

Non-invasive spinal cord stimulation approaches and effects on motor pathways in humans

By

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Abstract

Spinal cord injury (SCI) is a central nervous system injury that can lead to motor, sensory and autonomic dysfunction. Electrical stimulation of the spinal cord has been increasingly recognized as a method to facilitate motor function after SCI. Two types of non-invasive stimulation have been used, including transcutaneous electrical pulsed stimulation (EPS) and direct current stimulation (DCS). Although DCS and EPS increased excitability of spinal motoneurons, these types of stimulation have never been directly compared in terms of effects when applied in the same person. Moreover, effects of EPS and DCS on spinal networks were assessed near the site of stimulation. Therefore, we sought to compare effects of EPS and DCS when applied in the same person; and to determine effects of lumbar stimulation on excitability of motoneurons innervating forearm muscles. Participants with SCI and an intact spinal cord were recruited. Spinal electrical stimulation was applied over the T11-L1 vertebrae for 15 minutes during sham EPS, EPS, sham DCS, and DCS. Input-output curves of H-reflexes and motor-evoked potentials (MEP) induced by transcranial magnetic stimulation in the flexor carpi radialis (FCR) muscle were recorded before and after spinal stimulation.

Inter-session analysis demonstrated a trend for decreasing H-reflex in the SCI group and for increasing MEP in both the SCI and intact groups following the DCS intervention. Our results demonstrated no significant changes in the excitability of spinal motoneurons or the corticospinal pathway after 15 minutes of EPS and DCS. Within-session analysis revealed a significant increase in the mean FCR H-reflex: after sham EPS in SCI group and after EPS and sham DCS in the intact group but these results were not confirmed by another standard H-reflex analysis. Additionally, in the SCI group after DCS, systolic blood pressure (BP) increased and heart rate (HR) decreased by 15.7% and by 8.6% pre-DCS values, respectively.

Overall, increased BP and HR and the lack of excitability changes after DCS and EPS should be interpreted with caution due low number of participants and variable injury levels in the SCI group.

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List of Abbreviations

AD- Autonomic dysreflexia

BB- Biceps brachii

BP- Blood pressure

CPG- Central pattern generator

DCS- Direct current stimulation

EES- Epidural electrical stimulation

EMG- Electromyography

EPS- Electrical pulsed stimulation

FCR- Flexor carpi radialis

HR- Heart rate

MEP- Motor-evoked potential

MSO- Maximum stimulator output

PSN- Propriospinal neuron

SCI- Spinal cord injury

SCS- Spinal cord stimulation

TMS- Transcranial magnetic stimulation

1. Introduction

1.1 Epidemiology of spinal cord injury

Spinal cord injury (SCI) is a central nervous system injury that can result in sensory loss, motor and autonomic dysfunction (1). Traumatic spinal cord injury can be due to motor vehicle accidents, falls, violent injuries, and sports injuries (2). Non-traumatic spinal cord injury can be caused by spondylosis, spinal cord compression by tumors, congenital diseases and inflammatory diseases such as MS (3). It was shown that each year 1,237 individuals are affected by traumatic spinal cord injuries in Canada (1). The incidence of non-traumatic spinal cord injury is 1.65 times more than traumatic SCI (1). SCI, according to the severity and damage below the level of injury, can be classified as incomplete or complete (4). Most motor complete injuries are related to traumatic SCI, while non-traumatic SCI usually causes partial loss of motor function (3).

1.2 Spinal cord injury stages and general treatment strategies

Spinal cord injury affects each individual's physical and psychosocial condition, thus creating a significant burden for themselves, their family, the community, and health care system. A key challenge in managing SCI is activating or reorganizing the surviving neuronal network and promoting axonal regeneration of the descending connections originating from above the spinal cord and neurons within the spinal cord (4). In this regard, it is essential to review the pathological changes that happen after spinal cord injury.

Spinal cord injury consists of two phases. In primary or acute phase, the direct mechanical trauma leads to injury and disruption of neural and tissue connection, laceration, and hemorrhage in the spine (2,5). The point at which an acute phase becomes chronic is not clearly defined, but generally, one to four weeks is often used to differentiate between these phases (6). The primary injury triggers a secondary chain of events, including ischemia, ionic dysregulation, neurotransmitter release, calcium accumulation and excitotoxicity. In the subacute stage, inflammation, neuronal demyelination, and scar formation begin. Later, in the chronic stage, axonal degeneration, glial scar maturation and cyst formation happen (5). The SCI lesion site will undergo changes and may contain multilocular cavities in addition to the regenerated axon bundles passing through the cavity (7). The clinical severity of the SCI can not be identified until three weeks or more after injury.

1.3 Treatment strategies following SCI

During initial rehabilitation, people with SCI are often managed and treated by the allied work of various medical disciplines such as neurosurgery, neurology, pain specialist, and rehabilitation professionals such as occupation and physical therapists (2). Currently, standard of care treatment aims to achieve a return to independent community living for each person, achieved with appropriate mobility-aids, medical supplies and any social or health supports needed.

Three major research approaches have been tested to try to improve neurological function in people with SCI. Firstly, pharmacological treatments are thought to increase neuronal regeneration and also offer neuroprotective effects. These medications include riluzole, minocycline, ketorolac, and magnesium, which showed promising neuroprotective effects in early experiments and currently ongoing clinical trials aim to confirm their efficacy after SCI (8). Secondly, cell transplantation interventions support stem cell plasticity and promote regeneration of neurons and supporting tissue such as glia and astrocytes (9). However, more clinical trials and basic research are required to achieve meaningful therapeutic potential. Thirdly, non-pharmacological treatments, which can be divided into early and late-stage interventions, have been proposed. In the acute (early) stage, treatments consist of decompression surgery, stabilization and blood pressure augmentation (8). In the chronic (late) stage, non-pharmacological treatments include rehabilitation, functional electrical muscle stimulation and neurostimulation (2,8). Neurostimulation, either by the sole use of electrical stimulation or by the combined use of electrical stimulation with chemical (drug) applications, is an emerging method to excite remaining neural networks below the level of injury in efforts to improve function (2).

In most people with SCI, spontaneous recovery is expected to some extent, which is much more substantial after incomplete lesions than after clinically complete lesions (10). Spontaneous recovery can be expected up to one year after injury. However, some studies reported recovery from clinically complete to incomplete even after five years of injury (7,11). This recovery could be attributed to plasticity and some regeneration which is not well understood (7). This regeneration could be partially attributed to the existence of cells and axons bypassing the lesion even after a year, supporting the idea of implementing restorative therapy to improve the remaining network as a general strategy for improving functional recovery.

1.4 Plasticity after SCI

Studies on complete spinal cord injury showed that sometimes the fibers around injured grey matter could survive after complete spinal cord injury. Further experiments showed these fibers can convey neural signals between cervical and lumbar enlargements (12). One of the first pieces of evidence of the existence of intact long-projecting axons after clinically complete injury stemmed from the observation made by Faganel and Dimitrijevic (12). They showed that noxious electrical stimulation of different spinal cord segments ipsilaterally and bilaterally had an effect on ankle jerk in people with complete spinal cord lesions. They attributed these excitations to the fibers that could survive after spinal cord injuries, such as the axons of propriospinal and primary sensory neurons (12). Later experiments showed that recovery after lesions were linked to this neural plasticity (10).

1.5 Central pattern generators

Previous experiments in several animal species showed a group of neurons in the spinal cord that control rhythmic motor activities resembling walking and swimming (13). Locomotion is controlled by networks of interneurons in the spinal cord, defined as a central pattern generator or CPG network that controls alternating flexor and extensor movements. CPG neurons can maintain locomotor-like patterns without supraspinal assistance and peripheral sensory feedback (14). Intraneuronal recordings showed that this rhythmic activity can be generated in the absence of sensory input or activated by a variety of sensory afferents.

Previous studies in humans showed an oscillatory pattern of activity thought to be generated by spinal neural networks integrated with sensory afferents in the lumbar area without supraspinal input (15). One of the strong evidence regarding the presence of CPG in humans was provided by Dimitrijevic et al., who showed that electromyography (EMG) activity resembling locomotion was elicited when tonic electrical stimulation was applied to the L2 segment in a person with a complete spinal cord injury (16). These muscle activities could be modulated by sensory input; these were the consequence of peripheral stimuli interacting with the activity generated within specific spinal segments by the electrical stimulation of the cord (15).

It has been suggested that lumbar locomotor networks undergo plasticity, reorganization and cellular-level alterations after SCI (13,17). In animal models, immediate early gene expression, which has been suggested to play a role in central nervous system plasticity, increased in the area below injury within hours to a few days after trauma. This alteration in gene expression in CPG

neurons after SCI is proposed to increase excitability (13). As an example, the gene expression for the glycine and GAD-67 receptor decreased in CPG, and the 5-HT_{1A/7} receptor increased (13).

Locomotor patterns of muscle activity can be generated by pharmacological and electrical interventions that stimulate CPG neurons (10). Therefore, these properties make the CPG a good target for applying a combination of rehabilitative strategies for locomotion improvement in persons with SCI.

1.6 Afferent input

Although rhythmic activity can be elicited in deafferented mammals, sensory input, especially muscle group I afferent input, has a great role in the CPG network. (14). In spinally transected mammals, sensory information interacts and alter excitability of CPG neurons and can induce stepping movements. Sensory input can alter the phase duration and transition without changing cycle rhythm of induced locomotion (14). It was shown that load receptors in both legs could play a significant role in regaining locomotion after SCI in humans (15). Proprioceptors innervating extensor muscles and conveying load information of the foot sole, tendons and stretching of muscle fibers can stimulate neurons in the spinal cord to induce locomotor-like activity (15). It was demonstrated that hip afferent input is needed to transition from the stance to the swing phase (15). The results of several studies, mainly in cats, showed that combinations of different sensory afferent input could be applied to induce locomotor activity by CPG neurons in an isolated spinal cord with no descending locomotor drive (10,15).

1.7 Propriospinal interneurons

Propriospinal neurons (PSN) are interneurons that originate and project axons entirely within the spinal cord. They can be very long-projecting neurons connecting the cervical enlargement to sacral segments or project just over one or a few segments (18). Moreover, they can link the ventral to the dorsal horn and have bilateral projections to the right and left sides of the cord (10). Several pools of PSNs are known to receive projections from bulbospinal pathways and a couple of descending commands with sensory afferent feedback throughout the spinal cord (18,19). PSNs likely play a crucial role in CPG control, including CPGs for respiration and autonomic processes (19). During locomotion, data from animal experiments suggested PSNs with ascending projections coupled the forelimb/hindlimb movements, while PSNs with descending axons were biased to control interlimb coordination and propagating sensory information for smooth locomotion (20). As an example, a human study provided evidence that there is neural coupling

between lower limb CPG and the cervical area, which could be mediated by propriospinal networks (21).

In addition to contributing to locomotion, the PSNs play a crucial role in the recovery of function after spinal cord injury (10,18–20). Some previous experiments showed that they had undergone plasticity and changes just within days after injury (10,19). Moreover, it may be easier for PSNs in the spinal cord to regenerate and bridge short segments of the injured spinal cord lesion compared to long axons from the brainstem (18,20). Therefore, PSNs may be a good target for movement recovery after spinal cord injury. A previous study showed that PSNs were capable of relaying a descending locomotor command signal when two different levels of cord hemisection were performed and locomotor activity was triggered by brainstem stimulation (18). Other studies showed that disruption of the corticospinal tract following spinal cord injury led to a new connection between corticospinal fibers and PSNs that bridged the lesion and synapses between PSNs and lumbar motor neurons doubled over eight weeks after hemi section (20).

1.8 Neuromodulation

The main challenge after SCI is activating remaining networks below or above the lesion and/or inducing appropriate fiber growth and synapses across the lesion. During SCI, decreasing the descending commands may reduce synapses and neurotransmitter efficacy in the residual neural networks (10). Changes in the state of residual neural network excitability can be achieved by neuromodulation. Neuromodulation is defined as a change in excitability state or function of neurons in a neural network, induced either chemically or electrically (4,10). Neuromodulation leads to the change in the concentration and release of neurotransmitters and neuromodulators such as serotonin, glycine, GABA and catecholamine in the spinal cord (10). Moreover, at the ionic level in the presynaptic axons, it can have effects on the Ca^{++} -activated K and Na^+ channels.

In addition, chemicals and electrical stimulation have effects on upregulation and downregulation of the receptors in the spinal neurons. As an example, using glycinergic inhibitor such as strychnine led to the upregulation of glycine receptors in the motoneurons (10). Electrical stimulation, on the other hand, increased mGluR receptors on motoneurons and postsynaptic NMDA receptor levels (10).

Such neuromodulation may be achieved by using implanted or non-implanted devices that deliver chemical or electrical stimulation (4).

1.9 Chemical and electrical neuromodulation

Several studies showed that exogenous pharmacological substances could induce locomotor activity in animals. Studies used noradrenergic/adrenergic, serotonergic and dopaminergic substances applied to the spinal cord (21).

This kind of neuromodulation is believed to alter or regulate communication within locomotor CPG networks (22). However, electrical stimulation seems more effective than chemical neuromodulation. This is attributed to three reasons. Firstly, electrical stimulation causes a release of a variety of neurotransmitters by activating or suppressing different types of neurons in the spinal cord. Secondly, electrical stimulation may increase neurotransmitter release at each activated synapse. Thirdly, in contrast to chemical neuromodulation, pulsed electrical stimulation induces a patterned release of neurotransmitters. This phasic electrical stimulation decreases receptor desensitization and fatigue and may induce larger synaptic potentiation than chemicals (10).

Therefore, given the above information, electrical stimulation may have advantages compared to chemical stimulation, as a means to provide a locomotor-inducing environment.

Electrical stimulation is one of the methods to promote communication in neural networks and restore function (23). In electrical stimulation, an electrical field is produced between the anode and cathode across the spinal tissue. Spinal electrical stimulation with electrodes was first performed in humans by Sherwood et al. in 1980, who used epidural electrical stimulation in 28 participants with upper motor neuron disease (24). The main mechanisms thought to explain improved motor activity during spinal stimulation include 1) changes in segmental reflex circuits; 2) facilitatory effects of sensory fiber input on the spinal state (25). Studies showed that electrical stimulation could increase motor activity and improve autonomic functions, such as cardiovascular and respiratory output (26). Electrical stimulation can be integrated with other methods, such as peripheral nerve stimulation, mechanical assistance by the practitioner, or other motorized devices (22).

1.10 Epidural electrical stimulation

In 1988, for the first time, Dimitrijevic et al. showed that epidural electrical stimulation (EES) at the lumbosacral area could produce step-like activity in participants with complete SCI (16). After that, many studies showed that stimulation of cervical and lumbar areas in cats and rats with a

spinal transection could induce quadrupedal and bipedal locomotion (23). In bipolar epidural stimulation, it is estimated that 78% of the current is distributed through the CSF and 11% is distributed in the spinal cord (27). In epidural stimulation, the afferent fibers can be excited better at the dorsal root entry zone to the spinal canal or their longitudinal path near the cathode (27) than at any other region. Harkema et al. showed that continuous epidural electrical stimulation of the lumbosacral segment could facilitate rhythmic contractions of the lower limbs and enable participants to walk and stand independently even several years post SCI (28). However, later studies showed that continuous spinal cord stimulation might evoke greater afferent fiber excitation than motoneuron excitation (29). Low amplitude but high frequency electrical stimulation delivered in bursts led to temporal summation of excitatory postsynaptic potentials and this summation could elicit Ia fiber excitation near the electrode better than other types of stimulation patterns. These low amplitude high frequency bursts decreased the motor pool threshold, leading to higher excitability and facilitation of motoneuron output during limb movements (29).

There are two types of electrodes used for epidural stimulation. One is cylindrical and can be applied with a minimally invasive procedure through a hollow needle. The other uses paddle-type electrodes, which need laminectomy for implantation. The latter type provides more stability in the long term (30). Both procedures may have some complications, such as hematoma and paralysis developing after insertion. The risk of complications is higher when the placement involves the cervical area than when within the lumbar region. Moreover, tissue damage caused by the pressure and movement of the stimulation electrode and neural irritation or subsequent damage caused by dislocated, broken electrodes, has been reported (30).

1.11 Non-invasive spinal cord stimulation

Although epidural stimulation delivers a more focused electrical current than transcutaneous stimulation, it requires an invasive procedure, as described above, with some potentially serious complications (31). Moreover, its' high costs impede widespread adoption. Transcutaneous transspinal electrical stimulation is a non-invasive and cost-effective substitute believed to be a safe method for spinal cord stimulation (32). This type of stimulation consists of electrodes placed over the skin instead of within the epidural space (33). Stimulation of the cord in this non-invasive manner influenced the same neural networks recruited by epidural stimulation (34). In a study by Hofstetter et al., posterior root muscle responses (PRMs) were assessed in subjects using

transcutaneous pulses and epidural stimulation. The shape, amplitude and other properties of reflex-evoked responses were similar. However, the electrical field created by transcutaneous stimulation is diffuse and non-focused because of the heterogenous properties of the tissue layers between the cathode and anode acting as a conductor (35).

When stimulus intensity is high, great amounts of current could be generated directly beneath the electrode, leading to contraction of paravertebral and abdominal muscles as well as exciting cutaneous fibers (35). Deeper in the body, currents penetrate subcutaneous layers and deep muscles, which have great conductivity compared to the vertebral bones. Computational stimulation models estimated that 8% of the total current reaches the cerebrospinal fluid and about 1% is distributed in the spinal cord. With this type of stimulation, the afferent and efferent fibers could be depolarized much more at the entry or exit points from the spinal canal than at other regions (27).

It has been demonstrated that spinal electrical stimulation can activate spinal levels far from the electrodes simultaneously, which could be attributed to the modulation of propriospinal interneurons (36). Furthermore, individuals who underwent repeated sessions of transspinal lumbar stimulation achieved improvements in postural control, suggesting that spinal stimulation modulates neurons across multiple segments. (32,37).

Non-invasive spinal cord stimulation is thought to modulate spinal and supraspinal networks including within the autonomic system (26,38). Changes in the autonomic nervous system after SCI can result in significant daily issues such as impaired cardiovascular and temperature regulation, limited or absent metabolic mobilization, and extreme reductions in ability to exercise (26). In an individual without spinal cord injury, the autonomic response is managed by the hypothalamus and the brain stem which exert an appropriate and coordinated drive on spinal interneurons. The sympathetic system activates pre and post-ganglionic neurons that induce proper responses in various target tissues, such as the heart, vasculature, bowel, adrenal gland, sweat glands, etc. The spinal neurons comprising the sympathetic nervous system are located predominantly between T1 to L2 segments. In high-level spinal cord injuries above T6, specifically those above T3, the autonomic centers may not elicit proper heart regulation responses (26). In the absence of appropriate descending control of the sympathetic nervous system, noxious stimuli, such as pain or a full bladder, can induce vasoconstriction and an increase in blood pressure. An

increase in BP induces the baroreceptors in the aortic arch and carotid sinus. Baroreceptors activate the parasympathetic system to decrease the heart rate as a normal response to high BP. In SCI people with lesions above T6, the ability of cardiovascular center to inhibit sympathetic system below the lesion is disrupted, which leads to paradox of high BP and low heart rate. This phenomenon is called autonomic dysreflexia (AD).

A literature review conducted by Flett et al. revealed that all participants with orthostatic hypotension (defined as an inability to adequately increase BP upon moving from supine to sitting or standing) experienced an improvement in their blood pressure (BP) following spinal stimulation by 20 – 40 mmHg (26). However, the BP reverted to low levels again after stimulation. People with SCI with lower-level injuries did not get any benefit from spinal stimulation in terms of their blood pressure response (26). It was proposed that spinal cord stimulation activates spinal preganglionic neurons and has shared neurons with the lumbar CPG (26). The stimulation at the thoracolumbar area could also regulate BP better by the ill-defined ascending spinal interneurons to upper-level sympathetic preganglionic neuron (26). Moreover, the thoracic and high-lumbar areas stimulation by activation of lower limb preganglionic sympathetic neurons can lead to the lower limbs' cutaneous vasculature vasoconstriction. This vasoconstriction can result in return of blood to the main cardiac system and can be considered as one of the reasons of increase BP by stimulation. The BP during stimulation could also be affected by other sensory stimuli, posture, or the amount of synaptic input and excitability of ascending, descending and interneurons of the spinal cord with connections to the sympathetic system (26).

