular function and structure are associated with increased risk of stroke as well as cognitive dysfunction [119, 120]. As stroke risk is 200-300% times higher, and cognitive dysfunction occurs in up to 60% of those with SCI [93, 121], a critical appraisal of the association between autonomic dysfunction and cerebrovascular health is needed to improve understanding and elucidate potential preventative and therapeutic targets. In two seminal clinical trials, improving orthostatic hypotension using midodrine improves cerebrovascular function, orthostatic tolerance, and cognition [122, 123]. 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 presser pharmacological agents after SCI, and partially restore normal cerebrovascular function and cognition in those living with SCI.

Respiratory function after SCI: Breathing is an essential life-sustaining activity that requires the contraction of respiratory muscles, coordinated by the respiratory motor control system which, when healthy, integrates input from the brain, brainstem, spinal cord and peripheral nerves. Since respiratory and cardiovascular systems are inter-related and impact one another through the respiratory-cardiovascular (also known as cardio-respiratory) coupling mechanism [105, 124], respiratory dysfunction plays role in the development of arterial hypotension [125, 126], autonomic dysreflexia [127], and stroke [128]. Respiratory neuroplasticity, defined as a persistent morphological and functional change in neural control based on prior experience, is critically dependent on the establishment of necessary preconditions, the stimulus paradigm, balance between opposing modulatory systems, age, gender, and genetics. Investigating respiratory muscle activation during voluntary inhalation in SCI individuals, we observed that respiratory muscles below clinically motor complete injury levels can be activated when this task is assisted by epidurally implanted stimulator at the lumbosacral spinal cord level [2]. Our previous work showed that abnormal respiratory-cardiovascular interactions in patients with SCI-induced orthostatic hypotension are associated with respiratory and autonomically-mediated cardiovascular deficits which can be improved using activity-based rehabilitative approach [129]. We have

found that these interventions lead to respiratory motor [129, 130] and autonomic plasticity in association with improved baroreflex responses and better orthostatic tolerance [131]. However, the effectiveness of this intervention is limited, due to decreased excitability of spinal networks secondary to disruption of the drive from supraspinal centers [132]. Our recent studies demonstrated that tonic scES at the lumbar level in individuals with SCI lead to voltage-dependent changes in breathing pattern, in association with improved respiratory-cardiovascular functional state may suggest that scES can modulate respiratory-cardiovascular plasticity.

C. Bladder, Bowel and Sexual Function

Bladder, bowel and sexual dysfunctions rank among the top disorders affecting quality of life after SCI [133, 134]. 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 (i.e. nerve growth factor) 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 scES 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.

Urinary Bladder Dysfunction in SCI: Development of a neurogenic bladder is a cause of significant morbidity and mortality in the SCI population [135, 136]. 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 [137, 138]. SCI individuals often exhibit chronic vesicoureteral 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 [139]. With the common use of anticholinergic medications, side effects such as dry mouth and constipation further exacerbate the underlying urologic dysfunction, making compliance difficult [156]. Up regulation of neurotrophic factors post-SCI [140] is responsible for the re-emergence of the spinal voiding reflex within 2 weeks of injury [141], but chronic changes in nerve growth factor appear to be responsible for bladder afferent hypersensitivity, hypertrophy, and sprouting of axons [142], all of which lead to bladder over-activity. Nerve growth factor and brain derived neurotrophic factor have been implicated as key factors in a variety of bladder dysfunctions [143]. Nerve growth factor delivery (intrathecal infusion at L6-S1 spinal level in adult rats) causes bladder dorsal root ganglion afferents to become hyper-excited and results in detrusor hyperreflexia [144], while nerve growth factor 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 [145, 146]. Sequestering brain-derived neurotrophic factor (daily tail vein administration of TrkB-Ig_2, which specifically binds brain-derived nerve growth factor and neutralizes it) has been shown to improve bladder function in a chronic cystitis model [147]. Exercise therapies also influence neurotrophin expression in visceral target organs, not just skeletal muscle [148, 149]. 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.

Bowel Dysfunction in SCI: Gastrointestinal problems are also a cause of significant morbidity and mortality in the SCI population [150, 151]. Stool evacuation is a complex process involving relaxation of the pelvic floor, contraction of the recto-sigmoid, and the inhibition of the anal sphincters [152]. 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 [150, 153-155]. Synergistic

activity between colonic smooth muscle and the striated anal sphincter muscle is lost [156]. Dysmotility, decreased transit time and loss of tissue compliance may be associated with the loss of descending modulation of the sympathetic supply [156].

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 [157]. Most individuals with SCIs above T10 demonstrate reflexogenic erections of varying degrees in response to very slight stimulation of the penis [158, 159]. 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) [160, 161] 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 [159, 162] 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 [163], 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 [164]. In females with SCI, impairments in genital responses and sexual arousal have been documented [165-167]. Fertility in females is not an issue, although there are special issues to address during pregnancy [165, 166, 168].

II. Research Approach

A. General Approach

We propose to understand the role of scES activating spinal circuits on the recovery of below injury voluntary control of movements, standing, stepping and the autonomic nervous system function in humans with severe paralysis.

Our approach for early investigational studies encompasses three primary *systems* (**Figure 5**):

- Motor Control (MC);
- Cardiovascular and Respiratory (CVR);
- Bladder, Bowel and Sexual Function (BB).

Within each of the three *systems* our investigations can be specific to:

- Proof of Principle (new implants, PP);
    - Proof of Principle is the method that demonstrates an individual effect of epidural stimulation with the aim of verifying that there is practical potential without confounding factors of other training or stimulation effects. Thus, the Proof of Principle early feasibility studies are those that involve new implants and research participants complete these studies prior to enrolling in any inter-system studies.
- Inter-System Participation (already implanted participants, IS)
- Advancement of Technology (already implanted participants, AT)

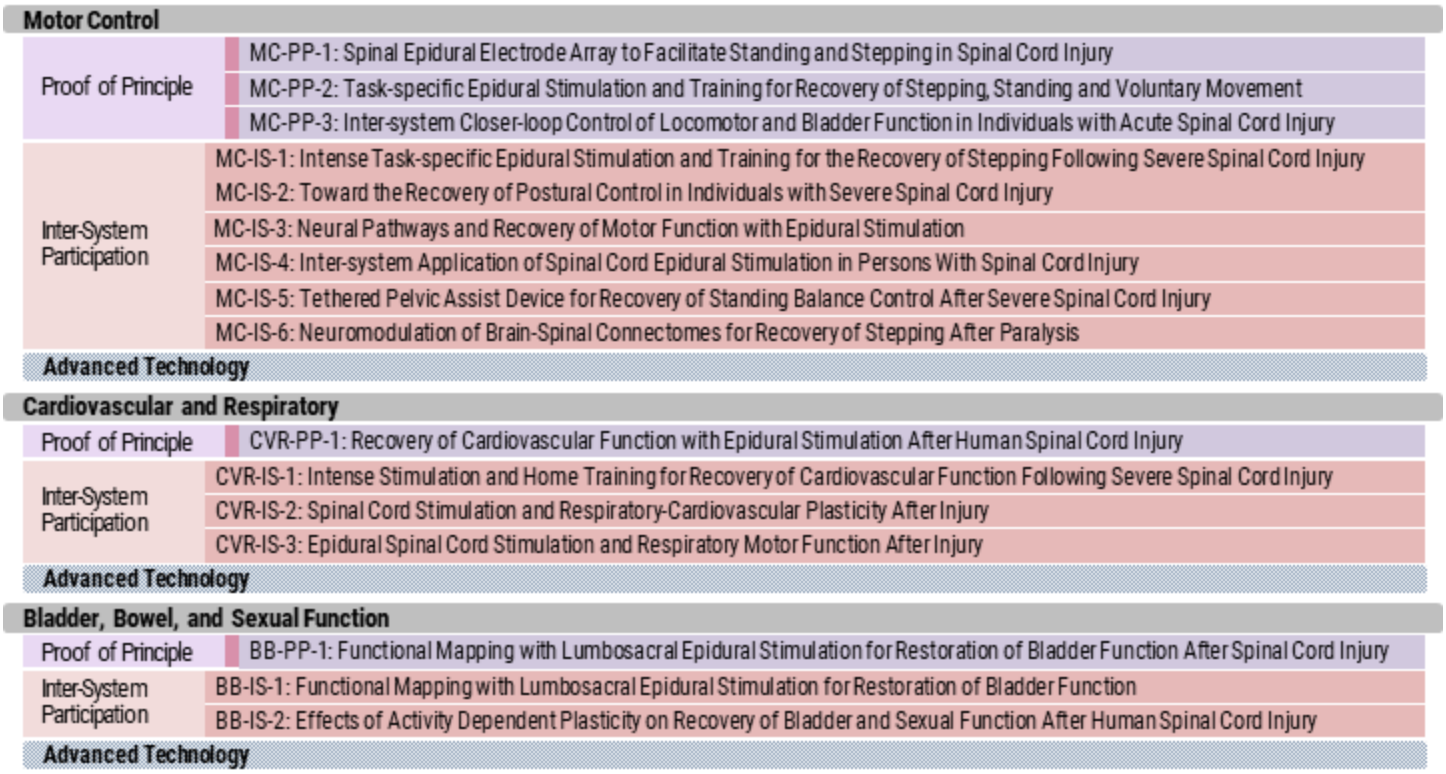


Figure 5: Schematic of overall Program Organization and Current Early Feasibility Studies with scES. Organizational Structure which encompasses three systems (grey): Motor Control, Cardiovascular and Respiratory, and Bladder, Bowel and Sexual Function. Each system contains three categories: Proof of Principle (purple horizontal bars, includes new implants-pink vertical bar), Inter-system Participation (orange horizontal bars), and Advanced Technology (blue horizontal bars). Titles are included in the figure for current studies. There are currently no proposed ***advanced technology projects as indicated by dotted coloring.***

Project specific aims will define specific training paradigms which will be discussed in detail below (sections IIC-E). Using this approach, we are focusing on 1) minimizing risks, obtaining a larger study sample number without the risk of surgery, and 2) maximizing benefits, allowing implanted individuals to participate in multiple consecutive interventional paradigms.

Our approach differs from 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 in the context of the 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, respiratory, urogenital and bowel control. In the case of standing and stepping, 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, 169, 170]. 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 an 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. These studies will further our understanding of the mechanisms underlying the restoration of walking, 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 and complete the interventions in up to 68 research participants who have sustained a spinal cord injury. Forty-eight individuals would have already been implanted with a neurostimulator and 5-6-5 Specify electrode at T11-L1 vertebral level (corresponds with the L1-S1 spinal cord levels), and twenty individuals will receive the implant as part of the study (**Figure 6**).

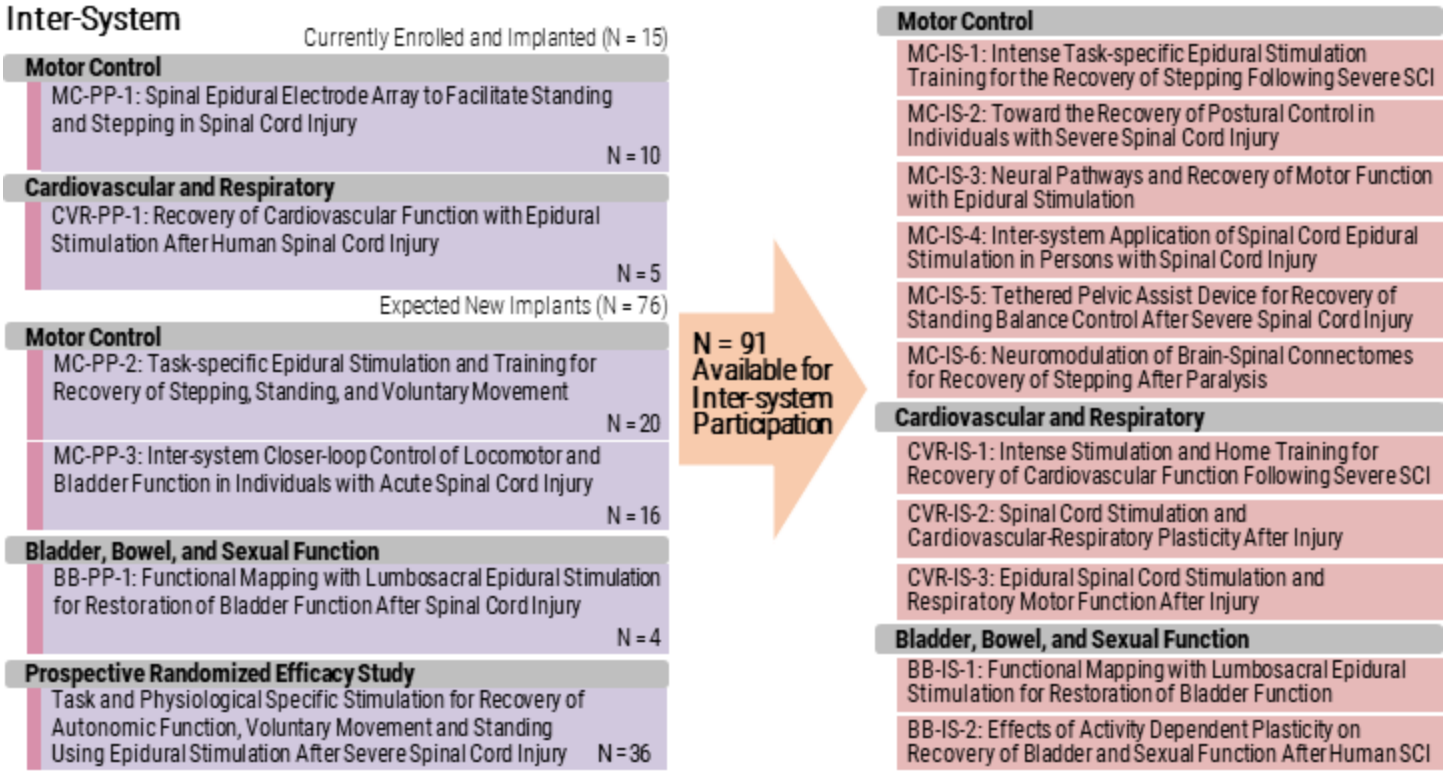


Figure 6: Enrollment strategy for proof of principle and inter-system studies. Left hand side of diagram depicts current and enrolled participants (N=15) and expected new implants (N=76). Right hand side of diagram lists current possibilities of inter-system study enrollment options for these 91 implanted participants.

We anticipate we will need to screen three times the number of potential research participants to reach our enrollment numbers (for new implants only). Once a participant completes a proof of principle study in a system, they may continue to participate in an intersystem study (**Figure 7**). Our novel approach of conducting repeated experiments with comprehensive assessments in a smaller cohort of participants, rather than a more traditional approach of including a large number of participants 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, and**

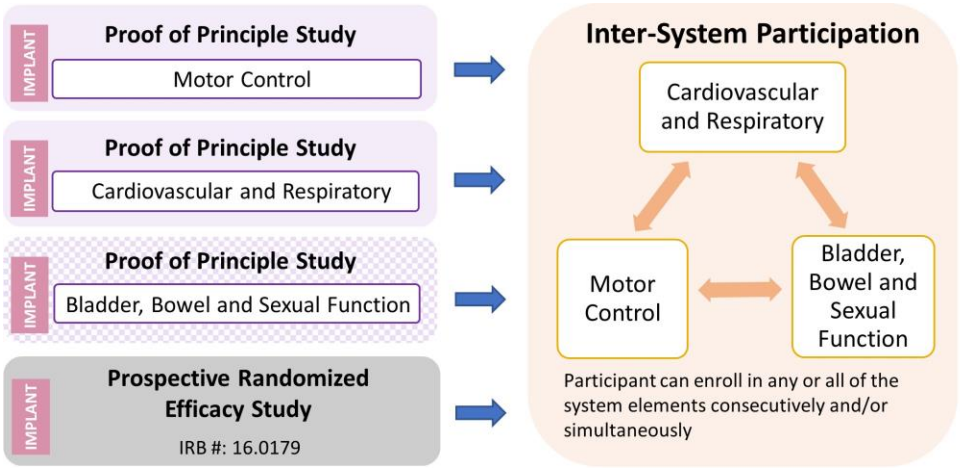


Figure 7: Overview of framework of participant path through epidural research program. Participants are enrolled and implanted in one proof of principle study (purple) of a specific system element (Motor Control, Cardiovascular and Respiratory, or Bladder, Bowel and Sexual Function) or a prospective randomized efficacy study (gray). After completion of the initial study, a participant can enroll in one or multiple inter-system studies (orange). Inter-system studies do not have new implants. ***Currently there is no ongoing proof of principle study in the Bladder, Bowel and Sexual Function (checkered purple).***

screening

a. Recruitment.

Recruitment for each proof of principle study will be based on specific inclusion/exclusion criteria (Section III.A); current availability (study being open to enrollment) and order of registration in our research database. 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. 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 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 the Kentucky Spinal Cord Injury Research Center with an investigator, research coordinator, and/or the research manager. During the info session research personnel will describe the time commitment, the requirement of surgery, the general assessments and interventions involved 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.

For Inter-System (IS) Participation and technology advancement, participants will be identified from the previous proof of principle studies. Individuals who have been implanted the longest will be contacted first. These individuals would not need to go through the pre-screening or screening process as all the information normally collected during pre-screening and screening (below) would have been collected during their previous participation and involvement in the proof of principle study.

***b. Pre-Screening*.**

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 Neuroscience Collaborative 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. Participants may require additional blood work, a urine test, nasal and peri-rectal swabs to determine infection risk for surgical implantation (i.e. if participant tests positive for MRSA, they may not be eligible for immediate participation). 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 pre-paid 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 research nurse and she/he may request consultation from the research physician, neurosurgeon and/or cardiologist. The research nurse and/or physician(s) will provide a medical recommendation to the principal investigator as to whether they should be invited to the screening process. If the principal investigator approves 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 the Neuroscience Collaborative Center at the Kentucky Spinal Cord Injury Research Center in Louisville.

c. Screening.

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 at the Kentucky Spinal Cord Injury Research 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 will participate in the initial screening of the research participants. All eligible SCI research participants will be invited to discuss the complete protocol and its risks and benefits with the principal investigator. The investigator(s) 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. The investigator(s) 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 (cardiovascular and respiratory studies only) and physician or nurse practitioner. The physician will conduct a medical history and physical examination and determine medical eligibility based on the clinical inclusion and exclusion criteria (see section III.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 physician or nurse practitioner, the potential research participant will undergo specific assessments. Potential study eligibility will be determined by the investigators based on the evaluation of these assessments to be consistent with the inclusion criteria (see section III.A below). In the case of screening for cardiovascular and respiratory studies, 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: Some studies might have the intent to avoid nicotine and drug users. After implantation, we will conduct random nicotine and drug testing using a urine sample and levels will be recorded. The individual will not be removed from the study after implantation, but nicotine and drug levels will be monitored and if detected, levels will be reported to the cardiologist for consideration in interpretation of the cardiovascular assessments.

The investigator 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. The investigator 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. If the individual meets all the inclusion and exclusion criteria and is given medical clearance by the study from the cardiologist (if applicable) and the study physician and is recommended for possible implantation by the study neurosurgeon, then they will complete the specific study assessments.

2. Surgery

a. Proof of principle studies: New implants

If the research participant decides to enroll in the surgical implantation and intervention phases of a study (defined below), the research participant will return to the Neuroscience Collaborative Center - Kentucky Spinal Cord Injury Research Center. The investigator will meet with the potential research participant to discuss the potential risks of the study. Pre-implant assessments will be repeated to match the assessments previously performed (pre-usual care or pre-training as specified by the study protocol). 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 complete the pre-implant assessments as specified by each study. The research participant will be assessed by the research team's neurosurgeon, cardiologist (if applicable) and physician or nurse practitioner. The neurosurgeon will review all aspects of the surgery including the potential risks specific to the surgery and the medical device with the individual. The investigators of the specific projects will review the results and collaborate on whether the individual is medically and scientifically eligible for the study. The study neurosurgeon will determine eligibility for surgical implantation. The investigator 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. The investigator 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 with the study, they can let the study team know and there will be no penalty. They can remain in our database, if desired, for recruitment in future studies.

1. 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 adapt its facilitating effects toward different functional activities, such as standing, stepping or voluntary movement or physiological responses such as blood pressure regulation

The procedure will be performed by the study neurosurgeon at University of Louisville Hospital to implant the epidural stimulating electrodes and neurostimulator in a single surgical procedure under general anesthesia following standard medical procedures. As per standard practice, a Medtronic representative will bring the neurostimulator to the operating room and provide technical support throughout the implantation procedure. The MRI compatible 5-6-5 Specify electrode, and neurostimulator, (MEDTRONIC, Minneapolis, MN, USA) will be implanted at the T11-L1 vertebral level which corresponds with the L1-S1 spinal cord levels (lumbosacral spinal cord), guided by fluoroscopy. Neurophysiological mapping will then occur to check appropriate placement of the electrode. The lead wires will be tunneled subcutaneously to the abdominal area. 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 in the abdominal area.

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 vertebral levels. Bilateral surface electromyography electrodes from the quadriceps, hamstrings, tibialis anterior, medial gastrocnemius and soleus, and fine-wire electromyography electrodes of the iliopsoas will also be used to record the multi-segmental motor responses induced by 2 Hz stimulation. The research team 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 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 placed in a TYRX antibacterial pouch and 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.

2. Postoperative Care

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 2-6 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 the study physicians. 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 III.B below).

b. All Systems. Replacement of Technology

There are three situations that might warrant replacement of an implanted stimulator and/or electrode.

1. Neurostimulator End of Service
2. Malfunctioning unit
3. Availability of better technology

The 12 participants implanted from Projects 1 and 2 who have been transferred to Project 4 (MC-PP-1, CVR-PP-1) will be given the option to have the stimulator and/or electrode replaced if their current implanted device is reaching end of service (EOS) or it is malfunctioning. The device replacement can be any approved device listed in this protocol, which can include the same device initially implanted or the latest technology available. If EOS has not been reached but significant advances have been achieved with the latest technology, participants may elect to have their stimulator and/or electrode replaced as well, as long as the benefits outweigh the risks of surgery.

If the research participant decides to have their neurostimulator and/or electrode replaced, the research participant will return to the Neuroscience Collaborative Center - Kentucky Spinal Cord Injury Research Center. The investigator will meet with the research participant to discuss the potential risks of the study. Pre-surgery assessments may be performed to obtain a baseline prior to surgery. The research participant will sign the consent form for device replacement.

The research participant will be assessed by the research team's neurosurgeon, cardiologist (if applicable) and physician or nurse practitioner. The neurosurgeon will review all aspects of the surgery including the potential risks specific to the surgery and the medical device with the individual. The study neurosurgeon will determine eligibility for surgical implantation. If any new information is identified that would determine they are ineligible for a device replacement, the reasons will be explained. If they are eligible, then all the potential risks will be discussed again. The investigator 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 have their stimulator and/or electrode replaced.

1. Surgical Implantation.

The procedure will be performed by the study neurosurgeon at University of Louisville Hospital. The epidural stimulating electrodes (if applicable) and neurostimulator battery will be implanted in a single surgical procedure under general anesthesia following standard medical procedures. As per standard practice, a Medtronic representative will provide technical support throughout the implantation procedure. Research personnel will bring the new neurostimulator battery and/or electrode to the operating room. The MRI compatible 5-6-5 Specify electrode (if applicable), and neurostimulator battery, (MEDTRONIC, Minneapolis, MN, USA) will be implanted at the T11-L1 vertebral level which corresponds with the L1-S1 spinal cord levels (lumbosacral spinal cord), guided by fluoroscopy. Neurophysiological mapping will then occur to check appropriate placement of the electrode.

Scenario 1: Replacement of both neurostimulator battery and electrode in an individual with neurostimulator battery in a posterior location (back area).

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 wide prepping of the thoracolumbar surgical field including site of the previous battery and any new intended battery sites will be performed using alcohol chlorhexidine disinfectant solution. The previous midline incision will be opened using the surgical scalpel and bovie electrocautery will be used to dissect through subcutaneous tissue to the electrode. The anchoring sutures will be excised, and scar overlying implant will be dissected off the implant. Loosening the electrode may require additional bony removal via laminectomies or laminectomy. After removal of the old epidural electrode, a new epidural electrode will be placed in the location of the prior electrode at 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 vertebral levels. Bilateral surface electromyography electrodes from lower extremity muscles, and fine-wire electrodes of the iliopsoas will also be used to record the multi-segmental motor responses induced by 2 Hz stimulation. The research team 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.

After confirmation of optimal electrode placement, the incision overlying the neurostimulator battery will be reopened to exposure of the battery. The distal end of the old electrode will be disconnected from the neurostimulator battery and the old epidural electrode and battery will be removed. The wires of the new implanted epidural electrodes will be tunneled under the skin and connected with the new neurostimulator battery. The new neurostimulator battery pack will be placed in a TYRX antibacterial

pouch and buried in the subcutaneous tissue directly external to the muscle or fascial tissue. The neurostimulator battery pack will be sutured to muscle or fascial tissue in order to prevent its migration. The surgical field will be irrigated using antibiotic solution to minimize infection. A multiple layer closure of the incision will be performed using Vicryl sutures.

Scenario 2: Replacement of both neurostimulator and electrode in an individual with neurostimulator battery in an anterior location (abdomen).

The surgery will begin in the supine position and the abdominal area overlying previous incision will be prepped using alcohol chlorhexidine. The incision overlying the battery will be reopened to exposure of the neurostimulator battery. The old battery will be disconnected from the electrode wires and removed. The free end of the electrodes will be left in the wound. The wound will be irrigated with antibiotic saline solution and closed in multiple layers. The research participant will then be transferred prone onto a Jackson surgical table and the entire thoracolumbar area and site of new neurostimulator battery placement in the paraspinal area between posterior iliac crest and last rib will be prepped and draped. The previous thoracolumbar incision is reopened, and the previous epidural electrode array will be removed as described above Scenario 1. Full removal will occur by pulling the previously disconnected distal ends subcutaneously from the abdominal site to the back site. A new epidural neurostimulator battery is placed as described above (Scenario 1). Next an approximately 4-inch incision is made at the paraspinal area close to posterior flank approximately midway between the posterior iliac crest and the last rib either on the left or ride side depending on research participant and/or surgeon preference. A subcutaneous pocket approximately 2.5-3 cm deep and big enough to fit the new neurostimulator battery will be created. The new epidural electrode is then tunneled to the new neurostimulator battery site and connected as previously described in Scenario 1.

Scenario 3: Replacement of neurostimulator battery only (either abdominal or back location)

If the prior stimulator was implanted on the abdomen, the individual will be placed in the supine position on the operating table with all pressure points being well-padded. If the prior stimulator was placed on the back, the individual will be placed in the prone position on the operating table with all pressure points being well-padded. The surgical field (abdomen or back) will be prepped using alcohol chlorhexidine disinfectant solution. An incision will be made on the same location as previous scar. The old neurostimulator battery will be disconnected from the wires of the implanted epidural electrodes and removed. The wires of the implanted epidural electrodes will be connected to the new neurostimulator battery pack that will be placed in the abdominal area. The neurostimulator battery pack will be placed in a TYRX antibacterial pouch and buried in the subcutaneous tissue directly external to the muscle or fascial tissue. The neurostimulator battery pack will be sutured to muscle or fascial tissue in order to prevent its migration. The surgical field will be irrigated using antibiotic solution to minimize infection. A multiple layer closure of the incision will be performed using Vicryl sutures.

2. Postoperative Care

The research participant will be taken from the operating room and transferred to the recovery room. The research participant will be kept in the recovery room for 2-6 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 research participant will be monitored for blood pressure, pulse, and temperature changes. Research participant will

receive postoperative antibiotics per protocol. A thoracolumbar postoperative xray will be obtained on postoperative day 1. 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 the study physicians. 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 III.B below).

For neurostimulator battery replacements only the research participant might be discharged on the same day. During the home recovery period, approximately 7-10 days, the research nurse will monitor the research participant with daily communication and periodic visits to inspect the wound healing and recovery. The research nurse will continuously update the study physicians. If any complications arise, the study physicians will be informed immediately, and the research participant will be provided with medical care.

3. Post-Implant Assessments

a. Proof of principle (PP) studies:

After recovery from surgery, post-implant, pre-intervention motor, bladder, pulmonary and cardiovascular assessments may be conducted. Mapping of the motor evoked responses related to spatial electrode selection, amplitude and/or frequency [81] will be conducted and the specific configurations and parameters optimal for stepping, voluntary movement, standing and cardiovascular function will be identified. 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 frequency parameters affect different spinal circuits, the array can be reconfigured, within limits, to adapt its facilitating effects toward different functional activities, such as stepping, standing or voluntary movement or physiological responses such as blood pressure regulation, respiratory and bladder function. Participants will continue to participate in their system's intervention training paradigm. Assessments will be repeated after each training intervention.

b. Inter-System (IS) and Advanced Technology (AT) Studies:

Once a participant completes a proof of principle study in a system, they may continue to participate in an intersystem study. Mapping of the motor evoked responses related to spatial electrode selection, amplitude and/or frequency will be conducted and the specific configurations and parameters optimal for stepping, voluntary movement, standing, cardiovascular, respiratory and bladder function will be identified. 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 frequency parameters affect different spinal circuits, the array can be reconfigured, within limits, to adapt its facilitating effects toward different functional activities, such as stepping, standing or voluntary movement or physiological responses such as blood pressure regulation, respiratory and bladder function. Participants will continue to participate in their system's intervention training paradigm. Assessments will be repeated after each training intervention.

c. Replacement of Technology:

After recovery from surgery, post-implant, pre-intervention motor, bladder, pulmonary and cardiovascular assessments may be conducted. Mapping of the motor evoked responses related to spatial electrode selection, amplitude and/or frequency will be conducted and the specific configurations and parameters optimal for stepping, voluntary movement, standing and cardiovascular function will be identified. 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 frequency parameters affect different spinal circuits, the array can be reconfigured, within limits, to adapt its facilitating effects toward different functional activities, such as stepping, standing or voluntary movement or physiological responses such as blood pressure regulation, respiratory and bladder function.

Assessments will be performed to ensure new configuration can be obtained to perform tasks similar to those using the old device. The individual will be allowed to participate in daily training sessions to become accustomed to the new unit and demonstrate its safe use. This process can take approximately 3 months.

The participants may elect to enter an intersystem study following surgery and initial mapping assessments. Participants will continue to participate in their system's intervention training paradigm as described in project section. Assessments will be repeated after each training intervention.

4. Interventions

a. Intervention Summary:

Depending on the project, participants will undergo different training interventions with epidural stimulation, including voluntary movement (Vol-scES), cardiovascular (CV-scES), stand (Stand-scES), step (Step-scES), bladder (UB-scES), and respiratory (RT-scES). If applicable to the project, participants may complete a training intervention without epidural stimulation prior to implantation or training with epidural stimulation. Each project (section IIC) describes the specific intervention that will be used in the study. Training interventions are described in section IIE. Assessments may be conducted during the intervention period.

b. Post-Intervention (proof of principle studies only):

After the training intervention and final assessments, the individual will then be asked whether they would like to have the epidural stimulator removed. The neurosurgeon will remove the stimulator if requested by the research participant. If the research participant would like to continue scES, they will be provided with stimulation configurations that they can demonstrate safe use of patient programmer during specific tasks.

5. Follow-up

Participants will be asked to return for a 6-month follow-up visit to repeat assessments. We will then check-in with participants via phone every 6-months for up to 2-years. Participants may return for revaluation of configurations if needed. After 2 years, as required by the FDA, we will be available for long-term support of subjects implanted with the device until either the device is removed, or the device is approved for the investigational indications for use. Participants may contact us at any time regarding the device and configuration evaluations.

C. Projects

1. Motor Control. Proof of Principle

a. MC-PP-1: Spinal Epidural Electrode Array to Facilitate Standing and Stepping in SCI

We propose to determine the level of functional gain, below the injury for recovery standing and stepping function as a result of activation of spinal circuits with scES in humans with severe paralysis. Training will consist of practicing stepping, standing and voluntary movements in the presence of specific scES configurations designed specific for stepping (Step-scES), specific for standing (Stand-scES) and for the voluntary movements of the legs and trunk (Vol-scES). Ability to step, stand, and move voluntarily will be assessed in these individuals with chronic severe spinal cord injury.

Participants and their data enrolled under IRB# 07.0066 will be moved to this comprehensive protocol. We anticipate no future enrollment as part of this study. Those enrolled and currently under study will continue with their training interventions and assessments.

i. Specific Aims

Specific Aim: Determine whether an integrated rehabilitation approach combining ES with body-weight-supported stand or step training will lead to better performance in functionally complete SCI individuals than can be achieved by either ES or robotic training alone.

ii. Training Interventions

Pre-Implantation phase: Research participants will receive approximately 80 sessions of step training and stand training (described below). These sessions can occur once a day (stand and step will alternate days) or twice daily (stand and step on the same day), 5 days a week for 1 to 1.5 hours.

Post-Implantation phase: Spinal cord epidural stimulation (scES) will be administered during training interventions. The pattern of electrically active electrodes, as well as electrode voltage, stimulating frequency, and stimulating pulse width may be remotely programmed using Medtronic's Clinician programmers. 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 stepping, standing or voluntary movement. After determining the configurations for stepping (Step-scES), standing (Stand-scES) and voluntary activity (Vol-scES), the individual will then begin training in the laboratory.

Research participants will receive approximately 160 sessions of step training with Step-scES, stand training with Stand-scES, in addition to daily voluntary training with Vol-scES (described below). These sessions can occur once a day (stand with Stand-scES and step with Step-scES will alternate days) or twice daily (stand with Stand-scES and step with Step-scES will alternate days), 5 days a week for approximately 1 hour. If stand with Stand-scES and step with Step-scES occur on the same day they will be approximately 2 hours apart. Voluntary training with Vol-scES will occur daily in addition to stand and/or step with scES. Independence levels and functional gains will be evaluated periodically. Surface electromyography (as described in assessment section II.D below) might be used during training sessions to evaluate motor activity during changes in stimulation configurations.

b. MC-PP-2: Task-specific epidural stimulation and training for recovery of stepping, standing and voluntary movement following severe spinal cord injury

We propose to determine the level of functional gain, below the injury for voluntary control of movements, and recovery standing and stepping function as a result of activation of spinal circuits with scES in humans with severe paralysis. Training will consist of practicing stepping, standing and voluntary movements in the presence of specific scES configurations designed specific for stepping (Step-scES), specific for standing (Stand-scES) and for the voluntary movements of the legs and trunk (Vol-scES). Ability to step, stand, move voluntarily, as well as cardiovascular, respiratory, bladder, bowel and sexual function will be assessed in these individuals with chronic severe spinal cord injury.

Following medical clearance for the assessments and participation in the pre-implant training portion of the study by the physician, the potential research participant will undergo behavioral, neurophysiological, cardiovascular and respiratory assessments (**Appendix MC-PP-2**) and will then initiate stand/step training for at least 80 sessions (Pre-implantation Training). If the research participant is unable to walk overground unassisted following the pre-implantation training, they will qualify to continue with the next phases of the study (surgical implantation and intervention phases). If the research participant decides to enroll in the surgical implantation and intervention phases of the study, the research participant will complete the *pre-implant* assessments. *Post-implant, pre-intervention* assessments will be conducted as described in Appendix MC-PP-2. Mapping of the motor evoked responses related to spatial electrode selection, amplitude and/or frequency [171] will be conducted as specified in the post-implant assessment section above. After determining the configurations for stepping, standing and voluntary movement, the individual will then begin training with scES. Assessments will be repeated mid-point, post training and during follow-up visits (**Appendix MC-PP-2**).

i. Specific Aims

Specific Aim 1: Demonstrate whether locomotor patterns improve in response to Step-scES and activity-based therapy.

Hypothesis 1.1: Step-scES and step training will significantly modulate locomotor patterns to a greater extent than step training with no stimulation.

Hypothesis 1.2: Step-scES and step training will enable independent stepping through parts of the step cycle.

Specific Aim 2: Demonstrate whether standing ability can improve in response to Stand-scES and activity-based therapy.

Hypothesis 2.1: Stand-scES and stand training will enable independent standing in individuals with severe spinal cord injury

Specific Aim 3: Demonstrate whether the ability to move can improve in response to Vol-scES and activity-based therapy.

Hypothesis 3.1: Vol-scES and voluntary training practice will improve movement patterns in individuals with severe spinal cord injury

ii. Training Interventions

Pre-Implantation phase: Research participants will receive approximately 80 sessions of step training and stand training (described below). These sessions can occur once a day (stand and step will alternate days) or twice daily (stand and step on the same day), 5 days a week for 1 to 1.5 hours.

Post-Implantation phase: Spinal cord epidural stimulation will be administered during training interventions. The pattern of electrically active electrodes, as well as electrode voltage, stimulating frequency, and stimulating pulse width may be remotely programmed using Medtronic's Clinician programmers. 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 stepping, standing or voluntary movement. After determining the configurations for stepping (Step-scES), standing (Stand-scES) and voluntary activity (Vol-scES), the individual will then begin training in the laboratory.

Research participants will receive approximately 160 sessions of step training with Step-scES, stand training with Stand-scES, in addition to daily voluntary training with Vol-scES (described below). These sessions can occur once a day (stand with Stand-scES and step with Step-scES will alternate days) or twice daily (stand with Stand-scES and step with Step-scES will alternate days), 5 days a week for approximately 1 hour. If stand with Stand-scES and step with Step-scES occur on the same day they will be approximately 2 hours apart. Voluntary training with Vol-scES will occur daily in addition to stand and/or step with scES. Independence levels and functional gains will be evaluated periodically. Surface electromyography and near-infrared spectroscopy optodes (as described in assessment section II.D below) might be used during training sessions to evaluate motor activity during changes in stimulation configurations.

c. MC-PP-3: Inter-System Closed-Loop Control of Locomotor and Bladder Function in Individuals with Acute Spinal Cord Injury

We propose to determine the level of functional gain, below the injury for motor control, including recovery standing and stepping function, and bladder function, improved capacity and voiding efficiency, as a result of activation of spinal circuits with scES in humans with

subacute severe paralysis. In Phase 1 of this proposal individuals will be assigned to either a locomotor training intervention (Group A) or a bladder training intervention (Group B). In Group A, training will consist of practicing stepping, standing and voluntary movements in the presence of specific scES configurations designed specific for stepping (Step-scES), specific for standing (Stand-scES) and for the voluntary movements of the legs and trunk (Vol-scES). Data about stimulation parameters and position changes will be collected throughout all training interventions to develop learning algorithms to integrate in Phase 2.

