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The Role of Voluntary Descending Control in Enhancing Motor Function Via Transcutaneous Spinal Cord Stimulation

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by
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Tai Yoon Kim

Washington University in St. Louis

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Dedicated to family and friends.

ABSTRACT OF THE THESIS

The Role of Voluntary Descending Control in Enhancing Motor Function

Via Transcutaneous Spinal Cord Stimulation

by

Tai Yoon Kim

Master of Science in Biomedical Engineering

Washington University in St. Louis, 2024

Professor Ismael Seáñez, Chair

Spinal cord injury (SCI) is a life-changing event that causes lasting motor impairments. Transcutaneous spinal cord stimulation (tSCS), a non-invasive form of neuromodulation in which electrodes are placed on the skin and used to stimulate the spinal circuits via an electrical current, has demonstrated positive effects on motor function recovery in individuals who have had SCIs. However, the precise mechanism of how tSCS interacts with voluntary descending drive remains poorly understood. This study aims to investigate the role of voluntary descending control in influencing reflex responses triggered by tSCS.

Electromyography (EMG) recordings were performed in ten unimpaired individuals while they engaged in torque control tasks with varying amplitudes of tSCS. Muscle responses recorded during isometric dorsiflexion and plantarflexion tasks using varying levels of torque were analyzed. The peak-to-peak muscle responses at the motor threshold amplitude at rest were examined to compare the muscle responses during different levels of voluntary pre-activation.

Results demonstrate that dorsiflexion of the ankle inhibits ipsilateral extensors - the medial gastrocnemius and soleus - while ankle plantarflexion inhibits the ipsilateral medial gastrocnemius and contralateral rectus femoris and the contralateral soleus. Additionally,

findings indicate smaller muscle responses during dorsiflexion compared to plantarflexion. Limitations of this study include the relatively small sample size which yielded non-significant p-values after correction for multiple comparisons. Future directions involve examining the relationship between task intensity and muscle inhibition, the impact of ankle torque direction at tSCS delivery on the extent of muscle inhibition, and the amplitude of maximum muscle responses across different task intensities.

Overall, this study provides insights into the interaction between volitional movements and tSCS, contributing to the customization of rehabilitative strategies tailored to individual needs.

Chapter 1: Introduction

1.1 Spinal Cord Injury

1.1.1 Introduction to Spinal Cord Injury

Spinal cord injury (SCI) refers to any damage to the bundle of nerves and nerve fibers responsible for transmitting signals to and from the brain[1]. SCI may result in temporary or lasting changes in sensation and motor functions below the site of the injury. The manifestations of SCI vary according to the position and severity of the injury[1]. A larger portion of the body is affected if the injury occurs higher on the spinal cord. Such injuries in the upper spinal cord may lead to paralysis, known as tetraplegia or quadriplegia, that influences all limbs[2]. Conversely, injuries occurring in the lower spinal cord may only impact the lower limbs, resulting in paraplegia[2]. Injuries where all sensation and mobility are lost below the site of injury are referred to be complete. Injuries with some sensation and mobility remaining below the affected area are referred to be incomplete[3].

1.1.2 Statistics on SCI Populations

SCI often leads to permanent disability[4]. Typically stemming from major trauma, primary injury tends to be irreversible[5]. Traumatic SCI results in lasting motor impairments and various complications that increase both morbidity and mortality risks in affected individuals[6]. Economically, SCI results in a lifetime financial burden ranging from 2 to 4 billion dollars[7-8]. According to the 2023 Spinal Cord Injury US Statistics, approximately 18,000 new SCI cases occur every year in the United States[9]. The primary causes of SCI are automobile crashes (31.5%) and falls (25.3%), with gunshot wounds (10.4%), motorcycle crashes (6.8%), diving incidents (4.7%), and medical/surgical complications (4.3%) also contributing significantly[10]. Together, these factors account for 83.1% of all SCIs.

1.1.3 Challenges and Impact of SCI

SCI results in sudden and often severe damage to the central nervous system, leading to adverse effects across musculoskeletal, integumentary, digestive, urinary, cardiovascular, and reproductive systems[11]. Individuals with SCI have to relearn basic skills like eating, bathing, and driving and often rely on adaptive technologies such as wheelchairs or ventilators, which significantly impact their quality of life[11]. Moreover, they face increased risks of secondary complications including neurogenic bowel and bladder issues[12], respiratory problems[13], cardiovascular complications[14], pressure ulcers[15], altered sexual functioning[16], and psychiatric comorbidities such as depression[17] and anxiety[18].

The suicide rate among the individuals affected with SCI is significantly higher, ranging from two to six times that of the unaffected population[11]. Additionally, 35-50% of individuals with traumatic SCI experience concurrent cognitive difficulties[19-20]. Unemployment also remains

a serious issue within the SCI community, with fewer than 40% of individuals under the age of 65 returning to employment[21].

Collectively, these factors contribute to a significantly decreased quality of life for people with SCI, which underscores the need for effective treatments targeting SCI which affects a substantial portion of the population.

1.1.4 Currently Available Treatment Options for SCI

Presently, there is no known complete cure for SCI nor ways to reverse damage to the spinal cord[22]. Therefore, current treatment for SCI primarily focuses on preventing further injury and enabling individuals with SCI to reintegrate into active and productive lifestyles.

Treatment for acute SCI (<14 days after injury), a medical emergency caused by trauma such as gunshot, motor vehicle accidents, or falls[23], may include surgery to remove fluid or tissue compressing the spinal cord, fusion of broken spinal bones, or application of spinal braces[22]. Following acute treatment, individuals with SCI undergo comprehensive rehabilitation to optimize recovery and regain functional independence[24]. It has been shown that active rehabilitation that involves the usage of remaining mobility is crucial to the recovery of some lost mobility and prevention of secondary impact from paralysis[25]. Rehabilitation for SCI can be divided into the acute (<14 days), subacute (14 days to 6 months), and the chronic (>6-12 months) phase. The majority of motor recovery occurs during the first 6-9 months post-injury, with the fastest recovery rate observed during the first three months[26], while recovery plateaus at the chronic stage[27]. Rehabilitation typically involves physical therapy tailored to muscle strengthening, mobility enhancement, and communication skills development, along with the use of assistive devices like wheelchairs, walkers, and leg braces[23].

However, it's important to acknowledge that these rehabilitation strategies can significantly improve quality of life and functional abilities, but they may not completely reverse the effects of SCI. Additionally, the majority of improvements in neurological recovery typically occur within the first six months to a year following injury[28]. Beyond this initial period, particularly after one year in the chronic stage, the likelihood of significant changes in motor

function diminishes, which highlights the need for interventions that aim to restore motor function even after the initial one-year recovery period.

1.2 Transcutaneous Spinal Cord Stimulation

1.2.1 Mechanisms of Transcutaneous Spinal Cord Stimulation

Transcutaneous spinal cord stimulation (tSCS) is a form of non-invasive neuromodulation in which electrodes are used to stimulate spinal circuits to facilitate a motor response[29]. Studies have shown that electrical pulses administered through spinal cord stimulation primarily excite sensory afferents within the posterior roots, eliciting a motor reflex response called the posterior root-muscle (PRM) reflex[30]. By examining the PRM reflex as an alternative to the H-Reflex, researchers are able to broaden the neurophysiological evaluation of sensory-motor transmission across multiple muscle groups[31].

tSCS is believed to be different from direct stimulation of the motor efferents as seen in conventional nerve or muscle stimulation methods, and rather activates the proprioceptive afferents[32]. It is also theorized that tSCS can modulate inter-neuronal spinal excitability, and that this may account for the observed motor recovery when used in individuals with SCI[33, 34, 35].

1.2.2 tSCS Enhances Spinal Neural Excitability

Studies indicate that tSCS can enhance the excitability of spinal neural circuits, leading to improved voluntary performance in patients with incomplete SCI (iSCI)[36]. When combined with training, tSCS can induce functional changes in iSCI patients comparable to those achieved with epidural spinal cord stimulation (eSCS)[37]. This suggests that tSCS is a promising addition to existing physical rehabilitation interventions that can offer clinical utility without the surgical implantation risks associated with eSCS.

1.2.3 Challenges and Limitations of Current State of Research

Studies investigating the effects of tSCS on motor rehabilitation in chronic SCI have reported improved functioning in the lower limbs[38-39], trunk[40], and upper limbs[41]. However, as this approach is still in its early phases of exploration with injured individuals, there is much to uncover regarding its application and clinical potential[29].

Several challenges need to be addressed to optimize tSCS as a therapeutic intervention, including the lack of comprehensive understanding of its physiological mechanisms and its immediate effects on neuronal circuits within intact and injured spinal cords[42]. The long-term outcomes of tSCS also remain uncertain, particularly whether tSCS is able to sustain improvements in motor function.

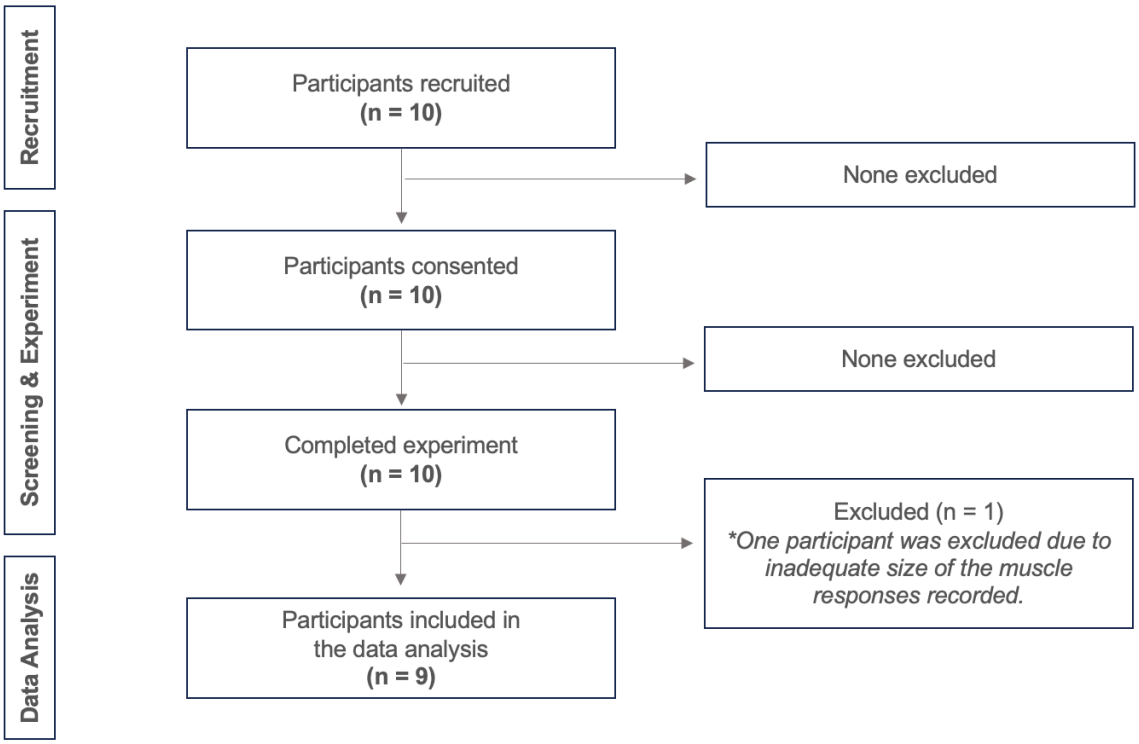
Moreover, the precise mechanism through which tSCS facilitates voluntary movement control post-injury remains poorly understood. The primary objective of this study is to investigate how voluntary descending control contributes to improving motor function through tSCS by comparing the muscle responses elicited during voluntary contractions with those recorded during the resting state. Understanding how muscle responses are affected during voluntary contraction is essential for refining current tSCS interventions for individuals with

SCI. For instance, if a particular muscle response is increased during voluntary contraction, rehabilitation strategies can be adjusted accordingly. This may involve incorporating specific movements, modifying the intensity of the movement, or targeting particular muscles to achieve desired outcomes. By testing various stimulation amplitudes and task intensities during voluntary contractions across 12 muscles, the results can be utilized to tailor therapy to individual needs, leading to more effective treatment for individuals with SCI.