Generally, non-invasive spinal cord stimulation protocols vary from lab to lab without standardized recommendations for the pulse intensity, duration frequency and or the location of the stimulation electrodes (26,36,39). From a broad perspective of methodology, two types of non-invasive electrical stimulation techniques can be differentiated, as recently reviewed by Nardone et al. (38). One type of electrical stimulation is when the current is delivered in short bouts with pulses at durations on the milliseconds to seconds. Scale and frequencies vary greatly from lab to lab. This method is referred to as transcutaneous electrical pulsed stimulation (EPS) and is different from the other method that is referred to as transcutaneous direct current stimulation (DCS) when the current is constant and delivered for several minutes to even an hour, albeit kept at very low intensity. Interestingly, these methods have been around for about the same time, but to our

knowledge, no studies compared effects evoked by these methods in the same group of people, neither with intact nor injured spinal cords. This represents a significant gap in knowledge about non-invasive spinal cord stimulation.

1.12 Transcutaneous electrical pulsed spinal cord stimulation (EPS)

In EPS, stimulation with varied pulse widths with different intensities and frequencies are delivered through the skin. This technique, like epidural stimulation, can evoke a posterior root reflex. EPS can be delivered using a single pulse or train of pulses and can be applied with or without a so called “carrier wave”. The carrier wave refers to another set of pulses with a frequency varying from lab to lab in the range of 2.5 kHz to 10 kHz, delivered to decrease discomfort and pain sensations resulting from the high-intensity pulses intended to influence motoneurons (40). However, it has been found that the current amplitude required for transcutaneous epidural spinal stimulation (EPS) in combination with a carrier wave needs to be higher than that of pulses without a carrier wave in order to induce a motor response (40). In other words, the carrier wave has some theoretical capacity to decrease discomfort, but in actuality, due to the higher intensity required to evoke motor responses, the carrier is not very effective in reducing pain sensations (32,40).

The single pulse pattern of stimulation usually employs rectangular pulses up to 90 Hz frequency, pulse width ranging from 0.4 to 2 msec and intensities up to 170 mA (32,36). EPS applied as a biphasic or monophasic rectangular pulse at 30–50 Hz frequency has demonstrated therapeutic potential for enhancing voluntary motor activity, trunk stability, gait function, and reduction in spasticity (41). However, other studies showed that a single session of short-duration pulses (100-400 µsec) had no effect on spinal (posterior root reflexes) or corticospinal evoked reflexes (motor-evoked potentials) (42,43).

Most of the studies used a cathode placed at T11-L1 spinous process to stimulate the lumbar area and at C6-C7 or C7-T1 to excite cervical motor networks. However, the ideal stimulation site varies depending on the muscle group or rehabilitation strategy targeted (32,36). The anode may be placed over the iliac crest or the abdominal area near the umbilicus for lumbar stimulation or on the clavicle and anterior of the neck in case of cervical stimulation (32).

1.13 Transcutaneous Direct current stimulation (DCS)

Direct current stimulation was first used for brain neuromodulation. Constant current at a low stimulation intensity (1-2.5 mA), below the sensation threshold, was applied for 15-25 minutes.

The first study on the application of DCS to the spinal cord was done by Cogiமானian et al., who evaluated somatosensory-evoked potentials after anodal transspinal DCS in healthy subjects (44). DCS consists of placing two sponge electrodes to create a current path; one over the spinal cord and one over the umbilicus for lumbar stimulation or over the shoulder blade for cervical stimulation (38). It has the ability to depolarize or hyperpolarize the neurons based on the polarity and placement of electrodes (38). Based on the reference electrode, it can affect different parts of the spinal cord. As an example, while the cathode is positioned at T10, positioning the reference electrode on the right shoulder, umbilicus and head have been shown to affect the thoracic cord, t cauda equina and the cervical spinal cord, respectively (45). It was shown that effects induced by DCS could last from minutes to hours (38).

1.14 Mechanisms behind the effects of electrical spinal cord stimulation

Many studies showed that spinal cord stimulation could excite the dorsal root ganglion cells, the dorsal root fibers, the anterior ascending fibers, or dorsal column axons, as well as interneurons in the spinal grey matter (10,27,32,35,46–49). A modeling study by Holsheimer reported it is unlikely that epidural stimulation directly excites the lateral corticospinal tract as the threshold for stimulating the pyramidal tract could be 6.9-fold higher than the threshold for exciting dorsal column fibers. Moreover, this study stated that it is unlikely that the ventral spinothalamic tract cells are recruited by dorsal stimulation (49).

Dorsal root activation during epidural stimulation was recorded as the antidromic volley in the peripheral nerves (50), with large diameter myelinated proprioceptive fibers having the lowest threshold. This low depolarization threshold in the cathodic electric field depends on two things: the sudden shift from high to low tissue conductivity in the surrounding nerve (27); and the axon's spatial orientation according to the electrical field's polarity (27,51). A computational model showed that a convex curvature of fibers leads to a lower threshold for cathodic stimulation. On the other hand, for anodic stimulation, a concave orientation of neural fibers decreases threshold. Therefore, the hot spot site for depolarization in the cathodic electrical field is where the posterior rootlet orientation changes from longitudinal to transverse orientation and enters the spinal cord (27).

Low intensities of electrical stimulation excite low threshold afferent fibers, and by increasing intensity, other fibers with higher threshold are subsequently recruited (47). Large diameter Ia

afferents that convey information from primary muscle spindles are activated first, along with group Ib afferents, which originate from Golgi tendons. Both types of stimulation (epidural and transcutaneous) can cause paresthesia and tingling sensations, which are attributed to the recruitment of medium-diameter fibers (32,35,47). Paresthesia induced by transcutaneous stimulation is also due to activation A β fibers of group II afferents in skin (49). North et al. showed that sensory fibers in the ligamentum flavum (i.e., ligaments connecting two adjacent vertebral bodies) might be excited by transcutaneous spinal stimulation. These fibers might be responsible for the non-radiating discomfort sensation when the stimulation intensity reaches above the paresthesia threshold (52).

Hofstoetter et al. showed that paired electrical stimulation, using either epidural or transcutaneous stimulation, could evoke a post-activation depression response in leg muscles. This suggested the reflex nature of transspinal evoked potentials and they proposed that the homonymous connection of group Ia fibers to motor neurons evoked these reflexes (35,53). Other studies demonstrated that transcutaneous stimulation of several posterior roots could also lead to heteronymous reflexes of Ia fibers and contribute to these reflex responses (32). In an animal model used for studying epidural stimulation, Moraud et al. showed that responses from muscle spindle fibers and reciprocal inhibitory pathways could excite the locomotor CPG. The stimulation had synergistic effects on muscle spindle fibers which evoked motor neuron firing or modulated motor activity in the absence of cortical control (48). In addition, polysynaptic connections between cutaneous mechanoreceptors of the skin can play a role in the excitability of motor pools as mechanoreceptors under the electrode could excite polysynaptic pathways at different spinal levels (32).

The order of depolarization from spinal stimulation with increasing intensity is as follows: posterior root, anterior root fiber, and finally, posterior column neurons (54). The posterior column consists of ascending axons of neurons which also provide perpendicular collaterals that travel to the grey matter. Parameters that play a role in the recruitment of dorsal column fibers include the diameter of the collateral and fiber type (i.e., myelinated or not) and the distance between electrode and axon (46). Computational modeling studies showed that superficial fibers had the lowest threshold, while fibers at the anterolateral location of the posterior columns had the highest threshold. The longitudinal fiber excitation threshold is generally three times higher than the intensity required for excitation of posterior afferent fibers. Branching reduces the longitudinal

fiber threshold such that increasing the number of branches decreases threshold. Most of the longitudinal fibers can be depolarized below the stimulating electrode. Small increases in transcutaneous stimulation intensity above the posterior column fiber threshold (estimated as 45.4 V in one study) can induce activity in the posterior columns at multiple segments (54).

The polysynaptic nature of afferent fiber input could lead to modulation in remote or distant segments of the spinal cord through activation of propriospinal interneurons (32). In the complete absence of supraspinal input, electrical stimulation could induce rhythmic well-coordinated, multi-limb locomotion, which was attributed to activation of PSNs (55). An animal model study found that epidural stimulation could not recruit dorsoventrally transversing and commissural fibers in the grey matter, where most PSNs are located (25). These researchers believe that electrical stimulation indirectly, via sensory input, activates PSNs in the spinal cord (25,48). However, other computational modeling demonstrated that intraspinal networks, including spinal interneurons that ascend or descend, may be recruited at high intensities.

They proposed that spinal stimulation at high intensity could increase interneuron excitability and lead to muscle contractions without directly activating any action potentials (47). Electrical stimulation can decrease the depolarization threshold of efferent fibers, making them more excitable and able to respond with minimal input from supraspinal and afferent fibers (32,47). Stimulation at higher intensity, especially epidural, could excite motor axons even at resting potential. Regardless of frequency, this high-intensity stimulation can disrupt natural walking in an animal model, bypassing spinal circuits and interfering with agonist and antagonist alternation during movement (48).

DCS could affect motoneurons and spinal cord neurons by different mechanisms. It has been proposed that cathodal DCS can improve cortical network excitability, increase plasticity in neural networks and promote skilled motor movement in SCI animal models. It may affect spinal networks by altering glutaminergic neurotransmitter release (45). The effectiveness of network excitability using continuous subthreshold electric currents like DCS may result from temporal summation of weak stimuli but may also result from subthreshold electric current synchronizing neural circuits (56). Even a small subthreshold cell membrane depolarization can influence cell properties and modulate neurotransmitter levels and release (10).

Electrical stimulation of the spinal cord is likely to affect supporting cells such as the abundant astrocytes and glial cells in the spinal cord. Supporting cells are connected tightly to each other to form a network in the cord. Despite the fact that glial cells are typically believed to be non-excitabile or incapable of generating action potentials, their excitability is associated with variations in their intracellular calcium levels. Therefore, calcium waves travel rapidly throughout the astrocytic network. The increase in calcium levels in astrocytes leads to modulation of neural networks by three mechanisms, including reuptake of neurotransmitters, balancing extracellular ionic concentrations and release of glial cell neurotransmitters. For example, the release of adenosine by astrocytes regulates motor patterns (10). These all could be affected by spinal electrical stimulation and currently, little knowledge exists about how these actions might affect spinal neural activity.

1.15 The H-reflex and motor-evoked potentials as classical methods for studying the neural control of movement

The H-reflex or Hoffmann reflex, refers to a monosynaptic reflex evoked by stimulating Ia afferents that synapse on alpha motor neurons. In 1940, the H-reflex technique was introduced as a way to measure motor neuron excitability in the spinal cord of humans (57). During movement, this neuronal circuit plays a crucial role in regulating muscle tone and posture. Hyperexcitability of these reflexes leads to abnormal increases in muscle tone and spasticity, which is an adverse event in many neurological diseases, including spinal cord injury (57). H-reflex excitability modulation correlates with restored motor skills after spinal cord injury and often an increase in motor excitability (58,59). Its modulation in the spinal cord plays a crucial role in mediating smooth movements of muscles and limbs. Therefore, its measurement is an important tool when developing therapeutic procedures for clinical rehabilitation of people with neurological illnesses, such as spinal cord injury or stroke (57). The H-reflex is used extensively as a physiological tool when comparing the effects of various interventions (60–67) and predominantly for examining the excitability of spinal motor neuron pools (68).

When testing muscle responses, usually an input-output curve is generated using several stimulation intensities. The H-reflex has an “ascending and descending” limb, which represents as the range of amplitude responses. By stimulating below motor fiber threshold, Ia fibers with a lower threshold are activated. These fibers orthodromically convey the pulse to the spinal cord. Monosynaptic connections then excite alpha motoneurons. By increasing the current, more Ia

fibers are recruited, and a higher amplitude H-reflex is evoked (ascending phase of H-reflex recruitment curve). By increasing current above the motor fiber threshold, the M wave becomes the initial response visible by surface EMG, which results from activation of motor fibers by peripheral nerve stimulation in the efferent direction. The motor response is elicited earlier than the time it takes for the monosynaptic response to travel from the afferents to the spinal cord and to the electromyographic (EMG) recording site. By increasing the current to activate approximately 40-50 % Mmax, the H-reflex amplitude reaches its highest amplitude (69). After this point, increasing the intensity leads to a decrease in the H-reflex size. This decrease is attributed to the collision of reflex discharges with antidromic responses of current in the motor axons (69). Therefore using the H-reflex as a measure of excitability in its descending limb would be inaccurate due to this collision and comparing the facilitation and inhibition in the H-reflex after a test condition is inaccurate (69).

In this method, the peripheral mixed nerve is stimulated, and the peak-to-peak amplitude of the waveform is measured. The EMG amplitude of the reflex depends on many factors, such as the number of afferent fibers evoked by stimulation, pre and postsynaptic inhibition that can modulate the afferent response and motoneuron excitability (70). Moreover, subject-dependent factors such as height, age and nerve injury can affect the H-reflex amplitude. In addition, technical factors can influence the H-reflex amplitude, such as: quality of the recording electrode contact, distance to EMG recording site, location of recording electrode on the designated muscle, quantity of subcutaneous tissue beneath the stimulation electrode, and stimulation electrode location (71). Therefore, when comparing changes in H-reflex excitability in different subjects, the absolute size of the responses was not used. Rather, the H-reflex is presented as a percentage of the maximal motor response or Mmax, which represents the maximal amplitude possible due to the electrode's positioning and subject-based factors (69). H-reflex/Mmax ratio is recommended for comparing H-reflex amplitudes in different settings and across different participants (72,73).

Another way of examining motoneuron activity is by motor-evoked potentials or MEPs. MEP amplitude is also monitored by EMG responses much like the H-reflex, but results from stimulation of the cortex. Corticospinal tract stimulation can be elicited by electrical or magnetic stimulation over the scalp. Transcranial magnetic stimulation or TMS was introduced in 1985 (74) and has been widely accepted as the least invasive method for corticospinal motor pathway

excitation in humans. During TMS, a short-lasting magnetic field over the cortex generates an electrical field that excites neurons within the field. TMS is commonly used in studies because of its safety and simplicity in accessing the cortex and corticospinal pathways of various muscles (74). The MEP amplitude and onset latency measurements are considered the gold standard and most sensitive neurophysiological means for evaluating changes in excitability of corticospinal pathways (41,61,75–77). Changes in MEP amplitudes at a given stimulation intensity is an indicator of excitability changes in corticospinal tract cells or in modulation of excitability of interneuron activity between cortical neurons and spinal motor neurons (76).

It has been speculated that electrical stimulation may affect spinal network function and improve interaction with cortical input (36). Transspinal stimulation activates Ia fibers that send afferent information to the somatosensory cortex. Then, the transcortical connection between the somatosensory and motor cortex can facilitate the motor cortex and corticospinal pathways (76). In addition, sensory input, especially in cutaneous pathways, can display long latency responses following electrical pulse stimulation (25). It has been proposed that this long latency response could be because of spinal stimulation-based effects on polysynaptic pathways including of neurons in the spinal cord (35) or transcortical responses evoked by cutaneous input (41). Moreover, it is plausible that sensory input may excite propriospinal neurons, which in turn may facilitate motor responses and increase excitability of locomotor-related neurons in subcortical or supraspinal areas (20,78).

2. Hypothesis

Thus, the current project aimed to compare these two stimulation methods in the same participants.

We hypothesized that thoracic non-invasive spinal cord stimulation at T11 vertebral level, either by DCS or EPS changes both spinal excitability and corticospinal excitability in people with intact or injured spinal cord.

To test this hypothesis, firstly, the H-reflex recruitment curve (H_{MAX}/M_{MAX} curve) of flexor carpi radialis (FCR) was evoked to measure spinal motor neuron excitability changes before and immediately after (less than 5 minutes) EPS or DCS for 15 minutes. Secondly, we elicited motor-evoked potentials (MEPs) by transcortical magnetic stimulation (TMS) to measure corticospinal excitability changes in the FCR muscle and biceps brachii (BB) before and immediately after (less than 15 minutes) stimulation.

In order to evaluate the potential effects of EPS and DCS on the autonomic nervous system, we also recorded and compared changes in heart rate and blood pressure before, during and after spinal stimulation interventions. Moreover, we recorded surface EMGs from multiple muscles as it is unknown if stimulation at lower thoracic levels can influence arm and trunk motor neurons as well as leg motor neurons.

3. Methods

The experiments were performed on neurologically healthy intact and spinal cord injured volunteers. All participants gave informed written consent before the study in accordance with the protocol, which conformed to standards of the Declaration of Helsinki and was approved by the local Institutional Review Board at the University of Manitoba (Protocol HS23666). Lumbar metal implants, electronic implants, allergy to the electrode material, epilepsy, and pregnancy were exclusion criteria for recruitment.

3.1 Experimental procedures and general set-up of lab visits

Each participant contributed by participating in four sessions, consisting of 1) sham EPS, 2) EPS, 3) sham DCS, and 4) DCS sessions. All participants underwent the same order of sessions as listed 1-4, i.e., sessions were not randomized. Each session was conducted on a separate day, 6- 7 days apart from another one. During each session, experiments consisted of a) baseline measurements, b) spinal stimulation intervention and c) post-spinal stimulation measurements. Baseline and post-intervention measurements included cubital fossa stimulation to evoke FCR H-reflexes and TMS for evoking FCR motor-evoked potentials (Fig. 1). Each spinal cord stimulation intervention lasted for 15 minutes. All experiments were conducted while the participant was seated comfortably, and hands were placed on a table with adjustable height with or without a cushion being used under the hands.

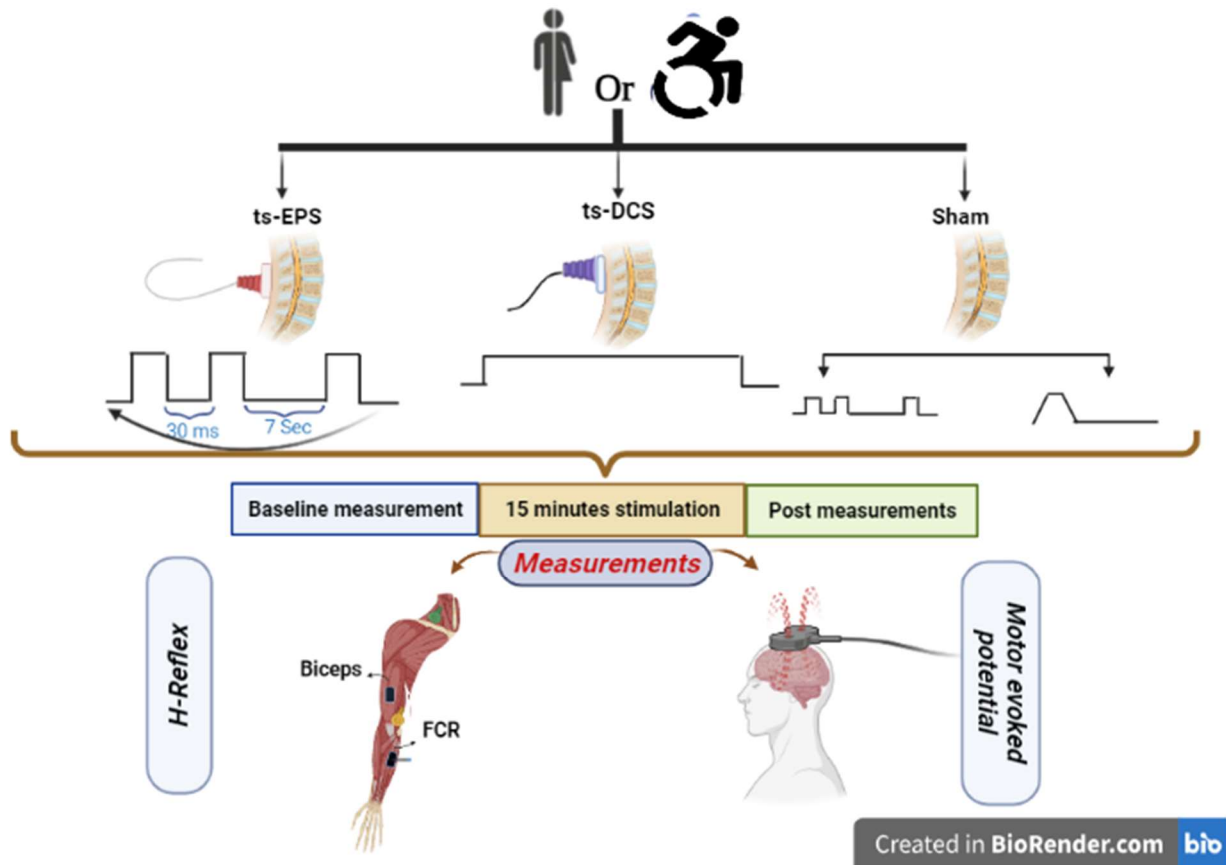


Figure 1. Study design and assessment.

