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Evaluating Neuromuscular Function of the Biceps Brachii after Spinal Cord Injury: Assessment of Voluntary Activation and Motor Evoked Potential Input-Output Curves Using Transcranial Magnetic Stimulation

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Evaluating Neuromuscular Function of the Biceps Brachii after Spinal Cord Injury: Assessment of Voluntary Activation and Motor Evoked Potential Input-Output Curves Using Transcranial Magnetic Stimulation

A dissertation submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy at Virginia Commonwealth University.

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List of Abbreviations:

BAC: Baclofen

CNS: Central Nervous System

CV: Coefficient of Variation

tDCS: Direct Current Transcranial Stimulation

EEG: Electroencephalography

EMG: Electromyography

FDI: First Dorsal Interosseous

GABA: Gamma-Aminobutyric Acid

ICC: Intraclass Correlation Coefficient

ICF: Intracortical Facilitation

ISI: Inter-stimulus Interval

ISNCSCI: International Standards for Neurological Classification of SCI

LICI: Long Interval Intracortical Inhibition

MEP: Motor Evoked Potential

MRI: Magnetic Resonance Imaging

MSO: Maximal Stimulator Output

MVA: Motor Vehicle Accident

MVC: Maximal Voluntary Contraction

NMDA: N-methyl-D-aspartate

OX: Oxybutynin

RMS: Root Mean Square

RMT: Resting Motor Threshold

SCI: Spinal Cord Injury

SD: Standard Deviation

SEM: Standard Error of the Mean

SICI: Short Interval Intracortical Inhibition

SIT: Superimposed Twitch

TMS: Transcranial Magnetic Stimulation

VA: Voluntary Activation

VA_{PNS}: VA measured with Peripheral Nerve Stimulation

VA_{TMS}: VA measured with Transcranial Magnetic Stimulation

Abstract

Activation of upper limb muscles is important for independent living after cervical spinal cord injury (SCI) that results in tetraplegia. An emerging, non-invasive approach to address post-SCI muscle weakness is modulation of the nervous system. A long-term goal is to develop neuromodulation techniques to reinnervate (i.e. resupply nerve to) muscle fiber and thereby increase muscle function in individuals with tetraplegia. Towards this goal, developing monitoring techniques to quantify neuromuscular function is needed to better direct neurorehabilitation. Assessment of voluntary activation (VA) is a promising approach because the location of the stimulus can be applied cortically using transcranial magnetic stimulation (TMS) or peripherally (VA_{PNS}) to reveal what levels of the nervous system are disrupting the innervation of muscle fibers. Voluntary activation measured with TMS (VA_{TMS}) can indicate deficits in voluntary cortical drive to innervate muscle. However, measurement of VA_{TMS} is limited by technical challenges, including the difficulty in preferential stimulation of cortical neurons projecting to the target muscle and minimal stimulation of antagonists. Thus, the motor evoked potential (MEP) response to TMS in the target muscle compared to its antagonist (i.e. MEP ratio) may be an important parameter in the assessment of VA_{TMS}. Using current methodology, VA_{TMS} cannot be reliably assessed in patient populations including individuals with tetraplegia. The overall purpose of this work was to investigate novel TMS-based methods to evaluate neuromuscular function after spinal cord injury. First, we developed and evaluated new methodology to assess VA_{TMS} in individuals with tetraplegia. The objective of the first study was to optimize the biceps/triceps MEP ratio using modulation of isometric elbow flexion angle in nonimpaired participants and participants with tetraplegia following cervical SCI (C5-C6). We hypothesized that the more flexed elbow angle would increase the MEP ratio. The MEP ratio was only modulated in the nonimpaired group but not across the entire range of voluntary efforts used to estimate VA_{TMS}. However, we established that VA_{TMS} and VA_{PNS} in individuals with tetraplegia were repeatable across days. In a second study, we aimed to optimize MEPs during the assessment of VA_{TMS} using paired pulse TMS to elicit intracortical facilitation and short-interval intracortical inhibition. We hypothesized that intracortical facilitation would lead to an

increased MEP ratio compared to single pulse and that short-interval intracortical inhibition would lead to a lower MEP ratio. The MEP ratio was modulated in both groups but not across the entire range of voluntary efforts, and did not affect VA_{TMS} estimation compared to single pulse TMS. Paired pulse TMS outcomes revealed abnormal patterns of intracortical inhibition in individuals with tetraplegia. Further, VA_{TMS} was sensitive to the linearity of the voluntary moment and superimposed twitch relationship. Linearity was lower in SCI relative to nonimpaired participants. We discuss the limitations of VA_{TMS} in assessing neuromuscular impairments in tetraplegia. In a third study, we aimed to collect MEP input-output curves of the biceps in SCI and nonimpaired and evaluate curve-fitting methodology as well as their repeatability across sessions. We hypothesized that slopes would be greater in the SCI group compared to nonimpaired. Slopes obtained with linear regression were greater in tetraplegia compared to nonimpaired participants, suggesting compensatory reorganization of corticomotor pathways after SCI. Linear regression accurately represented the slope of the modeled data compared to sigmoidal function curve-fitting method. Slopes were also found to be repeatable across days in both groups. In a fourth study, we aimed to implement a low-cost navigated TMS system (< $3000) that uses motion tracking, 3D printed parts and open-source software to monitor coil placement during stimulation. We hypothesized that using this system would improve coil position and orientation consistency and decrease MEP variability compared to the conventional method when targeting the biceps at rest and during voluntary contractions across two sessions in nonimpaired participants. Coil orientation error was reduced but the improvement did not translate to lower MEP variability. This low-cost approach is an alternative to expensive systems in tracking the motor hotspot between sessions and quantifying the error in coil placement when delivering TMS. Finally, we conclude and recommend future research directions to address the challenges that we identified during this work to improve our ability to monitor neuromuscular impairments and contribute to the development of more effective neurorehabilitation strategies.

1 Chapter 1: Introduction

1.1 Neuromuscular Function and Spinal Cord Injury

1.1.1 Background

Chronic spinal cord injury (SCI) is a condition of the central nervous system (CNS) that may involve motor impairment through partial or complete paralysis [1]. Currently, an estimated 288,000 individuals are suffering from a chronic spinal cord injury in the United States, with an additional 17,700 new cases occurring each year ("Facts and Figures at a Glance," 2018). The most common form is incomplete tetraplegia, which can be caused by damage to the low cervical section of the spinal cord (C5-C8), causing deficits in the upper limb neuromuscular function. Upper limb function is crucial for daily activities and is often rated as the single most desired ability to be recovered by individuals with tetraplegia to improve their quality of life [2].

Functional reorganization of the neuromuscular system following SCI takes place across multiple sites of the nervous system [3]. Lesions to the spinal cord will cause adaptive responses leading to the reorganization of the cortical representations of muscles, where changes occur immediately after injury [4], [5] and on a long-term scale [6]. Most patients will experience some degree of spontaneous functional recovery within the first year post-injury, but motor function will typically stagnate thereafter [7]. This suggests that while the CNS has innate repair mechanisms, they are alone insufficient to reach higher levels of motor function recovery after large lesions. Thus, *understanding neuroplasticity following SCI has implications for the development of rehabilitation programs that will take motor function recovery further* [8].

Neurorehabilitation has the potential to address muscle weakness, spasticity, and fatigability after SCI [9]–[12]. Repeated activation of the spared motor pathways leads to the strengthening of existing neuromuscular circuits and the sprouting of new fibers and connections between the cortex, the brainstem, and the spinal cord [13]–[15]. Following this principle, motor training-based approaches (e.g., physical therapy) after SCI have shown promising results, especially in combination with neuromodulation [9], [10]. For instance, repetitive transcranial

magnetic stimulation interleaved with the practice of a hand function task led to clinical improvements in grasp strength and the performance of a fine motor task in the target hand of individuals with SCI (effect sizes reported for grasp strength; treatment: 0.67 versus sham: 0.39, Jebsen-Taylor Hand Function Scores: treatment: 0.85 versus sham: 0.42)[11]. Effects were also found in the contralateral hand, implying transfer of the training effects to the non-trained hand. Additionally, maximal intensity resistance training in the lower limb of incomplete SCI participants was shown to increase volitional function and strength [12]. Spasticity is caused by abnormal supraspinal influence over spinal reflex circuits [16]. Operant conditioning of spinal reflexes such as the H-reflex (a form of neurorehabilitation) has the potential to decrease spasticity in the lower and upper limb of SCI individuals [17], [18]. Repetitive TMS protocols have also been used to reduce spasticity in chronic SCI [19], [20]. Finally, previous work demonstrates a decrease in fatigue resistance of muscle affected by SCI [21], [22]. This is especially relevant to our work since the technique we evaluated in chapters 2 and 3 have direct implications for the assessment of neuromuscular fatigue.

1.1.2 Biceps Brachii function after Spinal Cord Injury

In the upper limb, distal muscles (e.g., muscles in the hand) have been the primary focus of neuromodulation research to date [23]. One reason is that hand muscles such as the first dorsal interosseous (FDI) have greater cortical representations (i.e. higher neuron count and connections associated with controlling the muscle fibers) compared to proximal muscles which make them easier targets for neuromodulation [3], [24], [25]. However, proximal muscles, such as those spanning the elbow, are critical for upper limb function. The ability to extend and flex the elbow increases the workspace of the hand and enhances a person's ability to grasp and manipulate objects [26], [27]. One of these muscles, the biceps brachii, remains relatively functional after an SCI at the cervical level [28], [29]. In fact, a recent report showed similar MEPs (SCI: 45.4 ± 25.7% of M-max, controls: 47.0 ± 24.3% of M-max) and EMG activity during maximal voluntary contractions (MVCs) between individuals with incomplete cervical SCI and healthy controls [30]. The authors also suggest that enhanced reticulospinal inputs to the biceps brachii may be responsible for the biceps' greater potential for spontaneous recovery compared to its

antagonist (the triceps). Relatively spared muscle groups after cervical SCI, such as the biceps brachii, may be particularly responsive to neuromodulation. Plasticity of the neuromuscular system preferentially occurs when existing circuits are solicited via motor training or external stimulation [13]–[15]. Consequently, a focus on muscle groups relatively preserved can yield long-term benefits for SCI patients by maximizing the function of remaining neuromuscular circuits.

Although the biceps brachii is relatively preserved after cervical SCI compared to more distal muscles, the biceps brachii is typically affected by the consequences of SCI. Namely, the biceps brachii function after SCI at the C5-C6 level is commonly characterized by decreased strength (i.e., muscle weakness)[28], increased spasticity [31], and decreased fatigue resistance compared to nonimpaired muscle [32]. In particular, reciprocal inhibition between the biceps brachii and its antagonist the triceps brachii is altered following an SCI, leading to increased triceps activation during elbow flexion [33]. Moreover, previous work investigated the properties of single motor units during voluntary contraction of the biceps brachii of SCI patients [34]. They found a decreased firing rate compared to healthy controls, which may contribute to increased fatigability. Finally, spasticity affecting the biceps brachii may be improved by intervention [31]. Operant conditioning training experiments in SCI patients have shown that biceps brachii spinal stretch reflex can be down-regulated after 8 weeks of training [18].

Tendon transfer surgery is another reason why the biceps brachii function after a SCI is important [35], [36]. The biceps can be surgically transferred to insert at the tip of the olecranon such that the biceps performs the function of the more paralyzed triceps [37]. After surgery, the individual undergoes rehabilitation aimed at motor re-education, which is to say learning how to use the biceps in its new function, to extend the elbow. However, substandard results after tendon transfer have been reported, which may be due to poor motor re-education, tendon rupture, stretching, and altered biomechanics [38]. Thus, improved ways to evaluate the biceps brachii function prior to and after this surgery may help predict clinical outcomes of tendon transfer.

The biceps brachii is part of the elbow flexor group. Flexion-extension of the elbow is critical for activities of daily living and preserving or restoring its proper function in individuals with tetraplegia is ranked as a top priority [39]. Currently, clinical assessment of muscle function in individuals with SCI is subjective; a clinician follows a manual muscle testing protocol, grading each muscle on a scale from 0 (total paralysis) to 5 (normal active movement)[40]. However, manual muscle testing is a poor predictor of voluntary strength and cannot precisely elucidate the amount of innervated muscle fiber [41]. A more comprehensive and quantitative evaluation of the biceps brachii function post-SCI will pave the way towards more effective neuromodulation protocols aimed at recovering and optimizing arm function for individuals with tetraplegia.

1.2 Transcranial Magnetic Stimulation

1.2.1 Background

Transcranial magnetic stimulation (TMS), a type of non-invasive brain stimulation, can be used to evaluate and modify cortical physiology and the condition of motor pathways [42], [43]. When TMS is delivered to the motor cortex (M1) region of the brain, the resulting action potentials travel down the pyramidal tract and through the spinal cord, where it can be recorded with implanted epidural electrodes as D-waves and I-waves (Figure 1-1). I-waves reflect the indirect activation of pyramidal neurons via interneuron recruitment, which are preferentially recruited as stimulus intensity increases [43]. D-waves reflect the activation of axons directly from stimulation (i.e. shortest pathway for action potentials). The motoneurons in the spinal cord then activate muscle fibers through the neuromuscular junction to elicit a muscle response (Figure 1-2). The response known as a motor evoked potential (MEP) can be recorded using

electromyography (EMG) sensors and used to evaluate changes to the excitability of the corticospinal motor pathway [44], [45].

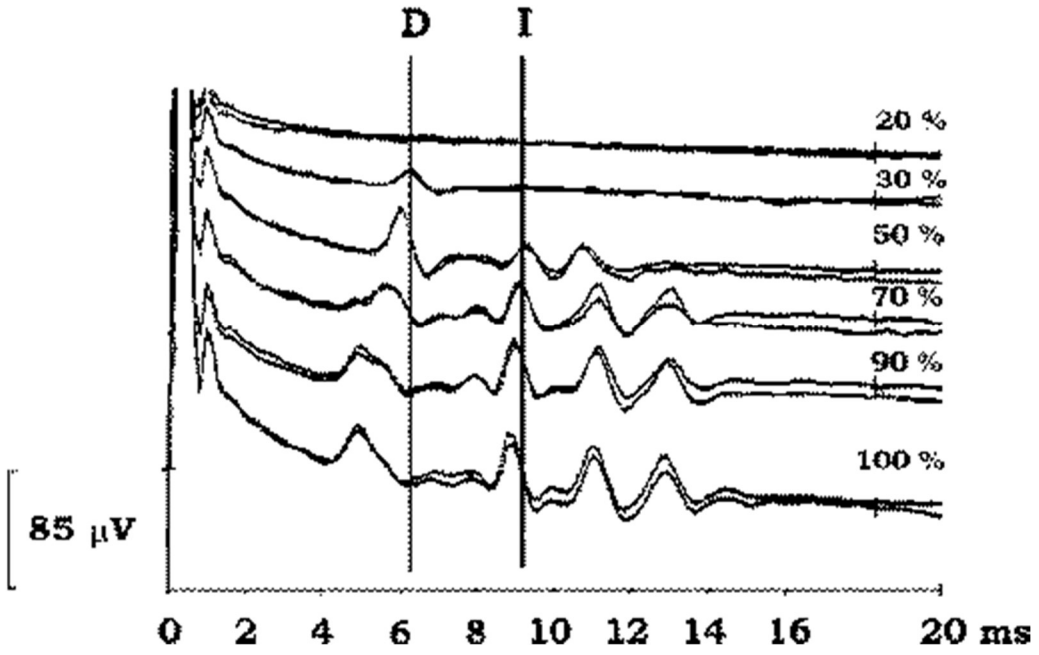


Figure 1-1. Schematic representing epidural recording of D-waves and I-waves following TMS delivered at various stimulator intensities. More/stronger I-waves are induced with higher intensities (100% corresponds to 750 volts of stimulator output) (modified from CNS Clinic - Jordan - Munir Elias).

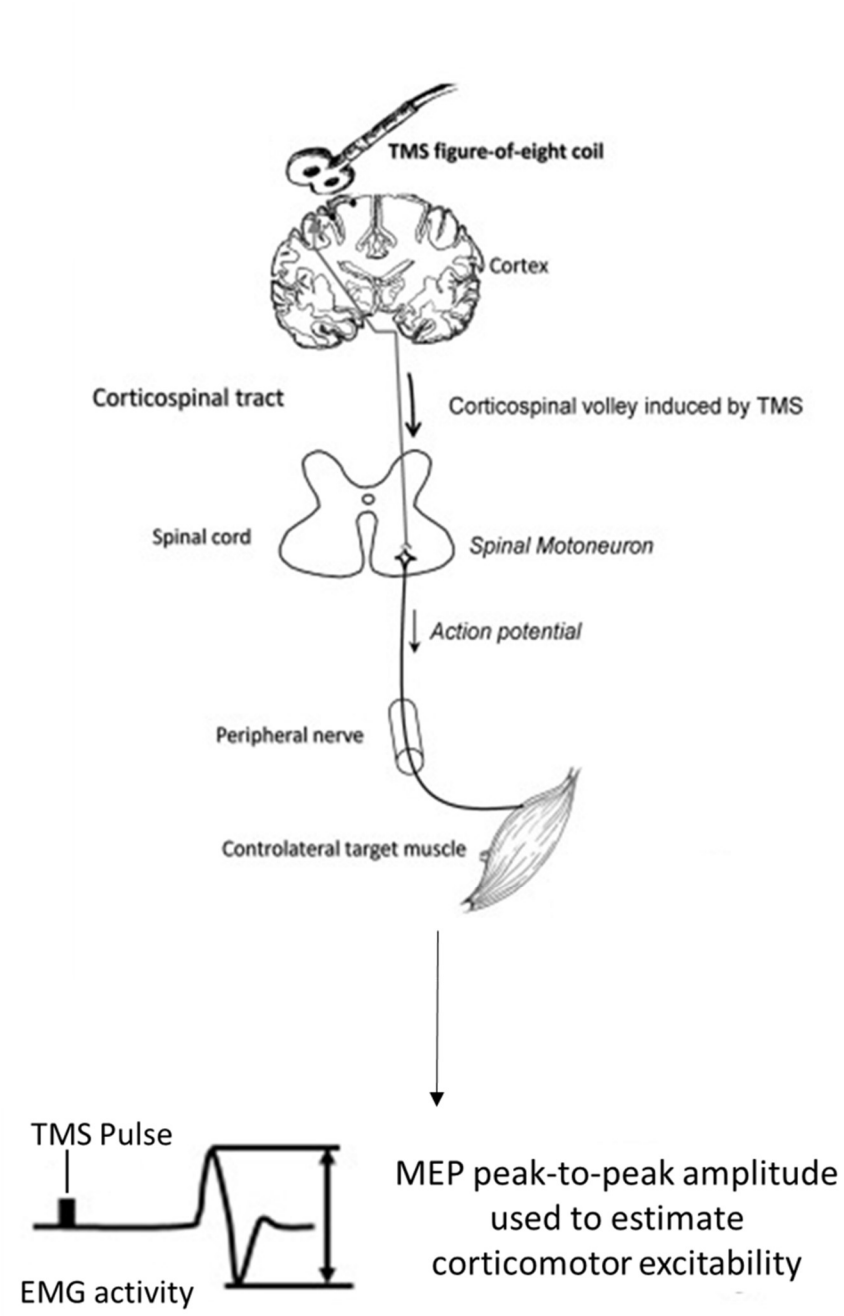


Figure 1-2. Schematic illustrating how TMS activates the descending motor pathways. Recruited neurons by TMS evoke a descending volley of signals down the corticospinal tract, ultimately causing the contralateral muscle to contract. The motor evoked potential (MEP) can be used to evaluate corticomotor excitability (modified from Klomjai, Katz, & Lackmy-Vallée, 2015).

The amplitude of MEPs can be used to assess the integrity of motor output to the spinal cord, changes within M1, and changes in cognitive processes that influence M1 [46]. The assumption is that MEPs are correlated to motor performance itself such as accuracy of movement [47] and force generation [48]. However, MEPs are highly variable and sensitive to multiple parameters such as the level of activation of the target muscle [49], reliable positioning of the TMS coil [50], [51], and fluctuations in the existing activity of neurons and interneurons at the time of stimulation [52].

TMS metrics can predict functional outcomes in patient populations [53]–[55]. For example, in post-stroke patients, the presence of MEPs in the biceps brachii and FDI muscles were associated with higher joint coordination (Fugl-Meyer Scores), higher likelihood of muscle contractions, and greater strength [56]. When combined with electroencephalography, TMS has the potential to be a marker of post-stroke upper-limb motor function [44], [57]. TMS outcomes can also be used for the diagnosis of neurodegenerative dementia by using TMS parameters for classification instead of other validated markers (e.g. cerebrospinal fluid analysis, amyloid positron emission tomography) [58]. Together, this suggests that TMS can be a promising diagnosis and monitoring tool in patient populations. Our goal is to harness advanced TMS-based techniques to improve the ability to evaluate neuromuscular impairments in individuals with tetraplegia.

1.2.2 Advanced Transcranial Magnetic Stimulation Techniques

TMS parameters can be modified and/or combined with other tools to exploit the full potential of its capabilities as a tool for probing motor pathways. TMS research features several experimental techniques that have been employed to characterize different aspects of motor pathway function following SCI; the following are the techniques relevant to this work.

Paired-pulse TMS:

Paired-pulse TMS can investigate the balance between excitatory and inhibitory pathways within the brain [59]. When two stimulators are connected to the same coil, it is possible to fire

two TMS pulses with various inter-stimulus intervals. When the first pulse is subthreshold (i.e. lower intensity than necessary to elicit a MEP), it can be used as a conditioning stimulus that will cause either facilitation or inhibition of the subsequent MEP response (Figure 1-3) [44], [60]. An inter-stimulus interval (ISI) between 10 and 30 ms will produce intracortical facilitation (ICF) that will cause increased MEP amplitudes. On the other hand, a very short inter-stimulus interval of 1-2 ms will result in short interval intracortical inhibition (SICI) and decreased MEP amplitudes. The mechanisms behind ICF are mostly related to the N-methyl-d-aspartate (NMDA) receptors mediated facilitation [61] while SICI is thought to be related to GABA-A receptor-mediated inhibition [62]. In the SCI population, studies involving sub-threshold TMS have been used to study the balance between excitatory and inhibitory processes in the brain. A reduced activity of intracortical inhibitory circuits (via SICI) was observed that may act as a compensatory mechanism enabling more cortical reorganization [63]–[65].

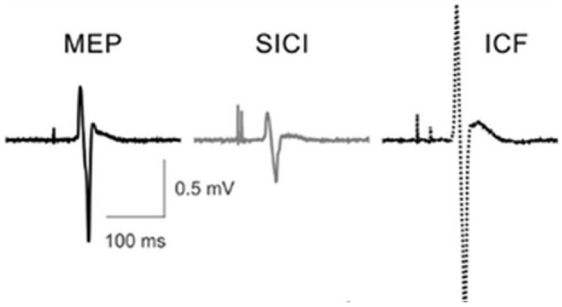


Figure 1-3: Paired-pulse TMS affects MEP amplitudes. From left to right: single pulse TMS, paired-pulse TMS with ISI 1-2 ms, paired-pulse TMS with ISI 10-30 ms (modified from Auriat et al, 2015).

MEP input-output curves:

MEP input-output curves, otherwise referred to as stimulus-response or recruitment curves, are another way to characterize corticomotor excitability by probing the motor cortex with increasing stimulus intensities (Figure 1-4) [66]. MEP input-output curves are collected by delivering TMS across a range of stimulator intensities, ranging from subthreshold to suprathreshold. Plotting the MEPs against TMS intensity, curves typically resemble a sigmoidal waveform that plateaus at higher stimulus intensities [66]. The slope of the curve indicates the

excitability of the targeted neuromuscular circuit across multiple neuronal populations, such as direct corticospinal projections and inter-cortical neurons [39]. Increased slope values represent greater overall level of excitability throughout the corticospinal tract of the target muscle [68]. Furthermore, compared to a single MEP response, which is highly variable and state-dependent, the slope is extracted from multiple MEPs elicited over the course of several minutes and at various stimulation intensities, reflecting a more comprehensive and non-instantaneous measure of corticomotor excitability.

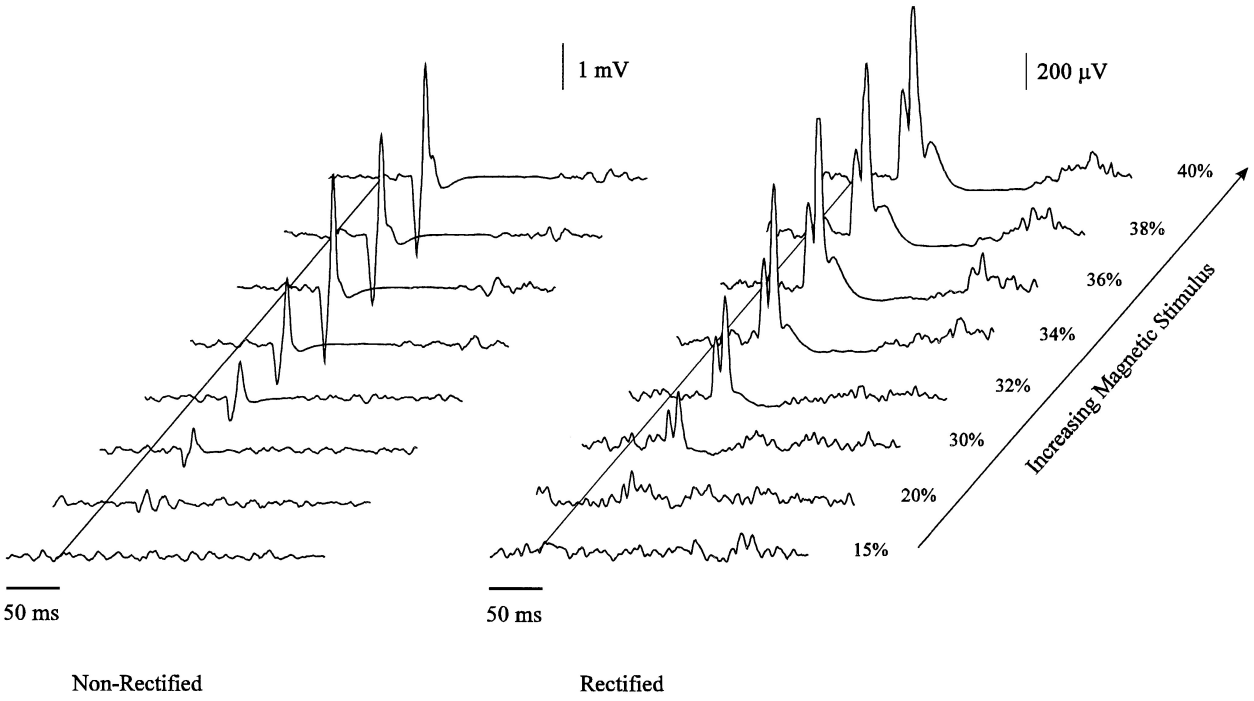


Figure 1-4. Representation of typical EMG recordings of MEPs as a function of TMS intensity that are used to construct the MEP input-output curve (From Devanne et al. 1997).

