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Spinal Excitability Changes Following Sensory Electrical Stimulation of The Forearm

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Abstract

Sensory electrical stimulation can be used to suppress tremor in Parkinson's disease. This study investigated the central mechanism underlying this suppression in healthy participants. Reciprocal inhibition (RI) of the wrist flexors before and after a session of sensory electrical stimulation (SES) applied to the antagonistic extensor muscles was assessed using electromyography. It was hypothesized that a 15-minute session of SES, rated by participants as a 3 on a 0-10 pain scale, would produce an increase in RI. Seven of the 18 participants experienced an increase in RI at 0-5 minutes post stimulation, which returned to baseline at 10-15 minutes. The findings of the present experiment suggest that increases in RI are only observed in a "responders" subgroup consisting of predominantly male participants. More research is needed to understand the optimal stimulation intensity and any sex-linked factors that are important to produce reliable changes in reciprocal inhibition following sensory electrical stimulation.

Keywords

Sensory Electrical Stimulation, Tremor Suppression, Spinal Excitability, Reciprocal Inhibition, Flexor Carpi Radialis, Extensor Carpi Radialis Longus, Parkinson's Disease

Summary for Lay Audience

Tremor is an involuntary rhythmic movement that is commonly found in the forearm of people with Parkinson's disease. These rhythmic movements at the joint are generated by contractions of the two opposing sets of muscles called "extensors" and "flexors", creating a back-and-forth motion. One approach to reduce tremor is to apply weak electrical stimulation to the muscle in a pattern to oppose the movement. It has been thought that inhibition in the spinal cord may play an important role in this process. By electrically stimulating the extensor muscles with weak electrical current that does not produce muscle contraction i.e. sensory electrical stimulation, it is possible to reduce the contraction of the flexor muscles. To test this mechanism in the wrist muscles, the level of inhibition present in the wrist flexors was measured before and after a sensory electrical stimulation was applied to the wrist extensors. The electrical stimulation was mild causing slight discomfort (3 on a 0-10 pain scale, with 0 for no pain and 10 being the worst pain imaginable) to the participants. It was expected that after a15-minute session of this stimulation the inhibition to the flexors would increase. Although there was a significant inhibition present in the flexors for all participants, the expected increase in inhibition after the sensory electrical stimulation of the extensors was found only in a subgroup of primarily male participants. A possible explanation is that the intensity of the sensory electrical stimulation was not strong enough for some participants to produce the expected changes in inhibition. More research is needed to understand the best stimulation intensity and any sex-linked factors that influence the inhibition following sensory electrical stimulation.

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Chapter 1

1 Introduction

1.1 Background and Rationale

Parkinson's disease (PD) is a debilitating neurodegenerative disease that currently has no cure (Elias & Shah, 2014). One of the most common symptoms of PD is tremor, which is an involuntary and repetitive contraction pattern usually observed in the hands. Tremor has proven challenging to treat and often resists medication regimens (Flora, Perera, Cameron, & Maddern, 2010). Surgical approaches can affect tremor positively, but have inherent risks and are usually reserved for very advanced PD patients (Rascol et al., 2000). Because of these limited treatment options, the development of wearable tremor suppression devices (WTSDs) has been researched as a safe and noninvasive means of providing relief from tremor.

These WTSD devices aim to counteract tremor by applying forces out of phase to the tremor motion (Hara, Ogawa, & Muraoka, 2006; Rocon et al., 2007). Neuromuscular electrical stimulation (NMES) is one of the technologies used to achieve the desired canceling effect. Two studies in particular have investigated the ability to cancel tremor movement using sensory electrical stimulation (SES), which is stimulation that is below the level required to produce a muscle contraction (Dosen et al., 2015; Heo et al., 2015). The results show that, when applied with the same antiphase timing, SES has the ability to suppress tremor as much as 88%. One explanation to this phenomenon is that the SES is capable of producing reciprocal inhibition (RI), possibly mimicking the effect of stretch receptors in the muscle (Rosenbaum, 2010). Heo et al. (2015) also demonstrated that the suppression effect lasts for a minimum of five minutes following the application of SES; an intriguing finding, as this suggests that changes in excitability may reflect transient plasticity in the spinal cord.

Transient changes in reciprocal inhibition have been demonstrated in the soleus muscles following SES of the common peroneal nerve in healthy controls with the inhibitory effect also persisting at five minutes post stimulation (Perez, Field-Fote, & Floeter, 2003). Interestingly, Perez et al. (2003) also demonstrated the necessity for SES to be applied in a patterned manner in order to modulate the RI response. It is not clear if there is an optimal pattern of stimulation for PD tremor suppression, but Perez et al. (2003) achieved significant results by mimicking the type of sensory feedback that would be produced from muscle afferents during a regular gait cycle.

The measurement techniques used in WTSD studies assess the devices efficacy based on movement characteristics but fail to highlight the underlying physiological mechanism governing these changes. It remains unclear whether SES using patterns that mimic tremor influences reciprocal inhibition in the forearm. Investigating the effect that patterned SES has on levels of reciprocal inhibition within the ipsilateral limb may help strengthen our understanding of WTSD control strategies and could potentially assist in the design of WTSD systems.

1.2 Objective and Hypothesis

The purpose of this study is to explore whether SES can produce changes in RI. This study aims to answer the question: Can reciprocal inhibition be increased following a SES session using a set of stimulation parameters that have previously been shown to suppress Parkinsonian tremor movement characteristics in the forearm? It is hypothesized that the spinal cord will exhibit a transient increase in RI of the Flexor Carpi Radialis (FCR) muscle following a session of patterned SES applied to the antagonist Extensor Carpi Radialis Longus (ECRL) muscle.

Chapter 2

2 Literature Review

2.1 Parkinson's Tremor and Treatments

Parkinson's Disease is neurodegenerative disease that affects roughly 100,000 people in Canada, according to estimates from Parkinson Canada (www.parkinson.ca). Parkinson's Disease is understood to arise from the degeneration of dopaminergic cells in the nigrostriatal pathway, and is characterized by progressive tremor, bradykinesia, rigidity, and cognitive impairment (Calne, 1993). Parkinson's Disease ranks second only to Alzheimer's as the most prevalent chronic neurological disease among the elderly (Elias & Shah, 2014). Among the symptoms of PD, tremor has proven to be very challenging to treat and often resists medication regiments (Rascol et al., 2000). Tremor is defined as an involuntary and repetitive contraction pattern usually observed in the hands and is found in 75% of PD patients (Elble & Koller, 1990). The cornerstone of PD symptom management consists of a variety of medications designed to stimulate dopamine receptors in the brain (Calne, 1993). Medication treatments for tremor vary in effectiveness and are often associated with side effects such as drowsiness, confusion, nausea, and hallucinations (Rascol et al., 2000). In advanced cases, neurosurgical approaches have been shown to be effective. However, there are inherent risks with neurosurgical procedures, limiting its application to only severe disease (Rascol et al., 2000).

Persistent tremor has been shown to impact PD patients by limiting their ability to perform activities of daily living (Rosen & Aldenstein, 1981). Additionally, PD patients often experience significant social stigmatization due to the presence of their tremor (Puschmann & Wszolek, 2011). The limited number of treatment options has inspired the development of a range WTSDs to offer a safe and effective means of alleviating the burden of PD tremor (As'arry, Md Zain, Mailah, & Hussein, 2013; Hara et al., 2006; Rocon et al., 2007).

2.2 Wearable Tremor Suppression Devices

Wearable tremor suppression devices offer a non-invasive treatment option that suppresses tremor based on the mechanical and electrical characteristics of the tremor (Zhou, Jenkins, Naish, & Trejos, 2016). WTSDs are designed to intelligently track, predict, and cancel tremulous movements. To achieve this, forces are generated across the joints of interest out of phase to the tremor movements. Two main approaches have been used to achieve the desired canceling effect: mechanical suppression systems and electrical suppression systems. A variety of mechanical suppression systems have been tested in recent literature, including motors, pneumatic cylinders, and vibration exciters, to name a few (Ando et al., 2012; Lavu & Gupta, 2009; Rocon, Gallego, Moreno, Pons, & Kheng, 2009). Although effective, mechanical loadings systems are often limited by their size and weight, making them less practical for everyday use.

Electrical suppression systems typically utilize a specific type of Functional Electrical Stimulation (FES) to achieve tremor suppression. Functional Electrical Stimulation uses electrical currents to evoke a contraction force within a muscle of interest. Functional Electrical Stimulation systems are lighter and more power efficient than mechanical suppression systems but have a set of limitations that should be considered. Firstly, because a force is generated from the musculature, the use of FES can produce fatigue and discomfort in the user over an extended period. Secondly, the presence of both an electrical and mechanical delay in response to electrical stimulus necessitates more advanced prediction software (Zhang, Poignet, Widjaja, & Tech Ang, 2011). Thirdly, as the muscle fatigues, its force output capacity declines, ultimately making control systems more challenging to develop (Allen, Lamb, & Westerblad, 2008).

In addition to FES systems, a small subset of studies has examined the use of SES for suppressing tremor. When applied in an antiphase manner, SES has been shown to be effective, suppressing as much as 88% of tremor peak power (Heo et al., 2015). The main advantage of using SES is that the lack of a physical contraction eliminates the risks of fatigue production in the muscles. This ultimately makes SES systems a more practical option for daily use and simplifies their control systems. The physiological mechanism of how SES systems achieve tremor suppression is less clear, but researchers speculate that

it may operate through the ability of SES to modulate excitability at the spinal level (Dosen et al., 2015; Heo et al., 2015).

2.3 Electromyography: Hoffmann Reflexes and M-Waves

Electromyography (EMG) is a technique in which the electrical activity of muscles is recorded using electrodes placed either superficially or inserted into the muscle. Recording EMG responses to peripheral nerve stimulation is vital for the assessment of spinal excitability and RI.

The Hoffmann reflex (H-reflex) is an electrically induced spinal reflex. The pathway for the H-reflex is identical to the stretch reflex observed in humans, only it bypasses the muscle spindles (Schieppati, 1987). Electrical stimulation of the peripheral nerve activates primary afferent fibres (Ia afferents) that synapse with their corresponding alpha motor neurons in the spinal cord. The action potential then travels down the efferent pathway and through the neuromuscular junction to the muscle fibres, where it elicits an electrical response that can be detected with electromyography (EMG). The EMG response that is detected is the H-reflex and is representative of a compound action potential from several muscle fibres innervated by the excited alpha motor neurons.