The study chart includes types of stimulation and measurements for assessing spinal and corticospinal excitability. After recruiting participants in the study, they underwent four sessions of stimulation. Before and after each stimulation, measurements were taken, including H-reflexes by peripheral nerve stimulation and motor-evoked potential by transcranial magnetic stimulation of the flexor carpi radialis muscle.

3.2 Transcutaneous electrical pulsed stimulation (EPS) of the spinal cord

Transcutaneous electrical pulsed spinal stimulation was timed by Pulse Master A300 (World precision instrument) and generated by the DS8R Bipolar constant current stimulator (Digitimer, Medtel, NSW, Australia). Rectangular (5x10 cm) self-adhesive neurostimulator electrodes were used (ValueTrobe, Axel GAARD, Denmark). Cathode electrode was placed at the T11- L1 spinous process, which was defined by counting the spinous process from L4 at the same level as the iliac crest. Two anodes (connected by a bifurcating cable to function as one unit) were placed horizontally at the iliac crest on both sides. At first, spinal stimulation (rectangular pulses with 1 ms width) was delivered every 3-5 s with intensities ranging from 10 mA to 130 mA or to the

highest intensity each participant could tolerate comfortably. Each stimulation intensity was repeated three times, and this was used to determine the intensity of stimulation to be applied during the 15 min intervention. During the 15 min EPS, single and paired pulses alternated (Fig. 1). These paradigms were the same for sham EPS but the intensity used for stimulation was different during sham and actual EPS sessions. Intensity selection was based on the sensory threshold in each participant in the sham EPS session. This was done by increasing intensity starting from 2 mA until intensity that the participant could sense the stimulation. Average intensity of sham stimulation was 5.9 ± 1.9 mA, while actual stimulation intensity of EPS, on average, was 51.08 ± 20.3 mA.

3.3 Transcutaneous direct current stimulation (DCS)

DCS stimulation was delivered by a low-intensity constant current stimulator (1300A Model, Sotrix Medical) through a rubber pad electrode covered with a saline-soaked (0.9% normal saline) sponge (3x4.75 cm). A cathode was placed at the T11 to L1 spinous process while an anode was placed on the abdomen over the umbilicus. The cathode and anode were secured by Tegaderm transparent film. The stimulation intensity was ramped up to reach 2 mA in 30 seconds and remained constant for 15 minutes. After 15 min, intensity ramped down to 0. Current density delivered through the actual DCS session was 0.14 mA/cm² for 15 min and a total charge of 2.1 C/cm². Sham stimulation consisted of ramping up current until 2mA was reached and then immediately current rapidly ramped down within a minute at the start and the end of 15 min based on a protocol standardized within the stimulator, but no current was effectively delivered for the rest of the sham trial (79).

3.4 EMG recordings

Surface electromyographic (EMG) signals were recorded from biceps brachii (BB), triceps brachii (TB), flexor carpi radialis (FCR), erector spinae (ES) at L4 level, vastus lateralis (VL) and tibialis anterior (TA), bilaterally. The skin over the muscle belly was first prepared by prep tape and 70% alcohol to clean the skin. This decreased background noise and improved signal-to-noise ratio on the EMG electrode recordings. Each electrode (DELSYS, Bagnoli EMG system, US) was covered by a sensor adhesive interface and then placed over the muscle belly (according to international standardized recommendations). EMG signals were amplified (x100), digitalized at 2 KHz and recorded by the Nexus software (Vicon/Nexus, Motek, AD converter, The Netherlands) with the software gain set at level 10.

3.5 Nerve stimulation for H-reflex measurements

During reflex testing, each person's forearm rested on a soft cushion or table without any contractions (i.e., subjects were asked to relax their arm) and the elbow remained at about 90 degrees flexion. A 5x5 cm square-shaped self-adhesive electrode (ValueTrove, Axel GAARD, Denmark) was placed on the olecranon process as a ground. The FCR muscle belly was identified by flexion and radial deviation of each participant's wrist (or if necessary, by using passive movement of the wrist). The flexor carpi radialis (FCR) H-reflex was evoked by median nerve stimulation at cubital fossa (in the right arm for intact participants and in the functionally stronger arm of people with SCI). Two rounded surface self-adhesive electrodes (Neuroline 720, Ambue) were used as cathode and anode stimulating electrodes. Brachial artery pulse was palpated at the cubital fossa, and a cathode electrode was placed medial to the artery and an anode was placed at about 2 cm proximal to the cathode. To secure contacts of all stimulating and recording electrodes for the entire session; a thin bandage was wrapped around each elbow and arm. During stimulation, in order to decrease forearm and hand movement, which might cause movement artifacts in EMG recording, a light-weight (5 lbs), sandbag was gently placed on the hand of each participant. Peripheral nerve stimulation was done by using a DS8R stimulator triggered by D flow software (Motek Medical, Netherland) via a trigger box (Motek Medical, Netherland). Rectangular pulses with 300-350 ms width at 0.2 Hz starting at 4 mA with 2mA incremental steps (and each intensity repeated three times) delivered until the motor response (M wave) reached maximum size.

3.6 Evoking motor-evoked potentials by transcranial magnetic brain stimulation

A transcranial magnetic stimulator (Magstim200, Whitland, United States) was used to stimulate the contralateral motor cortex to evoke motor-evoked potentials (MEPs) in the FCR. To find the optimal stimulation site, the distance between the nasion and the occiput and the distance between left and right tragus was measured to establish vertex (Cz). Then, 3.5-4.5 cm lateral to the vertex was identified as FCR motor cortex (80) and marked on a cap that was placed over each participant's head. Stimulation was applied by placing a magnetic coil (figure 8 Alpha flat coil, Magstim, Whitland, United States) on the marked spot. MEPs were monitored by an oscilloscope (TDS 3014, Tektronix) and the site at which FCR MEP with amplitude higher than 50 uV could be evoked at 65-75% maximum stimulator output (MSO) was identified as the stimulation hot spot. Intensity was started from 40% MSO and increased by 5% steps with 3 repetitions of stimulation at each intensity until maximal MEP or 100% MSO was reached. During this

procedure, some participants were asked to flex their wrists gently against a lightweight (5 lbs) sandbag to help induce a measurable response. To decrease the flexion variability between participants, they were asked to contract to the amount that the contraction waves could not be seen in the oscilloscope with 20 μ V sensitivity.

3.7 Blood pressure and heart rate monitoring

Blood pressure (BP) and heart rate (HR) were measured before each trial and during spinal stimulation at 5 min intervals in each session. An automatic cuff-inflate machine (Omron, model BP72CANN) was used on an arm which was not stimulated during FCR reflex testing (always the left arm in intact participants).

3.8 Data analysis

Blood pressure (BP) and heart rate (HR) measurement during spinal stimulation were generally normalized to BP and HR before each spinal stimulation intervention (at baseline) for each participant using Microsoft Excel on a personal computer (Microsoft Windows 10). This was done because each participant's baseline BP and HR varied so widely. EMG recordings were analyzed using Spike 2 software (Cambridge Electronic Design, Cambridge, UK). Stimulation pulses delivered by the electrical and magnetic stimulators were identified using tag pulses to mark the exact time of each stimulus. The time window between stimulation onset and M, H and MEP responses on the muscle of interest were measured, and quantitative data of EMG features were transferred into Excel for further analysis as described below.

3.9 Methodological considerations for H-reflex comparisons

In order to interpret results correctly based on normalized H-reflexes for the pre and post-spinal stimulation intervention in each group, the consistency of maximum M response needed to be verified. Absolute amplitude of maximum motor response (Mmax) reflects the stability of the stimulation electrode and recruitment of the same (or close to same) pool of motoneurons before and after each intervention. The Mmax amplitude was compared for all sessions, as shown in Fig. 2. Although there were some variations in the size of the Mmax, no significant differences were found between pre and post-intervention for each participant (one-way, rm-ANOVA).

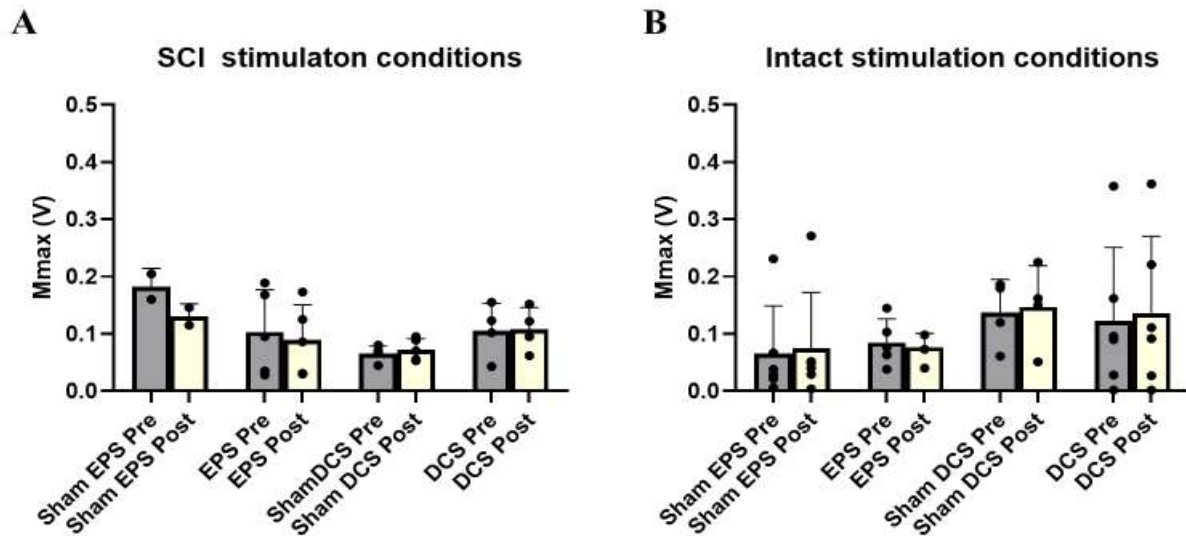


Figure 2. Consistency of FCR M-responses of pre and post-spinal stimulation intervention during each experimental session.

A, B. Absolute amplitude of the maximum motor response (Mmax) before and after each type of intervention in pre (grey) and post (yellow) intervention in SCI and intact groups. The interventions were: electrical pulsed stimulation (EPS) or sham EPS, direct current stimulation (DCS) or sham DCS. Individual data points represent different participants; bars show the mean and error bars as standard deviation.

These H-reflex measurements were normalized to Mmax as demonstrated from data obtained in one participant in Fig. 3. Initially, the amplitude of M and H-responses were measured as peak-to-peak amplitudes of raw EMGs. Average absolute amplitude of 2-3 trials delivered at the same intensity for M and H-reflexes was plotted with respect to stimulus intensity (in Excel). Plots are shown for before (Fig. 3A) and after (Fig. 3B) spinal cord stimulation. Then the H-reflex amplitude in pre and post-intervention were normalized and expressed as a percentage of the respective Mmax value, and the pre and post-intervention results were overlaid (Fig. 3C).

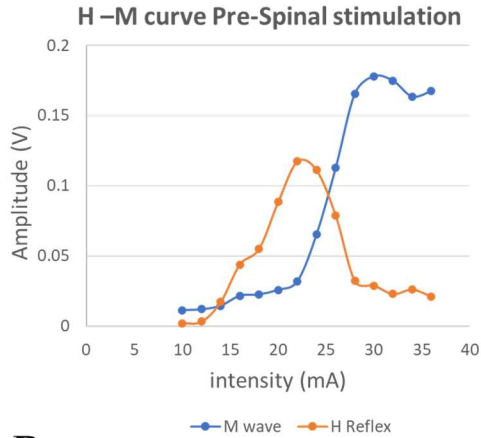
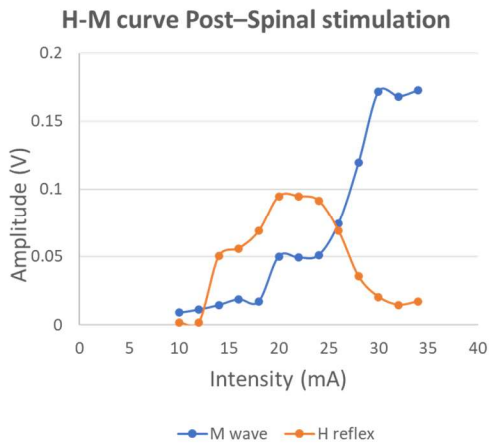
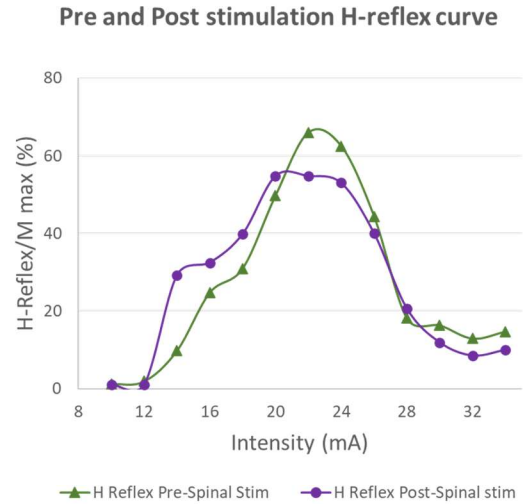
A**B****C**

Figure 3. Single participant example of FCR H-reflex input and output curve generation.

A. H-M curve of pre-spinal stimulation; B. H-M curve of the same participant post-spinal stimulation; C. Pre and post-spinal stimulation H-reflex amplitude normalized to Mmax overlaid for each tested intensity.

3.10 Sigmoid transition

An average absolute amplitude was determined from 2-3 trials delivered at the same intensity for the M and H-reflexes and then plotted. Plots were generated for trials before and after the intervention. The H-reflex amplitude evoked at each stimulation intensity was normalized to the maximal M wave (% of Mmax). Sigmoid transition was used as described in previous studies (63).

The H-reflex before and after spinal stimulation intervention was recalculated by $H(s) =$

$\frac{H_{Max}}{1+e^{m(S_{50}-s)}}$ formula; in which H(s) is H-reflex amplitude at a given stimulation intensity, m is the

slope parameter of the H-reflex curve, Hmax is the upper limit of the curve, S₅₀ is the stimulus intensity used to evoke 50% of Hmax. If the post-stimulation sigmoid curve shifts to the left or right (i.e., when comparing curves generated before and after spinal stimulation intervention), it reflects an increase or decrease in excitability of FCR motoneurons, respectively (Fig. 4). The H-reflex sigmoid curve was generated to take into account each stimulation intensity used. After calculating the sigmoid function for each intensity, the intensity was normalized to S₅₀-Hmax to generate an adjusted X axis of the sigmoid recruitment curve and to combine data from all participants.

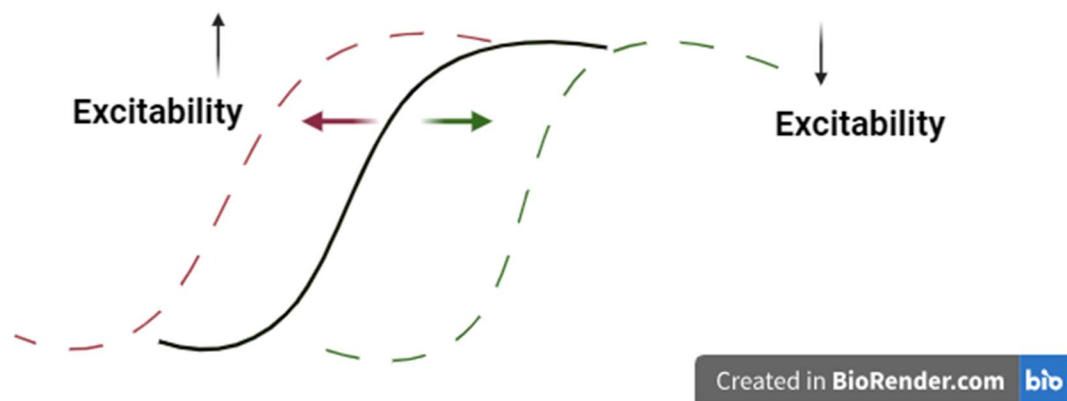


Figure 4. Sigmoid transition.

Change in excitability is shown by the transition in post-measurement sigmoid with respect to pre-intervention measurements.

3.11 “30% of Mmax” method

In addition to the sigmoid transfer, another type of H-reflex analysis was performed. The intensity at which the M wave amplitude was approximately 30% Mmax (in the ascending portion of the recruitment curve) was identified. Then this intensity was chosen to determine the FCR H-reflex before and after spinal stimulation intervention for the sake of comparison. The H-reflex, which is identified at the level of 30% Mmax, is similarly susceptible to facilitation or inhibition after intervention (69). Therefore, it represents an ideal indicator of potential changes.

3.12 “50% MEP max” method

The MEP recruitment curve follows a Boltzmann sigmoid function equation. The highest rate of change in MEP amplitude is detected at an intensity that is half the intensity required to evoke

MEP max (S50). Change in MEPs after each intervention was measured by subtracting the MEP amplitude obtained after intervention from that obtained before intervention in each session for each participant. The difference was expressed as a percent of MEP obtained before spinal stimulation intervention (baseline conditions).

3.13 Statistical analysis

Each type of comparison, such as intragroup (within group) and intergroup (between groups), was done by analysis of variances (ANOVA) using SPSS software (SPSS, version 28). A two-way repeated ANOVA was used to determine significant differences before and after each spinal stimulation intervention for H-reflex measurements. Regarding the H-reflex sigmoid function, only two stimulation intensities were consistently analyzed, since data were collected from all participants at these intensities (0.9 and 1 x S50-Hmax intensity).

A one-way ANOVA analysis was performed to determine statistically significant differences between H-reflex and MEP amplitudes under different stimulation conditions (DCS, EPS and sham for each) and different groups (SCI and intact group). For each ANOVA, normality (Shapiro-Wilk test) and Levene's test of homogeneity of variances were conducted. In case of outliers and violation of normality, nonparametric tests (Friedman for repetitive measurements and Kruskal-Wallis H test for non-repetitive measurements) were used with post hoc tests for multiple comparisons between groups. All data are presented as mean \pm standard deviation unless otherwise stated and p values <0.05 were used to define a significant difference.

Blood pressure and heart rate had some missing data points during spinal stimulation (n=10/96 time points in SCI and n=20/96 in intact participants), so a linear mixed model for repeated intragroup measurement (i.e., analysis between each time point at each session of spinal stimulation) was used for statistical comparisons.

4. Results

4.1 Participant information

Experiments were conducted on 6 SCI participants (3 women and 3 men) and 6 intact spinal (5 women and 1 man). The mean age for the SCI group was 55.84 ± 11.56 years, and 45.5 ± 16.5 ($p=0.06$) for intact participants. Age and other characteristics are shown in Table 1.

Table1.Characteristics of the participants in the SCI and intact groups.

Spinal cord injury group			Intact spinal group	
Sex	Age	Level of Injury	Sex	Age
F	33	C5-C6	F	46
M	62	C6-C7	F	29
M	58	T4, Syrinx at C6	F	34
F	62	T12	F	65
F	56	C7-C8	F	33
M	64	C5-C6	M	66
	55.8 ± 11.6			45.5 ± 16.5

F: Female, M: Male

4.2 H-reflexes in FCR after sham and spinal stimulation interventions

Data for H-reflexes was not collected from all participants, as shown by Table 2. The H-reflex could not be evoked in some participants because of technical issues such as alterations of electrodes placement or contact pre versus post-spinal intervention.

Table2. The number of analyzed data sets for FCR H-reflexes in each condition in each group

Group	Sham EPS	EPS	Sham DCS	DCS
Spinal cord injury	3	5	5	4
Intact	6	6	4	5

After normalizing H-reflexes in FCR muscles and combining data from all participants using a sigmoid transfer process, results showed that FCR H-reflex amplitude, on average, was consistently in the range of 20-70% of Mmax in all groups across all sessions (Fig. 5, 6). The mean amplitude of FCR H-reflexes had a nearly complete overlap in spinal intact participants (Fig. 6) compared to those with SCI (Fig. 5) when pre and post-interventions were compared by overlaying

the data. However, the differences observed between pre and post-intervention were significant in SCI sham EPS (at 1.1 x S50-Hmax) and intact EPS (at 1 x S50-Hmax).