Chapter 2: Methods

2.1 Participant Recruitment

Ten neurologically intact participants were recruited, adhering to the inclusion and exclusion criteria as specified on the informed consent form. Individuals under the age of 18 or over 65, as well as those with a history of neuro-motor impairments impacting leg mobility, were excluded from participation. Additionally, individuals with implanted metal or current medical issues were also excluded. Prior to participating in the study, all participants were provided with an explanation of the experiment protocols and were asked to provide consent. The study was reviewed and approved by the Institutional Review Board of Washington University in St. Louis. Out of the ten participants who consented and completed the study, one participant was excluded from the data analysis due to insufficient muscle recruitment caused by relatively lower tolerance to spinal cord stimulation (Figure 2.1). Three participants were excluded from the analysis for specific conditions because their data was missing due to incorrect data saving format. However, they were not removed from the overall data analysis, which is not reflected in Figure 2.1.



Note. Outliers and missing trials are not included in the flowchart.

Figure 2.1 Participant recruitment and exclusion.

The demographics of 9 study participants who were included in the final data analysis are presented in Table 2.1.

Table 2.1

Participant demographics.

Variable	Participants (N = 9)
Age (years)	
Mean (SD)	26.11 (4.31)
Gender	
Male (n, %)	3 (33.33)
Female (n, %)	6 (66.67)
Height (m)	
Mean (SD)	1.70 (0.09)
Weight (kg)	
Mean (SD)	63.35 (10.48)
Race	
White (n, %)	5 (55.56)
Asian (n, %)	3 (33.33)
Asian, White (n, %)	1 (11.11)
Ethnicity	
Hispanic or Latino (n, %)	1 (11.11)
Not Hispanic or Latino (n, %)	8 (88.89)

Note. One participant did not fill out the weight demographic and was excluded from the weight mean and standard deviation calculation.

2.2 Overview of the Experimental Setup

The study aims to elucidate the interaction between voluntary descending control and muscle recruitment during tSCS. Using wireless EMG sensors, the EMG activity of 12 muscles was recorded while paired pulses of tSCS were administered under different conditions: rest, dorsiflexion, and plantarflexion. A cathode stimulation electrode was attached on the participant's vertebrae, and two return electrodes were placed symmetrically on the abdomen. For the dorsiflexion and plantarflexion tasks, participants' maximum ankle torque was measured by pushing or pulling against the fixed Biodex Isokinetic dynamometer footplate (Figure 2.2). Various proportions of this maximum torque were then exerted while tSCS of different stimulation amplitudes was delivered. Muscle responses were evaluated as a function of stimulation amplitude as participants applied ankle torques to reach designated targets displayed

on a screen, allowing for the identification of the effects of voluntary contraction during tSCS on muscle recruitment.

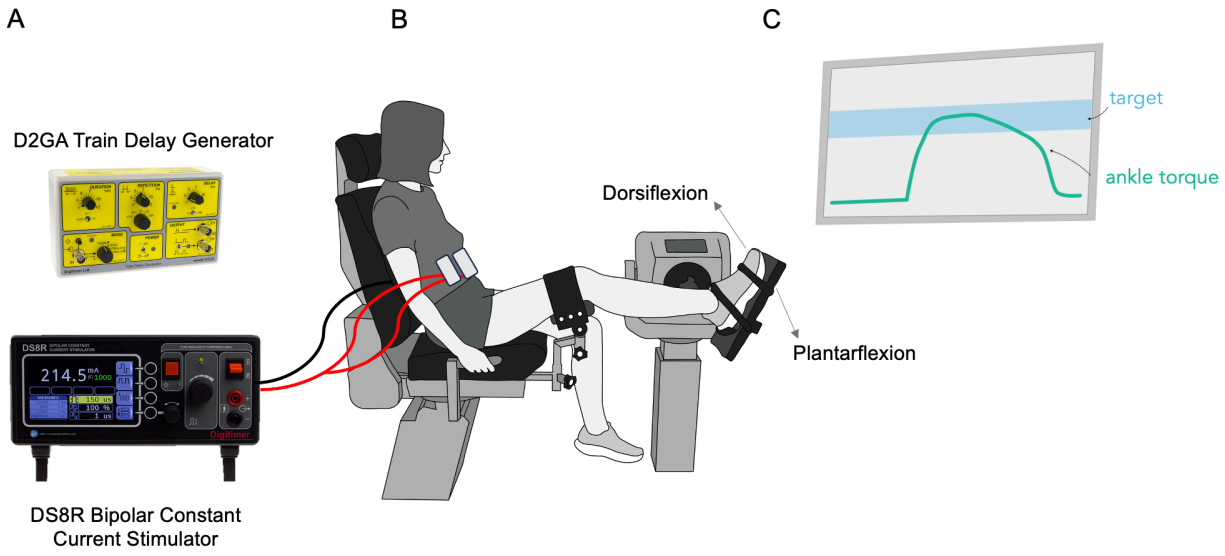


Figure 2.2 Overall experimental setup. **(A)** Hardware used for tSCS administration. **(B)** Participant sitting on the Biodex Isokinetic Dynamometer with their foot secured to the footplate, either pushing or pulling against it. **(C)** The target that the participant has to reach by dorsiflexing or plantarflexing was displayed on the screen in front of them.

2.3 Transcutaneous Spinal Cord Stimulation

2.3.1 Stimulation Electrodes

Vertebral segment T12/L1 was identified using manual palpation. Despite the fact that most common cathode placement when targeting the lower extremities is over the T11/T12 spinous process, the electrode placement was moved a segment down to better recruit the distal muscles of target, including the tibialis anterior, medial gastrocnemius, and the soleus[43].

With the participant lying on their abdomen, the posterior iliac crest was located to mark a transverse line towards the midline's center, where a surgical marker was employed to indicate the L4 spinous process. Palpation was used to identify interspinous ligaments from the L3/L4 level to T11/T12, which were then marked accordingly. The skin along the midline from T12 to

L1 was prepared by applying abrasive gel (NuPrep®, Weaver and Co. USA) using a Q-tip® in circular motions, followed by wiping with alcohol prep pads (Medline Industries Co., Ltd., USA). A single 5 X 9 cm rectangular neurostimulation electrode (Axelgaard Manufacturing Co., Ltd., USA) was positioned centrally over the T12/L1 vertebral segment for stimulation.

Two interconnected rectangular PALS neurostimulation electrodes measuring 7.5 x 10 cm (Axelgaard Manufacturing Co., Ltd., USA) were placed bilaterally on the abdomen, extending horizontally from the navel, serving as return electrodes. To improve conductivity, all electrodes were coated with conductive spray (Signa® Spray, Parker Laboratories, Inc., USA). Once applied to the skin, the electrodes were secured in place using transparent film dressing (TegadermTM, 3M Co., Ltd., USA) (Figure 2.3.1).

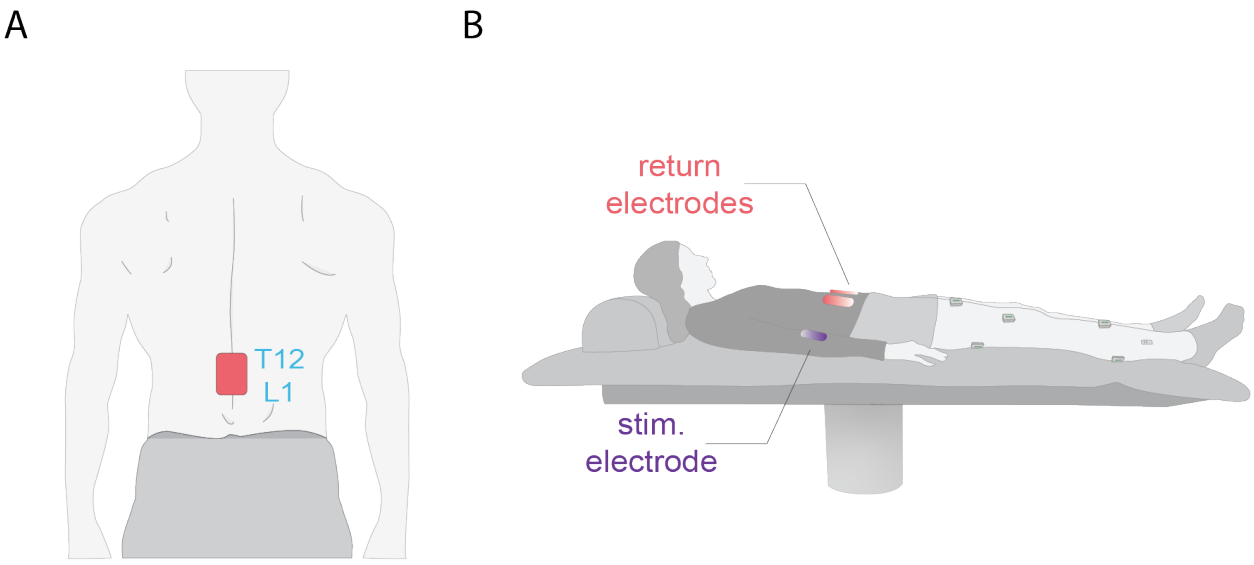


Figure 2.3.1 The schematic of electrodes placement. **(A)** One stimulating electrode affixed on the participant's back aligned at the center of the T12/L1 vertebral segment. **(B)** Two return electrodes affixed symmetrically on the participant's abdomen.

2.3.2 Wireless Electromyography Sensors

Muscle activity during torque control tasks was recorded using a 16-channel wireless electromyography (EMG) system (Trigno® Avanti, Delsys Inc., USA) with a sampling frequency of 2000 Hz. The wireless EMG sensors were placed bilaterally on the rectus femoris (RF), vastus lateralis (VL), semitendinosus (ST), tibialis anterior (TA), medial gastrocnemius (MG), and soleus (SL), following SENIAM guidelines (Figure 2.3.2). The same skin preparation procedure used for stimulating electrodes was applied. An additional wireless sensor (Trigno® Analog Input Adapter, Delsys Inc., USA) was connected to the biphasic stimulator's sync output using a BNC cable to synchronize the stimulation pulses offline. Control over stimulation pulse amplitude and triggering was facilitated through a data acquisition board (NI USB 6009, National Instruments, USA). Real-time EMG data for all 12 muscles was displayed on custom software developed in Python V3.10.

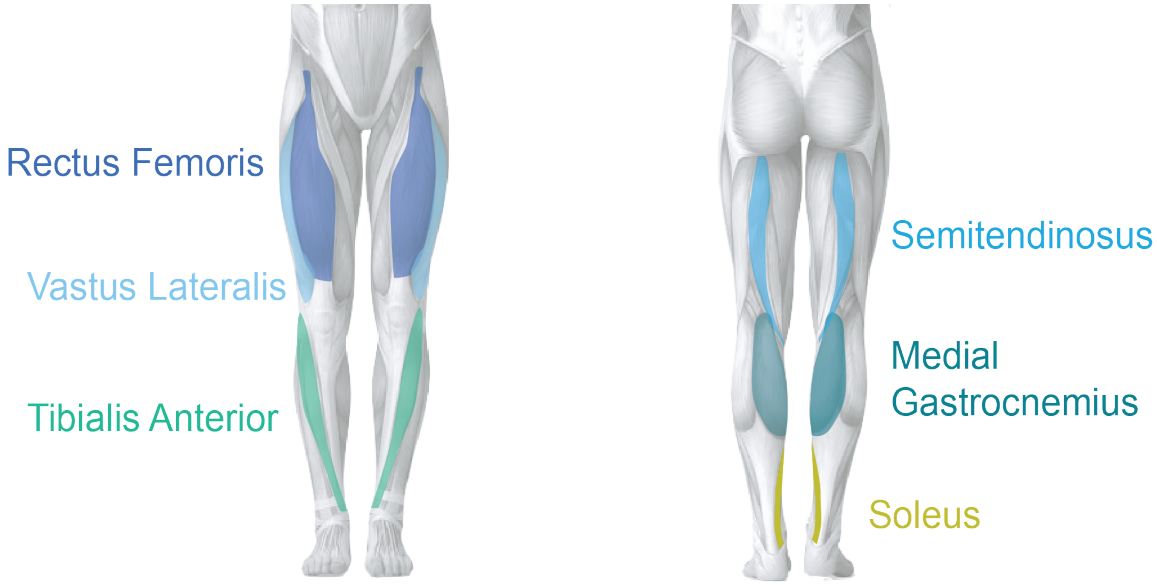


Figure 2.3.2 The location of EMG sensors.