In addition to the reflex pathway, peripheral stimulation of the nerve can cause direct activation of motor axons, generating a muscle compound action potential (M-wave) (Palmieri, Ingersoll, & Hoffman, 2004). The M-wave response is representative of a synchronous sum of action potentials produced by the muscle fibres within the recording range of the EMG. The EMG recordings of M-waves and H-reflexes during an H/M recruitment curve can be differentiated based on an estimation of the transmission delay after the stimulus application for a specific testing muscle. Given the length of transmission for both, M-waves and H-reflexes have been shown to appear at 1-5 ms and 14-20 ms, respectively, after the stimulus is given to the flexors and extensors of the forearm (Day, Marsden, Obeso, & Rothwell, 1984; Inglis, Christie, & Gabriel, 2007).

Based on the intrinsic properties of human Ia afferents, the type of stimulation used to evoke H-reflexes must be chosen carefully. The use of longer pulse durations has been shown to recruit relatively more sensory axons, making pulse durations of 0.5-1ms the most effective for evoking H-reflexes (Panizza, Nilsson, & Hallett, 1989). Based on their transmission pathway, H-reflexes are subject to antidromic collisions that can cancel out their EMG signatures entirely. H-reflex tracings initially appear on EMG recordings at low levels of stimulation intensity. With increases in stimulation intensity, H-reflexes gradually increase in size until antidromic depolarizations from peripheral muscle responses begin to cancel them out. This progression is visualized as an H-reflex/M-wave (H/M) recruitment curve and ends when stimulus intensities elicit a maximal M-wave (M-max) response.

Testing of the H-reflex should be done where the H-reflex response is most stable, preferably on the ascending portion of the H-reflex curve, before substantial M-waves are elicited (Grosprêtre & Martin, 2012). M-waves have been shown to be sensitive to small changes in intensity, meaning tests of the H-reflex done on the descending portion of the H-reflex curve could be subject to varying levels of antidromic interference (Pinniger, Nordlund, Steele, & Cresswell, 2001). Electromyogram tracings on the ascending region of the H-reflex curve should appear as a small M-wave response and a larger H-reflex response at their respective timings. H-reflex tracings that appear in conjunction with substantial M-waves could indicate the potential for higher antidromic interference and are less reliable.

The spinal cord has been implicated as an important site for studying changes in the central nervous system (CNS) in response to various sensory inputs (Wolpaw & Tennissen, 2001). The amplitude of the H-reflex is used as an estimate of spinal excitability and is therefore can be used an important indicator of plasticity at the spinal level.

2.4 Reciprocal Inhibition

Reciprocal inhibition (RI) describes the ability of antagonistic muscle pairs to actively suppress one another during various movements (Day et al., 1984). The structural connectivity underpinning this phenomenon is known as reciprocal innervation (figure 1). Reciprocal innervation describes the disynaptic connectivity of antagonistic muscle pairs through an inhibitory interneuron. Early experiments in animals revealed that part or all of RI arises from the excitation of Ia inhibitory interneurons residing in the spinal cord (Baldissera & Hultborn, 1981). The activity of these Ia interneurons is modulated by two main inputs: descending input from the brain and afferent input from peripheral Ia afferents residing in the muscle spindles (Day et al., 1984). The presence of these inhibitory pathways is necessary for the achievement of the coordinated contraction and relaxation of antagonistic muscle pairs (Crone, Hultborn, Jespersen, & Nielsen, 1987)

The use of the monosynaptic H-reflex pathway is the most common means of understanding the role of RI on the control of antagonistic muscle groups in humans (Crone et al., 1987). The amount of RI mediated by peripheral Ia afferents can be estimated from the depression of a test H-reflex combined with an appropriately timed conditioning stimuli applied to the antagonist nerve. The timing of the conditioning stimuli with respect to the evocation of the H-reflex is an important factor in maximizing inhibition. In the forearm, applying the conditioning stimuli simultaneously with the H-reflex testing stimuli has been shown to elicit the most inhibition (Day et al., 1984). Additionally, the amount of RI has been shown to be dependent on the intensity of the conditioning stimuli (Day et al., 1984). It is common that the conditioning stimuli will be applied at or just above the motor threshold (Crone et al., 1987).

It has been speculated that disynaptic reciprocal inhibition could be the mechanism underlying the changes in tremor movement characteristics following SES (Dosen et al., 2015; Heo et al., 2015). This hypothesis stems from the fact that SES-based tremor suppression studies utilize the same out of phase timing initially developed for force producing FES-based systems.

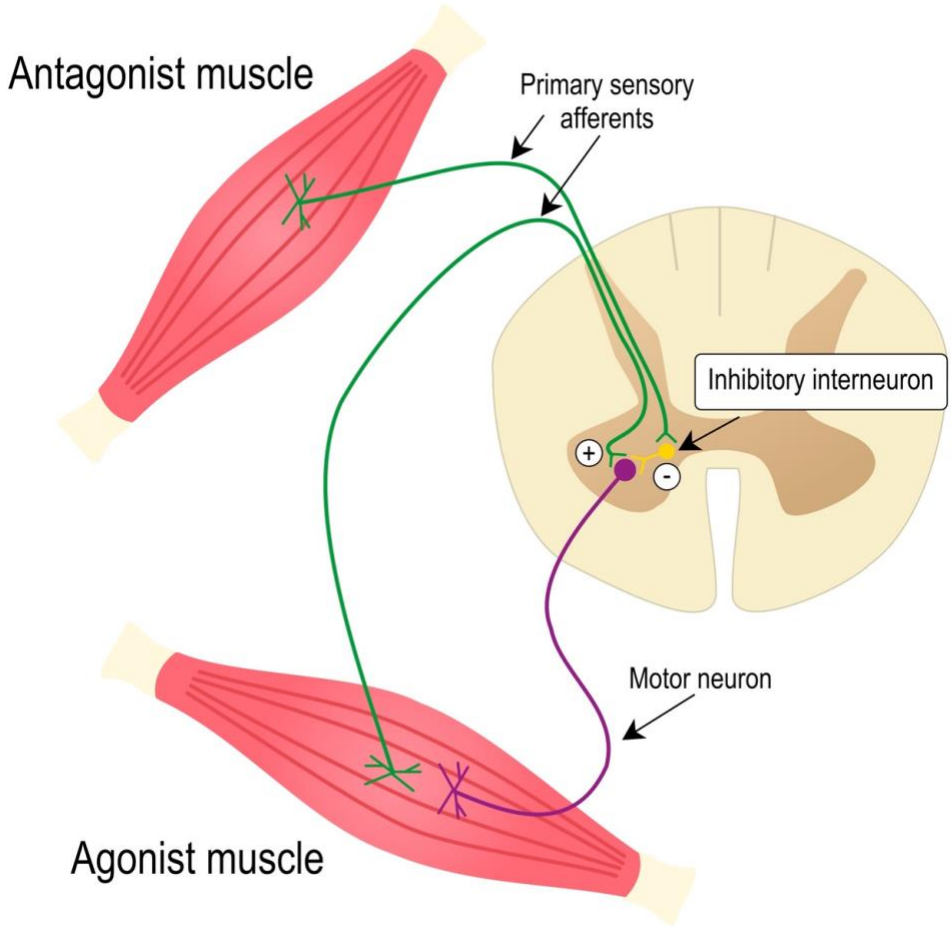

Figure 1. Connectivity diagram illustrating the nerve pathways governing reciprocal inhibition in the forearm. The monosynaptic pathway mediating the H-reflex response is illustrated by the green sensory afferent originating from the agonist muscle and the purple motor neuron.

2.5 The Role of Sensory Electrical Stimulation in Spinal Plasticity

The role of sensory afferents in the production of use-dependent spinal plasticity has been proposed as a means of facilitating motor skill learning in a variety of rehabilitation applications (Wolpaw & Tennissen, 2001). Use-dependent spinal plasticity has been demonstrated in both animal and human studies, and suggests that peripheral input could be an important site for adaptive changes (McCrea, 2001; Pearson, 2000). Furthermore, it

has been shown that SES can produce plasticity in the reciprocal Ia inhibitory pathway in the absence of descending input from the brain, suggesting that peripheral stimulation alone could be sufficient to produce functional adaptations at the spinal level (Perez et al., 2003). However, Perez et al (2003) also showed that spinal plasticity in the lower limb could only be achieved if SES was applied in a pattern that mimicked the afferent feedback that the spinal cord would receive during a regular gait cycle, suggesting that the type of SES for inducing spinal plasticity is highly use-dependent. The optimal pattern of SES application for those with PD tremor has not been investigated, but a reasonable starting point is to mimic the type of patterned feedback the spinal cord would receive during a typical tremor cycle. It has been recently observed that sensory stimulation regimens can create significant changes in tremor characteristics for at least 5 minutes following SES (Heo et al., 2015). If induced spinal plasticity following a session of SES is the underlying mechanism, tremor movement characteristics may represent a surrogate or secondary measure of these changes.

Chapter 3

3 Methods

This study was conducted at Western University in the Wolf Orthopedic Biomechanics Laboratory (WOBL). All materials and methods used in this study were approved by the Western University Health Sciences Research Ethics Board (Appendix A). All participants provided informed written consent prior to their participation.

3.1 Study Design

This study follows a Before/After cross-sectional comparison design, in which each participant served as their own control. Participants attended a single testing session lasting approximately 90 minutes. No compensation was provided to study participants.

3.2 Participants

This study included 25 healthy adult volunteers recruited from the Western University community. Seven participants were not included in the analysis due to a lack of reliable H-reflex responses (see Results). Of those with reliable H-reflexes, eleven males and seven females between the ages of 19-36 years participated in the study. To be eligible, participants had to meet the following criteria: 1) be over 18 years of age; 2) have the cognitive capacity to provide consent; 3) be proficient in English; and 4) have no prior history of neurological disease or damage.