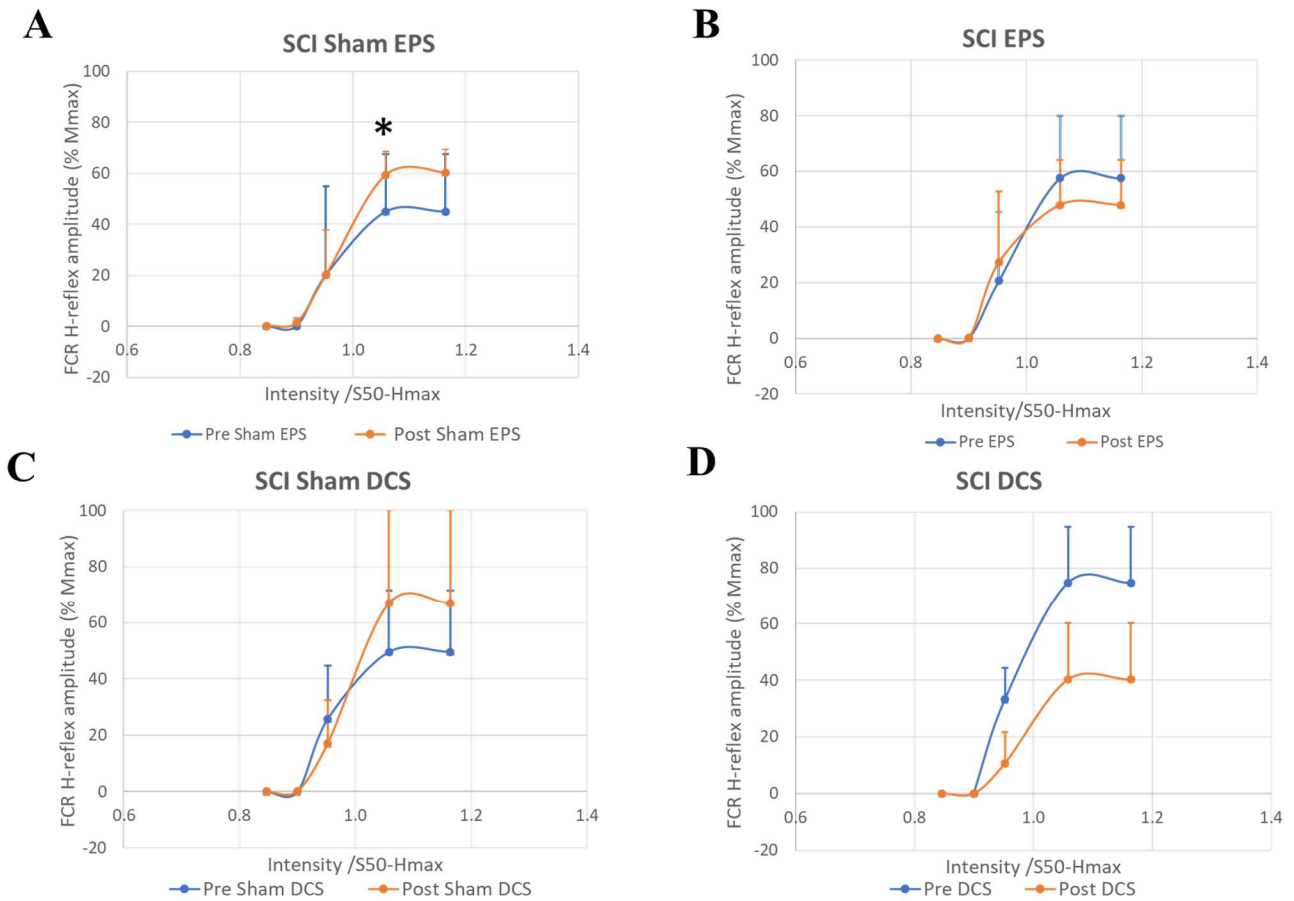


Figure 5. Flexor carpi radialis (FCR) H-reflex sigmoid curves show facilitation in sham stimulations and suppression in EPS and DCS.

FCR H-reflex size as % of Mmax after stimulation at different intensities after sigmoid transfers of results collected in the group with spinal cord injury (SCI). After A. Sham electrical pulsed stimulation (Sham EPS); B. Electrical pulse stimulation (EPS). C. Sham direct current stimulation (Sham DCS); D. Direct current stimulation (DCS). Asterisk shows p value <0.05.

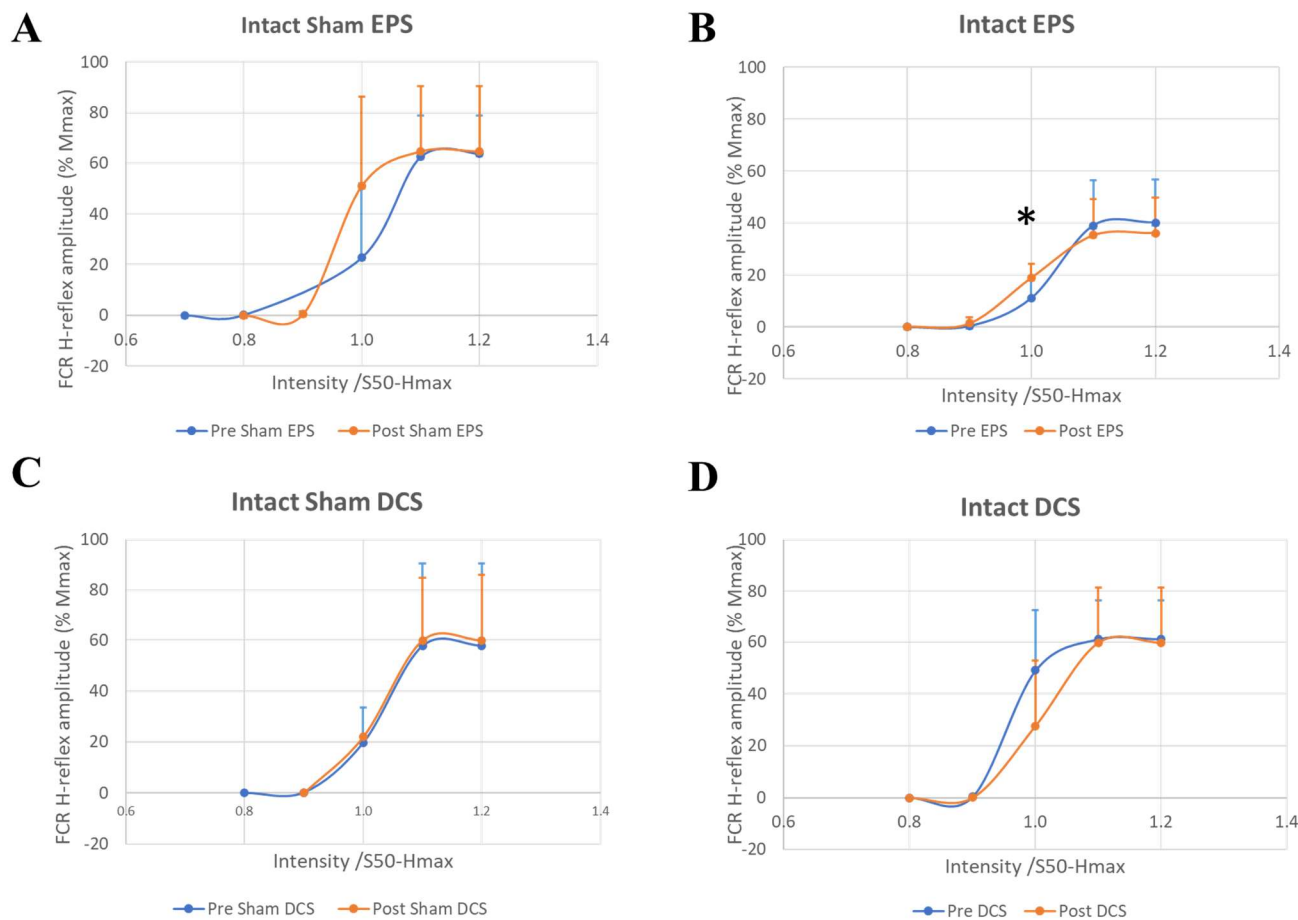


Figure 6. Flexor carpi radialis sigmoid curve in the intact group shows less variability compared to SCI participants.

FCR H-reflex size as % of Mmax after stimulation at different intensities after sigmoid transfers of results collected in the group with intact spinal cord (intact). A. Data after sham electrical pulsed stimulation (Sham EPS); B. after electrical pulse stimulation (EPS). C. after Sham direct current stimulation (Sham DCS); D. after direct current stimulation (DCS). Asterisk shows the p value <0.05.

In order to illustrate the responses for each individual, data obtained from the sigmoid transfer process were plotted to show pre and post-spinal stimulation data at stimulus intensities of 1 and 1.1 x S50max. These results are illustrated in Fig. 7 for participants with SCI. There was a significant increase in the size of the FCR H-reflex from $44.9 \pm 20.4\%$ Mmax to $59.3 \pm 8.2\%$ Mmax after sham EPS at a stimulus intensity of 1.1 x S50- Hmax (one-way rm-ANOVA, p value=0.037, star in Fig. 7A). No significant differences were observed for other stimulation intensities (1 or 1.1 x S50-Hmax) during either EPS, DCS or sham DCS in the SCI group (Fig. 7). However, as you

can see in Fig. 7A, individual responses were highly variable. Note that in participants with SCI, sham DCS increased FCR H-reflex amplitude in 3/5 participants, whereas DCS decreased H-reflex amplitude in $\frac{3}{4}$ participants at 1.1 x S50-Hmax.

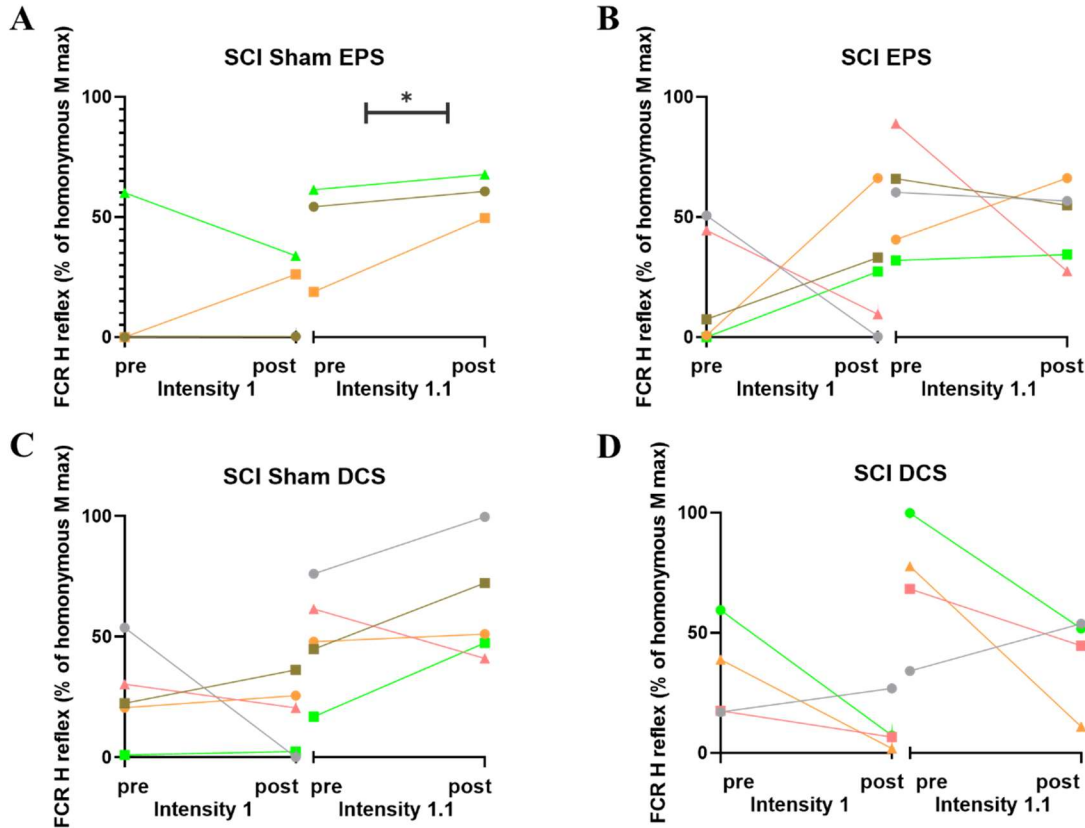


Figure 7. FCR H-reflex amplitude after sigmoid transfer was re-plotted for each participant for pre and post comparison illustrating high variability of individual responses.

FCR H-reflex amplitude expressed as % of Mmax from data obtained before (symbols on the left) and after (symbols on the right) spinal stimulation intervention. Each symbol represents results from a different participant at 1 and 1.1 x S50-Hmax. Results from the SCI group after sham EPS stimulation in A and EPS in B; sham DCS in C, and DCS in D. Each line represents data from the same participant pre and post-stimulation intervention as indicated. Asterisk shows p value <0.05.

The analysis of H-reflex amplitudes at the 30% Mmax point, which is the most sensitive to changes, showed a similar result in the SCI group. FCR H-reflexes at this point showed no significant changes after any of the interventions, as illustrated in Fig. 8 (one-way rm-ANOVA).

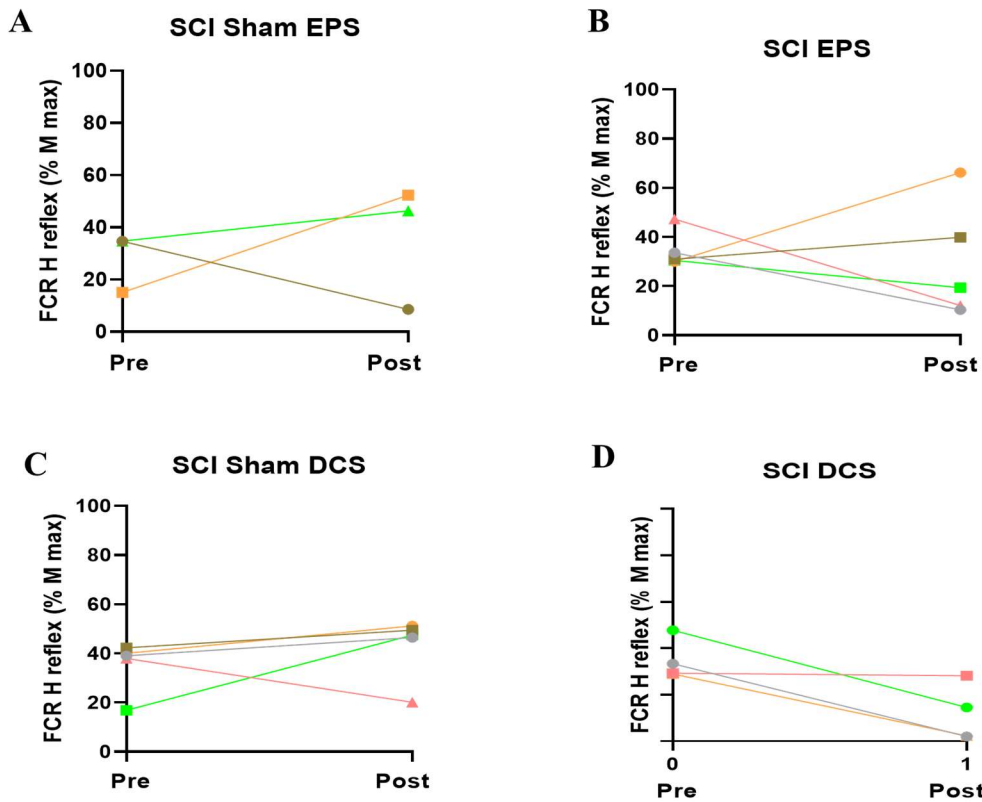


Figure 8. FCR H-reflex changes based on analysis at 30% Mmax point in the SCI group show high variability of individual responses.

FCR H-reflex amplitude expressed as % of Mmax from data obtained before (symbols on the left) and after (symbols on the right) spinal stimulation intervention. Each symbol represents results from a different participant in SCI group. Data after sham EPS stimulation in A and EPS in B; and sham DCS in C, and DCS in D. Each line represents data from the same participant pre and post-stimulation.

Results from the intact group after the sigmoid transfer process showed that the FCR H-reflex did not differ significantly pre and post-spinal stimulation for sham EPS, sham DCS and DCS (Fig. 9A, C and D). The FCR H-reflex size after EPS at 1 x S50-Hmax was $19 \pm 5.2\%$ Mmax, which was 8 % higher than H-reflex before EPS ($11.2 \pm 7.5\%$ Mmax) as shown in Fig. 9B (one-way rm-ANOVA, $F_{1,5}=9.62$ and p value=0.027) indicating statistically significant difference (asterisk Fig. 9B). The interaction between the spinal stimulation intervention and the stimulus intensity used for evoking the H-reflex was significant only in the EPS condition ($F_{2,10}=7.2$ and p value =0.011).

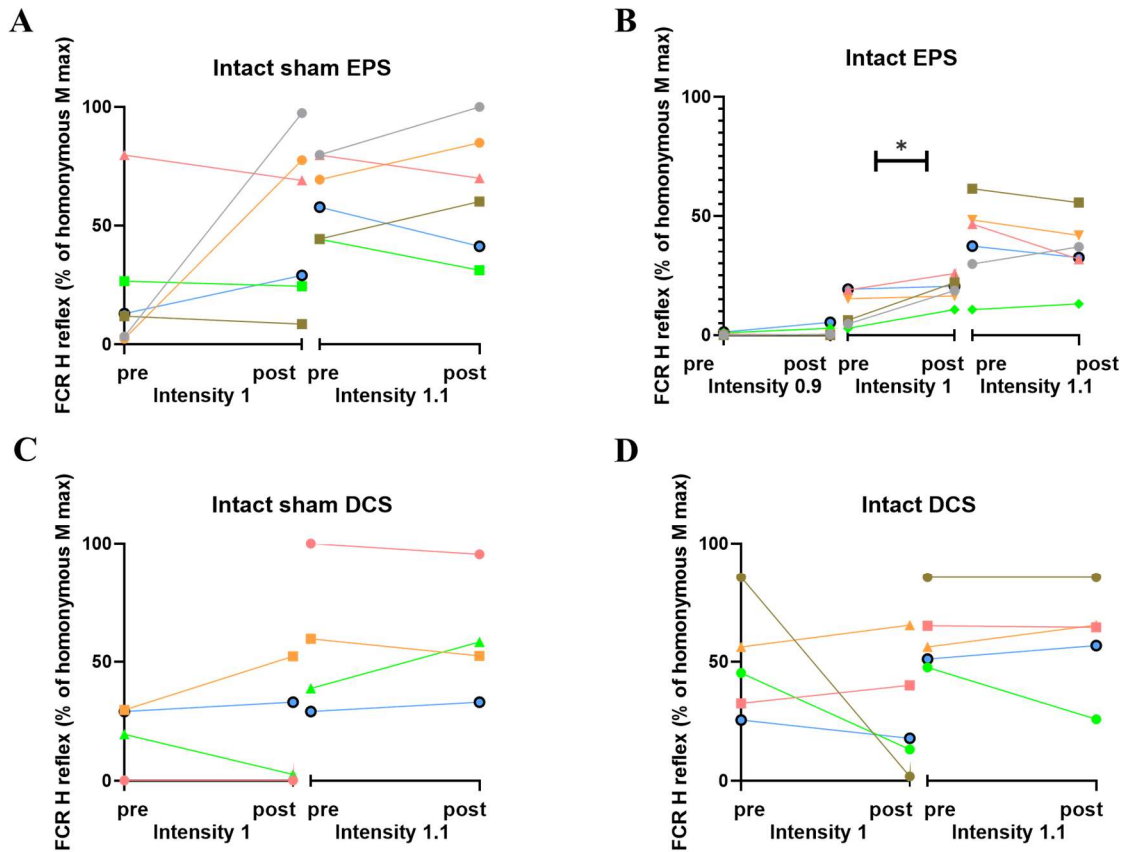


Figure 9. FCR H-reflex amplitude after sigmoid transfer re-plotted for each participant for pre and post comparison show high variability responses with an increased H-reflex after EPS.

FCR H-reflex amplitude expressed as % of Mmax from data obtained before (symbols on the left) and after (symbols on the right) spinal stimulation intervention. Each symbol represents results from a different participant at 0.9, 1 and 1.1 x S50-Hmax. Results from the intact group after sham EPS stimulation in A and EPS in B; sham DCS in C, and DCS in D. Each line represents data from the same participant pre and post-stimulation. Asterisk shows p value <0.05.