Those assigned to Group B will receive specific configurations and parameters optimal for continence (storage) and micturition (voiding) phases of bladder function will be identified during filling cystometry. Data about stimulation parameters and blood pressure regulation during storage and voiding will be collected throughout all training interventions to develop learning algorithms to integrate in Phase 2.

Following medical clearance for the assessments and participation in the study by the physician, the potential research participant will undergo behavioral, neurophysiological, and autonomic assessments (**Appendix MC-PP-3**). Individuals will be assigned to either Group A or B prior to implantation and based on the recruitment timeline. Following implantation, mapping of the motor evoked responses related to spatial electrode selection, amplitude and/or frequency [171] will be conducted as specified in the post-implant assessment section above. After determining the configurations for stepping, standing and voluntary movement (Group A) or capacity and voiding (Group B), the individual will then begin training with scES. Assessments will be repeated post training (**Appendix MC-PP-3**).

i. Specific Aims

Specific Aim 1: To evaluate the use of position based sensors internal to the neurostimulator to modulate stimulation parameters used in the training for motor function recovery following severe SCI.

Hypothesis 1.1: scES parameters modulated through position feedback will significantly improve standing and stepping ability following locomotor raining with scES, to a greater extent than scES without position sensor modulation and to no scES.

Specific Aim 2: To identify the scES parameters, using physiological feedback (continuous measures of systolic and diastolic pressure and heart rate), that improve bladder storage and emptying while controlling blood pressure following severe SCI.

Hypothesis 2.1: scES parameters that maintain normotensive blood pressure will significantly improve bladder storage and emptying, extending the time between catheterizations and reducing the incidences of autonomic dysreflexia and symptoms of blood pressure dysregulation following bladder training with scES, to a greater extent than scES without blood pressure regulation and to no scES.

ii. Training Interventions

Group A

Spinal cord epidural stimulation will be administered during training interventions. 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 stepping, standing or voluntary movement. After determining the configurations for stepping (Step-scES), standing (Stand-scES) and voluntary activity (Vol-scES), the individual will then begin training in the laboratory.

Research participants will receive approximately 160 sessions of step training with Step-scES, stand training with Stand-scES, in addition to daily voluntary

training with Vol-scES (described below). These sessions can occur once a day (stand with Stand-scES and step with Step-scES will alternate days) or twice daily (stand with Stand-scES and step with Step-scES will alternate days), 5 days a week for approximately 1 hour. If stand with Stand-scES and step with Step-scES occur on the same day they will be approximately 2 hours apart. Voluntary training with Vol-scES will occur daily in addition to stand and/or step with scES. Independence levels and functional gains will be evaluated periodically. Surface electromyography might be used during training sessions to evaluate motor activity during changes in stimulation configurations.

Group B

Steps to select parameters: Initial configurations will be selected based on the history of mapping data and responses observed for cardiovascular and motor changes. The configurations will be consistent with those that provide cardiovascular control without motor responses.

1. Frequency and voltage responses will be assessed to select the specific configurations for bladder capacity and voiding.
2. Before the start of bladder filling from empty, voltage will be ramped up slowly (0.1V increments) and the effects on motor evoked responses will be monitored. The ramp up on voltage will continue until muscle contraction is present as a result of the stimulation (then lowered 0.1 V – sub-threshold).
3. Filling the bladder with physiologic saline at a fixed 20 ml per minute rate (see cystometry below) will be done (reclined position) with stimulation on. Per Figure 8 example, filling will be stopped if an uninhibited bladder contraction that results in a leak occurs, if signs of autonomic dysreflexia occur, bladder filling pressure greater than 60cmH2O, or if bladder filling reaches 600 ml. (Please refer to the II.C.5: Adequacy of Protection Against Risk: Bladder and Bowel Assessment section for details regarding the monitoring of autonomic dysreflexia and the steps taken to minimize risk in the research participants)
4. The electrode configuration will be modified by changing the distance between the anodes and cathodes.

Steps to select parameters (during cystometry for storage versus voiding phase)

1. Configurations (anode and cathode selection, frequency, voltage and pulse width) will be varied at voltage sub-threshold motor levels to see if improvements in bladder capacity can be obtained. If an increase in capacity is not achieved with the initial selection, steps 1-4 will be repeated with a different electrode combination.
2. Configurations (anode and cathode selection, frequency, voltage and pulse width) will be varied at voltage sub-threshold motor levels to see if improvements in bladder voiding can be obtained. If an increase in voiding is not achieved with the initial selection, steps 1-4 will be repeated with a different electrode combination. If voiding is achieved, the post-void residual volume will be obtained (target is below 50 ml – consensus among urologists is: residual volumes between 50 and 100 ml constitute the lower threshold of abnormal whereas above 300 ml is considered at risk for upper urinary tract problems [193]).

2. Motor Control. Inter-System Participation

a. MC-IS-1: Intense task-specific epidural stimulation and training for recovery of stepping following severe spinal cord injury

We propose to determine the level of functional gain, below the injury for recovery of stepping function as a result of activation of spinal circuits with scES in humans with severe paralysis. Intense training will consist of practicing stepping in the presence of specific scES configurations designed specific for stepping (Step-scES).

Participants recruited for this study will already have a neurostimulator and 5-6-5 Specify electrode implanted and would have previously participated in an interventional study. Following medical clearance for the assessments and participation the potential research participant will undergo behavioral, neurophysiological, cardiovascular and respiratory assessments (**Appendix MC-IS-1**). Mapping of the motor evoked responses related to spatial electrode selection, amplitude and/or frequency [171] will be conducted as specified in the post-implant assessment section above. After determining the configurations for stepping, the individual will then begin training with scES. Assessments will be repeated at mid-point, post-training and during follow-up visits (**Appendix MC-IS-1**).

i. Specific Aims

Specific Aim 1: To determine the effects of intense and prolonged stepping with step scES training after traumatic complete SCI in humans on recovery of independent walking.

***Hypothesis 1.1*:** A minimum of 100 sessions of task specific step training with Step-scES will be required for the emergence of bilateral independent steps in the BWST.

***Hypothesis 1.2*:** Intense task specific step training with Step-scES and concurrent intent driven by the individual will lead to independent overground walking in >50% of the individuals with previous Step or Stand with scES training experience.

Specific Aim 2: Understand the interaction between spinal and supraspinal plasticity resulting from intent to step during stepping with Step-scES.

Hypothesis 2.1: Independent steps will have higher lower extremity motor output and alternating muscle activation of extensors and flexors of the same leg that will only occur with appropriate concurrent Step-scES and intent driven by the individual.

Hypothesis 2.2: Independent walking will have bilateral activation of extensors and flexors coordinated to generate alternation of right and left leg propulsion and alternation of same limb extensors and flexors that will only occur with appropriate concurrent Step-scES and intent driven by the individual.

ii. Training Interventions

Spinal cord epidural stimulation will be administered during training interventions. The pattern of electrically active electrodes, as well as electrode voltage, stimulating frequency, and stimulating pulse width may be remotely programmed using Medtronic's Clinician programmers. After determining the configurations for stepping (Step-scES), the individual will then begin training in the laboratory.

Participants will be trained in the laboratory daily to step with Step scES and BWST and progressed to overground stepping if sufficiently independent. Those participants that are able to stand with Stand-scES at home will continue with their daily routine. Those unable to stand independently at home will stand in the laboratory. Participants will receive approximately 250 sessions (5 days/week, 1-6

hours/day, 50 weeks) of step training with step scES. Participants will return to the laboratory multiple times a day to complete a session, as long as they can maintain an appropriate kinematic pattern. Session lengths will vary and likely increase as the research participant becomes more independent. The number of sessions might be reduced if safe independent use of the stimulator is demonstrated by the participant for independent walking in the community. Parameters for stimulation will be modified accordingly to promote the highest level of independence possible. Independence levels and functional gains will be evaluated periodically. Surface electromyography and near-infrared spectroscopy optodes (as described in assessment section D below) might be used during training sessions to evaluate motor activity during changes in stimulation configurations. Assessments might be performed monthly to gain an understanding on the course of plasticity occurring with training and intent.

b. MC-IS-2: Toward the recovery of postural control in individuals with severe spinal cord injury.

Spinal cord epidural stimulation (scES) combined with activity-based rehabilitation can promote the recovery of standing and walking in motor complete spinal cord injured (SCI) individuals. However, no improvements in postural control have been observed yet. We hypothesize that sensory stimulation via muscle vibration can contribute to the recovery of postural control in these individuals during motor functions enabled by scES, leading to significant mobility improvement. Muscle vibration stimulation will be applied for: (Aim-1) identifying vibration parameters (amplitude, frequency, location) optimal for eliciting muscle group-specific activation, and (Aim-2) characterizing postural responses to vibration stimulation during standing with scES. Five SCI individuals who are already implanted with a scES unit will participate in this study. Electromyography of leg muscles will be recorded in supine while different vibration parameters are applied to primary leg muscles. Combinations of vibration parameters (peak to peak amplitude between 1.0 and 2.5 mm delivered at frequencies between 20 and 150 Hz) will be applied in a randomized order targeting the dominant side. Individual-specific epidural stimulation parameters previously identified to facilitate standing will be delivered at a near-motor threshold intensity.

Optimal vibration parameters will be subsequently used to influence standing postural control, which will be assessed via electromyography, kinematics and kinetics. Effects of vibration during standing will be tested: i) on single muscle groups separately; ii) on different combinations of muscle groups (i.e. primary proximal and distal antigravity muscles; anterior muscle chain; posterior muscle chain) (**Appendix MC-IS-2)**.

i. Specific Aims

The overall objective of this mechanistic research proposal on human subjects is to provide a proof of principle that optimized muscle vibration stimulation can selectively influence postural control during standing enabled by spinal epidural stimulation in motor complete SCI individuals.

Specific Aim 1: Identify optimal vibration parameters (amplitude, frequency and site) for eliciting muscle-specific activation (N = 5 research participants).

Hypothesis 1.1: Vibration delivered at an amplitude of 2.5 mm will provide greater and more selective activation of the targeted muscle group compared to other amplitudes.

Hypothesis 1.2**:** Vibration delivered at a frequency of 100 Hz will provide greater and more selective activation of the targeted muscle group compared to other frequencies.

***Hypothesis 1.3*:** Vibration stimulation at the muscle-tendon junction will provide greater and more selective activation of the targeted muscle group compared to vibration stimulation at the muscle belly.

Optimal vibration parameters defined at this stage will be used for Specific Aim 2.

Specific Aim 2: Characterize motor responses to muscle vibration stimulation during standing with epidural stimulation (N = 5 research participants).

***Hypothesis 2.1*:** Vibration stimulation will result in increased activation of the targeted muscle group(s).

***Hypothesis 2.2*:** Vibration stimulation will result in postural adaptations specific of the targeted muscle group(s).

ii. Training Interventions

This project does not include a training intervention for enrolled participants. Participants can be participating in other projects while enrolled in this project assessments.

c. MC-IS-3: Neural Pathways and Recovery of Motor Function with Epidural Stimulation

We will assess whether the corticospinal tract, reticulospinal tract, vestibulospinal tract and long propriospinal pathways provide effective descending input to the lumbosacral spinal cord in a cohort of participants who have received epidural stimulation implants and training. Approximately twenty-four participants with motor complete SCI who received spinal cord epidural stimulation (scES) and task-specific training will return to the lab to assess the integrity of various supraspinal and spinal pathways. During their visit they will attend the lab on five separate occasions (**Appendix MC-IS-3)** to assess the efficacy of neural transmission along the i) corticospinal pathway (FNPA and TMS study), ii) reticulospinal pathway (ASR study), iii) vestibulospinal pathway (GVS study) and iv) propriospinal pathways. This will be assessed both in the presence and absence of epidural stimulation (ES) configurations that have been optimized for either voluntary movement (Vol-scES), standing (Stand-scES), cardiovascular function (CV-scES) or stepping tasks (Step-scES). It is our goal to determine whether recovery of function is dependent on the variable recovery or amplification of descending signals within the four pathways identified in the proposed study. Additionally, we aim to determine whether within the same individual, the various scES configurations (Vol-scES, Stand-scES, CV-scES or Step-scES, Stand-scES, Vol-scES) differentially amplify these types of descending input to the lumbosacral spinal cord.

i. Specific Aims

We hypothesize that all participants who have exhibited some form of motor recovery will have at least one type of effective descending input to the lumbosacral spinal cord, however, the strength of each type of descending input may vary with recovery of function. Lastly, we hypothesize that all descending input to the lumbosacral cord will be more efficacious in the presence of scES.

Specific Aim 1: To determine which descending pathways contribute to the recovery of *independent, overground* stepping with scES and training.

Hypothesis 1: We hypothesize that strengthening of all residual descending inputs to the lumbosacral cord will contribute to the highest level of motor recovery exhibited thus far following scES and training, namely the ability to walk independently overground. Given the importance of volitional input, automatic

and synergistic locomotor functions, balance and interlimb coordination during this task we hypothesize that corticospinal, reticulospinal, vestibulospinal and propriospinal inputs will be more effective in individuals who have recovered the ability to walk independently. We also hypothesize that the efficacy of these inputs will be dependent on the scES configuration, and that the optimal Step-scES configuration for each individual will provide the most efficacious amplification of these descending signals to the lumbosacral cord.

Specific Aim 2: To determine which descending pathways contribute to the recovery of *treadmill stepping* with scES and training.

***Hypothesis 2*:** We hypothesize that the recovery of treadmill stepping will rely mainly on integrated spinal networks. However, given the increasingly important role of the reticulospinal tract in the generation of movements after SCI, we hypothesize that the amplification of *reticulospinal input* to the cord may contribute to this recovery of function. In addition, given the natural quadrupedal nature or "coupling" between the cervical and lumbar enlargements during locomotion [32] and consequently strong interlimb coordination required during stepping we also hypothesize strengthening of *propriospinal connections* that bridge the lesion to contribute to this type of recovery of function. We also hypothesize that these inputs will be amplified in the presence of Step-scES configurations.

Specific Aim 3: To determine which descending pathways contribute to the recovery of *independent standing* with ES and training.

Hypothesis 3: We hypothesize that strengthening of the reticulospinal (gross motor output) and vestibulospinal (balance) inputs to the lumbosacral cord will be primarily responsible for the recovery of independent standing. We also hypothesize that these inputs will be amplified in the presence of Stand-scES configurations.

Specific Aim 4: To determine which descending pathways contribute to the recovery of *voluntary movement* with ES and training.

***Hypothesis 4*:** We hypothesize that strengthening of corticospinal inputs to the lumbosacral cord will be primarily responsible for the recovery of isolated, voluntary movements. Additionally, corticospinal input will be amplified in the presence of Vol-scES.

ii. Training Interventions

This project does not include a training intervention for enrolled participants. Participants can be participating in other projects while enrolled in this project assessments.

d. MC-IS-4: Inter-system Application of Spinal Cord Epidural Stimulation in Persons with Spinal Cord Injury

This study aims to expand the knowledge and the capacity of epidural stimulation (scES) to facilitate recovery of multiple systems synergistically (Inter-System scES) in individuals with chronic motor complete SCI. The ability to transfer scES knowledge and expertise to other sites is important to foster the long-term availability and viability of this promising therapeutic approach to facilitate functional gains in motor and autonomic nervous system recovery. This proposal is based on an inter-system study approach and has three important objectives: 1) to extend the findings of these early feasibility trials, 2) to gain an

understanding as to whether functional improvements in multi-systems can occur in individuals with severe chronic SCI and 3) to implement similar intervention strategies at multi-sites across different regions of the United States.

We will enroll 36 research participants (Specific Aims 1-3) and 12 research participants (Specific Aims 4-5) who have already received an epidural stimulation unit (Intellis Neurostimulator and 5-6-5 Specify electrode array, Medtronic) as part of a currently funded efficacy study at U of L (IRB #16.0179, N=36, and IRB #17-1024 MC-PP1 and CVR PP-1, N=12). Individuals will be recruited **after** they have completed their interventions (Voluntary+Stand; or Cardiovascular+Stand) with scES for their specific motor and/or physiological task. Specifically, 36 participants currently being enrolled at KSCIRC for IRB# 16.0179 will have been randomized and completed the following combined interventions. (1) Stand-scES + CV-scES (n=18) or (2) Stand-scES + Vol-scES. For this clinical proposal, IRB# 17.1024 MC-IS-4, KSCIRC and Kessler will recruit - participants from each of the 2 groups for a total of 36 participants (see Figure 8 below). The research participants will receive 80 sessions of the scES intervention that they have not previously received (Voluntary or Cardiovascular) for Specific Aims 1-3. For Specific Aim 4, research participants will have completed step and stand interventions using epidural stimulation and for Specific Aim 5 will have completed stand, voluntary and cardiovascular using epidural stimulation. This approach will provide longitudinal data on the durability of the effects because the participants will serve as their own control, and data collected as part of the prior project will be used in conjunction with the data collected in this study. Before and after the intervention the outcome measures will be completed to address each aim/hypothesis.

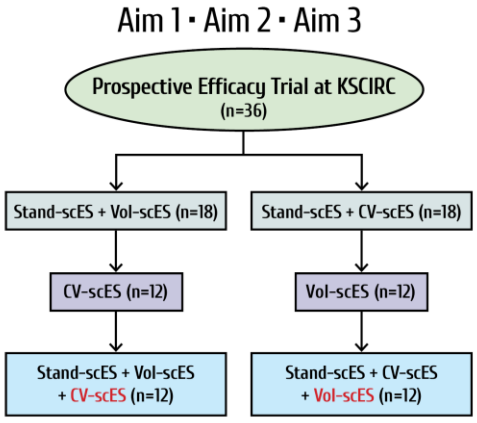


Figure 8: Aims 1-3: Flowchart of the number of research participants available. Thirty-six individuals have completed the Prospective Randomized Efficay Trial at KSCIRC, IRB #16.0179. These participants will have completed two study groups: "Stand-scES + CV-scES" OR "Stand-scES + Vol-scES." These participants will then be available for recruitment into this MC-IS-4; The participants will receive either Vol-scES or CV-scES, whichever they did not receive in the Efficacy Trial, while maintaining their other epidural stimulation either at home or in the laboratory as desired. All participants at the end of the project will have received all possible interventions of cardiovascular, voluntary movement, and standing.

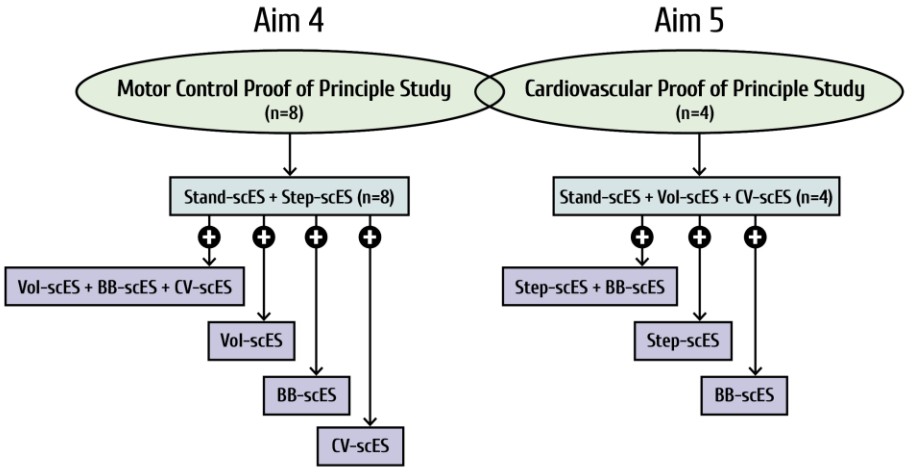


Figure 9: Aims 4-5: Flowchart of the number of participants available. Twelve participants will have completed the Motor Control Proof of Principle (MC-PP-1) or Cardiovascular Proof of Principle (CVR-PP-1) studies at KSCIRC. These participants will have completed two training interventions: Stand-scES and Step-scES or Stand-scES + Vol-scES + Stand-scES. These participants will then be available for recruitment into MC-IS-4 Aim 4 or 5. Participants enrolled in Aim 4 will continue Stand-scES + Step-scES while also adding Vol-scES + BB-scES + CV-scES or they can add each one singularly. Participants enrolled in Aim 5 will continue Stand-scES + Vol-scES + CV-scES while adding Step-scES + BB-scES or they can add each one singularly.

i. Specific Aims

Specific Aim 1: To determine if voluntary leg movement can be significantly recovered with targeted voluntary scES (VOL-scES) in participants with severe chronic SCI who previously received CV-scES and Stand-scES interventions.

Hypothesis 1.1: Independent lower leg voluntary movement can be recovered with 80-sessions of targeted VOL-scES and voluntary training as measured by a significantly higher number of consecutive repetitions of the hip, knee, ankle and toe joints.

Specific Aim 2: To determine if CV targeted scES (CV-scES) training will improve cardiovascular stability in participants with severe chronic SCI who previously received VOL-scES + Stand-scES

Hypothesis 2.1**:** The percentage of systolic blood pressure recordings over 2-hours between 110 and 120 mmHg will be significantly greater after CV-scES Training.

Hypothesis 2.2**:** The percentage of systolic blood pressure recordings between 110-1120 mmHg will be significantly increased during a 30-minute 70° head-up tilt maneuver after 80 sessions of CV-scES.

Specific Aim 3: To determine if the current state of recovery of the initial systems was maintained after targeted CV-scES or Vol-scES training.

Hypothesis 3.1**:** The level of independent standing using epidural stimulation will not be significantly different after 80 sessions of targeted CV-scES training as measured by the amount of time within a consecutive standing bout (maximum of 30 minutes) without assistance using Stand-scES.

Hypothesis 3.2**:** The level of independent standing using epidural stimulation will not be significantly different after 80 sessions of targeted Vol-scES training as measured by the amount of time within a consecutive standing bout (maximum of 30 minutes) without assistance using Stand-scES.

***Hypothesis 3.3*:** The level of independent lower leg voluntary movement will not be significantly different after 80 sessions of targeted CV-scES training as measured by the number of repetitions of the hip, ankle and toe joints.
***Hypothesis 3.4*:** Cardiovascular function using epidural stimulation will not be significantly different after 80 sessions of Vol-scES as measured by the stability of systolic blood pressure in the range of 110 and 120 during a two-hour period after stimulation and for 30 minutes during a head-up tilt maneuver to 70°.

Specific Aim 4: To determine if the current state of recovery of motor systems will be maintained or improved with task specific training using Motor-scES while also using configurations for BB-scES for storing and voiding with training to improve bladder function and/or Vol-scES.

***Hypothesis 4.1*:** The level of independent standing and stepping using epidural stimulation will not be significantly different or will be significantly greater after 80 of Vol-scES and training, CV-scES and while also undergoing BB-scES for bladder storing and voiding.

***Hypothesis 4.1*:** The level of independent standing and stepping using epidural stimulation will not be significantly different or will be significantly greater after 80 of CV-scES.

***Hypothesis 4.2*:** The level of independent standing and stepping using epidural stimulation will not be significantly different or will be significantly greater after 80 of Vol-scES and training.

***Hypothesis 4.3*:** The level of independent standing and stepping using epidural stimulation will not be significantly different while also undergoing BB-scES for bladder storing and voiding.

Specific Aim 5: To determine if the current state of recovery of standing, voluntary and cardiovascular stability will be maintained or improved with task specific training while also using configurations for BB-scES for storing and voiding with training to improve bladder function and/or using Step-scES with locomotor training.

***Hypothesis 5.1*:** The level of independent standing and voluntary movement and cardiovascular stability using epidural stimulation will not be significantly different or will be significantly greater while also using configurations for BB-scES for storing and voiding with training to improve bladder function and using Step-scES with locomotor training.

***Hypothesis 5.2*:** The level of independent standing and voluntary movement and cardiovascular stability using epidural stimulation will not be significantly different or will be significantly greater while also using configurations for BB-scES for storing and voiding with training to improve bladder function.

***Hypothesis 5.3*:** The level of independent standing and voluntary movement and cardiovascular stability using epidural stimulation will not be significantly different or will be significantly greater while also using Step-scES with locomotor training.

iii. Training Intervention (Specific Aims 1-3)

Participants will receive either 6 hours of Voluntary Intervention with Vol-scES or Cardiovascular Intervention with CV-scES, whichever they did not receive in the Efficacy Trial (IRB# 16.0179) for approximately 80 sessions. Participants will maintain their original epidural stimulation training either at home or in the laboratory as desired (i.e. Vol-scES or CV-scES, and Stand-scES). Total stimulation is up to 14 hours per day (e.g. original training intervention: Vol-scES or CV-scES up to 6 hours,

and Stand-scES up to 2 hours; new training intervention: Vol-scES or CV-scES up to 6 hours). All participants at the end of the project will have received all possible interventions of cardiovascular, voluntary movement, and standing. Section IIE describe the training interventions for cardiovascular, voluntary and standing with scES.

iv. Training Intervention (Specific Aim 4-5)

Bladder intervention is conducted under (BB-IS1). For Aim 4, participants will receive standing Intervention with Stand-scES, and Step-scES on the treadmill with BWS or overground as they did in (MC-IS1) in the laboratory if needed to maintain their level of ability. They may conduct these motor tasks at home if possible. Total stimulation is up to 14 hours per day (e.g. motor and bladder scES interventions). All participants at the end of the project will have received all possible interventions of bladder storage and voiding, stepping, voluntary movement, and standing. Section IIE describe the training interventions for, voluntary and standing, stepping and bladder with scES.

a. MC-IS-5: Tethered Pelvic Assist Device for Recovery of Standing Balance Control after Severe SCI

We propose to improve the effectiveness of stand balance training in individuals with motor complete SCI implanted with an scES device using a cable-driven robotic device that will: i) provide active control of balance to the subjects during early training; ii) provide controlled perturbations throughout training; and iii) quantitatively measure forces applied on the participants for assistance and perturbation, as well as their motor responses. The Tethered Pelvic Assist Device (TPAD) is a light-weight cable-driven robot which is designed so that (i) it can be programmed to provide corrective forces/moments to the trunk, pelvis, and knees in response to motion perturbations of these body segments and (ii) it can apply external forces and moments to the trunk and pelvis to perturb them from their nominal configuration during standing [311]. This technology has been adapted from its previous version to well suit the SCI population by adding features that allow balance control of the trunk and pelvis, and assistance for knee extension to avoid buckling during standing. The TPAD, which consists of all commercially available parts, will support participants weighing up to 400 pounds. The University of Louisville IRB as well as Columbia University IRB have approved the use of this TPAD version for studying and training standing balance control in individuals with SCI as well as healthy individuals (University of Louisville IRB #17.0587; Columbia University IRB#-AAAR6780). The data collected in this research project will allow us to obtain comprehensive, quantitative, and sensitive neurophysiological outcomes that will improve our understanding of the physiology of SCI and scES-promoted motor recovery, helping us to design better therapeutic interventions for individuals with SCI implanted with a scES unit.

The study will include a total of 20 participants with SCI who are already implanted with an scES unit and are not able to stand independently without scES as well as upper limb support. There will be two groups: Group 1 (n=10), will include SCI participants who will be evaluated without receiving any intervention. Group 2 (n=10) will include SCI participants who will be evaluated before and after receiving stand training assisted with the TPAD. The eligibility criteria for both groups is the same. Participants who are willing to participate in the study for approximately four months would be enrolled in Group 2, while those who are unable or unwilling to participate for that long would be enrolled in Group 1 (approximately 3-week participation).

i. Specific Aims

Specific Aim 1: To examine the muscle activation patterns generated during stable and perturbed standing in spinal cord injured individuals using scES and TPAD-assistance or self-assistance for balance.

***Hypothesis 1.1*:** Standing with TPAD-assistance for balance will promote:
H1a) Lower activation of shoulder and upper limb muscles compared to self-assistance for balance provided by research participants' upper limbs placed on a fixed frame.
H1b) Higher activation of trunk and lower limb muscles compared to self-assistance for balance.

Specific Aim 2: To examine the effectiveness of TPAD-assisted stand training in the improvement of postural control during stable and perturbed standing in spinal cord individuals using scES.

***Hypothesis 2*:** After TPAD-assisted stand training with trunk and hip perturbations:
H2a) The level of assistance at knees, hips, and trunk required to maintain stable standing will be lower than before training.
H2b) The level of balance assistance required to maintain standing, in response to posture perturbation, will be lower than before training.
H2c) Motor patterns generated at the trunk and lower limbs during standing in response to posture perturbations will be organized differently than before training, being more similar to those generated by non-disabled individuals, with long continuous bouts of independent standing.

Specific Aim 3: To examine the effectiveness of TPAD-assisted stand training with scES in the improvement of postural control during stable and perturbed sitting in spinal cord injured individuals.

***Hypothesis 3*:** After TPAD-assisted stand training with hip perturbations:
H3a) The level of assistance at the trunk required to maintain stable sitting will be lower than before training.
H3b) The level of balance assistance required to maintain sitting, in response to posture perturbation, will be lower than before training.
H3c) Motor patterns generated at the trunk during sitting in response to posture perturbations will be organized differently than before training, being more similar to those generated by non-disabled individuals, with longer continuous bouts of independent sitting.

ii. Training Intervention

Research participants will perform stand balance training assisted by the TPAD. TPAD will be used at the trunk, pelvis and knees to assist as needed and maintain appropriate pelvic tilt, trunk and knee extension to promote appropriate standing posture. Manual assistance provided by trainers may be also added if needed. Self-assistance for balance provided by the participant's upper limbs as well as external assistance at different body segments will be removed as appropriate to allow participants to safely stand with minimal assistance. Each participant will have "independence from assistance" goals throughout training. Trunk independence with free hands will be considered the first level of independence to be achieved, followed by knees and hips. Upper extremity mobility, dynamic tasks (i.e. self-initiated weight shifting) and small perturbations delivered by the TPAD will be integrated during the session to challenge posture. Participants will undergo 5 training sessions a week, for a total of 80 stand training sessions. Each session will last approximately 1 hour. Participants will be encouraged to stand for as long as possible throughout the training session, with the

goal to stand for 60 minutes with the least amount of assistance. Seated resting periods will occur when requested by the individuals.

b. MC-IS-6: Neuromodulation of brain-spinal connectomes for recovery of stepping after paralysis

Recent research has shown convincing evidence that after spinal cord injury (SCI) the spinal neuronal networks can be transformed from a dormant to a functional state to enable partial recovery of voluntary movement by using epidural stimulation in individuals with chronic complete motor paralysis [2] [172]. Our research group has been able to train two individuals with a chronic motor complete spinal cord injury (SCI) to walk independently overground and on the treadmill [173]. This occurred following intense locomotor training with constant spinal cord Epidural Stimulation (scES). In addition, one other group showed the ability of a motor complete individual to ambulate overground with minimal assistance [174]. It has been suggested that such spinal neuromodulation strategy can form new functional connections among neural networks in the spinal cord and that these functional connections are dynamic and activity-dependent. We have previously reported that multisite transcutaneous stimulation applied to cervical and lumbar spinal cord modulate the activity of lumbar networks more effectively than transcutaneous stimulation of lumbar spinal cord alone [18]. Recently, [175] it has also been demonstrated that transcutaneous stimulation of cervical spinal cord neuromodulate the lumbar neuronal circuitry. Thus, the potential for engaging cervical spinal cord networks through transcutaneous stimulation to enhance cervical lumbar connectivity during rehabilitation is remarkable. The main goal of this project is to assess the acute changes in locomotor ability when a combination of lumbosacral scES and scTS at cervical spinal cord is applied.

Four individuals with clinically motor or sensory-motor complete SCI who already have been implanted with an epidural stimulation unit will participate in Group 1 (Aims 1-3) of this study over a four-week period. Participants will be recruited from those that have not received a locomotor training intervention with scES (n=2) and those that have received a locomotor training intervention prior with scES (n=2). There are no treatment sessions in the Group 1 part of this protocol. Participants will do 2 to 4 assessments in the research laboratory per week. Based upon participant availability, it is estimated that it would take a maximum of four weeks for participants to complete the Group 1 part of the study.

Up to eight individuals with clinically motor or sensory-motor complete SCI who have already been implanted with an epidural stimulation unit will participate in Group 2 (Aims 4-6) of this study over a six to eight-month period. Participants have completed the Group 1 part of the study as well as those who have previously received a locomotor training intervention prior with scES and those that have not received a locomotor training intervention prior with scES will be recruited. These participants will complete assessments and step training and voluntary movement training with epidural stimulation and transcutaneous stimulation.

The Spinal Stimulator, BioStim-5, will be used for transcutaneous electrical stimulation of the spine (see Appendix Spinal Stimulator, BioStim-5). scTS will be done in the lab by trained and skilled research personnel using the following stimulation parameters below. Transcutaneous stimulation will target the cervical spine above the injury site (C4 – C7) and the injury site which will vary but will be above T5. More specifically, stimulating electrodes will be placed in the spinal midline between the spinal processes of the vertebrae. Placement will be guided by palpation of anatomical landmarks (vertebral processes and other bony structures). Reference electrodes (anodes) will be placed bilaterally on bony landmark based on the location of the cathodes.

Mode	Single Pulse and/or Continuous

Stimulation Type	Mono-Phasic and/or Bi-Phasic
Stimulation Frequency	0 – 100 Hz
Modulated Frequency	4 – 10 kHz
Current or Intensity	0 – 200 mA
Pulse Duration	0.5 – 1 ms
Spinal Sites of Stimulation	C3 – T5

i. Specific Aims

Specific Aim 1: To define the effectiveness of epidural spinal cord stimulation (scES) and transcutaneous spinal cord stimulation (scTS) to facilitate nonweight-bearing modulation of stepping patterns in paralyzed individuals.

***Hypothesis 1.1*:** Absence of proprioceptive feedback to the spinal circuitry in gravity neutral position of the legs provides optimal conditions for highly sensitive tuning to find optimal scES parameters to initiate nonweight-bearing stepping.

***Hypothesis 1.2*:** scTS applied above the level of SCI facilitates nonweight-bearing rhythmic stepping patterns initiated by specifically configured scES.

Specific Aim 2: To determine the effectiveness of scTS and scES with voluntary enhancement in facilitating weight bearing treadmill stepping.

***Hypothesis 2.1*:** Multi-modal spinal cord stimulation (scTS+scES) in combination with voluntary enhanced stepping-related behavior facilitates weight-bearing treadmill stepping leading to a larger number of successful independent steps when compared to scES alone.

***Hypothesis 2.2*:** Multi-modal spinal cord stimulation (scTS+scES) during weight-bearing treadmill stepping will result in a larger number of successful independent steps in individuals previously trained when compared to non-step trained individuals.

Specific Aim 3: To determine which descending pathways contribute to the recovery of independent stepping following locomotor training with scTS+scES.

***Hypothesis 3.1*:** Motor recovery resulting from locomotor training with scTS+scES will strengthen all residual descending inputs (corticospinal, reticulospinal, vestibulospinal and propriospinal inputs) to the lumbosacral cord.

***Hypothesis 3.2*:** The efficacy of the corticospinal, reticulospinal, vestibulospinal and propriospinal inputs will be dependent on the scTS+scES configuration, with the optimal configuration for each individual providing the most efficacious amplification of these descending signals to the lumbosacral cord.

Specific Aim 4: To determine the effectiveness of scTS and scES with voluntary enhancement in facilitating weight bearing treadmill stepping and overground walking.

***Hypothesis 4.1*:** Multi-modal spinal cord stimulation (scTS+scES) in combination with voluntary enhanced stepping-related behavior facilitates weight-bearing treadmill stepping leading to a larger number of successful independent steps when compared to scES alone when combined with locomotor training.

***Hypothesis 4.2*:** Multi-modal spinal cord stimulation (scTS+scES) during weight-bearing treadmill stepping will result in a larger number of successful independent steps overground when compared to scES alone when combined with locomotor training.

Specific Aim 5: To determine the effectiveness of scTS and scES with voluntary enhancement in facilitating weight bearing standing.

***Hypothesis 5.1*:** Multi-modal spinal cord stimulation (scTS+scES) in combination with voluntary enhanced stepping-related behavior facilitates weight-bearing standing leading to a longer period of time independent knees, hip and or trunk during standing when compared to scES alone when combined with stand training.

Specific Aim 6: To determine the effectiveness of scTS and scES with voluntary enhancement in facilitating voluntary leg extension and flexion of the legs.

***Hypothesis 6.1*:** Multi-modal spinal cord stimulation (scTS+scES) in combination with intentional movement facilitates independent voluntary movement leading to a larger number of successful independent trunk, hip, knee and ankle extension and flexion movements when compared to scES alone.

***Hypothesis 6.2*:** Multi-modal spinal cord stimulation (scTS+scES) in combination with intentional movement facilitates independent voluntary movement leading to a larger number of successful independent trunk, hip, knee and ankle extension and flexion movements when compared to scES alone and when combined with daily voluntary trunk and leg training.

ii. Training Intervention (Aims 4 – 6)

Stimulation (scES and scTS) will be administered during training interventions. The pattern of electrically active electrodes, as well as electrode voltage, stimulating frequency, and stimulating pulse width may be remotely programmed using Medtronic's and Biostim 5 Clinician programmers, respectively. After determining the configurations for stepping (Step-scES, Step-scTS), (Stand-scES, Stand-scTS), and (Vol-scES, Vol-scTS) and then the individual will begin training in the laboratory.