3.3 Experimental Procedure

3.3.1 Initial Setup

Participants laid supine on the testing bed. Given the sensitivity of excitability measures to small alterations in the physical environment, participant comfort was required to minimize the risk of body position shifts during the protocol (Hopkins, Ingersoll, Cordova, & Edwards, 2000). The participants' right arm was placed into an arm apparatus mounted on a small testing table. The arm apparatus held the arm at a constant angle of 45 degrees of shoulder abduction and full elbow extension. The space between the testing table and the arm apparatus provided access to the upper arm for electrode

placement. Within the arm apparatus was a large dowel for participants to rest their palm and to maintain their forearm angle. Participants were instructed to not contract their hand around the dowel to limit voluntary contraction during the testing session. A schematic of initial setup is shown in figure 2.

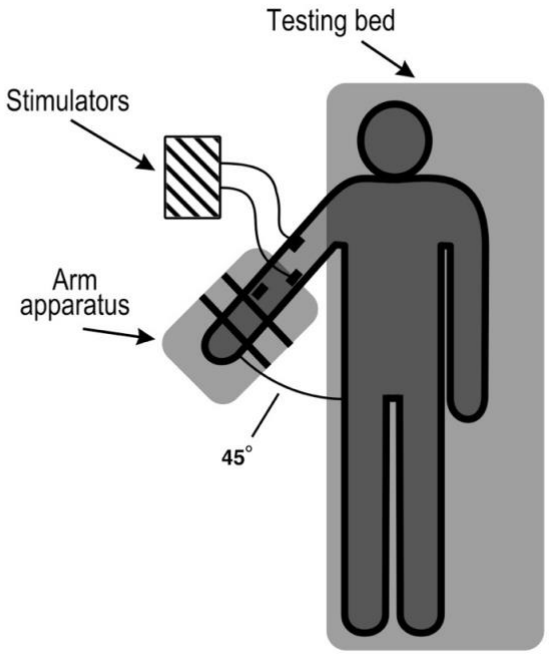

Figure 2. Overhead schematic of the testing setup. Participants laid supine on a large testing bed with their right arm abducted at a 45-degree angle. The right arm was supported by an arm apparatus on an adjacent testing table. The gap between the two testing tables provided direct access to the stimulating sites on the upper arm.

3.3.2 Stimulating Electrode Placement

All recording and stimulating sites were cleaned with an alcohol-based cleansing wipe. Electrical stimulations to the median and radial nerves were delivered using electrodes that consisted of two stainless steel prongs (~2mm in diameter) spaced 2.5 cm apart, embedded in a custom plastic padded brace. Velcro straps attached to the plastic brace held the electrodes in place when secured and electrodes were oriented parallel to the predicted path of the stimulated nerve. Median and radial nerve stimulations were delivered using a Digitimer DS7A, and a Digitimer DS7AH respectively (Digitimer, Welwyn Garden City, UK). All stimulating sites are illustrated in figure 3.

The median nerve stimulation electrode was placed along the medial border of the biceps muscle, approximately 1/3 of the length of the humerus proximal to the elbow joint (figure 3A). The stimulation site was found by manually triggering mild stimulations while gathering feedback from the participants. Participants were asked to report the strength of the stimulation and whether it felt localized or spread distally down the arm. The sensation of stimuli spreading down the arm indicated the proximity of the electrodes to the nerve. The radial nerve simulation site was located on the lateral side of the arm, midway down the humerus (figure 3B). The stimulating electrode was placed between the lateral head of the triceps and the brachialis muscle, which was identified by asking participants to fully supinate the arm and internally rotate the shoulder.

An adhesive bipolar silver/silver chloride surface electrode with a diameter of 1 cm and an interelectrode distance of 2 cm was used to administer sensory stimulation to the extensor carpi radialis longus muscle (ECRL). This electrode was placed just distal to the ECRL EMG electrode, parallel to the direction of the muscle fibres.

3.3.3 EMG Electrode Placement

Two wireless bipolar surface EMG electrodes with a 10mm interelectrode distance were placed on the participants arm (Trigno Avanti wireless sensors, Delsys Inc., Natick, Massachusetts, USA). The digital signals from the EMG sensors were recorded using Spike2 v.9.03 software via Delsys Talker (Cambridge Electronic Design Ltd., Milton, Cambridge, UK) at 2000 Hz. The muscle bellies of the Flexor Carpi Radialis (FCR) and Extensor Carpi Radialis Longus (ERCL) muscles were identified through palpation and voluntary contractions of the arm. Once identified, the EMG electrodes were adhered to the skin and left in place for the remainder of the testing session (figure 3 A and B).

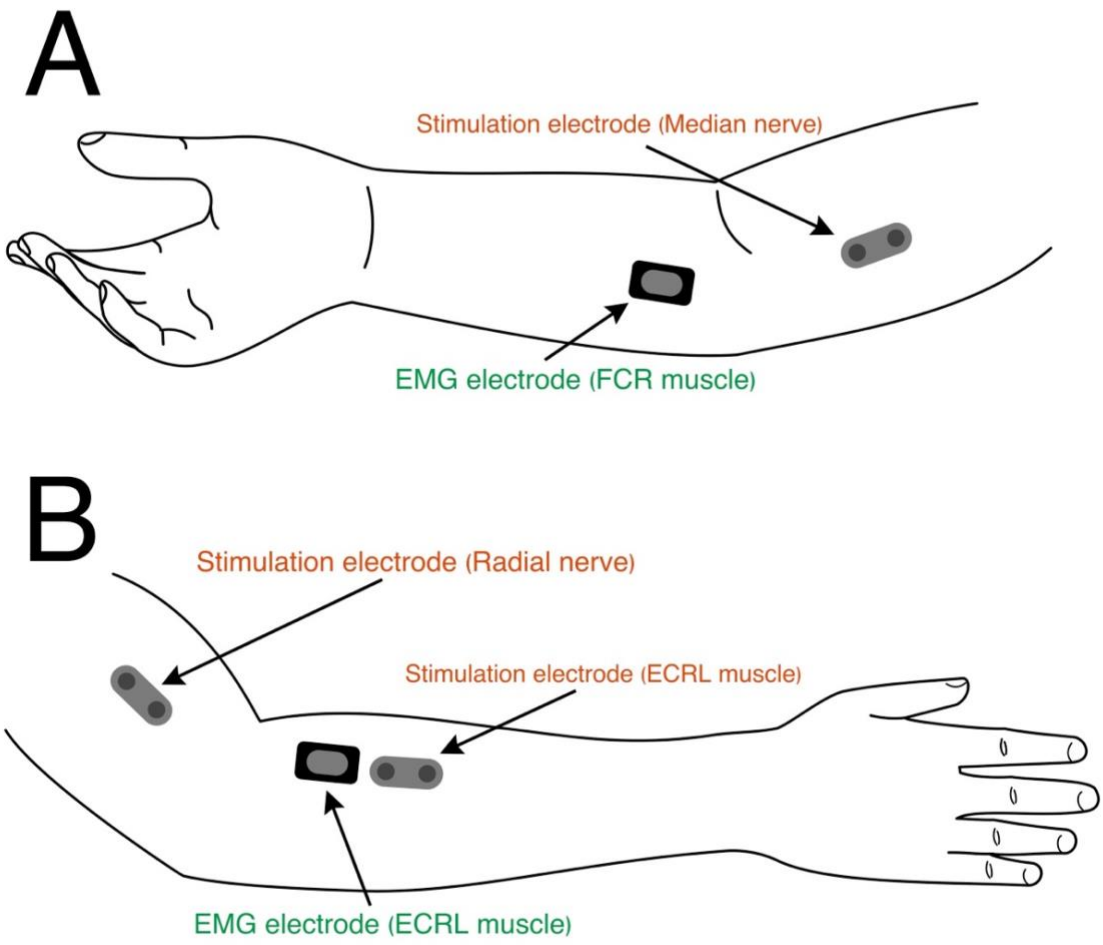

Figure 3. Diagram of recording and stimulating sites. (A) anterior aspect of the right arm, depicting the electrodes used for median nerve stimulation and FCR EMG recording. (B) Posterior aspect of right arm, depicting the electrodes used for radial nerve and ECRL muscle stimulation, and ECRL EMG recording.

3.3.4 Baseline Measures

Following the placement of stimulating and recording electrodes, a series of stimulations were given to obtain baseline measures. One millisecond single pulse stimuli were given to the median nerve to obtain a full H/M recruitment curve and M-max from the FCR muscle. To begin, median nerve stimulation was given in small increasing increments until a visible H-reflex was observed. The H-reflex was identified based on the EMG

latency within a timing window of 14-20 ms after the stimulus artifact (Inglis et al., 2007). Once the smallest H-reflex was detected, stimuli were given in eight second intervals with increasing intensity until the M-max was reached. A minimum of ten stimulations were given within the entire H-M recruitment range. During the recruitment curve, horizontal and vertical cursors were placed by the recording software to identify the amplitude and corresponding stimulation intensity associated with H-max and M-max. Following this, the ECRL M-max was obtained using 200 microsecond single pulse stimulations. Both M-max values for FCR and ECRL were confirmed using a supramaximal stimulation intensity (120% of maximal stimulation intensity).

Peak to peak amplitude measurements were taken from the M-max of both FCR and ECRL recordings. Decreases in the M-max amplitude could mean that the electrode stimulating the nerve of interest has shifted to a less optimal location. The resulting changes in EMG signal could be due to less current being picked up by sensory axons. To combat these factors, all H-reflex and M-wave responses are reported as a relative percentage of the M-max value (Palmieri et al., 2004).

Conditioning stimuli were maintained between 10-15% of M-max for the ECRL muscle. During the H/M-recruitment curve, the stimulus intensity at which H-max was elicited was also identified. Median nerve testing stimuli was delivered at an intensity that was on the ascending part of the H-reflex recruitment curve, just prior to the H-max value.

3.3.5 Testing Intervals

With baseline measures obtained, a series of measurement intervals were initiated. Testing intervals occurred prior to, and at 0-5, 10-15, and 20-25 minutes post sensory stimulation (striped rectangles; figure 4). At each testing interval, reciprocal inhibition was assessed using a FCR H-reflex conditioning test paradigm. That is, the FCR test stimuli applied to the median nerve was conditioned by the ECRL conditioning stimuli delivered to the radial nerve. The conditioning stimulus to the ECRL was used to produce reciprocal inhibition to the FCR. The timing between testing and conditioning stimuli was set at 0s for all participants to maximize the potential for disynaptic inhibition (Day et al., 1984). The FCR H-reflex (unconditioned) was alternated with stimuli in which the

ECRL and FCR stimulation were delivered simultaneously (conditioned). A total of ten conditioned and ten unconditioned reflexes were elicited in an alternating fashion eight seconds apart. M-max measurements for the FCR muscle were used to ensure no muscle fatigue occurred during the testing session. Flexor carpi radialis M-max were elicited after each group of 10 unconditioned/conditioned H-reflexes except at the 10–15-minute post time.