Analysis of FCR H-reflexes in the intact group at the most sensitive part of the ascending limb showed a significant increase in post-stimulation H-reflex amplitude after sham DCS. As illustrated in Fig. 10. The H-reflex post sham DCS was $55.65 \pm 18.83\%$ Mmax in comparison to $29.95 \pm 9.43\%$ Mmax before sham DCS that showed a significant difference (one-way rm-ANOVA, $F_{(1,3)}=22.24$ and p value= 0.018).

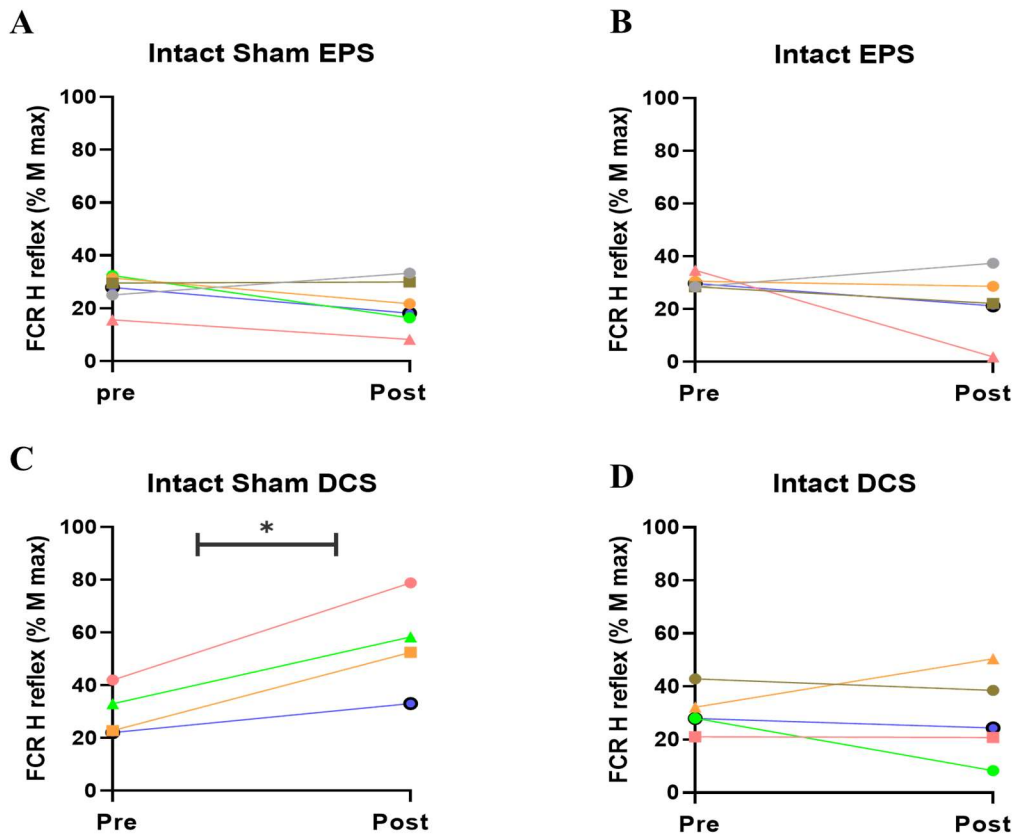


Figure 10. FCR H-reflex responses based on analysis at 30% Mmax point in the intact group show minimal changes after stimulation, except for an increase after sham DCS.

FCR H-reflex amplitude expressed as % of Mmax from data obtained before (symbols on the left) and after (symbols on the right) spinal stimulation intervention. Each symbol represents results from a different participant in the intact group. Data after sham EPS stimulation in A and EPS in B; and sham DCS in C, and DCS in D. Each line represents data from the same participant pre and post-stimulation interventions as indicated (EPS, DCS or sham). Asterisk shows p value <0.05.

The changes in FCR H-reflex expressed as the mean % Mmax at 1.1 x S50-Hmax in SCI group were extracted after the sigmoid transfer process for each intervention and graphed in Fig. 11A. FCR H-reflex in SCI group increased by $61.5 \pm 87.5\%$ Mmax after sham EPS, decreased by $4.3 \pm 47.4\%$ after EPS, increased by $55.2 \pm 80.6\%$ after sham DCS and decreased by $29.8 \pm 62\%$ after DCS as a percentage of change of pre-spinal stimulation H-reflex amplitude. Interestingly, after EPS and DCS, there was a tendency for mean FCR H-reflex to decrease in comparison to

respective sham sessions, but DCS reduced the FCR H-reflex more than EPS. These changes, however, were not statistically significant (Friedman test, p value=0.17).

In the intact group, mean change in the FCR H-reflex at $1.1 \times S50-H_{max}$ was $2.1 \pm 29\%$ after sham EPS, $-3.4 \pm 22.1\%$ after EPS, $11 \pm 28.3\%$ after sham DCS and $-4.8 \pm 28.1\%$ after DCS (as a percentage of change of pre-spinal stimulation H-reflex amplitude) (Fig. 11B). Both EPS and DCS showed a tendency to decrease FCR H-reflex compared to respective sham stimulations. The suppression following the DCS was very similar to suppression following EPS (only 1.4% larger). The data showed no statistically significant differences between any of spinal stimulation conditions (one-way rm-ANOVA, $F_{3,16} = 0.31$ and p value=0.81).

One interesting observation was that in some participants of the SCI group, there could be a large facilitation (nearly doubling) or a large suppression (nearly halving), while in intact group, changes of FCR H-reflex were smaller and more uniform than changes in the SCI group.

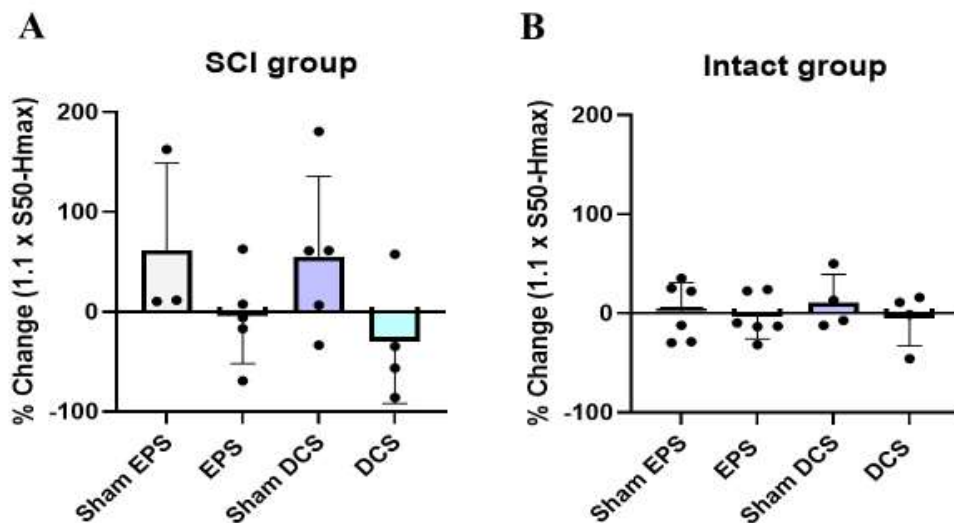


Figure 11. Summary of mean changes in FCR H-reflex in intact and SCI groups after sigmoid transfer show higher variability in the SCI group, with reduced responses after EPS and DCS and an increasing trend in sham conditions.

Mean changes in FCR H-reflex amplitude (as a percentage of M_{max} pre-intervention at $1.1 \times S50-H_{max}$) after different spinal cord stimulation interventions. A. Data from the SCI group ($n=3$ in

sham EPS, n=5 in EPS and sham DCS and n=4 in DCS). B. Data from the intact group (n=6 in sham EPs and EPS, n= 4 in sham DCs and DCS). Height of the bar represents the mean, and error bar represents standard deviation. For individual values, see Appendix Figure 1.

In the SCI group, changes in FCR H-reflex expressed as mean % Mmax at 30% Mmax before spinal stimulation were also extracted for each participant and graphed in Fig. 12A. The mean changes in the SCI group were $45 \pm 126.5\%$ after sham EPS, $-7.6 \pm 79\%$ after EPS, $39.5 \pm 84.4\%$ after sham DCS and $-64.8 \pm 42.2\%$ after DCS (Fig. 12A). The FCR H-reflex after EPS and DCS had a tendency to decrease which was greater after DCS stimulation than EPS. However, these changes were not statistically significant (Friedman test, p value=0.12).

In the intact group, changes in FCR reflex amplitude were $-21.38 \pm 32.4\%$ after sham EPS, $-24 \pm 45.9\%$ after EPS, $86.1 \pm 33.4\%$ after sham DCS and $-7.5 \pm 5.2\%$ after DCS (Fig. 12B). Change in the FCR H-reflex after sham EPS ($21.38 \pm 32.4\%$ decrease) was significantly different when compared to change after sham DCS that was an increase by $86.1 \pm 33.4\%$ (one-way rm-ANOVA, pairwise comparisons with a Bonferroni correction, p value=0.003). The FCR H-reflex was not statistically significantly different between EPS, DCS, and related sham conditions.

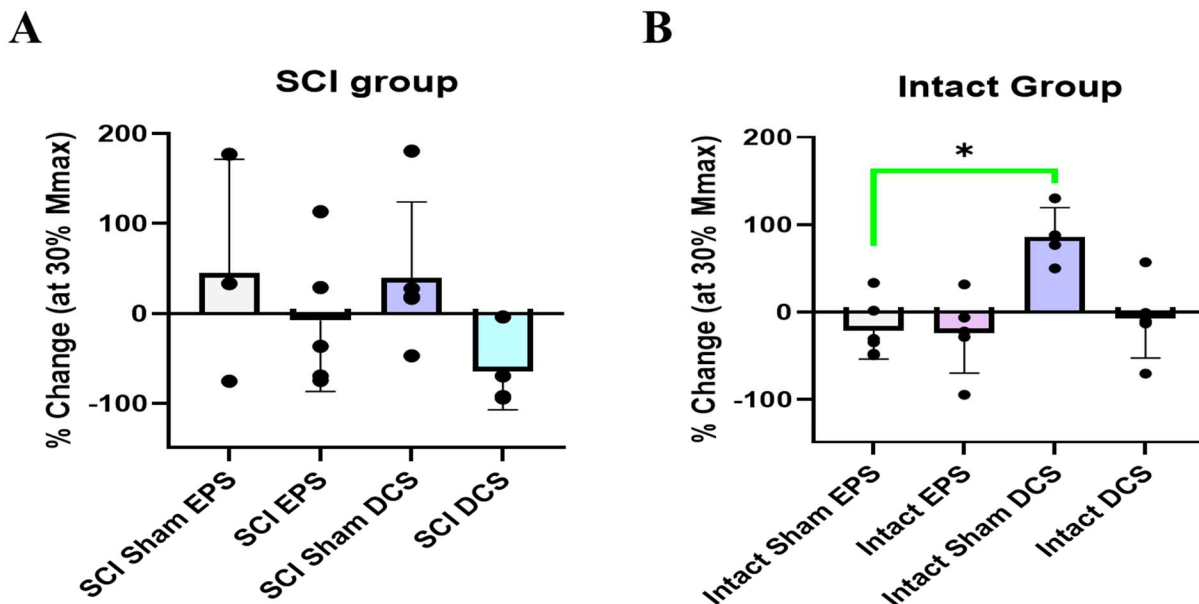


Figure 12. Summary of mean changes in FCR H-reflex at 30% Mmax in intact and SCI groups show a decreasing trend after EPS and DCS stimulation.

Changes in FCR H-reflex amplitude (as a percentage of Mmax pre-intervention at an intensity evoking 30% Hmax response) after different spinal cord stimulation interventions. A. Data from the SCI group (n=3 in sham EPS, n=5 in EPS and sham DCS and n=4 in DCS). B. Data from the intact group (n=6 in sham EPs and EPS, n= 4 in sham DCs and DCS). Height of the bar represents the mean, and the error bar represents standard deviation. For individual distribution see Appendix Figure 2. Asterisk shows p value <0.05.

Percentage of changes in the FCR H-reflex at 1.1 x S50-Hmax in sigmoid recruitment curves and the 30% FCR H-reflex at ascending portion of the curve in SCI and intact groups were compared to each other by one-way ANOVA and Kruskal-Wallis test.

To allow comparisons between SCI and intact groups, changes in the FCR H-reflex were replotted and compared at 1.1 x S50-Hmax. In both SCI and intact groups, FCR H-reflex tended to be suppressed after EPS and DCS (see Fig. 13). Although the FCR H-reflex was suppressed after DCS in both groups, the suppression was 25% larger in the SCI group. Facilitation of H-reflexes were observed after sham EPS and sham DCS in both groups, but the facilitation was larger in those with SCI. No significant differences were found between intact and SCI groups after any of the interventions (by one-way ANOVA and Kruskal-Wallis test, for EPS and DCS, p value=0.62, and for sham EPS and sham DCS, p value=0.59).

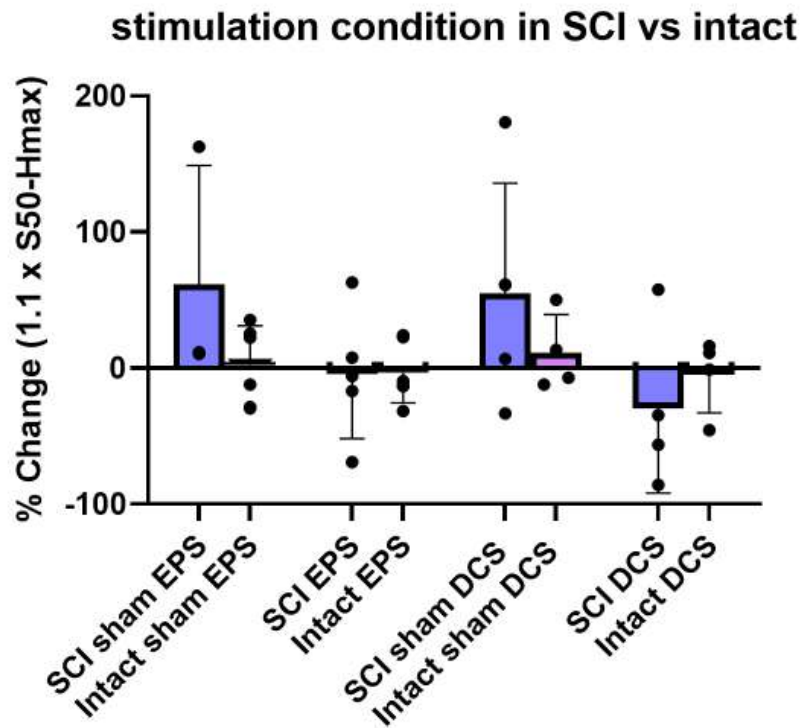


Figure 13. No significant differences were seen in mean FCR H-reflex amplitude after sham or spinal stimulation in either SCI or intact groups (at 1.1× S50-Hmax).

FCR H-reflexes at 30% Mmax were reduced in both SCI and intact groups after EPS and DCS (see Fig.14). Reflexes after EPS were 16.4% more reduced in the intact group compared to those with SCI group. In contrast, after DCS, reflexes were 57.2% more reduced in the SCI group compared to intact participants. However, these differences were not significant (Kruskal- Wallis test for EPS and DCS, p value =0.45, and for sham EPS and sham DCS, p value=0.08).

Stimulation condition SCI vs Intact

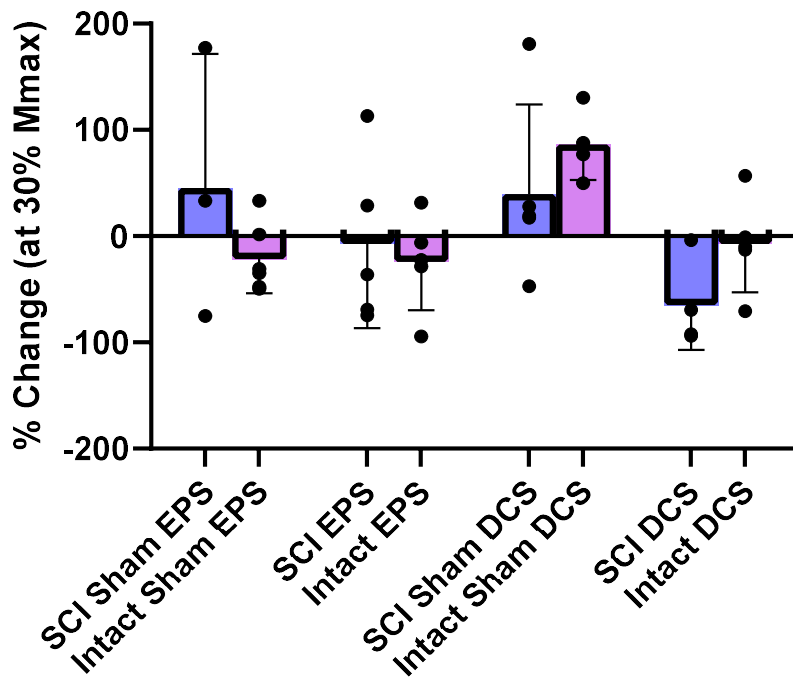


Figure14. No significant differences were seen in mean FCR H-reflex change after any stimulation condition when the SCI group was compared to the intact group (30% Mmax).

4.3 Motor-evoked potentials in BB or FCR after sham and spinal stimulation interventions

We were unable to record MEPs in all participants in each condition, due to discomfort or technical issues; numbers of participants with MEP data are shown in Table 3.

Table3. Number of participants with MEP data in the SCI and intact groups

Group	Sham EPS		EPS		Sham DCS		DCS	
	BB	FCR	BB	FCR	BB	FCR	BB	FCR
SCI (N)	4	3	2	4	2	3	3	4
Intact (N)	5	4	5	6	4	4	5	4

FCR MEP

Changes expressed as a percentage of pre-stimulation FCR MEP amplitude were measured at stimulation intensities that induced a response measuring approximately 50% of MEP max. This

point on the MEP input-output curve is thought to be the most sensitive for demonstrating either suppression or facilitation due to spinal stimulation interventions.

Overall, in the SCI group, FCR MEPs tended to increase after DCS; decrease after sham EPS; and remain unchanged after EPS and sham DCS. The mean changes in the SCI group were $-15.9 \pm 33.5\%$ after sham EPS, $-3.4 \pm 43.6\%$ after EPS, $-2.2 \pm 31.2\%$ after sham DCS and $15.1 \pm 37.15\%$ after DCS (see Fig. 15A) but none of these were statistically significant.

Similarly, in the intact group, there was a trend for MEP to increase after DCS; decrease after sham EPS; and remain unchanged after EPS and sham DCS. The mean change in FCR MEP amplitude after each intervention was $-18.6 \pm 40.7\%$ after sham EPS, $3.3 \pm 78.4\%$ after EPS, $8 \pm 21.5\%$ after sham DCS and $13.2 \pm 34.3\%$ after DCS (Fig. 15B). No statistically significant differences were found after any of the interventions (one-way ANOVA).

Statistical comparison of changes between SCI and intact groups showed no significant differences between modulation of FCR MEPs after any tested interventions (one-way ANOVA).

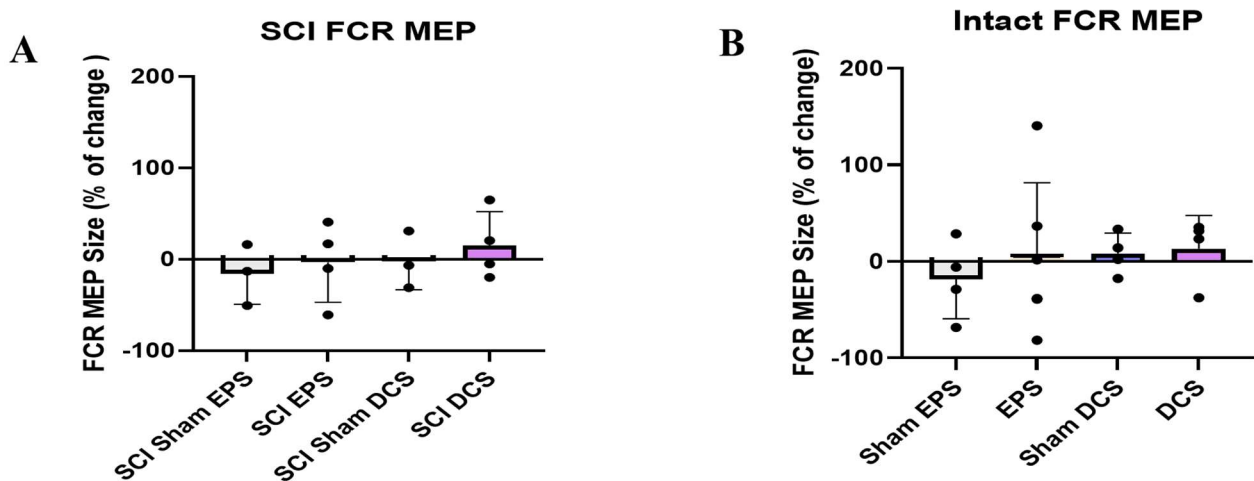


Figure 15. Summary of mean changes in FCR MEP amplitude in the SCI and intact groups show a trend to increase after DCS.