2.4 Experimental Procedure

2.4.1 Basic Recruitment Curves Recording

Recruitment curves were computed as the muscle response amplitude as a function of stimulation amplitude. Before administering tSCS, participants were positioned on the Biodex Isokinetic Dynamometer with their right leg extended and foot placed on the footplate. The angles of the hip, knee, and ankle were adjusted to ensure they were within ±10 degrees of 120, 160, and 110 degrees, respectively.

Once seated, stimulating and return electrodes on the participant's back and abdomen were connected to an isolated constant current stimulator (DS8R, Digitimer Ltd, UK). Single pulse tSCS was then delivered at increasing amplitudes to determine the motor and saturation thresholds. The motor threshold was identified as the stimulation amplitude where the first muscle response larger than 0.05 mV was observed, while the saturation threshold was defined as the amplitude where all muscles exhibited responses or when the participant could no longer comfortably tolerate further increases in stimulation amplitude.

Following the determination of motor and saturation thresholds, an amplitude sweep of six different amplitudes ranging from 50% of the motor threshold up to the saturation threshold was defined. For the recording of recruitment curves during rest, participants placed their foot on the footplate without making voluntary contractions, and ten paired pulses were delivered at each step of the amplitude sweep, with stimulation amplitudes decreasing from the highest to the lowest.

Paired pulse tSCS was administered using the isolated constant current stimulator (DS8R, Digitimer Ltd, UK). The stimulation protocol consisted of a pair of charge-balanced, anodic leading, biphasic pulses, with each phase lasting 1 ms. The inter-stimulus interval was set to 60 ms using a train generator (DG2A, Digitimer Ltd.).

The basic recruitment curves recorded at rest were subsequently used in the data analysis to compare muscle recruitment during different levels of effort. An overview of the overall experimental procedure is presented in Figure 2.4.1.

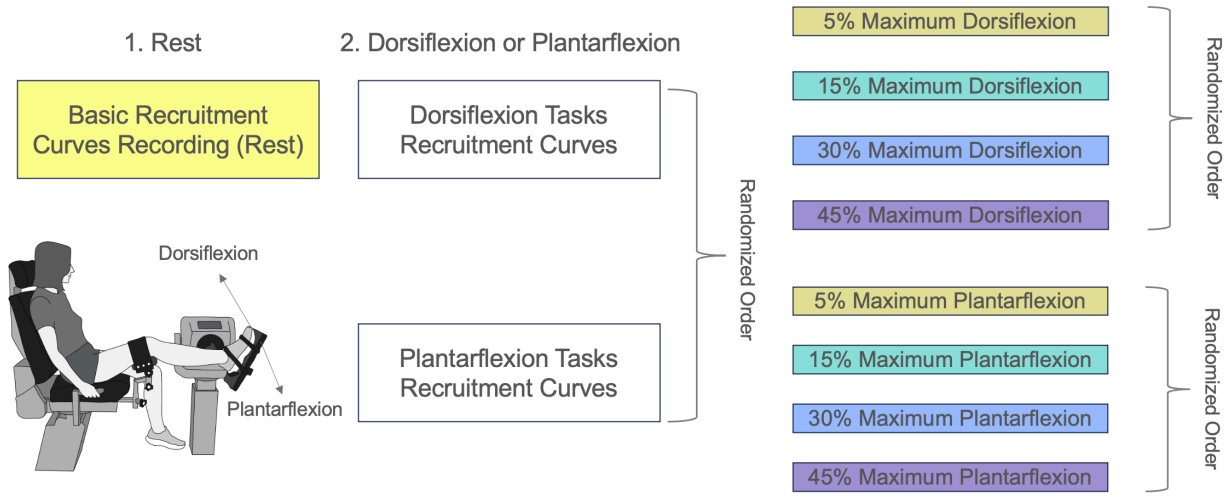


Figure 2.4.1 The overall experimental procedure.

2.4.2 Dorsiflexion and Plantarflexion Tasks

After the recruitment curves recording during rest, participants were instructed to either dorsiflex or plantarflex their toes to exert maximum force. The corresponding maximum force value was measured and documented. This maximum force was used to determine their torque task levels corresponding to 5%, 15%, 30%, and 45% of their maximum torque.

In each block (5%, 15%, 30%, and 45%), participants aimed to dorsiflex or plantarflex their foot to reach a designated target area displayed on a screen using custom software. Ten paired pulses of tSCS were administered while participants were in the target range for each of the 6 amplitudes throughout the amplitude sweep, ranging from the saturation threshold down to 50% of the motor threshold. Following each stimulation, participants returned to their resting position for a 5-second interval before the next stimulation. The order of blocks was randomized.

2.5 Data Processing and Analysis

2.5.1 Quantification of Motor Response and Recruitment Curves

Data analysis was conducted offline using custom software developed in Python. For all nine recruitment curve recordings - including rest, four dorsiflexion tasks (5%, 15%, 30%, and 45%), and four plantarflexion tasks (5%, 15%, 30%, and 45%) - evoked responses were averaged across the 10 repetitions, resulting in one average response waveform for each of the six stimulation amplitudes.

Peak-to-peak detection was applied to the EMG data collected from the wireless sensors with a sampling frequency of 2000 Hz. The EMG signals were appropriately amplified using a data acquisition system (Trigno® Avanti, Delsys Inc., USA) with a gain of 30 and a band-width of 20-450 Hz. Data for each of the nine tasks was saved separately. For each stimulation amplitude and muscle, traces of 10 EMG responses were averaged. From these averaged traces, peak-to-peak values were identified using Python. Peak-to-peak values were determined by calculating the difference between the maximum positive peak and the maximum negative peak within a selected time window.

The peak-to-peak values of the evoked responses for each muscle across all stimulation amplitudes were normalized to the maximum peak-to-peak value for that muscle. Data normalization was performed separately for each recruitment curve recording, resulting in separate recruitment curves for each condition (Figure 2.5.1).

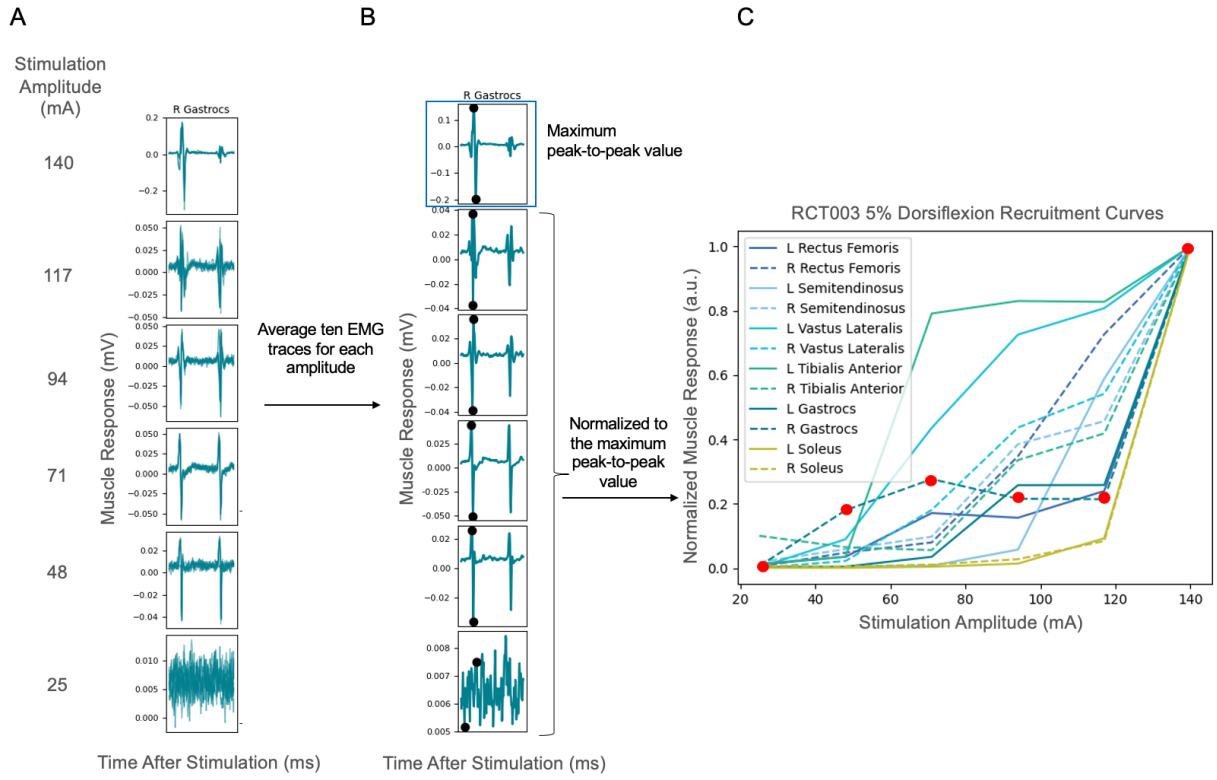


Figure 2.5.1 Generation of recruitment curves. **(A)** Overlayed traces of EMG response at 10 repetitions of tSCS for each of the 6 stimulation amplitudes for one muscle. **(C)** The averaged traces of 10 EMG responses at each stimulation amplitude for one muscle with peak-to-peak values detected. **(D)** The resulting recruitment curves for a single participant from the normalized peak-to-peak values (red circles) of the 5% dorsiflexion trial with 12 muscles.

2.5.2 Identification of the Rest Threshold Amplitude

The stimulation amplitude during the rest recruitment curves, where the first response exceeding 0.05 mV was elicited (the rest threshold amplitude), was pinpointed and isolated for group-level statistical analysis (Figure 2.5.2). This approach was chosen because the study aimed to compare the muscle response with the rest condition. Statistical analysis was carried out independently for dorsiflexion and plantarflexion tasks.