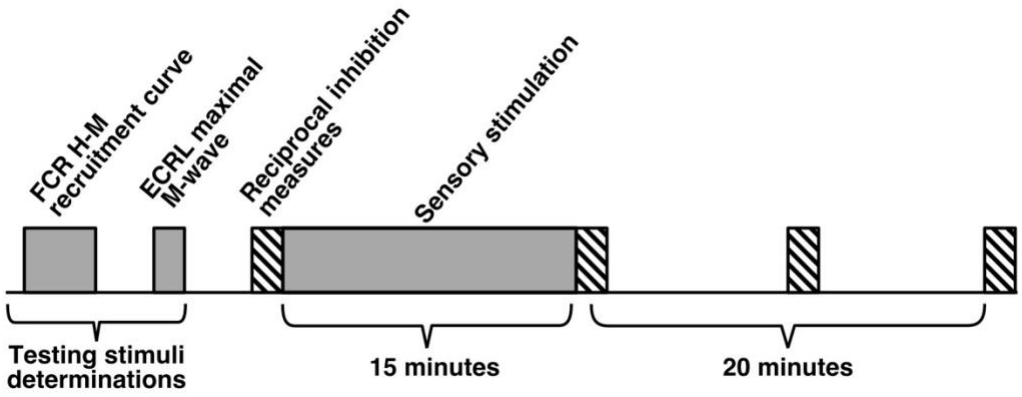

Figure 4. Protocol timeline of events. H-reflex recruitment curves were obtained from the FCR muscle to determine testing stimuli intensity. Maximal M-wave was obtained from the ECRL muscle to determine conditioning stimuli intensity. Striped rectangles indicate when RI measures were assessed (Pre stimulation Baseline and between 0-5 minutes, 10-15 minutes, and 20-25 minutes post-stimulation).

3.3.6 Sensory Stimulation Protocol

Sensory electrical stimulation was administered using a Digitimer DS7A (Digitimer, Welwyn Garden City, UK) constant current stimulator through an adhesive surface electrode placed on the belly of the ECRL muscle adjacent to the EMG electrode (figure 3B). Stimulation parameters were similar to those administered in previous tremor suppression literature and maximized the involvement of Ia afferents residing in the muscle (Bergquist et al., 2011; Dosen et al., 2015). Accordingly, the SES was delivered in 4-pulse bursts with the following parameters:

- ~4.6 Hz burst frequency (i.e. burst delivered every 217 ms)
- 100 Hz pulse frequency (duration of the burst 40 ms)

- 1 ms pulse width

The total duration of the SES session was set at 15 minutes, delivering a total of 16,560 stimulations. Pulse amplitude was set to the same subjective comfort level for all participants. This level was found by achieving a motor response, followed by incremental 1mA decreases until the participant verbally rated the intensity of the sensation during stimulation as a 3 on a 0-10 numerical rating scale (Downie et al., 1978).

3.4 Data Analysis

Bipolar surface EMG recordings were analyzed using Spike2 v.9.03 software. Raw EMG signals were bandpass filtered (Butterworth, 2nd order, 20-450 Hz cut-off). All M-wave and H-reflex magnitudes were measured from peak-to-peak. The ten conditioned and ten unconditioned H-reflex measurements at each testing time point were inspected for outliers based on 1.5X interquartile range method (Tukey, 1977). Outliers, not more than 1 or 2, present in some of the sequences were removed and the remaining conditioned and unconditioned values were averaged thus producing one conditioned and one unconditioned H-reflex value per testing time point for each participant. Flexor M-max values were taken from baseline, 0-5-, and 20-25-minute time point recordings, and were used to normalize data and ensure peripheral fatigue was not produced. Extensor conditioning stimuli were normalized to the M-max value determined at baseline. Reciprocal inhibition (H-reflex suppression) was calculated as the difference between the averaged unconditioned and conditioned H-reflexes at the same time point. Changes in reciprocal inhibition following SES were calculated as the difference between H-reflex suppression at 0-5-, 10-15-, 20–25-minute time point post sensory stimulation and the baseline suppression, normalized to the baseline (initial) suppression for each participant.

3.5 Statistical Analysis

All statistical analyses were performed using SPSS v.25 (IBM Corp, Armonk NY). Statistical outliers were calculated from the ten measurements at each time point and removed prior to averaging as described above. Tests of normality using Shapiro-Wilk Test were performed on all data. A two-way repeated measures analysis of variance

(rANOVA) with condition (unconditioned, conditioned) and time (baseline, prior to SES, and 0-5-, 10-15-, 20-25-minutes after SES) was performed on the amplitude of H-reflexes, comparing the effects of ECRL sensory stimulation on FCR reciprocal inhibition. To ensure that testing stimuli were held constant at all time points, one-way rANOVAs compared FCR M-max, FCR unconditioned H-reflexes, and radial nerve conditioning stimuli amplitude. To determine the association between the H-reflex initial suppression and suppression changes at the 0–5-minute post time point a Spearman's rank correlational analysis was performed. An additional Spearman's rank correlational analysis was performed between the baseline unconditioned H-reflex amplitude and the suppression changes at the 0-5 minute post time point. Based on the correlation analysis, responders and non-responders subgroups were identified and further analyses were performed. Paired t-tests were used to compare changes in RI between baseline and 0-5 minutes after SES on both responders and non-responders as dictated by the smaller sample size for each group. The alpha level for all statistical tests was set at $p \leq .05$ and all data are reported as mean ± SD unless otherwise noted.

Chapter 4

4 Results

4.1 Baseline Measurements and Stability of Recordings

4.1.1 Sample Characteristics

Reliable H-reflexes could not be obtained for all 25 participants recruited. Seven of the participants (all female) did not have measurable H-reflexes at rest and were excluded from the analysis. Additionally, seven of the 18 participants had M-waves present throughout the entire H-reflex curve; for the other 11, H-reflexes could be isolated without M-waves at lower stimulation intensities. Examples of each are shown in figure 5. The presence of M-waves during H-reflex testing were not associated with any differences in outcome.

4.1.2 H/M Recruitment Curves

H/M recruitment curves were gathered for all participants prior to the SES session. Figure 6 shows an individual example of this recruitment curve. For all H/M recruitment curves, smaller increases in intensity were used to obtain the initial part of the curve, until maximal H-reflex was elicited, after which larger increases were used to reduce the total number of stimulations given to obtain the maximal M-wave. Flexor carpi radialis M-max amplitudes ranged from 0.77mV to 6.53mV with an average of 2.38 ± 1.39mV. There was no difference in the amplitudes of FCR M-max elicited at baseline, 0-5- and 20-25 minutes post SES as shown by a one way repeated measures ANOVA ($F(2, 17) = .406$, $p = .670$). An individual example of the FCR M-max across all time points is illustrated in figure 7A. Flexor carpi radialis H-max amplitudes expressed relative to M-max ranged from 6.3% to 52.1% with an average of 25.3 ± 13.4%.

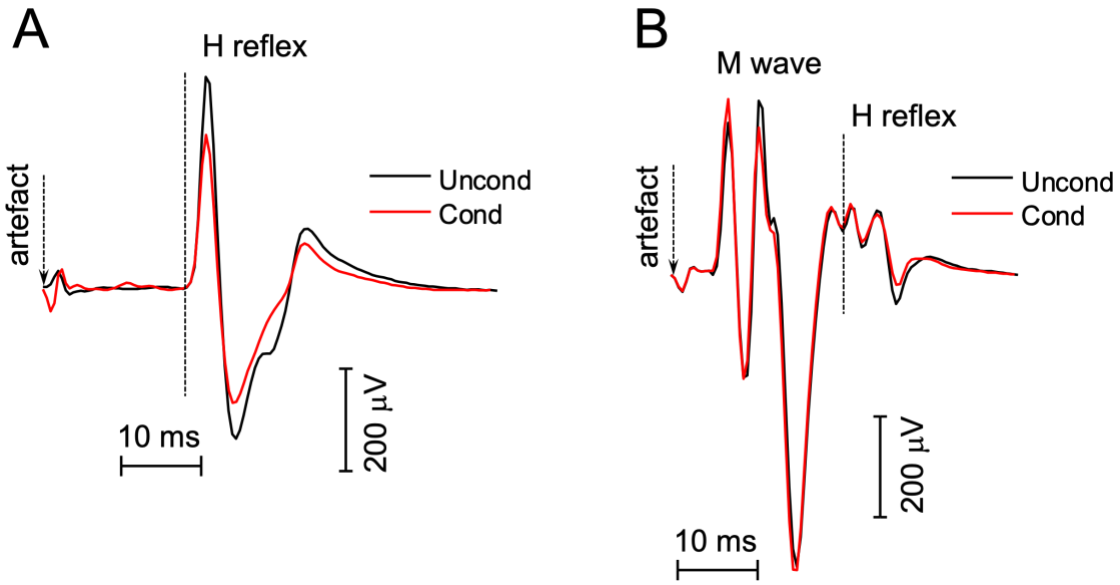

Figure 5. FCR H-reflex recordings. A) Example of a conditioned and unconditioned FCR H-reflex response with no M-wave present. B) Example of a conditioned and unconditioned FCR H-reflex response with M-wave present. Dotted lines indicate the beginning of the H-reflex waveform.