Participants will be trained in the laboratory daily to step with (Step-scES, Step-scTS) and BWST and progressed to overground stepping if sufficiently independent. Those participants that are able to stand with (Stand-scES, Stand-scTS) at home will continue with their daily routine. Those unable to stand independently at home will stand in the laboratory. Participants will receive approximately 80 sessions (5 days/week, 1-6 hours/day, 16 weeks) of step training with (Stand-scES, Stand-scTS). Participants will return to the laboratory multiple times a day to complete a session, as long as they can maintain an appropriate kinematic pattern. Session lengths will vary and likely increase as the research participant becomes more independent. The number of sessions might be reduced if safe independent use of the stimulator is demonstrated by the participant for independent walking in the community. Parameters for stimulation will be modified accordingly to promote the highest level of independence possible. Independence levels and functional gains will be evaluated periodically. Surface electromyography and near-infrared spectroscopy optodes (as described in assessment section D below) might be used during training sessions to evaluate motor activity during changes in stimulation configurations. Assessments might be

performed monthly to gain an understanding on the course of plasticity occurring with training and intent.

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

3. Motor Control. Advanced Technology

No current proposed studies

4. Cardiovascular and Respiratory. Proof of Principle

a. CVR-PP-1: Recovery of cardiovascular function with epidural stimulation after human spinal cord injury

We propose to determine the functional gain that can be achieved in voluntary control of movements below the level of injury and autonomic nervous system function as a result of activation of spinal circuits with epidural stimulation (ES) in humans with paralysis.

Participants and their data enrolled under IRB# 13.0625 (Recovery of cardiovascular function with epidural stimulation after human spinal cord injury) will be transferred to this comprehensive protocol. One participant enrolled and under study at the time the protocol was transferred will continue with their training interventions and assessments and will be asked to sign an addendum informed consent for this project. The participant has completed interventions 1 and 2 and will continue with intervention 3. Additional assessments specific to this project include: bioelectrical impedance spectroscopy, blood draws, MG and soleus H-reflex, corticospinal pathways, vestibulospinal pathways, reticulospinal pathway, long propriospinal pathway, and somatosensory evoked potentials (refer to **Protocol Addendum 1** for descriptions of these assessments).

i. Specific Aims

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

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

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

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

a. End systolic and end diastolic volumes

b. Systolic function and ejection fraction

c. Cardiac Output

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

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

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

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

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

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

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

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

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

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

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

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

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

ii. Training Interventions

Intervention 1: 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 allowed to perform the stimulation at home for one day. 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 2-6 hours daily (7 days a week) for approximately 80 CV training sessions.

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

Intervention 3: The final intervention will include daily stand training sessions once or twice a day (1-hour each) with ES stimulation parameters optimized for standing for approximately 80 sessions. Research participants will participate in 2-6 hours of cardiovascular and 2-4 hours of voluntary parameters outside of the stand training session. Total daily stimulation time will be 5-12 hours. All stand training with ES sessions will be conducted in the laboratory. Approximately every 10 sessions the cardiovascular and voluntary sessions may be conducted in the laboratory (monitoring purposes, if necessary). Then the final clinical and neurophysiological assessments will be conducted.

5. Cardiovascular and Respiratory. Inter-System Participation

a. CVR-IS-1: Intense stimulation and home training for recovery of cardiovascular function following severe spinal cord injury

We propose to understand the role of long-term higher intensity home cardiovascular spinal cord epidural stimulation CV-scES on cardiovascular function. We will enroll up to 12 research participants previously implanted who have sustained a severe SCI to participate in the cardiovascular training. These participants will undergo daily CV-scES at home to maintain their blood pressure within normative ranges. In our pilot data from the first three individuals we observed that in two individuals as they continued stimulation they were able to maintain normal blood pressure levels without stimulation for some periods of time. In one individual, the stimulation was always needed to maintain systolic blood pressure within normal ranges, however if allowed to stimulate for longer periods of time they may have reached normal ranges without needing ES. Thus, the protocol now allows CV-scES time periods customized to the actual modulation of the blood pressure for each individual. This allows us to expand the number of research participants to help understand recovery of cardiovascular and respiratory

function with CV-scES without the additional risks of surgical implantation. These individuals would have already been implanted with the same device in the same location. Participants will undergo mapping experiments and begin ES optimized for cardiovascular function (CV-scES).

i. Specific Aims

Specific Aim 1. Demonstrate whether cardiovascular function improves in response to long-term home training with CV-scES.

Hypothesis 1.1: CV-scES can consistently maintain resting systolic blood pressure (SBP), diastolic blood pressure (DBP) and heart rate (HR) within normal ranges for up to 12 hours during daily life.

Hypothesis 1.2: Resting SBP, DBP will be significantly higher and HR will be significantly lower after daily home CV-scES.

Hypothesis 1.3: Orthostatic hypotension in response to postural stress will be significantly reduced after daily home CV-scES

Hypothesis 1.4: SBP, DBP and HR will remain within normal ranges significantly longer during the day after CV-scES when there is no epidural stimulation.

ii. Intervention

The individual will then undergo CV-scES optimizing cardiovascular function for at least 80 sessions for up to 12 hours per day. The individual will monitor their blood pressure and use the stimulator during those periods when their systolic blood pressure is outside the targeted range. The individual chooses the time periods during the day they will use the stimulator. CV-scES is extended to up to 12 hours each day and limited to when the individual is awake. Each session of scES continues unless the blood pressure remains within the targeted range without stimulation. Stimulation periods will be conducted and observed in the laboratory before being approved to be done in the home. Assessments will be performed before, mid, post-training and follow-up visits (**Appendix CVR-IS-1**).

b. CVR-IS-2: Spinal Cord Stimulation and Respiratory-Cardiovascular Plasticity after Injury

Evaluation of the modulations of respiratory and cardiovascular responses to scES will have important implications for the development of specific therapeutic strategies to mitigate complications of SCI. A primary objective of the project is to investigate whether scES can significantly increase activation of motor and autonomic networks. This work will be done by electrophysiological characterization of respiratory, autonomic, baroreflex and hemodynamic responses as assessed simultaneously at rest and in response to the orthostatic and respiratory challenges in SCI individuals with orthostatic hypotension (**Appendix CVR-IS-2**). Changes in functional measures will be assessed when scES device is turned “off” and “on”. To evaluate “dose-dependent” effects, changes in electromyography magnitude and baroreflex effectiveness / sensitivity, and aortic/femoral pulse wave velocity will be assessed when epidural stimulation is used during maximum expiratory efforts. The extent to which the stimulation is associated with the largest change in respiratory and cardiovascular parameters will be implemented as a strategy to be used during stimulation in combination with Respiratory Training. We will apply scES during our original approach of the Respiratory Motor Control Assessment (RMCA) [176, 177] and orthostatic stress test to characterize respiratory motor control as a function of the respiratory pump. The rationale for this aim is that successful completion of the proposed research will contribute fundamental elements to our basic knowledge of the role of scES for respiratory and cardiovascular functions. When the proposed studies for Aim 1 have been completed, we expect that respiratory, autonomic and cardiovascular inter-related characteristics affected by scES in

participants with SCI will be better understood. Such findings would be important because it will allow us to evaluate the ability of scES to enhance the state of spinal network activity for the potential for multifunctional rehabilitation.

Participants recruited for this study will already have a neurostimulator and 5-6-5 Specify electrode implanted and would have previously participated in an interventional study. Mapping of the motor evoked responses related to spatial electrode selection, amplitude and/or frequency [171] will be conducted as specified in the post-implant assessment section above. Selection of parameters will be based on the dynamics of blood pressure towards normalization in multiple body positions while optimizing the respiratory/trunk muscle activity and minimizing the generation of the leg muscle contractions. Optimal stimulation parameters will be those that achieve normal (approximately 110-120 mmHg of systolic blood pressure) or will be selected based on the participants' baseline blood pressure and to promote trunk activity that supports respiratory activity.

Step to select parameters:

1. Global configurations will be tried first. A global configuration is defined by selecting anodes and cathodes at opposite ends of the array generating either a caudal or rostral flow of current. The first configuration will be with a rostral cathode and caudal anode.
2. A frequency of 30 Hz with 450 us pulse width will be used first.
    a. frequency and voltage will be adjusted to facilitate trunk activity, minimize leg activity and maintain normal systolic blood pressure.
3. Frequency and amplitude will be varied to maintain improvements in Respiratory and Cardiovascular parameters.
4. Based on previous experience, electrode configuration and voltage will be the most critical parameters. If optimal respiratory motor and/or cardiovascular parameters are not achieved with the initial selection, steps 1-4 will be repeated with a different electrode combination.

i. Specific Aims

Specific Aim 1: Evaluate the effects of scES on respiratory-cardiovascular interactions and identify their underlying physiological mechanisms.

Hypothesis 1.1: scES excites respiratory motor and autonomic networks leading to amplified respiratory and cardiovascular responses.

Specific Aim 2: Evaluate the effectiveness and therapeutic mechanisms of scES combined with RT.

Hypothesis 2.1: compare to Respiratory Training alone, Respiratory Training assisted by scES leads to enhanced use-dependent plasticity of both respiratory motor and autonomic networks, leading to respiratory-cardiovascular functional recovery.

ii. Training Interventions

Spinal cord epidural stimulation will be administered during training interventions. Respiratory Training alone will be performed 7 days per week for one month. After another round of assessments, a 3-month combined Respiratory Training and scES will be performed, also 7 days per week. If stable cardio-respiratory parameters are obtained, the same electrode configuration, voltage and frequency specified for stimulation in combination with respiratory training will be used for 3 consecutive days. This will occur in the laboratory setting and cardio-respiratory parameters will be monitored. If 3 consecutive stable days are

achieved the participant will begin home stimulation and training for 1 day. They will return to the lab the following business day for monitoring. If recordings show stable cardio-respiratory parameters they will be allowed to train at home for 3 consecutive days, prior to returning to the lab. If recordings show stable cardio-respiratory parameters they will be allowed to train at home for 4 consecutive days, prior to returning to the lab. If recordings show stable cardio-respiratory parameters they will be allowed to train at home for 5 consecutive days, prior to returning to the lab. On the return visit, if their cardio-respiratory parameters remain stable they will be able to start the home program of 6 days a week and 1 day in lab training. At any point if the parameters need to be modified, the participants will return to the 1 home, 1 lab, 3 home, 1 lab, 4 home, 1 lab, 5 home, 1 lab training schedule. Assessments will be repeated after 1 month of Respiratory Training (RT) and then after 3 months of Respiratory Training with stimulation (RT+scES).

c. CVR-IS-3: Epidural Spinal Cord Stimulation and Respiratory Motor Function after Injury

Due to the respiratory deficits associated with altered respiratory motor control, individuals with SCI are highly susceptible to respiratory complications including COVID19-related, which are among the leading causes of morbidity and mortality in this patient population. Despite the clear need for a solution, there are currently no specific rehabilitative strategies that are recommended as a standard of care for respiratory motor deficits in individuals with chronic SCI. We have reported previously that these conditions can be improved by using our original Respiratory Training (RT) paradigm. However, due to disruption of supraspinal input and decreased excitability of spinal motor networks below the lesion, the effectiveness of this intervention is limited by the functional capacity preserved after injury. We propose that this limitation can be minimized with the use of scES by activating motor spinal networks specific for respiration. We expect that our new and significant contribution outlined in this study will be a detailed physiological characterization of respiratory motor and interdependent autonomically-mediated cardiovascular responses to the scES and the rehabilitative capacity of RT when combined with individually configured stimulation will be determined. Based on this work, specific therapeutic interventions can be implemented not only in individuals with SCI, but in other populations with respiratory, cardiovascular, and neurological disorders. The proposed study will contribute to a broader understanding of the neurophysiological modulations in response to stimulation and training as an interventional approach fundamentally advancing the field by providing new avenues for multifunctional rehabilitation.

***Overall Experimental Plan*.** Our goal is to determine and evaluate the effects of scES optimized for each scES participant (Aim 1) and to define the efficacy of neuromodulatory strategy when this individually configured scES is combined with RT (Aim 2) in individuals with motor discomplete SCI at the neurological level T1 and above, and 2 years after injury. Twenty participants with SCI and an implanted epidural stimulator will be recruited. Another ten non-scES participants will be recruited under a different IRB protocol (IRB# 21.0564). After participants are deemed eligible and medically cleared to participate, they will enter the study. After screening and recruitment, primary and secondary outcome measurements will be obtained in the Lab at the following time points: 1) Pre-Training Pre-Mapping; 2) Pre-Training Post-Mapping (participants with scES, n=20); 3) Mid-Training (post session #40,after 8 weeks of intervention), 4) Post-Training (post session #80 after 16 weeks of intervention); and 5) Follow-up (16 weeks after Post-Training time point). In total, it is anticipated that 5 assessment timepoints will be obtained for each participant with an

implanted epidural stimulator and 4 assessment timepoints for participants without an epidural stimulator implant. (**Figure 1**).

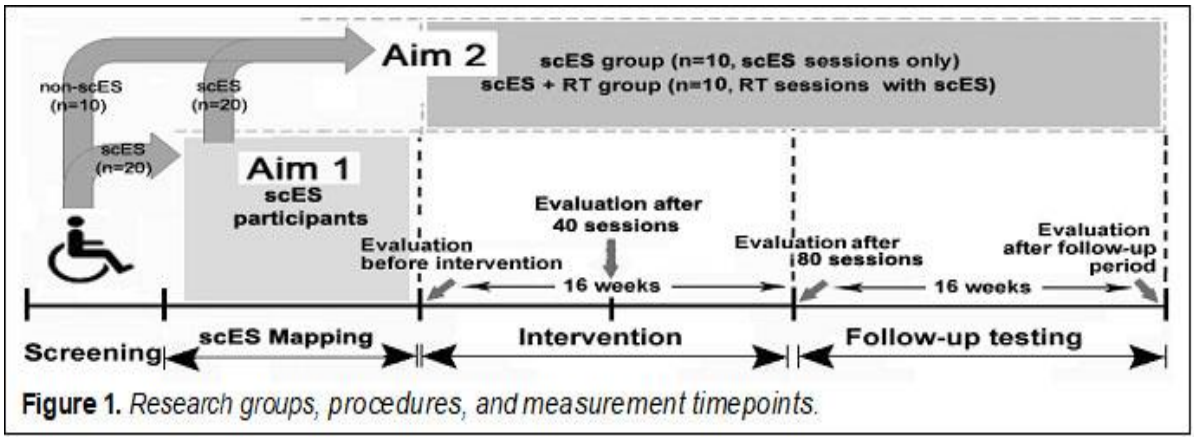


Figure 1. *Research groups, procedures, and measurement timepoints.*

i. Specific Aims

Specific Aim 1: Evaluate the acute effects of epidural spinal cord stimulation on respiratory and cardiovascular functional, motor, and autonomic control properties.

> ***Hypothesis 1.1:*** scES increases spinal motor and autonomic networks excitability leading to increased respiratory and cardiovascular outcomes due to enhanced activation of neural networks specific for respiration and cardiovascular function.

We will characterize respiratory and cardiovascular responses to various scES configurations by using pulmonary function tests, electromyography and recordings of trunk kinematics assessed during respiratory efforts. During these assessments, beat-to-beat blood pressure and heart rate / respiratory rate variability will be assessed to evaluate respiratory-cardiovascular interactions.

Data collection plan for Specific Aim 1. To define optimal scES configuration, Maximum Expiratory Pressure (PE_{max}) and associated respiratory sEMG magnitude (Mag) and Similarity Index (SI) will be assessed repeatedly during stimulation with various combinations of the anodes/cathodes settings, pulse widths, amplitudes, and frequencies optimal for maximal respiratory functional and sEMG performance. Individual optimal scES pattern will be remotely programmed and will be activated during Respiratory Motor Control Assessment (RMCA) and Valsalva maneuver to evaluate the respiratory-cardiovascular effects.

Specific Aim 2: Evaluate the effectiveness of scES combined with respiratory training.

> ***Hypothesis 2.1:*** Specifically configured scES combined with respiratory training will allow for enhanced use-dependent neural plasticity for respiration and cardiovascular function.

Our hypotheses will be confirmed if the respiratory training combined with scES results in significantly improved functional outcomes when compared to the measures obtained from either scES-only or respiratory training-only groups. The physiological characterization of respiratory functional and motor control responses in these groups will be used to identify specific therapeutic effects. This work will be done by using methods outlined in Aim 1 and additional clinical measures all assessed before/after stimulation and/or training and during the follow-up period.

Data collection plan for Specific Aim 2: For this part of the project, we will have 3 experimental groups: participants who will undergo RT only (RT group), scES only (scES group), and those who will undergo the RT in combination with scES (scES+RT group). All three groups will be measured at time $t_0 = 0$ which corresponds to before intervention (RT or scES or RT+scES), then at t_1 = after 40 intervention sessions, t_2 = after 80 sessions and then at t_3 = 16 week after secession of the interventional period.

6. Cardiovascular and Respiratory. Advanced Technology

No current proposed studies

7. Bladder, Bowel and Sexual Function. Proof of Principle

a. BB-PP-1: Functional Mapping with Lumbosacral Epidural Stimulation for Restoration of Bladder Function after Spinal Cord Injury: Simulation-Based Modeling and Interactive Programming Integration for Bladder Home-Training

i. Specific Aims

Specific Aim 1: To determine novel stimulus patterns and parameters (electric field distribution, anode-cathode arrangement, frequency, intensity, pulse width) and the neural structures (spinal cord location) that are effective for bladder function using a computational simulation-based model built upon our existing database of bladder-specific configurations in newly implanted participants with chronic spinal cord injury.

Hypothesis 1.1. Spinal cord anatomy and positioning of the surgically implanted paddle array relative to the segments of the lumbosacral enlargement vary from participant to participant.

Hypothesis 1.2: Stimulation configurations found effective for bladder storage and bladder voiding will have the focused electric field corresponding to spinal cord segments L1-L2 and S2-S4, respectively.

Hypothesis 1.3. Stimulation configurations found effective for cardiovascular stimulation will have the focused electric field corresponding to spinal cord segments L3-L4.

Hypothesis 1.4. Stimulation configurations found effective for both bladder and cardiovascular function (intersystem stimulation) will target overlapping spinal segments synergistically.

Hypothesis 1.5. The use of multiple stimulation cohorts requires electrode steering in order to direct the electric field direction and intensity towards distinct regions of the array and spinal neural elements.

Hypothesis 1.6. Simulation-based bladder mapping includes cohorts that maintain low bladder pressure (<10 cmH_2O) during filling, cohorts that maintain storage volume, cohorts that promote contraction of the detrusor, cohorts that promote relaxation of the external urethral sphincter, cohorts that promote a more normalized bladder pressure (<40 cmH_2O) during emptying, and cohorts that stabilize blood pressure.

Specific Aim 2: To improve the transition from bladder storage to voiding in the home-setting by flexibly adjusting the preset parameters, in accordance with real-time physiological responses to bladder distention (e.g. changes in blood pressure, sensations of fullness) using Telehealth.

Hypothesis 2.1. Stimulation parameters such as duration, frequency, and amplitude will vary during home-training with scES.

Hypothesis 2.2. Stimulation parameters that maintain blood pressure stability during bladder storage will prevent the occurrence of autonomic dysreflexia, thereby allowing for significantly greater time-intervals between catheterizations or extended duration of suprapubic clamping intervals without incontinence triggered by detrusor over-activity.

Hypothesis 2.3. Sensations of bladder fullness (without the triggering of autonomic dysreflexia) during home-training with scES will be used to guide the transition from storage to voiding resulting in on-demand, efficient control of voiding with significant improvements (reduction) in post-void residual volumes.

Hypothesis 2.4. Improvements in quality of life (QOL) (per SCI-QOL) will be associated with decreased occurrence of incontinence and bladder/bowel complications impacting daily life.

Hypothesis 2.5. Semi-structured interviews will identify common facilitators and barriers in the context of bladder management and participant experience during home training with scES.

ii. Bladder Training

Storage to voiding in the home-based training environment using Bladder-scES. After determining the configurations for bladder capacity-scES and bladder voiding-scES from mapping, the individual will initially begin training in the laboratory (as detailed above). Once in the home-setting, participants will train independently with bladder-scES for up to 8 hours a day. During that period, the research nurse and research team members will use Telehealth to interact with participants and monitor home-training progress. A 1-2 hour window will be reserved 2x/day during Monday – Friday to assess their progress. Participants will train independently on the weekends, but the research nurse and participant's advocate will be available should the participant require assistance. At the start of training, the research nurse and investigator will initially acquire a baseline lifestyle and bladder log (dietary habits, intake/output, bowel program time, estimation of urinary rate from Urodynamic testing) and ambulatory blood pressure monitoring over the course of 1 week to estimate the timing of storage to voiding as well as symptoms of autonomic dysreflexia. During home-training, monitoring of fluid intake/output and bladder sensations will be completed daily using the iUflow App and a smart water bottle (i.e. HydrateSpark). The Autonomic Dysfunction Following SCI (ADFSCI) questionnaire will be used to assess individual symptoms of blood pressure instability during home-training. Participants will return to the lab after 20 sessions of training for monitoring, including collection of urine (for urinalysis), urodynamics, and study questionnaires.

8. Bladder, Bowel and Sexual Function. Inter-System Participation

a. BB-IS-1: Functional Mapping with Lumbosacral Epidural Stimulation for Restoration of Bladder Function after Spinal Cord Injury

Locomotor Training (LT) has shown a range of benefits on health and function in both human [178-190] and animal [191, 192] models and we are currently studying its effects on bladder and sexual function. However, the effect of scES alone on bladder function is not known and the parameters used to date have been directed toward the locomotor and cardiovascular system. Thus, this proposal will map the effects of scES on storage and voiding phases of bladder function and then test the effects of training with scES specifically for bladder (urinary bladder=UB-scES) as this phenomenon has not been studied. The discovery-driven insights of how scES affects urological function not only will lead to imminent treatments for those suffering with SCI but may influence the treatment of other neurological disorders such as multiple

sclerosis, Parkinson's, and stroke, and stimulate investigations on other systems such as sexual and bowel dysfunctions.

Participants recruited for this study will already have a neurostimulator and 5-6-5 Specify electrode implanted and would have previously participated in an interventional study. We will enroll approximately 12 participants in this study. Participants will undergo assessments as specified in **Appendix BB-IS-1**. Mapping of the motor evoked responses in response to spatial and amplitude/frequency responses [171] will be conducted and the specific configurations and parameters optimal for continence (storage) and micturition (voiding) phases of bladder function will be identified during filling cystometry (**Figure 8**).

Steps to select parameters: Initial configurations will be selected based on the history of mapping data and responses observed for cardiovascular and motor changes. The configurations will be consistent with those that provide cardiovascular control without motor responses.

1. Frequency and voltage responses will be assessed to select the specific configurations for bladder capacity and voiding.
2. Before the start of bladder filling from empty, voltage will be ramped up slowly (0.1V increments) and the effects on motor evoked responses will be monitored. The ramp up on voltage will continue until muscle contraction is present as a result of the stimulation (then lowered 0.1 V – sub-threshold).
3. Filling the bladder with physiologic saline at a fixed 20 ml per minute rate (see cystometry below) will be done (reclined position) with stimulation on. Per Figure 8 example, filling will be stopped if an uninhibited bladder contraction that results in a leak occurs, if signs of autonomic dysreflexia occur, bladder filling pressure greater than 60cmH_2O, or if bladder filling reaches 600 ml. *(Please refer to the II.C.5: Adequacy of Protection Against Risk: Bladder and Bowel Assessment section for details regarding the monitoring of autonomic dysreflexia and the steps taken to minimize risk in the research participants)*
4. The electrode configuration will be modified by changing the distance between the anodes and cathodes.

Steps to select parameters (during cystometry for storage versus voiding phase)

1. Configurations (anode and cathode selection, frequency, voltage and pulse width) will be varied at voltage sub-threshold motor levels to see if improvements in bladder capacity can be obtained. If an increase in capacity is not achieved with the initial selection, steps 1-4 will be repeated with a different electrode combination.
2. Configurations (anode and cathode selection, frequency, voltage and pulse width) will be varied at voltage sub-threshold motor levels to see if improvements in bladder voiding can be obtained. If an increase in voiding is not achieved with the initial selection, steps 1-4 will be repeated with a different electrode combination. If voiding is achieved, the post-void residual volume will be obtained (target is below 50 ml – consensus among urologists is: residual volumes between 50 and 100 ml constitute the lower threshold of abnormal whereas above 300 ml is considered at risk for upper urinary tract problems [193]).

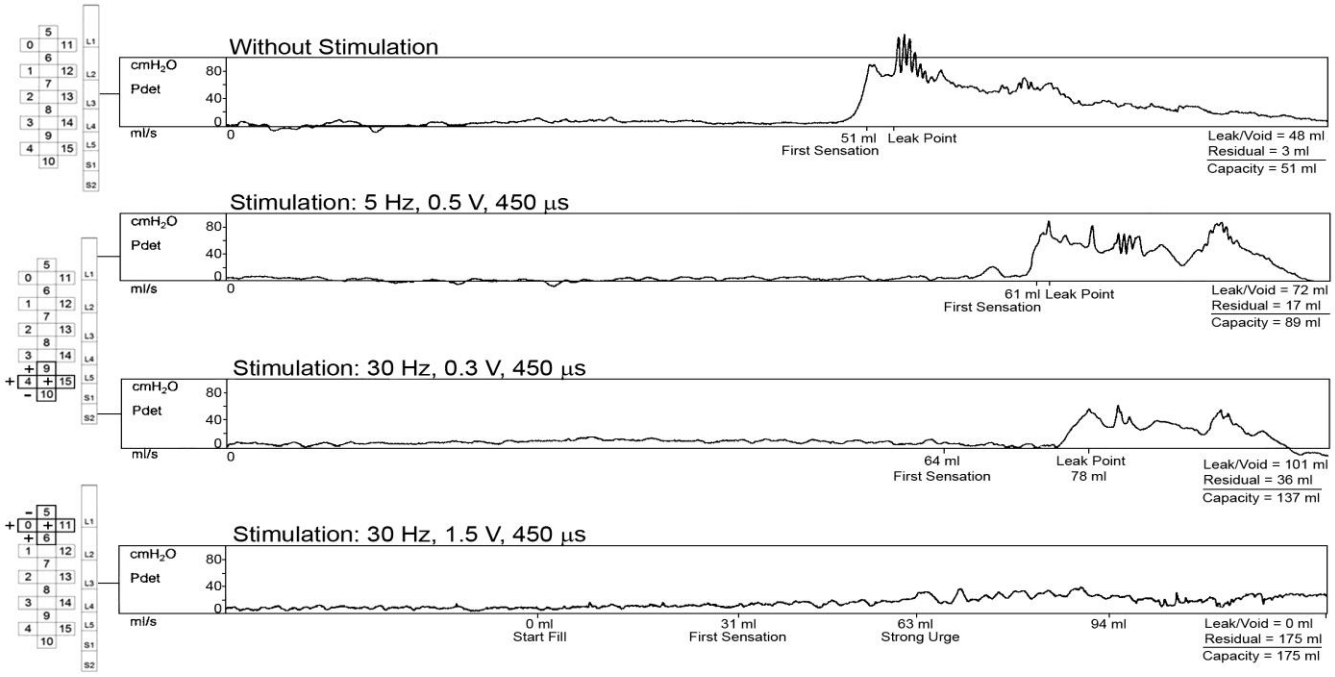


Figure 9. Detrusor pressure recordings from multiple fill/void cycles of a 24-year-old male (injured December of 2007) with a C4 neuro level AIS Grade A (motor and sensory complete) SCI. The electrode configurations are illustrated to the left of each of 4 cycles provided. Note the increase in voiding capacity with the L5/S1 configurations (middle two cycles; low frequency then higher frequency sub-motor threshold stimulus) and the total inhibition of the reflex void with the configuration at a different location (L1/2). Note that there is a possibility that the inhibition was due to a cumulative stimulation effect (perhaps the same total inhibition would have occurred if the same configuration was used at the same location for multiple fill/voids). In the current proposal, only one configuration will be tested in a given session/day (see General Method's section). Also note that the last fill/void cycle was stopped, not because of elevated detrusor pressure (remained low; less than 40 cmH2O is desirable), but because the research participant experienced autonomic dysreflexia common to individuals with SCI.

i. Specific Aims

Specific Aim 1: scES mapping study during cystometry (bladder filling) to determine the optimal parameters (cathode/anode configuration, stimulation frequency and voltage) and location (L1-S1) for each of the two phases of lower urinary tract function (storage and voiding phase). Note that any changes in bowel pressure recorded via the anal catheter during bladder mapping will be further evaluated with anorectal manometry.

Hypothesis 1.1: Bladder Capacity (BC)-scES with local rostral (L1/2) anode/cathode selection, lower frequencies and lower amplitudes will significantly increase bladder capacities toward normative values (350-600 ml) while still maintaining compliant detrusor pressures during the fill phase.

Hypothesis 1.2: Bladder Voiding Efficiency (BVE)-scES will significantly increase post-SCI voiding efficiencies toward normative values (> 90% or < 25 ml residual volume) with caudal (L5/S1) anode/cathode selection at higher frequencies using sub-threshold voltage levels while maintaining safe leak point pressures (< 40 cmH2O) during the void phase.

Specific Aim 2: The goal is to determine the long-term urinary benefits of scES bladder training, beginning with the most effective stimulation parameters for storage and voiding per Aim 1, as well as any secondary benefits (bladder medication usage, susceptibility to urinary tract infections, indirect cardiovascular benefits). Training paradigms will be implemented for continence and voiding on a daily basis and remapped/optimized when necessary over time.

Hypothesis 2.1: Daily training with BC-scES parameters for storage will yield normal capacity values at safe detrusor pressures thereby extending time between catheterizations and thus reducing the number of daily catheterizations without any incontinent episodes (per voiding diaries).

Hypothesis 2.2: Improvements in storage capacity with BC-scES bladder training will either reduce the dosage or eliminate the daily use of anticholinergic medications (which have undesirable side effects).

Hypothesis 2.3: Daily training with BVE-scES parameters for voiding efficiency will reduce post-void residual volumes and the dependence on self-catheterization over time by improving detrusor-sphincter coordination.

Hypothesis 2.4: A decrease in the frequency of self-catheterization with improved storage capacity and increase in voiding efficiency will reduce the frequency of urinary tract infections (weekly urinalysis).

Hypothesis 2.5: scES characterized for optimal bladder function will result in lower maximal systolic pressure relative to baseline, thereby decreasing episodes of autonomic dysreflexia.

Hypothesis 2.6: scES results in a measurable positive effect on urologic quality of life (per questionnaires).

*(Please refer to **Appendix BB-IS-1** for SPARC Project Milestones and timeline.)*

b. BB-IS-2: Effects of activity dependent plasticity on recovery of bladder and sexual function after human spinal cord injury

Participants recruited for this study will already have a 5-6-5 Specify Electrode implanted and would have previously participated in an interventional study using epidural stimulation. We will enroll approximately 7 participants in this study. Participants will undergo assessments as described in **Appendix BB-IS-2**.

i. **Specific Aims**

Specific Aim 1: Use scES for maintenance of normative blood pressure and heart rate in a controlled lab setting during CMG (bladder filling) and Anorectal Manometry (balloon distension in the rectum).

Hypothesis 1.1: Regulation of blood pressure and maintenance of heart rate to normative values (target systolic pressure of 110-120 mmHg) and bladder pressure below 10 cmH_2O during bladder filling up to the targeted pressure (fill volume of 400 mL) will be obtained using scES. Likewise, normative blood pressure and heart rate values will be maintained with scES during rectal balloon insertion and distention.

Specific Aim 2: Determine the long-term benefits of scES cardiovascular training in the at-home setting for maintenance of normative blood pressure and heart rate that be triggered from bladder filling and during bowel evacuation.

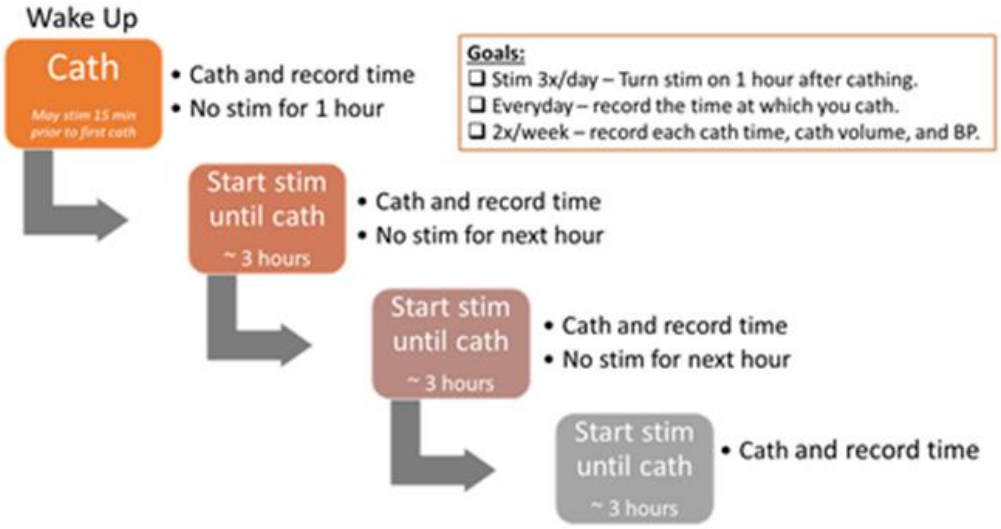
Hypothesis 2.1: The electrode configurations (L1-S1 cathode/anode location, stimulation frequency, voltage, pulse width) for cardiovascular parameters can be used for the storage phase of bladder function (one that increases capacity at safe detrusor pressures).

ii. **Training**

Seven participants will complete Aim 1 in a controlled lab setting by interactively identifying the optimal stimulation parameters (cathode/anode configuration,

stimulation frequency, voltage; placement of scES electrode is from L1-S1) for blood pressure and bladder pressure control to achieve a bladder storage and capacity within the recommended guidelines of the International Continence Society (target normative range of 400-450 mL).

After completion of Aim 1, the same seven participants will complete Aim 2. Beginning with the most effective stimulation parameters for maintaining continence values between 400-450 mL, scES will be used daily following a protocol (see figure 10 below). Specifically, during the home program, the voiding and residual volumes will be measured twice weekly (via urinal, catheter bag, or clear beaker) by the research participant and submitted to the research team via the voiding diary APP. Bowel information will be entered into the PoopTrack APP twice weekly.



Initially, research participants will be allowed to train at home for one month, prior to returning to the lab for monitoring, study questionnaires (bladder and bowel), and clinical urological and bowel evaluation including collection of urine (for urinalysis and biomarker evaluation), uroflow, cystometry, Anorectal Manometry (ARM), and ultrasound of the bladder and kidney. Once a participant is independent with portable blood pressure monitoring during training in the home setting, the participant will notify his/her advocate by phone as they initiate training and then once again when they complete the training session. The advocate will log the training duration. After one month of training and back in the laboratory, configurations will be reviewed and modified if needed. Mapping will be re-done as the optimal parameters may have changed with training. When recordings show stable outcomes for an additional 3 consecutive days in the lab, the research participant will be able to return to the home program and be allowed to train at home for another month, prior to returning to the lab for monitoring, study questionnaires (bladder and bowel function), and clinical urological and bowel evaluation including collection of urine (for urinalysis and biomarker evaluation), uroflow, cystometry, ARM and ultrasound of the bladder and kidney. During the home program, voiding and residual volumes will be measured twice weekly and submitted to the research team. The participant will continue to update his/her advocate during this period. The one-month home program will be repeated for a three-month period, returning after 3 months for monitoring, testing of urine, questionnaires and clinical evaluation. The research participant will then have the choice to continue or discontinue the home program. Follow up studies will then be

conducted 6 months and 1 year later (in lab monitoring, testing of urine, questionnaires and clinical evaluation).

9. <u>All Systems. Advanced Technology</u>

No current proposed studies

D. Outcomes

Tables for each study identify time points when assessments will be completed. Assessments will be performed with scES when appropriate.

1. <u>Motor Control Assessments</u>

a. Multisegmental Motor Responses (MMR)

<u>Procedure:</u> Multisegmental motor responses in different muscles can be evoked by non-invasive stimulation of the dorsal spinal cord [194-196]. Such responses are the basic components of the muscle responses that are elicited by epidural stimulation of posterior lumbar cord structures [197, 198]. Multi-Segmental Motor Responses will be evoked transcutaneously by using a constant current stimulator (NeuroEnabling Technologies/Cosma, Inc. or DigiTimer-DS5/DS7A) between the C2 and L4 spinous processes. For stimulation with the DigiTimer, the AgCl cathode (pre-gelled, soft surface electrodes) will be placed over the skin between C2 and L4 spinous processes and two 50 x 100 mm large anodes will be placed bilaterally over the anterior spine at different levels. The optimum site of stimulation will be located first. 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. A constant current stimulator will be used to produce a 1 ms square pulse. The stimulus intensities will be adjusted for each testing condition, i.e. supine, prone, and standing conditions. The transcutaneous stimulation, with the NeuroEnabling device, will be used to generate interferential stimulation with unique characteristics accommodating safe and comfortable stimulation patterns, the concept is utilizing two stimulating circuits: main (up to 30 Hz) and carrier (up to 10 kHz). The advantage of this approach is that stimulation can be used painlessly at relatively high amplitudes with minimized activation of the cutaneous sensory receptors under the electrodes while promoting electrophysiological response comparable with that induced by a regular mono- or biphasic stimulation waveform [19]. Multisegmental motor responses will be recorded bilaterally from leg muscles, arm muscles, and the trunk using low-noise, pre-amplified surface electromyography electrodes with fixed inter-electrode distance of 1.7 cm placed on skin over the muscle.

<u>Analyses:</u> The Multi-Segmental Motor Responses 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 Multi-Segmental Motor Responses 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)

<u>Procedure:</u> The FNPA assesses the motor capacity and control of the upper and lower extremities and trunk [199]. Bilateral low-noise pre-amplified surface electromyography electrodes are placed on the skin over multiple muscles of upper and lower extremity and trunk muscles including, but not limited to sternocleidomastoid, upper trapezius, biceps brachii, triceps brachii, extensor carpi radialis, flexor digitorum profundus, abductor digiti minimi, external intercostal (6^{th} intercostal space), rectus abdominis, erector spinae (lateral to T10 spinous process), rectus femoris, vastus lateralis, medial hamstrings, tibialis anterior, extensor digitorum longus and lateral soleus, in individuals with injuries above T2. In individuals with injuries below the T2 neurological level, and thus with no impairment of upper limb musculature, biceps brachii, triceps brachii, extensor carpi radialis, flexor digitorum profundus, and abductor digiti minimi are typically replaced with external oblique, erector spinae at L2, peroneus longus, medial gastrocnemius, and flexor hallucis brevis for greater resolution in the motor segments below the lesion. We may also use fine-wire electromyography to acquire activity from the ilio-psoas, extensor hallucis longus, extensor digitorum longus or other deep muscles.