MEP input-output curves can be used to evaluate the integrity and function of neuromuscular circuits in patient populations and guide rehabilitation. For example, after immobilization (90 days of bed rest) in healthy volunteers, the slopes of the MEP input-output curves collected in the leg and hand were decreased [69]. In stroke patients, the slope of the

curve in the FDI was negatively correlated to the magnitude of task-related brain activation (as assessed via functional MRI techniques) in several motor-related regions, such as the primary motor cortex and the supplementary motor area [70]. Another study found an association between graded increases in the slope and graded functional gains in recovery following a direct current transcranial stimulation (tDCS) protocol in the hand of post-stroke patients [71]. Together, this suggests that MEP input-output curves have the potential to be used as a tool to inform rehabilitation.

Navigated TMS:

One factor that contributes to the variability of TMS metrics is the coil positioning when targeting a specific cortical region and the preservation of this position during and across sessions [72], [73]. Navigated TMS systems were initially developed for neurosurgical planning and functional motor cortex mapping where they helped investigators maintain spatial accuracy of about 2 mm (intra-session stability) [74]. Here, we define spatial accuracy as the distance (in millimeter), from the visualized "hotspot" location to the location of stimulation. We define the hotspot as the location where the MEP response is highest and most specific (i.e. minimal activation of surrounding muscles). Some advanced navigation systems use patient-specific magnetic resonance images (MRI) to visualize individual anatomical structures of the brain (Figure 1-5). Intracranial E-field can then be calculated and used for subsequent navigation [75]. While MRI and E-field based navigation are advantageous since the stimulation of the actual cortical structures can be predicted and quantified, these navigated TMS systems are very expensive (> $50,000), add many preparatory steps, including individual MRI scans, to a TMS protocol which represent limiting factors for its use in the clinic and research [76]. It is possible to develop more affordable and user-friendly alternatives that may improve spatial accuracy (coil position and orientation) and the reliability of TMS outcomes [77]. In its simplest form, navigated TMS consists of reliably tracking the position of the coil relative to the head. Real-time 3D feedback is then provided to the technician to assist in the re-positioning of the coil over the

previously determined hotspot. To date, this novel, low-cost approach has only been evaluated in the FDI muscle in three participants.

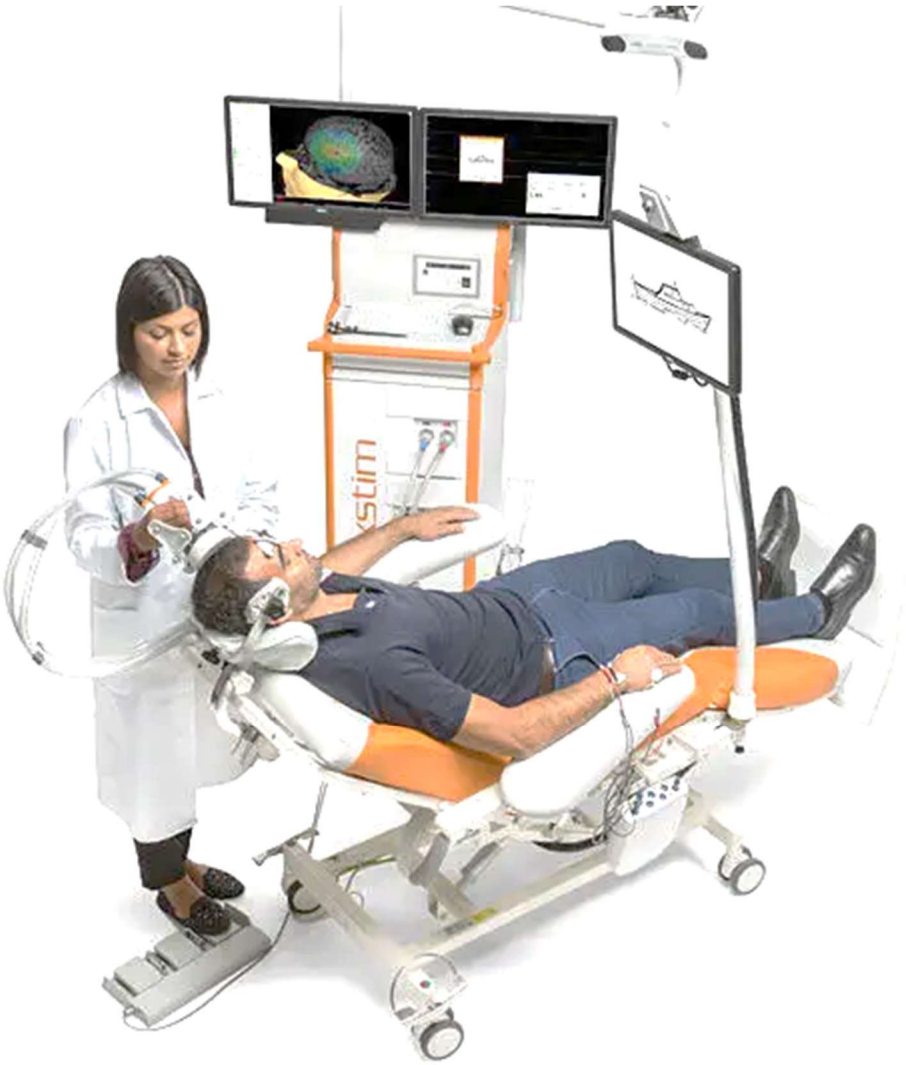


Figure 1-5. Example of commercial navigated TMS system (Nexstim NBS system 5) that uses MRI-guided navigation to ensure accurate and repeatable stimulation (From Nextstim.com).

Whether Navigated TMS is effective to decrease MEP variability is unclear and may depend on additional parameters such as the activation state of the motor pathways and the muscle tested [49], [78], [79]. In fact, previous work was not able to find an effect of navigated TMS on MEP amplitudes and their coefficient of variance in the FDI muscle at rest [80]. However, since

MEP variability largely depends on the target muscle and its level of voluntary activation [49], navigated TMS may be beneficial in a different context.

1.3 Voluntary Activation

Voluntary activation is a measure of the neuromuscular system's ability to activate muscle fibers [81]. It has namely been used in fatigue protocols to elucidate the specifics of neuromuscular fatigue, whether it takes place within the CNS (central fatigue) or is caused by changes at the muscle level [82], [83]. Voluntary activation is assessed using the interpolated twitch technique, which relies on recording measurable and repeatable muscle twitch forces in response to stimulation [81], [84]. The ratio between the twitch torque superimposed to a maximal voluntary contraction (MVC) and the twitch torque at rest can quantify the completeness of motor units' recruitment by the neural drive. When electrical stimulation is used peripherally, the collected measure is known as voluntary activation, which we will refer to as VA_{PNS}.

VA_{PNS} can be used in patient populations to investigate fatigue properties and neuromuscular impairments. For instance, VA_{PNS} deficits in voluntary activation of muscle were identified in upper limb muscles after stroke [85]. In incomplete SCI individuals, VA_{PNS} of the flexor carpi radialis muscle was lower compared to healthy controls [69]. This study also revealed that the deficit in central drive was responsible for a higher sensitivity to fatigue. Another study used VA_{PNS} to evaluate a paired corticospinal-motoneuronal stimulation paradigm in the biceps brachii of individuals with tetraplegia but they did not find any effect [87]. When nonimpaired subjects maximally contract muscle, nearly all innervated muscle fibers can be activated by voluntary effort (Figure 1-6). In contrast, in muscle affected by SCI composed of both innervated and denervated muscle fibers, only innervated muscle fibers can be activated by voluntary effort (Figure 1-6). When electrical stimulation is superimposed on maximum voluntary contraction by individuals with tetraplegia, the stimulation activates additional motoneurons resulting in a large twitch force [88]. VA_{PNS} is a reliable measure in the nonimpaired biceps brachii within an

individual across days [84]. In nonimpaired individuals, all muscle fibers are innervated and VA_{PNS} less than 100% is due to the limited rate at which motoneurons can be recruited.

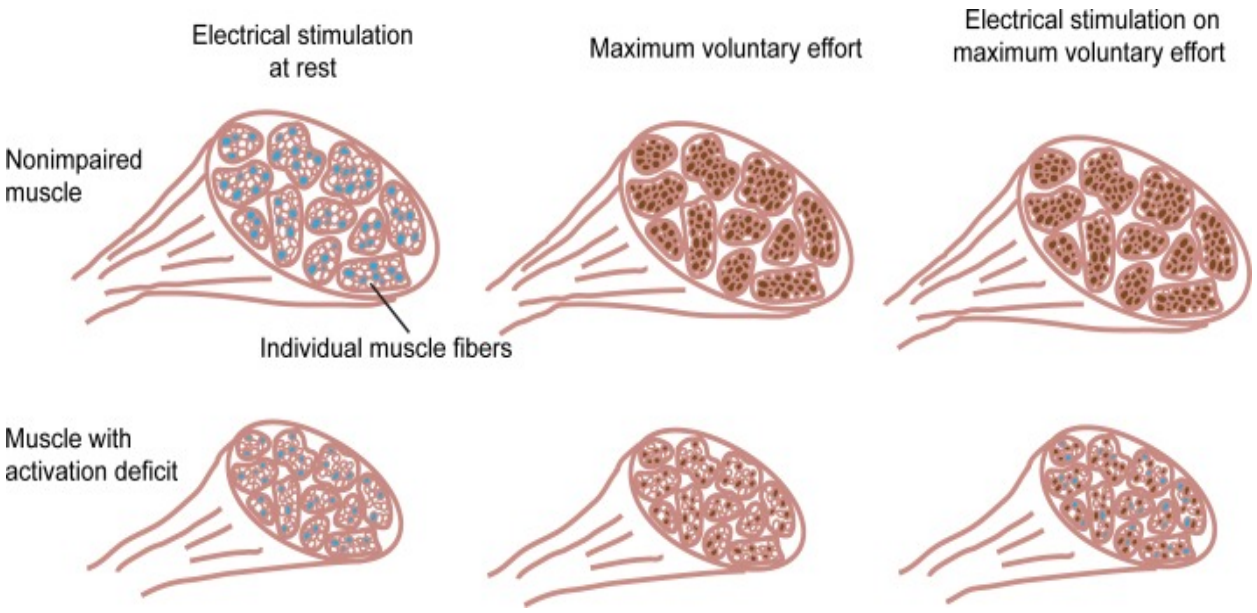


Figure 1-6: Cross-section illustration of muscle to demonstrate the recruitment of motor units during electrical stimulation, maximum voluntary effort, and electrical stimulation superimposed on maximum voluntary effort. Electrical stimulation with the muscle at rest recruits part of the motor pool (light blue fill represents muscle fibers innervated by motor units recruited by electrical stimulation). In nonimpaired muscle, nearly the entire motor pool can be voluntarily recruited at maximum effort (brown fill represents muscle fibers innervated by motor units recruited voluntarily); superposition of electrical stimulation results in additional recruitment of only a few motor units. In muscle with an activation deficit, only a percentage of the motor pool can be recruited during maximum voluntary effort; superposition of electrical stimulation recruits many additional motor units (Modified from Peterson et al, 2017).

The principle of the interpolated twitch technique can be used to assess voluntary activation at different neuromuscular sites [89]. TMS over the motor cortex can be used to assess voluntary activation, which we refer to as VA_{TMS}. A key difference in using the interpolated twitch technique to assess VA_{TMS} as opposed to VA_{PNS} is the site of stimulation. VA_{PNS} cannot indicate the site of deficit in voluntary drive within the pathway from the motor cortex to muscle. Using TMS to activate cortical neurons evokes a MEP, which produces a consequent twitch force that

can be recorded by a sensitive load cell. Thus, VA_{TMS} can provide additional insight on how optimally cortical neurons are voluntarily activated to generate descending action potentials to the muscles.

Over the past ten years, VA_{TMS} has been broadly used in various protocols to study the mechanisms of neuromuscular function and fatigue following physical training [90]–[94], in aging populations [95], [96], and in patient populations [97]–[101]. While most studies have focused on the upper limb [102], VA_{TMS} can also be assessed in the lower limb [91], [103] or even back muscles [100], [104]. Several studies also focused on the methodological aspects of assessing VA_{TMS} [102], [105]–[107]. VA_{TMS} can monitor physical therapy protocols. For example, VA_{TMS} was increased after eight weeks of local vibration training in the lower limb [108]. In another study, VA_{TMS} was reduced in athletes that had suffered a concussion, highlighting impairments that persist beyond the acute phase of the concussion [98]. VA_{TMS} is especially relevant in the context of neuromuscular impairments that affect individuals with tetraplegia since it offers a quantitative measure of the ability to generate neural drive to muscle. VA_{TMS} also constitute a more direct way of testing the level of neuromuscular impairment, as opposed to manual muscle testing. Identifying impairments in voluntary activation in individuals with tetraplegia is important because it allows quantifying to what extent the loss of function is directly due to neural factors. Additionally, using TMS over the motor cortex has the potential to provide insights on more specific neurophysiological mechanisms that affect motor function after an SCI. So far, VA_{TMS} has not yet been used to inform rehabilitation in patient population due to several technical limitations.

In patient populations, evaluating VA_{TMS} poses technical challenges [97], including individuals with cervical SCI [88]. Todd et al. established a protocol to reliably quantify VA_{TMS} in the elbow flexors of nonimpaired individuals [89], [109]. Using the same protocol in individuals with biceps paresis post-stroke, Bowden et al. were unable to quantify VA_{TMS} of the elbow flexors due to large variability in MEPs and twitch forces within subjects [97]. Also, in a study that attempted to quantify VA_{TMS} of the elbow extensors in individuals with tetraplegia, MEPs and

twitch forces were variable or absent in 83% of subjects such that VA_{TMS} could not be quantified [88]. The authors suggest that these findings were due to the high levels of muscle weakness and the lack of focality of cortical stimulation. In this current work, we evaluated the biceps brachii, a muscle that is typically less impaired than its antagonist following SCI, as a first step in applying the VA_{TMS} approach to muscle affected by SCI.

1.4 Motivation and Knowledge Gaps in the Field

1.4.1 Challenges of assessing VA_{TMS} in patient populations

According to Todd et al. (2016), reliably assessing VA_{TMS} of the elbow flexors requires several conditions to be met: First, we need to be able to elicit large MEPs in the elbow flexors relative to MEPs in the elbow extensors (Figure 2-1, A). In other words, a high MEP ratio recorded during stimulation is required. This can be achieved in nonimpaired individuals by selecting the optimal stimulus intensity that maximally activates cortical neurons projecting to the elbow flexors and minimally activates cortical neurons to elbow extensors. However, this is more difficult to achieve in patient populations [88], [97]. One possible explanation is that a higher TMS stimulus intensity is often required to elicit reliable MEPs. The necessity of a high stimulus intensity increases stimulus spread to other cortical areas including those that project to the triceps, thus influencing the biceps to triceps MEP ratio. We address this challenge through an innovative approach to exploit protocol modifications that can facilitate large MEPs in the contracting elbow flexors relative to the elbow extensors.

The first protocol modification consists of isometric modulation of the elbow flexion angle. Static changes in elbow flexion angle can modulate MEP amplitudes in the relaxed biceps [110]–[112]. Specifically, biceps MEPs were maximized and triceps MEPs were minimized at a more flexed elbow angle relative to more extended elbow angles [92]–[94]. Whether elbow angle can be prescribed to optimize the target/antagonist MEP ratio across the range of voluntary effort levels needed to estimate VA_{TMS} remains unknown. Thus, our approach will determine

whether careful prescription of the isometric elbow flexion angle can optimize the biceps/triceps MEP ratio and improve the measurement of VA_{TMS}.

The second protocol modification involves modulation of the stimulation paradigm from single pulse to paired-pulse TMS. Paired TMS pulse paradigm consisting of a low intensity conditioning pulse delivered prior to a second higher intensity pulse, causing intracortical facilitation (ICF), can increase MEP amplitudes in the targeted muscle. Previous work has shown that ICF can be elicited in patient populations [114], [115]. Thus, our approach is to deliver paired-pulse TMS instead of single pulse TMS to optimize the biceps/triceps MEP ratio in the assessment of VA_{TMS}. In these experiments, we also provide an understanding of activation-dependent modulation of cortical inhibition and facilitation.

Another condition of reliably assessing VA_{TMS} is the high linearity of the relationship between superimposed twitch torque and voluntary torque [102]. In nonimpaired individuals, previous work has shown that the superimposed twitch torque and voluntary torque relation is approximately linear during high voluntary contraction (Figure 1-7)[89]. Neuromuscular fatigue has been associated with decreased linearity of this relationship [116]. This is a concern since individuals with chronic SCI typically present decreased stamina and higher fatigability of motor units even in relatively spared muscle groups [32], [88]. Nevertheless, since no data on the linearity of the superimposed twitch torque and voluntary torque relation exists for individuals with tetraplegia, further investigation is warranted.

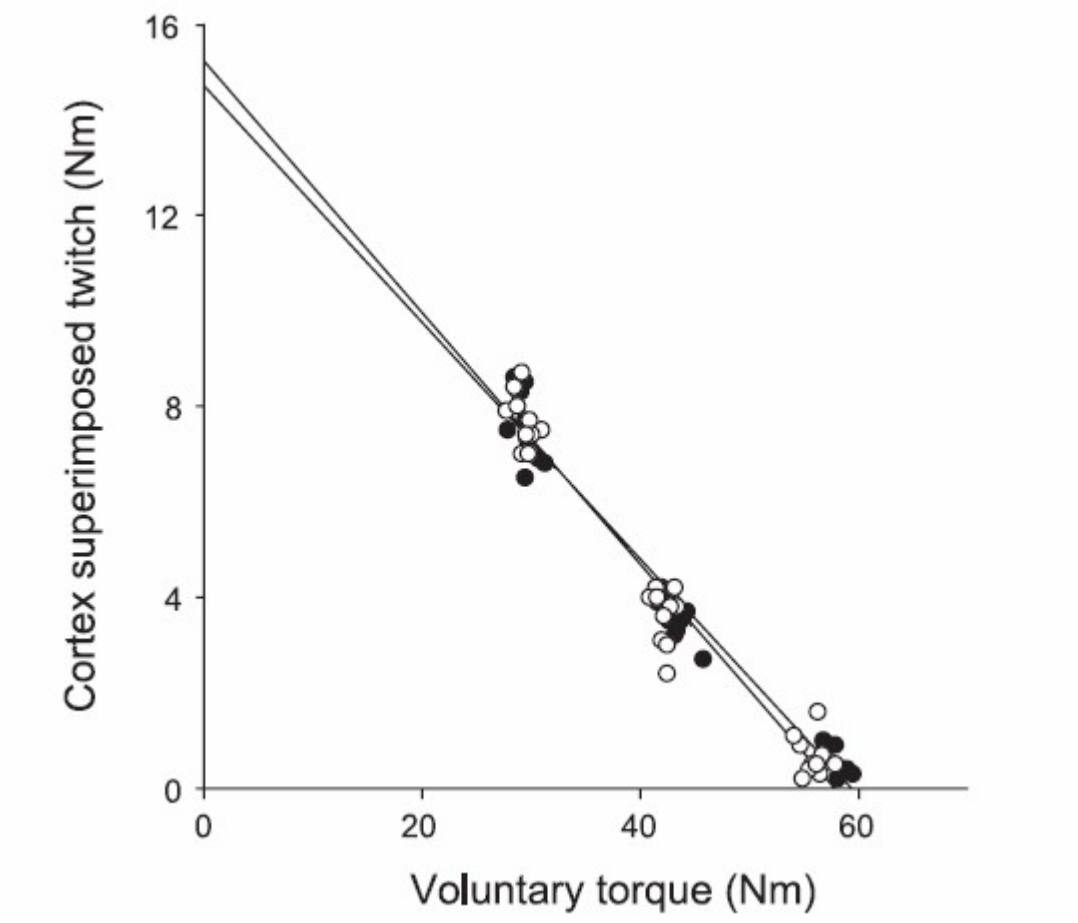


Figure 1-7. Amplitude of the superimposed twitch evoked by TMS over the motor cortex during 50, 75, and 100% MVC in the nonimpaired elbow flexors (From Todd et al., 2003).

1.4.2 TMS input-output curves in the biceps brachii of individuals with SCI

While MEP input-output curves have been studied in individuals with tetraplegia, previous work focused on upper limb distal muscles such as those of the hand [81]. However, TMS outcomes are largely dependent on the stimulation target [118]. While more proximal upper limb muscles such as the biceps often retain more function than distal muscles in patients with cervical SCI [88], [119], their neuromuscular circuits may be affected in different ways. A recent TMS motor mapping study found no differences in biceps cortical representation between chronic cervical SCI and nonimpaired participants [120]. However, the biceps is a difficult TMS target due to its relatively smaller motor cortex representations compared to hand muscles [24], [117], and

is less likely to provide reproducible MEP measures across sessions [121]. Therefore, investigating more proximal muscle groups is needed to get a more comprehensive picture of neuromuscular function after SCI.

Further, using a sigmoid curve fit method can yield reproducible outcomes when targeting the FDI muscle of nonimpaired individuals [89]. Yet, in patient populations such as stroke, analyzing MEP recruitment curves with linear regression was found to be as predictive as using a sigmoid function model [90]. Whether these findings hold when targeting the biceps brachii of individuals with cervical SCI is unknown.

1.4.3 Does low-cost TMS navigation decrease MEP variability?

Finally, low-cost navigated TMS approaches are viable tools for reliable coil placement during TMS procedures. So far, they have only been tested in the FDI muscles [77] and not on more challenging TMS targets such as the biceps brachii [121]. Additionally, protocols involving voluntary contractions add difficulty in maintaining the consistency of the cortical hotspot because of the participant's collateral motion. Whether a low-cost navigated TMS approach can be effective in reducing MEP variability in such context remains unknown.

1.5 Objectives

As a contribution to improving the monitoring of neuromuscular function in chronic SCI individuals via the advancement of TMS techniques, this work has three primary objectives (Figure 1-8). First, investigating innovative approaches to address the challenges of quantifying neural drive via voluntary activation measured with TMS in individuals with tetraplegia (elbow angle and stimulation paradigm modulation). Additionally, establishing the reproducibility across days of voluntary activation measured with TMS in individuals with tetraplegia. Second, improving the understanding of corticomotor plasticity following an SCI by comparing MEP input-output curves in the biceps brachii of individuals with tetraplegia and healthy controls. Further, establishing the reliability of MEP input-output curves in the biceps brachii of individuals with tetraplegia. Third, implementing a low-cost navigated system and testing its efficacy on MEP

variability in the biceps brachii of nonimpaired individuals during voluntary contractions to reduce variability in TMS outcomes.

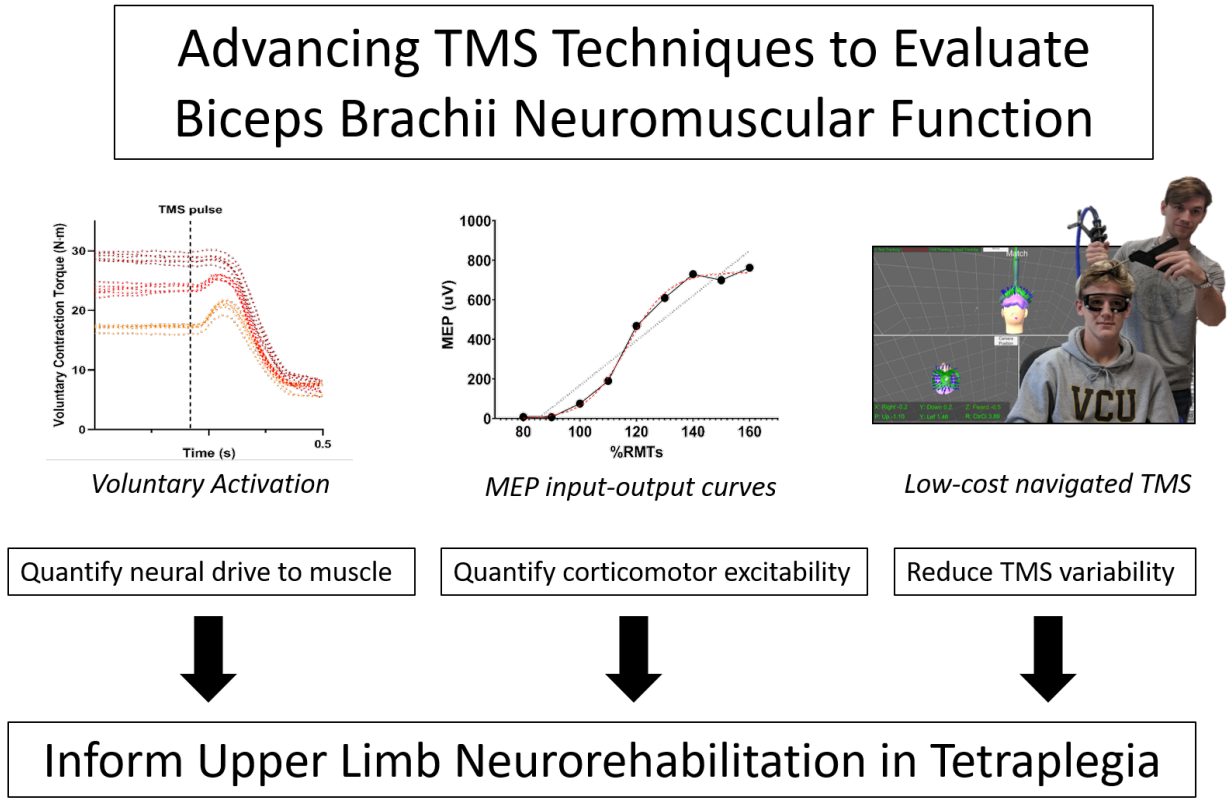


Figure 1-8: Illustration of objectives of this work. Our long-term goal is to improve upper limb neurorehabilitation therapies in individuals with tetraplegia. To that end, we use and improve upon TMS techniques to better evaluate neuromuscular function after a SCI. Voluntary Activation can quantify neural drive to muscle (orange, red, and dark red dashed traces indicate 50, 75, and 100% effort levels, respectively). MEP input-output curves can quantify excitability of the motor pathways. Finally, our implementation of a low-cost navigated TMS system may contribute to improving the reliability of TMS outcomes.