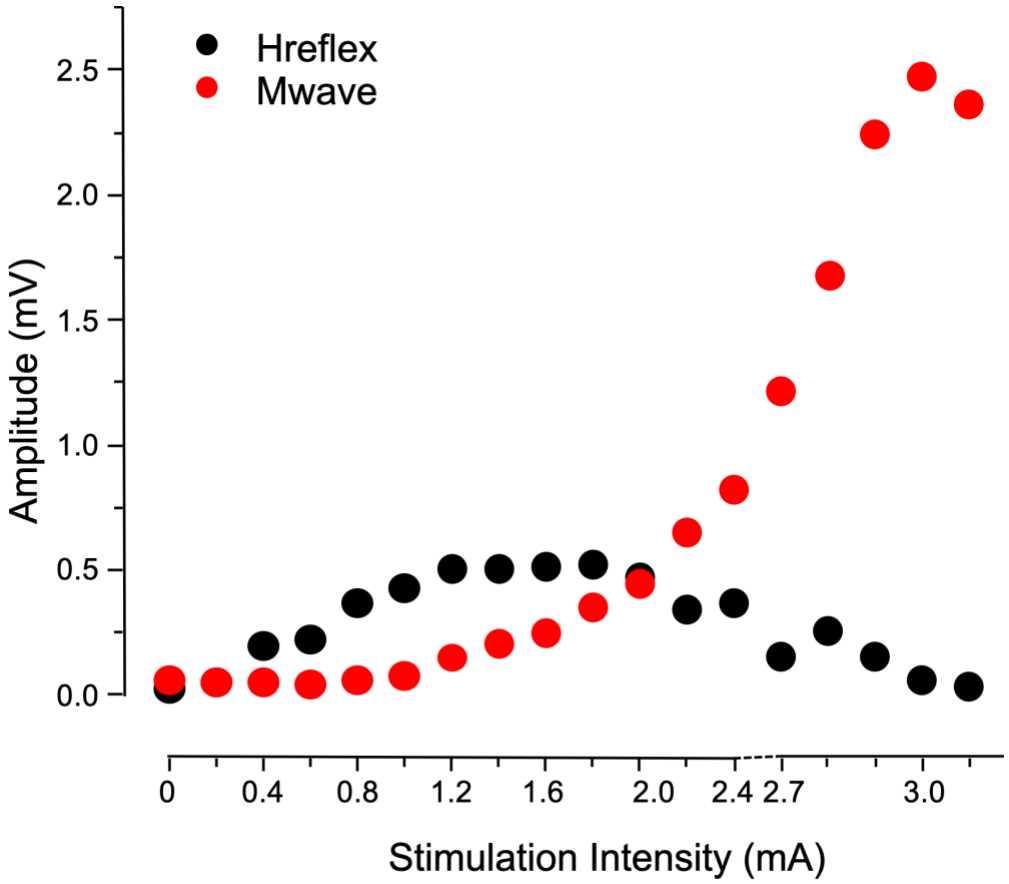

Figure 6. H/M recruitment curve of a representative participant. H-reflex amplitude is depicted by the black circles and M-wave amplitude is depicted by the red circles.

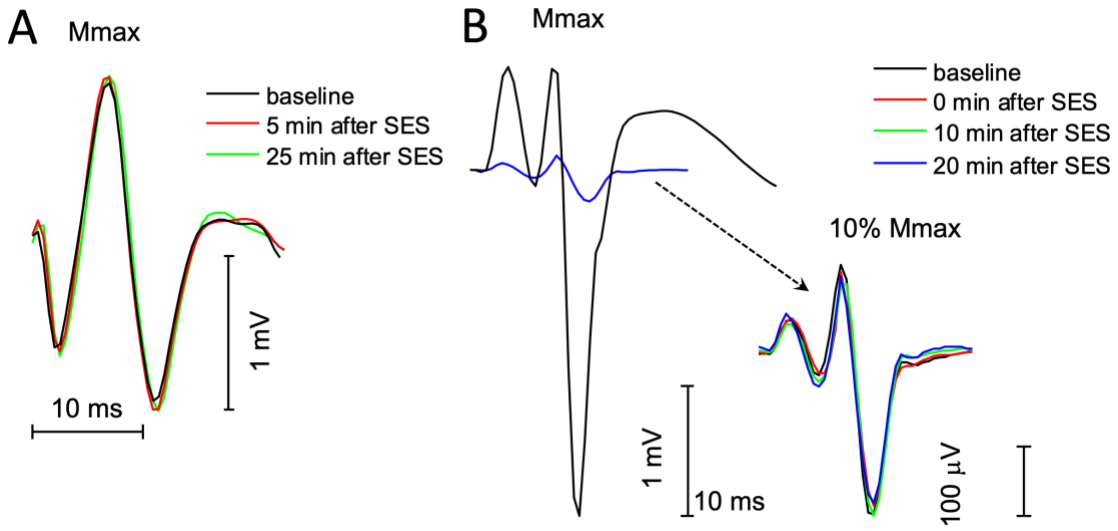

Figure 7. M-waves from FCR and ECRL from a representative participant. A) Overlayed FCR M-max waveforms at baseline, 5 min and 25 min after SES. B) ECRL M-wave of conditioning stimulus set at 10% of M-max. Overlayed waveforms show the stability of conditioning stimulus throughout the experiment.

4.1.3 Unconditioned H-reflexes

The stimulation intensity for the unconditioned H-reflex for each participant was selected with the goal of assessing spinal excitability on the ascending portion of the H-reflex recruitment curve. As such, for this confirmation only, unconditioned H-reflex testing amplitudes were expressed as a percentage of the H-max value measured at baseline. For all 18 participants, the unconditioned H-reflex amplitudes and stimulation intensities corresponded to the ascending portion of the H-reflex recruitment curve. Unconditioned H-reflex amplitudes ranged from 8.9% to 93.6% with an average of 53.0 ± 25.6 % of the H-max value. Participants with M-waves present during H-reflex testing stimulations were, on average, tested with a lower stimulation intensity and had smaller H-reflexes relative to H-max to reduce the size of the accompanying M-waves and prevent partially obscuring the H-reflex responses. Accordingly, average testing values for those with only H-reflexes was 62.1 ± 23.9% of H-max, whereas average testing values for those with M-waves present was 40.4 ± 21.8% of H-max. For all other outcome measures, unconditioned H-reflex testing amplitudes were normalized to the M-max value to reduce the between-participant variability of absolute amplitude measurements. Additionally, this normalization allows for any small changes in electrode positioning throughout the protocol, which would be reflected by a change in the M-max value. During the protocol, five participants had M-max values measured 0-5 minutes after SES that differed by more than ± 10% of baseline, of these three returned to baseline values at the 20–25-minute post time point. The unconditioned H-reflex amplitude at all follow-up time points was maintained at the level measured at baseline. A one-way repeated measures ANOVA revealed no statistically significant differences in the unconditioned H-reflex amplitude among all time points ($F(3, 17) = 1.502$, $p = .225$).

4.1.4 Conditioning M-Wave Amplitude

The conditioning stimulus throughout the experiment was selected to be approximately 10 – 15% of the ECRL M-max for each participant. Extensor carpi radialis longus M-max values ranged from 0.68mV to 3.49mV with a mean of 1.72 ± 0.84mV. Conditioning M-wave amplitudes were normalized with respect to the ECRL M-max value measured at baseline and were on average 11.1 ± 3.8% of M-max. Conditioning M-

wave amplitudes at all time points were maintained at the level measured at baseline. A one-way repeated measures ANOVA revealed no statistically significant differences in the conditioning M-wave amplitude between all time points ($F(3, 16) = 1.119$, $p = .351$). An individual example of the ECRL M-max and the conditioning M-waves across all time points is illustrated in figure 7B.

4.2 Reciprocal Inhibition

A two-way repeated measures ANOVA comparing the effects of conditioning stimuli and follow-up time on measures of H-reflex amplitude revealed a significant main effect of conditioning on the H-reflex response ($F(1, 17) = 21.386$, $p < .01$). No significant main effect of time or interaction effect were found ($F(3, 17) = 1.435$, $p = .243$; $F(3, 17) = .523$, $p = .668$). Mean peak-to-peak unconditioned and conditioned H-reflex amplitudes are shown in figure 8. Although RI was induced by the conditioning stimuli, the amount of reciprocal inhibition did not change over time; the observed change from 28.3 ± 23.1% at baseline to 32.1 ± 19.7% at 0-5 minutes post SES was not statistically significant.

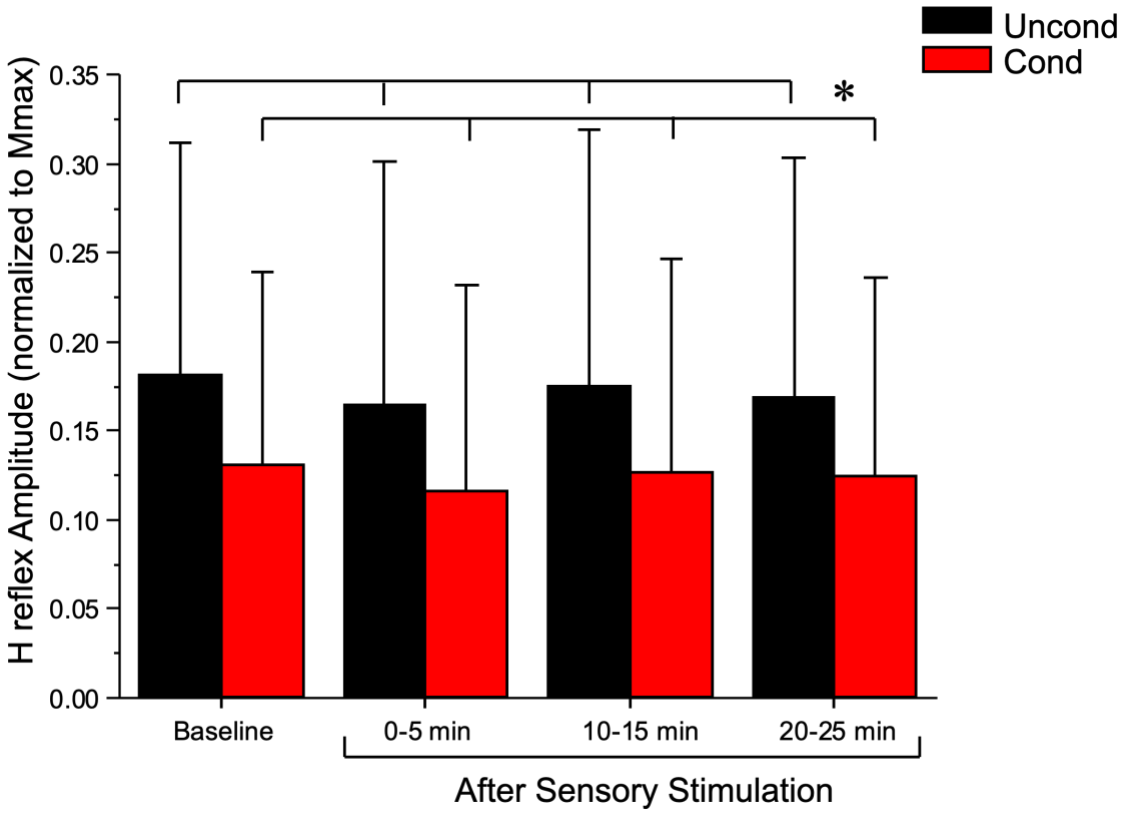

Figure 8. Amplitude of H-reflexes across all time points and conditions. Unconditioned (black bars) and conditioned (red bars) H reflexes for all 18 participants prior to sensory stimulation (baseline), immediately after the sensory stimulation (0-5 min) and at follow up (10-15 min and 20-25 min) are presented. Data are mean and SD. Asterisks denotes a significant difference between unconditioned and conditioned H-Reflexes ($p<0.05$).