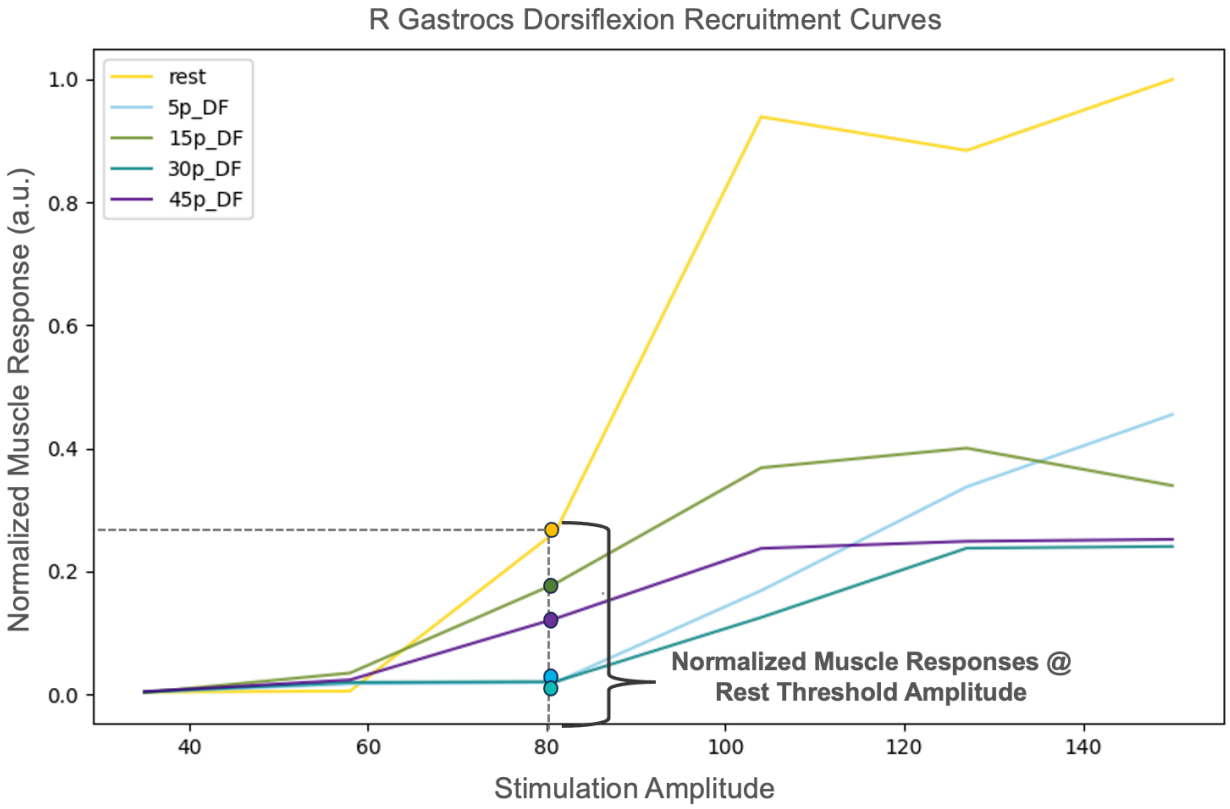


Figure 2.5.2 Identification of the rest threshold amplitude. The stimulation amplitude where the first normalized muscle response bigger than 0.05 mV at the rest recruitment curve was selected as the rest threshold amplitude.

2.5.3 Data Averaging and Outlier Detection

The peak-to-peak responses corresponding to the rest threshold amplitude for each muscle were normalized to the peak-to-peak responses of the rest trial for each participant (Figure 2.5.3).

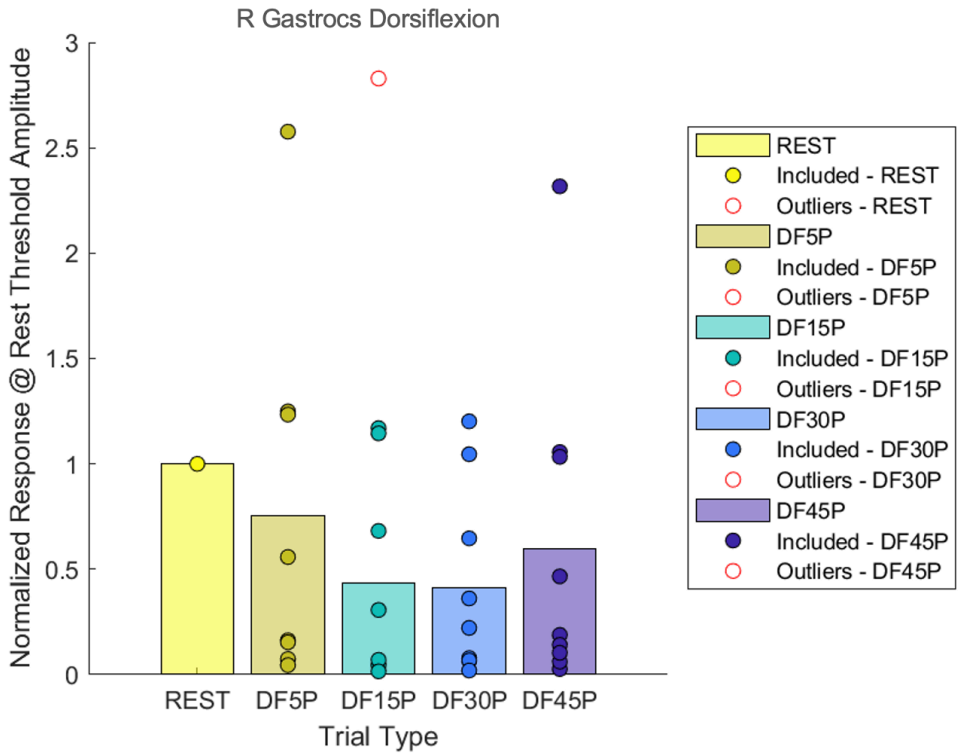


Figure 2.5.3 Peak-to-peak response for each group normalized to the rest.

Outliers were detected using the interquartile range method within each group comparing to rest (i.e., 5%, 15%, 30%, and 45% dorsiflexion or plantarflexion). If an outlier was identified within one of these comparison groups, the corresponding sample from the same participant was removed from the rest group to maintain consistent sample sizes for subsequent statistical analyses. Subsequently, statistical analyses were conducted on the data that remained unnormalized to the rest.

2.5.4 Statistical Tests

A repeated measures ANOVA test, with a significance level (alpha) set at 0.05, was applied to the group data for each muscle to assess if there were significant differences in group means among all groups (rest, 5%, 15%, 30%, and 45%) separately for dorsiflexion and plantarflexion tasks. Post-hoc analyses were conducted on the muscles for which the repeated measures ANOVA indicated a rejection of the null hypothesis, signifying a significant difference between group means.

For each pair of groups under comparison (i.e., 5%, 15%, 30%, and 45% dorsiflexion or plantarflexion), the Shapiro-Wilk test for normality was employed to determine the appropriate post-hoc statistical test. If either group exhibited a non-normal distribution, the Wilcoxon test was utilized. Conversely, if both groups displayed a normal distribution, the paired t-test was applied.

2.5.5 Power Analysis

For comparison groups where the post-hoc statistical tests yielded significant results, power analysis was performed using an online sample size calculator for adequate study power on the data normalized to rest[44]. Alpha of 0.05 was used to estimate the smallest sample size required to yield results with 80% power.

Chapter 3: Results

3.1 Effect of Dorsiflexion and Plantarflexion on Muscle Response Amplitude

Several muscles out of the 12 muscles examined were affected by either dorsiflexion or plantarflexion. Among these, all muscles that exhibited statistically significant differences from the resting condition showed inhibition in response amplitude. During dorsiflexion, the ipsilateral plantar flexors, including the right medial gastrocnemius and soleus, exhibited inhibited muscle response in one or more conditions compared to the resting state (Figure 3.1.1).

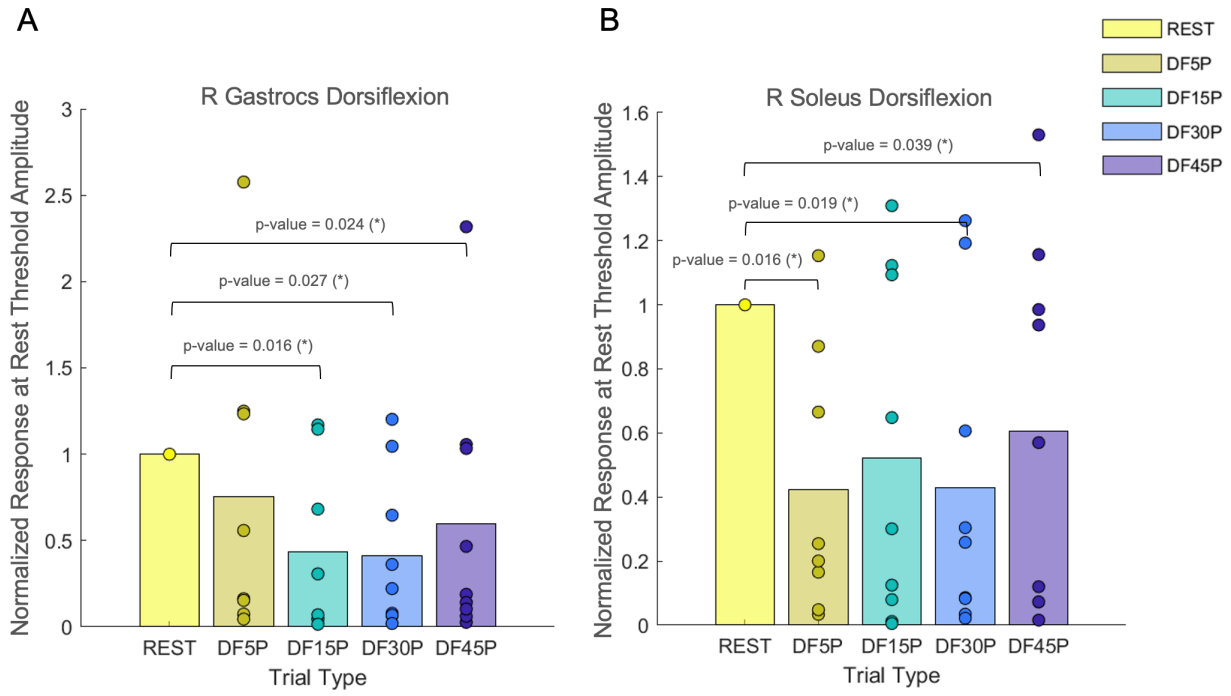


Figure 3.1.1 The effect of right TA pre-activation on **(A)** right gastrocnemius and **(B)** right soleus. P-values are shown for either the paired t-test or Wilcoxon test of each condition against rest, depending on data normality.

At 5% dorsiflexion, the response of the right soleus was inhibited (p-value = 0.016, effect size = 3.166). At 15% dorsiflexion, the response of the right medial gastrocnemius was inhibited (p-value = 0.016, effect size = 3.162). Furthermore, at 30% dorsiflexion, both the right soleus (p-value = 0.019, effect size = 3.000) and the right gastrocnemius (p-value = 0.027, effect size = 4.000) displayed inhibited responses. Similarly, at 45% dorsiflexion, both the right soleus (p-value = 0.039, effect size = 5.000) and the right gastrocnemius (p-value = 0.024, effect size = 2.772) showed inhibited responses.

Post-hoc statistical analysis indicated that the response of the right gastrocnemius muscle was inhibited during pre-activation in the right tibialis anterior at 15% ($p = 0.016$), 30% ($p = 0.027$), and 45% ($p = 0.024$), compared to the rest. Similarly, the response of the right soleus was inhibited during 5% ($p = 0.016$), 30% ($p = 0.019$), and 45% ($p = 0.039$) dorsiflexion. However, these p-values were not significant when corrected for multiple comparisons (Bonferroni correction for normally distributed data or Sidak correction for non-normally distributed data). The detailed statistical analysis results on the affected muscles are organized in Table 3.1.1. The remaining muscles were not affected during dorsiflexion.

Table 3.1.1

Summary of the statistical analysis performed on muscles affected during dorsiflexion.

	R Medial Gastrocnemius			R Soleus		
% Maximum Dorsiflexion	15	30	45	5	30	45
Post-hoc Analysis Type	Paired T-Test	Wilcoxon Test	Paired T-Test	Paired T-Test	Wilcoxon Test	Wilcoxon Test
Post-hoc Test Statistic	3.162	4.000	2.773	3.166	3.000	5.000
Post-hoc P-value (Non-corrected)	0.016 (*)	0.027 (*)	0.024 (*)	0.016 (*)	0.019 (*)	0.039 (*)
Sample Size Required with 80% Power	6	5	28	4	6	16

Regarding the affected plantar flexors during dorsiflexion, there appeared to be no clear relationship between the effect size (the degree of inhibition) and the level of voluntary contraction exerted (percentage of maximum torque during dorsiflexion). In both the right gastrocnemius and the right soleus muscles, 30% dorsiflexion led to a more pronounced inhibition in muscle response compared to lower levels of torque exertion, such as 5% and 15% dorsiflexion.