2 Chapter 2: Effect of Elbow Flexion Angle Modulation in the Assessment of Voluntary Activation

2.1 Background/Objectives

Voluntary activation is a measure that quantifies the level of voluntary neural drive that contracting muscles receive from the central nervous system [122]. Assessment of voluntary activation is useful in the study of mechanisms of neuromuscular fatigue [89], [116], [123] and neuromuscular impairments in clinical populations [99], [124], [125]. Measurement of voluntary activation involves superimposing supramaximal electrical stimulation of motoneurons upon an individual's voluntary effort to activate muscle. A deficit in voluntary activation is indicated when muscle force during maximum voluntary effort is further increased by electrical stimulation [126]. The increase in muscle force indicates that the stimulus recruited additional motor units beyond those already recruited via voluntary effort and/or that some motor units were discharging at subtetanic rates. When the stimulus is applied to peripheral motor nerve, we can assess what we will refer to as voluntary activation with peripheral nerve stimulation (VA_{PNS}). VA_{PNS} indicates the net voluntary drive, consisting of cortical drive and the transmission of the neural signal through corticospinal and lower motoneurons to innervate muscle [81]. VA_{PNS} reveals little about the site of deficit in voluntary drive within the pathway from the motor cortex to muscle. Thus, a technique to assess voluntary activation via transcranial magnetic stimulation (TMS) applied to the motor cortex was developed to indicate deficits in voluntary cortical drive [89]. We will refer to this measure as voluntary activation with TMS (VA_{TMS}). Measurement of VA_{TMS} may be particularly useful to characterize neuromuscular function after spinal cord injury (SCI). Muscle weakness, spasticity, and fatigability result from injury-induced changes at multiple sites of the nervous system [3] and have the potential to be addressed by neurorehabilitation [9]–[12]. Measurement of VA_{TMS} can better localize the deficit in voluntary drive, which may be useful in directing rehabilitation strategies.

Measurement of VA_{TMS} can be limited by technical challenges, [102], [105], [127] particularly in patient populations (e.g., spinal cord injury [88], post-stroke [97], which warrants

further investigation. Key challenges during stimulation are that TMS over the motor cortex may: 1) activate cortical neurons projecting to muscles other than the target muscle, including antagonists, and 2) not activate every motor unit in the target muscle. An ideal measure of VA_{TMS} would be obtained when TMS activates neurons that recruit all motor units not already recruited in the target muscle during maximum voluntary effort and does not recruit antagonist motor units. The ratio of the target muscle motor evoked potential (MEP) in response to TMS relative to the antagonist MEP (i.e., target MEP amplitude divided by antagonist MEP amplitude) can indicate how well the ideal measurement scenario is achieved. In the assessment of VA_{TMS} in nonimpaired individuals, adjustment of the TMS pulse intensity and selection of an appropriate target muscle can optimize the target/antagonist MEP ratio. For example, when the biceps brachii is the target muscle for assessment of VA_{TMS} in nonimpaired individuals, stimulus intensity can be adjusted to elicit a biceps MEP amplitude that is greater than 50% of the maximal M-wave (Mmax), and a triceps MEP less than 20% Mmax (i.e., corresponding to biceps/triceps MEP ratio greater than 2.5) [102]. However, this condition is likely more difficult to achieve in populations with neuromuscular impairments such as tetraplegia resulting from cervical SCI[88], [128]. In the assessment of VA_{TMS} in individuals with tetraplegia, for example, adjusting stimulus intensity alone could not elicit a large enough response in the triceps to estimate VA_{TMS} [88]. Thus, there is a need to investigate novel methodology that has the potential to optimize the target/antagonist MEP ratio. We focused on improving the methodology to assess VA_{TMS} of the biceps brachii in individuals with tetraplegia because: a) the biceps is innervated at the C5 and C6 levels such that considerable biceps function typically remains in many individuals with low cervical injuries (C5-C8) [28], [29], b) the biceps is important for upper limb function [26], and c) comparative data with single pulse TMS to assess VA_{TMS} exist in nonimpaired individuals [129], [130].

One methodological change is the careful prescription of isometric joint posture during measurement of VA_{TMS}. Static changes in elbow flexion angle modulate MEP amplitudes in the relaxed biceps in nonimpaired individuals [110]–[112]. Therefore, modulation of the isometric elbow angle may optimize the target/antagonist MEP ratio and enable the measurement of VA_{TMS}

in tetraplegia. In previous work assessing relaxed muscle, biceps MEPs were maximized and triceps MEPs were minimized at more flexed elbow angles relative to more extended elbow angles [113]. However, TMS is superimposed on voluntary contractions between 50-100% of the maximum voluntary contraction (MVC) to estimate VA_{TMS}. This is because extrapolation of the relationship between the voluntary effort and the superimposed twitch (SIT) moment is necessary to estimate VA_{TMS}. Whether elbow angle can be prescribed to optimize the biceps/triceps MEP ratio across the range of voluntary effort levels needed to estimate VA_{TMS} remains unknown.

In the current study, we focus on modulation of the isometric elbow flexion-extension angle in the assessment of VA_{TMS} of the biceps in nonimpaired individuals and individuals with tetraplegia. The primary objectives of this study were to determine the effect of the isometric elbow angle on: 1) the biceps/triceps MEP ratio across a range of voluntary efforts, and 2) VA_{TMS}. We hypothesized that the biceps/triceps MEP ratio would be greatest in a more flexed elbow angle at each level of voluntary effort. Further, we hypothesized that VA_{TMS} would depend on the biceps/triceps MEP ratio, based on our expectation that a greater biceps/triceps MEP ratio would indicate better targeting of the biceps relative to the triceps with TMS. A secondary objective of this study was to determine the repeatability of VA_{TMS} and VA_{PNS} of the biceps in individuals with tetraplegia when measured at 90^{o} of elbow flexion.

2.2 Methods

2.2.1 Human Participants:

Ten nonimpaired individuals (four females, six males, average age 22.7 ± 2.5 years) and ten individuals with tetraplegia (three females, seven males, average age 39.9 ± 10 years, see Table 2-1) were recruited to participate in three sessions. Inclusion criteria were C5 to C6 level of cervical spinal cord injury, at least a year post-injury. Exclusion criteria included any contraindication to receiving TMS and the inability to generate a visible contraction of the biceps. Data from one participant with SCI (#10) was excluded from the data analyses because TMS was

unable to elicit moment twitches from the elbow flexors. Participants were screened to ensure that they were eligible to receive TMS and provided informed written consent. The study was approved by the Institutional Review Board of Virginia Commonwealth University and complied with the 2013 update of the Declaration of Helsinki.

Table 2-1: Ten individuals with tetraplegia resulting from cervical SCI were recruited to participate. Maximum voluntary elbow moments presented here were measured at 90° of elbow flexion.

Participant #	Sex	Age	Injury Level	ISNCSCI	Time since SCI (years)	Cause of SCI	MVC (Nm)	Medications
1	F	52	C6	A	15	MVA	47.6 ± 1.89	BAC
2	F	53	C6	D	7	Spinal Stenosis	57.2 ± 2.40	BAC
3	M	42	C5	A	12	MVA	35.0 ± 1.93	BAC
4	M	45	C6	D	5	Transverse Myelitis	52.4 ± 2.69	None
5	F	54	C6	A	13	MVA	20.6 ± 2.27	BAC
**6	M	34	C5	A	16	MVA	21.0 ± 1.59	BAC, OX
**7	M	26	C6	A	6	Fall	80.7 ± 2.79	None
**8	M	33	C5	D	3	MVA	93.6 ± 2.84	None
9	M	32	C5	B	9	Fall	40.5 ± 1.68	None
**10	M	28	C5	B	4	MVA	2 ± 0.07	BAC

* ISNCSCI: International Standards for Neurological Classification of Spinal Cord Injury, MVA: Motor Vehicle Accident, BAC: Baclofen, OX: Oxybutynin

** Completed 2 out of 3 sessions

*** Excluded data

2.2.2 Experiment Overview:

In each session, participants completed trials to assess VA_{TMS} and VA_{PNS}; three sessions were conducted in order to assess between-session repeatability in a common elbow angle (90°, elbow flexion-extension angle defined in accordance with the International Society of Biomechanics [131]. Sessions were separated by at least one day, with no more than 7 days between sessions. Each session consisted of up to three experimental blocks; one block of nine

MVC trials to assess VA_{PNS} with the elbow positioned at 90° of flexion, one block to assess VA_{TMS} in 90^{o} elbow flexion, and one block to assess VA_{TMS} in either 45° or 120° elbow flexion. VA_{TMS} was not assessed at 45° and 120° in each session to minimize fatigue (i.e., the number of trials). After three sessions, each participant had completed one assessment of VA_{TMS} at 45°, one assessment of VA_{TMS} at 120°, and three assessments of VA_{TMS} and VA_{PNS} at 90° (Figure 2-1, B). The order of VA_{TMS} blocks was randomized.

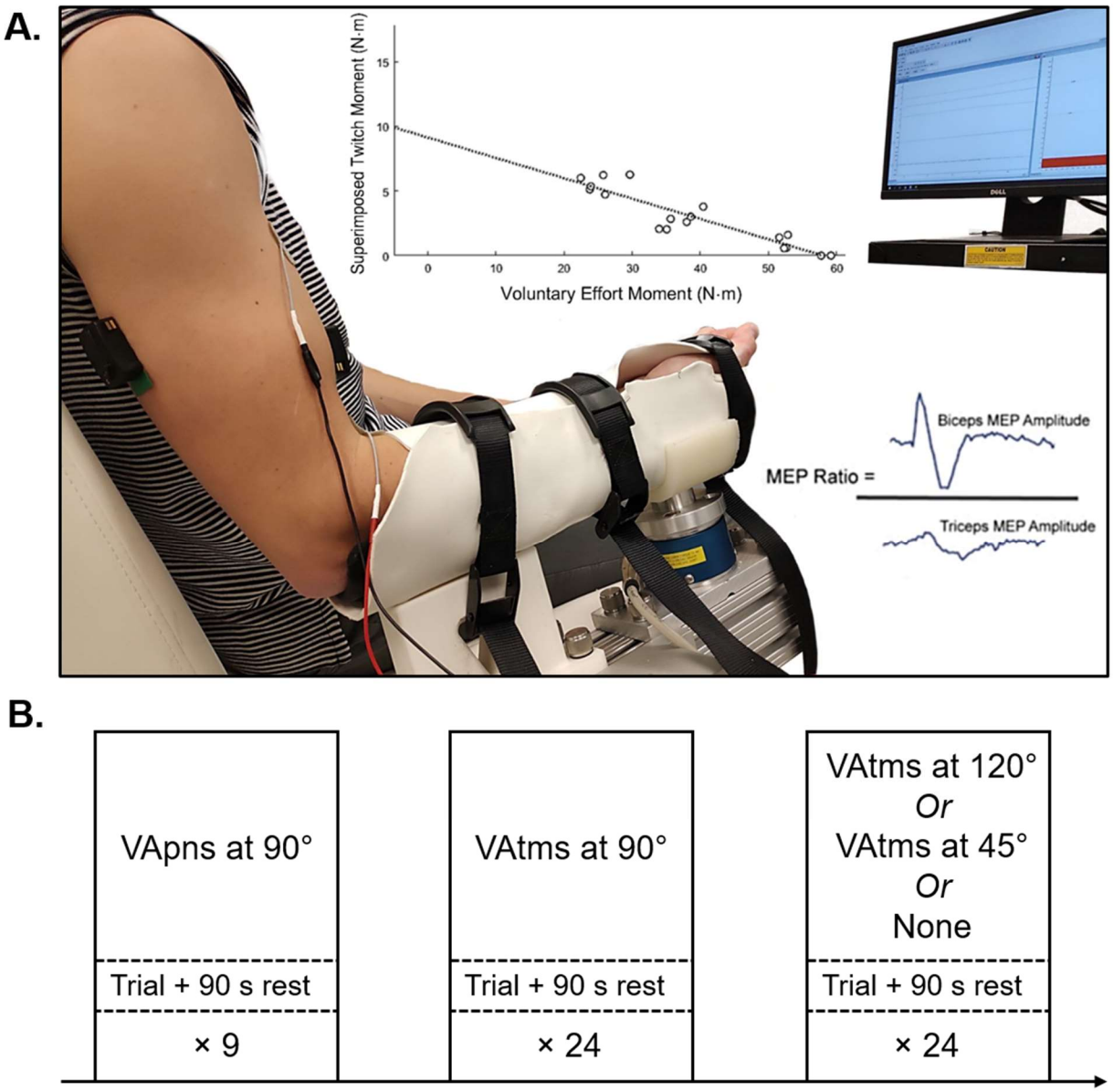


Figure 2-1: A. Participants received visual feedback of their voluntary elbow flexion moment as a thermometer-like gauge. VA_{TMS} trials were used to estimate the resting twitch via linear regression of superimposed twitch moments relative to the voluntary moments. Biceps and triceps EMG data were analyzed to calculate the MEP ratio for each trial. **B.** Experimental protocol schematic representing the randomized voluntary activation blocks (9 VA_{PNS} trials and 24 VA_{TMS} trials) completed by the participants in one session; each participant completed three sessions in total.

Before each block, participants followed a quick warm-up and familiarization phase, which consisted of repeated, brief, submaximal contractions for approximately two minutes.

Participants then performed three maximum voluntary contractions (MVCs) at each isometric elbow angle. For all trials, the participant's forearm was positioned in a custom brace attached to a six degree-of-freedom load cell (Model 30E15A4-I40-EF-100L, JR3, Woodland, CA) (Figure 2-1, A). Force and moment data were sampled at 2000 Hz. EMG data (Delsys Trigno Wireless Sensors) were also sampled at 2000 Hz and bandwidth limited to 20-450 Hz. Cambridge Electronic Design software and acquisition system were used to collect the data (CED Spike 2 and 1401).

The brachial plexus (i.e., Erb's point) was stimulated to measure both biceps and triceps maximal m-wave (Mmax) using a cathode on the skin in the supraclavicular fossa and an anode on the acromion process. Current pulses were delivered as a singlet (0.2 ms duration, SCI group ranged 80-200 mA, nonimpaired group ranged 80-180 mA, DS7AH, Digitimer, UK). Stimulus intensity was determined by increasing the stimulation current in 10 mA increments until the m-wave peak to peak amplitude reached a plateau [132]. Ten stimuli were delivered at 120% of the Mmax threshold stimulus intensity, separated by 5 s. This procedure was performed at each elbow angle.

2.2.3 Maximal Voluntary Contractions:

Participants performed three MVCs of the elbow flexors for three seconds while receiving real-time visual moment feedback and verbal encouragement (Figure 2-1, A). Each maximum effort was separated by at least 90 seconds of rest. The participant's MVC was calculated for each effort as the mean elbow moment maintained over ± 250 ms from the maximal moment achieved. The mean elbow flexion moment of three MVC trials was used for subsequent trials during which participants were asked to generate a voluntary moment to match a percentage of their MVC moment.

2.2.4 Assessment of VA_{PNS}:

Participants completed trials during which motor point electrical stimulation was superimposed on isometric MVCs in elbow flexion to estimate VA_{PNS}. For motor point stimulation, stimulating electrodes were placed over the biceps belly (anode) and distal tendon (cathode).

Stimulus intensity was determined by increasing the stimulation current in 10 mA increments until the moment response in the resting biceps reached a plateau. The threshold current (i.e., current corresponding to the start of the moment plateau) was recorded and motor point stimulation intensity was set at 130% of the threshold current [81]. Stimulation intensity ranged from 80 to 180 mA across both groups. Using visual moment feedback, participants were instructed to perform nine MVCs in elbow flexion during which stimulation was superimposed during and after the voluntary effort. Motor point stimulation with a single pulse (0.2 ms width, DS7AH, Digitimer, UK) was delivered after the participant maintained a voluntary moment ≥ 95% of their MVC moment for 0.5 seconds. A second stimulus event (same intensity and pulse width) was delivered 3 seconds after the first stimulus event while the arm was at rest. Each trial was followed by at least 90 seconds of rest to mitigate fatigue.

2.2.5 Assessment of VA_{TMS}:

Single pulse TMS was delivered using a 126 mm diameter double cone coil (Magstim DCC) and Magstim BiStim^2 stimulator. This coil was selected to ensure that motor thresholds could be found in all participants with SCI since compared to the figure-of-eight coil, the double cone results in lower motor thresholds [133]. Lower motor thresholds are beneficial in that they minimize the use of high stimulator intensities during VA_{TMS} blocks. The coil was held to induce a monophasic, posterior to anterior current across the central sulcus. Each session, motor cortex mapping was performed to obtain the location that evoked the largest peak-to-peak MEP in the biceps relative to the triceps at the lowest stimulation intensity [134]. This location was then marked on a cap secured to the participant's head; subsequent stimuli were delivered at that location. Resting motor threshold (RMT) was then determined as the lowest stimulus intensity able to induce biceps MEPs ≥ 50 μV in at least 5 out of 10 stimuli [135]. RMTs were used to normalize stimulation intensity to account for individual responsiveness to TMS in a given session. That way, each participant received the same relative amount of stimulation. In each session, participants completed a VA_{TMS} block with their elbow flexed at 90°. Participants also completed a block in which isometric arm posture was modified to be either 45° or 120° of elbow flexion. Modified elbow flexion conditions were presented in a randomized order. Each block

consisted of a set of 24 isometric contractions of the elbow flexors in randomized moment-matching trials of 0, 50, 75, or 100% MVC (6 of each effort level). Trials were separated by at least 90 seconds of rest to mitigate fatigue (Figure 2-1, B). A TMS pulse corresponding to 120% RMT was delivered when the participant achieved and maintained ± 2.5 percent of the target effort level for a sustained 0.5s. TMS intensity ranged from 31-76% of the maximal stimulator intensity (MSO) in the SCI group and 24-89% MSO in the nonimpaired group. Stimulation intensity was set using individual's RMT rather than based on the optimization of the MEP ratio as the purpose of this study was to observe an effect of the elbow flexion angle on the MEP ratio. Thus, using the MEP ratio as a parameter to set the TMS intensity would have been inappropriate.

2.2.6 Data Analysis:

VA_{PNS} and VA_{TMS} superimposed twitch moments were computed for each trial as the difference between the maximum moment occurring within 150 ms after the stimulus event and the pre-stimulus moment. The pre-stimulus moment was computed as the maximal 10 ms moving average moment maintained within 50 ms prior to the stimulus event. The potentiated resting twitch moment was also computed for each motor point stimulation trial. VA_{PNS} was calculated using Equation 1 from Allen et al. [81]:

$$\mathrm{VA_{PNS}} = \left[1 - \frac{\text{Superimposed Twitch Moment}}{\text{Potentiated Resting Twitch Moment}}\right] \times 100 \quad (1)$$

To compute VA_{TMS}, the resting twitch was estimated via linear regression using the methodology described by Todd et al. (2003). The linear regression function was derived from the amplitude of the superimposed twitch moments and the corresponding voluntary elbow moments at 50, 75, and 100% MVC (Figure 2-2, B). VA_{TMS} was calculated using Equation 2 from Todd et al. [89]:

$$\mathrm{VA_{TMS}} = \left[1 - \frac{\text{Superimposed Twitch Moment}}{\text{Estimated Resting Twitch Moment}}\right] \times 100 \quad (2)$$

In the nonimpaired group, VA_{TMS} blocks with poor linearity between voluntary moment and superimposed twitch were excluded ($r < 0.8$) similar to previous work assessing VA_{TMS} in nonimpaired individuals; 7 out of 50 blocks were excluded for this reason [102]. In the SCI group, VA_{TMS} blocks with poor linearity were not excluded as linearity was consistently poor ($r < 0.8$). MEP magnitude was calculated as the peak to peak amplitude of the EMG wave within a 50 ms window following stimulation. All MEPs recorded during a given session were normalized to the Mmax (biceps or triceps as appropriate, 90°, 120° or 45° elbow flexion as appropriate) value for the corresponding session in order to account for individual responsiveness to peripheral electrical stimulation and EMG sensor placement [136], [137]. The biceps/triceps MEP ratio for each trial was calculated as a percentage of the normalized biceps MEP divided by the normalized triceps MEP. To determine the effect of the MEP ratio on VA_{TMS}, MEP ratios were averaged across effort levels to represent its overall magnitude throughout any given block for each participant. Example EMG data, elbow flexion moment traces, and linear regression of superimposed twitch and voluntary moment from one VA_{TMS} block (90° elbow flexion) of representative nonimpaired and SCI participants are presented in Figure 2-2 and Figure 2-3, respectively.

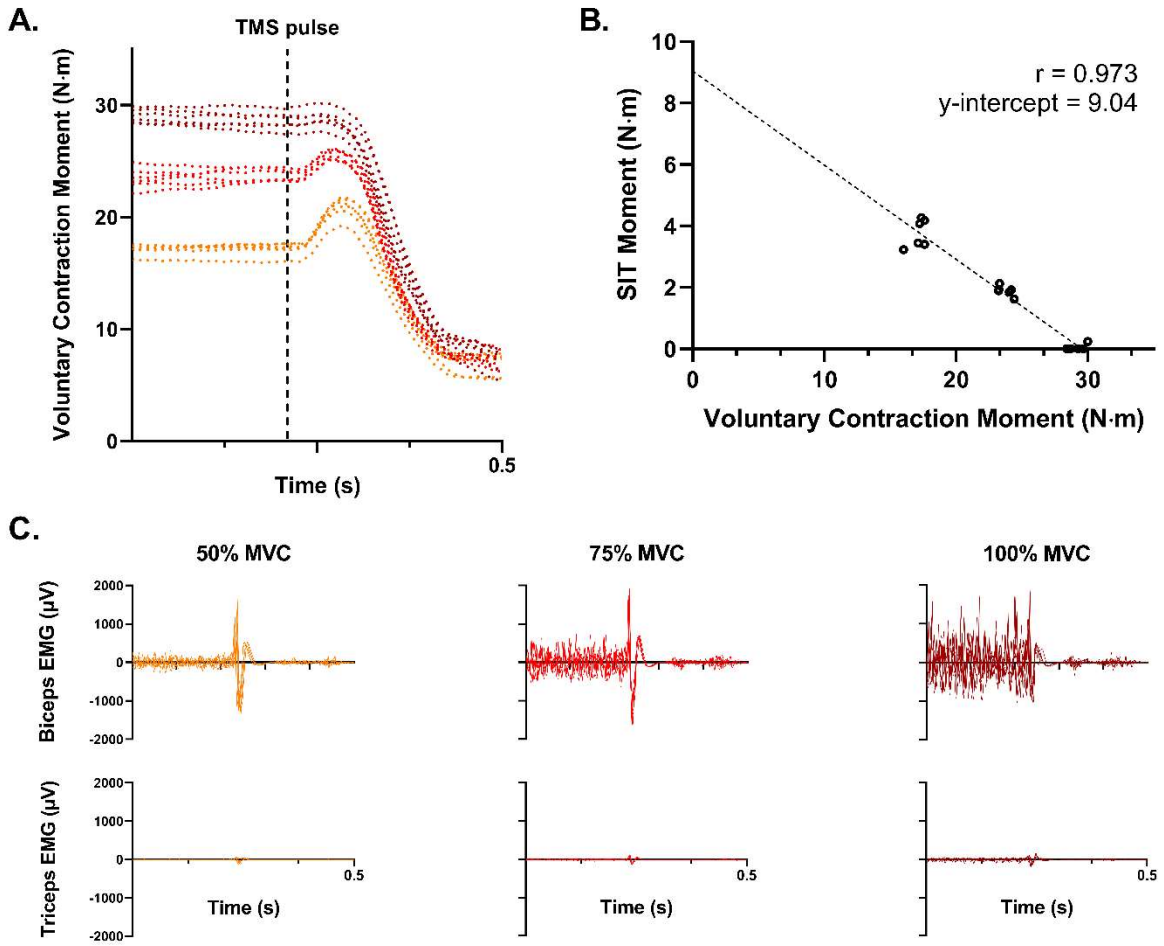


Figure 2-2: Example data from a nonimpaired participant**. A.** Moment traces collected during a VA_{TMS} block at 90° elbow flexion. **B.** Linear regression between SIT and Voluntary contraction moment obtained from the same VA_{TMS} VA_{TMS} block. **C.** EMG signals showing the biceps and triceps MEPs collected during the VA_{TMS} blocks at each effort level (6 MEPs are plotted per graph). Orange, red, and dark red traces indicate 50, 75, and 100% effort levels, respectively.

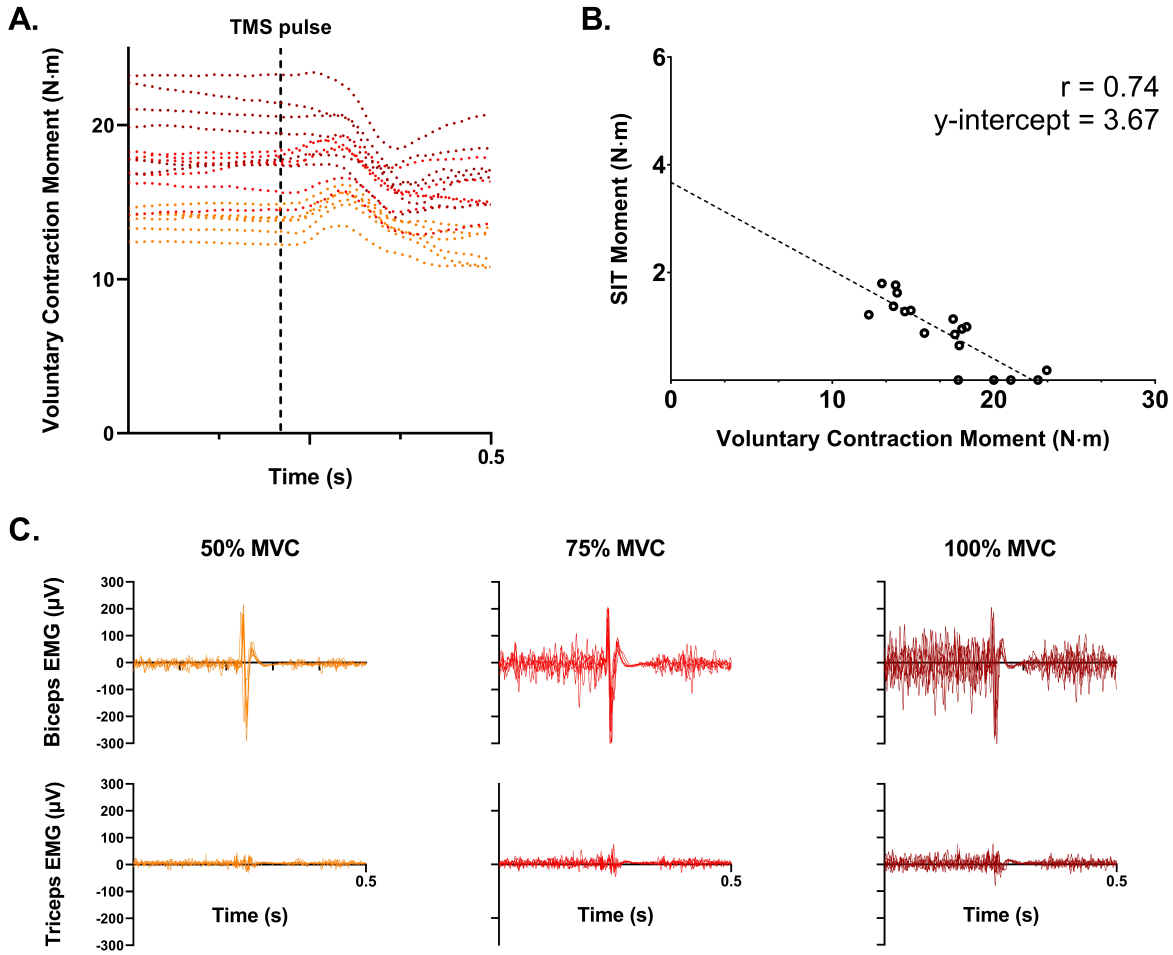


Figure 2-3: Example data from a SCI participant**. A.** Moment traces collected during a VA_{TMS} block at 90° elbow flexion. **B.** Linear regression between SIT and Voluntary contraction moment obtained from the same VA_{TMS} block. **C.** EMG signals showing the biceps and triceps MEPs collected during the VA_{TMS} blocks at each effort level (6 MEPs are plotted per graph). Orange, red, and dark red traces indicate 50, 75, and 100% effort levels, respectively.