Changes in FCR MEP amplitude after each spinal cord stimulation condition at 50% FCR MEP max. A. Data from the SCI group (n=3 in sham EPS and sham DCS, n=4 in EPS and DCS). B. Data from the intact group (n=4 in sham EPS, sham DCS and DCS, n=6 in EPS). Height of the bar represents the mean, and the error bar represents standard deviation. For individual values, see Appendix Figure 3.

BB MEP

The change, expressed as a percentage of pre-stimulation BB MEP amplitude, was measured at an intensity of stimulation that induced a response measuring approximately 50% of MEP max. In the SCI group, BB MEP showed a tendency to be suppressed after EPS and DCS but facilitated after sham DCS (Fig. 16A). The mean change in the SCI group was $-1.3 \pm 96.9\%$ after sham EPS, $-36.1 \pm 10.5\%$ after EPS, $37.1 \pm 17.3\%$ after sham DCS and $-13.9 \pm 35.7\%$ after DCS. However, these changes were not statistically significant in the SCI group (one-way ANOVA).

In intact group, BB MEP amplitude tended to be facilitated after sham DCS, and suppressed after sham EPS. Both EPS and DCS evoked no change in MEPs (Fig. 16B). Mean change of BB MEP was $-36 \pm 45.5\%$ after sham EPS, $-10.7 \pm 51.2\%$ after EPS, $50.1 \pm 89.1\%$ after sham DCS and $6.2 \pm 50.6\%$ after DCS interventions. Changes in BB MEP amplitude were not statistically significant in the intact group.

Statistical comparison of changes in BB MEP amplitude between the SCI and the intact groups showed no significant differences after sham or real stimulation (the Kruskal-Wallis test).

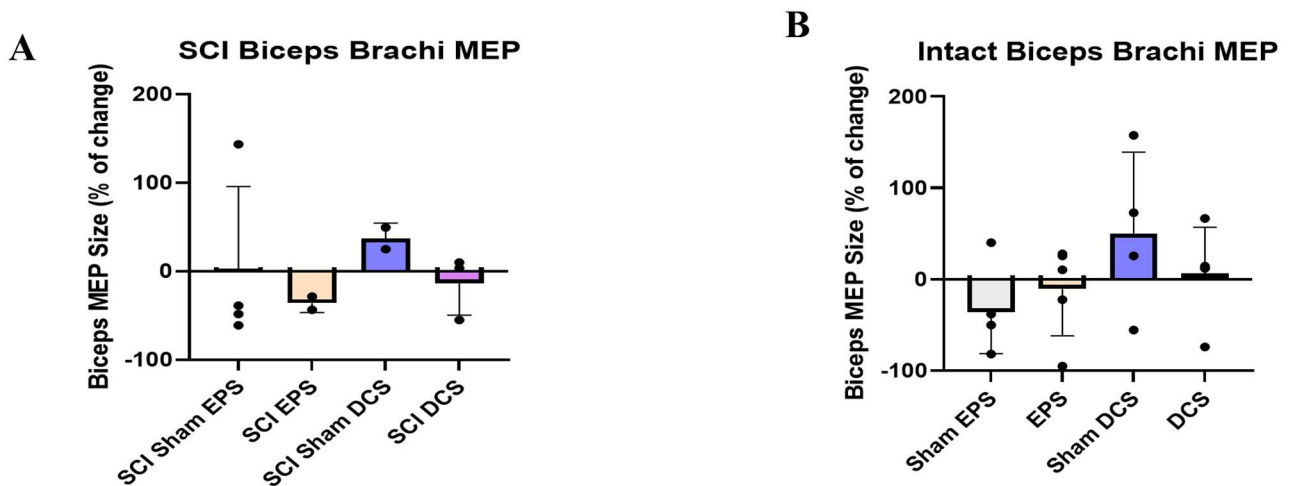


Figure 16. Summary of mean changes in BB MEP in SCI and intact groups show high variability in responses.

Changes in BB MEP after different spinal cord stimulation interventions at 50% FCR MEP max. A. Data from the SCI group (n=4 sham EPS, n=2 EPS and sham DCS and n=3 DCS). B. Data from the intact group (n= 5 sham EPS, EPS and DCS, n=4 the sham DCS). Height of column bar

represents the mean, and error bars represent standard deviation. For individual values, see Appendix Figure 4.

4.4 Systolic and diastolic mean arterial blood pressure and heart rate after spinal stimulation interventions

Changes in systolic and diastolic blood pressure and HR are expressed as a percentage of baseline values in Fig. 17-21. Systolic blood pressure in the SCI group after 10 min of DCS was significantly higher (15.7%) than at 5 min (linear mixed model, $F_{1,3}=6.3$ and p value= 0.008) (Fig. 17 and 18). In addition, HR in the SCI group 10 min after DCS was 8.6% lower than before DCS and this difference was statistically significant ($F_{1,3}=4.9$ and p value=0.02) (Fig. 20 and 21). However, other within-group analyses in SCI and intact groups showed no statistically significant differences.

Comparison of BP between different interventions in the intact group showed significant differences in diastolic BP after EPS and DCS. In this comparison, diastolic blood pressure during DCS was 9.15% higher than during EPS. ($F_{3,61}=3.7$ and p value=0.019). There was no significant time and intervention interaction in any group.

Systolic Blood Pressure

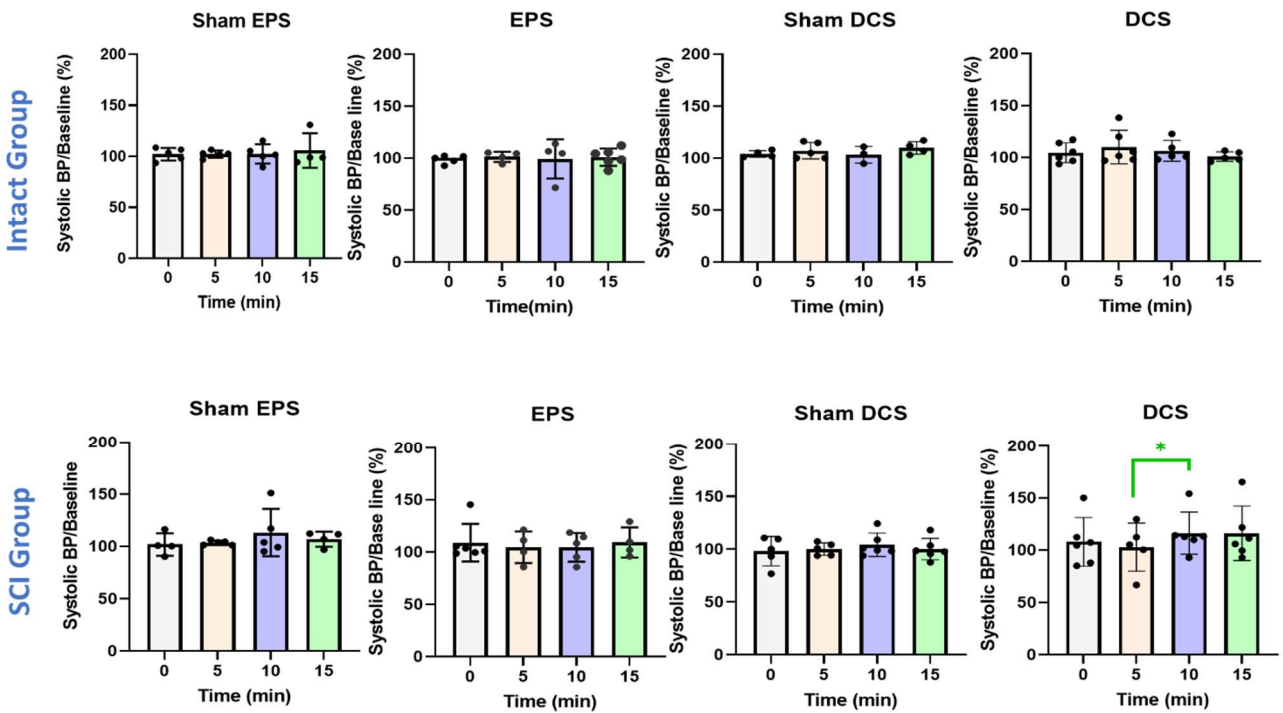


Figure 17. Systolic blood pressure in the intact and spinal cord injury groups was not affected by spinal stimulation except for DCS in those with SCI.

As seen in those with SCI, there was an increase after DCS between 5 minutes and 10 minutes post DCS. For individual values in the intact group, see Appendix Figure 5. Asterisk shows the P value <0.05.

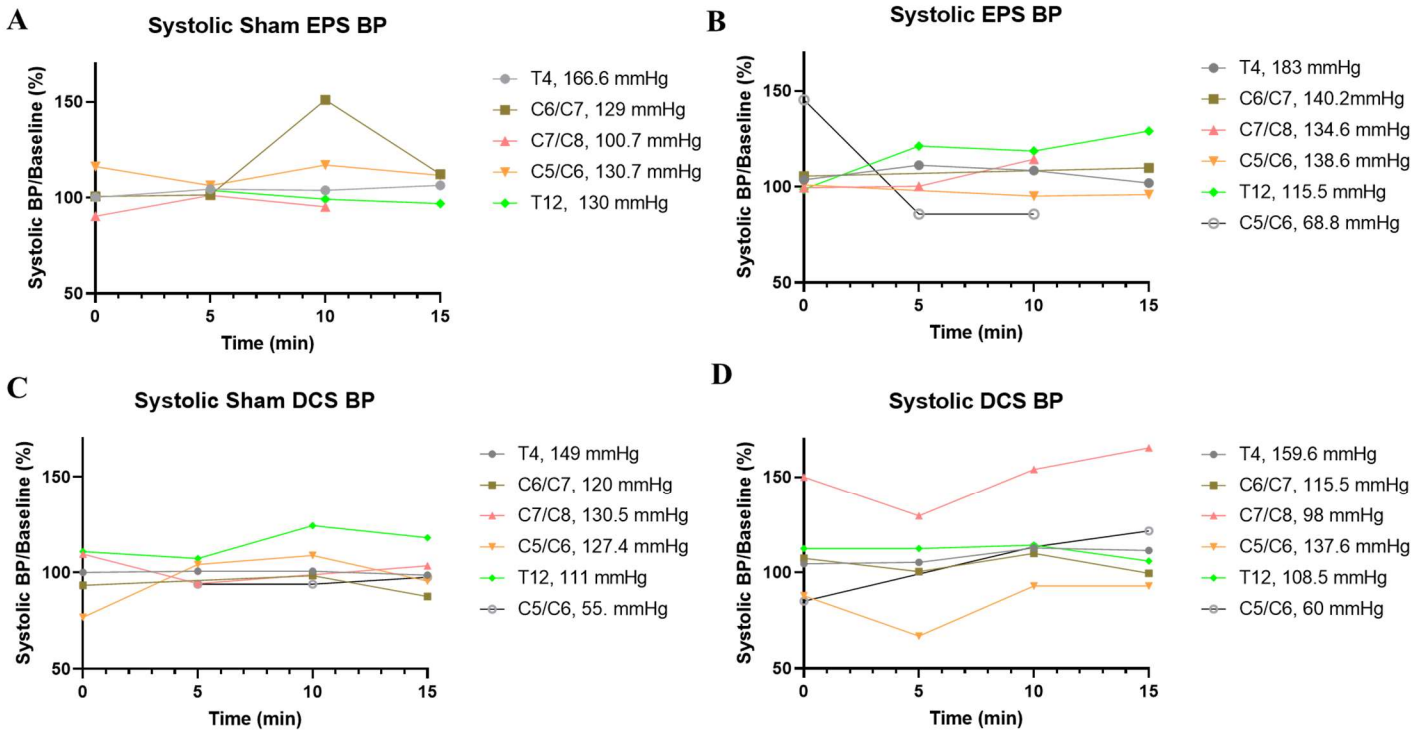


Figure 18. Systolic blood pressure values in participants with SCI were highly variable at baseline and during spinal stimulation.

Results showed variability in autonomic measures at baseline and during stimulation for participants with SCI (A. sham EPS, B. EPS. C. sham DCS. and D. DCS). See legend for level of SCI and absolute baseline systolic BP.

Diastolic Blood Pressure

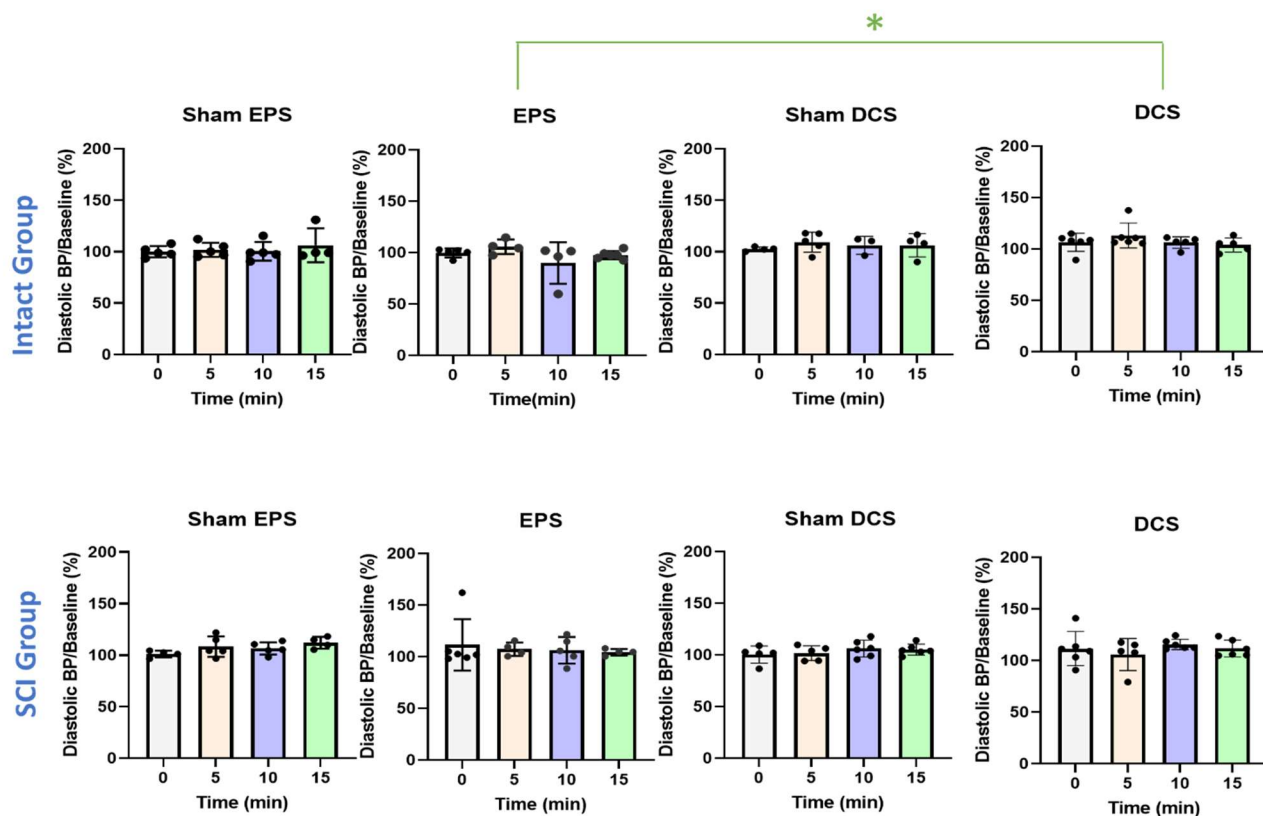


Figure 19. Diastolic blood pressure in the intact and spinal cord injury groups were not affected by spinal stimulation.

The diastolic BP in the intact showed a significant difference between EPS and DCS. There was no significant difference in within group stimulation. For individual participant values, see Appendix Figures 6 and 7. Asterisk shows the P value <0.05.

Heart Rate

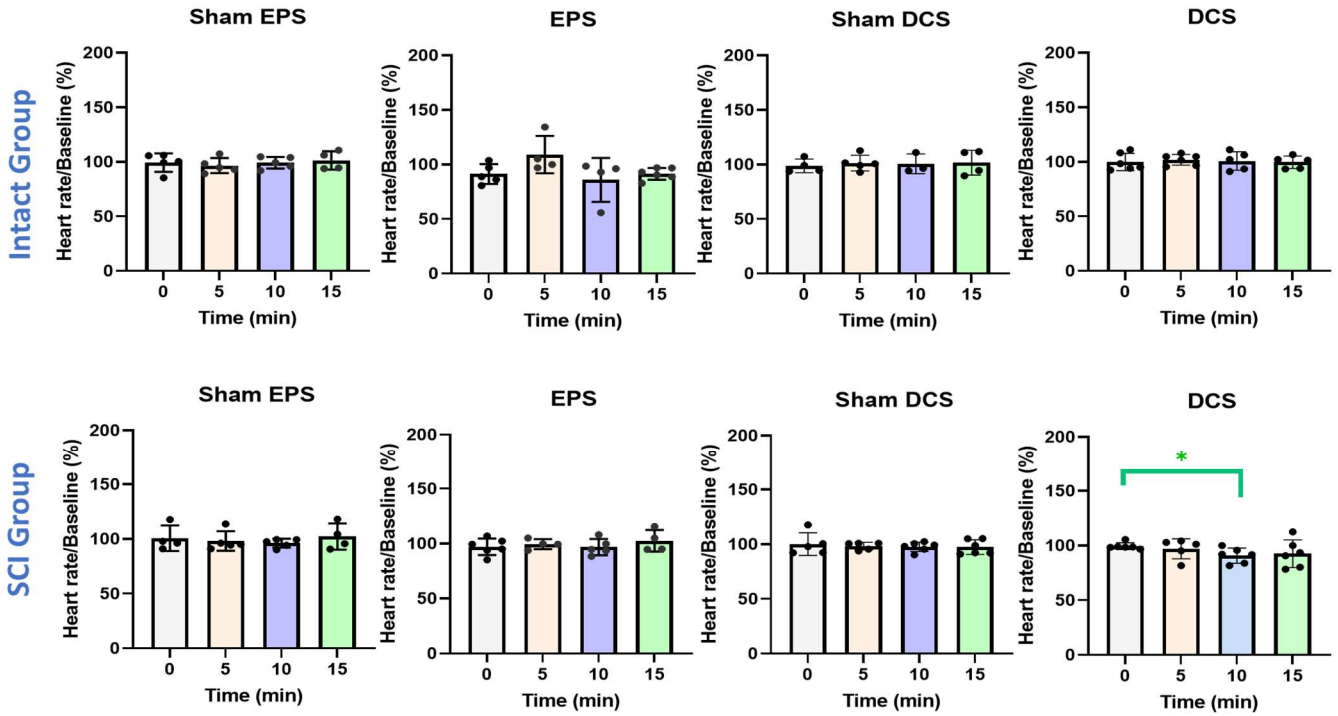


Figure 20. Heart rate in the intact and spinal cord injury groups were not affected by spinal stimulation except for DCS in those with SCI.

There was a significant difference after 10 minutes DCS in those with SCI. For individual values in the intact group, see Appendix Figure 8. Asterisk shows the P value <0.05.