The study protocol contains a variety of tasks that the research participant will be asked to perform. 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 study may also be performed with or without stimulation (including epidural stimulation).

Analysis: To quantify responses, data will be rectified and mean, integrated and root-mean-square values will be calculated for each attempt per muscle. Averages and standard deviations of electromyography measurements per muscle are calculated from three attempts in all events except relaxation. Relaxation will be divided into 30 second intervals. Average and standard deviation of mean and root-mean-square values will be determined from 10 intervals in 5 minute of relaxation per muscle per person. Advanced EMG analysis will also be performed utilizing custom-written Matlab programs. All analysis will be performed in Matlab.

c. Transcranial Magnetic Stimulation (TMS) (Corticospinal Pathway)

Procedure: Transcranial magnetic stimulation (TMS) of the cortex has been used extensively in human to assess the integrity of the descending corticospinal tract [41, 42, 200-206]. TMS of the cortex has been shown to have prognostic value after human spinal cord injury relating to recovery of function [202, 203, 207, 208]. We will probe corticospinal tract function by recording motor evoked potentials (MEPs) in various arm, trunk and leg muscles. We will administer transcranial magnetic stimulation pulses using a Magstim 200. We will use a variety of different coils depending on which muscles we are trying to activate (single 90 mm circular coil: 9784-00; figure-8 alpha coil flat range: 16613 (2010-10) and 16661 (2010-11); or 110 mm double cone coil: 9902-00). For those individuals who can maintain a voluntary contraction, an additional series of tests may be conducted with background EMG activity. Lastly, we may also pair TMS with various other types of stimulation, which could be the Hoffman reflex, multisegmental motor responses (MMRs), or in the presence of spinal cord epidural stimulation (scES) with/without spinal cord transcutaneous stimulation (scTS). Surface electrodes will be placed over the skin, and fine wire electrodes may also be used to record the activity of various arm, trunk and leg muscles. To assess changes in intracortical excitability pairs of TMS pulses may be used with different interstimulus intervals. This will be done using a Magstim Bistim generator. The same coils will be used as above and muscle responses will be recorded as above. In order to avoid transient magnetic fields near the abdomen if the TMS coils are moved while the device is on, the Magstim 200 will not be turned on until the coil is placed on the head. We will attempt to avoid stimulation of unintended structures using standard international TMS practices which include probing the cortex for optimal positioning of the coil over the primary motor cortex. To do so, we use low-intensity stimulation and move the coil laterally and antero-posteriorly from vertex in 1cm blocks until the responses obtained in the muscle of interest are maximal. This coil position is then marked on the participant's scalp to ensure correct and optimal positioning of the coil throughout the study. Published studies indicate that the figure 8 and double cone coils are able to activate cortical neurons at a depth of 1.5-3cm, therefore by determining the optimal positioning of the coils (as per above) we hope to primarily only activate cortical neurons of the primary motor cortex representations of our targeted muscles.

Analysis: We will perform MEP data analysis using custom-written Labview software, Matlab or Spike2/Signal software analysis. All evoked responses will be quantified as peak-to-peak amplitude or area, and plotted against stimulation intensity to construct recruitment curves for each muscle. A curve will be fitted to the recruitment curves, from which the rate of recruitment (slope of the recruitment curve), threshold, and maximum values will be obtained. If background EMG is elicited we will average the amplitude from a 100ms window prior to stimulation. All conditioned responses will be expressed as a percentage of test responses.

d. Acoustic Startle Reflex (ASR) (Reticulospinal Pathway)

Procedure: The reticulospinal pathway has been attributed to widespread muscle contractions in response to sudden unexpected auditory stimuli [98, 209, 210]. The latency, habituation after repeated stimuli and characteristics of muscular contraction suggest that the most likely mediating structure is the reticular formation

in the medulla and pons. Implementing an acoustic startle reaction has been shown to result in muscles responses in facial, arm and sometimes leg muscles that are attributed to the reticulospinal pathways [36, 57, 209, 211-214]. After SCI it appears that the reticulospinal pathway is strengthened as a compensatory mechanism [36, 57]. The reticulospinal pathway will be evaluated either while the participant is at rest or through paired conditioning studies. In conditioning studies the auditory stimuli will be paired with various types of stimulation which could be the Hoffman reflex, multisegmental motor responses (MMRs) or in the presence of epidural electrical stimulation with/without spinal cord transcutaneous stimulation (scTS). The auditory stimulus will be delivered using binaural earphones [36]. Muscle responses (electromyography signals, EMG) will be recorded through surface electrodes positioned over various facial, arm, trunk and leg muscles. Fine wire electrodes may be used at times to record intramuscular signals. EMG will be recorded from the orbicularis oculi muscle to confirm the acoustic startle response [36, 209].
Analysis: Amplitude of the reflex responses will be used to quantify the effects of the auditory stimulation. In paired conditioning studies, control reflexes will be evoked interleaved with those conditioned by auditory stimulation with a time separation of at least 2 minutes. A minimum of 5 responses of control and conditioned responses will be recorded during conditioning experiments. Peak to peak amplitude and the area of the potentials will be measured to quantify the response. Analysis will be performed using either Matlab, Labview, Spike 2 or Signal 6 software.

e. Galvanic Vestibular Stimulation (GVS) (Vestibulospinal Pathway)

Procedure: Galvanic stimulation has been a successful tool to probe vestibular function and the balance system in humans by delivering a perturbation at the receptor level [31, 97] during standing [63, 99, 100, 215-218] and walking [61, 219-222]. This non-invasive technique activates the vestibular cortices and adjacent cortical areas by application of weak direct currents, delivered by two electrodes attached to the mastoids. Muscle responses (EMG) are seen only in muscles engaged in balance indicating a task-dependent gating of descending vestibulospinal influences. Studies have shown that when subjects are sitting or supine, the amplitude of the soleus H-reflex is increased or decreased dependent on the polarity of the galvanic stimulation and influenced by the movement of the head [30, 31, 223, 224]. We will administer galvanic stimulation using electrodes placed over the mastoid processes for the assessment of the vestibulospinal pathways. Galvanic stimulation may be paired with various types of stimulation, which could be the Hoffman reflex, multisegmental motor responses (MMRs), or in the presence of epidural electrical stimulation with/without spinal cord transcutaneous stimulation (scTS). Research participants will either be lying with the head of the mat fixed at 30 degrees, or standing with appropriate support if able because posture influences the responses. Control responses will be evoked interleaved with those conditioned by galvanic stimulation during conditioning experiments.
Analyses: Peak to peak amplitude and area of the responses be calculated. For conditioning experiments, the conditioned responses will be expressed as a percentage of the control responses. Analysis will be performed either in Matlab, Labview, Spike 2 or Signal 6.

f. Propriospinal Pathway Assessment

Procedure: The propriospinal system has been extensively studied in humans by stimulating afferents from contralateral limbs and between upper and lower limbs [37, 38, 73, 75, 76, 225-230]. A study of individuals with a clinically complete spinal cord injury showed that spinal excitation of neural pathways of distant spinal segments (among thoracic, lumbar and sacral segments) exist even without detection of supraspinal pathways [228, 229]. Also, intersegmental reflexes and synaptic plasticity have been reported months after human spinal cord injury [37, 75, 76, 231]. A recent study in animals demonstrated recovery of supraspinal control of stepping by indirect propriospinal relay connections after spinal cord injury [67]. Previous studies demonstrated that electrical stimulation of the mixed nerve and mechanical stimulation of the upper limb muscles induced facilitation of the tendon reflex and H-reflex in the lower limb muscles [72, 232]. In this study, we will assess propriospinal pathways by electrically or magnetically stimulating a variety of peripheral nerves such as, but not limited to, the ulnar, superficial radial, common and superficial peroneal, and femoral nerves. We will then record interlimb reflexes/responses from various muscles. Additionally, nerve stimulation may be paired with various other types of stimulation, which could be the Hoffman reflex, multisegmental motor responses

(MMRs), or in the presence of epidural electrical stimulation with/without spinal cord transcutaneous stimulation (scTS). Conditioning of these responses will allow us to identify the effects of propriospinal pathways in the human spinal cord under conditions when we typically might not be able to see these responses in resting muscles.

Analysis: Peak to peak amplitudes and areas of the responses will be calculated. The propriospinal system will be evaluated at rest, or by examining the effects of peripheral nerve conditioning on other evoked responses. The reflexes will be elicited at a variety of different conditioning test intervals during conditioning experiments. The stimulus will be applied in a randomized order, with unconditioned test stimuli interleaved with conditioned responses. For conditioning experiments, peak to peak amplitudes and areas of the responses will be expressed as a percentage of test responses and will be analysed either in Matlab, Labview or Spike2/Signal 6 software.

g. Neuromuscular Recovery Scale (NRS) with Electromyography (EMG)

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 (Behrman 2012; Harkema 2016), comprised of fourteen motor tasks, and combined with electromyography, 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 electromyography depending on the physical status of the research participant. This will not affect the overall integrity of the data set.

Materials: Electromyography, 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 electromyography that may include but is not limited to the following combinations of muscles: sternocleidomastoid, upper trapezius, biceps brachii, triceps brachii, extensor carpi radialis, flexor digitorum profundus, abductor digiti minimi, external intercostal, rectus abdominis, external oblique, erector spinae, rectus femoris, vastus lateralis, medial hamstrings, adductor, tibialis anterior, peroneus longus, medial gastrocnemius, soleus, flexor hallucis brevis or longus, extensor hallucis longus, and extensor digitorum longus using the MA300 System (Motion Lab Systems, Baton Rouge, LA). We may also use fine-wire electromyography to acquire activity from the ilio-psoas, or other deep muscles, including the hand and feet muscles listed above. Standard needle insertion sites for each muscle will be used (Geiringer, 1999).

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(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 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. An optode may be placed on a muscle of the leg to measure muscle oxygenation and hemodynamics.

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

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

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

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

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

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

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

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

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

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

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

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

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

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

Step Adaptability: From a standing position over a treadmill with overhead body weight support, the research participant will be asked to maintain proper posture and natural arm swing while walking at speeds of 0.6-1.2 mph. The research staff will provide assist to provide proper posture and stepping kinematics only as needed. Body weight support will then be lowered until the research participate is unable to maintain proper posture or stepping kinematics. Depending on participant abilities, participant may be asked to step over objects, adjust to varying random treadmill speeds, and achieve a running pattern all while maintaining proper posture and stepping kinematics at various levels of body weight support.

Analysis: Based on the performance across categories, 4 phase scores can be assigned. Phase 1 represents the greatest impairment relative to normal movement patterns with most people being non-ambulatory. In Phase 2, people begin to stand and weight support independently. Phase 3 denotes walking with varying skill levels. Phase 4 reflects normal locomotor and transfer performance with marked adaptability to varying conditions. Electromyography 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 electromyography will assess the total electromyography 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.

h. Neuromuscular Voluntary Movement Assessments with Electromyography (EMG)

Procedures: Assess specific leg and trunk muscle electromyography and force generation during targeted intentional movements. Electromyography, 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 electromyography soleus, 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 [233]. 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. Electromyography 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 electromyography will assess the total electromyography 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 [172].

i. Standing Assessments with Electromyography (EMG)

Procedure: Assess the level of external assistance and leg and/or trunk muscle electromyography during standing. Electromyography, kinematic and kinetic analysis may be performed on the lower extremities and/or trunk during standing with and/or without scES. Lower extremity and trunk muscle activation patterns will be evaluated using electromyography that may include soleus, medial gastrocnemius, tibialis anterior, medial hamstrings, quadriceps, adductor muscles 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, and/or other deep muscles muscle. Standard needle insertion sites for each muscle will be used [233]. Limb kinematics may include arms, 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 using HRMat (TEKSCAN, Boston, MA) or forces during movement with a force transducer (Kistler, Amherst, NY). Standing assessment may include the placement of custom-made elliptical vibrators over the lower extremity muscle groups.

Participants will be placed either in a body-weight supported harness over a treadmill 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, if needed. Spinal cord epidural stimulation optimized for standing may be turned on when the participant is sitting. 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 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. An optode (Portamon, Artinis Medical Systems, The Netherlands) may be placed on a muscle of the leg to measure muscle oxygenation and hemodynamics. Oxygen consumption and carbon dioxide production may be measured at the mouth via indirect calorimetry using the Parvo Medic TrueOne 2400 system (Sandy, UT) while the research participant is breathing through a mouthpiece. Also, femoral and popliteal arteries blood flow may be assessed by Doppler ultrasound (Philips Epiq 7G). These assessments may be done with and without scES.
Analyses: Electromyography 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 electromyography will assess the total electromyography 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. The relationship between EMG parameters, ground reaction forces, upper limb support, center of mass displacement, lower limb joint angles and the level of external assistance required for lower limb extension will be analyzed.

j. Stepping Assessments with Electromyography (EMG)

Procedure: Assess the level of external assistance and leg and/or trunk muscle electromyography during stepping. Electromyography, kinematic and kinetic analysis may be performed on the lower extremities and/or trunk during stepping with and/or without Step-scES and scES+scTS. Lower extremity and trunk muscle activation patterns will be evaluated using electromyography that may include soleus, medial gastrocnemius, tibialis anterior, medial hamstrings, quadriceps, adductor muscles 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 and/or other deep muscles muscle. Standard needle insertion sites for each muscle will be used [233]. 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). We will measure individual ground reaction forces using a zebris FDM-T System (zebris Medical GmbH, Isny, Germany).

Participants will be placed on the treadmill in an upright position and suspended using a body weight support system (PowerNeurorecovery, Louisville, KY) via an overhead pulley attached to a harness (Robertson, Hendersen, NV) for weight bearing stepping. If appropriate the participant will step overground with or without an assistive device. A trainer positioned behind the research participant will aid in pelvis and trunk stabilization, as well as weight shifting and hip rotation, and trainers positioned at each limb will provide manual assistance using a customized technique developed by this research team that facilitates knee extension during stance and knee flexion and toe clearance during swing. Trainer(s) promote knee extension by applying gentle pressure at the tibial tuberosity and stimulation of the patellar and Achilles tendons. Trainer(s) promote knee flexion by applying gentle pressure at the posterior medial hamstring tendon and anterior tibialis tendon simultaneously while lifting the ankle. Trainer(s) assist proper positioning of the foot at ankle during initial foot placement. Trainer(s) standing behind the individual will provide stability and facilitate rotation at the pelvis by using customized handles on the lateral (sides) of the pelvic belt of the harness to provide assistance. In some cases, the trainer will provide pelvic and trunk assistance by using customized handles on the back of the pelvic belt and chest strap to stabilize and rotate the pelvis and avoid trunk flexion. Trainers provide assistance only when needed. 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 and without scES and scES+scTS.

Analyses: Electromyography 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 electromyography will assess the total electromyography 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.

k. Supine or Gravity Neutral Device (GND) Assessments with Electromyography (EMG)

Procedure: Assess the motor capacity and responses of extremities and trunk muscles. Participants will be in a supine position (lying face upward) on a padded table or, when using a GND, participants will be lying on their side with upper leg supported in a sling suspended in the air directly at the shank and the lower leg place on a free rotating brace segment attached to a horizontal board supported by vertical ropes secured to the ceiling. By using this GND, the participant's legs will be supported and allowed to move freely in the horizontal plane in a gravity-neutral manner.

Electromyography and kinematic analysis may be performed on the upper/lower extremities and/or trunk during tasks with and without epidural stimulation. This assessment may include vibrators applied on each of the four primary lower limb muscle groups in correspondence of the belly or the distal muscle-tendon junction. Distal muscle-tendon junction may be detected by ultrasound imaging. Muscle vibration stimulation will be performed with custom-made elliptical vibrators that can be widely modulated in amplitude and frequency.

Analyses: Data will be processed and synchronized using Labview software (National Instruments, Austin, TX) customized by our laboratory. EMG data will be filtered using a 4th order bandpass Butterworth filter (10 Hz - 500 Hz) and full wave rectified. Mean EMG represents the relative number and frequencies of the motor units recruited per a given event. Integrated EMG assesses the total EMG activity generated per activation period.

l. Lower Extremity Torque Assessment with Electromyography (EMG)

Procedure: Participant will be sitting or lying supine 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 either epidural stimulation or neuromuscular electrical stimulation [234]. Neuromuscular electrical stimulation will be applied via bifurcated leads and self-adhesive reusable surface electrodes. The electrodes will be applied over the motor points of different lower limb muscles. Two electrodes will be used for each muscle group. Stimulation parameters will be optimized to maximize muscle contraction and minimize fatigue. Stimulation will start at the threshold level for minimal contraction and will advance close to the maximum tolerable level of stimulation. If epidural stimulation is used 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 [233].

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 (40 Hz - 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.

m. Stable Standing Assessment

Procedure: Research participants will be asked to stand over ground with the least amount of assistance while receiving scES to facilitate standing. Assistance for standing will be provided at the trunk and hips and knees by the TPAD system. Briefly, TPAD is a light-weight cable-driven robot that can be programmed to provide assistive forces on the pelvis, trunk and knees in order to maintain proper standing posture. TPAD will be used in an "assist-as-needed" mode to maintain a proper standing posture. Also, trainers may provide additional manual assistance if needed. Assistance will be removed, as appropriate, to allow participants to safely stand with minimal assistance. The research participants will be encouraged to stand for as long as possible throughout the session, minimizing rest periods. The use of an assistive device (walker, frame or cane) may be also allowed. Research participants will also undergo a series of standing conditions such as with and without upper extremity assistance, performing upper extremity challenges such as reaching in multiple directions, shifting in multiple directions and performing tasks with eyes open and closed.

The participant will begin the sitting-to-standing transition using the handles of the TPAD system positioned in front of him/her for assistance and support; trainers positioned at the trunk, pelvis and knees will manually assist as needed during this transition. 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 by the TPAD system or manually by trainers to promote extension. If the participant's upper limb, lower limb and trunk control is insufficient to safely use TPAD system for standing, he/she will be further assisted using a body-weight support system with a harness to avoid trunk collapse and knee buckling. Research participants will take a break and rest at any time they feel the need to during the session.

EMG, kinematic and kinetic analysis will be performed on the lower extremities and/or trunk during standing. The pelvic, trunk and knee force and position controller data from the TPAD will be also collected during all acquisitions; these data will be synchronized with EMG, kinematic and kinetic data. Muscle activation patterns will be evaluated recording EMG from different muscles (i.e. soleus (SOL), medial gastrocnemious (MG), tibialis anterior (TA), medial hamstrings (MH), quadriceps (VL and RF), adductor (AD), obliques muscles (OB), erector spinae (ES), trapezius (TR), triceps brachii (TB), biceps brachii (BB)) 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 illio-psoas and/or other deep muscles muscle. Standard needle insertion sites for each muscle will be used. 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) with force transducer(s) (Kistler, Amherst, NY). Blood pressure and heart rate will be measured using either a manual blood pressure monitor (Dinamap V100, GE Medical) or by a finger cuff (Finapres Medical Systems). Temperature will be monitored using a customized sensor system.

Analysis: EMG data will be full wave rectified and filtered using a 4th order bandpass Butterworth filter (20 Hz - 500 Hz) representing the relative number and frequencies of the motor units recruited. Integrated EMG will assess the total EMG activity generated during specific phases of the motor tasks. Co-activation values of agonists and antagonist muscles will be also evaluated. EMG mean and integrated amplitudes from each muscle will be compared before and after training. The relationship between EMG parameters and limb load, directional changes, and forces applied for assistance at pelvis, trunk and knee will also be calculated.

n. Postural Perturbation Assessment (PPA)

Procedure: This assessment will require the same instrumentation and procedures as described above. Additionally, we will collect upper extremity kinematics to better investigate the neuromuscular mechanisms associated with grasping.

The TPAD will generate postural perturbations to induce loss of balance during standing. TPAD will also generate a counterforce to prevent a fall in the case a return to balance is not achieved by the research participant. Additional assistance will be provided by trainers if needed. Participants will be instructed to attempt regaining balance after posture perturbation with and without grasping a fixed object.

Analysis: We will compare EMG onset and burst duration relative to perturbation onset, loss of balance onset and return to balance if applicable. EMG mean and integrated amplitudes from each muscle will be compared before and after training. The relationship between EMG parameters, limb load, kinematics, magnitude and direction of perturbations, and independence levels will also be calculated.

o. Stable Sitting Assessment (SSitA)

Procedure: Research participants will be asked to sit on a padded table with the least amount of assistance possible while receiving scES to facilitate trunk control. Assistance for sitting will be provided at the trunk by the TPAD system (described above). TPAD will be used in an "assist-as-needed" mode to maintain a proper sitting posture. Also, trainers may provide additional manual assistance if needed. Assistance will be removed, as appropriate, to allow participants to safely sit with minimal assistance. While sitting, research participants will also challenge balance control, for example by reaching in multiple directions, shifting weight in multiple directions and performing tasks with their eyes open and closed. EMG and kinematic analysis will be performed on the lower extremities, trunk and upper extremities during sitting. The trunk force and position controller data from the TPAD will be also collected during all acquisitions; these data will be synchronized with EMG and kinematic data. Muscle activation patterns will be evaluated recording EMG from different muscles (i.e. medial hamstrings (MH), quadriceps (VL and RF), adductor (AD), obliques muscles (OB), erector spinae (ES), deltoid (DE)) using the EMG and kinematic data collection instrumentation and procedures described above. Temperature will be monitored using a customized sensor system.

Analysis: EMG data will be full wave rectified and filtered using a 4th order bandpass Butterworth filter (20 Hz - 500 Hz) representing the relative number and frequencies of the motor units recruited. Integrated EMG will assess the total EMG activity generated during specific phases of the motor tasks. Co-activation values of agonists and antagonist muscles will be also evaluated. EMG mean and integrated amplitudes from each muscle will be compared before and after training. The relationship between EMG parameters, directional changes, and forces applied for assistance at the trunk will also be calculated.

p. Postural Perturbation Assessment in Sitting (PPASit)

Procedure: This assessment will require the same instrumentation and procedures as described above for the Stable Sitting Assessment. The TPAD will generate postural perturbations at the trunk to induce loss of balance during sitting. TPAD will also generate a counterforce to prevent a fall in the case a return to balance is not achieved by the research participant. Additional assistance will be provided by trainers if needed.

Analysis: We will compare EMG onset and burst duration relative to perturbation onset, loss of balance onset and return to balance if applicable. EMG mean and integrated amplitudes from each muscle will be compared before and after training. The relationship between EMG parameters, kinematics, magnitude and direction of perturbations, and independence levels will also be calculated.

2. Cardiovascular and Respiratory

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 Ambulatory Blood Pressure Monitoring (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, diastolic blood pressure, and heart rate will be recorded automatically every 15 minutes during their daytime active period, then every 30 minutes 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. In addition to the 24-hour ECG recording, participants may also be asked to receive a 12-lead ECG recording in the supine position. This 12-lead ECG will last approximately 10 minutes.

Analysis: Twenty-four-hour ambulatory blood pressure monitoring data will be stored and analyzed upon completion of the assessment [235, 236]. 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 Systolic Blood Pressure and Diastolic Blood Pressure 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 [96, 102, 237-240]. 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, diagnose orthostatic hypotension, evaluate baroreflex responses, and assess beat-to-beat blood pressure and heart rate variability in individuals with SCI [241, 242]. Each participant will be assessed in the morning in a quiet, temperature-controlled (~72^{o}F/22^{o}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 for calibration and confirmation of the finger waveform. 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 a cardiac chair or into the head-up position on the tilt-table (20-90 degrees) [243, 244]. 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 beat-to-beat blood pressure and heart rate. The assessment will be stopped if: the participants experience symptoms of orthostatic intolerance that would lead to syncope, 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 symptomatic of orthostatic intolerance. The Orthostatic Intolerance Questionnaire is a self-reported, 0-10 scale (0 = no symptom and 10 = intolerable) that ranks the following symptoms: headache, dizziness, blurred vision, nausea, weakness, confusion, fatigue, ringing in ears, and risk of 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. To assess the catecholamine release during orthostatic stress, blood will be drawn at minutes 3 and 10, or 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 as the participant returns to the supine position.

In individuals with implanted epidural stimulator, cardiovascular parameters at rest and in response to orthostatic challenge (sit-up or head-up tilt) 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 sit-up/head-up tilt positions. During sit-up and head-up tilt, mean blood pressure and heart rate will be analyzed in 1-minute intervals during 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-minute systolic blood pressure interval; these outcomes may also be analyzed during the position change on the tilt table. We will track the duration in the head-up tilt position as an outcome measure reflecting 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 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 fast fourier transformation. Spectral power will be calculated for low (0.04-0.15 Hz) and high (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 identified 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 the Jewish Hospital, LabCorp, Quest Diagnostics, Dr. David Goldstein's analysis laboratory at the National Institute of Health, or other licensed biochemical laboratory.

c. Echocardiography

Procedure: Research participants will be positioned on an echocardiography table in the left-lateral decubitus position. There will be a 3-lead electrocardiography (ECG) during procedure connected to participant as well as a blood pressure cuff. The participants blood pressure 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, apical 4 chamber, apical 2 chamber, apical 3 chamber, and from the subcostal view. Using M-mode we may measure the Tricuspid Annular Plane Systolic Excursion. 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. 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 and the hepatic vein. The research participant is then repositioned to an 80-degree angle for much of the same measurements. This procedure may be done or repeated with scES.

Analyses: Left Ventricular dimensions will be taken from the parasternal long axis view. This will include left ventricular internal dimension in diastole and systole, the interventricular septal dimension in diastole and the left ventricular posterior wall dimension in diastole. The cross-sectional area of the left ventricular outflow tract will be measured here also. Volumetric measurements may be determined from the apical 4 chamber & apical 2 chamber view using the modified Simpson's method in 2D and 3D. The left ventricular 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 apical 4 chamber or apical 3 chamber 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 right ventricle strain. From the apical 4 chamber and apical 2 chamber view the left atrial volume will be measured along with the right upper pulmonary vein via pulse wave doppler. The right and left atrial volumes will be measured in the apical 4 chamber view. Left ventricular strain may be assessed using parasternal short-axis at the papillary level, along with the apical 4 chamber, apical 2 chamber and apical 3 chamber 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 left ventricle 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 [122]. Heart rate will be recorded using a single lead electrocardiogram (ECG) (ModalML 123, AD Instruments Inc, Colorado Springs, CO). This may be done or repeated 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. Pulmonary Function Test (PFT)

Procedure: Standard Spirometry [245] will be performed in the seated and/or supine position by using the preVentTM 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 [246]).
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 (PEmax) and Maximum Inspiratory Pressure (PImax). The maximum expiratory pressure will be measured during a maximal expiratory effort starting from total lung capacity and the maximum inspiratory pressure will be measured during a maximal inspiratory effort beginning at residual volume (American Thoracic Society/European Respiratory Society 2002; [247]. 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 [248, 249]. The assessment will require a sharp forceful effort to be maintained for 2 seconds.
Analysis: For the standard spirometry assessment, three acceptable spirograms will be obtained and the results (force vital capacity and forced expiratory volume-1sec) 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 [250]. The three best attempts varying by less than 20% will be averaged.

f. 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 (Motion Lab Systems, Inc, Baton Rouge, LA) of the muscles of respiration.
Respiratory muscle activation patterns will be evaluated using electromyography of respiratory-related muscles using the MA300 System (Motion Lab Systems, Baton Rouge, LA) [251]. Electromyography 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 electromyography-based measure of motor output from the central nervous system recorded during voluntary tasks attempted in the supine and sitting position [176, 177]. The protocol begins in the seated position and consists of the following maneuvers followed by 5 minutes of relaxation in supine position: spirometry, maximum expiratory and inspiratory pressure, maximum expiratory and inspiratory pressure 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. Surface electromyography 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 abdominis, obliques, and the paraspinals will be recorded using a multi-channel electromyography 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 procedure may be done or repeated with scES.
Analysis: The envelope of electromyography activity for each muscle will be calculated using a root mean square algorithm [177]. 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 electromyography magnitude (μV, Mag) and the similarity index, that quantitate the multi-muscle distribution of activation during maximum expiratory pressure task in research participants compared to that of healthy subjects will be calculated using a vector-based analysis as previously described [176, 177]. In brief: multi-muscle activity parameters will be calculated using averaged root mean square amplitudes from each SCI subject for comparison to group values from non-injured (NI) subjects. The resulting Mag parameter is the amount of combined electromyography activity during maximum expiratory pressure task calculated as a length of the resultant vector. The similarity index 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 non-injured 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; electromyography; breathing rate and chest wall kinematics will be monitored simultaneously by using Powerlab acquisition system (ADInstruments, Colorado Springs, CO). For the bursts analysis, electromyography 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 electromyography will assess the total electromyography 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.

g. 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 [252].
Analysis: Depending on the stability of the readings, the last 3-5 resting energy expenditure 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 resting energy expenditure values based the data trends and upon observation of the participant.

h. Aerobic Fitness Test (VO2 Peak)

Procedure: Participants will be asked to not perform any moderate or heavy exercise 12 hours prior to the test. Blood pressure (Dinamap Carescape V100; GE Healthcare, Buckinghamshire, UK) and respiratory measures (Parvomedics Truemax 2400, Sandy UT) will be collected two minutes prior to the beginning of the test. We may also record resting ECG before the beginning of the test. Heart rate will be monitored with a chest strap heart rate monitor (Polar T31 heart rate monitor, Polar Electro Inc., Woodbury, NY). Each participant will perform a graded exercise test on a total body recumbent stepper (NuStep T4 ergometer, Ann Arbor, MI) or a SCIFIT hand cycle (SCIFIT Pro1, Tulsa, OK) to measure VO2Peak. For participants with tetraplegia, who have limited grip strength, tensor bandages will be used to secure the hands to the handles on either machine. Participants will be asked to maintain a cycling rate of 60 rev/min for the duration of the test. Some participants will not be able to perform 60 rev/min, in this case the experimenter will work with the participant to find a suitable cycling rate. After the initial warm-up at 0Watts, power output will be increased at a rate of 5Watts/min for tetraplegics and 10Watts/min for paraplegics, until volitional exhaustion (i.e. the cycling rate can no longer be maintained). Participants will be asked to identify their perceived rate of exhaustion on the Borg Scale every minute until completion of the test.

Analysis: The highest 15 second average of oxygen uptake during the test will be recorded as the VO2Peak. These values are provided by the acquisition software and recorded by the experimenter along with additional notes from the experiment.

i. Flow Mediated Dilation

Procedure: Flow mediated dilation (FMD) and blood flow will be measured in the research participant's arm and leg, respectively, via Doppler ultrasound (Philips EPIQ 7 ultrasound system). Participants will be asked to report to the laboratory after refraining from alcohol, smoking, and caffeine for at least 12 hours prior to testing. This testing procedure will involve a participant lying in a supine or seated position while ultrasound is used to measure blood flow in the brachial and femoral arteries prior to, during, and after cuff occlusion via a blood pressure cuff (Carescape Dinamap V100, GE Healthcare, Chicago, IL) or a rapid inflator (E20 Rapid Cuff Inflator, D.E. Hokanson, Inc., Bellevie, WA) connected to a large-volume compressor (Hokanson AG101 Cuff Inflator Air Source, D.E. Hokanson, Inc., Bellevue, WA). Continuous beat-to-beat arterial blood pressure will be recorded from a cuff placed around the finger using a Finapres Medical Systems unit with 3-lead or 12-lead

ECG (AD Instruments). Periodic brachial arterial blood pressure measurements will be used for blood pressure monitoring and beat-to-beat pressure calibration. Blood flow will be measured in the femoral artery during a 4- and a 10-minute cuff occlusion and upon release of the cuff (Olive, 2003). Blood flow will be measured in the brachial artery during a 5-minute cuff occlusion and upon release of the cuff (Stoner 2006). The testing procedures should take approximately 1.5 to 2 hours to complete.

Analyses: Flow mediated dilation of the brachial artery will be measured after cuff occlusion of the forearm to determine if there is abnormal endothelium-dependent vascular function. Flow mediated dilation is measured by determining the amount that a vessel vasodilates in response to a short term (5 minutes) cuff occlusion. Blood flow will be measured in the femoral artery during a 4- and 10-minute cuff occlusion. 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.

j. 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 (Hwang 2017). 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 will 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)[253, 254]. 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 (Dd) and the maximum change in diameter during the cardiac cycle (ΔD) will be measured for each beat. Distensibility ($mmHg^{-1}$) will be calculated as $\Delta D/\Delta P/Dd$.

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.

k. Transcranial Doppler (TCD)

Procedure: The Transcranial Doppler assessment is used to measure cerebral blood velocity in order to assess neurovascular coupling, cerebral autoregulation, and cerebrovascular reactivity [255]. Participants will be assessed in a quiet, temperature-controlled laboratory at the Neuroscience Collaborative 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.
This assessment will utilize an ST3 Transcranial Doppler (Spencer Technologies) to measure middle and posterior cerebral artery 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, heart rate (via electrocardiography - ECG), and respiratory kinematics, as described using equipment and methods used during the Orthostatic Stress Test. We may also record end tidal CO2 (ETCO2) using a nasal cannula and gas analyzer (Gemini Respiratory Monitor, CWE Inc or 17515 CO2 Analyzer Gold Edition, VacuMed). The posterior cerebral artery will be insonated at depths of 60-75 mm, while the M1 segment of the medial cerebral artery 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 choose to record Transcranial Doppler during the Orthostatic Stress Test, the Cognitive Assessment Battery, Respiratory Training, scES, or portions of the Respiratory Motor Control Assessment which are described in this protocol.

Analysis: Raw cerebral blood flow velocity 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, end tidal CO2, blood pressure, and raw cerebral blood flow velocity data will be upsampled at 5 Hz with cubic spline interpolation and spectral analysis will be performed to identify a physiological mechanism for cerebral blood flow responses.

I. Cognitive Assessment Battery

Procedure and Analysis: The cognitive assessment battery includes the following assessments: The Stroop Color Word Test, WAIS IV Digit Span, Serial 7s (PAR, Inc., Lutz, FL), Oral Symbol Digit Modalities Test (WPS, Torrance, CA), Raven’s Standard Progressive Matrices (Pearson TalentLens, San Antonio, TX), Controlled Oral Word Association Test (PAR, Inc., Lutz, FL), Wisconsin Card Sorting Test (PAR, Inc., Lutz, FL), California Verbal Learning Test (CVLT) II, WRAT 4 Word Reading, Frontal Systems Behavioral Evaluation Self Report, and the Spinal Cord Injury Quality of Life (SCI QOL).While these tests comprise the full battery that would be performed, some tests may not be administered depending upon information gleaned from the initial discussion and behavioral observations of the participant. Reasons for administration adjustment would include: participants’ waning energy or motivational effort with testing tasks, or the nature of their injury preventing or inducing frustration with task completion. Decisions will be made on a case-by-case basis, and testing may be completed across more than one session if necessary and with agreement of the participant.

- The Stroop Color Word Test is a measure of mental flexibility. Participants are instructed to say the color of the ink in which a color word is printed rather than read the word itself. Color words (i.e. red, blue, green, yellow, etc.) are printed in various colors of ink that can be congruent or incongruent to the word itself. Participants are typically quicker to respond to congruent trials than incongruent trials and the difference in reaction time between them is a measure of mental flexibility, with a small difference indicating strong mental flexibility. We will record the amount of time needed to complete each round and sum the subsequent number of errors.

- WAIS IV Digit Span is a measure of attention and working memory. Participants are instructed to 1) repeat lists of numbers read by the examiner in the same order and 2) repeat lists of numbers read by the examiner in the reverse order. Trials increase in difficulty as the participant progresses. The test ends when a participant cannot correctly recall two lists of the same length. The number of digits in the last list correctly recalled indicates a participant's level of attentional and working memory performance.
- Serial 7s is an assessment of attention. In this test, participants are asked to begin with the number 100 and count backwards by increments of seven until asked by the experimenter to stop (after five increments). Alternative forms of the assessment include spelling the word "world" backwards and starting with the month of December and counting back months of the year by increments of three. The accuracy with which a participant counts backward by seven from 100 (or any other form of the test) indicates attentional performance (e.g., 93, 86, 79, 72, 65).
- Oral Symbol Digit Modalities Test is an assessment of psychomotor efficiency. Using a reference key, a participant has 90 seconds to pair geometric symbols with corresponding numbers as quickly and accurately as possible. The accurate number-to-symbol responses are totaled, and the number of incorrect responses is subtracted, and the final number indicates psychomotor performance.
- Raven's Standard Progressive Matrices is an assessment of problem solving. This test consists of multiple sets of test items that increase in difficulty as the test progresses. In a single test item, a matrix of geometric figures is presented with a blank final space. The participant should determine the logic for the matrix in order to make an accurate multiple-choice response to complete the matrix. The last accurate response made in a set is an indicator of problem solving skill, as it is the most difficult problem that the participant could solve.
- Controlled Oral Word Association Test is a measure of verbal fluency. This test has two parts: category fluency and FAS. In the category fluency test, a participant is asked to name as many animals as they can within a sixty-second-time limit. In the FAS test, a participant should name as many words as possible that begin with a given letter within a sixty-second-time limit. This is completed three times for different letters (e.g., F, A, and S). Response criteria for both tests include no proper nouns and not repeating the same root word with different tenses or plurality. The valid responses are totaled, the number of invalid responses is subtracted, and the final number indicates verbal fluency skill.
- Wisconsin Card Sorting Test is a measure of executive function. In this test, a participant must sort a virtual deck of 64 cards into four stacks. The participant will verbalize their choices and the experimenter will make a button-click response accordingly. This is done to assist participants with motor deficits. Initially, the participant does not know the parameters for sorting, but should use inter-trial feedback to develop a strategy. Sorting parameters include color, shape, or number. The sorting rule changes intermittently and the participant must rely on feedback to adapt their response strategy to continue sorting accurately. The average number of trials that it takes a participant to adjust response strategy after receiving negative feedback is calculated. Quickly changing response strategy after receiving negative feedback indicates high executive function, but preserving an old strategy that no longer works is indicative of low executive function (i.e., latency).
- California Verbal Learning Test (CVLT) II is a measure of learning and memory (i.e., cued recall and free recall). In this test, the examiner first reads a list of words and the participant will repeat these words back to the examiner immediately. Responses are recorded. The participant is given four chances to hear the list of words and immediately recall. After the fourth cued recall trial, there is a 30 second delay and the participant then will try to freely recall the list. Next, there is a 15 minute delay and the participant will try to freely recall the list again. Finally, the examiner will attempt to cue the participant's memory by asking them to recall all the words in the list that fit into three categories (e.g., fruits, clothing, and tools). The total number of correct and incorrect responses will be totaled across all trials and the score will indicate learning and memory performance.
- WRAT 4 Word Reading will be used as a tool to estimate premorbid intellectual functioning. Participants are instructed to read a list of words that get more difficult as they progress. The total number of words successfully read from the list will be totaled and indicate the participant's level of intelligence prior to their injury.