4.3 Correlational and Subgroup Analysis

Further analysis of the individual participants' data during visual inspection of unconditioned and conditioned H reflexes plotted for all testing periods revealed a possible relationship between the amount of reciprocal inhibition evoked prior to sensory stimulation (baseline suppression) and the amount of change in RI immediately after the SES. The observed relationship suggested that higher baseline suppression (above 20%) could be limiting the change in RI after SES (at the 0-5 min time point) (figure 9A). Figure 9 A shows that individuals who experienced smaller amounts of initial

suppression appear to have the greatest changes in excitability immediately following SES. Confirming this trend, a Spearman's rank correlational analysis showed a statistically significant moderate negative correlation ($\rho = -.531$; $p = .023$) between changes in RI and baseline suppression values. There was no relationship between the baseline unconditioned H-reflex amplitude and changes in RI at the 0-5 minute time point ($p = .363$).

Ordering the amount of change in reciprocal inhibition from baseline to immediately after the SES from the smallest to the largest (Figure 9B) showed the potential existence of 2 groups of participants – "responders" and "non-responders". The group of "responders" (n=7) exhibited a change in RI that was above the zero line (a line representing no change in RI between baseline and immediately after the SES) and a group of "non-responders" being at or below the zero line. This indicated that the group of "responders" experienced an increase in RI after the session of SES. The "responders" group had 2 females and 5 males, whereas the "non-responders" group had 5 females and 6 males. Based on the smaller size of these subgroups, paired t-tests were chosen to evaluate RI between baseline and 0-5 minutes post SES. Within the responders group, the mean RI increased significantly from 13.4% to 33.3% between baseline and 0-5 minutes post ($t(7) = -2.88$, $p = .028$), suggesting the SES session increased the level of RI for this group (Figure 10). Within the non-responders group, a smaller but statistically significant decrease in RI was observed from 37.7% at baseline to 31.4% at 0-5 minutes post ($t(11) = 2.57$, $p = .028$), suggesting the SES session decreased the level of RI for this group (Figure 10).

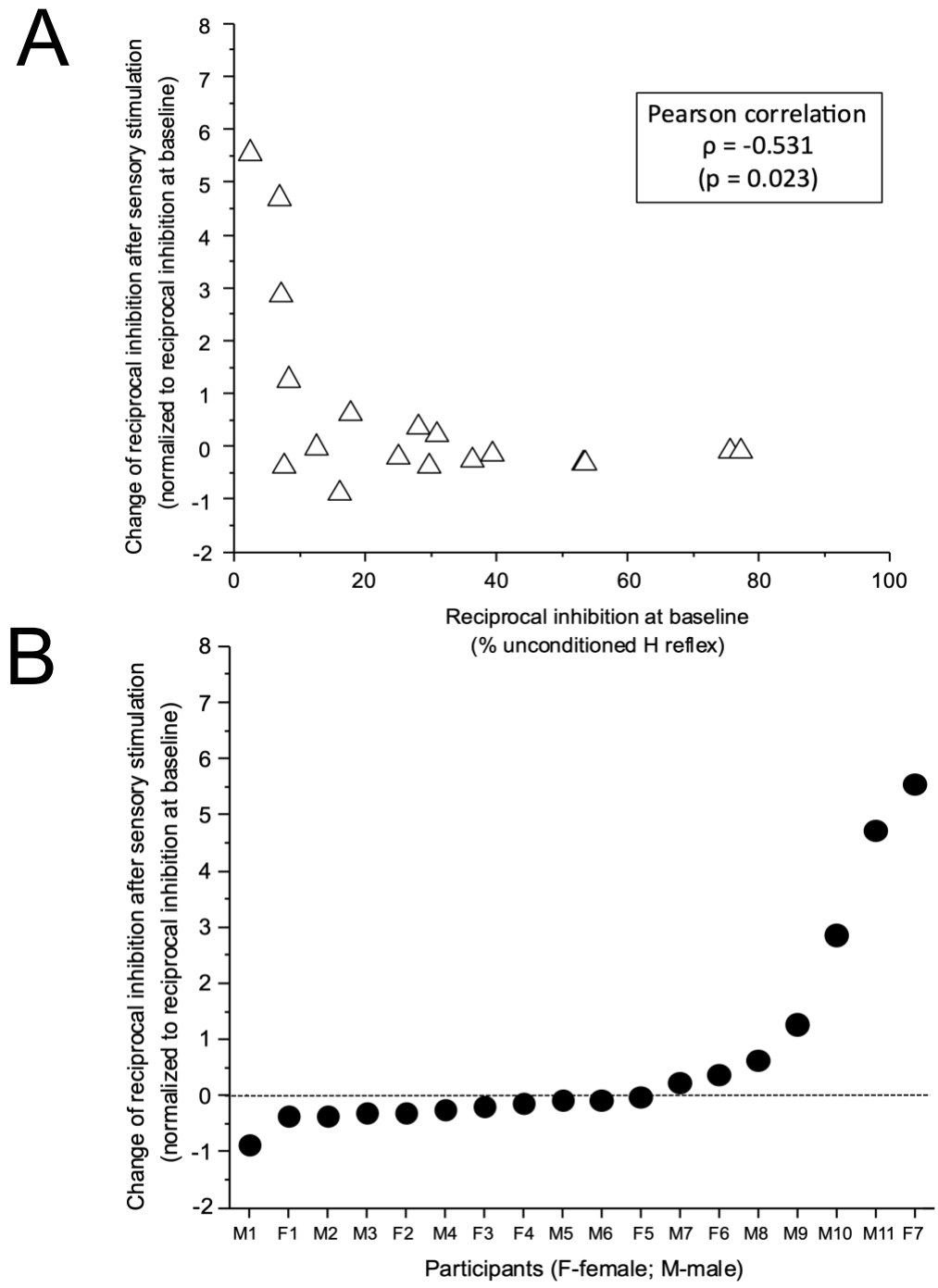

Figure 9. Reciprocal Inhibition A) Scatter plot showing changes in RI plotted against baseline suppression. The observed trend suggests a potential split in the responses to the SES session. B) Scatter plot ordered from smallest to largest change in RI between baseline and 0-5 minutes post SES. All participants above the zero line were included in the "responders" group, while "non-responders" were at or below the zero line.

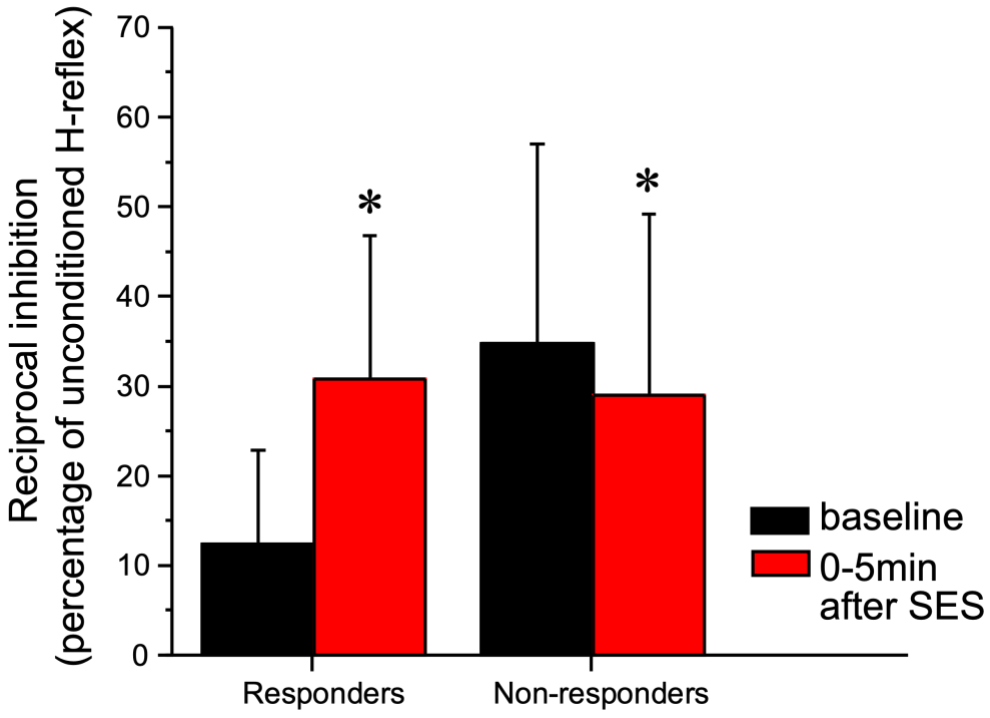

Figure 10. Reciprocal inhibition at baseline and 0-5 minutes post, expressed as a percentage of the unconditioned H-reflex for both "responders" and "non-responders." The asterisks denote significant changes in RI from baseline ($p<0.05$).