2.2.7 Statistical Analysis:

A linear mixed effect model was analyzed to determine the effect of independent variables on VA_{TMS} (the dependent variable). The independent variables were: isometric elbow flexion angle, block mean biceps/triceps MEP ratio, linearity of the voluntary moment and superimposed twitch relation (r-value), and RMT. In addition to excluding blocks with low

linearity ($r < 0.8$) in the nonimpaired group, we included linearity as an independent variable to test whether smaller ($0.8 < r < 0.99$) variations in linearity affected the estimation of VA_{TMS}. RMTs were added to the model as a continuous covariate in order to test whether individual responsiveness to TMS (as represented by RMTs) had an effect on VA_{TMS}. A random effect was added to account for individual differences that resulted in each participant being assigned a different intercept. P-values were obtained via the Kenward-Roger approximation for degrees-of-freedom implemented for linear mixed effect models. Comparisons were reported with respect to 90° elbow flexion, which is the common elbow angle used in previous studies investigating VA_{TMS} of the elbow flexors [109], [116]. The null hypotheses were that the independent variables (elbow angle, MEP ratio, linearity, and RMT) do not have an effect on VA_{TMS}. The alternative hypotheses were that increased elbow angle increases VA_{TMS} and decreased elbow angle decreases VA_{TMS}; increased MEP ratio increases VA_{TMS} and decreased MEP ratio decreases VA_{TMS}, decreased linearity leads to lower VA_{TMS}, and lower RMT increases VA_{TMS}.

A two-way ANOVA with repeated measures and a Fisher's least significant difference post-hoc test were used to compare the biceps/triceps MEP ratio across elbow angles for each target effort level. The same statistical test was performed to determine the effect of elbow angle on normalized MEP amplitudes of the biceps and triceps separately. The null hypothesis was that elbow angle does not have an effect on the MEP ratio or the individual biceps and triceps MEPs. The alternative hypothesis was that the MEP ratio increases with greater elbow flexion angle, across all effort levels. Another two-way ANOVA with no multiple comparisons was used to compare the linearity of the voluntary moment and superimposed twitch relation between the nonimpaired and SCI groups; this comparison was tested prior to excluding low linearity blocks in the nonimpaired data. The null hypothesis was that there are no differences in linearity between groups. The alternative hypothesis was that linearity is lower in the SCI group compared to the nonimpaired group. Intraclass correlation coefficients [138] were determined to assess the inter-session repeatability of VA_{PNS} and VA_{TMS}. A two-way mixed model, ICC(3,k) was used where sessions are considered fixed effects and participants were treated as random effects. Coefficients of variation (SD/mean) were computed per participant and per session then

averaged to represent within-session variability of VA_{TMS} measures. All data and statistical analyses were performed in Matlab (MathWorks, Inc, Natick, MA), R (R Core Team, Vienna, Austria) and Prism (GraphPad Software, La Jolla California USA) with custom-written code. Tests were evaluated at a significance level corresponding to $p < \alpha = 0.05$.

2.3 Results

Across all SCI participants, mean VA_{TMS} at 90° elbow flexion was 93.7 ± 6.3%, 92.6 ± 10.2%, and 97.5 ± 2.4% for sessions 1, 2, and 3, respectively. Mean VA_{TMS} was 95.2 ± 6.3% at 120° elbow flexion, and 94.2 ± 5.6% at 45° elbow flexion (Figure 2-4). Mean VA_{PNS} was 95.9 ± 4.3%, 94.1 ± 10.8%, and 97.9 ± 2.6% for sessions 1, 2, and 3, respectively. Across all nonimpaired participants, mean VA_{TMS} at 90° elbow flexion was 92.1 ± 6.8%, 94.6 ± 7.3%, and 93.8 ± 7.1% for sessions 1, 2, and 3, respectively. Mean VA_{TMS} was 90.3 ± 7.5% at 120° elbow flexion, and 89.6 ± 9.9% at 45° elbow flexion (Figure 2-4). Mean VA_{PNS} was 98.0 ± 3.2%, 95.9 ± 4.4%, and 97.8 ± 3.3% for sessions 1, 2, and 3, respectively. A summary of all key measures is presented in Appendix A. Supplementary and raw data can be accessed online: https://osf.io/r2sa6/.

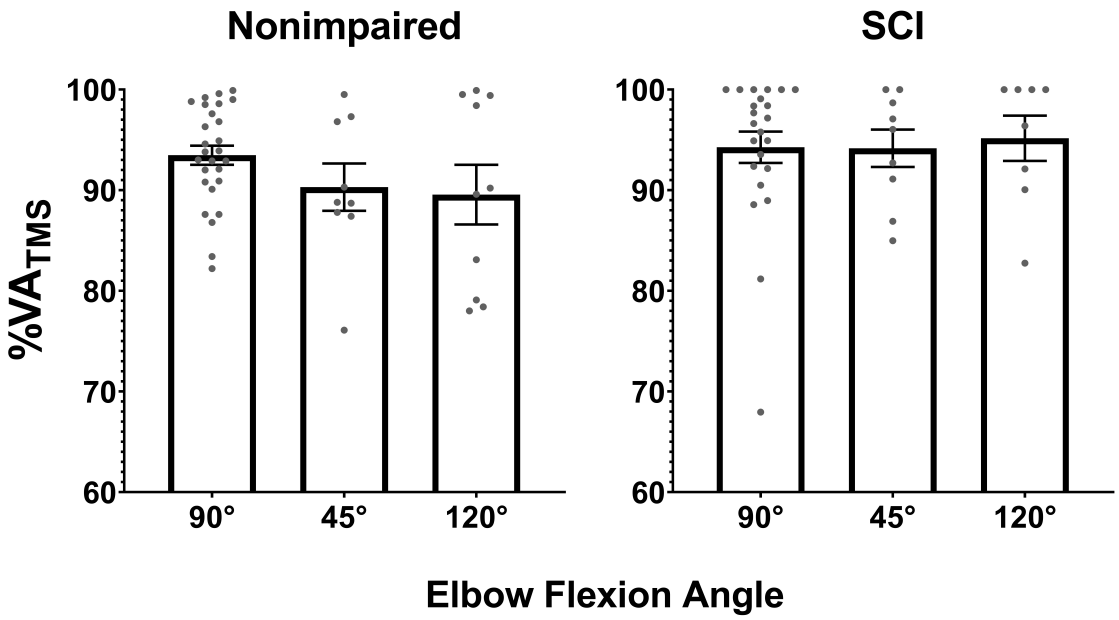


Figure 2-4: VA_{TMS} measures collected at each stimulation conditions in nonimpaired and SCI participants. Grey points represent individual mean VA_{TMS} (per block) and VA_{TMS} ranged from 67 to 99%. Error bars represent the standard error of the mean.

2.3.1 Effect of Elbow Angle on the Biceps/Triceps MEP Ratio:

In the SCI group, across all elbow angles, the biceps/triceps MEP ratio increased from 0 to 50% MVC, then remained unchanged from 50% to 100% MVC [$p < 0.001$]. No differences in MEP ratio were found across elbow angles at any effort level (Figure 2-5, B).

In the nonimpaired group, across all elbow angles, the biceps/triceps MEP ratio increased from 0 to 50% MVC, then decreased from 50% to 100% MVC [$p < 0.001$]. At rest (i.e., 0% MVC), there were no differences in the biceps/triceps MEP ratio due to the elbow angle. At 50% MVC, the biceps/triceps MEP ratio was greater in 120^o flexion relative to 90^o flexion [Figure 2-6, B, $p = 0.033$]. At 75% MVC, biceps/triceps MEP ratio in 120^o was greater relative to 90^o flexion [$p = 0.009$]. At 100% MVC, biceps/triceps MEP ratios did not differ across the elbow angles (Figure 2-6, B).

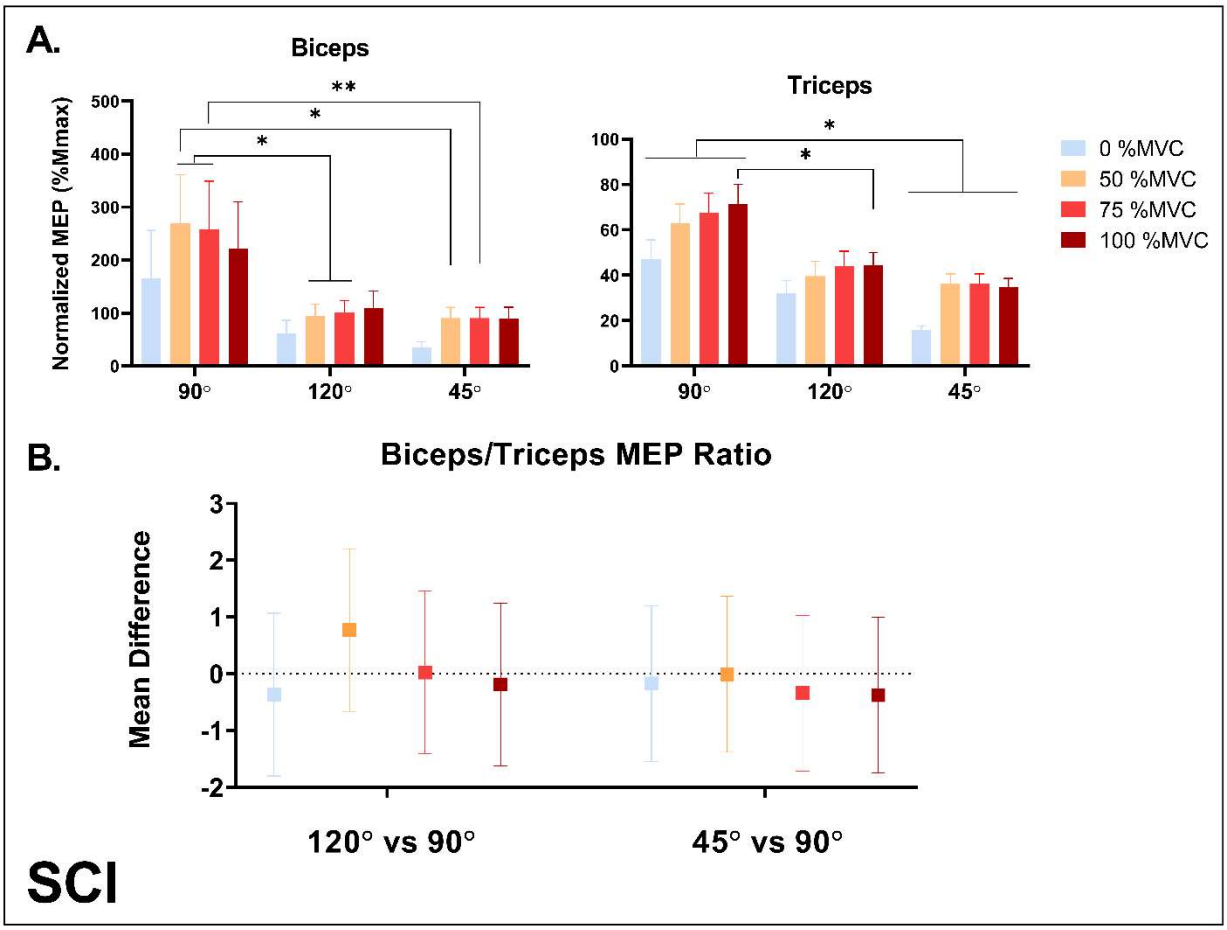


Figure 2-5: A. Average biceps and triceps normalized MEPs (normalized to corresponding Mmax) in the SCI group. At high but submaximal effort levels, biceps MEPs were decreased at 45 and 120^{o} of elbow flexion compared to 90^{o}. **B**. MEP ratio mean difference relative to 90^{o} in the SCI group. No differences were found**.** Errors bars show 95% confidence intervals. Asterisks indicate a significantly greater mean MEP ratio ([*] = $p < 0.05$, ([**] = $p < 0.01$) relative to the mean MEP ratio at 90^{o} elbow flexion.

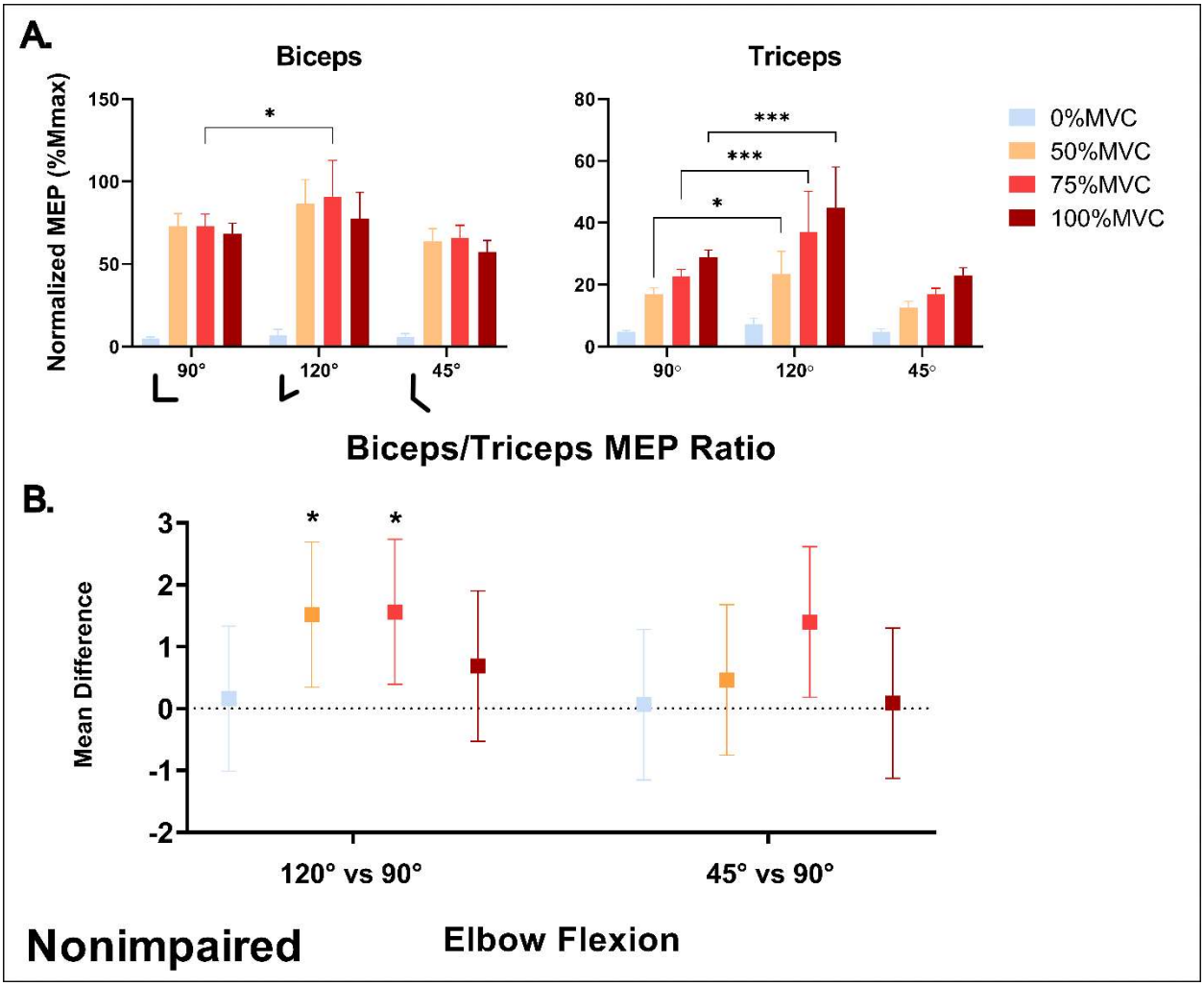


Figure 2-6: A. Average biceps and triceps MEPs (normalized to corresponding Mmax) across elbow angles and effort levels in the nonimpaired group. Biceps MEPs increased significantly from rest to 50% MVC then reached a plateau while triceps MEPs increased linearly with effort. Error bars represent 95% confidence intervals. **B**. MEP ratio mean difference relative to 90^o of elbow flexion across effort levels in the nonimpaired group. Asterisks indicate a significantly greater mean MEP ratio ([*] = $p < 0.05$, ([***] = $p < 0.001$) relative to the mean MEP ratio at 90^o elbow flexion.

2.3.2 Effect of Independent Variables on VA_{TMS}:

In the SCI group, the main effect of elbow angle on VA_{TMS} was not significant in the linear mixed-effects model [45^o: $t = -0.233$, $p = 0.82$, 120^o: $t = -0.038$, $p = 0.97$]. The main effect of the linearity of the voluntary moment and SIT relation on VA_{TMS} was significant in the linear mixed-effects model. For each 0.1 increase in linearity (for $0 < r < 0.99$), VA_{TMS} was predicted to increase

by 9.31% [t = 7.909, p < 0.0001]. Interaction analyses showed that this effect was dependent of the elbow flexion angle. Further analyses revealed that the mean MEP ratio and RMT had no significant main effects on VA_{TMS} as well as no interaction effects with elbow flexion angle.

In the nonimpaired group, the main effect of elbow angle on VA_{TMS} and the interaction effect of elbow angle and biceps/triceps MEP ratio on VA_{TMS} were significant in the linear mixed-effects model. VA_{TMS} assessed at an isometric elbow angle of 45^o flexion (i.e., the more extended elbow angle) was predicted 14.6 ± 4.2% lower relative to VA_{TMS} assessed in 90^o elbow flexion [t = -3.46, p = 0.0019]. This effect was dependent on the MEP ratio. For each unit increase in the biceps/triceps MEP ratio, VA_{TMS} at 45^o elbow flexion was predicted to increase by 2.84% [t = 3.08, p = 0.0035]. The main effect of RMT on VA_{TMS} was significant. The model predicted that VA_{TMS} would decrease 1.82% for each 10% maximum stimulator output (MSO) increase in RMT [t = 2.39, p = 0.033]. Interaction analyses showed that this effect was independent of the elbow angle, linearity, and MEP ratio. The main effect of the linearity of the voluntary moment and SIT relation on VA_{TMS} was significant. For each 0.1 increase in linearity (for 0.8 < r < 0.99), VA_{TMS} was predicted to increase by 4.4% [t = 2.34, p = 0.023]. This effect was independent of the elbow angle, MEP ratio, and RMT.

2.3.3 Repeatability and Variability of VA Estimates:

In the SCI group, an ICC of 0.78 [p = 0.004] resulted from the inter session analysis of VA_{PNS} assessed in 90^o elbow flexion. An ICC of 0.75 [p = 0.008] resulted from the inter session analysis of VA_{TMS} assessed in 90^o elbow flexion. (Figure 2-7, B). Mean within-session coefficients of variation for VA_{TMS} were 6.9 ± 7.4% at 90^o elbow flexion, 11 ± 17% at 120^o elbow flexion, and 6.3 ± 6.2 % at 45^o elbow flexion

In the nonimpaired group, an ICC of 0.64 [p = 0.03] resulted from the inter session analysis of VA_{PNS} assessed in 90^o elbow flexion. An ICC of 0.66 [p = 0.03] resulted from the inter session analysis of VA_{TMS} assessed in 90^o elbow flexion. (Figure 2-7, A). Mean within-session coefficients

of variation for VA_{TMS} were 6.2 ± 5% at 90^{o} elbow flexion, 14.7 ± 10.5% at 120^{o} elbow flexion, and 5.8 ± 4.9 % at 45^{o} elbow flexion.

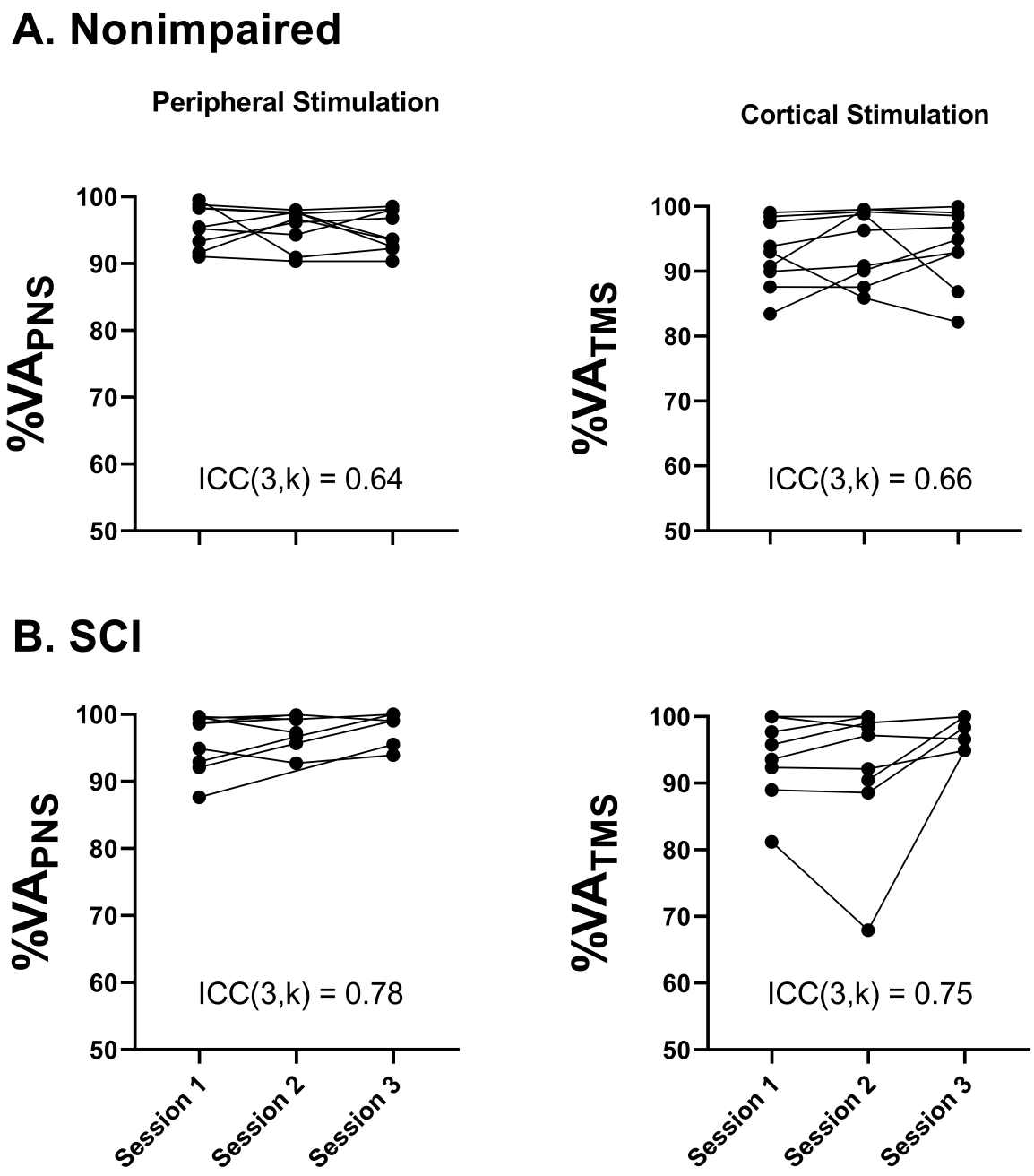


Figure 2-7: A. Interclass correlation coefficients (ICCs) suggest that VA_{PNS} (left) and VA_{TMS} (right) are repeatable at 90^{o} of elbow flexion across sessions in the nonimpaired group. **B.** ICCs suggest that VA_{PNS}

and VA_{TMS} are repeatable at 90° of elbow flexion across sessions in the SCI group. Each data point represents the mean VA estimate for a participant and session.

2.3.4 Effect of Elbow Angle on Biceps MEPs:

In the SCI group, no differences in biceps MEPs due to elbow angle were found at rest. At 50% MVC, 120° of elbow flexion [-174.9% Mmax, p = 0.014] and 45° of elbow flexion [-178.8% Mmax, p = 0.008] decreased biceps MEPs compared to 90° elbow flexion. At 75% MVC, 120° of elbow flexion [-156.8% Mmax, p = 0.027] and 45° of elbow flexion [-167.1% Mmax, p = 0.014] decreased biceps MEPs compared to 90° elbow flexion (Figure 2-5, A).

In the nonimpaired group, at 0% MVC (rest), there were no differences in biceps MEPs due to elbow angle. At 75% MVC, 120° of elbow flexion increased normalized biceps MEPs [+11.3% Mmax, p = 0.04] compared to 90° elbow flexion (Figure 2-6, A).

2.3.5 Effect of Elbow Angle on Triceps MEPs:

In the SCI group, at rest, 45° of elbow flexion led to lower triceps MEPs compared to 90° of elbow flexion [-31.4% Mmax, p = 0.017]. At 50% MVC, 45° of elbow flexion decreased triceps MEPs as well [-26.6% Mmax, p = 0.04]. At 75% MVC, 45° of elbow flexion decreased triceps MEPs as well [-31.5% Mmax, p = 0.016]. At 100% MVC, 120° of elbow flexion [-27.1% Mmax, p = 0.04] and 45° of elbow flexion [-36.7% Mmax, p = 0.005] decreased triceps MEPs compared to 90° elbow flexion (Figure 2-5, A).

In the nonimpaired group, at rest, there were no differences in triceps MEPs due to elbow angle. At 50% MVC, 120° of elbow flexion increased normalized triceps MEPs [+9.8% Mmax, p = 0.01] compared to 90°. Similarly, at 75% MVC, 120° of elbow flexion increased normalized triceps MEPs [+18.4% Mmax, p < 0.0001] compared to 90°. At 100% MVC, 120° of elbow flexion increased normalized triceps MEP amplitudes [+18.1% Mmax, p < 0.0001] compared to 90° of elbow flexion (Figure 2-6, A).