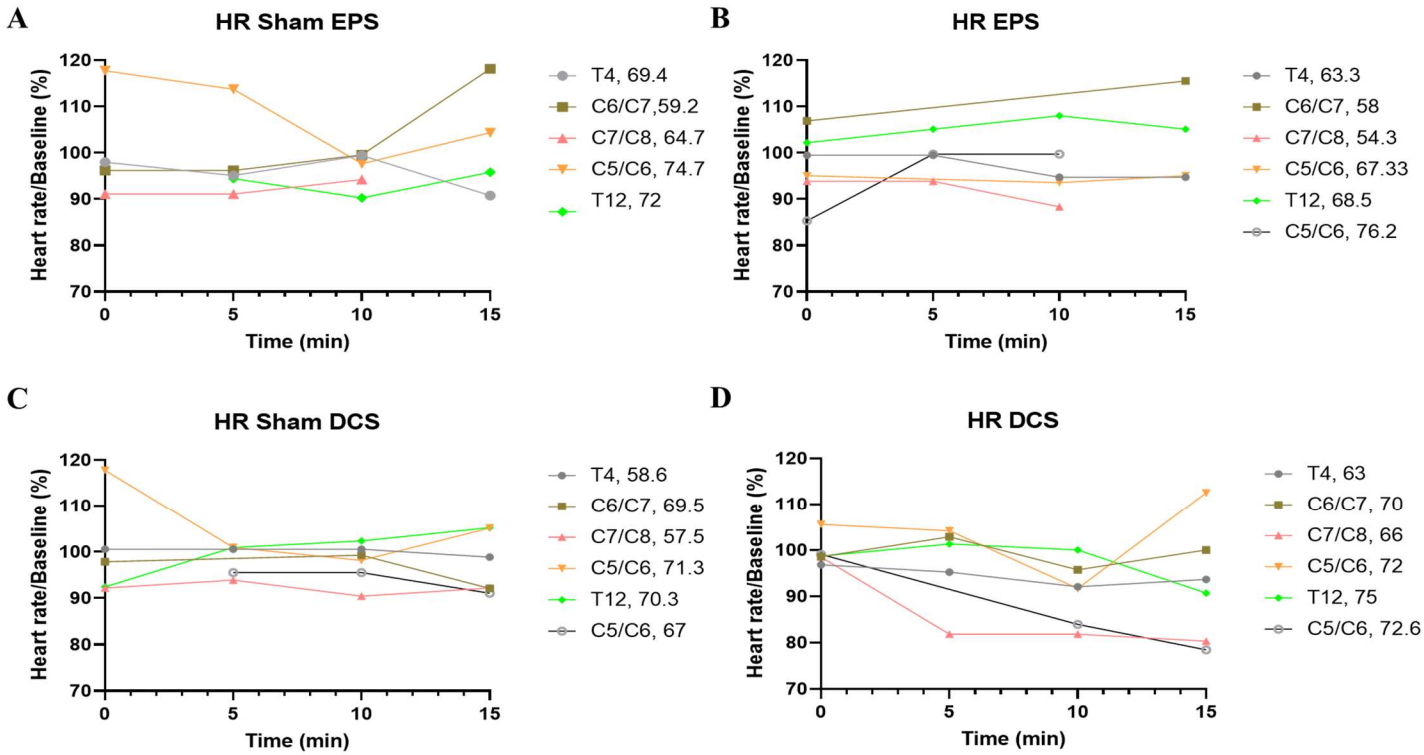


Figure 21. Heart rate at baseline and during stimulation in SCI individuals varies widely.
 A. sham EPS, B. EPS. C. sham DCS. and D. DCS. See legend for level of SCI and absolute baseline heart rate.

5. Discussion

5.1 Overview of Findings

This study aimed to compare two types of non-invasive lumbar spinal electrical stimulation applied at T11-L1 vertebral levels on the excitability of motoneurons innervating upper limb muscles. We compared effects of electrical stimulation with short pulses (EPS) and direct current stimulation (DCS) when applied for 15 minutes in a seated position. This is the first study, to our knowledge, that directly compared these two non-invasive spinal stimulation methods for 15 minutes in the same people and in the seated position. Previous studies have typically used longer (45-60 min) stimulation interventions in different positions (i.e., standing, walking, or lying on a bed).

Why did we use upper limb measurement in thoracolumbar electrical stimulation? It is evident that a network connection is present between the cervical and lumbar enlargements that bidirectionally transfers information between these two regions (31,61). Some research indicated that stimulation within the cervical area led to a change in excitability of H-reflexes in the lower extremities (61,79). However, it is unknown that stimulation at the lumbar and CPG area can have effects on the cervical and supracervical regions. To our knowledge, no study has compared the effects of thoracolumbar stimulation on FCR H-reflex and MEP. So, in this study, we measured H-reflex and MEPs in the upper limb before and after thoracolumbar spinal stimulation.

We conducted four independent sessions (two with actual EPS and DCS and two sham sessions) to evaluate flexor carpi radialis (FCR) motoneuron excitability in people with spinal cord injury (SCI group) and in another group of people with intact spinal cords (intact). We found that DCS and EPS for 15 minutes did not change H-reflex amplitude significantly compared to the respective sham interventions in either SCI or intact groups (Fig. 11 and 12). There were similar findings when examining motor-evoked potentials (MEP) after activating corticospinal neurons using TMS. Thus, FCR or BB MEP amplitudes were not changed significantly after 15 minutes of DCS or EPS compared to sham in either SCI or intact groups (Fig. 15 and 16). Our data showed that, overall, EPS and sham stimulations (both sham EPS and sham DCS) had no effect on blood pressure or heart rate, but a decrease in HR and an increase in systolic blood pressure were evident in the SCI group after DCS (Fig. 17-21).

Why did we use before and after stimulation measurements? We used before and after stimulation measurements to compare our results to previous studies using longer than 15 minutes of spinal

stimulation. Moreover, aftereffects outlasting stimulation period would likely represent functionally important effects. Although in some studies, excitability changes were assessed during transcutaneous spinal stimulation (61).

5.2 FCR H-reflex and interpretation of spinal excitability changes

Given that Mmax responses remained constant (Fig. 3), we have high confidence that our measurements accurately reflect changes in the excitability of spinal motoneurons (61). In order to achieve a consistent Mmax (which represents recruited efferent fibers), consistent placement and contact of stimulating and recording electrodes is required. Another methodological consideration during this study related to the level of activity in the tested motor pools. During H-reflex and MEP testing, we asked participants to keep their limbs in the same position since a change in position might result in a change in stretch of flexors and extensor muscles and affect H-reflex excitability in spinal motoneurons. Furthermore, we tried to keep background muscle activity at the same level while evoking MEPs to try to ensure descending drive and spinal excitability were maintained consistently.

We used two methods to evaluate H-reflex changes. One method employed a sigmoid transfer, while the other method involved only a single data point (measured at the intensity at which 30% Mmax is evoked). We used these two different methods because both have been used for evaluating effects of non-invasive spinal stimulation.

Why did we use the sigmoid transfer-based analysis? Previous studies showed that estimating the size of the H-reflex based on a sigmoid function is the most reliable way to estimate the ascending limb of an H-reflex recruitment curve when data is not sufficient or reliable for generating a complete input/output curve (63). The sigmoid transfer enables comparison of H-reflex amplitude between participants and testing sessions since it reliably normalizes the current needed to evoke the reflex on different testing days. By using the sigmoid equation, the physiologically based prediction of the input/output relation of the ascending limb of the recruitment curve of FCR H-reflex responses in all participants were obtained at two intensities ($1 \times S50\text{-Hmax}$ and $1.1 \times S50\text{-Hmax}$, Fig. 7 and 9). Other intensities above 1.1 or below $1 \times S50\text{-Hmax}$ were associated with interpolated values in most participants. Therefore, those were not included in our statistical comparisons.

Why did we use the reflex size at 30% Mmax-based analysis? The second method comparing the FCR H-reflex before and after spinal stimulation was based on the value of the FCR H-reflex input-output curve at only one intensity, which resulted in a reflex that amounted to about 30% of Mmax. The H-reflex between 10-30% of Mmax is equally susceptible to facilitation and inhibition (64). A recent study by Islam et al.(64) used this method and in order to allow comparisons of our results to the outcome of their study; we also applied this analysis (as shown in Fig. 8 and 10). In another recent study by Parhizi et al. a single point on the ascending limb of FCR H-reflex at 70% of the Hmax was used for measuring the effect of the EPS (61). Our data showed a significant increase in the FCR H-reflex after sham DCS in the intact group and a nearly significant reduction in the FCR H-reflex after DCS in the SCI group ($p=0.054$).

Unexpectedly, our findings of significant changes in the within-session analyses of FCR H-reflex were not consistent, based on the two comparison methods, i.e., the “sigmoid transfer” vs. “30% Mmax”. This suggests that other studies should be interpreted with caution when relying on only one type of analysis. We did not identify any papers using both methods, yet there are known limitations of each method.

Why did we perform intersession (between-sessions) comparisons? We hypothesized that non-invasive spinal cord stimulation at the T11 vertebral level, using either DCS or EPS, alters spinal and corticospinal excitability in people with intact or injured spinal cord. However, our results showed no significant differences between the SCI and intact groups after any intervention. The smallest modulation of the FCR H-reflex occurred after EPS in both SCI and intact groups. However, FCR H-reflexes showed a tendency to be suppressed after DCS in both, but especially in the SCI group. On the other hand, sham DCS facilitated the FCR H-reflex. This observation was similar after using both types of H-reflex analysis (the sigmoid or the 30% Mmax methods).

How did our results compare to other data from EPS-induced modulation of the H-reflex? Our data are in line with Knikou and Murray’s findings (60). The H-reflex showed no change after EPS in intact subjects (60). However, they found a decreased soleus (Sol) H-reflex in their SCI group after 60 min of EPS. Important to note that spinal stimulation in that study was applied at T10, but reflexes were assessed in a leg muscle (Soleus), unlike in our experiments with the reversed configuration. Our data is disagreement with the result of Parhizi et al. study who found facilitation (by 11%) of the FCR H-reflex after lumbar EPS (61).

One potential reason for finding no changes of the FCR H-reflex after EPS in the SCI group could be the low stimulus intensity, i.e., subthreshold for evoking motor response in the legs. In another study that used cervical EPS with subthreshold intensity in participants with non-injured spinal cord; no change in Sol H-reflex was observed (64). In addition, Subthreshold cervical EPS showed no significant change in FCR H-reflex in SCI or in healthy participants (81). Sasaki et al. revealed that a short duration of subthreshold cervical stimulation did not affect the posterior root reflex of several upper limb muscles in able-bodied participants (42). These three studies demonstrated evidence that subthreshold EPS has no effect on H-reflexes. In our study, the EPS used during the intervention could be considered subthreshold as it did not evoke contractions in leg muscles. Islam et al., from a recent study in the Knikou lab, pointed out that the supramotor threshold EPS at cervical levels facilitated H-reflexes in the lower limbs (64).

Overall, it may be concluded that pulsed subthreshold stimulation intensities may not be sufficient to modulate excitability of neurons at the intra-segment or multi-segment spinal levels. However, stimulation intensities that evoked muscle action potentials (i.e., suprathreshold) could evoke facilitation of H-reflexes, thought to be due to neuromodulation of propriospinal neurons located in the cervical and lumbar regions (64). In addition, other reasons for these discrepancies could be the frequency of spinal stimulation and the number of spinal stimulation sessions. Most studies reporting an increase in participants' arm function used higher frequency EPS stimulation (15-50 Hz) (41,80), while in our study, we used low frequency (0.3 Hz) stimulation. Other research showed that repetitive electrical stimulation (epidural or transcutaneous) enhanced inhibitory mechanisms in spinal networks, as was shown by a decrease in spasticity and stretch reflexes (82,83). In our study, a significant reduction was not observed after any intervention. Therefore, it may be that a single 15-minute session of spinal stimulation might not be sufficient to induce neuromodulatory effects.

How did our results compare to other data from DCS -induced modulation of the H-reflex? Our results are in agreement with Murray et al. study that revealed reductions in lower limb H-reflexes after DCS at T10-12 for 30 minutes with participants in seated or supine positions (84). We also found a trend of decreased FCR H-reflex after DCS in our SCI group (Fig.13-14). In our study, we observed a decrease in H-reflex amplitude after DCS in those with incomplete cervical SCI

individuals (in 30% Mmax method, Fig. A2), while a participant with incomplete lower thoracic SCI showed no changes.

In contrast to other studies that showed no effect on H-reflexes after sham DCS (84), we noticed facilitatory effects after sham DCS in our SCI and intact groups (Fig 13-14). As a potential explanation of the effect after sham DCS, we should consider cutaneous sensory pathways to the spinal cord (32); because the wet sponge was used during this session. Our thinking is that DCS, by constant current, may block large diameter afferent inputs (32), including cutaneous mechanoreceptor afferent input, which may lead to decreased spasticity. In contrast, in sham DCS, the spinal cord receives cutaneous afferent input from the wet sponge, which could lead to an increase in the alpha motoneurons' excitability and facilitation in FCR H-reflex.

There is great variability in H-reflex responses under different experimental conditions in the research literature (59,84–86). This variability could be the result of different protocols (39). The different findings after stimulation depend on the size of electrodes, direction of anodal and cathodal stimulation, location of the reference electrode, posture, stimulation intensity, as well as stimulation duration. For example, cathodal DCS in a supine position had a greater effect on spinal excitability than when delivered in a seated position (84). DCS is more dependent on intensity than duration of stimulation (67). Even a 3-minute cathodal DCS on lumbar area could increase power of plantarflexion in healthy participants (67).

5.3 MEP and interpretation of changes in cortico-spinal excitability

The peak slope of the input-output curve of MEPs is thought to reflect the excitability of the motor cortex. The peak slope is usually obtained at a stimulation intensity corresponding to one that elicits 50% of MEP max (also called S50) (87). So, we used the S50 of MEP max as the point at which changes in MEP size were examined. Moreover, at this intensity, MEP facilitation or inhibition can be detected accurately (61).

Our findings demonstrated that there were minimal, non-significant changes after EPS (-3% and 3% in SCI and intact groups, respectively) in FCR MEPs (Fig. 15). This result is in agreement with Parhizi et al. showing that lumbar EPS did not influence FCR MEPs, although they measured MEPs while delivering EPS, not after EPS as in our study (61). Sasaki et al. demonstrated that short-duration sub-motor threshold cervical EPS did not change FCR MEPs significantly, but the FDI MEPs had a tendency to be reduced after EPS (42).

Our data showed no significant changes after DCS in comparison to sham DCS; however, a trend for increased FCR MEPs in both SCI and intact participants was evident (by 15% and 13%, respectively, Fig. 15). With a higher number of participants in the study, this increase might have been a significant change. According to MEP changes after DCS, our findings agree with Murray et al. results. They showed that cathodal DCS of the thoracic area had a facilitatory effect on leg muscle MEPs (84). Murray and Knikou showed that repeated EPS and cathodal DCS work differently in terms of their effects on the corticospinal pathway (88). They revealed that EPS had a neuromodulation effect on the brain neural circuits and cortical interneurons, while DCS changed spontaneous cortical activity and membrane excitability of ascending pathways (88). In another study, it was proposed that spinal DCS could affect the cortex through afferent input and somatosensory pathways, resulting in changes that could last for 10 minutes after ceasing the stimulation (15 min duration) in some animal experiments (87).

5.4 Mean arterial blood pressure and heart rate after 15 min of EPS and DCS

There was no significant difference in systolic BP after any of the interventions in the intact group. However, in SCI group, DCS resulted in a 15.7 mmHg increase of systolic blood pressure at 10 min after the onset of DCS stimulation (Fig. 17). Diastolic BP showed no significant change when within-session analysis was done. Heart rate at 10 min after the onset of DCS stimulation was significantly reduced (by 8.5 beats/min) than the rate at the start of DCS stimulation in the SCI group (Fig. 20). When inter-session comparisons were performed, diastolic blood pressure was higher after DCS than after EPS (by 9.1 mmHg) in the intact group.

Our results were similar to the findings of Gad et al., demonstrating that thoracolumbar EPS caused a minimal increase in HR and no change in BP during their protocol (89). In contrast to our findings, another study showed low-frequency (0.2 Hz) EPS of upper thoracic segments had an increased effect on mean arterial pressure (by 20%); however, HR decreased in most of the participants (81). On the other hand, high-frequency spinal stimulation (30 to 120 Hz) of segments between T6 to L1 was deemed to evoke modulatory effects on the orthostatic intolerance (26); however, in our study, we used low-frequency EPS.

In contrast to our results that showed a decrease in the HR during DCS stimulation, Shaleykian et al. demonstrated an increase in the heart rate of participants with SCI after DCS (90). However,

they used 10 mA of current for 20-50 min with epidural stimulation which was delivered in several daily bouts.

The sympathetic nuclei in the spinal cord are known to receive impulses from serotonergic (5-HT) neurons from the brain stem (26). It could be plausible that DCS can affect not only spinal but also the supraspinal 5-HT neurons in the brainstem, which can increasingly activate the sympathetic system and contribute to the increase of systolic blood pressure.

During DCS session in the SCI group, the participants with cervical spinal cord injury had an increase in systolic BP and a decrease in HR; however, two participants with thoracic injury (T4 and T12) did not show these changes (Fig. 18, Fig. 21). It is plausible that transcutaneous stimulation can alter the BP and HR of participants who are susceptible to autonomic dysregulation (cervical spinal cord injury). This explanation was also supported by a recent scoping review by Flett et al. (26).

5.5 Limitations

Low number of participants had great effects on our results. A larger group of participants with uniform and similar SCI levels would have decreased the variability of our findings.

The penetration of electrical stimulation depends on the thickness of the tissue layers it should pass through. Although not objectively measured here, this study had great variability in terms of body composition and anatomical differences in the stimulated regions, especially in the SCI group, which should be taken into consideration in future studies.

In contrast to our study, most studies in the literature used 20-30 Hz frequency for the EPS intervention (39). Moreover, EPS intensity was usually above the threshold required to evoke lower limb muscle contractions (39). However, we elicited contractions in lateral vastus in only 2 participants during 15 min EPS stimulation, while in other participants, the EPS stimulation intensity was subthreshold to leg motor contraction.

TMS evoked responses from both FCR and BB in our experiments, even though it was aimed to excite only the FCR muscle. As TMS can activate a wide region throughout the motor cortex, multiple muscles often show MEPs. Typically, only those MEPs that correspond to the muscle for which precise positioning of the stimulation coil (hot spotting) was successful are measured. Nevertheless, here we analyzed MEPs in the BB in order to support trends observed in the FCR

MEPs, but slight similarities were found (Fig. 16). Variable results of BB MEP in comparison to the FCR MEP changes in both intact and SCI groups could be a result of suboptimal interneuron stimulation as we set the location of the TMS based on the FCR muscle and not the BB (except in one participant in intact group due to discomfort). Moreover, given the small data set, especially for the SCI group (n=2 in EPS and sham DCS), these comparisons should be interpreted with caution.

It should be noted that in 2/6 participants in the SCI group, we could not evoke MEPs because of spontaneous activity (spasticity waves) of the FCR muscle, which obscured the baseline EMG. Moreover, we noticed that despite precisely targeting the hotspot on the motor cortex, we seldom could evoke the MEP in some individuals. These participants used GABAergic and anticonvulsant medications routinely due to spasticity. The GABAergic medications were shown to depress the stimulus-response of corticospinal neurons (91). Therefore, the medication history should be considered in the study's results in future studies on MEP in participants with central nervous system injuries.

In this study, we did not measure the effect of SCS on H-reflexes in leg muscles because of the time limit for testing sessions and the long period of sitting imposed on participants. Quantitative assessment of body position to ensure that everyone has the same angle for elbow and wrist positioning would have also increased consistency and stability of pre and post-intervention data. One way to reduce the standard deviation of H-reflex amplitude is to strap the arm for stabilization which should be considered for future studies.

5.6 Clinical implications

People with SCI showed decreased spasticity during thoracolumbar stimulation and voluntary contraction, which was thought to be due to increases in presynaptic and postsynaptic inhibitory pathways (82). This observation was supported by recent research showing that a combination of hand training during cervical spinal stimulation increased hand function and reduced MEP thresholds (92). Thus, it may be that a combination of training and spinal stimulation can lead to decreased spasticity. Decreasing spasticity may help people perform exercise or activities of daily living in a more efficient manner. Based on these findings, future research should test whether integrating SCS with voluntary exercise and training improves quality of life and function after SCI.

5.7 Conclusions

In contrast to our hypothesis, our results demonstrated no significant differences in either spinal or corticospinal excitability after 15 min EPS or DCS, delivered while seated in intact and spinal cord participants. Although EPS delivered at a low frequency and subthreshold intensity did not change FCR H-reflex or MEP amplitudes, DCS showed an inhibitory trend on FCR H-reflex amplitude in those with SCI and facilitatory tendency in MEP amplitude in both groups. Our study demonstrated that DCS increased systolic blood pressure while decreasing the heart rate during stimulation. However, these results should be interpreted with caution due to the small sample size. Like other studies (32,60,63,72,76,92), our findings support the idea that transcutaneous spinal stimulation may affect spinal excitability at multiple spinal segmental levels. We demonstrated that stimulation of neurons within the lumbar spinal cord could alter excitability of neurons within cervical regions. So, electrical stimulation of the lumbar regions could influence cervical motor networks.