- Frontal Systems Behavioral Evaluation Self Report is an assessment of assorted behaviors that are mediated by frontal lobe processes. The participant will rate their own behaviors on a scale of one to five, strongly disagree to strongly agree. They will complete this test twice, once for themselves before injury and once for after injury. Although this test may not accurately reflect the changes that occurred in the participants' behaviors after injury, it will reflect the way that they perceive their post injury selves.
- Spinal Cord Injury Quality of Life (SCI QOL) measurement system

Conditional information: Cerebral blood flow by Transcranial Doppler (TCD), and/or peripheral blood pressure recordings may be measured continuously throughout administration of all cognitive tests. Cognitive assessments may be introduced during various interventions and training sessions as a way to test their effects on cognition. Behavioral performance on each of the tests may be correlated with cerebral blood flow velocity and peripheral blood pressure measures obtained during test performance. Correlations will indicate the strength of the relationship between neurovascular coupling and the performance on each cognitive domain.

m. Venous Occlusion Plethysmography.

Procedure and Analysis: We will use standard equipment; a rapid cuff inflator, plethysmograph, and a rubber strain gauge (D.E. Hokanson, Inc., Bellevue, WA, 98005). Participants will be in the supine position with their dominant-side leg elevated at heart level. A tape cloth will be used to measure the widest circumference on the lower leg. Then a strain gauge, 2cm smaller than the widest circumference, will be placed around the lower leg. A small inflatable cuff will be placed just above the ankle and a second cuff will be wrapped around the thigh at its midpoint. The ankle cuff will be inflated to a suprasystolic pressure (≈200mmHg) while the thigh cuff is rapidly inflated just below diastolic pressure (≈50mmHg). The strain gauge will measure the change in volume of the lower leg, which will be displayed in real-time on a computer screen and the data will be stored to our autonomic data collection cart for off-line analysis. The thigh cuff will remain inflated until a plateau in volume change is observed on the computer screen (about 3-4 minutes in), at which point the thigh cuff will be deflated, and 60 seconds later the ankle cuff will be released. We will continue monitoring the change in blood volume until it returns to resting values. The test may be repeated up to two times.

3. Bladder, Bowel, and Sexual Function

a. Voiding Diary

Procedure: A voiding diary with frequency/volume of each void and/or catheterization will be generated for two consecutive 24 hour periods once per week by using standard voiding diaries [256]. The number of voids, voided volume, and distribution between daytime and night-time will be obtained in order to capture the timing of any stimulation/training related effects during the course of study. A baseline voiding diary will be obtained prior to initiating stimulations.

Analysis: Bladder diary data will be analyzed from weekly logged entries by the participant. Reductions or increases in catheterizations, post-void residual volumes and/or incontinent episodes over the course of each training intervention will be noted and averaged across each time point.

b. Urodynamics

Procedure: The participant may be asked to refrain from taking any bladder medications 24-hours prior to Urodynamics. For the urodynamic study (necessary to determine bladder function/voiding pressures/degree of detrusor sphincter dyssynergia; [257, 258], 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 [259-261] (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, a uroflow can be obtained. Note that if the research participant is able to achieve a voluntary void, a standard uroflow is also performed prior to cystometry.
A 12 French straight catheter will be used to empty the bladder completely and a urine sample may be obtained to assess for the presence of blood, urobilinogen, glucose, ketones, bilirubin, protein, nitrites, leukocytes, pH, and specific gravity using DiaScreen reagent strips for urinalysis. The collected urine sample

may also be tested for biomarkers in the lab (described in more detail in section c below). Participants suspected of having a urinary tract infection will be triaged with the clinical research nurse as appropriate. Then a 7 French 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 physiologic saline. 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 saline 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 saline). 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. The Urodynamic equipment, and with a rectal balloon catheter in place, may also record pressure measurements of the anal sphincter muscles, the sensation in the rectum, and activity of the reflex pathways that are needed for normal bowel movements. In participants receiving urinary bladder (UB) spinal cord epidural stimulation (UB-scES), following the initial fill/void cycle, a second fill/void cycle will be assessed using continuous UB-scES and configurations optimized for bladder storage and voiding. A final post-UB-scES fill/void cycle may be performed.

During the time when cystometric evaluation is being done, surface patch electromyography electrodes will be placed on the skin at 3 and 9 o'clock positions alongside or just anterior to the anus in the lithotomy position (ground electrode placed on the hip) to capture muscle activity during the cystometrogram. The electromyography activity will evaluate coordination of the urethral and anal sphincters during the voiding phase and during possible uninhibited contraction episodes. Detrusor-sphincter dyssynergia may be evaluated and classified according to the Blaivas classification into: *type 1 detrusor sphincter dyssynergia* 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 detrusor sphincter dyssynergia* 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 detrusor sphincter dyssynergia*, characterized by external urethral sphincter contraction persists throughout the entire detrusor contraction (these Participants void with an obstructive stream or cannot void at all) [262, 263].

Blood pressure (BP) parameters may be monitored throughout urodynamic testing using either the Dinamap Carescape V100 (GE Healthcare) or continuous arterial BP acquired from a finger cuff placed around the finger or thumb (Finapres Medical Systems). Surface electromyography electrodes may be placed on the lower extremities to monitor motor output during filling and voiding cycles. After urodynamic testing, the skin of the participant will be inspected and ensured that all areas exposed during the experiment are dry. After the examination, participants may continue activities of daily living. Urodynamics should last approximately 1.5 to 2 hours. This procedure may be done or repeated with UB-scES.

Urodynamics may also include questionnaires (described in more detail in section f below: *The International Lower Urinary Tract Function and Urodynamic Basic Spinal Cord Injury Data Sets, The International Bowel Function Basic Spinal Cord Injury Data Set, Index of Erectile Function (IIEF), and The Female Sexual Function Index (FSFI) with the International SCI Female Sexual and Reproductive Function Basic data set*) regarding urogenital and bowel function that are provided to the participant to complete at the end of the testing session. The Autonomic Dysfunction Following SCI (ADFSCI) questionnaire will be used to assess individual symptoms of blood pressure instability during lab and home-based training. The ADFSCI is a 24-item questionnaire consisting of four main parts: (i) demographics, (ii) medication, (iii) autonomic dysreflexia, and (iv) hypotension. A 5-point scale is used to score the frequency and severity of either hyper- or

hypotensive symptoms (i.e. headache, goosebumps, dizziness, light-headedness). These procedures may take about 15 minutes to complete.

Analysis: Detrusor pressures will be calculated by subtracting the intra-abdominal pressure from the intra-vesical pressure. The volume of saline (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 will be calculated by the following equation: Voiding Efficiency= (amount leaked/voided)/(amount leaked/voided + post-void residual volume) * 100. Bladder compliance will be calculated by the change in detrusor volume divided by change in detrusor pressure and expressed as (ml/cmH2O)[264]. Filling sensations will be noted and are defined as: First sensation of fullness – the first sense that there is fluid in the bladder; First desire – the feeling that you would void at the next convenient moment; Strong desire – a compelling need to void that is less comfortable to postpone; Capacity – the feeling that voiding cannot be delayed any longer. Uroflow parameters will be recorded if the participant is able to void voluntarily [265]. Documentation of the flow rate (ml/sec), voided volume, residual urine and the pattern will be noted (Normal, bell-shaped curve; Obstructed, intermittent). Electromyography 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.

c. Urinalysis/Urinary Biomarkers

Procedure: Urine samples will be obtained by either using a sterile 12 French straight catheter that is inserted through the urethra into the bladder for cystometry or from the participant's first morning catheterization (stored appropriately on ice until the participant arrives to the laboratory for the urodynamics assessment). 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 [266-268]. 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 nerve growth factor and brain derived neurotrophic factor concentrations will then be determined on the thawed samples using the Emax ImmunoAssay System with specific ELISA kit per manufacturer instructions [266-268].

Analysis: The concentration of nerve growth factor and brain-derived neurotrophic factor in each urine sample will be extracted from a standard curve and normalized to the concentration of urinary creatinine (nerve growth factor/creatinine, brain-derived nerve growth factor/creatinine). All samples will be run in triplicate and the values averaged.

d. Bladder and Kidney Ultrasound

Procedure: An ultrasound 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 [269]. The participant will report to the Urogenital and Bowel Lab at Frazier Rehab Institute on the 11th floor to have ultrasound 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 ultrasound, 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 [270].

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.

e. Anorectal Manometry

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 to have anorectal manometry assessment using the Aquarius® LT system by Laborie Medical Technologies (Williston, VT, USA). Participants will be asked to either complete their bowel program or perform 2 Fleet® enemas 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 Urodynamics is performed in conjunction with anorectal manometry, 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 system 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, evaluates the nerve supply to the anal muscle. Anal sphincter electromyography 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 electromyography 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 air. 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 anorectal manometry assessment period, participants' blood pressure, heart rate and oxygen saturation will be monitored using the Dinamap Carescape V100 (GE Healthcare) or continuous arterial BP acquired from a finger cuff placed around the finger or thumb (Finapres Medical Systems). 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 [271-273]. 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.

f. Questionnaires

- ***Bladder Questionnaire.***

Procedure: The International Lower Urinary Tract Function and Urodynamic Basic Spinal Cord Injury Data Sets [274, 275] 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. Participants 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.

- ***Bowel Questionnaire.***

Procedure: The International Bowel Function Basic Spinal Cord Injury Data Set [276] 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.

- ***The Autonomic Dysfunction Following SCI (ADFSCI) questionnaire***

Procedure: The ADFSCI questionnaire will be used to assess individual symptoms of blood pressure instability during lab and home-based training. The ADFSCI is a 24-item questionnaire consisting of four main parts: (i) demographics, (ii) medication, (iii) autonomic dysreflexia, and (iv) hypotension.

Analysis: A 5-point scale is used to score the frequency and severity of either hyper- or hypotensive symptoms (i.e. headache, goosebumps, dizziness, light-headedness).

- ***SCI Urinary Tract Infection***

The NIH Common Data Elements International SCI Urinary Tract Infection Data Set contains information on bladder-emptying method(s), awareness of the need to urinate, urinary incontinence, medication usage and standardization of the collection and reporting of urinary tract infections. The collection of data related to UTI's will be captured with this form.

- ***Quality of Life***

Quality of life will be measured with the Spinal Cord Injury – Quality of Life (SCI-QOL) items relevant to bladder management and associated complications. SCI-QOL measurements were developed using IRT and scores range from 0-100. It includes 19 calibrated items banks and 3 fixed length scales spanning the broad domains of physical-medical health, emotional health, social participation and physical functioning.

- ***Sexual Function in Male Participants.***

Procedure: Measures of erectile function will be assessed using the full International Index of Erectile Function questionnaire which is readily available in many publications [277]. It has been validated in 32 languages and can be used cross-culturally [278]. Briefly, there are 15 questions that can be divided into 5 unique domains: erectile function, intercourse satisfaction, orgasmic function, sexual desire, and overall satisfaction [277]. 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 [279, 280]. 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. The questionnaire is used to document any off-target effects to sexual function, such as novel erections or ejaculation, as a result of the use of spinal cord epidural stimulation. We will advise the participant to follow-up with their primary care provider, as appropriate.
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).

- ***Sexual Function in Female Participants.***

Procedure: The Female Sexual Function Index along with the International SCI Female Sexual and Reproductive Function Basic data set will be used [157, 281, 282]. The 19 question Female Sexual Function Index 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. The questionnaire is used to document any off-target effects to sexual function, such as changes in arousal or lubrication as a result of the use of spinal cord epidural stimulation. We will advise the participant to follow-up with their primary care provider, as appropriate.

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	

g. SmartPill

Procedure: Assessment of overall gastrointestinal transit will be obtained non-invasively via the wireless motility/pH capsule - SmartPill™ (Medtronic) [44,45]. The US Food and Drug Administration approved the device for the evaluation of delayed gastric emptying in 2006 and for the evaluation of colonic transit in 2009. As a single-use, orally ingested, non-digestible, data recording capsule, it is capable of providing continuous measurements of luminal pH, temperature, and pressure during transit (via peristalsis) throughout the whole gut until exiting the body through the anus. The SmartPill is a nonradioactive alternative to radiopaque markers and scintigraphy scanning and can be conducted non-invasively in the clinic-based setting, capturing gastric emptying time (GET), small bowel transit time (SBTT), colonic transit time (CTT) and whole gut transit time (WGTT). Prior to the assessment, participants will be asked to refrain from taking any medications that may alter gastric pH (i.e. antacids, proton pump inhibitors) or GI motility (i.e. laxatives). On the day of the assessment, participants will be reminded that the SmartPill is not magnetic resonance (MR) compatible and in the instance of emergent medical event necessitating imaging, an alternative approach that does not require magnetic field may be used (i.e., computerized tomography, CT, or Ultasound, US). After an initial 6 hour fast, participants will be asked to consume a standardized meal (Smart Bar – provided by this study), followed by ingestion of the SmartPill capsule, approximately the size of a large vitamin, with 8 ounces of water. To obtain an accurate measurement of GET, the participant will need to wait 6 hours after ingesting the capsule before eating another meal. After which, usual schedule eating times may resume. Participants will also be asked to refrain from using laxatives, anti-diarrhea and other motility affecting medications until the capsule has passed. Measurements of luminal pH (range, 0.05–9.0), pressure (range, 0–350 mmHg), and temperature (range, 25–49°C) will be transmitted continuously from the capsule to a small portable receiver carried by the participant until the capsule is excreted during their bowel program. Normal colonic transport takes 12 to 30 hours from the ileocecal valve to the rectum [46]. During monitoring, participants are permitted to leave the lab with the receiver and resume daily life. The receiver is returned to the lab once the capsule is excreted and the data obtained will be downloaded to the SmartPill software.

Analysis: Based on previously reported outcomes in SCI [47], GET is defined as the time elapsed between capsule ingestion and the first abrupt rise in pH (>3pH units). An abrupt change from an acid gastric pH to an alkaline duodenal pH is associated with a burst of phasic contractions and indicates that the capsule has passed from the antrum through the pylorus and into the duodenum. CTT is defined as the time elapsed between the ileocecal junction (drop of at least 1 pH unit that is sustained for 10 minutes and occurs at least 30 minutes after gastric emptying) and capsule exit from the body (evidenced by an abrupt decrease in temperature). WGTT is defined as the time elapsed between capsule ingestion and its expulsion from the

body. Reference ranges for gastrointestinal transit: gastric emptying (2–5 hours), small bowel ***transit (2–6 hours), colonic transit (10–59 hours) and whole gut transit (10–73 hours) [48].***

h. G-Tech Gastrointestinal Monitoring system

Myoelectric signals from the gastrointestinal tract may be collected via g-tech wireless disposable patches, placed on the abdominal wall and transferred via Bluetooth to an iOS-based mobile device over a period of 3 days at 3 time points (prior to, mid-point, and post-home training). The patch system would be donated from Dr. Axelrod, who is a current SPARC awardee developing this tool. Pilot data collected will assess changes in gastrointestinal activity between the stomach, small intestine, and colon in response to training with scES and compared with motility data obtained via the SmartPill.

i. Semi-structured in-depth interviews

Procedure: Interviews will be conducted with participants to collect data on their perspectives on changes in bladder function and recovery over the course of this research project. This qualitative methodology constitutes a rigorous and objective method of uncovering the facilitators and barriers to bladder dysfunction within the SCI population. Each interview will last approximately 60-90 minutes. Interviews will be conducted prior to and following home-training. Interviews will be conducted by Ms. Carla Rich who has extensive experience with conducting both structured and semi-structured patient interviews designed to understand participant preferences as well as elicit beliefs, attitudes, and perceptions of care and interventions [37-39]. Moreover, Ms. Rich is not a part of the study care and intervention team, which avoids the researcher-participant bias. Analysis of this data will help us to construct an integrated “story” reflecting the perspective and perception of participants on bladder-related changes associated with scES.

Analysis: All interviews will be audio and/or video recorded for accuracy and facilitation of analysis. Participants will be informed and consented. Next, the interviews will be transcribed verbatim, and analyzed. The analysis process consists of, first, building a code book containing deductive codes based on the discussion guide and questions prompts. The code book is divided into topics which could be further sectioned into sub-topics. The latter emerge when there are recurring themes within larger topics and they can help gain more insight into complex topics [40,41]. The topics, sub-topics and the transcripts are then loaded into a qualitative analysis software. Using the Dedoose software, 2 coders will perform duplicative coding on an initial five transcripts in order to establish inter-rater reliability. If the inter-rater reliability is low, codes will be reviewed until a reliability of 90% is reached. At that point, the remaining interviews will be divided among the two coders and analyzed.

4. Metabolic Function and Quality of Life

a. 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.
Analyses: 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.

b. Muscle Oxidative Capacity by Near-Infrared Spectroscopy (NIRS)

Procedure: NIRS is a well-known non-invasive method used to measure muscle oxygenation and hemodynamics in vivo [283, 284]. This technology relies on the oxygen-dependent changes in near-infrared light absorption and scattering characteristics. Skeletal muscle oxidative capacity will be evaluated during a recovery kinetics test by using the NIRS technology [285-287].

Each subject will be placed on an adjustable chair and remain seated or supine throughout the duration of the testing session. The foot of the tested leg will be secured in the attachment of the Biodex Dynamometer in order to minimize motion artifacts during data collection and record torque exertion. Muscle and adipose tissue thickness will be measured by ultrasound (Philips L12-5 mm linear array transducer on a Philips EPIQ 7 ultrasound system) on the most prominent bulge of the plantarflexor muscle group and/or on the vastus lateralis, approximately two-thirds of the way down from its origin (greater trochanter) to its insertion (patella).

The recover kinetics test will be performed on the plantarflexor muscle group and/or on the vastus lateralis muscle. In the case that they are both performed, they will be done sequentially.

a) Plantarflexors: NIRS optode will be placed on the most prominent bulge of this muscle group. A blood pressure cuff will be placed on the distal portion of the thigh.
b) Vastus lateralis: NIRS optode will be placed on the muscle approximately two-thirds of the way down from its origin (greater trochanter) to its insertion (patella). A blood pressure cuff will be placed on the proximal portion of the thigh.

In both cases, the NIRS optode will be secured on the lower limb with Velcro straps. In addition, two self-adhesive reusable surface electrodes will be applied on the examined muscle group, one proximal and one distal to the NIRS optode, in order to induce submaximal intermittent muscle contractions for about 30 seconds. An electrical stimulator (DS7A Constant Current Stimulator, Digitimer Ltd, Hertfordshire, England) will be used to induce muscle contractions. If the individual is able to move with scES, epidural stimulation will be used instead of the electrical stimulator. After the last muscle contraction, a series of rapid cuff inflations (~3 to 20 seconds at 300 mm Hg) will be performed by means of a rapid inflator (E20 Rapid Cuff Inflator, D.E. Hokanson, Inc., Bellevie, WA), which will be connected to a large-volume compressor (Hokanson AG101 Cuff Inflator Air Source, D.E. Hokanson, Inc., Bellevue, WA). The longest cuff occlusion will last no more than 5 minutes and will be perform in order to obtain a physiological calibration for oxy- and deoxyhemoglobin signals. Subjects will rest for five minutes before the entire protocol is repeated.

Analysis: Muscle oxidative capacity will be assessed by analyzing the kinetics of muscle oxygen consumption as previously described in detail [288]. Briefly, oxy- and deoxyhemoglobin signals will be corrected based on the principle that total blood-volume remains constant during arterial occlusions. The linear slope of the decrease in oxyhemoglobin over time will be calculated as muscle oxygen consumption for each arterial occlusion, using a custom-made software interface programmed in Matlab. Post exercise (submaximal intermittent muscle contracts) muscle oxygen consumption measurements will be fit to an exponential curve and the skeletal muscle oxidative capacity will be calculated. This recovery kinetics corresponds to the phosphocreatine recovery kinetics measured by MRI spectroscopy, which quantified mitochondrial oxidative capacity.

c. BOD POD Body Composition.

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

Analysis: Body composition, fat mass and fat-free mass will be calculated by the Cosmed BOD POD software package based upon the measured or predicted thoracic gas volume and the equation of the chosen model. Resting Metabolic Rate is estimated from the thoracic gas volume and the activity level that the research

participant self-reports. The Brozek and Siri models (based on sex and ethnicity) are standard with the software package. Additional models can be built into the software.

d. SCIM, CHART, and ADFSCI Questionnaires.

Spinal Cord Independence Measure (SCIM) - The SCIM, version III, consists of 17 items from which two of the subscales, Self-Care and Mobility, will be were compared to the other outcome measures for the purposes of this study [289]. The most independent people would have scores of 20 in the Self-Care sub-scale and 40 for the Mobility sub-scale. The total SCIM score of 100 points includes another 40 points from four items that make up the Respiration and Sphincter Management sub-scale.

The Craig Handicap Assessment & Reporting Technique (CHART) – The CHART questionnaire is designed to measure the level of handicap in a community setting: physical independence, mobility, occupation, social integration, and economic self-sufficiency. CHART collects information on the degree to which the respondent fulfills the roles typically expected from people without disabilities and consists of 32 questions, 2 to 7 questions per subscale [290].

Dysfunction Following Spinal Cord Injury (ADFSCI) questionnaire- The ADFSCI questionnaire will be used to provide information about individual symptoms of the blood pressure instability. The ADFSCI is a 24-item questionnaire consisting of four parts: (i) demographics, (ii) medication, (iii) autonomic dysreflexia, and (iv) hypotension. Each item will be graded using a 5-point scale to score the frequency and severity of hyper- or hypotensive symptoms, such as headache, goosebumps or dizziness, light-headedness, and so on, under different circumstances including standing / sitting time of individuals before experiencing hypotensive symptoms [291].

SCI-QOL and EQ-5D-3L: 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. 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. 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.

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.

E. Electrode Configuration Selection and Interventions

1. Mapping

a) Spinal Cord Epidural Stimulation (scES)
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 amplitude at a given frequency, ramped frequencies at a given amplitude, 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. These will also be repeated when participants enroll in a new study (inter-system participation).

b) Spinal Cord Transcutaneous Stimulation (scTS)
Initially research participants will participate in mapping studies to identify the neurophysiological response to various stimulation sites at the cervical level, amplitude and frequency combinations to provide a basis for identifying the specific configurations for each intervention. A safety limit of 200 mA will be implemented. Other stimulation parameters are set at: 5 kHz carrier frequency, 1.0 ms pulse width duration, and 15 – 50 Hz burst frequency. Stimulation intensity for each stimulation modality will be determined as follows:
Continuous scTS: Stimulation intensity will start at 0 mA and increase in steps of 10 mA until EMG burst activity in arm muscles is observed. Stimulation will then be increased until rhythmic movement in upper limb is obtained. In the event that no rhythmicity is observed, the stimulation intensity inducing EMG bursting activity will be used.

2. Voluntary Intervention

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 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). Participants might receive Core voluntary training focusing on trunk exercises and sitting balance for 1 hour. Core voluntary training will be performed in the laboratory under supervision of trained research staff.

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 or via TeleHealth. Two consecutive days of stable cardiovascular parameters must be obtained in the laboratory prior to the participant being given the option to do training via TeleHealth. After the completion of the first 5 training sessions, if the participant's blood pressure is stable and the participant has demonstrated safe use of the programmer, they will train at home for one day to perform the stimulation. The participant will return to the laboratory or TeleHealth 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 or TeleHealth 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 2-6 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 Training Intervention

During the stand training sessions, participants may use the custom designed standing apparatus described above or a less assistive device such as walker or cane. 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 be encouraged to stand for as long as possible throughout the training session, with the goal to stand for 60 minutes with the least amount of assistance. Seated resting periods will occur when requested by the individuals.

5. Step Training Intervention

Research participants may be placed on the treadmill in an upright position and suspended in a harness by an overhead cable at the maximum load at which knee buckling and trunk collapse can be avoided (i.e. the BWST). All trainers are careful to provide manual assistance only when needed. A trainer positioned behind the research participant will aid in pelvis and trunk stabilization, as well as appropriate weight shifting and hip rotation during the step cycle. The trainer will ensure that the trunk and pelvis are not flexed or hyper-extended during stepping, and that the weight is shifted from the ipsilateral limb to the contralateral limb simultaneous with ipsilateral swing initiation. Trainers positioned at each limb will provide manual assistance using a customized technique developed by this research team that facilitates knee extension during stance and knee flexion and toe clearance during swing. Trainers promote knee extension by applying posteriorly directed gentle pressure at the patellar tendon. They will promote knee flexion and toe clearance by applying a gentle anteriorly directed force at the medial hamstring tendon during swing. Research participants will step at various body weight load and speed. Research participants will take a break and rest at any time they feel the need to during the session. If independence is achieved during stepping, some training might be performed overground with appropriate assistive device and manual assistance to maintain participant safety. Participants will be encouraged to step for 60 minutes with the least amount of assistance. Seated or standing resting periods will occur when requested by the individuals.

6. Bladder Training Intervention

See **Appendix BB-IS-1** for SPARC Project Milestones #7, 10, 11, 12, and 13 for details on bladder mapping and training intervention.

7. Respiratory Training Intervention

Respiratory scES mapping: The optimal configuration for respiratory function will be chosen during scES mapping sessions. During these sessions, we will assess the appropriate stimulation parameters including anodes/cathodes configurations, voltage, current, and frequency to achieve maximal respiratory motor functional performance as represented by pulmonary function test outcomes in association with increased electromyographic magnitude from key accessory respiratory muscles (pectoralis, intercostals, rectus abdominus, and obliques). Every research participant will be slowly acclimated to stimulation. Blood pressure and heart rate will be closely monitored throughout stimulation sessions in the Lab.

If stable parameters are obtained, the same electrode configuration, voltage and frequency specified for stimulation in combination with RT will be used for 3 consecutive days. This will occur in the laboratory setting and scES parameters will be monitored. If 3 consecutive stable days are achieved the participant will begin home stimulation and training for 1 day. They will return to the lab the following business day for monitoring. If recordings show stable cardio-respiratory parameters they will be allowed to train at home for 3 consecutive

days, prior to returning to the lab. If recordings show stable cardio-respiratory parameters they will be allowed to train at home for 4 consecutive days, prior to returning to the lab. If recordings show stable cardio-respiratory parameters they will be allowed to train at home for 5 consecutive days, prior to returning to the lab. On the return visit, if their cardio-respiratory parameters remain stable they will be able to start the home program of 6 days a week and 1 day in lab training. At any point if the parameters need to be modified, the participants will return to the 1 home, 1 lab, 3 home, 1 lab, 4 home, 1 lab, 5 home, 1 lab training schedule. Assessments will be repeated after 1 month of RT and then after 3 months of Respiratory Training with stimulation (RT+scES).

Steps to select scES parameters:

1. Global configurations as is defined by selecting anodes and cathodes at opposite ends of the array generating either a caudal or rostral flow of current will be used first starting with rostral cathode and caudal anode with frequency of 30 Hz with 450 us pulse width will be used first. Frequency and voltage will be adjusted to facilitate trunk activity, minimize leg activity and maintain normal systolic blood pressure.
2. Frequency and amplitude will be varied to maintain improvements in respiratory-cardiovascular parameters.
3. If optimal parameters are not achieved with the initial selection, steps 1 and 2 will be repeated with a different electrode combination.

During the training session, participants will be positioned in the hospital bed/chair or personal wheelchair with an approximately 45° head-up tilt with nose clip on. Standard threshold Positive Expiratory Pressure Device (PEP, Respironics Inc., Cedar Grove, NJ) or standard threshold Inspiratory Muscle Trainer (IMT, Respironics Inc., Cedar Grove, NJ) or combination of both will be used to breathe through with adjustable resistance ranging of 20 to 41 cm H_2O. All of these devices are in routine clinical use. The principal of combination of inspiratory and expiratory resistance in the respiratory muscle trainers is accepted for rehabilitation and research [249, 292]. Unlike other devices, our setting allows us to adjust the resistance. These devices will be assembled together using a T-shaped connector with flanged mouthpiece (Airlife 001504) (**Figure 10**).

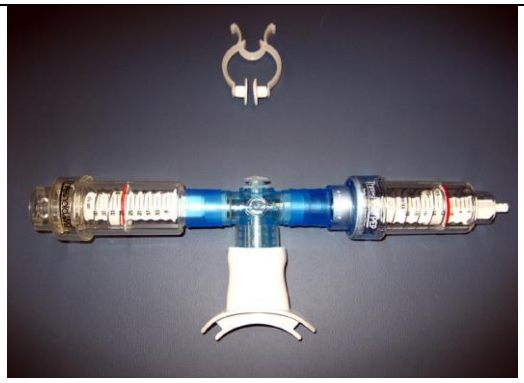


Figure 10. Respiratory Muscle Training Device with nose clip**:** Positive Expiratory Pressure Device (left) and Inspiratory Muscle Trainer (right) assembled together using a T-shaped connector (blue) with flanged mouthpiece (white).

The participants will be instructed to perform inspiratory and expiratory efforts against a resistive load [249, 293]. During inhalation, the subjects will be instructed to sustain the effort until their lungs feel full. During exhalation, the subjects will be instructed to sustain their effort until their lungs feel empty [249]. Participants will ideally be trained at the Neuroscience Collaborative Center for up to 2 hours per day, 5-7 days per week. However, in certain situations we may allow the training to be done from the participant's home. The Respiratory Training device is easy to assemble, portable, non-invasive, easy to clean, and carries a low cost. The Respiratory Training paradigm is simple, easy to learn, and can be done independently. Requiring volunteers to be present at the hospital every day for the Respiratory Training can be difficult and inefficient timewise in some scenarios. With these factors in mind, it would be reasonable to suggest that this research training would be suitable for in-home use. This "in-home training" would reduce the transportation burden and

stress placed on research volunteers, reduce reimbursement costs, and improve the efficiency of the training process. The goal would be to invite volunteers to the Neuroscience Collaborative Center to be instructed on how to use the Respiratory Training device and then monitor them from their homes via a web camera. Laboratory personnel would train volunteers to follow the proper protocol, effectively use the portable blood pressure monitor, and be trained in using web camera software. We would supply each volunteer with all of the necessary equipment, except for a personal computer. These items would include: a web camera, web camera software, the Respiratory Training device, and a portable blood pressure monitor. During each training session, the research participants will be monitored and coached by laboratory personnel. The supervising technician will be responsible for recording blood pressure readings throughout the training session as well as cueing the participant with regards to training and resting durations. The training will be initiated with a load equal to 20% of their individual maximum inspiratory and expiratory pressures with progressive increases as tolerated up to 60% of their baseline maximum inspiratory or expiratory pressures. The goal will be to have all participants training at 60% of maximum inspiratory and expiratory pressures during the last week of the training. An interval training protocol will be used with participants performing 6-8 work sets, 5 minutes in duration, separated by rest intervals lasting 1-3 minutes [294]. A research team member will monitor all training sessions. One training day per week, we may record respiratory and/or cardiovascular outcomes during the respiratory training session. We will continuously record arterial blood pressure acquired from a finger cuff placed around the middle finger/index finger /thump (Finapres Medical System). The hand will be placed in an arm sling and kept at the level of the heart throughout the training. Manual arterial blood pressure measurements using a digital blood pressure device will be taken at the beginning and during the training. A three-lead electrocardiogram (lead II; ML132, ADInstruments) and stretching belts will be placed for electrocardiogram monitoring. This data will allow us to study the cardiovascular changes observed in our research subjects throughout the training sessions.

F. Home and Community Integration

Participants will be asked to demonstrate safe use of the patient programmer for any activity that they will be allowed to continue to stimulate at home at the conclusion of the study.

Assistive devices will be designed to match the level of assistance required to safely translate the training to the home environment. Training of care givers or personal trainers to assist the participants in achieving safe and independent standing/stepping or core voluntary movement may be provided as appropriate.

G. 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 scES in regaining the ability to:

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

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 standing, stepping, 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.

III. 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 3x the number of individuals needed to reach our enrollment target. We anticipate we will need to pre-screen approximately 2x the number of individuals to identify those that will undergo our screening process. 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 is a minimum of 25% women to adequately represent the percentage in the SCI population.

For inter-system participation individuals *who have received a neurostimulator and electrode array as part of a previous study* will be approached for participation in new studies.

General Inclusion/Exclusion Criteria for all Studies (additional criteria are specific to each study).

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 (except MC-PP-3)
4) stable medical condition
5) fully vaccinated against Covid-19

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) pregnant at the time of enrollment or planning to become pregnant during the time course of the study.

Study specific criteria is listed below in addition to the above criteria

1. Motor Control. Proof of Principle

a. MC-PP-1: Spinal Epidural Electrode Array to Facilitate Standing and Stepping in SCI

Inclusion criteria:

1) Inability to stand and step independently
2) unable to voluntarily move all individual joints of the legs

3) no current anti-spasticity medication regimen
4) must not have received botox injections in the prior six months

b. MC-PP-2: Task-specific epidural stimulation and training for recovery of stepping, standing and voluntary movement following severe spinal cord injury

Inclusion criteria:

1) Inability to walk independently overground
2) unable to voluntarily move all individual joints of the legs

c. MC-PP-3: Inter-System Closed-Loop Control of Locomotor and Bladder Function in Individuals with Acute Spinal Cord Injury

Inclusion criteria:

1) Inability to stand and step independently
2) unable to voluntarily move all individual joints of the legs
3) no current anti-spasticity medication regimen
4) must not have received botox injections in the prior six months
5) Bladder dysfunction as a result of SCI
6) SCI between C6 and T10

2. <u>Motor Control. Inter-System Participation</u>

a. MC-IS-1: Intense task-specific epidural stimulation and training for recovery of stepping following severe spinal cord injury

Inclusion criteria:

1) Inability to walk independently overground without assistive devices

b. MC-IS-2: Toward the recovery of postural control in individuals with severe spinal cord injury.

Inclusion criteria:

1) Unable to stand independently without the use of upper limbs support

c. MC-IS-3: Neural Pathways and Recovery of Motor Function with Epidural Stimulation

Inclusion criteria: No additional inclusion criteria

Exclusion criteria: Specific to the TMS assessment only

1) personal or family history of seizures or epilepsy
2) individuals with increased intracranial pressure (which lowers seizure thresholds)
3) individuals taking medications which may lower the seizure threshold including tricyclic antidepressants, amphetamines, neuroleptics, dalfampridine, and buproprion
4) active or inactive implants including cardiac pacemakers, implantable defibrillators, ocular implants, deep brain stimulators, vagus nerve stimulator, and implanted medication pumps
5) conductive, ferromagnetic or other magnetic-sensitive metals implanted in their head
6) individuals who receive or anticipate receiving treatment with diathermy
7) intracardiac lines
8) history of stroke or other brain lesions
9) heavy alcohol consumption within past 48 hours

d. MC-IS-4: Inter-system Application of Spinal Cord Epidural Stimulation in Persons with Spinal Cord Injury

Inclusion criteria:

1) Received epidural neurostimulator and completed interventions from the prospective randomized efficacy study IRB# 16.0179 or IRB #17-1024 (MC-PP-1 or CVR-PP-1).

e. MC-IS-5: Tethered Pelvic Assist Device for Recovery of Standing Balance Control After Severe SCI

Inclusion criteria:

1) Unable to stand independently without scES and upper limb support

f. MC-IS-6: Neuromodulation of brain-spinal connectomes for recovery of stepping after paralysis

Inclusion criteria:

1) no current anti-spasticity medication regimen
2) must not have received botox injections in the prior six months

Exclusion criteria: Specific to the TMS assessment only

1) personal or family history of seizures or epilepsy
2) individuals with increased intracranial pressure (which lowers seizure thresholds)
3) individuals taking medications which may lower the seizure threshold including tricyclic antidepressants, amphetamines, neuroleptics, dalfampridine, and buproprion
4) active or inactive implants including cardiac pacemakers, implantable defibrillators, ocular implants, deep brain stimulators, vagus nerve stimulator, and implanted medication pumps
5) conductive, ferromagnetic or other magnetic-sensitive metals implanted in their head
6) individuals who receive or anticipate receiving treatment with diathermy
7) intracardiac lines
8) history of stroke or other brain lesions
9) heavy alcohol consumption within past 48 hours

3. Cardiovascular and Respiratory. Proof of Principle

a. CVR-PP-1: Recovery of cardiovascular function with epidural stimulation after human spinal cord injury

Inclusion criteria:

1) Injury aboveT1
2) 21-70 years of age
3) unable to voluntarily move all single joints of the legs
4) cardiovascular dysfunction including presence of persistent resting low blood pressures and/or symptoms of autonomic dysreflexia and/or orthostatic hypotension
5) at least 15%-deficit in pulmonary function outcomes detected by screening spirometry; and
6) presence of functional segmental reflexes.

Exclusion criteria:

1) severe anemia (Hgb<8 g/dl) or hypovolemia
2) HIV or AIDS related illness (self-reported or based on chart review)
3) Ongoing use of combustible drugs (Nicotine and illicit: methamphetamine, marijuana, cocaine, etc.); and
4) unable to wean off from anti-spasticity medications.