2.3.6 Post-hoc Evaluation of Biceps/Triceps MEP Ratio and Linearity:

Post-hoc evaluation was performed to further analyze the biceps/triceps MEP ratio. In both the nonimpaired and SCI groups, the more extended elbow angle (45^o) yielded to the highest percent of trials meeting the guideline criteria from Todd et al. (biceps MEP ≥ 50% Mmax and triceps MEP ≤ 20% Mmax) and our adjusted condition of a MEP ratio greater than 2.5 (Table 2-2). Across both groups and all elbow angles, the MEP ratio > 2.5 condition was met more often than the Todd et al. criteria. Finally, the linearity of the voluntary moment and SIT moment relation was on average lower in the SCI group compared to the nonimpaired group across elbow angles ($F(1, 86) = 6.208$, $p = 0.015$)(Table 2-2).

Table 2-2: Percent trials (between 50%-100% MVC) meeting the Todd et al. criteria (biceps MEP ≥ 50% Mmax and triceps MEP ≤ 20% Mmax), MEP ratio > 2.5 (where Biceps MEP is 2.5 larger than triceps MEP) and the average linearity of the voluntary moment and SIT moment. * indicates statistical differences ($p < 0.05$).

NONIMPAIRED				
ELBOW FLEXION ANGLE	**Todd et al. criteria (% met)**	**MEP ratio > 2.5 (% met)**	**Mean linearity**	**Total # of trials**
90^o	34.1	60.2	0.87	540
120^o	39.4	55.6	0.76	198
45^o	46.7	77.8	0.86	180
MEAN	**40.1**	**64.5**	**0.83***	918
SCI				
90^o	14.4	52.8	0.81	432
120^o	16.7	54.9	0.81	162
45^o	35.7	56.9	0.59	144
MEAN	**22.3**	**54.9**	**0.73***	738

2.4 Discussion

Our innovative approach was to evaluate the modulation of isometric elbow angle as a strategy to improve the measurement of VA_{TMS} through increasing the biceps/triceps MEP ratio. The objectives of this study were to determine the effect of elbow angle on: 1) the biceps/triceps MEP ratio across a range of voluntary efforts, and 2) the measurement of VA_{TMS} in the elbow flexors of nonimpaired and individuals with tetraplegia. We hypothesized that the biceps/triceps MEP ratio would be greatest in a more flexed elbow angle (120^{o} flexion) at each level of voluntary effort. In the SCI group, this hypothesis was not supported. In the nonimpaired group, this hypothesis was only supported at the 50% and 75% MVC effort levels (i.e., not at 100% MVC). Further, we hypothesized VA_{TMS} would depend on the biceps/triceps MEP ratio. This hypothesis was only supported in the more extended elbow angle (45^{o} flexion) of nonimpaired participants. Our results indicate that the bicep/triceps MEP ratio is not modulated by elbow angle across the full range of voluntary efforts in nonimpaired and SCI participants and does not improve the estimation of VA_{TMS}. Finally, both VA_{TMS} and VA_{PNS} measured in nonimpaired participants and individuals with tetraplegia at 90^{o} elbow flexion were repeatable across three days.

In the nonimpaired group, the more extended elbow angle led to lower VA_{TMS} measures compared to 90^{o} elbow flexion; this effect was dependent on the mean biceps/triceps MEP ratio (for each given block). In the more extended arm posture, a large biceps/triceps MEP ratio was associated with a greater VA_{TMS} estimate. This suggests a potential small effect of better targeting the biceps relative to the triceps with TMS, but considering this effect was only seen in one condition, other factors affecting VA_{TMS} estimation may be more important. The decreased VA_{TMS} in the more extended elbow angle is not due to changes in the moment-generating capacity of the biceps and triceps with elbow angle because VA is expressed as a ratio of a superimposed

moment response to TMS over the estimated resting moment response to TMS. Thus, VA is normalized by the moment-generating capacity at a given elbow angle.

Although the biceps/triceps MEP ratio may reflect the focality of cortical stimulation when targeting the biceps, its influence on the estimation of VA_{TMS} is limited. In order for the biceps/triceps MEP ratio to improve VA_{TMS} estimation, the ratio must be increased across the range of effort levels needed to assess VA_{TMS} (i.e., 50, 75 and 100% MVC). In the more flexed elbow angle (120^{o}) of nonimpaired participants, we observed increased MEP ratios at only 50% and 75% MVC. Furthermore, the increased MEP ratio in the more flexed elbow was not associated with a change in the magnitude of VA_{TMS}. The increased biceps/triceps MEP ratio in the more flexed posture occurred primarily via biceps MEP facilitation (Figure 2-6, A). On the other hand, in the SCI group, the modified elbow angle conditions led to lower biceps and triceps MEPs (Figure 2-5). However, this effect did not translate to MEP ratio modulation. Changes in MEP amplitudes due to isometric joint angle are mostly attributed to spinal mechanisms [112], [139], namely the influence of afferent feedback provided to the spinal cord [110]. However, central influence also plays a role [111]. In this study, we modulated the elbow angle while keeping other posture-related parameters (i.e. forearm orientation, shoulder and head position) unchanged. Therefore, in the nonimpaired group, the increased biceps MEP ratio was likely due to a combination of central and spinal facilitatory mechanisms affecting the shortened biceps. Additionally, triceps MEP facilitation was higher at 100% MVC compared to 50-75% MVC whereas biceps responsiveness to TMS reaches its peak early, at high but submaximal voluntary contractions, then deteriorates at near-tetanic state of the muscle [140]. This may explain why we could detect an increase in MEP ratio only at high but submaximal biceps recruitment. Finally, post hoc analyses of the MEP ratio were used to quantify how often a satisfactory MEP ratio condition was met. The criteria recommended by Todd et al. (biceps MEP ≥ 50% Mmax and triceps MEP ≤ 20% Mmax) was met more often in the nonimpaired group (40% of trials) compared to the SCI group (22% of trials) which supports previous findings that the optimal stimulation conditions are more difficult to achieve in patient populations [88], [97], [102]. A modified interpretation of the Todd criteria, where the biceps MEP is 2.5 larger than the triceps

MEPs, was met more often in both groups (Table 2-2). This condition could be used as a less restrictive way of monitoring stimulation quality during VA_{TMS} trials in patient populations where Mmax measures are low [141] and triceps co-activation more common [33], [142]. However, elbow angle modulation only marginally improved the frequency in which these conditions were met and ultimately did not improve the measurement of VA_{TMS}.

Good repeatability of VA baseline measures (i.e., pre-therapy) is critical for its utility in informing rehabilitation. Here, we established the repeatability of VA_{TMS} and VA_{PNS} measured at 90^{o} of elbow flexion in participants with SCI. In nonimpaired populations, the repeatability of VA_{TMS} and VA_{PNS} measured at 90^{o} across several sessions was consistent with previous reports in nonimpaired populations suggesting moderate/good reliability of these measures [143], [144]. Inter-session repeatability of VA_{TMS} had previously been established across two days in the elbow flexors [109], wrist extensors [145] and knees extensors [146]. VA_{PNS} assessed in the elbow flexors is reliable across five days [147]. While VA_{TMS} is often used in the context of fatiguing protocols [105], [116], this study focused on evaluating its potential to be used as a monitoring tool in patient populations. Thus, our experimental protocol was modified, compared to previous work [109], to include 90 seconds resting periods between every voluntary contraction trial in order to facilitate task completion and prevent fatigue. Here, we confirmed that our modified protocol yielded repeatable VA_{TMS} measures in both groups. Furthermore, while within-session variability was small for VA_{TMS} at 45^{o} (nonimpaired: Coefficient of Variation (CV) = 5.8 ± 4.9 %, SCI: CV = 6.3 ± 6.2 %) and 90^{o} (nonimpaired: CV = 6.2 ± 5%, SCI: CV = 6.9 ± 7.4 %) of elbow flexion and VA_{PNS}, the more flexed elbow flexion presented higher variability (nonimpaired: CV = 14.7 ± 10.5%, SCI: CV = 11 ± 17%).

VA_{TMS} measures collected at 90° of elbow flexion were underestimated compared to VA_{PNS} in both nonimpaired and SCI participants. Underestimation of VA_{TMS} compared to VA_{PNS} has been reported in both unfatigued and fatigued biceps [89], [116] and remains a methodological challenge when estimating VA_{TMS} [102]. Poor linearity of the voluntary moment and SIT relation may explain artificially low estimation of VA_{TMS}. In this study, modulating the

elbow angle had no impact on linearity. In the nonimpaired group, we identified a positive association between linearity and the magnitude of VA_{TMS}, even amongst blocks with satisfactory linearity ($r > 0.8$). In the SCI group, higher linearity was also associated with higher VA_{TMS} estimates. Moreover, participants with tetraplegia had on average lower linearity (0.73 vs 0.83) compared to nonimpaired participants. Increased muscle spasticity [31] and fatigability [32] following SCI may contribute to this outcome. Neuromuscular fatigue is associated with a decrease in linearity of the voluntary moment and SIT relation [116]. This suggests that fatigue may have a non-linear effect on the neuromuscular system across the range of voluntary effort. As the method used to estimate VA_{TMS} relies on the assumption of linearity between voluntary moment and the SIT, the validity of the measurement may be hindered in a context that affects linearity, such as fatigue. Together, this implies that VA_{TMS} is sensitive to the linearity of the voluntary moment and SIT relation in nonimpaired participants, even when linearity is high ($0.8< r < 0.99$), and in tetraplegia, where high linearity is more difficult to achieve.

A potential limitation of our approach is that we excluded blocks from the data analysis demonstrating poor linearity in the nonimpaired group ($r < 0.8$). Although excluding data because of poor linearity is a common approach [102], [148], [149], our exclusion rate was higher (14% versus 7-9%), which may be related to our modified experimental protocol featuring longer blocks, including 90 seconds resting periods between each trial (one per effort level). The modified protocol with frequent breaks was intended to minimize fatigue in the SCI group (Appendix I, Figure 2-9). We did not exclude blocks in the SCI group in order to preserve data since linearity was lower overall compared to the nonimpaired group. However, this likely did not affect our results since we did not compare VA_{TMS} and MEP data between groups. Another potential limitation of VA_{TMS} is the high variability of MEPs [79] that depends on the levels of muscle activation [49]. MEPs indicate how the corticomotor pathway responds to cortical stimuli. Thus, MEP variability may translate to SIT moment variability, which subsequently affects VA_{TMS}. A third limitation is that we normalized all MEPs to the Mmax collected at the start of a session to allow comparison of MEPs between participants and across days. However, Mmax can decrease over the course of a session despite constant isometric posture [150], [151] and is

sensitive to the level of voluntary contraction [152]. In order to limit the number of stimulus events, we collected Mmax at rest while MEPs were collected at various levels of muscle activation.

Our results indicate that modulating the elbow flexion-extension angle does not improve the measurement of VA_{TMS} in the biceps of nonimpaired individuals and individuals with tetraplegia. The biceps/triceps MEP ratio was not modulated by elbow angle across the full range of voluntary efforts in nonimpaired and SCI participants, and did not uniformly impact VA_{TMS}. Thus, a focus on increasing the biceps/triceps MEP ratio through modulation of the elbow angle does not further improve estimation of VA_{TMS}. In nonimpaired participants, VA_{TMS} was sensitive to elbow flexion angle which suggests that elbow angle should be carefully monitored and reproduced across trials, participants, and sessions when assessing VA_{TMS}. Finally, VA_{TMS} was sensitive to small changes in the linearity of the voluntary moment and SIT relation. In SCI participants, linearity was lower compared to nonimpaired which poses an additional challenge in the estimation of VA_{TMS} in individuals with tetraplegia. As modulating elbow flexion did not affect linearity of the voluntary moment and SIT relation, further research is needed to determine whether VA_{TMS} is a viable assessment of neuromuscular function in individuals with SCI.

2.5 Appendix I

Table 2-3: Data summary presenting all key measures collected on nonimpaired participants. When applicable, measures are presented as mean ± standard deviation.

90˚ elbow flexion	MVC (Nm)			%VA_{TMS}			Est. Resting Twitch (Nm)		
Nonimpaired	**Session 1**	**Session 2**	**Session 3**	**Session 1**	**Session 2**	**Session 3**	**Session 1**	**Session 2**	**Session 3**
1 (M)	58.7 ± 1.68	59.1 ± 2.03	49 ± 1.26	97.6 ± 4.81	98.8 ± 3.04	86.8 ± 3.55	12.8	11.6	12.5
2 (F)	19.5 ± 1.26	24 ± 3.09	29 ± 3.78	Excluded	Excluded	99.9 ± 0	N/A	N/A	2.9
3 (F)	27.6 ± 0.82	39.6 ± 2.03	41 ± 0.96	83.4 ± 9.82	90.1 ± 7.36	94.9 ± 7.42	6.52	8.43	10.9
4 (M)	Excluded	Excluded	Excluded	Excluded	Excluded	Excluded	25.8	N/A	N/A
5 (M)	90.1 ± 3.93	94.5 ± 6.12	84 ± 2.49	93 ± 4.19	Excluded	82.2 ± 4.07	27.8	N/A	11.9
6 (M)	58.2 ± 0.9	59.4 ± 1.4	59.4 ± 1.25	87.6 ± 3.97	87.6 ± 8.18	92.9 ± 4.52	14.3	16.3	24.1
7 (F)	31.4 ± 1.93	32.1 ± 2.24	27.6 ± 1.7	Excluded	90.9 ± 10.28	92.9 ± 7.84	N/A	7.62	4.62
8 (M)	56 ± 2.24	59.9 ± 1.01	49.9 ± 4.01	93.9 ± 5.27	96.3 ± 4.39	96.8 ± 3.71	19.7	17.9	16.8
9 (F)	33.8 ± 2.31	29 ± 0.81	31.7 ± 0.73	90.8 ± 5.2	99.6 ± 1.06	99 ± 1.5	10.7	9.07	8.66
10 (M)	47.1 ± 1.24	58.9 ± 2.39	56.1 ± 0.86	98.5 ± 2.08	99.2 ± 2.01	98.6 ± 2.44	14.5	13.32	12.19
Mean	50.36 ± 23.13	54.29 ± 22.56	49.22 ± 18.01	92.1 ± 6.82	94.6 ± 7.32	93.8 ± 7.1	16.3 ± 7.3	12.0 ± 3.9	11.3 ± 6.3

	% VA_{PNS}			Potentiated Twitch (Nm)			RMT (%MSO)		
Nonimpaired	**Session 1**	**Session 2**	**Session 3**	**Session 1**	**Session 2**	**Session 3**	**Session 1**	**Session 2**	**Session 3**
1 (M)	99.5 ± 1.5	99.4 ± 1.24	96.8 ± 5.04	11.5 ± 4.2	14 ± 0.75	6.2 ± 0.56	60	51	50
2 (F)	99.9 ± 0	95.3 ± 6.04	95.1 ± 3.7	4.2 ± 0.45	4.1 ± 2.92	4.3 ± 0.24	21	35	37
3 (F)	99.9 ± 0.25	99.7 ± 0.8	99.4 ± 0.93	4.8 ± 0.35	5.2 ± 0.77	3.7 ± 0.51	53	21	20
4 (M)	Excluded	Excluded	Excluded	N/A	N/A	N/A	71	53	46
5 (M)	98.7 ± 2.6	98.7 ± 1.9	97.9 ± 2.76	15.2 ±	12.2 ±	14.4 ±	48	74	74
6 (M)	94.4 ± 4.9	94.8 ± 5.6	95.6 ± 4.3	14.5 ±	10.3 ±	12.6 ±	40	56	50
7 (F)	94.2 ± 3.4	98.8 ± 2.52	97.0 ± 3.2	5.2 ± 0.5	6.3 ± 0.61	3.4 ± 0.14	37	47	47
8 (M)	97.9 ± 4.1	98.8 ± 1.9	99.9 ± 0.05	11 ± 2.35	8.7 ± 0.84	10 ± 1.01	37	47	39
9 (F)	99.6 ± 0.9	99.8 ± 0.4	99.5 ± 0.82	5.5 ± 0.63	4.8 ± 0.63	4.7 ± 0.26	34	33	34
10 (M)	98.8 ± 2.8	98.2 ± 3.0	99.1 ± 1.25	8.4 ± 0.64	8.5 ± 0.62	12.3 ±	51	32	32
Mean	98.0 ± 3.2	95.9 ± 4.4	97.8 ± 3.3	9 ± 4.04	8.5 ± 3.4	8.1 ± 4.14	45.2	44.9	42.9

120˚ elbow flexion	MVC (Nm)	% VA_{TMS}	Est. Resting Twitch (Nm)	RMT (%MSO)
NONIMPAIRED				
1 (M)	48.5 ± 2.1	87.4 ± 12.17	8.99	50
2 (F)	24.3 ± 2.13	Excluded	N/A	35
3 (F)	38.8 ± 1.46	97.3 ± 6.62	4.39	21
4 (M)	58.4 ± 2.36	88.7 ± 8.25	N/A	46
5 (M)	55.7 ± 2.36	76.1 ± 16.31	7.79	74
6 (M)	41.3 ± 3.36	88.8 ± 11.43	9.02	64
7 (F)	15.2 ± 1.15	Excluded	N/A	47
8 (M)	46.9 ± 1.48	87.8 ± 7.36	7.41	37
9 (F)	21.4 ± 1.89	99.5 ± 1.16	7.26	34
10 (M)	74.5 ± 1.8	96.8 ± 7.9	5.08	32
Mean	42.5 ± 17.68	90.3 ± 7.53	7.82	44

45˚ elbow flexion	MVC (Nm)	% VA_{TMS}	Est. Resting Twitch (Nm)	RMT (%MSO)
NONIMPAIRED				
1 (M)	56.2 ± 1.39	78.4 ± 5.95	11.13	50
2 (F)	17 ± 3.03	Excluded	N/A	60
3 (F)	25 ± 1.74	99.4 ± 1.55	4.94	21
4 (M)	63.9 ± 3.22	90.2 ± 5.62	N/A	53
5 (M)	75.8 ± 1.89	78 ± 12.15	15.01	74
6 (M)	57.6 ± 1.25	79.1 ± 4.98	15.74	56
7 (F)	26.3 ± 2.56	83.1 ± 9.63	4.79	40
8 (M)	56.8 ± 2.01	98.4 ± 2.47	17.09	39
9 (F)	23 ± 1.26	99.9 ± 0	6.3	34
10 (M)	56.7 ± 7.17	99.5 ± 1.14	23	32
Mean	45.8 ± 20.05	89.6 ± 9.96	13.7	45.9

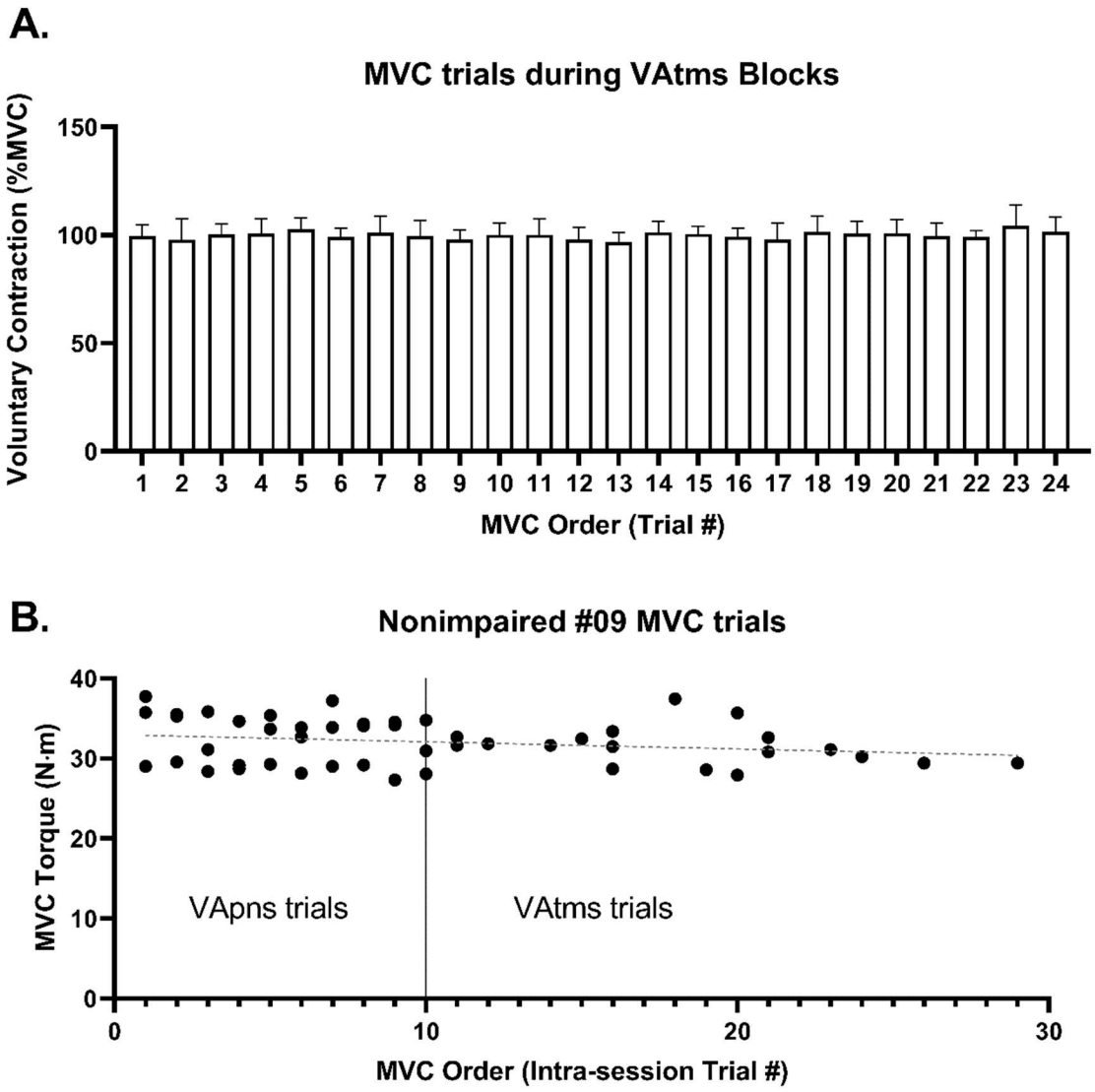
Table 2-4: Data summary presenting all key measures collected on SCI participants. When applicable, measures are presented as mean ± standard deviation.

90˚ elbow flexion	MVC (Nm)			%VA_{TMS}			Est. Resting Twitch (Nm)		
SCI	**Session 1**	**Session 2**	**Session 3**	**Session 1**	**Session 2**	**Session 3**	**Session 1**	**Session 2**	**Session 3**
1 (F)	90.84 ± 3.36	78.28 ± 2.09	62.95 ± 2.62	88.97 ± 12.82	88.56 ± 18.14	98.39 ± 3.93	5.39	2.93	8.68
2 (F)	57.2 ± 2.4	105.23 ± 5.54	102.37 ± 2.63	92.36 ± 7.44	92.14 ± 7.33	94.92 ± 5.81	9.12	15.8	6.28
3 (M)	74.47 ± 3	53.79 ± 2.37	24.41 ± 0.33	81.17 ± 18.86	67.95 ± 16.48	94.91 ± 4.05	6.23	7.14	2.83
4 (M)	22.9 ± 1.27	22.05 ± 2.55	26.16 ± 1.01	N/A	90.49 ± 10.65	99.99 ± 0	1.84	2.34	1.92
5 (F)	20.61 ± 2.27	15.68 ± 0.92	15.46 ± 1.19	95.77 ± 8.23	99.08 ± 1.46	99.99 ± 0	3.61	2.41	4.88
6 (M)	20.97 ± 1.59	26.67 ± 0.91	N/A	93.55 ± 7.72	97.17 ± 3.61	N/A	2.91	8.27	N/A
7 (M)	80.7 ± 2.79	77.2 ± 2.77	N/A	97.68 ± 3.6	99.99 ± 0	N/A	8.36	7.31	N/A
8 (M)	93.57 ± 2.84	73.97 ± 4.02	N/A	99.99 ± 0	99.99 ± 0	N/A	7.57	7.66	N/A
9 (M)	40.5 ± 1.68	46.23 ± 2.46	39.91 ± 1.5	99.99 ± 0	98.36 ± 4.02	96.62 ± 4.17	7.85	6.72	7.62
10 (M)	Excluded	Excluded	Excluded	Excluded	Excluded	Excluded	Excluded	Excluded	Excluded
Mean	55.8 ± 30.4	55.5 ± 30.47	45.2 ± 32.53	93.7 ± 6.33	92.6 ± 10.21	97.5 ± 2.34	5.88	6.73	5.37