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Appendix

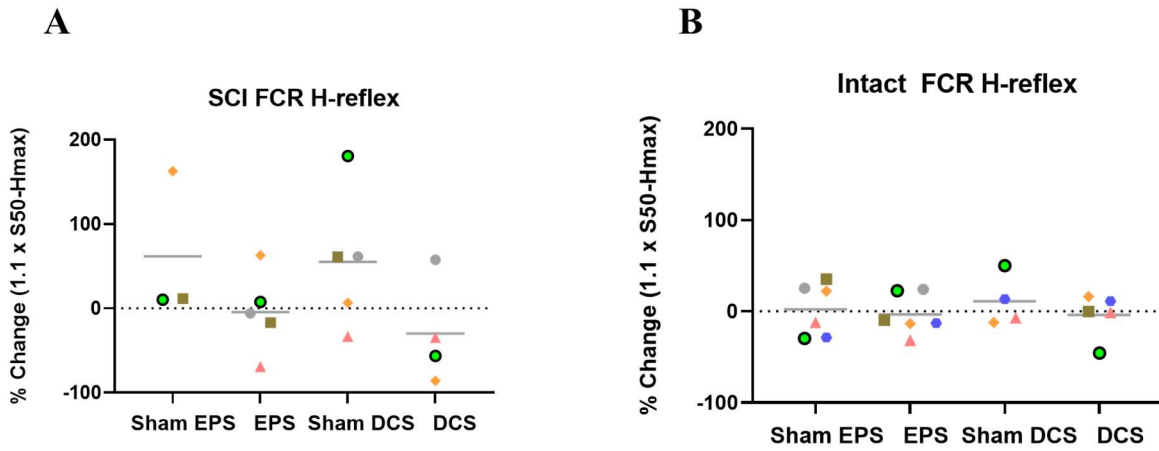


Figure A1. Replotted data from FCR H-reflex in intact and SCI groups after sigmoid transfer showed great variability in SCI individuals.

Each symbol indicating each participant (i.e., individual distribution). A. Data from SCI group. B. Data from intact group.

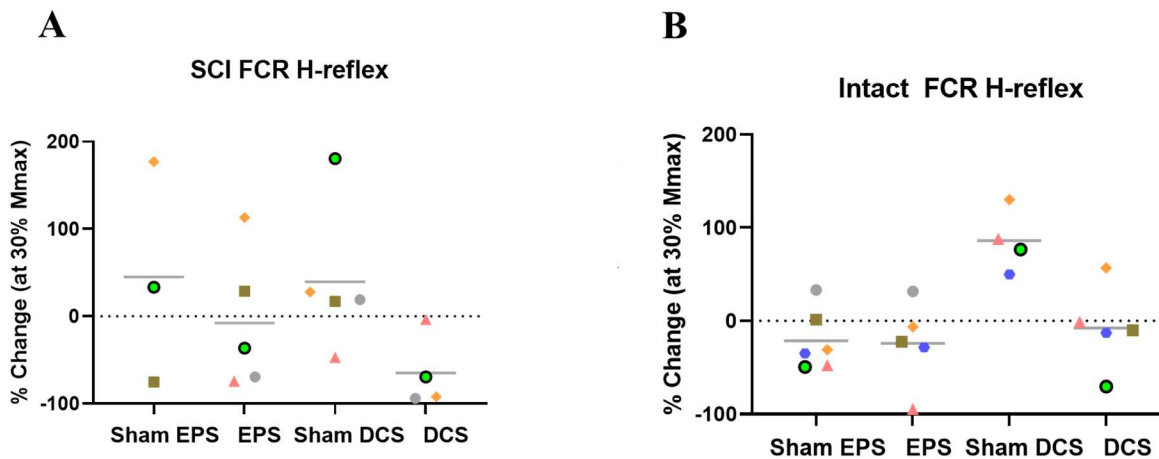


Figure A2. Replotted data from FCR H-reflex at 30% Mmax in intact and SCI groups showed great variability in SCI individuals.

Each symbol indicating each participant (i.e., individual distribution). A. Data from SCI group. B. Data from intact group.

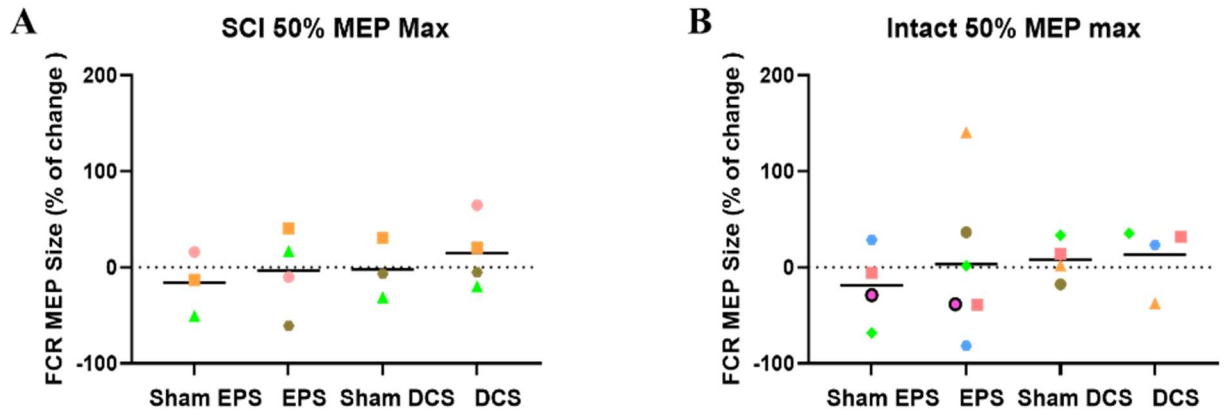


Figure A3. Replotted data from FCR MEP in intact and SCI groups showed variability in participants.

Each symbol indicating each participant (i.e., individual distribution). A. Data from SCI group. B. Data from intact group.

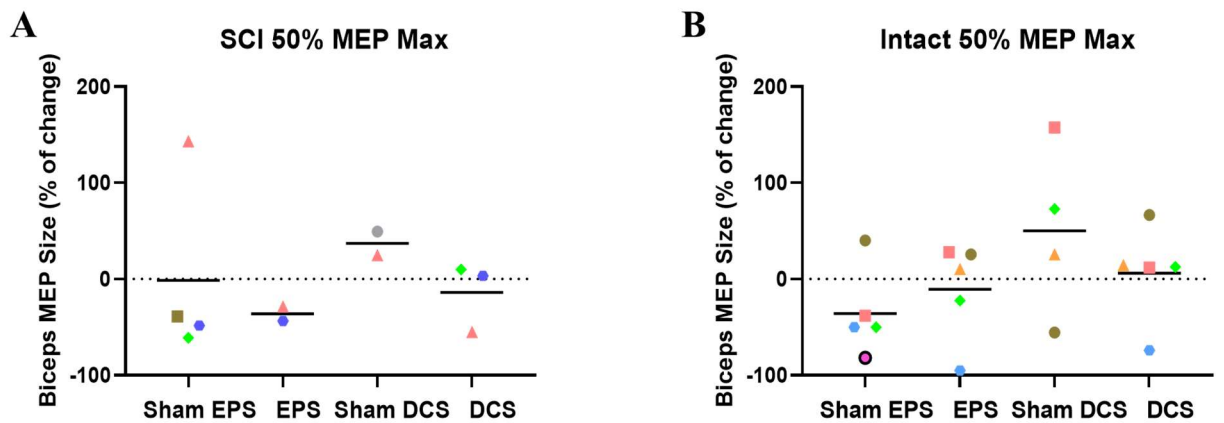


Figure A4. Replotted data from Biceps brachii MEP in intact and SCI groups showed great variability in individuals.

Each symbol indicating each participant (i.e., individual distribution). A. Data from SCI group. B. Data from intact group.

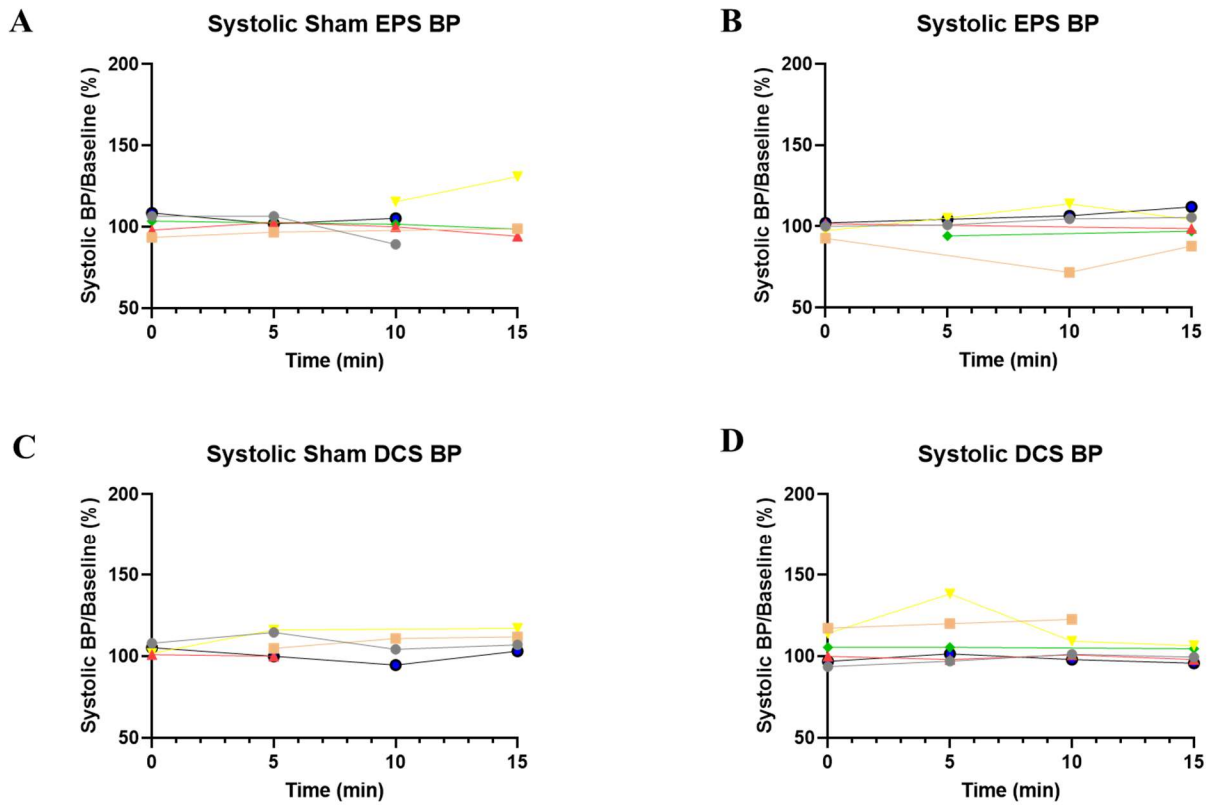


Figure A5. Data from systolic blood pressure during each electrical stimulation in the intact group at 4 time points showed consistent BP.

A. Results from sham EPS. B. Results from EPS. C. Results from sham DCS. D. Results from DCS

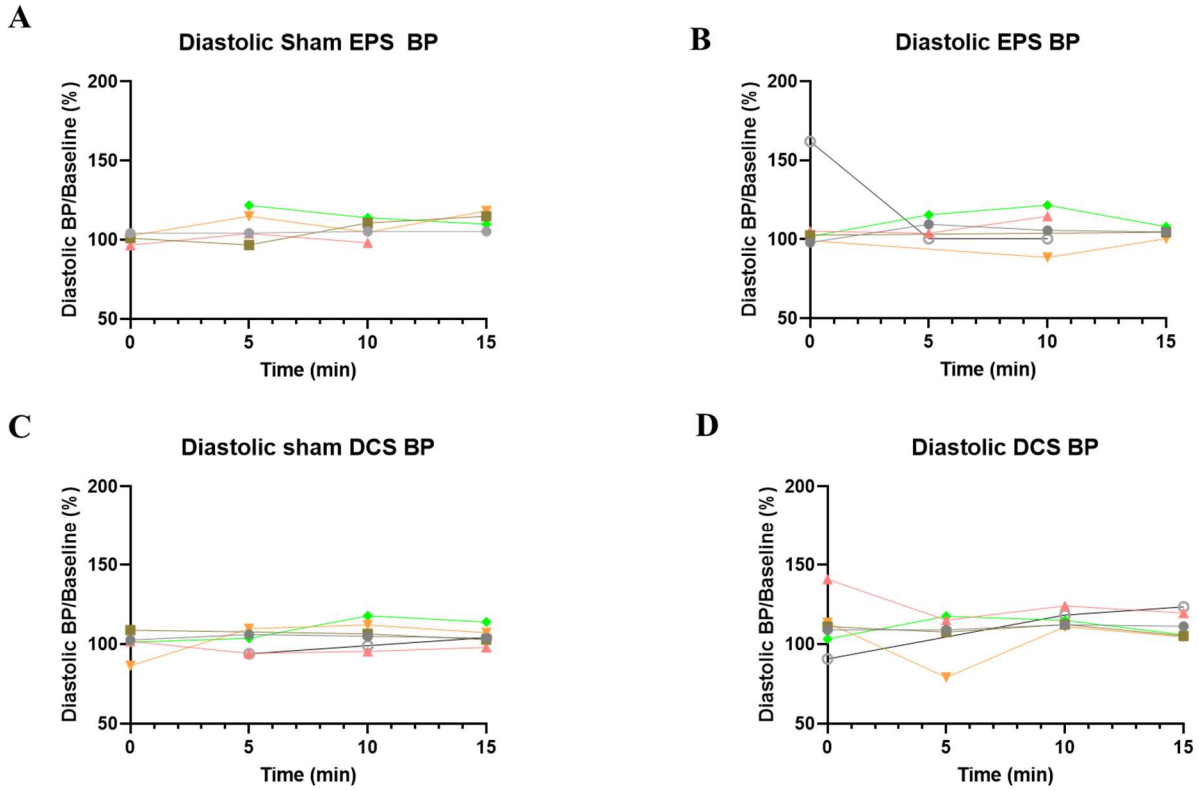


Figure A6. Data from diastolic blood pressure during each electrical stimulation in the SCI group at 4 time points showed less variability.

A. Results from sham EPS. B. Results from EPS. C. Results from sham DCS. D. Results from DCS

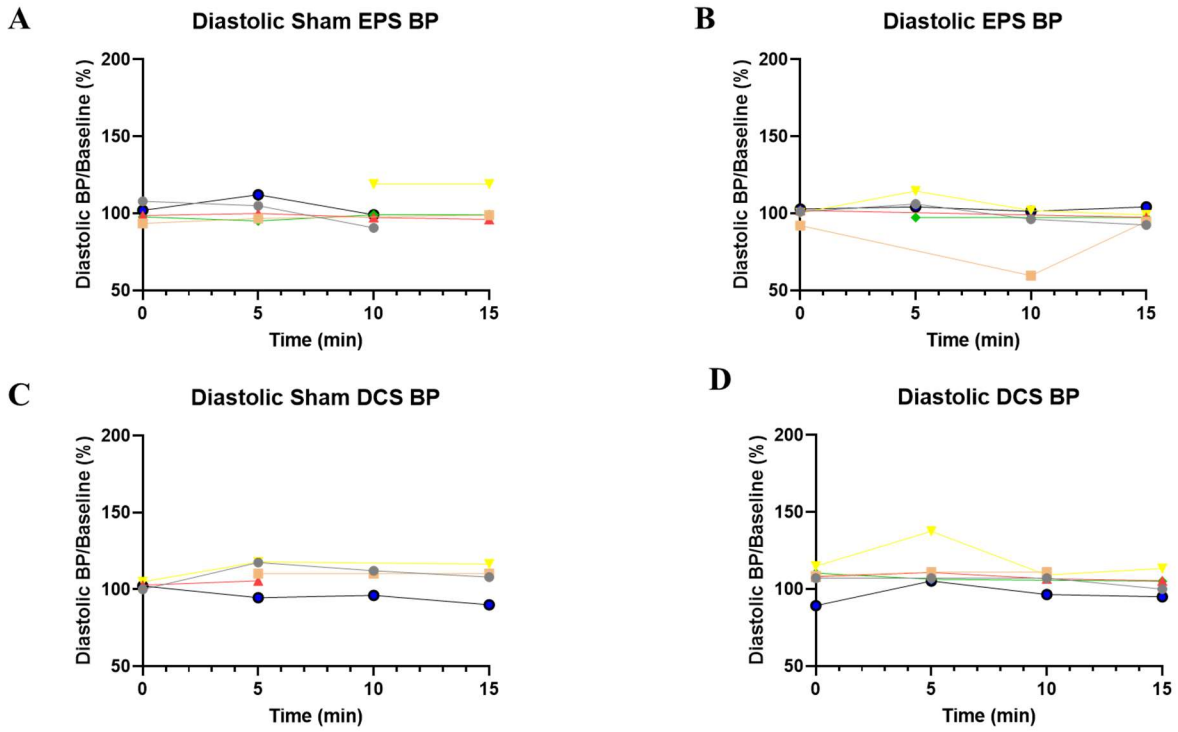


Figure A7. Data from diastolic blood pressure during each electrical stimulation in the intact group at 4 time points showed consistent BP.

A. Results from sham EPS. B. Results from EPS. C. Results from sham DCS. D. Results from DCS

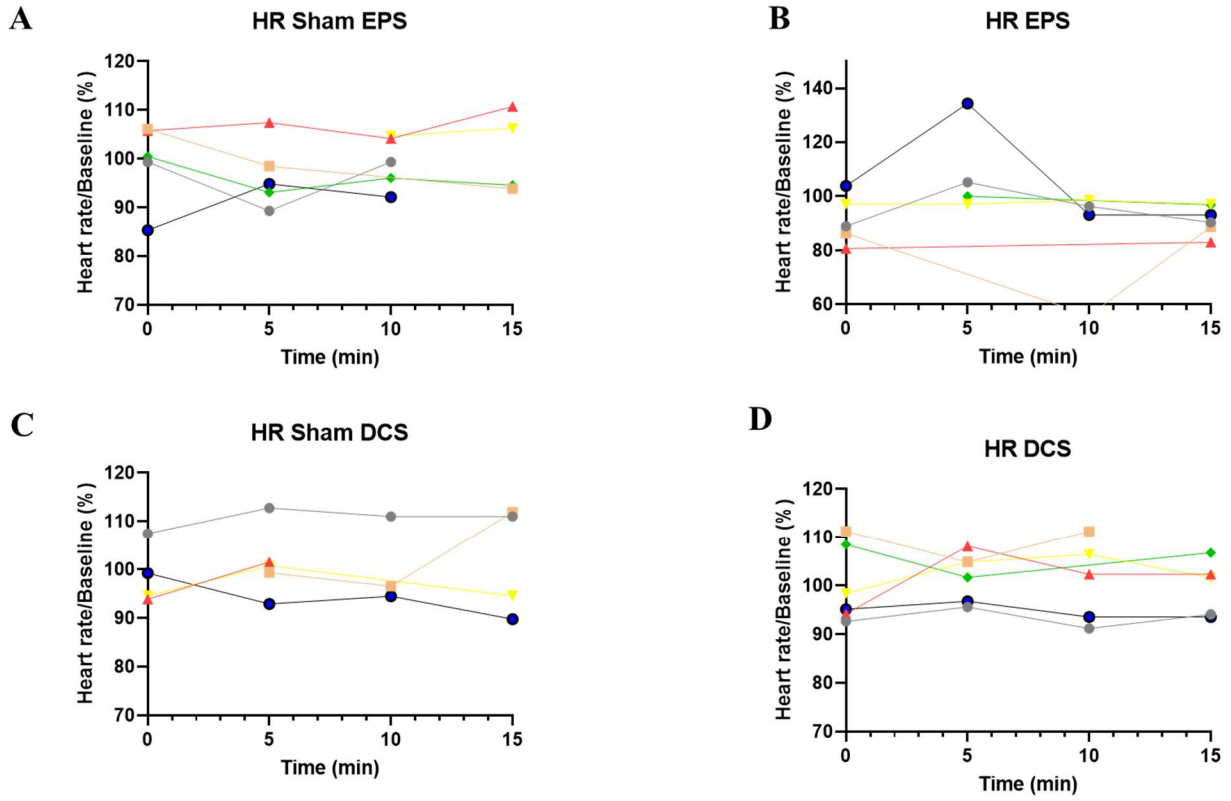


Figure A8. Data from heart rate during each electrical stimulation in the intact group at 4 time points showed variability in the individuals.

A. Results from sham EPS. B. Results from EPS. C. Results from sham DCS. D. Results from DCS.