4. **Cardiovascular and Respiratory. Inter-System Participation**

   a. ***CVR-IS-1: Intense stimulation and home training for recovery of cardiovascular function following severe spinal cord injury***

   Inclusion criteria:

   1) cardiovascular dysfunction including presence of persistent resting low blood pressures and/or symptoms of autonomic dysreflexia and/or orthostatic hypotension

   b. ***CVR-IS-2: Spinal Cord Stimulation and Respiratory-Cardiovascular Plasticity after Injury***

   Inclusion criteria:

   1) non-progressive C3-T1 AIS A-C SC
   2) at least 15%-deficit in pulmonary function outcomes (FVC and FEV1) detected by screening spirometry; and
   3) arterial hypotension and/or orthostatic intolerance detected by screening orthostatic stress test.

   Exclusion criteria:

   1) ongoing nicotine use

   endocrine disorders, malignancy, marked obesity, deep vein thrombosis, HIV/AIDS related illness, secondary causes of orthostatic hypotension (anemia, hypervolemia, endocrine and neurological diseases), and major esophageal/gastrointestinal problem or other major medical illness contraindicated for respiratory training or testing.

   c. ***CVR-IS-3: Epidural Spinal Cord Stimulation and Respiratory Motor Function after Injury (study HL150581)***

   Inclusion criteria:

   1) non-progressive C3-T1 SCI;
   2) sustained SCI at least 24 months prior to enrollment;
   3) at least 15%-deficit in pulmonary function outcomes expressed as FVC and/or FEV1 ≤ 85% of predicted values measured by screening spirometry.

   Exclusion criteria:

   1) Presence of major pulmonary or cardiovascular disease unrelated to SCI
   2) Endocrine disorders
   3) Malignancy
   4) Untreated secondary hypotension (anemia, hypervolemia, endocrine, and neurological diseases)
   5) Major esophageal/gastrointestinal disease or dysfunction
   6) Untreated contracture
   7) Untreated urinary tract infection
   8) Botox injections of the bladder less than 12 months prior to enrollment or musculoskeletal botox injections less than 6 months prior to enrollment
   9) Taking medication for muscle spasticity

5. **Bladder, Bowel, and Sexual Function. Poof of Principle**

   a. ***BB-PP-1: Functional Mapping with Lumbosacral Epidural Stimulation for Restoration of Bladder Function after Spinal Cord Injury***

   Inclusion Criteria:

1) Stable medical condition
2) No painful musculoskeletal dysfunction, unhealed fracture, contracture, pressure sore or urinary tract infection
3) No clinically significant depression or ongoing drug abuse
4) Clear indications that the period of spinal shock is concluded determined by presence of muscle tone, deep tendon reflexes or muscle spasms and discharged from standard inpatient rehabilitation;
5) At least 2 years post spinal cord injury;
6) AIS classification A-C;
7) Non-progressive supra-sacral SCI (i.e., upper motor neuron regarding urogenital circuitry);
8) bladder dysfunction as a result of SCI;

Exclusion Criteria:

1) Prior Botox injections of the bladder less than 12 months prior to implant and/or
2) Continent diversion procedure with our without bladder augmentation and/or
3) Diabetes diagnosis

6. <u>Bladder, Bowel and Sexual Function. Inter-System Participation</u>

a. BB-IS-1: Functional Mapping with Lumbosacral Epidural Stimulation for Restoration of Bladder Function after Spinal Cord Injury

Inclusion criteria:

1) Bladder dysfunction as a result of SCI

b. BB-IS-2: Effects of activity dependent plasticity on recovery of bladder and sexual function after human spinal cord injury

Inclusion Criteria:

1) AIS A to D
2) Presence of neurogenic bladder and bowel dysfunction
3) Use of intermittent catheterization for bladder emptying

Exclusion Criteria:

1) Prior Botox injections of the bladder and/or
2) Bladder augmentation surgery and/or
3) Colostomy

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 physical risks and/or discomforts:

1. <u>Surgical Risks</u>

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

Less Likely
- Skin infection at the incision site
- Infection at the incision site with washout

Rare
- Open wound
- Infection resulting in device removal
- Seroma- pocket of clear fluid that can develop in body after surgery
- Complications from anesthesia
- Lung Infection
- Bleeding into the spinal cord without compression
- Bleeding into the spinal cord requiring surgery
- Skin erosion
- Hematoma (swelling of clotted blood) without compression
- Hematoma (swelling of clotted blood) requiring surgery
- Cerebrospinal fluid leak results from a hole or tear in the dura – the outermost tissue that covers the spinal cord and brain
- 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
- Discomfort and/or pain from stimulation

2. <u>Risks of Assessments</u>

Likely
- Skin irritation from hand placements of trainers and/or TPAD attachments
- Skin irritation from adhesive tape, sensors, wires, and/or pads
- Skin irritation from vibrators
- Tingling feeling from the stimulation
- Dizziness during sitting, standing or stepping
- Skin irritation from vein needle and/or fine wire insertion
- Significant changes in blood pressure and heart rate during position change
- Slight discomfort during inflation of the pressure cuffs

Less Likely

- Bleeding and/or bruising from fine wire insertion and/or blood draw
- Skin abrasion from hand placements of trainers and/or TPAD attachments
- Feelings of fear of being in closed spaces
- Shortness of breath
- Significant changes in heart rate and/or blood pressure

- Muscle and joint soreness

Rare

- Chest pain
- Infection from blood draw
- Dizziness during blood draw
- Joint sprain or muscle strain
- Nausea
- Fall
- Broken bones requiring medical treatment
- Broken bones requiring surgical treatment and long-term medical follow-up

Transcutaneous Stimulation Risks:

- Tingling feeling from the stimulator (likely)
- Significant changes in hear rate and/or blood pressure (likely)
- Muscle soreness (likely)
- Skin irritation from stimulation pads (likely)
- Thermal burns (Rare)
- Fatigue (Rare)

Transcranial Magnetic Stimulation (TMS) Risks:

- Skin irritation from sensors and/or pads (likely)
- Tingling feeling from the stimulation (likely)
- Shortness of breath (less likely)
- Significant changes in blood pressure and heart rate (less likely)
- Headache or neck ache (less likely)
- Jaw muscle contractions resulting in biting of tongue (rare)
- Seizure (rare)
- Nausea (rare)
- Fatigue (rare)
- Chest pain (rare)
- Muscle and joint aches (rare)

MRI Risks: The only 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 affect by the magnet. Finally, because the imaging process produces loud pinging noises, the participant will be provided with earplugs to wear during the experiment. Participant may experience feelings of claustrophobia while in the MRI machine.

DXA Risks: Minimal amounts of radiation during the DXA scan (less than 200 millirem). The radiation dose that the participant may receive from this study is well below the levels that are thought to result in a significant risk of harmful effect and is below the average radiation dose we are exposed to from natural (sun, earth, etc.) and man-made sources (about 300 millirem per year).

Bladder Assessments

Likely

- Feeling of shyness
- Significant changes in heart rate/or blood pressure

- Autonomic dysreflexia symptoms (sudden high blood pressure) that resolves when the cause is removed (*individuals with an injury level above T6 and in those who have previously experienced these symptoms)

Less Likely
- Mild discomfort, especially during urination after assessments of the bladder
- Urinary tract infection requiring oral antibiotics
- Discomfort from lying still for ultrasound

Rare
- Autonomic dysreflexia symptoms (sudden high blood pressure) but the cause cannot be identified, and the high pressure does not resolve, and medical intervention is required (*individuals with an injury level above T6 and in those who have previously experienced these symptoms)
- Urinary tract infection requiring intravenous antibiotics
- Excessive pain, fever, chills

Bowel Assessments

Likely
- Feeling of shyness
- Significant changes in heart rate/or blood pressure
- Autonomic dysreflexia symptoms (sudden high blood pressure) that resolves when the cause is removed (*individuals with an injury level above T6 and in those who have previously experienced these symptoms)

Rare
- Autonomic dysreflexia symptoms (sudden high blood pressure) but the cause cannot be identified, and the high pressure does not resolve, and medical intervention is required (*individuals with an injury level above T6 and in those who have previously experienced these symptoms)
- Bleeding of the rectum
- Bowel infection requiring oral antibiotics
- Bowel perforation (tearing) in those with previous rectal surgery, bowel inflammation, or bowel obstruction

Sexual Function Assessments (Questionnaire)

Likely
- Feeling of shyness

3. Interventions

Risks of Stand, Step and Voluntary Training Interventions

Likely
- Skin irritation from hand placements of trainers and/or TPAD attachments
- 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 and/or TPAD attachments
- 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

Risks of Respiratory Training Intervention

Less Likely

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

Rare

- Chest pain

Risks of Bladder Training Intervention

Likely

- Autonomic dysreflexia symptoms (sudden high blood pressure) that resolves when the cause is removed (*individuals with an injury level above T6 and in those who have previously experienced these symptoms)
- Significant changes in heart rate/or blood pressure

Rare

- Autonomic dysreflexia symptoms (sudden high blood pressure) but the cause cannot be identified, and the high pressure does not resolve, and medical intervention is required (*individuals with an injury level above T6 and in those who have previously experienced these symptoms)

C. Data Sharing

This study may utilize de-identified data from procedures in IRB #s 07.0224, 07.0066, 13.0625, 16.0179, 16.0781, 17.0204, 17.0135, 19.1194, and 20.1143. A complete waiver will be utilized for the sharing of data among these studies collected from matching assessments.

Our program is a highly collaborative center addressing the myriad of consequences of those with spinal cord injury and by integrating findings among faculty we have been able to understand many mechanisms that would not otherwise be possible. In addition, due to the unique nature of the epidural stimulator implant, all research participants have chosen to keep the stimulator after the study has been completed, so we follow research participants for many years (to date, on individual has been followed for a decade). In some cases, the valuable utilization of data across studies is recognized long after the initial study. The complete waiver would be used to gain access to de-identified data from previous studies.

The spinal cord injury field is interested in the long-term effects of neuromodulation on neuromuscular function, health and quality of life, and as additional studies become funded, participants may choose to enroll, and so we have strategically utilized complete waivers as these studies emerge. The longitudinal data we acquire across studies has been of tremendous value both scientifically and clinically.

D. 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 the Neuroscience Collaborative Center to discuss the complete protocol, including risks and benefits with the principal investigator 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, surgical, main and interventions informed consent(s) and discuss the study with their physician, family and friends, before signing each of the three IRB approved informed consents. Potential participants for implantation will sign consents in the following order: pre-screening and/or screening, surgical consent, main consent, and applicable intervention consent. Participants who already have an implant will not need to sign the pre-screening, screening or surgical consents, however they will be asked to sign the main consent and applicable intervention 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 the principal investigator 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. 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. 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, nurse practitioner 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.

Each participant will be assigned an observer to accompany the research participant to assessments 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.

3. Surgery

a. Surgical Implantation

The 5-6-5 Specify electrode, (MEDTRONIC, Minneapolis, MN, USA) and neurostimulator, (MEDTRONIC, Minneapolis, MN, USA) will be surgically implanted. For this clinical study related to spinal cord injury, this implantable technology will be used off-label. The patient will be apprised of the surgical risks by the study neurosurgeon. To minimize risks of infection each patient will be administered intravenous antibiotics throughout the operation and for 48 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. 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. There may be discomfort from the operation which will be treated with pain medications as required.

b. Protection Against Infectious Disease from Surgery

An expanded set of screening laboratory tests will be done prior to surgery (see table below) to assess the risk of infection prior to surgical implantation based on the recommendation from the Infection Prevention and Control Department (ULH) and Division of Infectious Diseases, Dept. of Medicine (UofL). The study physician will oversee treatment for any positive urine culture and infections in consultation with the Division of Infectious Diseases Department. A checklist with all preventive measures will be created for each participant and will require two staff members to verify each item was completed and reviewed. A research team member will be present for surgery and will ensure and verify precautions are met per infectious disease protocol. The principal investigators will review the completed checklist to confirm all precautions were adequately completed.

If a patient tests positive for MRSA, de-colonization will be recommended and monitored by the infectious disease team in collaboration with the study physicians. Treatment plan includes the following to be administered for 7 days: 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. Following initial treatment, the participant will be retested for MRSA. If the participant still tests positive, then treatment will include repeated decolonization as well as the following: rifampin (600 mg PO daily) plus either doxycycline (100 mg twice daily) or trimethoprim/sulfamethoxazole (one double strength twice daily). The participant will be retested following this second treatment. If the participant still tests positive, then they should be excluded from the study. The physicians will manage the medical treatment for infections and for any immediate safety concerns.

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) 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. 4) Surgical antibiotic prophylaxis will be prolonged to 48 hours. 5) Patients will be pre-screened with the laboratory tests in the table below. 6) MRSA patients will be decolonized, retested and repeated if necessary. 7) Each patient will be instructed to clean their skin (neckline to toes) with a 2% chlorhexidine bath wipe one week prior to surgery (at home) and the day of surgery in the hospital by a nurse. 8) Vancomycin powder will not be used. 9) 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 decolonize and use vancomycin as pre-operative antibiotics	Approximately 4 weeks prior to scheduled surgery to allow for time for treatment, re-test + treatment and final re-test.
Peri-rectal swab for MDRO Gram-negatives	To determine if colonized and, based on organism, consider decolonization and adjustment of pre-operative antibiotics	
urinalysis	To identify infection for clearance prior to procedure	Within 30 days of scheduled surgery; may need to be repeated closer to surgery date
urine culture		
C-reactive protein (CRP)	To obtain for baseline measure	
Erythrocyte sedimentation rate (ESR)		
Procalcitonin		
Prealbumin/Transthyretin	To verify appropriate status for procedure	
Albumin (included in CMP)		
25-Hydroxy Vitamin D		
CBC with differential		
CMP		
Pregnancy Test		

4. Epidural Stimulation

Principal investigator 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 nurse practitioner 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 their 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.

5. Transcutaneous Stimulation

The Principal Investigator and/or research team members will continually assess the stimulation parameters, including configurations, pulse width, and frequency. Stimulation parameters used during the assessments will be monitored closely by the research team. Electrodes will not be reused or shared between participants. This will ensure that the adhesion of each electrode to the participants' skin is optimized and will reduce the risk of charge density and patient burns. The participants' skin will be monitored closely throughout scTS. If signs of a potential burn are noticed (i.e., skin redness) then the stimulation will stop immediately until the signs have resolved. Stimulation parameters will be changed and stimulation will be attempted again. If signs of potential burns are still present then the stimulation will be stopped and will not be attempted again. Additionally, specific pulse configurations with long lasting terminal phases will be used which will prevent thermal burns. scTS will be applied at the cervical level above the site of injury and therefore, the intensity of the stimulation will not be as strong. Transcutaneous stimulation will only be used in the laboratory and applied by trained and skilled research personnel.

6. Assessments

a. Motor Control Assessments: Mapping, Supine, Standing, Stepping, Voluntary, FNPA, Postural Perturbation, Stable Sitting, LE Torque and Supine

The skin integrity will be checked, and the joints will be examined for swelling or redness after every assessment. Before and after every experiment, 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, hand placements of trainers or muscle vibrators, electrode, hand and vibrator 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 sprain, joint sprain or fracture (e.g. increased temperature, redness, swelling and/or spasticity). If the individual displays signs or discomfort 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 nurse practitioner will be notified and will also examine the individual. Immediate medical care will be provided when necessary.

Blood pressure and heart rate will be routinely measured. If the individual displays symptoms of syncope (dizziness, light headedness, darkening of vision) during the assessment, they will be immediately returned to sitting position and if necessary, their legs raised above the heart. If the individual displays symptoms of autonomic dysreflexia (sudden pounding headache; sweating, generally above level of lesion; unease; flushed/reddened face; goose-bumps below the level of the lesion; cold/clammy skin below the level of the lesion; stuffy nose) during the assessment, the activity will be ceased immediately. Staff will Identify and relieve the noxious stimulus (i.e. stop stimulation, check catheters, check harness, check shoes and shocks). If the stimulus cannot be identified the study physician or nurse practitioner will be notified and will also examine the individual. Immediate medical care will be provided when necessary.

b. Transcranial Magnetic Stimulation (TMS) Assessment

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. An additional TMS study screen will be performed to determine eligibility for TMS assessments. Every research participant will be slowly acclimated to the stimulation. This may help the research participants avoid experiencing significant blood pressure and heart rate fluctuations. However, if these conditions should occur, stimulation will be modified or stopped. The research team will continually assess the appropriate stimulation parameters.

In order to avoid transient magnetic fields near the abdomen if the TMS coils are moved while the device is on, the Magstim 200 will not be turned on until the coil is placed on the head. Epidural electrode array impedances will be measured prior to the start of the assessment, following the initial set of TMS stimulations, at the end of the stimulus response curve and at the conclusion of the testing session. Changes in electrode impedances

over 20% will result in stopping the TMS. There is no safety concern in combining TMS with epidural stimulation or TMS causing IPG unit malfunction as the IPG unit is implanted in the abdomen.

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. Blood pressure and heart rate will be continuously monitored throughout assessment. Research staff will use an Orthostatic Intolerance Questionnaire periodically throughout the assessment and rate symptoms, on a 0-10 scale, which may result from the seated upright or head-up tilt positions. If symptoms persist or at the participant's request, participants will be returned to the supine position and allowed to recover before ending the assessment.

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 Assessments

Assessments will be performed by either a licensed registered nurse, or by a certified sonographer (for ultrasound), under the supervision of the principal investigator(s). 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. The observer will communicate with the research participant and monitor for signs of autonomic dysreflexia, risks and other signs of discomfort. If the participant displays symptoms of autonomic dysreflexia and/or with a rapid rise in systolic blood pressure and decreased HR, the following steps will be taken:

- Stop the assessment
- Identify and relieve the noxious stimulus (i.e. stop bladder filling, empty bladder, remove catheters)
- Move the participant to an upright position and continue to monitor blood pressure
- Monitor blood pressure and heart rate until values return to the participant's established normal limits.

- If the autonomic event is not resolved, the study physician will be called to evaluate the participant. If the autonomic event is still not resolved, immediate emergency medical care will be provided. There is an emergency room located on the first floor of the building.

There may be circumstances where a participant has been pre-prescribed nitrate medication by the physician that he/she would have available for the research nurse to administer in the case an autonomic dysreflexic event was not mitigated by means of our standard protocol. In this instance, the nurse would administer the participant's prescribed medication while monitoring his/her blood pressure. If blood pressure did not resolve in response to medication, emergency protocol would be instated, as described above.

Prior to initiating cystometry, the research participant's blood pressure and heart rate will be obtained to establish his/her pre-fill values. Once the cystometrogram has begun, continuous beat-to-beat arterial blood pressure will be recorded from a cuff placed around the finger using Finapres Human Non-Invasive Blood Pressure Medical Systems unit. Brachial arterial blood pressure measurements will be taken periodically with a Dinamap V100 (GE Medical Systems) device for calibration purposes. Throughout the fill cycle, the research team will also assess the participant for symptoms of autonomic dysreflexia (see list of symptoms below). If rapid rising of systolic pressure as well as fluctuations in heart rate are observed, the fill cycle will be stopped, and the participant's bladder will be immediately emptied, followed by the removal of both the vesical and abdominal catheters. The participant's head will be elevated while blood pressure will be continuously measured until values return to the pre-fill status.

The signs and symptoms of autonomic dysreflexia include:

- A sudden, significant increase in both the systolic and diastolic blood pressure above their usual levels, frequently associated with bradycardia
- Pounding headache
- Reflex bradycardia (note that this may be a relative slowing such that the heart rate is still within the normal range)
- Profuse sweating above the level of the lesion, especially in the face, neck, and shoulders, or possibly below the level of the lesion
- Piloerection or goosebumps above or possibly below the level of the lesion
- Flushing of the skin above the level of the lesion, especially in the face, neck, and shoulders, or possibly below the level of lesion
- Blurred vision
- Appearance of spots in the visual fields
- Nasal congestion
- Feelings of apprehension or anxiety
- Minimal or no symptoms, despite a significantly elevated blood pressure (silent autonomic dysreflexia)

Note that a research participant may have one or more of these signs and symptoms when experiencing an episode of autonomic dysreflexia. Symptoms may be minimal or even absent, despite an elevated blood pressure. An episode of autonomic dysreflexia will be documented in the research participant's medical record including the presenting signs and symptoms during the assessment, what treatment was instituted, a copy of the logged blood pressure and pulse recordings, the participant's response to treatment, and resolution of the incident (i.e. final recording demonstrating that the blood pressure and pulse rate have returned to a participant's established normal limits).

The urodynamic assessments will be conducted by our research urology nurse who is specifically trained and experienced in managing bladder dysfunction in the spinal cord injury population, including recognizing signs and symptoms of autonomic dysreflexia as well as administering appropriate treatment to resolve the event. The study physician and/or physician assistant is also present in the building during the time urodynamics is conducted. The principal investigator is present in the room during the assessment as well as other members of the research team who will also be monitoring the participant's blood pressure and response to bladder filling/stimulation.

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 damper 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. Research participants may also feel uncomfortable answering questions regarding their bladder management. A private room will be used to complete the questionnaires and it will be explained to the participant that he/she may refuse to answer any question that makes him/her feel uncomfortable.

g. Bowel Assessments

Assessments will be performed by either a licensed registered nurse, under the supervision of the principal investigator(s). 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. The observer will communicate with the research participant and monitor for signs of autonomic dysreflexia, risks and other signs of discomfort.

If the participant displays symptoms of autonomic dysreflexia (pounding headache; profuse sweating, generally above level of lesion; goose-bumps above or possibly below the level of the lesion; flushed/reddened face, neck, and shoulders, or possibly below the level of lesion; blurred vision and/or appearance of spots in the visual fields; nasal congestion; feelings of anxiety or apprehension) and/or with a sudden rapid rise in blood pressure, frequently associated with reflex bradycardia, the following steps will be taken:

- Stop the assessment
- Identify and relieve the noxious stimulus (i.e. stop rectal distention, remove rectal catheter)
- Move the participant to an upright position and continue to monitor BP
- Monitor BP and HR until values return to the participant's established normal limits.
- If the autonomic event is not resolved, the study physician will be called to evaluate the participant. If the autonomic event is still not resolved, immediate emergency medical care will be provided. There is an emergency room located on the first floor of the building.

There may be circumstances where a participant has been pre-prescribed nitrate medication by their physician that he/she would have available for the research nurse to administer in the case an autonomic dysreflexic event was not mitigated by means of our standard protocol. In this instance, the nurse would administer the participant's prescribed medication while monitoring his/her blood pressure. If blood pressure did not resolve in response to medication, emergency protocol would be instated, as described above.

During the manometry assessment the anorectal pressure will be closely monitored, and balloon inflation will not exceed a maximum pressure of 200 ml of air pressure.

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. 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. Research participants may also feel uncomfortable answering questions regarding their bowel management. A private room will be used to complete the questionnaires and it will be explained to the participant that he/she may refuse to answer any question that makes him/her feel uncomfortable.

h. Sexual Function Assessments

Research participants may feel uncomfortable answering questions regarding sexual health and function. A private room will be used to complete the questionnaires and it will be explained to the participant that he/she may refuse to answer any question that makes him/her feel uncomfortable.

i. Safety Features and Additional Assistance During Standing with TPAD

A. *Hardware*:

a. *Emergency Stops*: Three emergency stops are available to stop the power to the motors, in case of an adverse event. These are available to the research participant, PT/Physician, and trainers. In case of an unexpected situation, the power to the motors can be shut down abruptly. In this case, the tension in the motors stops at its current value. The user and/or personnel can back drive the motor to free the user from the cables and allow the individual to sit.

b. *Device Exit*: The device is designed with an "easy exit" configuration. In case of an emergency or need for quick sitting and exit, the motors can be turned off and the individual can perform an assisted transition from standing to sitting onto their wheelchair within approximately 30 seconds. Also, the belts can be subsequently removed within a minute.

c. *Ergonomics*: The TPAD attachments for knees, pelvis and trunk are cushioned to ensure comfort and appropriate gripping while wearing. This reduces any contact stress and/or pinching. All parts near the user will be covered with protective foam and all electrical wires are mounted on the side of the aluminum frame, away from the user.

d. *Additional external assistance:* Trainers will have immediate access to reach the research participant and provide additional manual assistance if needed. Moreover, if the participant's lower limb, trunk, and upper limb control is insufficient to safely use the TPAD system for standing, he/she will be further assisted using a safety harness, which is a backup for fall prevention. In case of motor failure, user slippage or fall, the safety harness is capable of providing body weight support up to 300lbs or 136 kg. Body weight support may be also applied if needed. If so, it will be gradually decreased and removed, as appropriate, to allow participants to safely stand with minimal assistance.

e. *Electrical Outage*: The system consists of all commercially available parts, and is powered by an AC outlet. In case of power failure or outage, the power to the motors and system are automatically shut off, thus turning the system off. In this case, the motors stop at their current configuration and can be manually manipulated by the user or personnel. The motors used in this system are high quality DC motors. All electrical components are grounded and properly insulated. The connectors and cables are properly shielded. The motors are mounted to an aluminum frame which is also grounded to reduce electrical interference. The only part in contact with the patient is a PVC (plastic) coated steel cable, which reduces any possible electric conductivity.

B. *Software:*

The device is controlled by a computer program. Safety guidelines are programmed to ensure safe functionality of the motors/system. These ensure that forces applied to the user stay within a specified limit. This limit is controlled and determined by the operating personnel. This can be in the form of percent body weight force, limiting all forces to be applied within safe limits. The forces can also be turned off immediately with a click of a button.

7. Interventions

a. Stand, Step and Voluntary Intervention

The skin integrity will be checked, and the joints will be examined for swelling or redness after every assessment and training session. 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 sprain, joint sprain or fracture (e.g.

increased temperature, redness, swelling and/or spasticity). If the individual displays signs or discomfort 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 nurse practitioner will be notified and will also examine the individual. Immediate medical care will be provided when necessary.

Blood pressure and heart rate will be routinely measured. If the individual displays symptoms of syncope (dizziness, light headedness, darkening of vision) during standing, stepping or voluntary assessments, they will be immediately returned to sitting position and if necessary their legs raised above the heart. If the individual displays symptoms of autonomic dysreflexia (sudden pounding headache; sweating, generally above level of lesion; unease; flushed/reddened face; goose-bumps below the level of the lesion; cold/clammy skin below the level of the lesion; stuffy nose) during standing, stepping or voluntary assessments, the activity will be ceased immediately. Staff will Identify and relieve the noxious stimulus (i.e. stop stimulation, check catheters, check harness, check shoes and shocks). If the stimulus cannot be identified the study physician or nurse practitioner will be notified and will also examine the individual. Immediate medical care will be provided when necessary.

b. Cardiovascular Training Intervention

Participants will stimulate at home based on configurations that have been studied and determined safe in the lab. In the laboratory environment all configurations will be tested with EMG and continuous blood pressure and ECG monitoring to evaluate the effects of the stimulation on cardiovascular parameter. Research personnel will set limits on frequency and voltage of stimulation to make sure participants remain within safe ranges of stimulation. We do not anticipate any added risks of stimulation in the home environment as stimulation parameters will be limited to those thoroughly tested in the lab. If participants present unstable or inconsistent responses to stimulation in the laboratory they will be required to train in the lab under the supervision of a research staff member. Home training will not be allowed until the effects of stimulation on the cardiovascular system is well understood for each participant and the research team is confident that stimulation at home will be safe.

c. Respiratory Training Intervention

Prior to each respiratory training day, brachial arterial blood pressure, heart rate, oxygen saturation, and assess of participant readiness will be obtained. We also request and check that each participant has catheterized bladder before the training day begins. The standard training day regimen involves six breathing sessions that are five minutes in duration while the participant is seated upright in their personal wheelchair. We assess additional blood pressure, heart rate, and oxygen saturation levels after each five-minute training periods during the standard three-minute break between sessions. We allow enough rest time for the blood pressure and heart rate to return to baseline before beginning the next five-minute session. On one training day per week we will record beat-by-beat blood pressure and heart rate continuously to observe changes during each five-minute breathing sessions. Since functional ability varies between participants, we monitor how each participant tolerates the standard training regimen. If a participant has trouble completing six full five-minute sessions, we decrease the number and duration of the training sessions until the participant acclimates to the standard regimen. Additionally, the pressure threshold level on the training device is increased gradually and in accordance with participant performance and readiness. During respiratory training sessions, participants will be closely monitored for any signs of dizziness, lightheadedness, shortness of breath, fatigue, and significant changes in blood pressure. Should any of these symptoms occur, the training session will be stopped, and the participant will be allowed as much time as needed to recover until the symptoms have abated before beginning again. Each occurrence and abatement of symptoms will be recorded in the training notes. If any training related symptoms persist and/or worsen, the training day will be cancelled, and the research medical team will be contacted. At this point the research nurse and/or study physician will examine the participant and manage care appropriately. Each medical event will be documented and followed up on by the medical and regulatory compliance teams.

d. Bladder Training Intervention

Individuals who will be enrolling in this study already have epidural stimulator implants and have been using them successfully at home, including monitoring their blood pressure and heart rate. Participant ability to fully

engage and self-monitor home programs independently and/or with a caregiver are reviewed in the laboratory prior to the initiation of the home training phase.

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. Individuals will conduct the bladder training programs during an entire day within the laboratory prior to being cleared for home training programs. Participants will be instructed to call their 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 and training 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 or nurse practitioner. If stimulation parameters cannot be found to eliminate the onset of adverse effects, the study will be stopped.

8. Drug Testing and Medication/Supplement Review

Research participants will have drug testing completed at each of the study time points (including follow-up time points) and will be randomly tested throughout the study.

Each participant will provide their current medication and supplement list to the study research nurse. The study physician will review the medication and supplement list with them at each study physician visit. Research participants will immediately advise their research nurse of any changes to their medications and/or supplement use. If a participant is taking a medication and/or supplement that is deemed by the study physician(s) to increase their risk, the study physician(s) will discuss this with the research participant, and they may be asked to stop using epidural stimulation if the medication or supplement is a necessity.

9. Long-Term Follow-Up

Once a research participant completes the study and any specified follow-up assessments and they choose to keep the epidural stimulator, they will enter long-term follow-up. A research nurse will follow-up with them via TeleHealth six months after completing the protocol specified follow-up and then yearly. During these TeleHealth visits, the research nurse will collect medication and/or supplement lists, epidural stimulation usage, and ask questions about the participant's overall health. Medical records will also be requested.

10. Covid-19

The protection against risk from Covid-19 for those with chronic spinal cord injury and the research staff are of utmost importance. Individuals with chronic spinal cord injury are immunosuppressed and are at higher risk for infection, higher risk of hospitalization due to infection, and higher risk of death due to infection. The nature of the study requires close contact between research participants and research staff during assessments and training interventions, making the transmission of Covid-19 possible. Additionally, the main intervention of this study, neuromodulation, directly targets and affects the cardiovascular and nervous systems, both of which are targets of Covid-19. The interaction of Covid-19 and neuromodulation is unknown.

All newly enrolled research participants will be required to be fully vaccinated against Covid-19. Those who are currently enrolled will be provided the opportunity to become fully vaccinated. For those participants who are currently enrolled and choose not to be vaccinated, the following safety measures will be implemented:

a) Wearing a mask within all research areas;
b) Testing twice weekly and providing the results to the research nurse;
c) being scheduled at times when greater than 6 feet social distance from other people with chronic spinal cord injury in the research program is feasible.

As twice weekly testing will be required for currently enrolled participants who choose not to be fully vaccinated, the research study will cover the cost of the testing. Participants can choose the location for the testing but will need to provide the result to their research nurse.

E. 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 or nurse practitioner 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 nurse practitioner), the principal investigator, and the study physical therapists.

F. Data and Safety Monitoring Board

- **Suzanne Groah, MD, MSPH** (Chairperson of DSMB)
  - Physiatrist
  - Professor, Rehabilitation Medicine, Georgetown University
    Director of Spinal Cord Injury Research Program, MedStar NRH
- **Glenn Hirsch, MD** (Primary Safety Monitor of DSMB)
  - Cardiologist
    Chief, Unified Division of Cardiology
    Medical Director, Cardiovascular Service Line National Jewish Health and St. Joseph Hospital
- **Anton Emmanuel, MD**
  - Neurogastoenterologist
  - Professor, Neuro-gastroenterology, University College London
- **Linda Jones, PT, MS**
  - Physical Therapist
  - Craig H. Neilsen Foundation
- **Ann Parr, MD**
  - Neurosurgeon
  - Department of Neurosurgery University of Minnesota

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. 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.

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V. Protocol Addendum 1

Cardiovascular and Respiratory. Proof of Principle

CVR-PP-1
One participant in 13.0625 had not completed the study when the study was transferred to this comprehensive protocol. The subject will transfer to the comprehensive protocol and will continue with their training interventions and assessments.
The following additional assessments are specific to CVR-PP-1 group only.

Metabolic Parameters (Specific Aim 1)

Bioelectrical impedance spectroscopy: Body fat content will be measured using bioelectrical impedance spectroscopy. This is a non-invasive procedure in which subject lies supine, while two self-adhesive skin electrodes are placed on the hands and feet. A single channel, tetrapolar device scans 256 frequencies between 4kHz and 1000kHz. As a small insensible (cannot be felt) constant current is passed through the body, fat mass, fat free mass, and intra and extracellular fluids may be measured. This procedure will take less than 5 minutes. Body composition via skinfold measurements will also be taken.

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

MG and Soleus H-reflex (Specific Aim 4): All EMG data will be collected at 2000 Hz with custom-written acquisition software (National Instruments, Austin, TX, USA). We will record bilateral EMG (Motion Lab Systems, Baton Rouge, LA, USA) from same muscles as above. The soleus H-reflex will be evoked by monopolar electrical stimulation of the posterior tibial nerve at the popliteal fossa using a 1-ms pulse, generated by a constant current stimulator (DS7A, Digitimer, UK) and will be recorded by surface monopolar differential electrodes placed over the soleus muscle. A minimum 10 control and conditioned reflexes will be

recorded in every trial. The indifferent electrode will be placed above the patella for selective stimulation of the nerve trunk. The EMG signal will be amplified and band-pass filtered (10 Hz-500 Hz) before being sampled at 2 kHz (1401 plus running Spike 2 software). The digitized EMG signals will be rectified and the size of M-wave and H-reflex responses will be measured as the area under the full-rectified waveforms. Soleus H-reflexes will be recorded as designated by each specific supraspinal pathway protocol (described in detail below). For all conditioning experiments, amplitude and latency changes of the soleus H-reflex will be used to quantify the effects of the TMS, galvanic, auditory or ulnar nerve stimulation. Control H-reflexes will be evoked interleaved with those conditioned by the respective stimulation.

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

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

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

Reticulospinal pathway (Specific Aim 4): The reticulospinal pathway will be evaluated using soleus H-reflex amplitude under conditioning stimulation via auditory stimulus (30 ms tone of 90 dB at 700 Hz) that will be delivered using binaural earphones. EMG will be recorded from the sternocleidomastoid muscle to confirm the startle response (10). The soleus H-reflex will be elicited 50 ms after the sound to peak after 75-125 ms and

return to baseline values after 250 ms (24). Amplitude changes of the soleus H-reflex will be used to quantify the effects of the auditory stimulation. Control H-reflexes will be evoked interleaved with those conditioned by auditory stimulation with a time separation of at least 2 minutes. A minimum of 5 responses of control and condition will be measured peak-to-peak and averaged with conditioned responses expressed as percentage of control values.

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

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

Analyses: Average latencies will be calculated for N20 & P23 peaks for the Median nerve and N45 & P37 peaks for the Tibial nerve using Natus (Viking). The peak to peak amplitude can also be calculated using Natus (Viking).