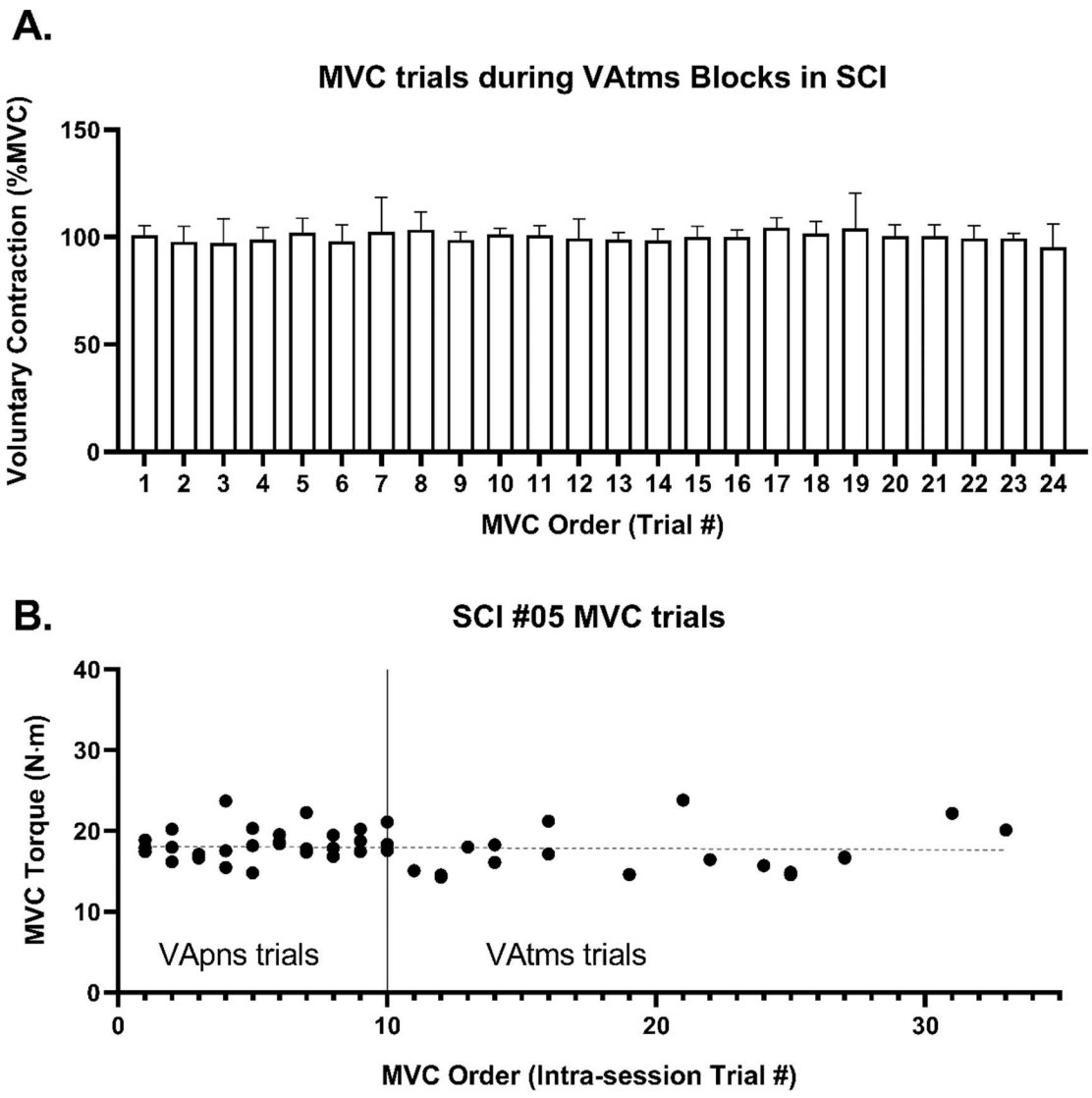
	%VAPNS			Potentiated Twitch (Nm)			RMT (%MSO)		
SCI	**Session 1**	**Session 2**	**Session 3**	**Session 1**	**Session 2**	**Session 3**	**Session 1**	**Session 2**	**Session 3**
1 (F)	92.93 ± 1.33	96.7 ± 3.23	99.99 ± 0	15.29 ± 1.7	12.33 ± 1.65	5.37 ± 0.75	63	52	52
2 (F)	92.09 ± 6.9	95.7 ± 6.49	99.06 ± 1.17	6.21 ± 0.29	5.77 ± 0.34	4.59 ± 1.51	34	32	28
3 (M)	87.66 ± 22.41	65.88 ± 37.29	95.51 ± 6.75	2.88 ± 0.3	1.04 ± 0.21	1.57 ± 0.3	37	39	38
4 (M)	94.88 ± 5.77	92.71 ± 6.38	93.93 ± 5.68	10.18 ± 0.91	6.6 ± 3.41	8.07 ± 1.33	43	42	36
5 (F)	99.66 ± 1.01	99.27 ± 2.29	99.99 ± 0	9.36 ± 0.67	2.27 ± 0.28	3.97 ± 0.48	49	54	52
6 (M)	99.43 ± 1	99.91 ± 0.23	N/A	7.92 ± 0.88	8.8 ± 0.65	N/A	28	30	N/A
7 (M)	98.64 ± 1.54	99.4 ± 1.18	N/A	13.47 ± 1.42	8.57 ± 0.82	N/A	32	27	N/A
8 (M)	99.56 ± 1.12	97.28 ± 2.59	N/A	11.66 ± 1.46	10.97 ± 0.91	N/A	26	32	N/A
9 (M)	98.73 ± 2.64	99.98 ± 0.06	99.01 ± 1.62	19.86 ± 1.81	19.8 ± 4.53	16.74 ± 3.21	26	26	26
10 (M)	Excluded	Excluded	Excluded	Excluded	Excluded	Excluded	Excluded	Excluded	Excluded
Mean	95.95 ± 4.3	94.09 ± 10.84	97.92 ± 2.56	10.76 ± 5.06	8.46 ± 5.64	6.72 ± 5.34	37.56	37.11	38.67

120˚ elbow flexion	MVC (Nm)	%VATMS	Est. Resting Twitch (Nm)	RMT (%MSO)
SCI				
1 (F)	40.7 ± 2.39	99.99 ± 0	1.75	52
2 (F)	55.44 ± 3.17	100 ± 0	2.84	32
3 (M)	50.26 ± 2.17	90.05 ± 6.27	5.69	39
4 (M)	10.09 ± 2.98	92.11 ± 19.22	0.22	36
5 (F)	7.62 ± 0.43	99.99 ± 0	1.01	52
6 (M)	N/A	N/A	N/A	N/A
7 (M)	59.24 ± 0.99	99.99 ± 0	0.95	27
8 (M)	34.95 ± 1.71	96.39 ± 8.83	3.32	32
9 (M)	25.1 ± 1.83	82.76 ± 42.21	0.92	26
10 (M)	Excluded	Excluded	Excluded	Excluded
Mean	35.4 ± 19.78	95.2 ± 6.37	2.09	37

45˚ elbow flexion	MVC (Nm)	%VATMS	Est. Resting Twitch (Nm)	RMT (%MSO)
SCI				
1 (F)	86.6 ± 10.31	86.91 ± 17.85	3.82	63
2 (F)	96.86 ± 3.6	96.03 ± 6.6	6.94	28
3 (M)	24.8 ± 0.55	91.1 ± 4.36	3.28	38
4 (M)	23.19 ± 6.64	97.08 ± 4.82	3.21	42
5 (F)	14.58 ± 0.74	99.99 ± 0	2.78	52
6 (M)	26.12 ± 0.6	92.71 ± 5.9	7.54	30
7 (M)	78 ± 1.45	98.68 ± 3.23	6.73	27
8 (M)	71.48 ± 4.04	84.97 ± 8.41	8.52	32
9 (M)	49.21 ± 0.9	99.99 ± 0	8.43	26
10 (M)	Excluded	Excluded	Excluded	Excluded
Mean	52.3 ± 31.45	94.2 ± 5.58	5.69	37.56



Supplementary Figure 2-8: Experimental procedure limited fatigue in nonimpaired participants**. A.** All 100% MVC trials for nonimpaired participants demonstrates there was no impact of within block trial order on the mean MVC (normalized to corresponding reference MVC for each session/participant) across all nonimpaired participants. Error bars represent the standard deviation. **B.** MVC moment data from one representative participant collected during VA_{PNS} and VA_{TMS} blocks across all three sessions.



Supplementary Figure 2-9: Experimental procedure limited fatigue in participants with SCI**. A.** All 100% MVC trials for SCI participants demonstrates no impact of within block trial order on the mean MVC (normalized to corresponding reference MVC for each session/participant) across all participants with SCI. Error bars represent the standard deviation. **B.** MVC moment data from one representative participant collected during VA_{PNS} and VA_{TMS} blocks across all three sessions.

3 Chapter 3: Effect of Paired-Pulse Stimulation in the Assessment of Voluntary Activation

3.1 Background/Objectives

Voluntary activation (VA) quantifies the level of voluntary neural drive to the target muscle during voluntary effort [153]. Assessment of VA relies on the assumption that during a maximal voluntary contraction (MVC) the motoneuron pool is maximally recruited and unable to produce any additional force with artificial electrical stimulation [153]. VA of a specific muscle or muscle group can be measured via electrical stimulation of the peripheral nerve (VA_{PNS} superimposed on MVC's) [154], [155]. However, VA_{PNS} cannot reveal the extent to which deficits in VA are due to central (i.e. originating from the motor cortex) versus peripheral factors (i.e. function of spinal motoneurons and neuromuscular junction) [153], [156]. One way to assess the central factors that may affect VA is with transcranial magnetic stimulation (TMS) of the motor cortex superimposed on voluntary contraction [129], [157], [158]. VA assessed with TMS (VA_{TMS}) can elucidate the site of impairment in voluntary drive [159], which may be particularly useful to characterize neuromuscular function after cervical spinal cord injury (SCI). After SCI, injury-induced functional changes occur at multiple sites of the nervous system [3] and lead to motor impairments such as weakness, spasticity [31], and fatigability [28] that affect relatively spared muscles (e.g. the biceps brachii). Existing rehabilitation protocols have limited potential to address these impairments [9]–[12], and thus would benefit from the better quantification and localization of neuromuscular deficits that VA_{TMS} can provide.

While VA_{TMS} can assess neuromuscular deficits, technical challenges in measuring VA_{TMS} exist [129], [130], [156], [160]–[164]. An important limitation of VA_{TMS} is the recruitment of muscles other than the target muscle because TMS over the motor cortex can stimulate neighboring cortical neural pathways projecting to agonistic and antagonistic muscles [165]. This lack of precision is due, in part, to the high stimulation intensities needed to evoke measurable force/moment responses, especially in patient populations [130], [160], [166]. Greater stimulus intensities are associated with greater stimulus spread in the brain [167]. The motor evoked

potential (MEP) in response to TMS of the target muscle compared to its antagonist's MEP can indicate, to some degree, the focality of stimulation and excitability of pathways projecting to the target muscle relative to its antagonists [160], [168]–[170]. Isolated recruitment of the target muscle with TMS, while ideal, is difficult with currently available TMS devices [160], [171], [172]. As such, Todd et al. (2016) suggest a realistic compromise of isolated recruitment of the target muscle when its MEP amplitude reaches ≥ 50% of the muscle's maximal compound motor action potential (Mmax) and the antagonist MEP amplitude is ≤ 20% of Mmax [160]. This compromise can be achieved in nonimpaired, non-fatigued individuals by adjusting TMS intensity when targeting certain muscle groups, such as the elbow flexors [160], [164], [165]. However, this is more difficult in patient populations – specifically individuals with spinal cord injury (SCI) [166], [173]–[175]. For example, in individuals with tetraplegia after cervical SCI, the TMS intensity could not be adjusted to elicit appropriate responses in the triceps to estimate VA_{TMS} of the elbow extensors [166], [176]. Additional considerations and modifications to existing VA_{TMS} protocols may be needed to assess VA_{TMS} in individuals with tetraplegia. As a first step, we focused on improving the methodology to assess VA_{TMS} of the biceps brachii in individuals with tetraplegia because: a) the biceps is innervated at the C5 and C6 levels such that considerable biceps function typically remains in many individuals with low cervical injuries (C5-C8) [28], [29], b) the biceps is important for upper limb function [26], and c) comparative data with single pulse TMS to assess VA_{TMS} exist in nonimpaired individuals [129], [130].

One approach to modify VA_{TMS} protocols to assess biceps neuromuscular function in patient populations is to increase the motor response to TMS in the biceps relative to the triceps; the degree to which that is achieved can be measured by the ratio of the biceps MEP relative to the triceps MEP (i.e., biceps MEP divided by triceps MEP). Paired pulse TMS techniques can modulate MEP amplitudes and can potentially optimize the biceps/triceps MEP ratio in the assessment of VA_{TMS} [177]–[179]. Paired pulse TMS techniques consist of a conditioning stimulus followed by a test stimulus with a specific inter-stimulus interval (ISI) between the two stimuli [180]. At ISIs ranging from 10-30 ms, MEPs are typically increased in the biceps relative to single pulse TMS through the physiologic mechanism referred to as intracortical facilitation (ICF)[181].

At shorter ISIs (ranging from 2 to 5 ms), biceps MEPs are decreased in the biceps relative to single pulse TMS through the physiologic mechanism referred to as short intracortical inhibition (SICI)[60], [181].

Low levels of muscle contraction (≤ 20% MVC) modify responses to paired pulse TMS [182], [183], but how higher levels of muscle contraction (> 20% MVC) modify MEPs in response to paired pulse TMS remains unknown. Thus, whether and how paired pulse TMS techniques can modulate the biceps/triceps MEP ratio across different higher levels of voluntary contractions (i.e., 50 to 100% of MVC) needs to be investigated. The effect of paired pulse TMS on biceps and triceps MEPs at higher levels of voluntary contraction needs to be established because VA_{TMS} is assessed by superimposing TMS on voluntary effort levels ranging from 50 to 100% MVC to extrapolate the linear relationship between voluntary moment and superimposed twitch moment required to estimate VA_{TMS} [129], [154], [159]–[161]. Further, it is unknown whether an increased biceps/triceps MEP ratio across effort levels can improve the estimation of VA_{TMS} in individuals with tetraplegia.

The purpose of this study was to determine the relationship between VA_{TMS} and the biceps/triceps MEP ratio in individuals with low cervical SCI and nonimpaired individuals. Modulation of the TMS paired pulse ISI was tested as a method to modulate the biceps/triceps MEP ratio across effort levels of 0, 50, 75, and 100% MVC. We hypothesized that the ICF (facilitatory modulation) paired pulse TMS condition would increase the biceps/triceps MEP ratio across effort levels relative to single pulse TMS in both participant groups. Conversely, we hypothesized that SICI would decrease the biceps/triceps MEP ratio across effort levels relative to single pulse TMS in both participant groups. Finally, we hypothesized that the biceps/triceps MEP ratio would affect VA_{TMS}. The rationale behind this hypothesis is that the biceps/triceps MEP ratio may indicate the amount of cortical stimulation to the biceps relative to the triceps, with greater biceps cortical stimulation perhaps contributing to greater excitation of the biceps and larger superimposed elbow moment twitches during voluntary contraction.

3.2 Methods

3.2.1 Experiment Overview:

Elbow joint force and moment data, and elbow flexor (biceps brachii) and extensor (triceps brachii) electromyographic (EMG) data were collected from ten individuals with cervical SCI (Table 2-1) and ten nonimpaired individuals. Inclusion criteria required SCI participants to be between the ages of 18 and 65 years old with a low cervical spinal injury at levels C5-C8 as indicated by the International Standards for Neurological Classification of Spinal Cord Injury (ISNCSCI), at least one year post-injury. Exclusion criteria included metal implants in the head and the inability to generate a visible contraction of the biceps. Data from one participant with SCI (#10) was excluded from the data analyses because TMS was unable to elicit measurable moment twitches from the elbow flexors. Participants completed up to three sessions. During each session, participants were seated in a chair with their dominant arm supported in an isometric posture, against gravity, and the forearm supinated, elbow flexed at 90° (Figure 3-1). Participant's biceps and triceps maximal M-wave responses, elbow flexion MVC, and VA_{TMS} were measured during each of the three sessions; only one of the three paired pulse VA_{TMS} protocols were assessed each session to limit fatigue and the number of stimulus events. This study was approved by the Virginia Commonwealth University Institutional Review Board.

3.2.2 Electromyographic and Kinetic Recordings:

All data were recorded via a custom Spike2 script and a data acquisition system (CED 1401, Cambridge, UK). EMG data were recorded using wireless EMG sensors (Delsys Trigno, Natick, Massachusetts) placed on the participant's biceps and triceps in a muscle belly tendon arrangement. EMG data were sampled at 2000 Hz and bandwidth limited to 20-450Hz. The participant's forearm was positioned in a custom brace attached to a multi-axis load cell with a measurement range of ± 400 N and digital resolution of 0.1 N (JR3 30E15A4, Woodland, California). A different load cell was used for participants with weaker elbow flexors (JR3 30E12A4, measurement range of ± 100 N and digital resolution of 0.025 N). Three-dimensional

force and moment data were recorded at 2000 Hz and transformed to the elbow joint using standard coordinate transformations to determine the elbow flexor moment [184].

3.2.3 Compound Motor Unit Action Potential Recording:

Electrical stimuli were delivered using a constant current stimulator (Digitimer DS7AH, Fort Lauderdale, Florida) at 200 V with a 200 μs pulse width. The current delivered ranged from 5 mA (threshold of detection) to 150 mA (procedural maximum). Rectangular 3.3 x 5.3 cm neurostimulation electrodes (Axelgaard 891200, Fallbrook, California) were placed at Erb's point (cathode) and the acromion (anode). M-wave recruitment curves were obtained individually for the biceps and then the triceps starting from zero at intervals of 10 mA until a plateau in the M-wave amplitude was reached. Five supramaximal stimuli of 120% of the threshold current were delivered to obtain the Mmax for the biceps and triceps at rest.

3.2.4 Transcranial Magnetic Stimulation:

Motor cortex stimulation was delivered using a 126 mm double cone coil and Magstim BiStim^2 (Magstim, Whitland, United Kingdom). Motor mapping of the cortical hotspot was performed during each session to obtain the location that evoked the largest peak-to-peak MEP in the biceps relative to the triceps using the lowest stimulation intensity [134]. The hotspot location was then marked on a silicone or plastic cap secured to the participant's head. Resting motor threshold (RMT) was then determined as the lowest stimulus intensity able to induce MEPs of ≥ 50μV in at least 50% of ten stimuli and expressed as a percentage of the maximum stimulator output (%MSO) [185]. To reduce the number of stimuli, RMT was identified using maximum likelihood adaptive parameter estimation [186]. Paired pulse stimuli were delivered as a conditioning pulse set to 90% RMT followed by a test pulse at 120% RMT separated by an ISI of 1.5, 10, or 30 ms. Stimuli with 1-4 ms ISIs have been shown to elicit SICI while ISIs of 10-30 ms have been shown to elicit ICF [60], [180], [187].

3.2.5 Protocol:

Participants started with a quick familiarization phase and warm-up, which consisted of brief submaximal contractions for two minutes. Participants were then instructed to perform three sustained, isometric contractions of the elbow flexors at maximum effort to determine their MVC moment. Contractions were sustained for 3 seconds while participants received both real-time visual moment feedback, and auditory encouragement (Figure 3-1, B). Real-time visual elbow moment feedback was displayed on a nearby monitor as a thermometer-like bar. The MVC was calculated as the mean elbow flexion moment occurring within a 0.5 s window from the maximal moment value. The average of all three MVC efforts was used in the following VA_{TMS} trials where participants generated a voluntary moment to match a percentage of their MVC moment. Each MVC was separated by 90 seconds of rest.

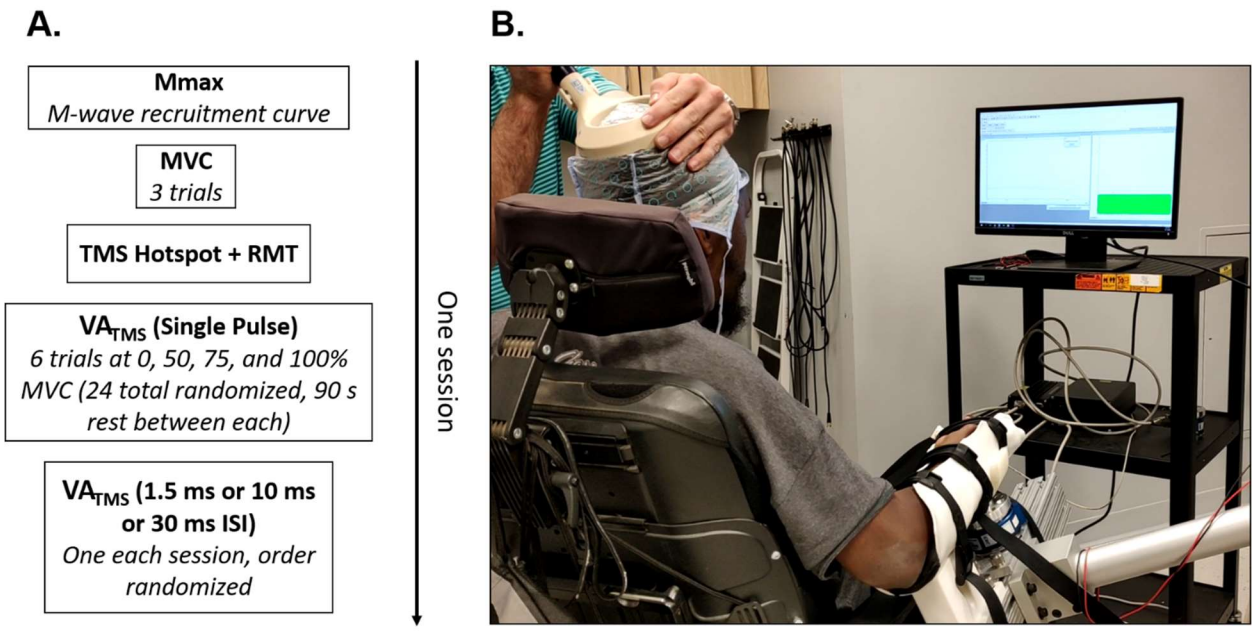


Figure 3-1: A. Experimental protocol block diagram representing the data collected during a single session. Participants completed three sessions in total. **B.** Experimental setup: Participants received visual feedback of their voluntary elbow flexion moment as a thermometer-like gauge.

After locating the cortical hotspot and establishing RMT, VA_{TMS} was assessed. Baseline (single pulse) and modified (paired pulse) VA_{TMS} protocols were assessed in a randomized order, with at least one baseline and one modified protocol per session (Figure 3-1, A). VA_{TMS} protocols

consisted of a set of 24 isometric contractions of the elbow flexors during randomized moment-matching trials of 0, 50, 75, or 100% MVC. Trials were separated by at least 90 s of rest to mitigate fatigue. To obtain the superimposed twitch moment in the single pulse protocol, a supramaximal (i.e. 120% RMT) TMS pulse was automatically delivered after the participant achieved and maintained ± 2.5% of the target effort for 0.5 s. In the paired pulse protocols, a conditioning pulse set to 90% RMT followed by a test pulse at 120% RMT separated by an ISI of 1.5, 10, or 30 ms was used.

3.2.6 Data and Statistical Analysis:

Force, moment and EMG data were post-processed using a custom MATLAB script. MEPs were determined as the peak-to-peak EMG signal within 100 ms of cortical stimulation and subsequently normalized to the Mmax of each session; all MEPs were visually inspected. The MEP ratio for each trial was calculated as the normalized biceps MEP divided by the normalized triceps MEP. EMG traces of representative participants from the nonimpaired group and the SCI group are presented in Figure 3-2. VA_{TMS} was calculated as a percentage using the interpolated twitch technique: VA_{TMS} (%) = (1 − superimposed twitch at 100% MVC)/(estimated resting twitch) × 100 [129]. The resting twitch was estimated using linear regression of the 50-100% MVC efforts (see Todd et al., 2004, method 1 for detailed explanation) [159]. Finally, the pre-TMS stimulation EMG activity of the biceps and triceps was calculated as the root mean square of the signal during the 50 ms directly before stimulation.

A linear mixed effect model was analyzed to determine the effect of independent variables on VA_{TMS} (the dependent variable). The independent variables were defined as follows: stimulation pulse (single pulse vs paired pulse conditions), block mean biceps/triceps MEP ratio, linearity of the voluntary moment, and superimposed twitch relation (R-value), and RMT. Blocks with low linearity ($r < 0.8$) in the nonimpaired group were excluded [160], but no exclusion was done in the SCI group to preserve data. Linearity was included as an independent variable to test whether variations in linearity affected the estimation of VA_{TMS}. RMTs were added to the model as a continuous covariate to test whether individual responsiveness to TMS (as represented by

RMTs) affected VA_{TMS}. A random effect was added to account for individual differences that resulted in each participant being assigned a different intercept. P-values were obtained via the Kenward-Roger approximation for degrees-of-freedom implemented for linear mixed effect models [188]. Comparisons were reported with respect to single pulse VA_{TMS} measures. Two-way ANOVAs with repeated measures were used to compare MEP ratios of single pulse to paired pulse VA_{TMS} conditions (1.5 ms, 10 ms or 30 ms ISI) across effort levels (0, 50, 75, and 100% MVC). Two-way ANOVAs were also used to compare biceps and triceps MEPs between single pulse and paired pulse VA_{TMS} conditions. The null hypotheses were that paired-pulse stimulation conditions do not have an effect on VA_{TMS} nor the MEP ratio, when compared to single pulse stimulation. The alternative hypotheses were that the SICI condition will decrease the MEP ratio and decrease VA_{TMS}, while both ICF conditions will increase the MEP ratio and increase VA_{TMS}. Another two-way ANOVA was used to compare the linearity of the voluntary moment and superimposed twitch relation between the nonimpaired and SCI groups; this comparison was tested before excluding low linearity blocks in the nonimpaired data. Finally, we reported the percent of trials that met the Todd et al. (2016) criteria (biceps MEP ≥ 50% Mmax and triceps MEP ≤ 20% Mmax) and an adjusted condition (MEP ratio ≥ 2.5) to account for the triceps being in a higher susceptibility state during VA_{TMS} trials. All data are presented as mean ± standard error of the mean unless stated otherwise. Statistical significance was set at the $p < 0.05$ level.

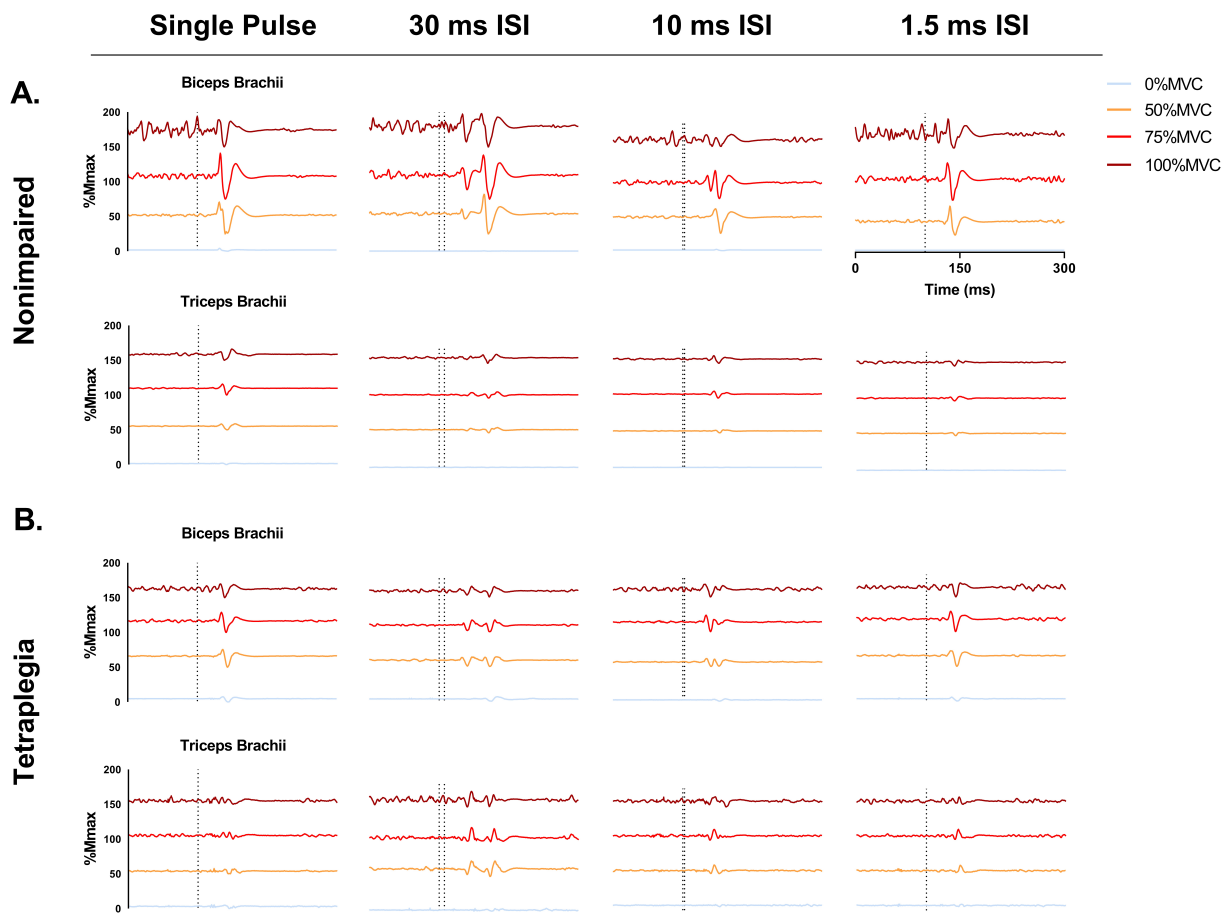


Figure 3-2: EMG traces showing representative MEPs over a 300 ms window across effort levels and stimulation pulses (single pulse 1.5 ms, 10 ms, and 30 ms ISI). **A.** EMG recordings from the biceps brachii and triceps brachii of a representative nonimpaired participant. **B.** EMG recordings from the biceps brachii and triceps brachii of a representative SCI participant. EMG signals shown were averaged across six trials and normalized to the Mmax of the corresponding session/participant. EMG traces presented within the same subdivision were offset from one another for presentation. The dotted vertical line in each subdivision represents the onset of stimulation.