However, while the effect size for 45% dorsiflexion was smaller than that for 30% dorsiflexion in the right gastrocnemius, it was larger than 30% in the right soleus. This observation could be attributed to the fact that maintaining stability within the target for tSCS administration was most challenging during 45% dorsiflexion and the increased background noise in the EMG data due to participants' feet constantly moving up and down to remain within

the target, in contrast to the relatively easier tasks like 5% and 15% dorsiflexion. However, this possible relationship was not quantified.

The recruitment of the right gastrocnemius was inhibited during plantarflexion compared to when no voluntary contraction was present. Post-hoc statistical analysis indicated that the response of the right gastrocnemius muscle was inhibited during pre-activation in the right gastrocnemius and soleus at 15% ($p = 0.023$, effect size = 2.898) compared to the rest, but not at 5% ($p = 0.200$), 30% ($p = 0.099$), and 45% (0.074) plantarflexion (Figure 3.1.2 A).

Additionally, two muscles on the left leg –rectus femoris and soleus – demonstrated significant differences between group means. The muscle response for the left rectus femoris was inhibited during 15% plantarflexion compared to rest ($p = 0.029$, effect size = 3.000), and similarly for the left soleus at 15% ($p = 0.016$, effect size = 1.000) and 30% ($p = 0.047$, effect size = 3.166) (Figure 3.1.2 B-C). The details of the statistical analyses of the affected muscles during plantarflexion are organized in Table 3.1.2. The remaining muscles were not affected during plantarflexion.

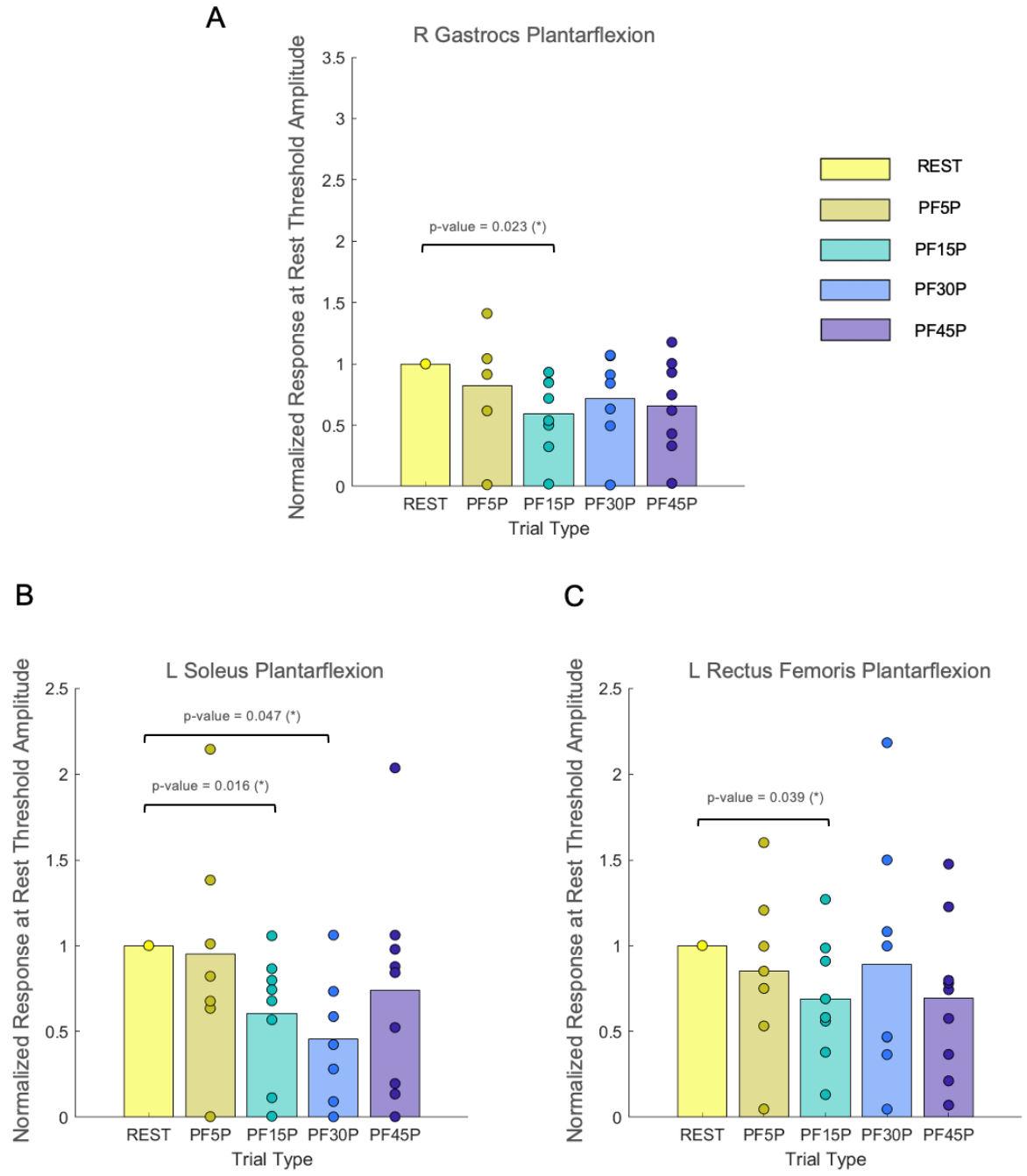


Figure 3.1.2 The effect of right MG and SL pre-activation on **(A)** right gastrocnemius, **(B)** left soleus, and **(C)** left rectus femoris. P-values are shown for either the paired t-test or Wilcoxon test of each condition against rest, depending on data normality.

Table 3.1.2

Summary of the statistical analysis performed on muscles affected during plantarflexion.

	R Medial Gastrocnemius	L Rectus Femoris	L Soleus	
% Maximum Plantarflexion	15	15	15	30
Post-hoc Analysis Type	Paired T-Test	Wilcoxon Test	Wilcoxon Test	Wilcoxon Test
Post-hoc Test Statistic	2.898	3.000	1.000	3.166
Post-hoc P-value (Non-corrected)	0.023 (*)	0.039 (*)	0.016 (*)	0.016 (*)
Sample Size Required with 80% Power	4	11	7	4

The effect size for each affected muscle were normalized to the maximum effect size across all conditions (5%, 15%, 30%, and 45%) for dorsiflexion and plantarflexion, separately (Figure 3.3).

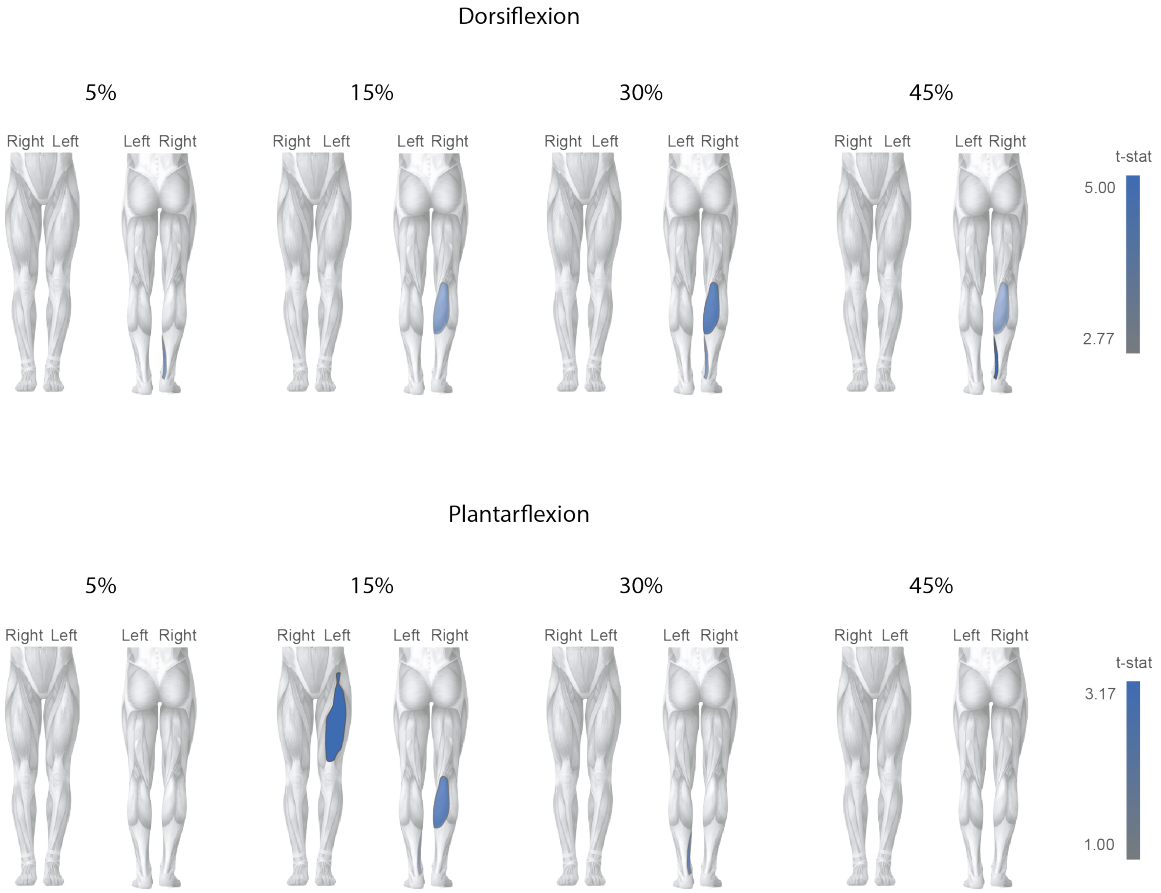


Figure 3.1.3 Muscles with inhibited response during dorsiflexion (top) and plantarflexion (bottom) shaded in blue. The opacity of the shade for each muscle is the percentage of maximum effect size during dorsiflexion and plantarflexion, separately.

During dorsiflexion, the right soleus exhibited the highest effect size (5.0) at 45%, succeeded by the right medial gastrocnemius at 30% (80% of the maximum effect size), the right medial gastrocnemius at 15% (63.24% of the maximum effect size), the right soleus at 30% (60% of the maximum effect size), and the right medial gastrocnemius at 45% (55.44% of the maximum effect size), ranked in descending order. This suggests that the right soleus experienced the most significant inhibition at 45% dorsiflexion, while the right medial gastrocnemius was the least inhibited at 45% dorsiflexion. The other muscles were affected in the sequence of the right medial gastrocnemius first, from higher intensity to lower (30% to 15%), followed by the right soleus at 30%. No clear pattern correlating order of the muscle or the task intensity with the degree of inhibition was evident. However, this may be attributed to the difficulty participants faced in executing dorsiflexion at 45% while maintaining stability within the target during tSCS administration, resulting in considerable variability in the data that may have influenced the outcomes.

In plantarflexion, the left soleus displayed the highest effect size (3.166) at 30%, followed by the left rectus femoris at 15% (representing 94.76% of the maximum effect size), the right medial gastrocnemius at 15% (with 91.54% of the maximum effect size), and the left soleus at 15% (representing 31.59% of the maximum effect size). Generally, among the groups where muscles exhibited significant differences from the rest, there appeared to be a trend of greater muscle inhibition as task intensity increased (observed at 30% plantarflexion), and lesser inhibition at lower task intensities (notably at 15% plantarflexion). However, no muscles were significantly affected to the extent of rejecting the null hypothesis in the 5% and 45% plantarflexion groups.

Chapter 4: Discussion

4.1 Voluntary Contractions During tSCS Results in Inhibition of Several Muscles

This study investigated the influence of voluntary descending control on muscle recruitment during tSCS. Our results demonstrate that dorsiflexion of the right ankle leads to the inhibition of ipsilateral extensors, specifically the medial gastrocnemius and the soleus. Conversely, ankle plantarflexion results in the inhibition of the ipsilateral medial gastrocnemius and the contralateral flexor muscle, namely the rectus femoris, along with the extensor muscle, the soleus.