VI. Appendices

MC-PP-2: Frequency of Outcomes

System: Motor Control Type: Proof of Principle
Task-specific epidural stimulation and training for recovery of stepping, standing and voluntary movement following severe spinal cord injury

Category	MC-PP-2 Assessments # = with and without stim	Pre-Implant				Post Implant				
		Pre-Screen	Screen	Pre-Training	Post-Training	Pre-Training	Inter-Training (periodic throughout intervention)	Mid-Training (80 sessions)	Post-Training (160 sessions)	Follow-up(s)
Medical	Spinal Cord MRI	X or X			X					
	ISNCSCI		X		X			X	X	X
	Medical Evaluation and Physical Therapy Evaluation		X		X	Physician only		X	X	X
	Pregnancy	X or X			X					
	Protection Against Infectious Disease Laboratory Tests	X or X			X					
	Drug Testing	X	X	X	X	X	X	X	X	X
Assessments	Epidural Mapping					X#	X#	X#	X#	X#
	Multisegmental Monosynaptic Reflexes (MMR) Stimulation		X		X					
	Functional Neurophysiological Assessment (FNPA)		X	X	X			X#	X#	X#
	Neuromuscular Voluntary Movement with Electromyography (EMG)					X#	X#	X#	X#	X#
	Standing Assessment with Electromyography (EMG)		X	X	X	X#	X#	X#	X#	X#
	Stepping Assessment with Electromyography (EMG)		X	X	X	X#	X#	X#	X#	X#
	Lower Extremity Torque Assessment with Electromyography (EMG)			X	X		X#	X#	X#	X#
	Orthostatic Stress Test		X or X		X			X#	X#	X#
	Respiratory Motor Control Assessment (RMCA)		X or X		X			X#	X#	X#
	Resting Metabolic Rate (RMR)			X	X			X	X	X

Category	MC-PP-2 Assessments # = with and without stim	Pre-Implant				Post Implant				
		Pre-Screen	Screen	Pre-Training	Post-Training	Pre-Training	Inter-Training (periodic throughout intervention)	Mid-Training (80 sessions)	Post-Training (160 sessions)	Follow-up(s)
	Urodynamics			X	X	X#		X#	X#	X#
	Dual Energy X-ray Absorptiometry (DXA)		X or	X	X			X	X	X
	BOD POD Body Composition			X	X			X	X	X

MC-PP-3: Frequency of Outcomes

Category	MC-PP-3 Assessments # = with and without stim	Pre-Implant		Post Implant			
		Pre-Screen	Screen	Pre-Training	Inter-Training (periodic throughout intervention)	Mid-Training (80 sessions)	Post-Training (160 sessions)
Medical	Spinal Cord MRI	X or X					
	ISNCSCI		X			X	X
	Medical Evaluation and Physical Therapy Evaluation		X	Physician only		X	X
	Pregnancy	X or X					
	Protection Against Infectious Disease Laboratory Tests	X or X					
	Drug Testing	X	X	X	X	X	X
Assessments	Epidural Mapping			X#	X#	X#	X#
	Multisegmental Monosynaptic Reflexes (MMR) Stimulation		X				
	Functional Neurophysiological Assessment (FNPA)		X				X#
	Neuromuscular Voluntary Movement with Electromyography (EMG)			X#	X#	X#	X#
	Standing Assessment with Electromyography (EMG)		X	X#	X#	X#	X#
	Stepping Assessment with Electromyography (EMG)		X	X#	X#	X#	X#
	Lower Extremity Torque Assessment with Electromyography (EMG)			X#	X#	X#	X#
	Urodynamics		X	X#	X#	X#	X#
	Bladder and Kidney Ultrasound		X			X	X
	ABPM		X		X	X	X
	Questionnaires		X	X#	X#	X#	X#

MC-IS-1: Frequency of Outcomes

System: Motor Control Type: Inter-System
Intense task-specific epidural stimulation and training for recovery of stepping following severe spinal cord injury

Category	MC-IS-1 Assessments # = with and without stim	Post Implant			
		Pre-Training	**Inter-Training (periodic throughout intervention)**	**Post-Training**	**Follow-up(s)**
Medical	ISNCSCI	X (if one was not completed within 1 year)		X	X
	Medical Evaluation and Physical Therapy Evaluation	X		X	X
	Drug Testing	X		X	X
Assessments	Epidural Mapping	X#	X#	X#	X#
	Functional Neurophysiological Assessment (FNPA)	X#		X#	X#
	Neuromuscular Voluntary Movement with Electromyography (EMG)	X#	X#	X#	X#
	Standing Assessment with Electromyography (EMG)	X#	X#	X#	X#
	Stepping Assessment with Electromyography (EMG)	X#	X#	X#	X#
	Lower Extremity Torque Assessment with Electromyography (EMG)	X#	X#	X#	X#
	Orthostatic Stress Test	X#		X#	X#
	Respiratory Motor Control Assessment (RMCA)	X#		X#	X#
	Resting Metabolic Rate (RMR)	X		X	X
	Urodynamics	X#		X#	X#
	Dual Energy X-ray Absorptiometry (DXA)	X		X	X
	BOD POD Body Composition	X		X	X

MC-IS-2: Frequency of Outcomes

System: Motor Control Type: Inter-System
Toward the recovery of postural control in individuals with severe spinal cord injury

Category	MC-IS-2 Assessments # = with and without stim	Post Implant
Medical	Medical Evaluation	X
Assessments	Supine Assessment with Electromyography (EMG)	X#
	Standing Assessment with Electromyography (EMG)	X#

MC-IS-3: Frequency of Outcomes

System: Motor Control Type: Inter-System
Neural Pathways and Recovery of Motor Function with Epidural Stimulation

Category	MC-IS-3 Assessments # = with and without stim	Post Implant
Medical	ISNCSCI	X (if one was not completed within 1 year)
	Medical Evaluation	X
Assessments	Functional Neurophysiological Assessment (FNPA)	X#
	Transcranial Magnetic Stimulation (TMS) (Corticospinal Pathway)	X#
	Acoustic Startle Reflex (ASR) (Reticulospinal Pathway)	X#
	Galvanic Vestibular Stimulation (GVS) (Vestibulospinal Pathway)	X#
	Propriospinal Pathways Assessment (PP)	X#

MC-IS-4: Frequency of Outcomes

System: Motor Control Type: Inter-System
Inter-system Application of Spinal Cord Epidural Stimulation in Persons with Spinal Cord Injury

Category	MC-IS-4 Assessments # = with and without stim	Post Implant			
		Pre-Training	Inter-Training (periodic throughout intervention)	Post Training	Follow Up(s)
Medical	ISNCSCI	X (if one was not completed within 1 year)		X	X
	Medical Evaluation and Physical Therapy Evaluation	X		X	X
	Drug Testing	X	X	X	X
Assessments	Epidural Mapping	X#	X#	X#	X#
	Functional Neurophysiological Assessment (FNPA)	X#		X#	X#
	Neuromuscular Recovery Scale (NRS) with Electromyography (EMG)	X#		X#	X#
	Neuromuscular Voluntary Movement with Electromyography (EMG)	X#	X#	X#	X#
	Standing Assessment with Electromyography (EMG)	X#	X#	X#	X#
	Lower Extremity Torque Assessment with Electromyography (EMG)	X#	X#	X#	X#
	Ambulatory Blood Pressure and Heart Rate Monitoring	X#	X#	X#	X#
	12-lead ECG	X		X	X
	Orthostatic Stress Test	X#	X#	X#	X#
	Echocardiography	X#		X#	X#
	Arterial Pulse Wave Velocity	X#		X#	X#
	Venous Occlusion Plethysmography	X		X	X
	Pulmonary Function Test (PFT)	X		X	X
	Respiratory Motor Control Assessment (RMCA)	X		X	X
	Resting Metabolic Rate (RMR)	X		X	X
	Vascular Ultrasound	X#		X#	X#
	Urodynamics (includes Urinalysis/Biomarkers)	X		X	X
	Bladder and Kidney Ultrasound	X		X	X
	Anorectal Manometry	X		X	X
	Bladder, Bowel, and Sexual Function Questionnaires	X		X	X
	Dual Energy X-ray Absorptiometry (DXA)	X		X	X
	Questionnaires	X		X	X

MC-IS-5: Frequency of Outcomes

System: Motor Control Type: Inter-System
Tethered Pelvic Assist Device for Recovery of Standing Balance Control After Severe SCI

Category	MC-IS-5 Assessments Group 1 # = with and without stim and TPAD	Post Implant
Medical	Medical Evaluation	X
Assessments	Stable Standing Assessment (SSA)	X#
	Postural Perturbation Assessment (PPA)	X#

Category	MC-IS-5 Assessments Group 2 # = with and without stim and TPAD	Post Implant		
		Pre-Training	**Inter-Training** (periodic throughout intervention)	**Post-Training**
Medical	Medical Evaluation	X	X	X
Assessments	Stable Standing Assessment (SSA)	X#	X#	X#
	Postural Perturbation Assessment (PPA)	X#	X#	X#
	Postural Perturbation Assessment in Sitting (PPASit)	X#		X#
	Stable Sitting Assessment (SSA)	X#		X#
	Neuromuscular Recovery Scale (NRS)	X#		X#

MC-IS-6: Frequency of Outcomes

System: Motor Control Type: Inter-System
Intense task- Neuromodulation of brain-spinal connectomes for recovery of stepping after paralysis

Category	MC-IS-6 Assessments Group 1 (Aims 1-3) # = with and without stim (scES and scTS)	Post Implant
Medical	ISNCSCI	X (if one was not completed within 1 year)
	Medical Evaluation	X
Assessments	Mapping (Epidural and Transcutaneous)	X
	Stepping Assessment with Electromyography (EMG)	X#
	Functional Neurophysiological Assessment (FNPA)	X#
	Transcranial Magnetic Stimulation (TMS) (Corticospinal Pathway)	X#
	Acoustic Startle Reflex (ASR) (Reticulospinal Pathway)	X#
	Galvanic Vestibular Stimulation (GVS) (Vestibulospinal Pathway)	X#
	Propriospinal Pathways Assessment (PP)	X#

Category	MC-IS-6 Assessments Group 2 (Aims 4-6) # = with and without stim (scES and scTS)	Post Implant		
		Pre-Training	Inter-Training (periodic throughout intervention)	Post-Training
Medical	ISNCSCI	X		X
	Medical Evaluation	X	X	X
Assessments	Mapping (Epidural and Transcutaneous)	X#	X#	X#
	Stepping Assessment with Electromyography (EMG)	X#	X#	X#
	Neuromuscular Voluntary Movement with Electromyography (EMG)	X#	X#	X#
	Lower Extremity Torque Assessment with Electromyography (EMG)	X#	X#	X#
	Functional Neurophysiological Assessment (FNPA)	X#		X#
	Acoustic Startle Reflex (ASR) Reticulospinal Pathway	X#		X#
	Transcranial Magnetic Stimulation (TMS) (Corticospinal Pathway)	X#		X#
	Galvanic Vestibular Stimulation (GVS) (Vestibulospinal Pathway)	X#		X#
	Propriospinal Pathways Assessment (PP)	X#		X#

CVR-IS-1: Frequency of Outcomes

System: Motor Control Type: Inter-System
Intense stimulation and home training for recovery of cardiovascular function following severe spinal cord injury

Category	CVR-IS-1 Assessments # = with and without stim	Post Implant			
		Pre-Training	**Inter-Training** (periodic throughout intervention)	**Post Training**	**Follow Up(s)**
Medical	ISNCSCI	X (if one was not completed within 1 year)		X	X
	Medical Evaluation and Physical Therapy Evaluation	X		X	X
	Drug Testing	X	X	X	X
Assessments	Epidural Mapping	X#	X#	X#	X#
	Functional Neurophysiological Assessment (FNPA)	X#	X#	X#	X#
	Neuromuscular Recovery Scale (NRS) with Electromyography (EMG)	X#	X#	X#	X#
	Ambulatory Blood Pressure and Heart Rate Monitoring	X#	X#	X#	X#
	Orthostatic Stress Test	X#	X#	X#	X#
	Echocardiography	X#	X#	X#	X#
	Arterial Pulse Wave Velocity	X#	X#	X#	X#
	Pulmonary Function Test (PFT)	X#	X#	X#	X#
	Respiratory Motor Control Assessment (RMCA)	X#	X#	X#	X#
	Resting Metabolic Rate (RMR)	X#		X#	X#
	Aerobic Fitness Test (VO_2 Peak)	X#		X#	X#
	Vascular Ultrasound	X#	X#	X#	X#
	Transcranial Doppler (TCD)	X#	X#	X#	X#
	Cognitive Assessment Battery	X#	X#	X#	X#
	Venous Occlusion Plethysmography	X	X	X	X

CVR-IS-2: Frequency of Outcomes

System: Cardiovascular and Respiratory Type: Inter-System
Spinal Cord Stimulation and Respiratory-Cardiovascular Plasticity after Injury

Category	CVR-IS-2 Assessments # = with and without stim	Post Implant			
		Pre-Training	**Inter-Training** (periodic throughout intervention)	**Post Training**	**Follow Up(s)**
Medical	ISNCSCI	X (if one was not completed within 1 year)		X	X
	Medical Evaluation and Physical Therapy Evaluation	X		X	X
	Drug Testing	X	X	X	X
Assessments	Epidural Mapping	X#	X#	X#	X#
	Ambulatory Blood Pressure and Heart Rate Monitoring	X#	X#	X#	X#
	Orthostatic Stress Test	X#	X#	X#	X#
	Echocardiography	X#	X#	X#	X#
	Arterial Pulse Wave Velocity	X#	X#	X#	X#
	Pulmonary Function Test (PFT)	X#	X#	X#	X#
	Respiratory Motor Control Assessment (RMCA)	X#	X#	X#	X#
	Resting Metabolic Rate (RMR)	X		X	X
	Aerobic Fitness Test (VO_2 Peak)	X		X	X
	Vascular Ultrasound	X#	X#	X#	X#
	Transcranial Doppler (TCD)	X#	X#	X#	X#
	Cognitive Assessment Battery	X#	X#	X#	X#
	SCIM, CHART, and ADFSCI Questionnaires	X		X	X
	Venous Occlusion Plethysmography	X		X	X

CVR-IS-3: Frequency of Outcomes

System: Cardiovascular and Respiratory Type: Inter-System
Epidural Spinal Cord Stimulation and Respiratory Motor Function after Injury

Category	CVR-IS-3 Assessments # = with and without stim	Post-Implant				
		Pre-Training Pre-Epidural Stim Config Mapping	Pre-Training Post-Epidural Stim Config Mapping	Mid-Training	Post-Training	Follow Up
Medical	Medical Evaluation	X			X	X
Assessments	Epidural Stim Config Mapping	X#	X#	X#	X#	X#
	Pulmonary Function Test	X#	X#	X#	X#	X#
	Respiratory Motor Control Assessment	X#	X#	X#	X#	X#
	ADFSCI, CHART, and SCIM Questionnaires	X			X	X

BB-PP-1: Frequency of Outcomes

System: Bladder, Bowel and Sexual Function Type: Proof of Principle
Functional Mapping with Lumbosacral Epidural Stimulation for Restoration of Bladder Function after Spinal Cord Injury: Simulation-Based Modeling and Interactive Programming Integration for Bladder Home-Training

Category	BB-PP-1 Assessments # = with and without stim		Post Implant			
		Screen	Pre-Training Pre-Implant	Inter-Training (periodic throughout intervention)	Post Training	Follow Up(s)
Medical	Spinal Cord MRI	X			X	X
	ISNCSCI	X			X	X
	Medical Evaluation and Physical Therapy Evaluation	X				
	Pregnancy Test	X				
	Drug Screen	X				
	Blood Panel	X	X			
	Protection Against Infectious Disease Laboratory Tests		X			
Assessments	Epidural Mapping		X#	X#	X#	X#
	Echocardiogram	X				
	Multisegmental Monosynaptic Reflexes (MMR) Stimulation	X				
	Functional Neurophysiological Assessment (FNPA)	X				
	Bladder Log (App)		X	X	X	X
	Urodynamics (includes Urinalysis/Biomarkers)		X#	X#	X#	X#
	Bladder and Kidney Ultrasound		X	X	X	X
	Anorectal Manometry		X	X	X	X
	SmartPill		X	X	X	X
	Ambulatory Blood Pressure Monitoring		X	X	X	X
	G-tech monitoring		X	X	X	X
	Semi-structured interviews		X	X	X	X
	Bladder, Bowel, and Sexual Function, QOL Questionnaires		X	X	X	X

BB-IS-1: Frequency of Outcomes

System: Bladder, Bowel and Sexual Function Type: Inter-System
Functional Mapping With Lumbosacral Epidural Stimulation For Restoration of Bladder Function after Spinal Cord Injury

Category	BB-IS-1 Assessments # = with and without stim	Post Implant			
		Pre-Training	Inter-Training (periodic throughout intervention)	Post Training	Follow Up(s)
Medical	ISNCSCI	X (if one was not completed within 1 year)		X	X
	Medical Evaluation and Physical Therapy Evaluation	X		X	X
Assessments	Epidural Mapping (Bladder Mapping)	X#	X#	X#	X#
	Voiding Diary/Log	X	X	X	X
	Urodynamics (includes Urinalysis/Biomarkers)	X#	X#	X#	X#
	Bladder and Kidney Ultrasound	X	X	X	X
	Anorectal Manometry	X#		X#	
	Bladder, Bowel, and Sexual Function Questionnaires	X	X	X	X

BB-IS-2: Frequency of Outcomes

System: Bladder, Bowel and Sexual Function Type: Inter-System
Effects of activity dependent plasticity on recovery of bladder and sexual function after human spinal cord injury

Category	BB-IS-2 Assessments # = with and without stim	Post Implant			
		Pre-Training	Inter-Training (periodic throughout intervention)	Post Training	Follow Up(s)
Medical	ISNCSCI	X		X	X
	Medical Evaluation and Physical Therapy Evaluation	X		X	X
Assessments	Epidural Mapping (Bladder Mapping)	X#	X#	X#	X#
	Voiding Diary/Log	X	X	X	X
	Urodynamics (includes Urinalysis/Biomarkers)	X#	X#	X#	X#
	Bladder and Kidney Ultrasound	X	X	X	X
	Anorectal Manometry	X#	X#	X#	X#
	Bladder, Bowel, and Sexual Function Questionnaires	X	X	X	X
	Bowel Diary	X	X	X	X

Appendix BB-IS-1: SPARC Milestones and timeline

Functional Bladder Mapping Infrastructure Plan

- Milestone #2: Regulatory IRB and FDA Human Approval

  Regulatory Approval for the study for all milestones has been initiated. Any changes to the milestones can be amended to the regulatory protocol throughout the project. Any significant correspondence with the IRB or FDA related to the protocol for this project will be forwarded to the NIH Project manager. The NIH Project Manager or Subject Matter Expert assigned to the project may participate in any meetings held with the FDA related to this NIH funded protocol. The most recent correspondence with FDA regarding an IDE is attached at the end of this document (letter dated July 31, 2017).

- Milestone #3: NIH Approval of Human Study

  Approval by NIH for the final protocol, informed consent document, case report forms, DSMB membership, and safety monitoring plan. The NIH Project Manager or Subject Matter Expert assigned to the project may participate in open sessions of the DSMB meeting. Minutes following each DSMB meeting will be forwarded to the NIH Project Manager within 7 days of the meeting. Note that any major changes related to this human subjects research will be provided to the NIH, including: amendments to the protocol, termination of the protocol, temporary suspension of the protocol, changes in informed consent or IRB approval status, temporary suspension or permanent termination of participant accrual, other problems or issues that could affect the safety of participants in the study. Notification of any of the above changes will be made within three (3) working days by e-mail, followed by a letter from the Principal Investigator, detailing the change of status notification to the local IRB and a copy of any responses from the IRB. Changes will not be implemented without written approval of the NIH Project manager assigned to this project unless needed to prevent immediate harm to a participant.

- Milestone #5: Registration of Clinical Study

  Our clinical study will be registered on the Clinicaltrials.gov website.

- Milestone #6: Recruitment and Enrollment for Human Study

  Recruitment will be from research participants who have already been enrolled in prior studies that involved epidural stimulation implantation. Currently, 11 individuals have received implants. One additional research participant will undergo surgery in the next few weeks, so there will be 12 individuals eligible for recruitment for Year 1 of the study. The targeted timeline for recruitment is two participants in the latter part of Year 1 and 4 additional participants during Years 2-3 (thus 6 in total over a three-year period).

- Milestone #7: Human Mapping, Phase Ia - for Bladder Capacity

  Mapping for capacity in the latter part of Year 1 will include two research participants. Two additional participants will be recruited in the mid-to-latter part of Year 2 and early part of Year 3 (6 total). We anticipate 6 – 8 weeks per participant for bladder mapping to identify stimulation configurations (location and optimal frequencies) for increasing bladder capacity using sub-motor threshold voltages (measured with EMG of lower extremities). The quantitative metric for capacity will be the % change in either residual volume alone if no leak occurred or residual volume plus leak volume if a reflex void occurred upon reaching capacity. Other indirect measures related to bladder capacity will include % change in maximum detrusor pressure and % change in bladder compliance. Off-target effects of epidural stimulation will include measures of rectal pressure (% change in amplitude and duration of contraction from the abdominal pressure T-DOC air-charged catheter channel) and % change in systolic blood pressure and heart rate. A baseline clinical urological evaluation will be conducted, including collection of urine (for urinalysis and biomarker evaluation), urodynamics, ultrasound of the bladder and kidney, an anorectal manometry assessment, a voiding diary or log of cath volumes, times of catheterization and blood pressure values, as well as study questionnaires (for bladder, bowel and sexual function). Participants will begin the voiding diary/log of blood pressure and cath volumes before bladder training is initiated and will continue to log throughout the intervention. The ultrasound assessment will be repeated prior to mapping for voiding and following bladder training for capacity and voiding. The anorectal manometry assessment will be repeated following bladder training for capacity and voiding.

- Milestone #10: Human Bladder Training, Phase IIa – Lab Based

  We will initially conduct daily training for capacity in a supervised on-site lab setting. Optimal configurations, identified from algorithms generated to detect and visualize data mapped during Phase I of the study, will be used at first. The storage phase configuration (Phase Ia - capacity) will be used until the time for voiding/catheterization. Quantitative metrics for storage outcomes includes voiding and/or residual volume (ml) measured via urinal, catheter bag or clear beaker by the research participant. Off target effects include measures of blood pressure and heart rate, which will be monitored in the laboratory setting using a portable device (Meditech ABPM-04 or Qardio Arm). The training procedure will be repeated for up to 8 hours daily on-site until three consecutive stable days of maintaining a consistent capacity values are achieved and deemed safe. The participant will be sent home for one day to perform the stimulation at home. In the home setting, participants will record blood pressure values, voided and residual volumes. Participants will return to the lab the following day for monitoring and testing. If assessments show stable outcomes, participants will be allowed to train at home for 7 consecutive days, prior to returning to the lab for clinical evaluation (urodynamics and questionnaires). On the return visit, if their storage/voiding outcomes remained stable over the 7 days, they will be able to start the home-based training for bladder capacity.

- Milestone #11: Human Mapping, Phase Ib - for Voiding Efficiency and Sphincter Control

  Mapping for voiding efficiency and sphincter control in Phase 1b will include the research participants from Phase 1a. The participant continues his/her training for capacity during this Phase. We anticipate 6 – 8 weeks per participant for bladder mapping to identify stimulation configurations (location and optimal frequencies) for coordinated voiding using sub-motor threshold voltages (measured with EMG of lower extremities). The quantitative metric for voiding will be the % change in efficiency (leak volume divided by leak plus residual volumes). Other indirect measures related to voiding will include % change in maximum amplitude of contraction, % change in contraction duration and area under the curve, % change in detrusor leak point pressure, and % change in timed activity between detrusor contraction and sphincter relaxation. Off-target effects of epidural stimulation will include measures of rectal pressure (% change in amplitude and duration of contraction from the abdominal pressure T-DOC air-charged catheter channel) and % change in systolic blood pressure and heart rate. A baseline clinical urological evaluation will be conducted prior to mapping for voiding, including collection of urine (for urinalysis and biomarker evaluation), urodynamics, bladder and kidney ultrasound, and study questionnaires (for bladder, bowel and sexual function).

- Milestone #12: Human Bladder Training, Phase IIb – Lab Based

  We will conduct training for voiding efficiency in a supervised on-site lab setting while the participant continues his/her training for capacity. Optimal configurations, identified from algorithms generated to detect and visualize data mapped during Phase Ib of the study, will be used at first. Voiding without catheterization will be attempted. Quantitative metrics for outcomes obtained via uroflow procedure for those participants that are able to void will include volume voided (mls), flow parameters (flow time versus void time, average flow rate, maximum flow rate), and residual volume (catheterization post- uroflow). Off target effects include measures of blood pressure and heart rate, which will be monitored in the laboratory setting using a portable device (Meditech ABPM-04 or Qardio Arm).

  The training procedure will be repeated for up to 8 hours on-site until three stable days of maintaining a consistent voiding efficiency are achieved and deemed safe. The participant will be sent home for one day to perform the stimulation at home. In the home setting, participants will record blood pressure values, voided and residual volumes. Participants will return to the lab the following day for monitoring and testing. If assessments show stable outcomes, participants will be allowed to train at home for 7 consecutive days, prior to returning to the lab for clinical evaluation and questionnaires. On the return visit, if their storage/voiding outcomes remained stable over the 7 days, they will be able to start the home program integrating bladder training for capacity and voiding.

- Milestone #13: Human Bladder Training, Phase III – Home Based

  During the home program blood pressure values and the voiding and residual volumes will be measured weekly (via urinal, catheter bag or clear beaker) by the research participant and submitted to the research team. Initially, they will be allowed to train at home for capacity, consisting of at least 20 sessions , prior to returning to the lab for monitoring, study questionnaires (bladder, bowel and sexual function), and clinical urological evaluation including collection of urine (for urinalysis and biomarker evaluation), uroflow, and urodynamics. Configurations will be reviewed and modified if needed throughout the training intervention. Mapping will be re-done as the optimal parameters may have changed with training.
  Participants will return after at least every 20 sessions of training for monitoring, including collection of urine (for urinalysis and biomarker evaluation), urodynamics, and study questionnaires (for bladder, bowel and sexual function).An ultrasound of the bladder and kidneys and an anorectal manometry assessment will be conducted post-training for capacity and voiding (after 160 sessions). The research participant will then have the choice to continue or discontinue the home program. Follow up studies will then be conducted 6 months and 1 year later to include: in lab monitoring, bladder mapping if needed, urodynamics, collection of urine (for urinalysis and biomarker evaluation), bladder and kidney ultrasound, and questionnaires.

- Milestone #15 Comparative Evaluation – Human versus Animal Data

  Following collection of all data sets, a comparison will be made to determine similarities and differences between animal and human outcome metrics.

- Milestone #16 SPARC Data and Resource Center Core Collaboration

  As stated in the SPARC Material Sharing policy, SPARC-funded investigator-derived material such as data, metadata, and resources (digital resources, computational models, animal models, tools, techniques, methods and procedures) generated from the animal and human mapping experiments will be made available to the DAT-Core and MAP-Core immediately upon completing the associated milestone. In this effort, research data obtained from human subjects will follow compliance with data privacy regulations and policies. The DAT-Core will be targeted as providing a framework to store, organize, manage, and track access of data and resources generated by this project. The MAP-Core will be targeted to link study-derived data into an interactive and modular multi-layered visualization of nerve-organ anatomy and function and continually updated, as appropriate, to assist in the development and evolution of the anatomical circuit map. Our center's research engineering core will be responsible for ensuring data is annotated with metadata per the Minimal Information Standards, developed by the SPARC Data Standards Subcommittee. In collaboration with the MIS-Physiology working group, led by the Data Standards Committee Members, Jeff Ardell and Bernard de Bono we will identify, adapt and test minimum information standards for the study specific data collected from our mapping experiments. Such data may represent electrode configurations, spinal stimulation locations and stimulation parameters acquired from both human and rat mapping studies. We will also interact closely with experts in informatics and data science from other SPARC-funded teams, such as Janet Keast, who is conducting functional bladder mapping in the rat as well.

Separate from External Research Partnership contracts between Medtronic and the University of Louisville, there is also an additional Collaboration Agreement among Medtronic, University of Louisville, and Kessler Foundation where Medtronic is paid to develop software applications and services that support and enable neurostimulation therapies for functions affected by spinal cord injuries. The funding for our engineers to develop the software and the User Interface is included in the budget for this project and the deliverables.

BB-IS-1: Timeline of Milestones

Year 1	**Months 1-7**	**Months 8-9**	**Months 10-12**
	Milestone #2, #3, #5 Regulatory - IRB/FDA, NIH Approval Registration of Clinical Study	Milestone #6 Recruitment and Enrollment	Milestone #7 Phase Ia Mapping – Capacity Group A (N=2)
Year 2	**Months 1-3**	**Months 4-8**	**Months 9-12**
	Milestone #10 Bladder Training Group A Milestone #11 Phase Ib Mapping, Voiding Efficiency and Sphincter Control Group A	Milestone #12 Bladder Training Group A (Lab) Milestone #13 Start Bladder Training Group A (at Home) Milestone #7 Phase Ia Mapping - Capacity Group B (N=2)	Milestone #13 Bladder Training Group A (at Home) Milestone #10 Bladder Training Group B (in Lab) Milestone #11 Phase Ib Mapping, Voiding Efficiency and Sphincter Control Group B
Year 3	**Months 1-9**	**Months 10-12**	
	Milestone #12 Bladder Training Group B (Lab) Milestone #13 Start Bladder Training Group B (at Home) Milestone #7 Phase Ia Mapping - Capacity Group C (N=2) Milestone #10 Bladder Training Group C (in Lab) Milestone #11 Phase Ib Mapping, Voiding Efficiency and Sphincter Control Group C Milestone #12 Bladder Training Group C (Lab) Milestone #13 Start Bladder Training Group C (at Home)	Analysis and integration of cumulative data including comparative evaluation (Milestone #15).	

Appendix MC-IS-6: Spinal Stimulator BioStim-5

SPINAL STIMULATOR

BioStim-5

Moscow & St. Petersburg

2016

TABLE OF CONTENTS

1. Purpose and principle of operation

1.1. Stimulator BioStim-5, Fig. 1.1. is the device for transcutaneous electrical stimulation of the different areas of the human body, and first of all - various areas of the spinal cord.

Fig. 1.1. General view

1.2. The stimulator can provide stimulation in five independent channels.

1.3. The output of each channel - two wires, plus (anode) and minus (cathode). With the bipolar pulse shape plus and minus indicates the polarity of the first half-cycle modulated pulse. The outputs of the different channels can be combined in the stimulus electrodes in any combination. Fig. 1.2. shows an example of arrangement of electrodes on the patient's body and the corresponding switching stimulator outputs. Positive outputs of all channels (anodes) are combined and connected with the two passive electrodes (25 and 26 in Fig.
1.2.).

The negative outputs (cathodes) are connected individually each channel with its active electrode (20, 21, 22, 23 and 24 in Fig. 1.2.).

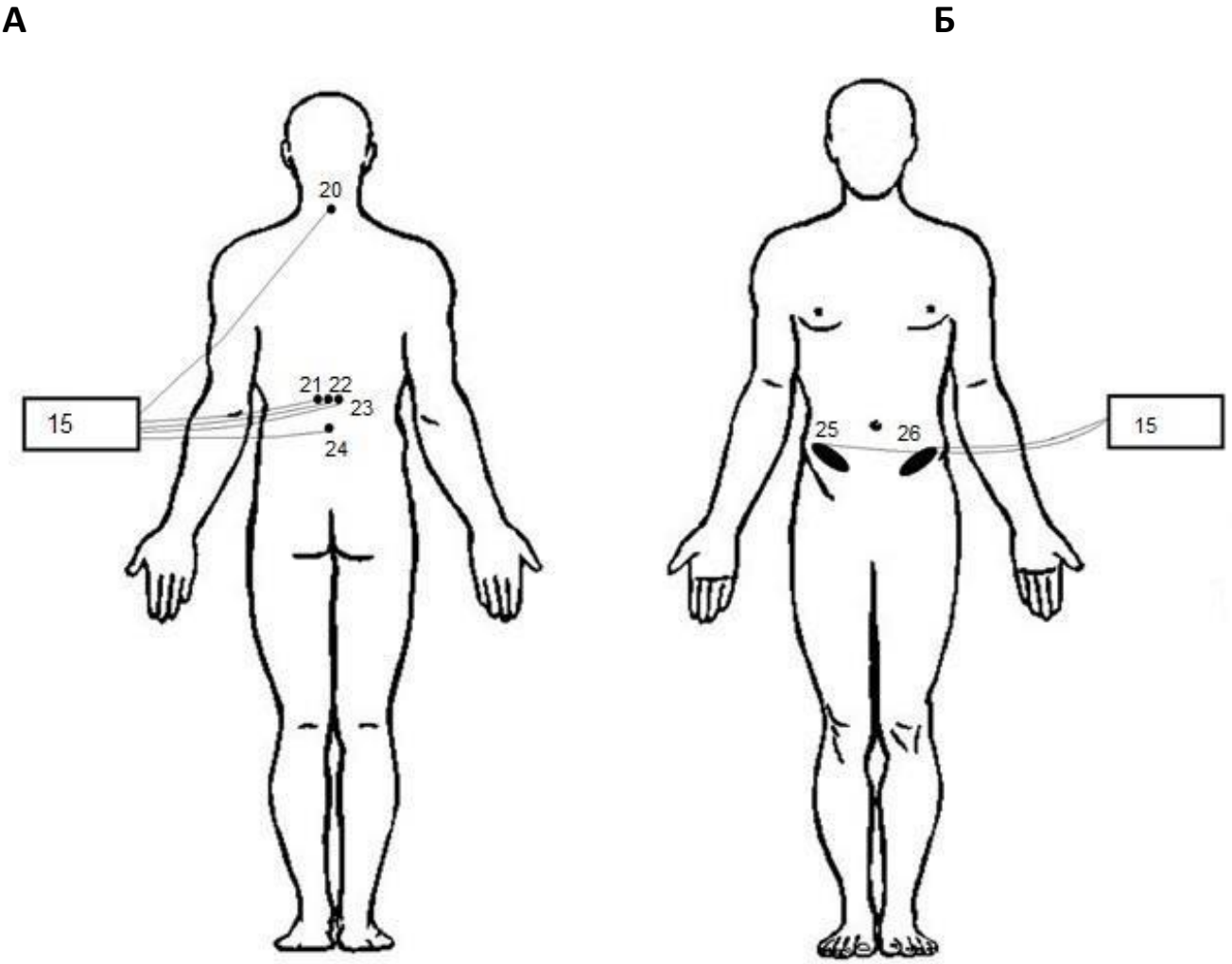


Fig. 1.2. Electrode localization on the human body. A - Rear view, B - Front view. Pos. 15 - stimulant. Pos. 20-24 - The active electrodes (cathodes) Pos. 25, 26 - Passive electrodes (anodes)

1.4. The stimulator sends pulses of current with preset value, duration and pulse frequency. Fig. 1.3. shows the oscillogram - example of the one channel.

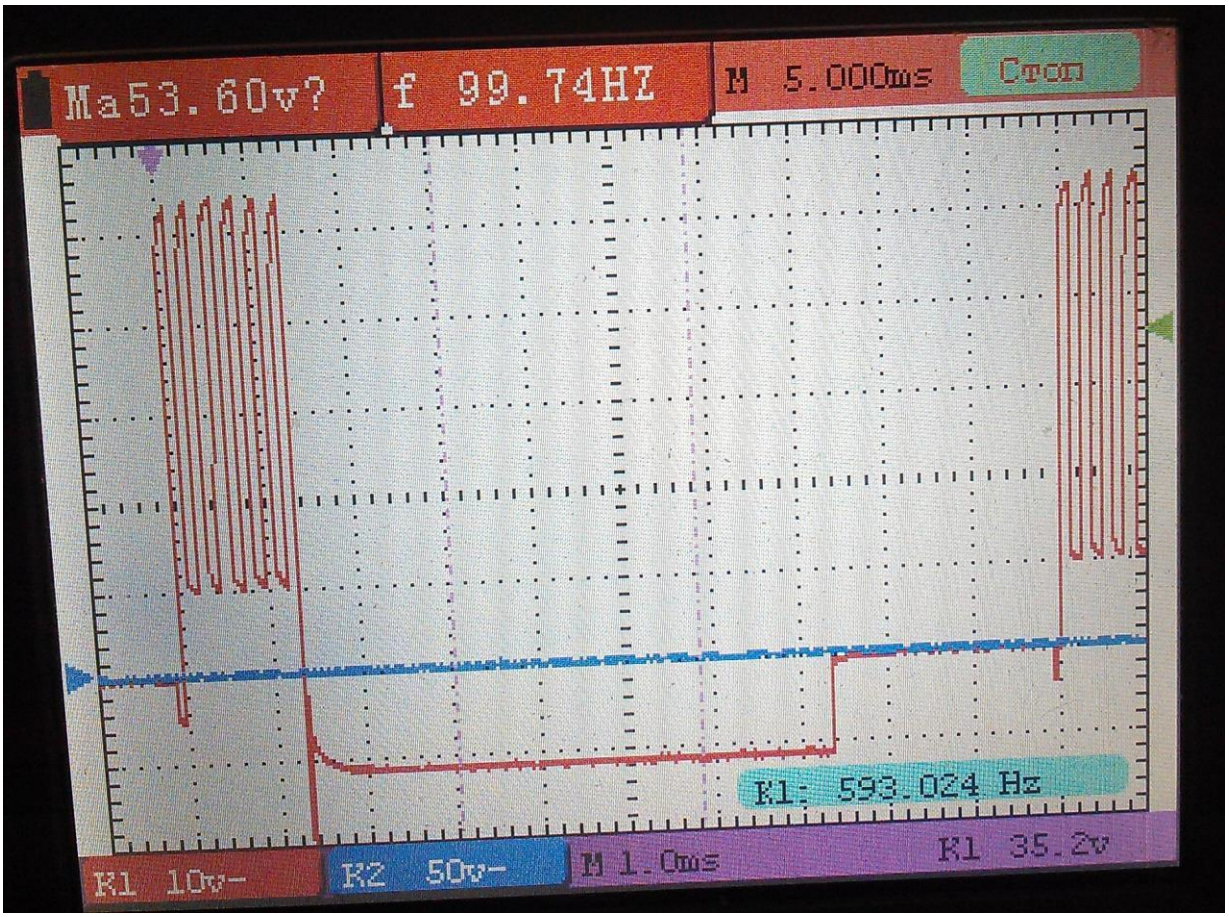


Рис. 1.3. Output voltage oscillogram of a single channel. Waveform - modulated unipolar; the modulation frequency of 4 kHz; Pulse duration - 1.5 ms; pulse frequency - 99 Hz; current - 100 mA; impedance - 510 ohms.

1.5. The pulse may be one of the following three forms: a nonmodulated, modulated unipolar and modulated bipolar (see Section 4.).

1.6. There are six possible triggering stimulation modes: single, continuous, single with the launch from another channel, periodic, external single and external continuous (see section 3.).

1.7. All stimulator channels have the same features and independently adjustable, including pulse shape and run modes of different channels which can be different. There are two restrictions on the ability to run different channel modes, these restrictions are described below in paragraphs 3.4. and 3.5.

1.8. Average current outputted from each channel stimulator (DC component) is zero. Therefore, unmodulated and modulated unipolar pulses have depolarizing phase of reverse polarity current. Two types of depolarizing phases are in use: passive or active. The passive phase is characterized by

• small depolarizing current;

• large duration of depolarizing phase: constant current decay time is 2 seconds.

The active depolarizing phase is characterized by

• relatively large depolarizing current, i.e. 1/4 of the set current pulse for baseband and 1/8 for the set current modulated unipolar pulse;

• relatively short duration of the depolarizing phases, i.e. a pulse duration of 4.

With single mode startup is set passive depolarization phase, and for continuous - active.

1.9. At any given time can be active only one channel. Time of channel activity consists of the pulse duration and the active depolarizing phase, if it is there. Thus, the operating frequency is limited by the total of all the channels.

1.10. Stimulator control is autonomous from the controllers and indicators located on the device.

1.11 With autonomous control for each channel can be set:

• current value

• pulse duration,

• repetition rate in continuous startup mode

• Type of start: single from "ON" button, or continuous,

• Select one of three possible forms of pulse: unmodulated, modulated unipolar or bipolar,

• In stand-alone control pulse modulation frequency is 5 kHz.