3.3 Results

Across all nonimpaired participants, mean VA_{TMS} collected with single pulse TMS was 91.1%. For paired pulse stimulation, mean VA_{TMS} was 84.5% with the 1.5 ms ISI condition, 90.2% with the 10 ms ISI condition, and 85.1% with the 30 ms ISI condition (Figure 3-3). For the SCI participants, mean VA_{TMS} collected with single pulse TMS was 94.3%. Mean VA_{TMS} was 92.7% with

the 1.5 ms condition, 88.9% with the 10 ms ISI condition, and 89.7% with the 30 ms ISI condition (Figure 3-3).

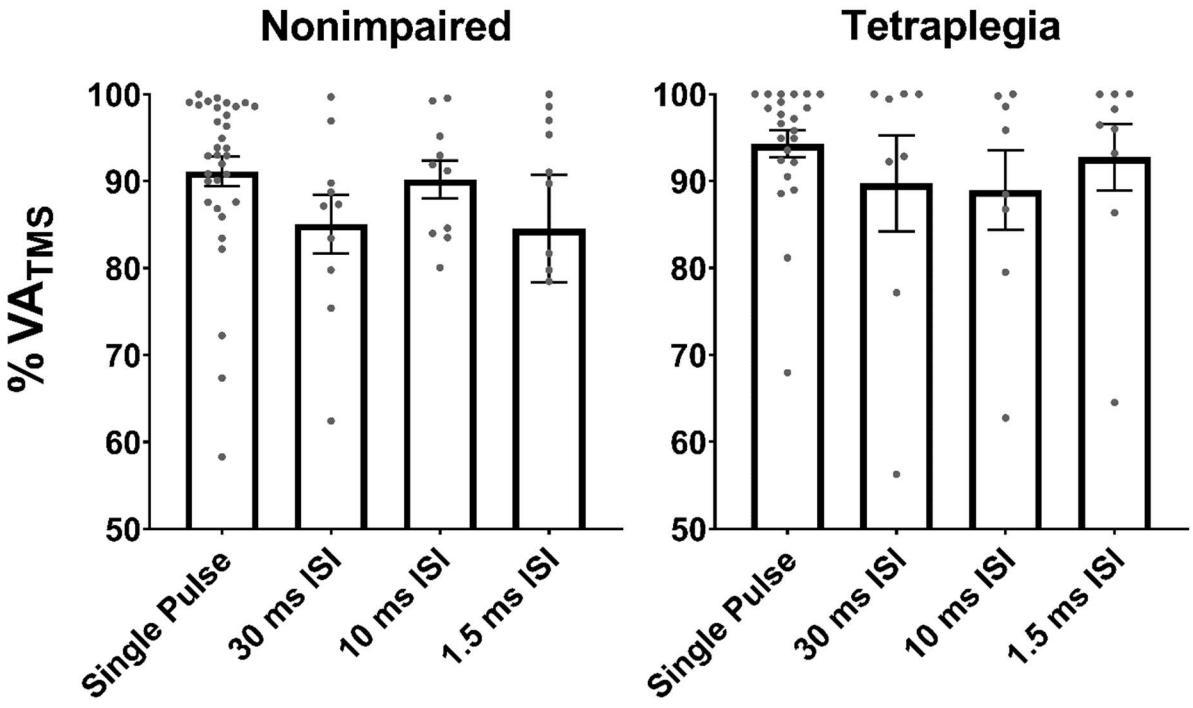


Figure 3-3: VA_{TMS} measures collected during the single pulse and paired pulse conditions in nonimpaired and SCI participants. Grey points represent individual mean VA_{TMS} (per block).VA_{TMS} ranged from 56 to 99%. Error bars represent the standard error of the mean.

3.3.1 Effect of Stimulation Pulse on the Biceps/Triceps MEP Ratio:

In the SCI group, the biceps/triceps MEP ratio was increased in the 10 ms ISI condition relative to the baseline single pulse condition at 50% MVC ($t = 2.205$, $p = 0.02$) and 75% MVC ($t = 3.571$, $p = 0.0004$) (Figure 3-4). The MEP ratio was also decreased in the 30 ms ISI condition relative to the baseline single pulse condition at 50% MVC ($t = 3.851$, $p = 0.0001$) and 75% MVC ($t = 3.506$, $p = 0.0005$) (Figure 3-4, B).

In the nonimpaired group, the biceps/triceps MEP ratio was increased in the 1.5 ms (t = 3.849, p = 0.0001) condition relative to the baseline single pulse condition only at 75% MVC (Figure 3-5, B).

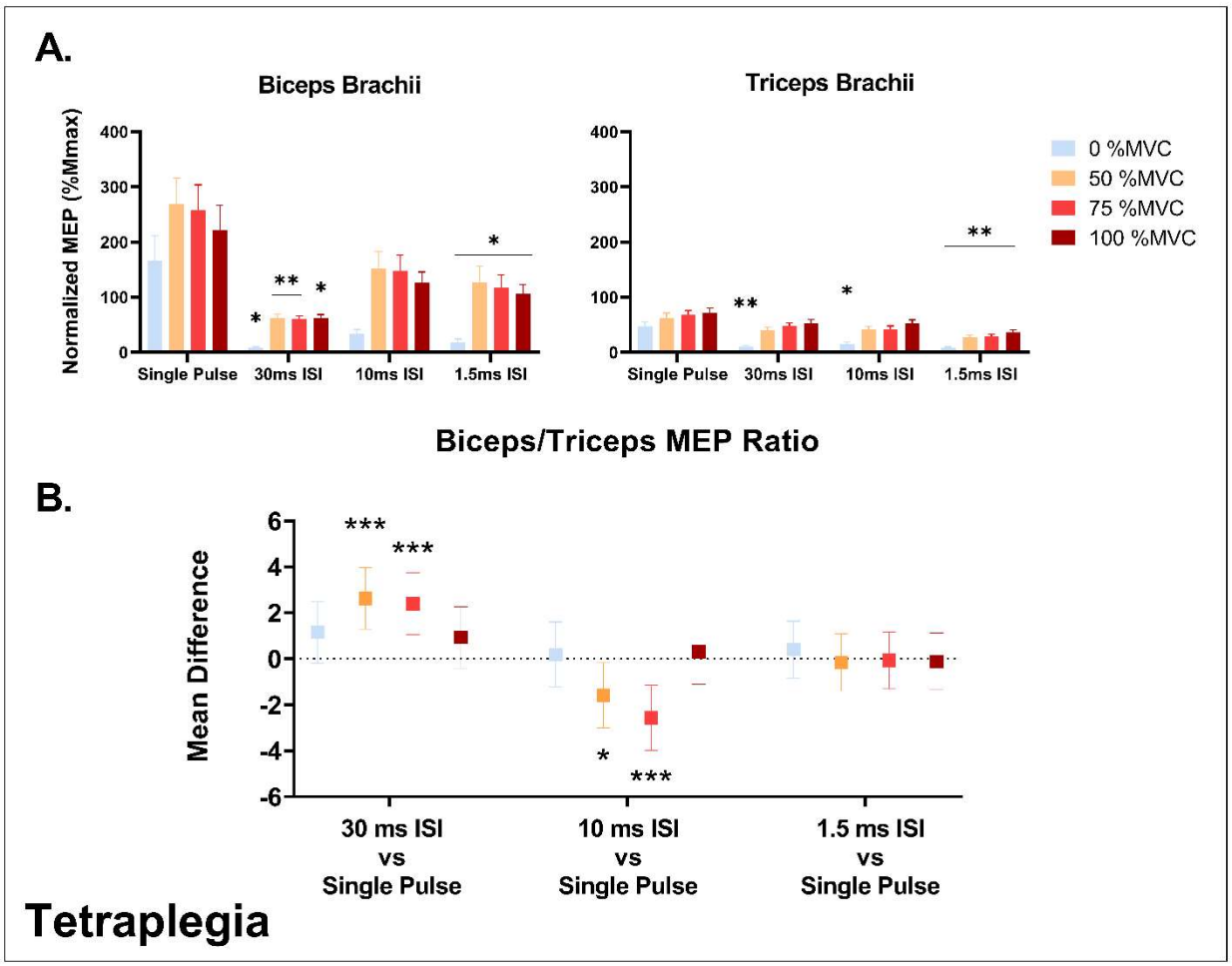


Figure 3-4: A. Average biceps and triceps normalized MEPs (normalized to corresponding Mmax) in the SCI group. In the biceps, a significant decrease was observed for MEP amplitudes in 30 ms ISI and 1.5 ms ISI conditions compared to single pulse. In the triceps, 30 ms ISI and 10 ms ISI conditions led to lower MEPs but only at rest while 1.5 ms ISI led to lower MEPs across all effort levels. Error bars represent the standard error of the mean. Asterisks indicate statistical significance compared to the single pulse condition. **B.** Biceps/ Triceps MEP ratio mean difference relative to single pulse TMS in the SCI group. Errors bars show 95% confidence intervals. Asterisks indicate a significantly different mean MEP ratio ([*] = $p < 0.05$, ([**] = $p < 0.01$, ([***] = $p < 0.001$) relative to the mean MEP ratio with single pulse TMS.

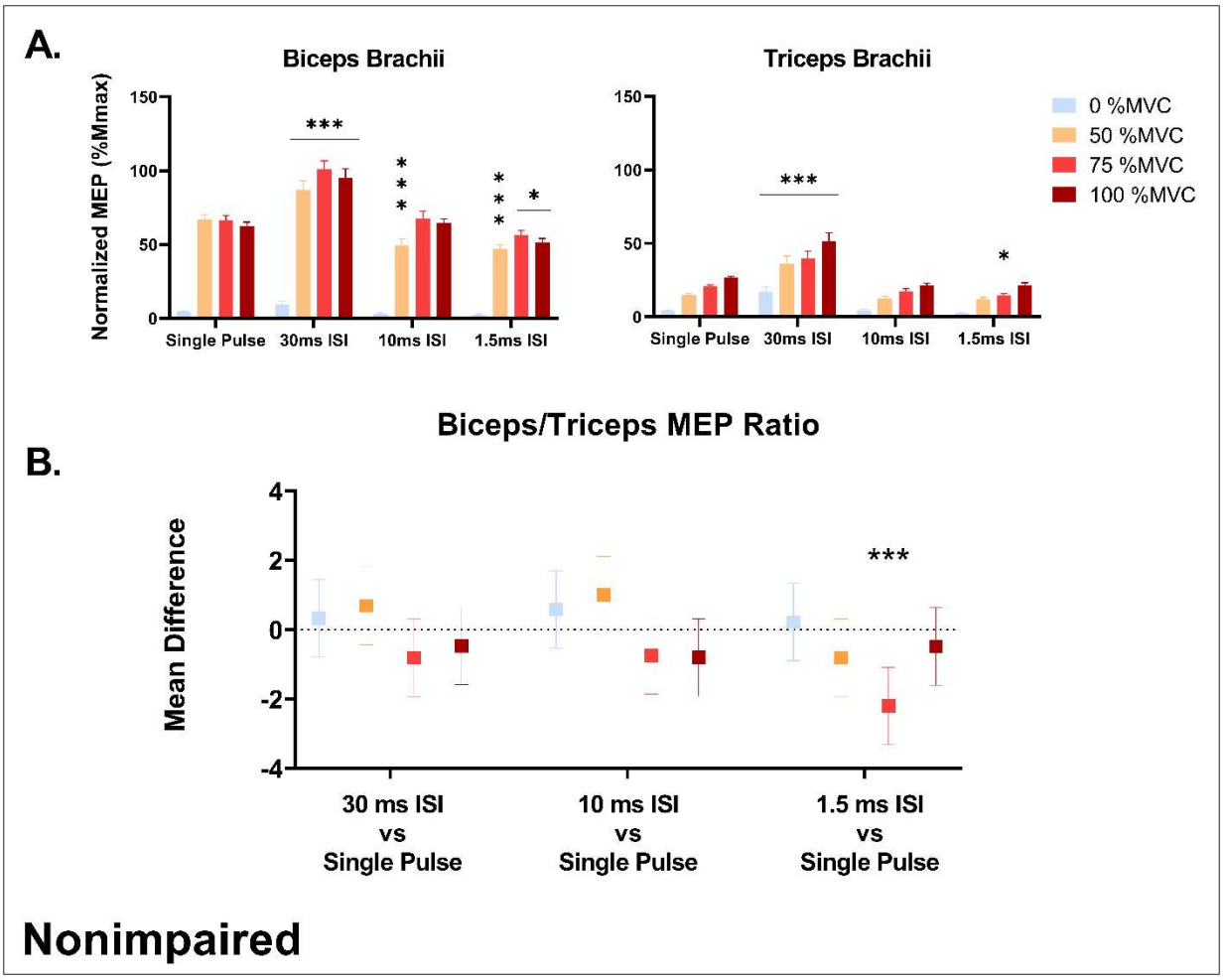


Figure 3-5: A. Average biceps and triceps MEPs (normalized to corresponding Mmax) across stimulation conditions and effort levels in the nonimpaired group. Biceps and triceps MEPs were increased during the 30 ms ISI condition while 10 ms ISI and 1.5 ms ISI led to lower MEPs but not across all effort levels. Error bars represent the standard error of the mean. Asterisks indicate statistical significance compared to the single pulse condition. **B.** MEP ratio mean difference relative to single pulse TMS across effort levels in the nonimpaired group, only 1.5 ms ISI led to an increased MEP ratio at 75% MVC. Errors bars show 95% confidence intervals. Asterisks indicate a significantly different mean MEP ratio ([*] = $p < 0.05$, ([***] = $p < 0.001$) relative to the mean MEP ratio with single pulse TMS.

3.3.2 Effect of Independent Variables on VA_{TMS}:

In the SCI group, the main effect of the stimulation pulse (1.5 ms, 10 ms, and 30 ms ISI compared to single pulse) on VA_{TMS} was not significant in the linear mixed-effects model. The main effect of the linearity of the voluntary moment and superimposed twitch moment relation

on VA_{TMS} was significant in the linear mixed-effects model. For each 0.1 increase in linearity (for $0 < r < 0.99$), VA_{TMS} was predicted to increase by 7.5% [$t = 7.005$, $p < 0.0001$]. Further analyses revealed that the mean MEP ratio, RMT had no significant main effects on VA_{TMS} as well as no interaction effects with the stimulation pulse.

In the nonimpaired group, the main effect of stimulation pulse (1.5 ms, 10 ms, and 30 ms ISI compared to single pulse) on VA_{TMS} was not significant in the linear mixed-effects model. Further analyses revealed that the mean MEP ratio, RMT, and linearity had no significant main effects on VA_{TMS} as well as no interaction effects with the stimulation pulse.

3.3.3 Effect of Stimulation Pulse on Biceps MEPs:

In the SCI group, 30 ms ISI [-158% Mmax, $p = 0.018$] and 1.5 ms ISI [-148% Mmax, $p = 0.016$] decreased biceps MEPs collected at rest. At 50% MVC, 30 ms ISI [-207% Mmax, $p = 0.0019$] and 1.5 ms ISI [-142% Mmax, $p = 0.02$] decreased biceps MEPs compared to single pulse. At 75% MVC, 30 ms ISI [-197% Mmax, $p = 0.003$] and 1.5 ms ISI [-140% Mmax, $p = 0.022$] decreased biceps MEPs compared to single pulse. At 100% MVC, only 30 ms ISI decreased biceps MEPs [-158% Mmax, $p = 0.017$] compared to single pulse (Figure 3-4, A).

In the nonimpaired group, stimulation pulse had no effect on biceps MEPs collected at rest (0% MVC). At 50% MVC, 30 ms ISI increased biceps MEPs [+20.04% Mmax, $p < 0.0001$] while 1.5 ms ISI [-20.2% Mmax, $p < 0.0001$] and 10 ms ISI [-17.7% Mmax, $p = 0.0003$] decreased biceps MEPs compared to single pulse. At 75% MVC, 30 ms ISI increased biceps MEPs [+34.3% Mmax, $p < 0.0001$] compared to single pulse. At 100% MVC, 30 ms ISI increased biceps MEPs [+32.6% Mmax, $p < 0.0001$] while 1.5 ms ISI decreased biceps MEPs 5 [-11.1% Mmax, $p = 0.023$] compared to single pulse (Figure 3-5, A).

3.3.4 Effect of Stimulation Pulse on Triceps MEPs:

In the SCI group, 30 ms ISI [-36.2% Mmax, $p = 0.004$], 10 ms ISI [-31.5% Mmax, $p = 0.019$], and 1.5 ms ISI [-38.9% Mmax, $p = 0.0009$] decreased triceps MEPs collected at rest. At 50% MVC,

only 1.5 ms ISI decreased triceps MEPs compared to single pulse [-35.7% Mmax, p = 0.002]. At 75% MVC only 1.5 ms ISI decreased triceps MEPs compared to single pulse [-39.1% Mmax, p = 0.0009]. At 100% MVC, only 1.5 ms ISI decreased triceps MEPs [-35.2% Mmax, p = 0.0026] compared to single pulse (Figure 3-4, A).

In the nonimpaired group, only 30 ms ISI increased triceps MEPs collected at rest [+12.7% Mmax, p < 0.0001]. At 50% MVC, 30 ms ISI increased triceps MEPs [+21.2% Mmax, p < 0.0001]. At 75% MVC, 30 ms ISI increased triceps MEPs [+19.1% Mmax, p < 0.0001] while 1.5 ms ISI decreased triceps MEPs 5 [-6.09% Mmax, p = 0.029] compared to single pulse. At 100% MVC, only 30 ms ISI increased triceps MEPs [+24.6% Mmax, p < 0.0001] compared to single pulse (Figure 3-5, A).

3.3.5 Post-hoc Evaluation of Biceps/Triceps MEP Ratio and Linearity:

In both the nonimpaired and SCI groups, the 10 ms ISI and 1.5 ms ISI pulses had a higher number of trials that met the guideline criteria presented by Todd et al. (biceps MEP ≥ 50% Mmax and triceps MEP ≤ 20% Mmax) and our adjusted condition of corresponding to a MEP ratio greater than 2.5 (Table 3-2). Across both groups and all stimulation pulses, the “MEP ratio > 2.5” condition was met more often than the Todd et al. criteria. Finally, the linearity of the voluntary moment and superimposed twitch moment relation was on average lower in the SCI group compared to the nonimpaired group across stimulation pulses [$F_{(1, 103)} = 7.043$, p = 0.0092](Table 3-2). Supplementary and raw data can be accessed online: https://osf.io /sdxj9/.

Table 3-1: Percent trials (between 50%-100% MVC) meeting the Todd et al. criteria (biceps MEP ≥ 50% Mmax and triceps MEP ≤ 20% Mmax), MEP ratio > 2.5 (where biceps MEP is 2.5 larger than triceps MEP), and the average linearity of the voluntary moment and SIT moment. * indicate statistically different values ($p < 0.05$).

NONIMPAIRED				
STIMULATION PULSE	**Todd et al. criteria (% met)**	**MEP ratio ≥ 2.5 (% met)**	**Mean linearity**	**Total # of trials**
SINGLE PULSE	34.1	60.2	0.87	540
PAIRED 1.5 MS ISI	42.8	68.9	0.78	180
PAIRED 10 MS ISI	41.9	69.7	0.81	198
PAIRED 30 MS ISI	39.4	61.7	0.81	180
MEAN	**39.5**	**65.1**	**0.83***	-
SCI				
SINGLE PULSE	14.4	52.8	0.81	432
PAIRED 1.5 MS ISI	20.0	68.9	0.65	180
PAIRED 10 MS ISI	27.8	60.4	0.74	144
PAIRED 30 MS ISI	15.3	34.0	0.71	144
MEAN	**19.4**	**54.0**	**0.73***	-

3.4 Discussion

We used facilitatory (ICF) and inhibitory (SICI) paired pulse TMS techniques to modify biceps MEPs, triceps MEPs, and the biceps/triceps MEP ratio across voluntary effort levels in individuals with SCI and nonimpaired individuals. We also evaluated the relationship between the biceps/triceps MEP ratio and VA_{TMS}. We hypothesized that ICF would increase the biceps/triceps MEP ratio across all effort levels relative to single pulse while SICI would decrease the MEP ratio. Our first hypothesis was not supported in either participant group (nonimpaired

and SCI). Further, we hypothesized that the biceps/triceps MEP ratio would affect VA_{TMS}. This hypothesis was also not supported. The biceps/triceps MEP ratio thus may not indicate the amount of cortical stimulation to the biceps relative to the triceps, and contribute to greater excitation of the biceps and larger superimposed elbow moment twitches during voluntary contraction. In the SCI group, VA_{TMS} was found to be sensitive to the linearity of the superimposed twitch moment and voluntary moment relation. Linearity was also lower in the SCI group compared to the nonimpaired group, which may pose a methodological limitation in the estimation of VA_{TMS} in tetraplegic patients. Further research is needed to determine whether VA_{TMS} is a viable assessment of neuromuscular function in individuals with tetraplegia.

While the target/antagonist MEP ratio may be an indication of cortical stimulation focality when muscles are at rest or low levels of activation, this relationship may not hold at higher levels of effort during stimulation. Increasing the level of muscle contraction leads to a greater proportion of large spinal motoneurons activated by TMS, which increases their sensitivity to changes in corticospinal excitability [189], [190]. However, when the biceps are highly activated (75-100% MVC), the triceps are not at rest, but experiencing low levels of activation (i.e. increased EMG activity compared to baseline as seen in Figure 3-2). The biceps during these high efforts, being at a near tetanic state, are not at optimal capacity to elicit a MEP response. Conversely, the triceps, being in a lower activation state, not only have an increased capacity to respond (i.e. are further from a tetanic state) but are in a state of higher susceptibility compared to rest (e.g. active motor thresholds are lower than RMT) [165], [170], [186]. Consequently, the Todd et al. (2016) criteria (i.e., biceps MEP ≥ 50% Mmax and triceps MEP ≤ 20% Mmax) were seldom met (Table 3-2, nonimpaired: 39.9% of trials, SCI: 19.4% trials). We proposed an adjusted condition (MEP ratio ≥ 2.5), more reflective of the relative responsiveness between both muscles to account for the triceps being in a higher susceptibility state during VA_{TMS} trials. This condition was met more often in both groups (nonimpaired: 65.1% of trials, SCI: 54% trials), especially in 1.5 ms ISI trials where both groups met the condition more than two-thirds of the time (see Table 3-2). However, neither paired pulse stimulation nor the MEP ratio affected the estimation of VA_{TMS}. Therefore, while the MEP ratio may be physiologically relevant at rest and efforts up to

about 20% MVC, its utility and importance are reduced at higher effort levels in which the target and antagonist muscles are disproportionately activated.

MEP ratio modulation did not occur across all effort levels in the nonimpaired group due to inconsistent SICI and ICF effects across effort levels, and MEP facilitation that occurred simultaneously in the biceps and the triceps. In the nonimpaired group, 30 ms ISI (ICF) increased both biceps and triceps MEPs across all effort levels compared to single pulse. Since MEP facilitation occurred simultaneously in the biceps and triceps, the biceps/triceps MEP ratio remained unchanged relative to single pulse. The only condition in which the MEP ratio was modulated in the nonimpaired group was at 75% MVC with an ISI of 1.5 ms in which SICI caused triceps inhibition. Unexpectedly, 10 ms ISI resulted in biceps MEP inhibition although such an ISI can elicit MEP facilitation [60], [191]. However, overall our results are largely consistent with previous reports where MEP responses were inhibited at 1-5ms ISIs and facilitated at higher ISIs [180], [187]. MEP inhibition via SICI is related to the activity of GABAergic receptors known to regulate the production of I-waves, especially later I-waves that occur when higher stimulator intensities are used [181], [192]. MEP facilitation via ICF is thought to be regulated by the activity of intracortical glutamatergic excitatory circuits [60], [193]. Previous research suggests that low amounts of voluntary activation (20% MVC) of the target muscle decrease SICI (i.e. less inhibition compared to rest) and ICF [182]. Our protocol involved high effort levels (50-100% MVC) where we observed both SICI and ICF.

MEP ratio modulation also did not occur across all effort levels in the SCI group due to inconsistent SICI and ICF effects across effort levels. Injury-induced reorganization of the corticospinal pathways after SCI affects inhibitory and facilitatory circuits and can lead to unpredictable paired pulse TMS outcomes [23], [174], [175], [194]. Unexpectedly, in our SCI group, the biceps/triceps MEP ratio was decreased in the 30 ms ISI condition compared to the single pulse protocol at 50% and 75% MVC; this occurred mostly via biceps MEP inhibition (-180% Mmax across all effort levels). Further, the MEP ratio was increased in the 10 ms ISI condition, which is the only condition that did not elicit significant biceps MEP inhibition compared to single

pulse. Following SCI, death of motoneurons and changes in the properties of remaining motoneurons will affect their behavior during voluntary efforts [195], [196]. Specifically, the excitability of motoneurons increases during voluntary contractions to a lesser extent than in nonimpaired [194]. SICI can be elicited in individuals with SCI during voluntary efforts but MEP inhibition is reduced compared to nonimpaired controls [197]. In our study, SICI occurred at 1.5 ms ISI in the biceps but also in the triceps, at high effort levels leading to an unchanged MEP ratio. As previously described, during MVC, the biceps is closer to its maximal firing rate and thus less sensitive to stimulation whereas the triceps is in a more receptive state. In such context, SICI appears to decrease triceps MEPs preferentially. This asymmetric response may also be influenced by increased reticulospinal inputs to the biceps and decreased corticospinal inputs to the triceps following SCI [30], [198].