A previous study by Hofstoetter et al. showed that ankle dorsiflexion in standing subjects significantly inhibits the ipsilateral semitendinosus and medial gastrocnemius[31]. Our study corroborates this finding, particularly in the inhibition of the right medial gastrocnemius during dorsiflexion. While we did not observe significant inhibition on the ipsilateral semitendinosus, we did observe an inhibited response in the right soleus.

In contrast to Hofstoetter et al., who found no significant overall tendency of muscle facilitation or inhibition during ankle plantarflexion, our study revealed inhibition in the ipsilateral medial gastrocnemius and the contralateral soleus and rectus femoris. This disparity in results may stem from differences in participant posture during voluntary contractions. While participants in our study were seated, those in Hofstoetter et al. were standing, potentially leading to variations in spinal cord curvature and the screening of the spinal cord due to different distances between adjacent vertebral processes.

Additionally, our study found a similar pattern to Hofstoetter et al. in that the magnitude of muscle responses in the affected muscles was smaller during dorsiflexion compared to plantarflexion. However, the magnitude of contraction in Hofstoetter's study was not controlled of the maximum contraction as in ours, leading to more chances of variability in the amount of contraction.

4.2 Limitations and Future Directions

This study examined the response at the rest threshold amplitude following the initial response from paired-pulse stimulation to assess whether muscle responses are significantly facilitated or inhibited compared to no voluntary contraction.

The primary finding of this study is that voluntary control of muscles in a seated position alters muscle responses at the rest threshold amplitude. However, due to the relatively small sample size, the statistical analysis yielded non-significant p-values after correction for multiple comparisons. Further participants, particularly at higher task intensities (45% dorsiflexion or plantarflexion), are necessary to fully uncover the effect of voluntary contraction on muscle recruitment during tSCS.

Future research could focus on the potential relationship between task intensity and the degree of inhibition in affected muscles. Additionally, investigating whether the direction of torque (i.e., downward or upward movement towards the target before stimulation) impacts the extent of inhibition could provide valuable insights. Examining at which amplitude the maximum muscle response occurs across different task intensities would also be beneficial. While the muscle responses at the rest threshold amplitude were reported in this study, responses at the maximum stimulation amplitude might have yielded a larger effect size.

In summary, this study provides insights into the interaction between volitional movements and tSCS in influencing muscle recruitment. Through examining various intensities of voluntary contraction throughout multiple muscles, the optimal intensity of dorsiflexion or plantarflexion at which muscle inhibition is most pronounced can be identified, and can be applied to refining the current rehabilitation methods that incorporate tSCS. Ultimately, this knowledge could contribute to the customization and refinement of rehabilitative strategies tailored to individual needs.

References

1. Spinal Cord Injury | National Institute of Neurological Disorders and Stroke. https://www.ninds.nih.gov/health-information/disorders/spinal-cord-injury.

2. Spinal cord injury - Symptoms and causes. *Mayo Clinic* https://www.mayoclinic.org/diseases-conditions/spinal-cord-injury/symptoms-causes/syc-20377890.

3. Spinal Cord Injuries and Their Impacts. *Cleveland Clinic* https://my.clevelandclinic.org/health/diseases/12098-spinal-cord-injury.

4. Bennett, J., Das, J. M. & Emmady, P. D. Spinal Cord Injuries. in *StatPearls [Internet]* (StatPearls Publishing, 2022).

5. Eckert, M. J. & Martin, M. J. Trauma. *Surgical Clinics of North America* **97**, 1031–1045 (2017).

6. Spinal Cord Injury (SCI) 2016 Facts and Figures at a Glance. *The Journal of Spinal Cord Medicine* **39**, 493–494 (2016).

7. Varma, A. K. *et al.* Spinal Cord Injury: A Review of Current Therapy, Future Treatments, and Basic Science Frontiers. *Neurochem Res* **38**, 895–905 (2013).

8. McDaid, D., Park, A.-L., Gall, A., Purcell, M. & Bacon, M. Understanding and modelling the economic impact of spinal cord injuries in the United Kingdom. *Spinal Cord* **57**, 778–788 (2019).

9. US Stats 2023 - Full.pdf. https://www.spinalcord.com/hs-fs/hubfs/US%20Stats%202023%20-%20Full.pdf.

10. Chen, Y., Tang, Y., Vogel, L. & DeVivo, M. Causes of Spinal Cord Injury. *Topics in Spinal Cord Injury Rehabilitation* **19**, 1–8 (2013).

11. Tulsky, D. S. *et al.* Overview of the Spinal Cord Injury – Quality of Life (SCI-QOL) measurement system. *The Journal of Spinal Cord Medicine* **38**, 257–269 (2015).

12. Costa, P. *et al.* Quality of Life in Spinal Cord Injury Patients with Urinary Difficulties. *European Urology* **39**, 107–113 (2001).

13. Spungen, A. M., Grimm, D. R., Lesser, M., Bauman, W. A. & Almenoff, P. L. Self-reported prevalence of pulmonary symptoms in subjects with spinal cord injury. *Spinal Cord* **35**, 652–657 (1997).

14. Wecht, J. M. *et al.* Cardiovascular Autonomic Dysfunction in Spinal Cord Injury: Epidemiology, Diagnosis, and Management. *Semin Neurol* **40**, 550–559 (2020).

15. Sugarman, B. Infection and pressure sores. *Arch Phys Med Rehabil* **66**, 177–179 (1985).

16. Fisher, T. L. *et al.* Sexual health after spinal cord injury: A longitudinal study. *Archives of Physical Medicine and Rehabilitation* **83**, 1043–1051 (2002).

17. Fuhrer, M. J., Rintala, D. H., Hart, K. A., Clearman, R. & Young, M. E. Depressive symptomatology in persons with spinal cord injury who reside in the community. *Arch Phys Med Rehabil* **74**, 255–260 (1993).

18. Kennedy, P. & Rogers, B. A. Anxiety and depression after spinal cord injury: a longitudinal analysis. *Arch Phys Med Rehabil* **81**, 932–937 (2000).

19. Roth, E. *et al.* A controlled study of neuropsychological deficits in acute spinal cord injury patients. *Paraplegia* **27**, 480–489 (1989).

20. Davidoff, G. N., Roth, E. J. & Richards, J. S. Cognitive deficits in spinal cord injury: epidemiology and outcome. *Arch Phys Med Rehabil* **73**, 275–284 (1992).

21. Tomassen, P., Post, M. & Van Asbeck, F. Return to work after spinal cord injury. *Spinal Cord* **38**, 51–55 (2000).

22. What are the treatments for spinal cord injury (SCI)? | NICHD - Eunice Kennedy Shriver National Institute of Child Health and Human Development. https://www.nichd.nih.gov/health/topics/spinalinjury/conditioninfo/treatments (2022).

23. Acute Spinal Cord Injury. https://www.hopkinsmedicine.org/health/conditions-and-diseases/acute-spinal-cord-injury (2021).

24. Peev, N., Komarov, A., Osorio-Fonseca, E. & Zileli, M. Rehabilitation of Spinal Cord Injury: WFNS Spine Committee Recommendations. *Neurospine* **17**, 820–832 (2020).

25. Seáñez-González, I. *et al.* Body-Machine Interfaces after Spinal Cord Injury: Rehabilitation and Brain Plasticity. *Brain Sciences* **6**, 61 (2016).

26. Kirshblum, S., Snider, B., Eren, F. & Guest, J. Characterizing Natural Recovery after Traumatic Spinal Cord Injury. *J Neurotrauma* **38**, 1267–1284 (2021).

27. Burns, A. S. *et al.* Type and Timing of Rehabilitation Following Acute and Subacute Spinal Cord Injury: A Systematic Review. *Global Spine J* **7**, 175S-194S (2017).

28. Ahuja, C. S. *et al.* Traumatic spinal cord injury. *Nat Rev Dis Primers* **3**, 17018 (2017).

29. Taylor, C. *et al.* Transcutaneous spinal cord stimulation and motor responses in individuals with spinal cord injury: A methodological review. *PLoS ONE* **16**, e0260166 (2021).

30. Danner, S. M., Hofstoetter, U. S., Ladenbauer, J., Rattay, F. & Minassian, K. Can the human lumbar posterior columns be stimulated by transcutaneous spinal cord stimulation? A modeling study. *Artif Organs* **35**, 257–262 (2011).

31. Hofstoetter, U. S. *et al.* Modification of reflex responses to lumbar posterior root stimulation by motor tasks in healthy subjects. *Artif Organs* **32**, 644–648 (2008).

32. Sayenko, D. G., Angeli, C., Harkema, S. J., Edgerton, V. R. & Gerasimenko, Y. P. Neuromodulation of evoked muscle potentials induced by epidural spinal-cord stimulation in paralyzed individuals. *J Neurophysiol* **111**, 1088–1099 (2014).

33. Minassian, K. *et al.* Spinal Rhythm Generation by Step-Induced Feedback and Transcutaneous Posterior Root Stimulation in Complete Spinal Cord–Injured Individuals. *Neurorehabil Neural Repair* **30**, 233–243 (2016).

34. Angeli, C. A., Edgerton, V. R., Gerasimenko, Y. P. & Harkema, S. J. Altering spinal cord excitability enables voluntary movements after chronic complete paralysis in humans. *Brain* **137**, 1394–1409 (2014).

35. Islam, Md. A. *et al.* Modulation of soleus H-reflex excitability following cervical transspinal conditioning stimulation in humans. *Neuroscience Letters* **732**, 135052 (2020).

36. Martin, R. Utility and Feasibility of Transcutaneous Spinal Cord Stimulation for Patients With Incomplete SCI in Therapeutic Settings: A Review of Topic. *Front. Rehabilit. Sci.* **2**, 724003 (2021).

37. Inanici, F., Brighton, L. N., Samejima, S., Hofstetter, C. P. & Moritz, C. T. Transcutaneous Spinal Cord Stimulation Restores Hand and Arm Function After Spinal Cord Injury. *IEEE Trans. Neural Syst. Rehabil. Eng.* **29**, 310–319 (2021).

38. Minassian, K. *et al.* Posterior root–muscle reflexes elicited by transcutaneous stimulation of the human lumbosacral cord. *Muscle and Nerve* **35**, 327–336 (2007).

39. Alam, M. *et al.* Reversing 21 years of chronic paralysis via non-invasive spinal cord neuromodulation: a case study. *Ann Clin Transl Neurol* **7**, 829–838 (2020).

40. Rath, M. *et al.* Trunk Stability Enabled by Noninvasive Spinal Electrical Stimulation after Spinal Cord Injury. *Journal of Neurotrauma* **35**, 2540–2553 (2018).

41. Inanici, F. *et al.* Transcutaneous Electrical Spinal Stimulation Promotes Long-Term Recovery of Upper Extremity Function in Chronic Tetraplegia. *IEEE Trans. Neural Syst. Rehabil. Eng.* **26**, 1272–1278 (2018).

42. Hofstoetter, U. S. & Minassian, K. Transcutaneous Spinal Cord Stimulation: Advances in an Emerging Non-Invasive Strategy for Neuromodulation. *J Clin Med* **11**, 3836 (2022).