1.12. The stimulator is powered by built-in rechargeable battery. The battery is charged from a network 220 V, 50 Hz. During battery charging the stimulation is not possible, for the normal operation must be disconnected from the 220 V.

2. Main Technical Specifications

2.1. The number of independent stimulation channels 5

2.2. Current adjustment range from 0 to 250 mA with the step of 1 mA.

The load is greater than 1 kOm may lead to the limited current stimulus, because the pulse voltage does not exceed 250 V.

Pulse width adjustment range of 0.1 to 1ms with step of 0.1 ms. The duration of the modulated pulse is an integer of modulation periods.

2.3. Modulation frequency is 4-10 kHz.

2.4. Range of adjustment for the pulse frequency from 1 to 99 Hz in 1 Hz steps.

When controlling from a computer program, it can be activated the coefficient reducing the pulse repetition rate by 10. When this coefficient is active, the pulse repetition frequency adjustment range becomes 0.1 to 9.9 Hz in 0.1 Hz increments.

2.5. The root-mean-square value of the current flowing through each channel is limited to 15 or 40 mA. If an attempt is made to increase the current, pulse width, or pulse repetition frequency, which results in violation of this limitation, an error message appears and the parameter does not change.

2.5. The average output current on each channel (the constant component of the current) is zero.

2.6. Time of continuous operation at the maximum parameters of stimulation and when fully charged new battery is at least 30 minutes. When stimulated with moderate currents - a few hours.

2.7. Battery charge time from 30% charge to fully charge is about 6 hours.

2.8. To charge the battery should be used a network of 110/220 V, 50 Hz

3. Trigger Modes of Stimulation

3.1. Each channel can be operated in one of the three single modes or in one of three continuous modes.

3.2. Single mode:

• Starting with the "ON." button. When you press the «ON» button, the channel provides a series of pulses.

• Starting with the delay from another channel, see. Figure 3.1. The delay time is measured from the end of a given ("other") channel.

3.3. In any mode of single pulse, stimulator produces a series of pulses at each start. The maximum duration of the series - 0.5s, minimum - one pulse. Time series with autonomous control (without computer) is 1 pulse. Minimum time of channel silence before the next launch - 1.5 seconds.

3.4. Modes continuous run:

 Constant run in which the channel is continuously displays current pulses with the specified parameters.

 Periodic start-up, see. Figure 3.2. In periodic mode launch, the first channel sets the rhythm of work: a series of pulses ("in-phase"), silence interval, a series of pulses "antiphase" channel, silence interval, a series of pulses, etc. The length of "In-phase" and "antiphase" series is the same and ranges from 0.1 to 10 seconds. The duration of intervals of silence is also the same and ranges from 0 to 10 seconds. The remaining channels run in batch mode or operate concurrently with the first channel (in-phase) or at the time "antiphase" series.

 External triggering.

3.5. Modes of different channels are selected independently, except in two cases:

• for selection on any channel mode "single with delay", the channel on which it will run must also be installed in one single operation modes.

• When on any channel is "periodic" mode, the first channel should also be set to "periodic" mode.

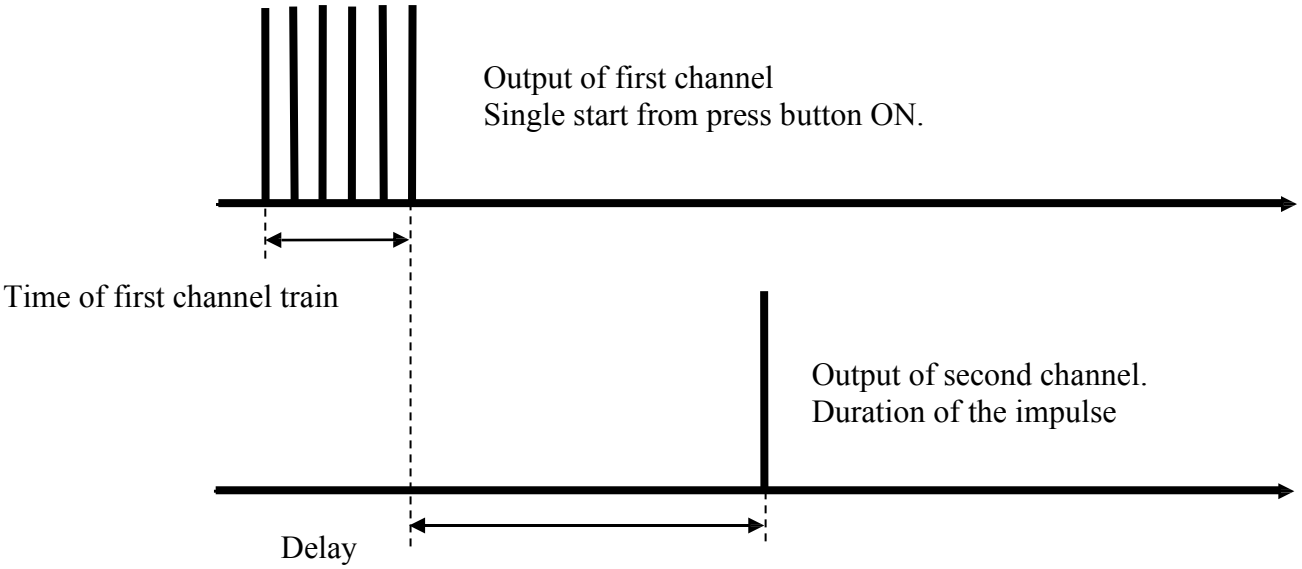


Рис. 3.1. Operation of second channel during single start with delay from first channel.

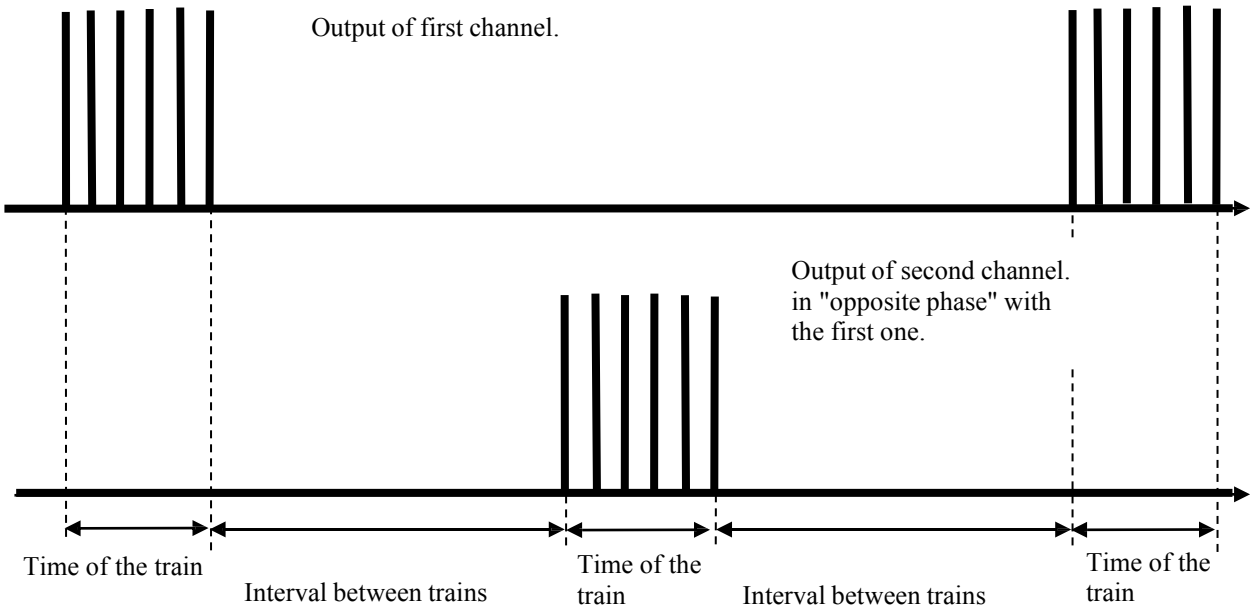
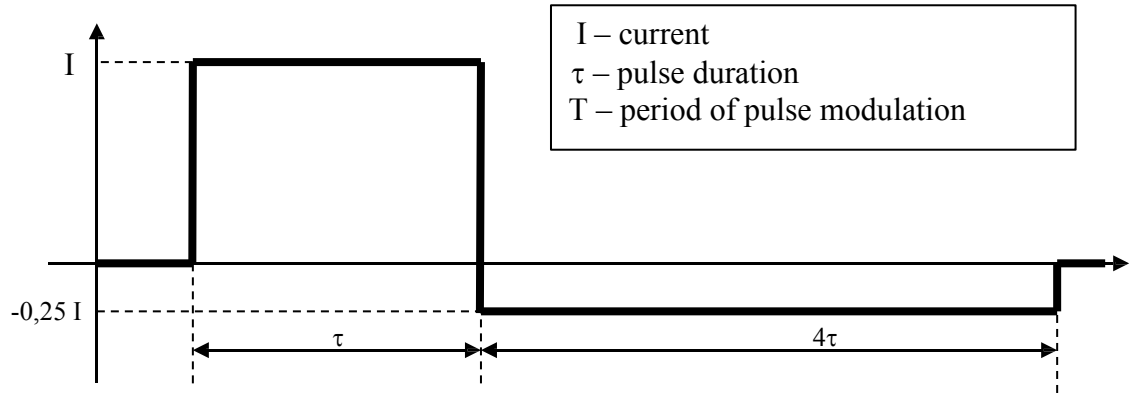
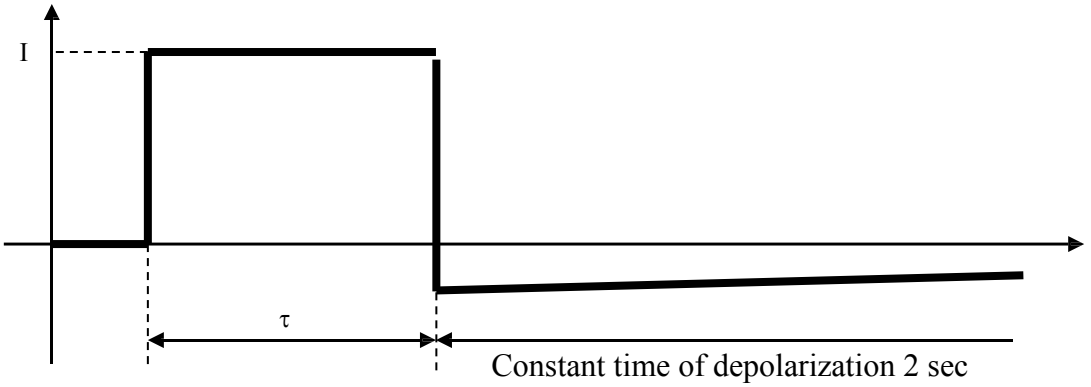


Fig. 3.2. Operation of the first and second channels in a periodic mode. The second channel works in "opposite phase" with the first one.

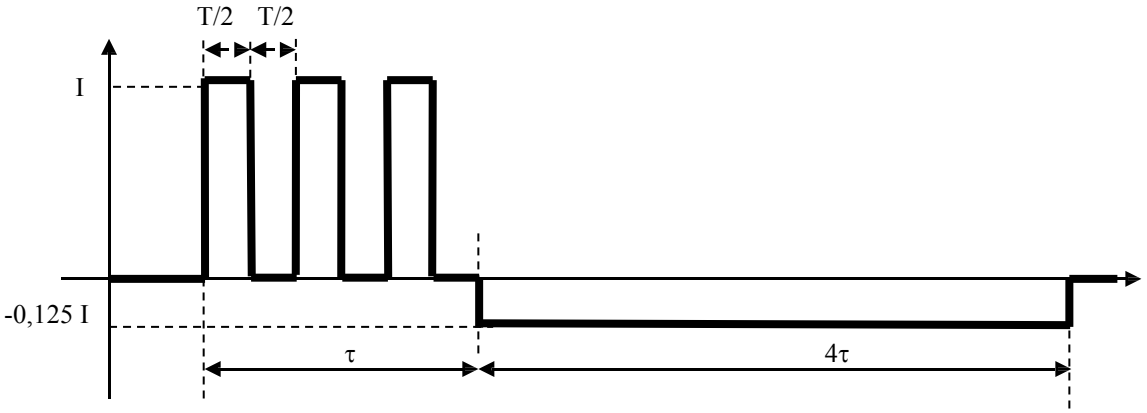
4. Possible Waveform



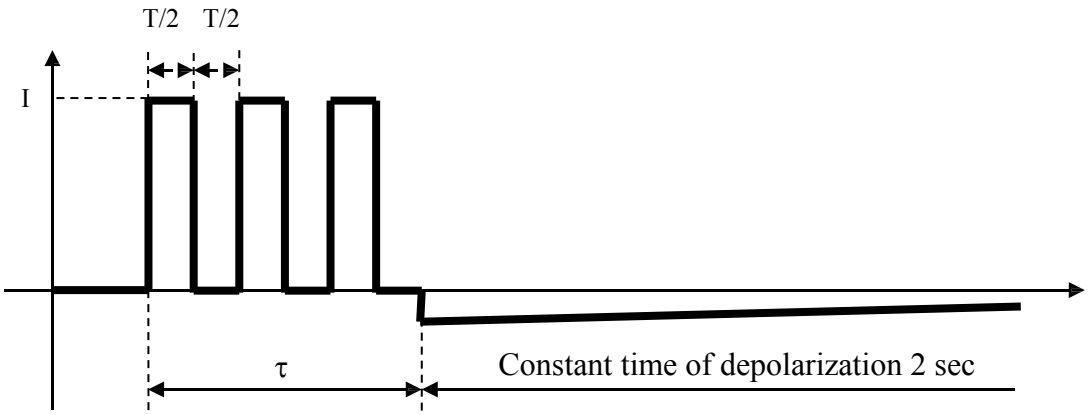
Non-modulated impulse with active phase of depolarization.



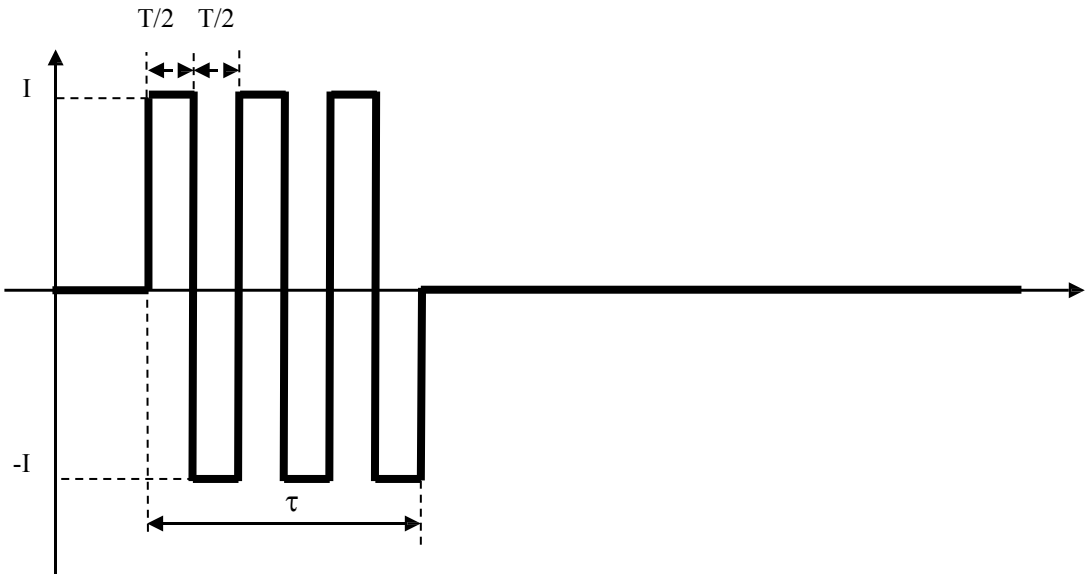
Non-modulated impulse with passive phase of depolarizaton.



Modulated monopolar pulse with active phase of depolarization.



Modulated monopolar impulse with passive phase of depolarization.



Modulated bipolar impulse.

5. The control and display elements of the system stimulator

5.1. The switch on / off of the stimulator and the connector for charging are located on the left side of the stimulator (Fig.5.1.).

ATTENTION! When the charger is connected, the stimulator turns off.

Fig. 5.1. The location of the connectors on the left side of the stimulator.
1 - Turning the stimulator on / off.
2 - Charger connector.

5.2. The input and output synchronization connectors are located on the right side of the stimulator (Fig.5.2).

Fig.5.2. The location of the connectors on the right side of the stimulator.

1 - Sync output connector.

2 - Sync input connector.

Output connectors for connection with stimulating electrode and the channel combining switch are located on the upper side surface of the stimulator

(Fig.5.3.).

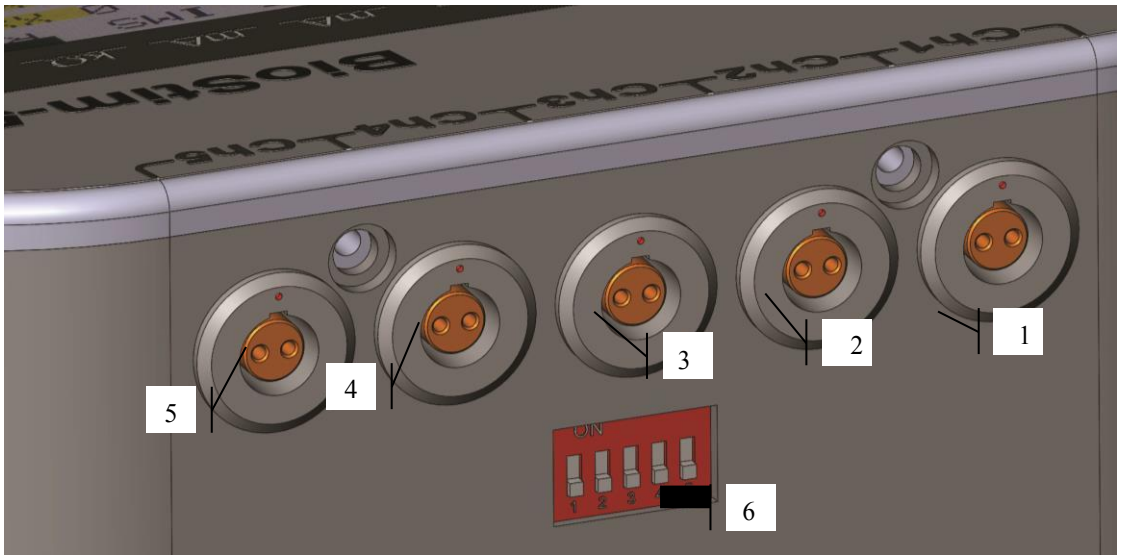


Fig.5.3. The location of the connectors and the switch on the upper side surface of the stimulator.
1, 2, 3, 4, 5 - Connectors for connection with stimulating electrodes.
6 - Channel combining switch.

To connect the cable to the connector, you must combine the red dot on the cable connector with the red dot on the stimulator connector (the dot is on top) and insert the cable connector into the stimulator connector until it clicks. To disconnect the cable, pull the connector of the patient cable by the ribbed holder.

The patient's cable consists of two wires: red - anode (plus) and blue - cathode (minus). The wires are terminated with male connectors to connect the stimulation electrodes to the corresponding connectors.

5.1. Table 5.1 shows the location of the signals on the output connector for connecting the patient cable. Table 5.2 shows the location of the signals at the synchronization output connector. Table 5.3 shows the location of the signals on the sync input connector.

Table 5.1. The composition of the signals on the output connector for connection with stimulating electrode. Connector type RM-EGG-1B-302-CLL (socket), return, cable, part RM-FGG-1B-302-CLAD52Z (plug)

Connector	Ch1		Ch2		Ch3		Ch4		Ch5	
№ contact	1	2	1	2	1	2	1	2	1	2
Signal	1+	1-	2+	2-	3+	3-	4+	4-	5+	5-

Notation: n + (-) means the positive (negative) output of channel n.

To connect together the anode pins of two or more stimulation channels, the switches of these channels must be switch ON. In this case, if two reference electrodes are located, for example, on the iliac crests, then to connect the anode pins of the first three channels, the red wire of the first channel can be connected to one reference electrode, the red wire of the second channel connected to the second reference electrode, and the red the wire of the third channel is left unconnected. If the connector is OFF then this channel is not connected to common (anode) electrode and the channel will work independently.

Table 5.2. Composition of signals on the synchronization output connector. Connector type: RM-EGG-1B-310-CLL (socket). Reverse, cable part: RM-FGG-1B-310-CLAD52Z (plug)

№ контакта	1	2	3	4	5	6	7	8	9	10
Color	White	Yellow	Green	Brown	Blue	Grey				
Signal	SI_1	SI_2	SI_3	SI_4	SI_5	0V	NC	NC	NC	NC

Symbols: SI_n means channel sync, n, NC is not used. Synchronization pulses, logic signals, duration 2 ms and amplitude 3.3 V are given out for each impulse of stimulation.

Table 5.3. Composition of signals on the synchronization input connector. Connector type: RM-EGG-1B-304-CLL (plug). Reverse, cable, part: RM-FGG-1B-304-CLAD52Z (socket)

№ контакта	1	2	3	4
Color	White	Yellow	Green	Brown
Signal	Sync1	Sync2	VDD	0V

Sync1 и Sync2 indicate external inputs of signal with amplitude from 3 to 5 V.

The following elements are located on the front panel of the stimulator (Fig.5.4.):

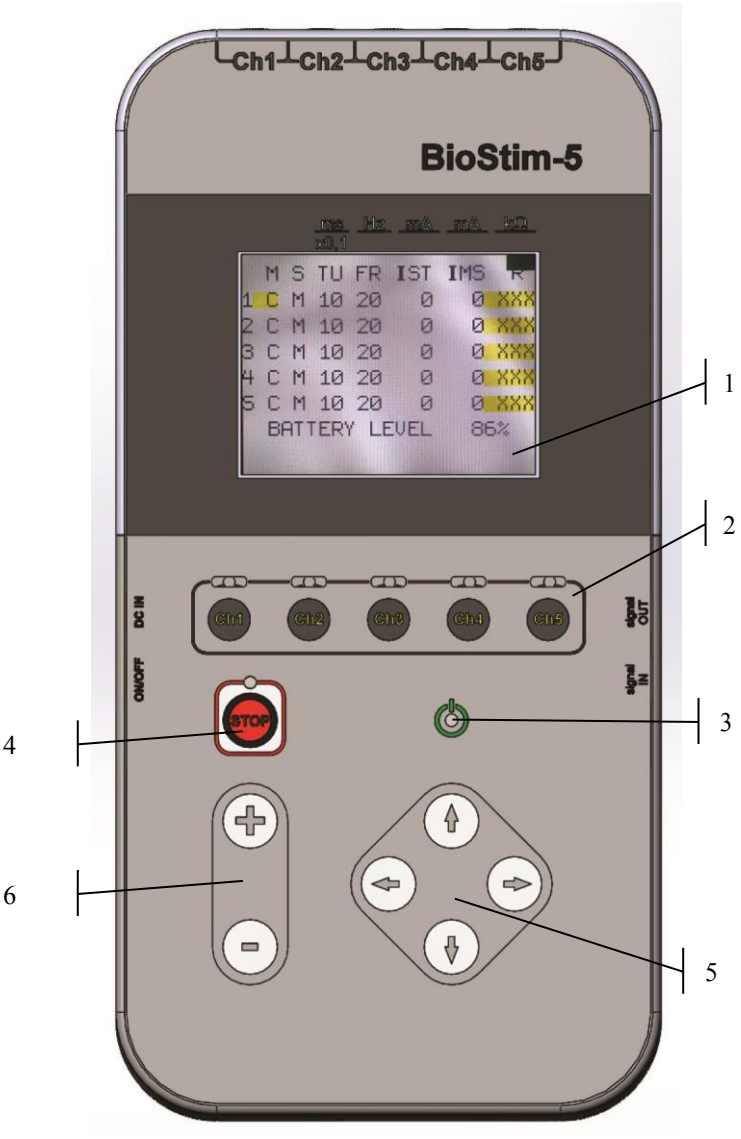


Fig.5.4. Arrangement of elements on the front panel of the stimulator.

1 - Screen;

2 - Buttons on / off channels;

3 - Indicator on the stimulator;

4 - Button for simultaneous deactivation of all stimulation channels;

5 - Navigation buttons on the screen: setting the cursor on the desired parameter;

6 - Buttons for changing the selected parameter.

5.5.1. The following information is displayed:

1 5 lines with simulation parameters, one line per channel;

2 A line with information about the level of charge of the battery;

3 A line with an error message.

5.5.1.1 The following information is displayed in the stimulation parameters line, from left to right:

4 Stimulation start mode: C - continuous; 1 - single from the button; X - one of the remaining 4 possible launch modes, set from the computer; Z - channel is not used.

5 Pulse shape: N - unmodulated, M - modulated unipolar, B - modulated bipolar.

6 The pulse duration is 0.1 ms.

7 Pulse repetition rate, Hz (or 0.1 Hz, if in the computer control program, see p. .., a coefficient of 0.1 is set).

8 Specified current, mA.

9 Measured current, mA,

10 Measured interelectrode resistance, kOhm.

6. Control of the stimulator using computer program

6.1. When operating with a computer program, you can also use the controllers located on the front panel of the stimulator.

6.2. The view of management program of BioStim-5 stimulator.

6.3. On the screen there are five identical windows to control each channel of the stimulator.

6.4. "Light" in the upper left corner of the window indicates the status of the channel: if the channel is OFF, then the color of the light bulb is gray, if the channel is ON but does not give pulses, then the color is white, if the channel is working, then the color of the light is green or red, whether the measured current is within the specified limits, or not.

6.5. The "Start" button duplicates the channel enable button on the front panel of the stimulator (see 5.3 and 5.3.2 of the previous section).

6.6. The "Test" button starts the channel operation in manual mode. "Test" button causes a constant output of pulses with the specified parameters. When the button is released, the stimulation stops.

6.7. Controller (current admin) indicate the stimulus current. The measured current is displayed to the right of this window. The measured interelectrode resistance is also shown.

6.8. Controller (current limit) indicate permissible current flowing through the electrodes. Initially, the maximum permissible value of the rms current is 15 mA, when the increased current limit is selected, this value becomes 40 mA. In this case, the color of the window in which the stimulus current is set becomes yellow (see Channel 2 in Figure 6.1).

6.9. There are controllers for:

• Pulse width, in 0.1 ms.

• Pulse repetition rate.

• Pulse modulation frequency (in case the modulated waveform is selected), kHz.

6.9.1. To decrease the frequency of stimulation you can select a factor of 0.1. The initial frequency ranged from 1 to 99 Hz. If the multiplier is set to 0.1, the frequency is set to 0.1 Hz and the frequency range is 0.1 to 9.9 Hz. In this case, the window elements describing the pulse repetition frequency are highlighted in yellow (see Channel 5 in Figure 6.1).

6.10. The controller for specification of the pulse shape is located below the interface elements described. If you choose an unmodulated pulse shape (as Channel 1 in Figure 6.1.) then yellow indication turns ONN which indicate that with the unmodulated pulse shape, stimulation can be painful.

6.11. Startup mode controller indicate the approach of stimulator activation.

6.11.1. If you select one of the single startup modes, a window for selecting the pulse duration is displayed (channels 2, 3 and 4 in Figure 6.1.).

6.11.2. If you select a trigger mode with a delay from another channel, a channel selection windows appear to indicate which channel will be the trigger and delay value.

6.11.3. When external trigger mode is selected, the window for selecting one of the two external signals and the signal polarity appear at which stimulation will occur.

6.11.4. If you select the periodic mode on any channel, channel 1 is also set to the periodic start mode. In this case, the window in the first channel allow you to set the duration of the stimulation interval and the duration of the silent interval. In the selected channel window (if it is not the first one) you have to indicate how this channel should work, in-phase or out-of- phase with the first one.

6.12. The channel settings are recorded in the patient database at the end of the procedure (at the end of the procedure by time or when the menu item "STOP" is selected) and restored at the beginning of the following procedure when selecting a patient from the list.

6.13. An error message is displayed in the lower right corner of the program window (Figure 6.1). In Fig. 6.1 the message "NO COMMUNICATION" is shown, meaning that the program "does not see" the stimulator. This message occurs, for example, if the stimulator is turned off, or is out of range of the radio adapter.

6.14. If the radio communication between the computer and the stimulator is normal, then in the lower right corner of the program window information is displayed on the charge level of the stimulator battery. If the charge level is 10% or less, the color of the label becomes red. At a charge level of 0%, the stimulator is automatically turned off.

6.15. There is a button for deactivating all stimulation channels simultaneously ("All STOP"). The "light bulb" to the left of the "All STOP" button lights up green if at least one stimulation channel is on.

6.16. At a large interelectrode resistance (chain termination) stimulation on this channel is disconnected.

7. General Operation of Stimulator

7. Only qualified and trained personnel can work with the stimulator.

7.1. During electrical stimulation procedure it is necessary to follow the course of the procedure. In case of malfunction, as well as in case of deterioration of the patient's condition or in case of occurrence of pain or discomfort, you should immediately stop work by button "Stop".

7.2. In order to increasing the battery life you should monitor battery condition. Do not start work when battery low. In case of prolonged stay with no function, recommended regularly to check the condition of the battery (at least every 2 months) and recharge it up to charge of 50 - 70%. Do not leave stimulator on for a long time.

7.3. To extend the life of the patient cable, it should be managed carefully. Do not add extra efforts attaching the connector to a stimulator or when disconnect from it. Do not bend the cable, do not pull the wire when disconnecting wires from the electrodes, and if you disconnect the cable from the stimulator.

7.4. If stimulator was staying at negative temperatures, before switching in functional mode it must be kept at room temperature for at least 2 hours.

Appendix MC-IS-6: Transcutaneous Device Parameters

Device Parameters:

Maximum output voltage at 500 Ω:	125V
Maximum output current at 500 Ω:	250mA
Pulse Width:	0.1ms to 1ms
Frequency:	1Hz to 99Hz
Maximum Current Density (mA/cm2) at 500 Ω:	250 mA / 7.9 cm2 = 49.4 mA/cm2 using electrode of diameter of 1.25 inch.
Maximum Power Density (W/cm2) at 500 Ω:	250 mA * 250 mA * 500 Ω / 7,9 cm2 = 3.96 W/cm2
Maximum phase charge (µC):	monophasic mode = 250 mA * 1 ms * 50% duty cycle = 125µC Biphasic mode = 0
Average Current Density at 500 Ω:	At max pulse amplitude of 250mA, Average current density equals 0.82mA/cm^2.
Average Power Density at 500 Ω:	At max pulse amplitude of 250mA, Average power density equals 0.1W/cm2.

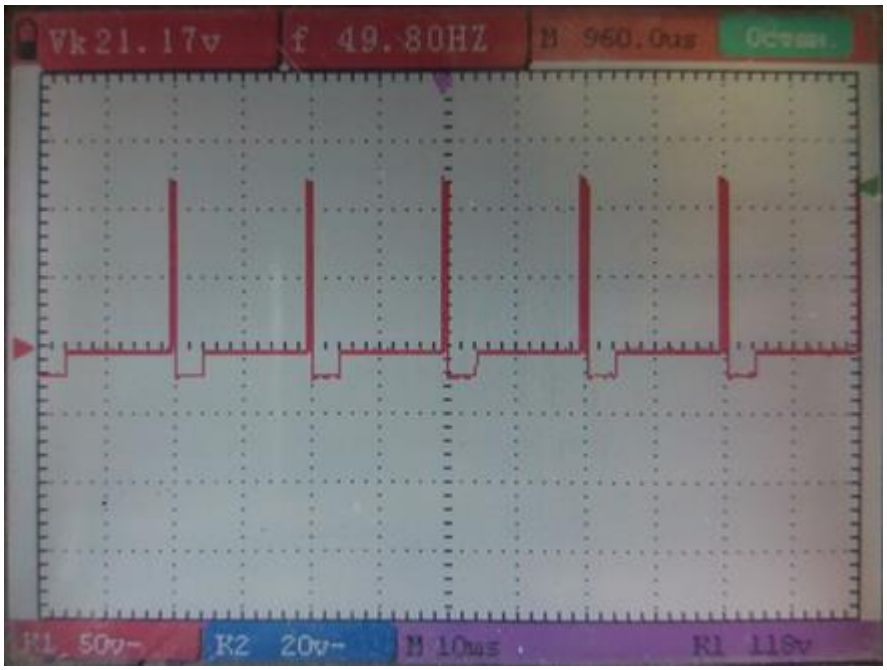
Device Parameters in Burst Mode7 (i.e., pulse trains)

Pulses per burst	5 to 10
Bursts per second	1 to 99
Burst duration (seconds)	0.1 to 1ms
Duty Cycle	0.1-9.99%

Oscilloscope tracings of the output waveforms delivered by BioStim-5 at 500 Ω:

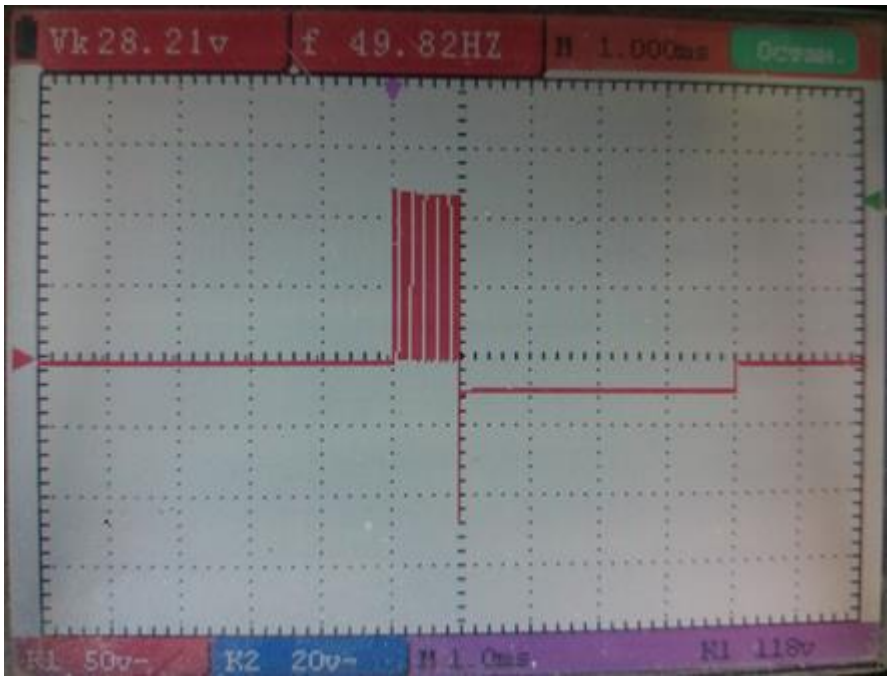
Stimulation Parameters
Mode: Monophasic
Amplitude: 250 mA
Burst Frequency: 50 Hz
Carrier Frequency: 10 kHz
Pulse Width: 1 ms

Scale
y axis: 50 v/div
x axis: 10 ms/div



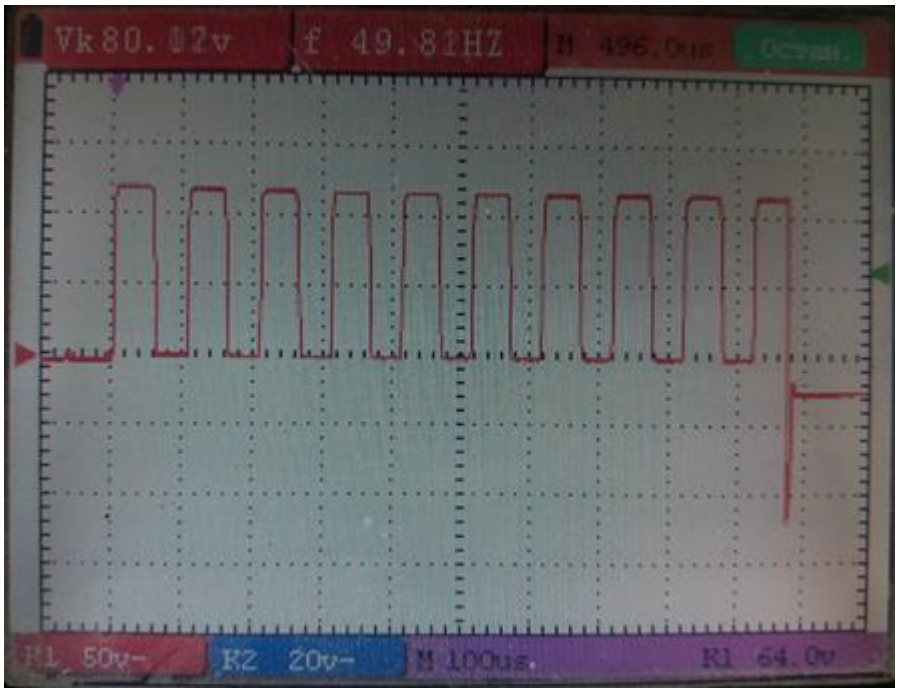
Stimulation Parameters
Mode: Monophasic
Amplitude: 250 mA
Burst Frequency: 50 Hz
Carrier Frequency: 10 kHz
Pulse Width: 1 ms

Scale
y axis: 50 v/div
x axis: 1 ms/div



Stimulation Parameters
Mode: Monophasic
Amplitude: 250 mA
Burst Frequency: 50 Hz
Carrier Frequency: 10 kHz
Pulse Width: 1 ms

Scale
y axis: 50 v/div
x axis: 0.1 ms/div



Sample calculations of derived values for the maximum and average current and power densities:

<u>Current Density</u>
Mean square is limited to 40 mA
Max pulse amplitude = 250 mA
Pulse duration = 1 ms
Electrode size = 7.9 cm^2

In this case pulse frequency = 40 mA * 40 mA / (250 mA * 250 mA * 1 ms) = 26 Hz
Average current density = 250 mA * 1 ms * 26 Hz / 7.9 cm^2 = 0.82 mA/cm^2.

<u>Power Density</u>
Mean square is limited to 40 mA

In this case, Average Power Density = 40 mA * 40 mA * 500 Ω / 7.9 cm^2 = 0.1 W/cm2