5 Discussion

5.1 Stability of EMG Data

5.1.1 Unanalyzed Participants

H-reflexes were not attainable in 7 participants and therefore were not included in the analysis. Based on previous literature, there are three possible explanations for this. First, it has been shown previously that FCR H-reflexes are attainable at rest in approximately 44% of participants, with the majority of participants requiring background voluntary EMG activity to evoke H-reflexes (Miller, Newall, & Jackson, 1995). In the present study, 18/25 participants recruited (72%) had H-reflexes at rest. The higher percentage of H-reflex responses at rest in the present study could be due to methodological differences, as the present study applied stimulations above the elbow compared to the cubital fossa location used in Miller et al. (1995). Second, the type of stimulating electrodes used in the present study could have limited the ability to evoke H-reflexes at lower stimulation intensities. The use of monopolar stimulation, with the cathode over the nerve and the anode on the opposite side of the limb, is recommended to excite Ia afferents at lower thresholds (Pierrot-Deseilligny & Mazevet, 2000). However, considerations for the type of stimulating electrode should be made based on the location of the nerve in relation to other nerves. In the upper arm, bipolar electrodes can be used to limit current from activating neighboring nerves (Pierrot-Deseilligny & Mazevet, 2000). Bipolar stimulating electrodes were used in the current study because the stimulation sites for the median and radial nerves are on opposite sides of the upper arm. The choice to use bipolar stimulating electrodes was made on the basis of isolating nerve activation but also knowing the potential tradeoff in the ability to evoke H-reflexes. Third, the presence of M-wave responses during FCR H-reflex testing is known to often obscure the H-reflex onset (Miller et al., 1995). Therefore, it is possible that the participants in this group had M-waves present that obscured H-reflex recognition.

Additionally, all 7 of the participants whose H-reflexes could not be attained were female. There is currently no evidence that suggests males and females differ in their capacity to respond to SES regimens. However, there could be factors relating to the methodology used, as well as the anatomical site of testing that created a sex-linked difference in the ability to record H-reflexes. Previous work has demonstrated sex differences in H-reflex and M-wave response thresholds. Females have been shown to exhibit higher thresholds for detecting an initial H-reflex compared to men, suggesting higher relative stimulation intensities must be used to elicit the H-reflex and M-wave responses (Dornowski, Kolosova, & Gorkovenko, 2017). Researchers have speculated that this could be due to higher electrical resistances caused by a greater amount of subcutaneous fat at the stimulation sites of females, compared to males (Kenney, Wilmore, & Costill, 2015).

5.1.2 Baseline H/M Recruitment Curves

H/M recruitment curves were measured for all 18 participants. Results show that seven of the 18 participants had M-waves present during the entirety of the H/M recruitment curve. Although not uncommon, the appearance of an M-wave on EMG recordings at low intensities suggests that the motor threshold was reached at stimulation intensities lower than that needed to excite the motor neurons in the spinal cord (Burke, Hallett, Fuhr, & Pierrot-Deseilligny, 1999). Monitoring the size of M-waves during H-reflex testing is suggested when performing dynamic movements, as they serve to indicate the stability of the stimulation conditions (Pierrot-Deseilligny & Mazevet, 2000). In the current study, M-waves were used to monitor stimulation conditions, however, because trials were static, significant changes in the stimulation conditions within a single testing interval were not observed.

Results show that all 18 participants were tested at an intensity corresponding to the ascending portion of the H-reflex recruitment curve (below the H-max). Because of the nature of antidromic collisions in the axons of motor nerves, testing of the H-reflex should be done on the ascending portion of the H-reflex recruitment curve. Because antidromic depolarizations from peripheral excitation are primarily from large diameter/fast motor units (Henneman& Mendell, 2011), the descending portion of the

curve is representative of only small motor neurons where collisions have not taken place (Pierrot-Deseilligny & Mazevet, 2000). Testing on the ascending portion of the H-reflex recruitment curve ensures that the H-reflex response is representative of the motor units that are most sensitive to excitation and inhibition (Pierrot-Deseilligny & Mazevet, 2000). Additionally, testing of the ascending portion of the H-reflex recruitment curve has been shown to be more reliable, as the M-wave response is most stable during this phase (Grosprêtre & Martin, 2012). The results suggest that the unconditioned H-reflex responses measured during the protocol concur with methodological considerations made by previous research.

5.1.3 Unconditioned H-reflexes

Previous research has shown mean FCR H-max values to be on average less than 37% of M-max (Pascual-Valdunciel et al., 2019). The present study supports this finding, as FCR H-max amplitudes were on average 25.26 ± 13.43% of M-max. Results showed no difference in unconditioned FCR H-reflexes at all time points, suggesting that the protocol successfully controlled for changes in unconditioned responses at all post SES time-points.

5.1.4 Conditioning M-wave Responses

It has been previously reported that the amount of RI measured in the FCR muscle is dependent on the intensity of the conditioning stimuli (Day et al., 1984), therefore it is imperative that the conditioning stimuli intensity are held constant at all time points. The ECRL M-wave amplitude in response to radial nerve conditioning stimuli was used to reflect the proportion of the motor neurons recruited. This method allows for normalization between participants, and accounts for potential changes in the stimulating conditions. Results showed no difference in radial nerve conditioning M-wave amplitudes at all time points, suggesting that the protocol successfully controlled for potential changes in stimulation conditions.

5.2 Reciprocal Inhibition

The results of the current study indicated that the radial nerve conditioning stimulation paradigm generated significant RI in the FCR muscle across all measured time points. Reciprocal inhibition of the FCR muscle was achieved through the simultaneous application of conditioning stimuli to the radial nerve consistent with short latency disynaptic RI observed in previous research (Day et al., 1984). Short latency disynaptic RI is known to arise through the excitation of inhibitory interneurons residing in the spinal cord (Day et al., 1984). In the present study, the inhibition of evoked FCR H-reflexes indicates that the conditioning stimuli paradigm used was successful in estimating the influence of the antagonistic inhibitory interneuron on FCR motor neuron excitability.

Contrary to previous research (Perez et al., 2003; Thompson, Lapallo, Duffield, Abel, & Pomerantz, 2011), the results of the present experiment showed no change in the level of RI between antagonistic muscle pairs following a session of SES. According to Yeh, Fong, and Huang (2015), such discrepancies could be due to differences in stimulation protocols, including the duration and intensity of stimulation used. In the present study, all parameters except the stimulation intensity were fixed. Based on potential applicability to WTSDs, the stimulation intensity was chosen to be anchored to subjective discomfort, rather than a physiological measure like the motor threshold. During the protocol, adjustments in the absolute intensity of SES were made to ensure participants remained at a level of 3 on a 0-10 numeric rating scale. This stands in contrast to Perez et al. (2003), who fixed SES intensity to the level of the motor threshold.

It is possible that the level of stimulation used in the current study was too low to influence measures of RI for all participants. Differences in the comfort of SES can be influenced by many factors outside of those collected in the present study, including participant hydration, subcutaneous fat content, and the type of electrodes used (Keller & Kuhn, 2009; Lyons, Leane, Clarke-Moloney, O'brien, & Grace, 2004; Vance, Rakel, Dailey, & Sluka, 2015). The goal of any SES regimen is to selectively activate primary sensory afferents, however, electrical currents will inadvertently activate pain receptors on the skin surface. With increasing stimulation intensities, activation of pain receptors

can produce discomfort, ultimately limiting the effectiveness of SES regimens (Gracanin & Trnkoczy, 1975). Because females typically have greater fat contents at the stimulating sites, a greater proportion of the stimulation current could have been picked up by pain receptors, rather than primary sensory afferents. Physiological differences between males and females could potentially mediate the responder and non-responder groups. In the split between responders and non-responders, females were much more likely to be in the non-responder group. While speculative, females could have been more likely to fall into the non-responder group because they reported a pain level of 3 at a lower absolute stimulation intensity. Unfortunately, we did not record the absolute stimulation intensity. Although not analyzed as a primary outcome, Lyons et al. (2004) showed a slight difference between males and females regarding their pain threshold in response to electrical stimulation, with reaching their threshold at higher relative intensities.

The influence of SES on FCR motor neuron excitability cannot be completely ruled out. Because participants were tested at a sub-H-max intensity, potential changes in the H-max amplitude could not be captured. Full H-M recruitment curves could not be obtained in a timely manner at each time point, allowing for the possibility that unconditioned stimuli could have been delivered at a different relative intensity because they were chosen based only on the H-max value obtained at baseline. The present study normalized H-reflex responses to their most recent M-max value to account for the potential influence of altered stimulating electrode conditions, however, this normalization method does not take into account potential changes in the H-max that would be reflective of maximum FCR motor neuron excitability. Changes in the H-max are representative of the maximal reflex activation (Palmieri et al., 2004), and would be assessed through an H-max to M-max ratio. While little to no changes in M-max were observed in the present study, a full H/M recruitment curve would need to be obtained to accurately estimate the H-max to M-max ratio.

Exploratory analysis identified a statistically significant negative correlation between the level of baseline suppression and the relative change in suppression at the 0–5-minute

post time point. The presence of this association suggests that the higher the baseline RI, the harder it is to produce or measure changes in RI from SES. Compounding the fact that some participants may have not received enough SES, this relationship suggests that the amount of SES needed may be based on the level of RI present at baseline, necessitating those with high baseline RI to receive higher intensity stimulation that would exceed a subjective level of 3 on the numeric rating scale. However, the role of the interstimulus interval may influence this relationship as well. Although a simultaneous delivery of the testing and conditioning stimuli was shown to be optimal in pilot testing, small individual differences in the optimal delivery (+1ms, - 1ms, etc….) may explain the variability in the amount of RI observed in this study.

Although whole group RI changes did not reach significance, the amount of RI appears to follow the same recovery trend reported in Perez et al. (2003), with slight increases at the 0–5-minute mark followed by a return to baseline levels by the 10-15- and 20-25-minute marks.

5.3 Role of Central Mechanisms

As described in Day et al. (1984), the activity of inhibitory interneurons in the spinal cord are determined by afferent feedback and descending input from higher levels of the CNS. Most research investigating the role of descending influence has been done in the context of various voluntary contraction patterns, as outlined by Crone and Nielsen (1994). As H-reflexes in the present study were elicited at rest, many of these established contributions are less applicable. However, Thompson, Chen, and Wolpaw (2009) found evidence that descending input can influence H-reflex size irrespective of normalized background EMG facilitation. The changes observed in Thompson et al. (2009) were generated through an operant conditioning regimen spread out over 10 weeks; therefore, it is highly unlikely that descending influences in the absence of EMG activity in the present study systematically influenced findings. As a result, it is more likely that the inhibitory effect of afferent feedback on spinal interneurons is the underlying mechanism for reciprocal inhibition measured in the current experiment.

5.4 Limitations and Future Research Directions

Although normalization of FCR H-reflexes to M-max values ensured the stability of stimulating site conditions, the lack of H/M recruitment curves at all time points means that we cannot rule out changes in FCR motor neuron excitability during the experiment. Future studies should aim to evaluate both RI and FCR motor neuron excitability immediately following SES. If there was a reduction in motor neuron excitability, we would expect to see this as a reduction in the amplitude of the unconditioned H-reflexes. There was no significant decrease in the unconditioned H-reflex after the SES session.