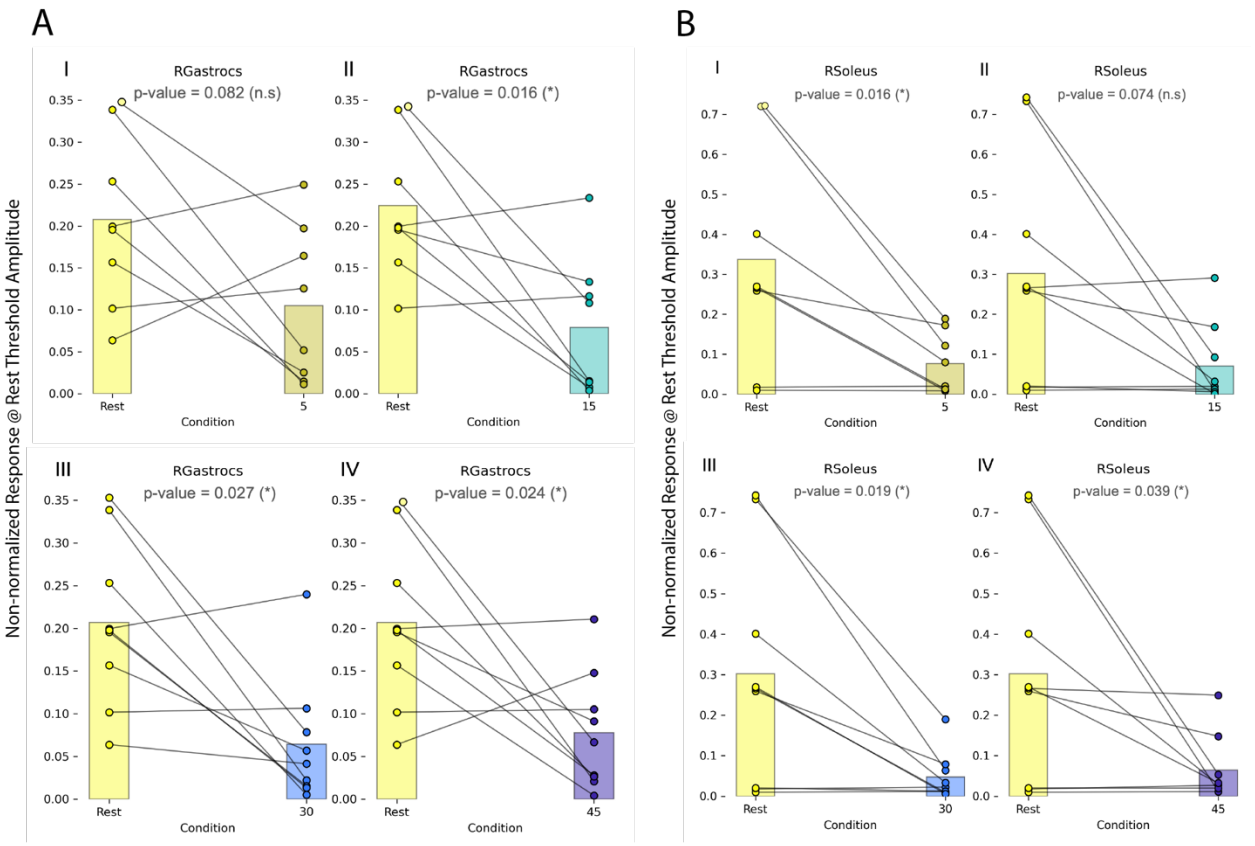
43. Bryson, N. *et al.* Enhanced selectivity of transcutaneous spinal cord stimulation by multielectrode configuration. *J. Neural Eng.* **20**, 046015 (2023).

44. Sample Size Calculator. https://clincalc.com/stats/samplesize.aspx.

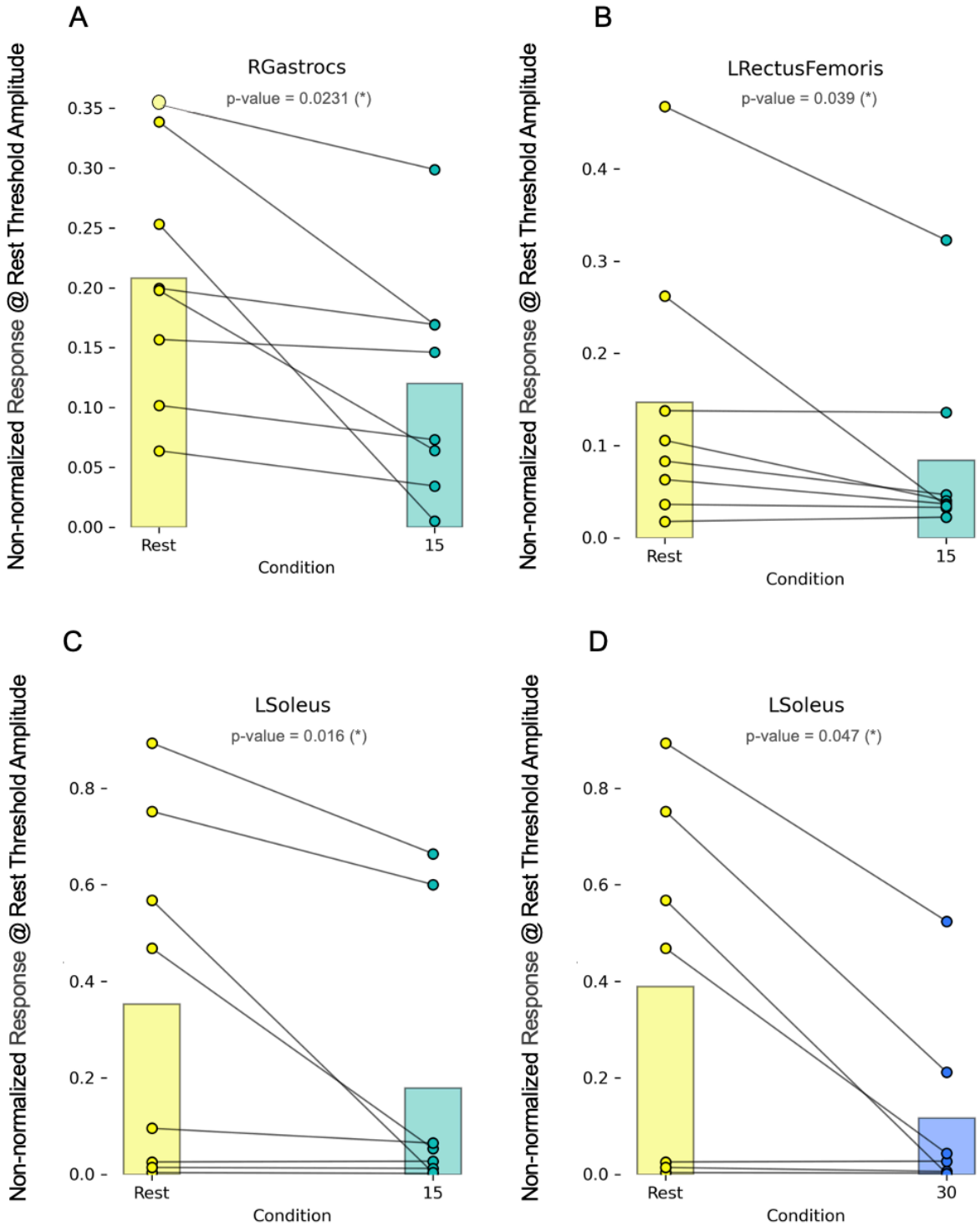
45. ChatGPT v3.5 was used to assist in the writing of this thesis for clarity and sentence structure. However, all content was my original idea.