The ability of Ia afferents to transmit the conditioning stimuli during the SES session presents a limitation. It has been shown that axonal hyperpolarization could effectively lower the excitability of both motor and cutaneous afferents following high frequency electrical stimulation (Kiernan, Lin, & Burke, 2004). If differences in the ability of Ia afferents to conduct the conditioning stimuli to the ECRL motor neuron were present, this could reduce the amount of RI at the 0-5 minute post time point for some participants.

Further, evidence suggests facilitation of the FCR H-reflex through voluntary activation could allow earlier detection of the H-reflex, something that appeared to limit the current study's results, particularly in female participants. Future studies conducted in the forearm should elicit FCR H-reflexes in conjunction with normalized voluntary activation. However, background voluntary EMG activity can obscure the H-reflex in the forearm, particularly with bipolar stimulating electrodes. Therefore voluntary activation should be employed with great caution.

Finally, the existence of subgroup responses in the present study suggests that the method of setting SES intensity used may not produce reliable changes in RI for all participants. To remove participant subjectivity, future studies should aim to standardize SES intensity to a physiological indicator such as the motor threshold. Regarding the development of a WTSD, the present experiment suggests that stimulation intensities that participants ranked as a pain level of 3 on a 0-10 scale may not be intense enough to produce changes in RI for all wearers but a higher level of discomfort would be a deterrent to the long-term use of a WTSD.

5.5 Conclusion

It was hypothesized that the spinal cord would exhibit a transient increase in RI of the FCR muscle following a session of patterned SES applied to the antagonist ECRL muscle. The findings of the present experiment suggest that increases in RI are only observed in a “responders” subgroup consisting of predominantly male participants. For these responders, RI was significantly increased 5-minutes following SES as compared to baseline RI prior to SES. Although increases in RI were present in some participants, more research is needed to better understand the optimal SES intensity required to produce reliable changes in RI. Physiological differences between males and females may explain this relationship, but more research is needed to investigate this.

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Appendices

Appendix 1. Ethics approval

Western Research

Date: 11 November 2020

To: Dr. Jayne Garland

Project ID: 116007

Study Title: Spinal Excitability Changes Following Sensory Electrical Stimulation of The Upper Limb

Application Type: HSREB Initial Application

Review Type: Delegated

Full Board Reporting Date: 01/Dec/2020

Date Approval Issued: 11/Nov/2020 11:52

REB Approval Expiry Date: 11/Nov/2021

Dear Dr. Jayne Garland

The Western University Health Science Research Ethics Board (HSREB) has reviewed and approved the above mentioned study as described in the WREM application form, as of the HSREB Initial Approval Date noted above. This research study is to be conducted by the investigator noted above. All other required institutional approvals must also be obtained prior to the conduct of the study.

Documents Approved:

Document Name	Document Type	Document Date	Document Version
Sensory stimulation research plan V1	Protocol	01/Oct/2020	1
Data collection form V1	Other Data Collection Instruments	01/Oct/2020	1
Sensory stimulation online poster V2	Recruitment Materials	09/Nov/2020	2
Direct Recruitment Email Script V2	Email Script	09/Nov/2020	2
Email Script for MASS Recruitment V2	Email Script	09/Nov/2020	2
Sensory stimulation LOIC V3	Written Consent/Assent	10/Nov/2020	3

No deviations from, or changes to, the protocol or WREM application should be initiated without prior written approval of an appropriate amendment from Western HSREB , except when necessary to eliminate immediate hazard(s) to study participants or when the change(s) involves only administrative or logistical aspects of the trial.

REB members involved in the research project do not participate in the review, discussion or decision.

The Western University HSREB operates in compliance with, and is constituted in accordance with, the requirements of the TriCouncil Policy Statement: Ethical Conduct for Research Involving Humans (TCPS 2); the International Conference on Harmonisation Good Clinical Practice Consolidated Guideline (ICH GCP); Part C, Division 5 of the Food and Drug Regulations; Part 4 of the Natural Health Products Regulations; Part 3 of the Medical Devices Regulations and the provisions of the Ontario Personal Health Information Protection Act (PHIPA 2004) and its applicable regulations. The HSREB is registered with the U.S. Department of Health & Human Services under the IRB registration number IRB 00000940.

Please do not hesitate to contact us if you have any questions.

Sincerely,

Ms. Nicola Geoghegan-Morphet , Ethics Officer on behalf of Dr. Joseph Gilbert, HSREB Chair

Note: This correspondence includes an electronic signature (validation and approval via an online system that is compliant with all regulations).

Appendix 2. Letter of Information and Consent Form

Western

Letter of Information

Title: Spinal Excitability Changes Following Sensory Electrical Stimulation of The Upper Limb

Dr. Jayne Garland, BScPT, MClSc, PhD (Principal Investigator)
Dean, Faculty of Health Sciences, Western University.
Western University, [redacted]

Co-Investigators: Mary Jenkins, Devin Box, Anita Christie, Tanya Ivanova, Ana Luisa Trejos, Yue Zhou

You are being invited to participate in a research study to determine the effects of sensory stimulation on spinal excitability of the upper limb. This research will form the basis of Devin Box's thesis. You are being invited because you are 18 years of age or more, have no known neurodegenerative disorders or damage, have the capacity to provide consent, and are proficient in the English language. We are seeking 20 participants for this study. One of the investigators on this project will review this letter of information with you, describe the procedure in detail, and answer any questions you may have.

Background:

The objective of this research is to determine the effects of sensory stimulation on spinal excitability of the upper limb. Your participation in this research study will aid in the development of control systems that will enable wearable devices to suppress tremor and improve voluntary motion.

Procedures:

Experiments will be conducted in the Wolf Orthopaedic Biomechanics Laboratory, 3M Centre at Western University. You will be asked to attend a single session lasting approximately one and a half hours. It is requested that you refrain from taking any stimulants, e.g. coffee, or suppressants, e.g. alcohol within 4 hours prior to the testing session. You will be lying in a comfortable position on a testing bed. The skin of your arms will be cleansed using electrode paste, after which noninvasive electrodes will be placed on various locations of your arm. Brief testing electrical stimulation will be delivered to your arm to establish the point at which the stimulation causes the muscles of your forearm to contract, both the minimum and the maximum value.

Next, a 15-minute bout of sensory-level stimulation will be applied to the extensor group of your forearm. These stimuli will not cause your muscle to contract and will not be painful. Immediately after this sensory stimulation bout, the same testing electrical stimulation will be obtained and again after 10 and 20 minutes of rest.

The experiment is expected to take 1.5 hours to complete. Your participation in this study is voluntary, and you can withdraw and have your data destroyed at any time. By participating in the study, you acknowledge that you are 18 years of age or more and have no known neurological diseases or damage.

Risks:
In rare cases, the electrodes used to stimulate the muscles may cause some redness or irritation on the skin in some individuals. Please let the investigators know if you feel itching under the electrodes at any time during the experiment. If you are injured as a result of this study, your medical care will be provided at no cost.

Benefits:
Although you may not benefit directly from this study, your participation will contribute to our knowledge of how to control wearable mechatronic devices designed to suppress tremor.

Participants will not be compensated for participating in this study. Participants will not be reimbursed for expenses incurred, such as transportation or parking.

Confidentiality:
The digital data collected during the experiment will be immediately uploaded to Western University OneDrive, where it can be accessed by research staff for analysis. Digitally collected data will be immediately destroyed from the data collection computer following its upload to Western University OneDrive and will not be physically transported at any point. The confidentiality of your data will be ensured by the assignment of an alphanumeric code to your information. This code will be associated with your “Data Collection Form” containing your subject number, age, and sex. The master list connecting your name to your “Data Collection Form” will be kept in a locked filing cabinet that only Devin Box, Jayne Garland, and Mary Jenkins have access to. Your data will be retained for seven years. The Western University Health Sciences Research Ethics Board and may require access to identifiable study records to monitor the conduct of the study and for quality assurance purposes.

You are not waiving any legal rights by agreeing to participate in this study. Your participation in this study is voluntary, and you can withdraw and have your data destroyed at any time until publication. If you have any questions about the conduct of this study or your rights as a research participant you may contact the Office of Human Research Ethics [redacted] A copy of this information package is yours to keep for your personal records.

Western

If you have any questions or concerns regarding participation in our study, please contact Dr. Jayne Garland [redacted]

Western

CONSENT FORM

Title of Research: **Transient Spinal Excitability Changes Following Sensory Stimulation of The Upper Limb**

Principal Investigator: Dr. Jayne Garland

Co-Investigators: Mary Jenkins, Devin Box, Anita Christie, Tanya Ivanova

For the Participant:

I have read and understand the above information describing this study. I have had the purposes, procedures and technical language of this study explained to me. I have been given sufficient time to consider the above information and to seek advice if I chose to do so. I have had the opportunity to ask questions which have been answered to my satisfaction. I am voluntarily signing this form. I will receive a copy of this consent form for my information.

If at any time I have further questions, problems or adverse events, I can contact Dr. Jayne Garland, the principal investigator of the project, at [redacted] or any of the investigators and collaborators on the project.

If I have any questions about your rights as a research participant or the conduct of this study, I may contact The Office of Research Ethics [redacted]

By signing this consent form, I am indicating that I agree to participate in this study.

Name of Participant (please print)	**Signature of Participant**	**Date**
Name of Person Obtaining Informed Consent	**Signature of Person Obtaining Informed Consent**	**Date**

Curriculum Vitae

Name:	Devin Box
Post-secondary Education and Degrees:	The University of Western Ontario London, Ontario, Canada 2015-2019 B.Sc. Honours Specialization in Kinesiology The University of Western Ontario London, Ontario, Canada 2019-2021 M.Sc. Candidate, Kinesiology, Integrative Biosciences
Honours and Awards:	Graduate Distinction The University of Western Ontario 2015-2019 Province of Ontario Graduate Scholarship 2020-2021
Related Work Experience	Graduate Teaching Assistant The University of Western Ontario 2019-2021
Presentations:	Spinal Excitability Changes Following Sensory Electrical Stimulation of The Forearm *Oral Presentation* Kinesiology Graduate Student Research Conference The University of Western Ontario, London ON 2021