Appendix

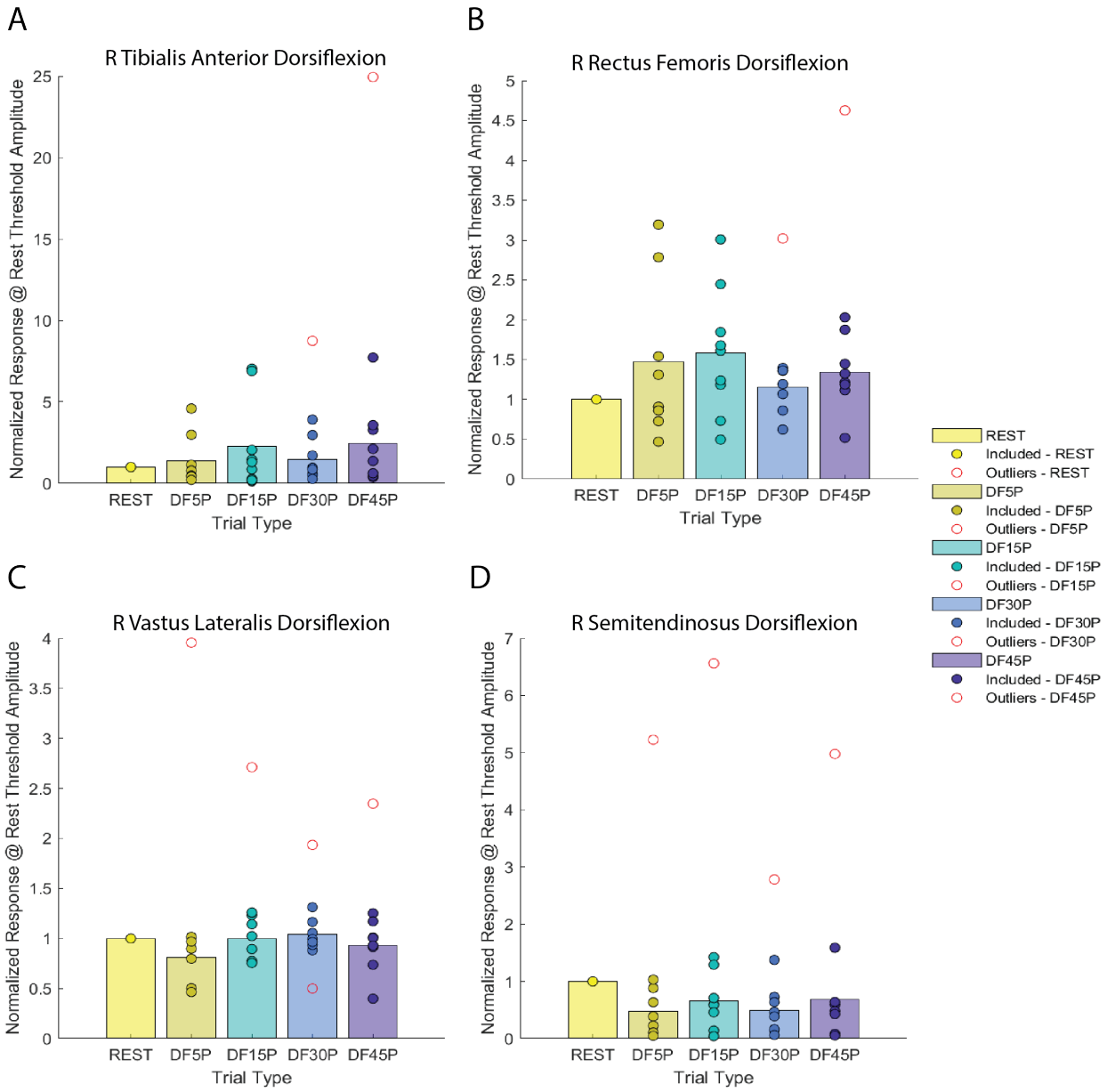
Supplementary Figures



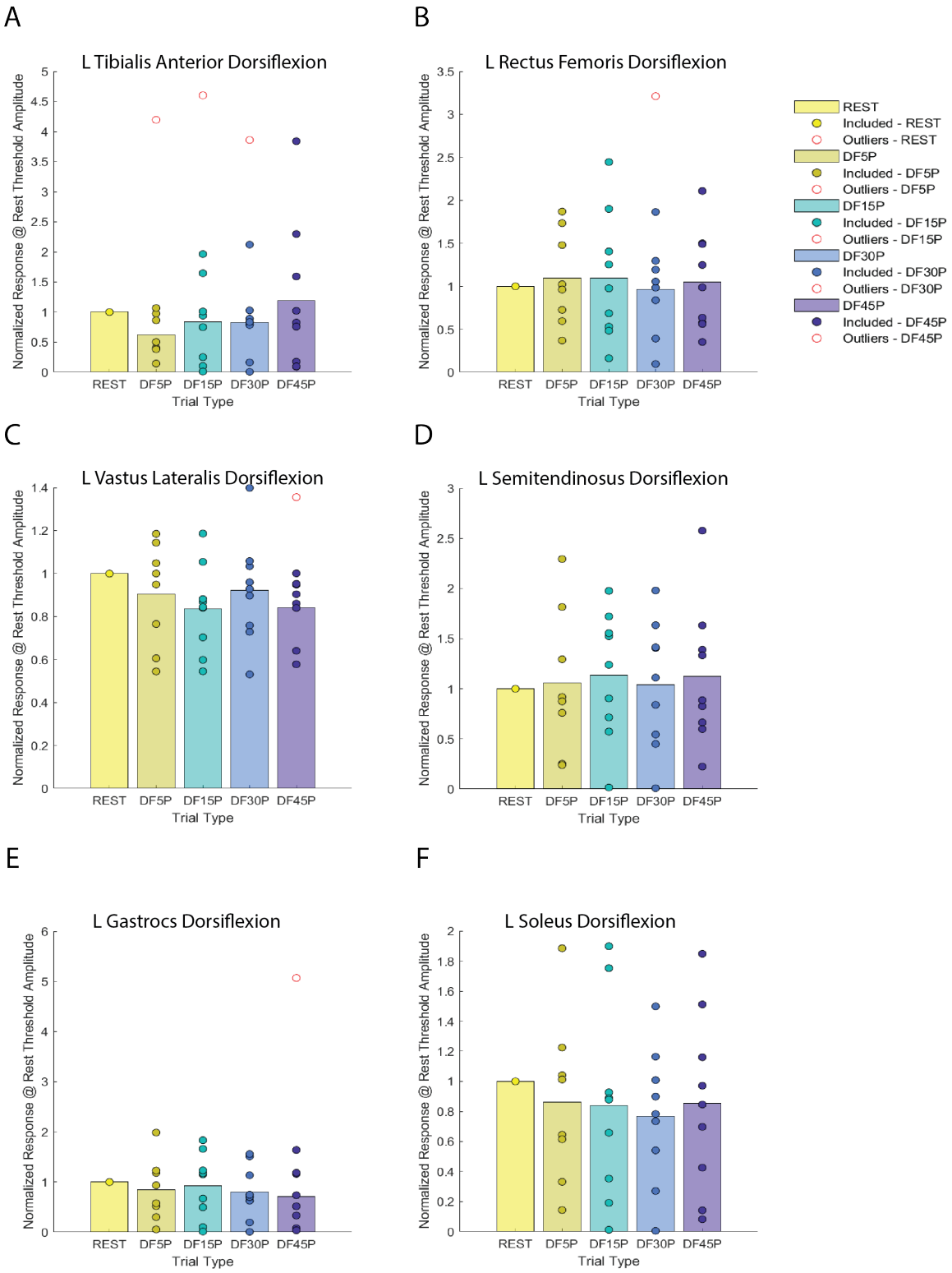
Supplementary Figure 1 Post-hoc statistical analyses on muscles affected during dorsiflexion: **(A)** right medial gastrocnemius, **(B)** right soleus. *(I-IV)* Comparison of muscle response in each group to rest with non-corrected p-values.



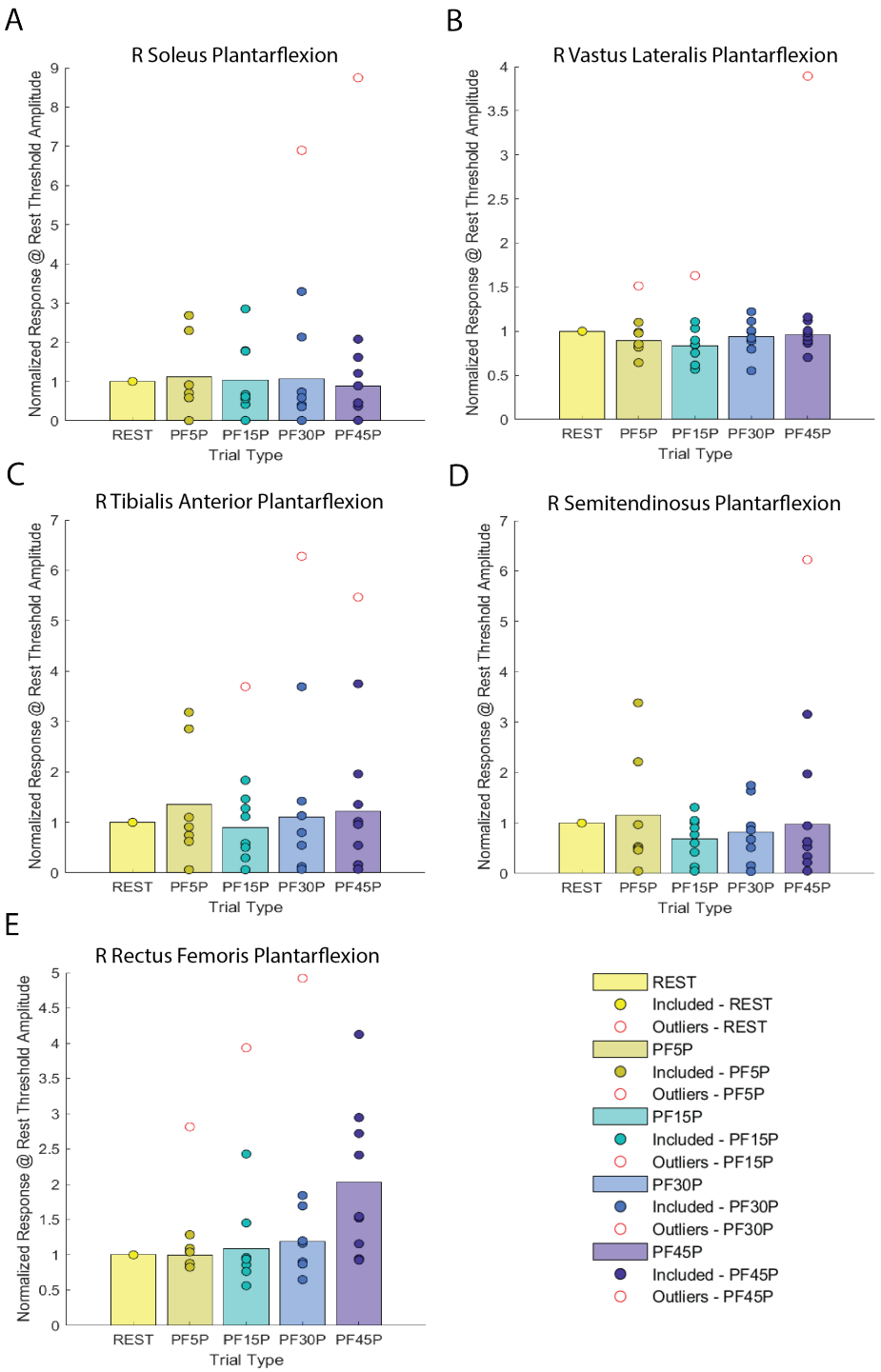
Supplementary Figure 2 Post-hoc statistical analyses on muscles affected during plantarflexion: **(A)** right medial gastrocnemius (rest vs. 15%), **(B)** left rectus femoris (rest vs. 15%), **(C)** left soleus (rest vs. 15%), and **(D)** left soleus (rest vs. 30%).



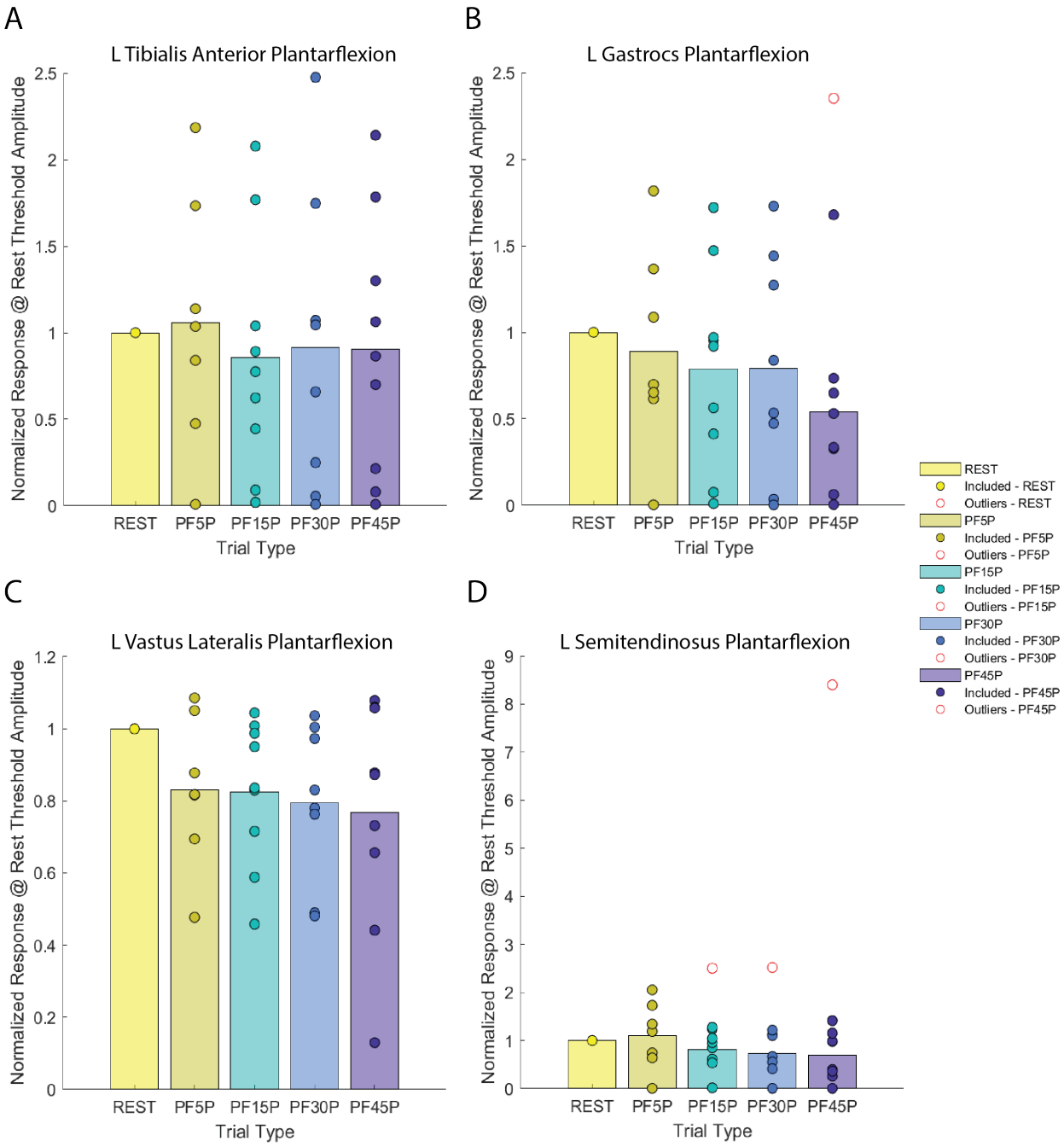
Supplementary Figure 3 Ipsilateral muscles that did not display responses that are significantly different compared to rest during dorsiflexion: **(A)** right tibialis anterior, **(B)** right rectus femoris, **(C)** right vastus lateralis, **(D)** right semitendinosus.



Supplementary Figure 4 Contralateral muscles that did not display responses that are significantly different compared to rest during dorsiflexion: (**A)** left tibialis anterior, **(B)** left rectus femoris, **(C)** left vastus lateralis, **(D)** left semitendinosus, **(E)** left medial gastrocnemius, **(F)** left soleus.



Supplementary Figure 5 Ipsilateral muscles that did not display responses that are significantly different compared to rest during plantarflexion: **(A)** right soleus, **(B)** right vastus lateralis, **(C)** right tibialis anterior, **(D)** right semitendinosus, **(E)** right rectus femoris.



Supplementary Figure 6 Contralateral muscles that did not display responses that are significantly different compared to rest during plantarflexion: **(A)** left tibialis anterior, **(B)** left medial gastrocnemius, **(C)** left vastus lateralis, **(D)** left semitendinosus.