In the SCI group, we observed abnormal MEP inhibition in paired pulse trials at 30 ms ISI that elicited facilitation in nonimpaired participants. While there is evidence to suggest that the excitability of inhibitory circuits mediated by the activity of GABA-A receptors is reduced after SCI as a compensatory mechanism [199]–[201] [174], [197], [200], [201], the effects of ICF neurophysiology following SCI have not been well documented. One possible interpretation is that 30 ms ISI caused long interval intracortical inhibition (LICI) in our SCI cohort instead of ICF. LICI is mediated by the activity of GABA-B receptors [202] and is typically elicited at ≥ 50 ms ISIs and with suprathreshold conditioning pulse [181]. However, animal studies have shown that the expression of GABA-B receptor is altered following SCI [203]. Further, five of our SCI participants were chronic users of baclofen (GABA-B agonist) which may also have contributed to the atypical occurrence of LICI (Table 2-1). Mechanisms below the cortical level may be involved as well. After SCI, the presence of axonal dysfunction of the descending corticospinal tract and peripheral motor axon dysfunction indicates that both central and peripheral pathways can contribute to aberrant modulation of MEPs [204], [205]. Finally, individuals with chronic SCI are more sensitive to neuromuscular fatigue [32]. As intracortical facilitatory and inhibitory circuits become less excitable with neuromuscular fatigue [187], [206], the effects of LICI, SICI, and ICF are reduced and/or become less predictable.

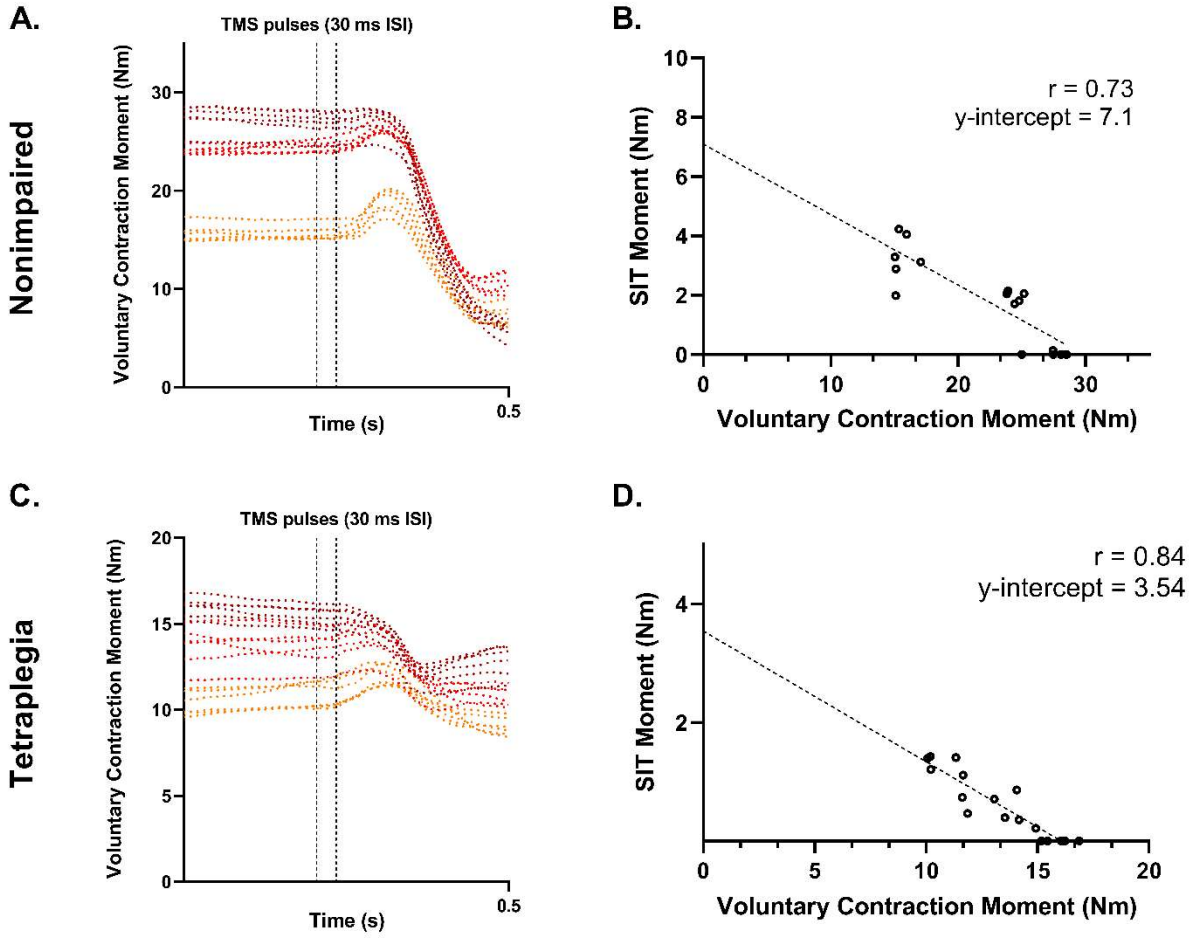
Linearity of the superimposed twitch moment and voluntary moment relation was lower in the SCI group compared to the nonimpaired group (0.73 vs 0.83, see Table 3-2), which reveals a methodological issue that may limit the interpretation of VA_{TMS} in populations with neuromuscular impairments. However, we were able to quantify VA_{TMS} in nonimpaired individuals and individuals with SCI using single pulse and paired pulse stimulation. Similar to data reported by Todd et al. (2003) (VA_{TMS} = 93.6 ± 5.6%), single pulse VA_{TMS} was in the 90-95% range on average, in both groups (nonimpaired: 91.1%, SCI: 94.3%). Paired pulse stimulation did not affect the estimation of VA_{TMS} compared to single pulse (Figure 3-3). Lower linearity, on the other hand, decreased estimation on VA_{TMS} in the SCI group. This is consistent with previous report where higher fatigue in the nonimpaired biceps was associated with underestimation of VA_{TMS} [130]. Todd et al. (2016) recommend a linear relationship ($r \geq 0.9$) for effort levels of 50-100% MVC to extrapolate the resting twitch moment and properly calculate VA_{TMS}. Thus, interpretation of VA_{TMS} may be especially difficult in a context that affects linearity, such as fatigue.

There are limitations in the current study. The sample size is small and there was a wide range of biceps function among the SCI participants. Some participants presented more overall remaining biceps motor function as indicated by greater maximum elbow flexor moments. Yet, VA_{TMS} measures across SCI participants and groups were in the same range (see Figure 3-3) which suggests that VA_{TMS} alone did not detect differences in motor impairments. While the number of motor units and their maximal firing rates may decrease in the biceps after SCI resulting in lower force-generating capacity [32], the relative amount of innervated muscle fibers able to receive descending voluntary neural drive may be unchanged. However, in a context where corticomotor transmission and excitability are affected such as in tetraplegia, TMS capacity to elicit moment twitches may be reduced, potentially resulting in overestimation of VA_{TMS}. Thus, interpretation of this outcome is difficult. Another limitation is that we did not exclude data based on linearity in the SCI group since linearity was already lower than in nonimpaired. However, we did not perform comparisons between groups other than linearity, which was done before data exclusion. Although we designed our experiment to have more rest, more often (90 s rest between each trial), between voluntary effort trials compared to previous work [130], [160],

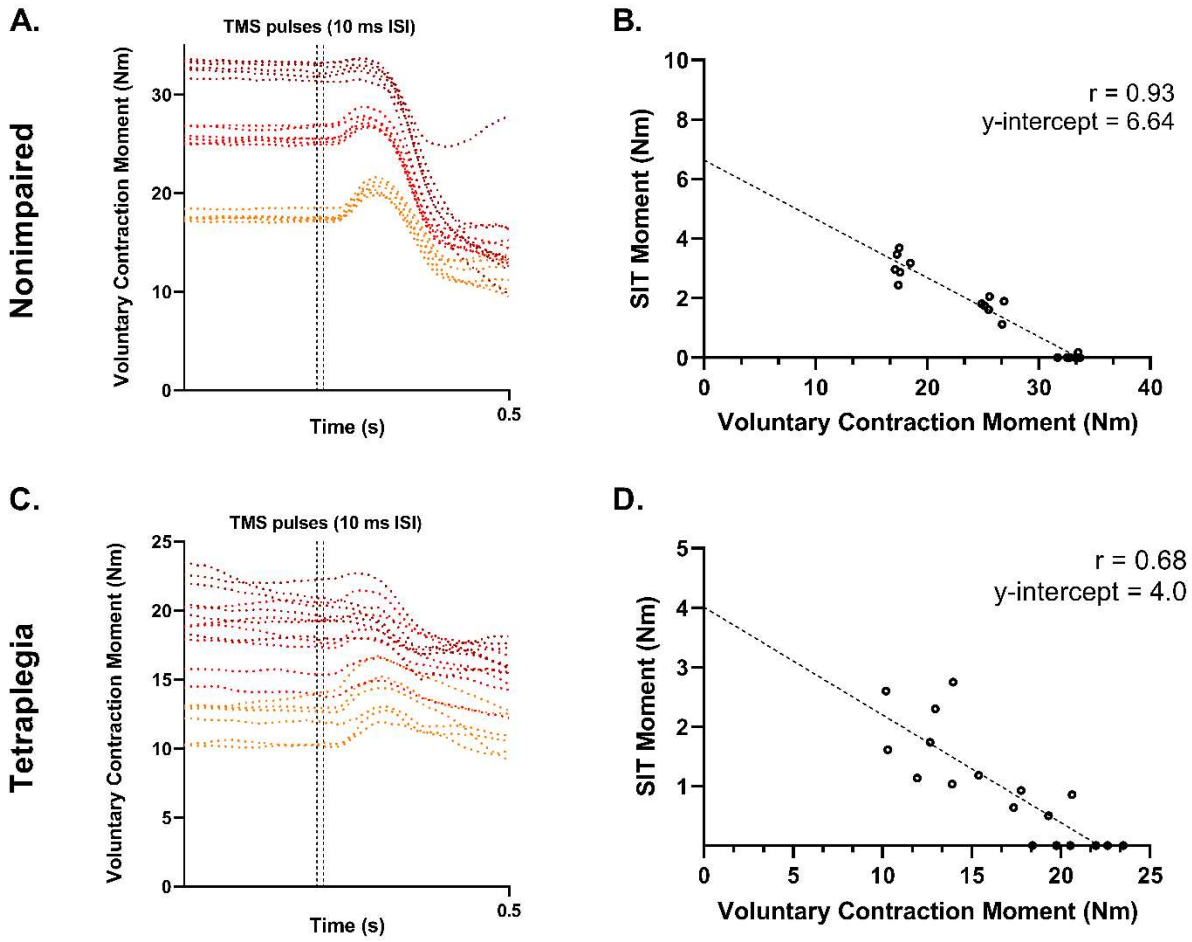
fatigue and attention may have affected our results, especially in SCI participants as previously discussed. Participants were not age-matched, however, no direct comparisons were made between groups when analyzing MEPs and VA_{TMS} and there is evidence to suggest that age does not influence outcomes of SICI and ICF [207].

As a novel contribution, we collected VA_{TMS} measures using paired pulse TMS in nonimpaired and individuals with tetraplegia although methodological issues remain that may limit its clinical application in monitoring neuromuscular function. Paired pulse TMS did not modulate the biceps/triceps MEP ratio across the full range of voluntary efforts in nonimpaired and SCI participants and did not affect the estimation of VA_{TMS}. Thus, a focus on increasing the biceps/triceps MEP ratio via paired pulse stimulation does not improve the estimation of VA_{TMS}. In participants with tetraplegia, paired pulse stimulation outcomes revealed different patterns of intracortical inhibition relative to nonimpaired participants that may be due to injury-induced corticospinal reorganization and alterations in the activity of GABA-B receptors following SCI. More comprehensive paired pulse TMS experiments (particularly facilitatory protocols) are needed to further our understanding of neuroplastic changes and functional reorganization after SCI. Finally, VA_{TMS} was sensitive to changes in the linearity of the voluntary moment and superimposed twitch moment relation in SCI participants. Linearity was also lower compared to nonimpaired, which constitute an additional challenge in the estimation of VA_{TMS}. Further research is needed to determine whether VA_{TMS} is a viable assessment of neuromuscular function in individuals with tetraplegia.

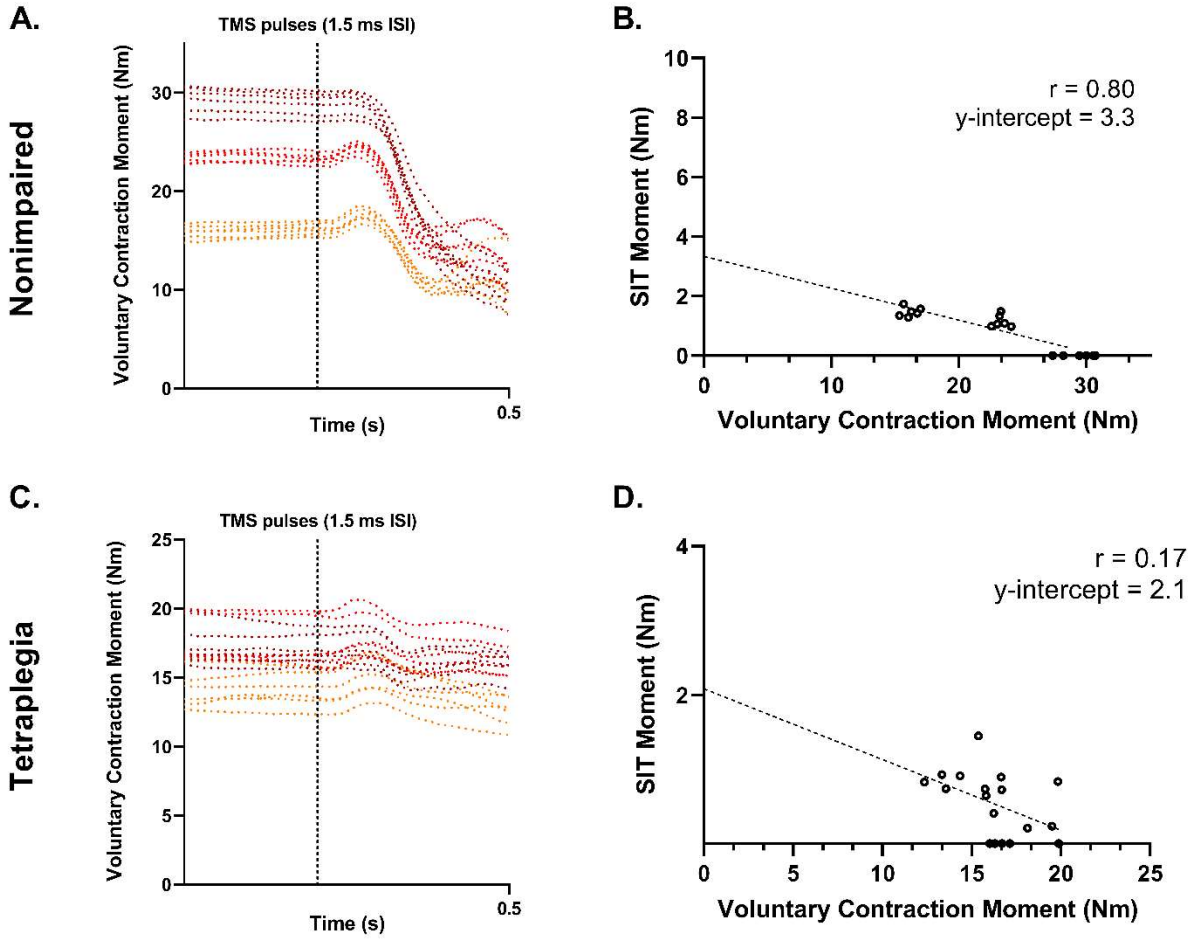
3.5 Appendix II



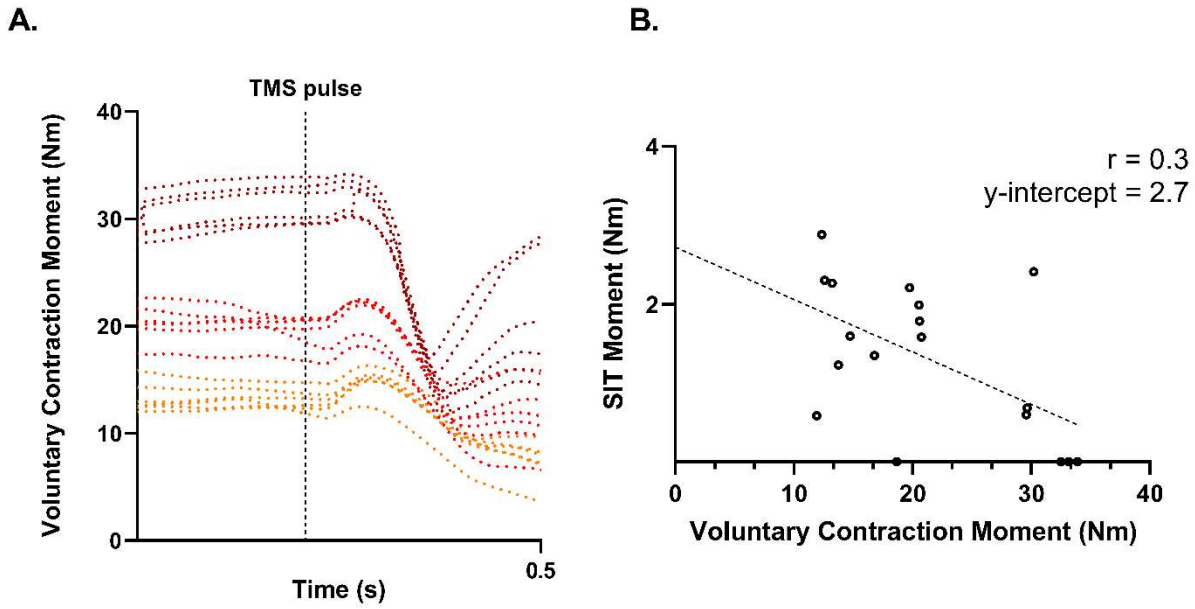
Supplementary Figure 3-6: VA_{TMS} collected with paired pulse TMS at 30 ms ISI example data. **A.** Moment traces collected during a VA_{TMS} block in representative nonimpaired participant. **B.** Linear regression between SIT and voluntary contraction moment obtained from the same VA_{TMS} block. **C.** Moment traces collected during a VA_{TMS} block in representative participant with tetraplegia. **D.** Linear regression between SIT and voluntary contraction moment obtained from the same VA_{TMS} block. Orange, red, and dark red dotted lines represent 50, 75, and 100% MVC trials, respectively.



Supplementary Figure 3-7: VA_{TMS} collected with paired pulse TMS at 10 ms ISI example data. **A.** Moment traces collected during a VA_{TMS} block in representative nonimpaired participant. **B.** Linear regression between SIT and voluntary contraction moment obtained from the same VA_{TMS} block. **C.** Moment traces collected during a VA_{TMS} block in representative participant with tetraplegia. **D.** Linear regression between SIT and voluntary contraction moment obtained from the same VA_{TMS} block. Orange, red, and dark red dotted lines represent 50, 75, and 100% MVC trials, respectively.



Supplementary Figure 3-8: VA_{TMS} collected with paired pulse TMS at 1.5 ms ISI example data. **A.** Moment traces collected during a VA_{TMS} block in representative nonimpaired participant. **B.** Linear regression between SIT and voluntary contraction moment obtained from the same VA_{TMS} block. **C.** Moment traces collected during a VA_{TMS} block in representative participant with tetraplegia. **D.** Linear regression between SIT and voluntary contraction moment obtained from the same VA_{TMS} block. Orange, red, and dark red dotted lines represent 50, 75, and 100% MVC trials, respectively.



Supplementary Figure 3-9: Example of a single pulse VA_{TMS} block with low linearity collected in participant with tetraplegia. **A.** Moment traces where orange, red, and dark red dotted lines represent 50, 75, and 100% MVC trials, respectively. **B.** Linear regression between SIT and voluntary contraction moment obtained from the same $VA_{TMS\ TMS}$ block.

4 Chapter 4: Investigate Neuroplasticity via Motor Evoked Potentials Input-Output Curves

4.1 Background/Objectives

Motor evoked potential (MEP) input-output curves, otherwise referred to as stimulus-response or recruitment curves, can evaluate corticomotor excitability in humans [66], [208]. Input-output curves are collected using transcranial magnetic stimulation (TMS): a noninvasive brain stimulation technique that uses magnetic induction to stimulate cortical neurons. By targeting the motor cortex, TMS can induce muscle responses and the associated MEP that can be measured with electromyographic (EMG) sensors. The input-output curve can be evaluated by plotting the MEP responses across a range of increasing stimulator intensities [66]. The slope of the curve indicates the excitability of the targeted neuromuscular circuit across multiple neuronal populations, such as direct corticospinal projections and inter-cortical neurons [67], [68]. MEP input-output curves can reflect motor function and performance [209], [210]. For example, in a motor skill training study, increased MEP input-output curve slopes in the ipsilateral hand of subjects corresponded to improved performance of the non-trained hand, and was attributed to increased corticomotor excitability of the ipsilateral circuit [210]. Pathological conditions have also been shown to influence MEP input-output curve parameters [117], [211]. For example, input-output curves collected in the first dorsal interosseous (FDI) muscle was increased in individuals with chronic spinal cord injury (SCI) compared to nonimpaired controls, suggesting injury-induced increased corticomotor excitability of the FDI neuromuscular circuit [117]. Since MEP input-output curves can predict motor function improvement [210], they have the potential to be used as a monitoring tool during rehabilitation programs aimed at recovering motor function, which may be particularly useful in SCI populations.

Other TMS-based techniques have been employed to study neuromuscular function in patient populations but they have limitations in their ability to comprehensively capture neuroplasticity. For example, studies involving sub-threshold TMS have shown a reduced activity of intracortical inhibitory circuits in tetraplegia, likely as a compensatory mechanism enabling

cortical reorganization [63]–[65]. Similarly, motor mapping studies using TMS have reported an increased representational surface area in the motor cortex for muscles rostral to the spinal lesion site [3], [24]. Unlike input-output curves, motor mapping studies and sub-threshold TMS do not use a range of stimulator intensities during TMS trials, thus neglecting the activity of later I-waves that preferentially occur at high stimulator intensities [62]. Late I-waves are important in their role as part of the descending volley and neuromodulation-related excitation [212]. Furthermore, compared to a single MEP response, which is highly variable and state-dependent, the slope is extracted from multiple MEPs elicited over several minutes and at various stimulation intensities, reflecting a more comprehensive and non-instantaneous measure of corticomotor excitability.

The optimal method to collect and analyze MEP input-output curves in populations with neuromuscular impairments, particularly tetraplegia, remains non-standardized. In nonimpaired participants, MEP input-output curves with a sigmoid curve fit method can yield reproducible outcomes when targeting the FDI muscle [208]. Yet, in chronic post-stroke individuals, analyzing MEP input-output curves with linear regression was found to be as predictive (goodness of fit) as using a sigmoid function model [213]. It is important to note that most of these studies have focused on upper limb distal muscles such as those of the hand. However, TMS outcomes are largely dependent on the stimulation target [118]. While more proximal upper limb muscles such as the biceps brachii often retain more function than distal muscles in patients with cervical SCI [88], [119], their neuromuscular circuits may be affected in different ways. In contrast to the FDI [3], [24], a recent TMS motor mapping study found no differences in biceps brachii cortical representation between chronic cervical SCI and nonimpaired participants [120]. However, the biceps brachii is a difficult TMS target due to its relatively smaller motor cortex representations compared to hand muscles [24], [117], and is less likely to provide repeatable MEP measures across sessions [121]. Therefore, further investigation of proximal muscle groups such as the biceps brachii is needed to get a more comprehensive picture of neuromuscular function after SCI and help inform neurorehabilitation protocols.

Our primary aim was to collect and compare MEP input-output curves of the biceps brachii from nonimpaired participants and participants with tetraplegia (injuries from C5-C6 region) to investigate differences in corticomotor excitability between groups and evaluate injury-induced alterations of the biceps brachii neuromuscular circuits. We hypothesized that the MEP input-output curves would be greater in participants with tetraplegia compared to nonimpaired. A secondary aim was to compare curve-fitting methods such as linear regression and sigmoid function and to investigate the repeatability across sessions of input-output curves collected with TMS. We hypothesized that sigmoidal curve-fitting would result in higher accuracy of prediction compared to linear regression and slopes would be repeatable across days. The repeatability of MEP input-output curves of the biceps brachii in individuals with incomplete tetraplegia is currently not well characterized.

4.2 Methods:

4.2.1 Participants:

Ten nonimpaired individuals (four females, six males, aged 22.7 ± 2.5) and ten individuals with chronic SCI (three females, seven males, aged 39.9 ± 10.6) were screened and recruited to participate in the study. Inclusion criteria were C5 to C6 level of cervical spinal injury, at least a year post-injury. Exclusion criteria included any contraindication to receiving TMS and the inability to generate a visible contraction of the biceps. Participants with SCI characteristics are shown in Table 4-1.

Table 4-1: Ten individuals with tetraplegia following cervical SCI were recruited to participate in the study.

Participant #	Sex	Age	Injury Level	ISNCSCI	Years since SCI	Cause of SCI	Medications
1	F	52	C6	A	15	MVA	BAC
2	F	53	C6	D	7	Spinal Stenosis	BAC
3	M	42	C5	A	12	MVA	BAC
4	M	45	C6	D	5	Transverse Myelitis	None
5	F	54	C6	A	13	MVA	BAC
6	M	34	C5	A	16	MVA	BAC, OX
7	M	26	C6	A	6	Fall	None
8	M	33	C5	D	3	MVA	None
9	M	32	C5	B	9	Fall	None
10	M	28	C5	B	4	MVA	BAC

*SCI: Spinal Cord Injury, ISNCSCI: International Standards for Neurological Classification of Spinal Cord Injury, MVA: Motor vehicle accident, BAC: Baclofen, OX: Oxybutynin.

4.2.2 Experiment Overview:

Each session consisted of preliminary TMS assessments to determine resting motor thresholds (RMT) and biceps motor hotspot prior to collecting a MEP input-output curve of the dominant biceps using computerized TMS control. Participants were seated in an upright position and had their dominant forearms immobilized in a custom brace, supinated, and positioned at 90 degrees of elbow flexion. Before the experiment, participants provided informed consent and completed a screening for TMS usage. The Institutional Review Board of Virginia Commonwealth University approved the study.

4.2.3 Materials:

EMG data were recorded using Wireless EMG sensors (Delsys Trigno, Natick, Massachusetts) positioned on the muscle belly of the biceps brachii. EMG data were band-pass filtered to 20-450 Hz and sampled at 2000 Hz. All data were recorded in Spike 2 software and a

data acquisition system (CED 1401, Cambridge CED). Custom scripts were written to control the TMS device and automatize the MEP input-output curve protocol.

4.2.4 Compound Motor Unit Action Potential Recording:

Electrical stimuli were delivered using a constant current stimulator (Digitimer DS7AH, Fort Lauderdale, Florida) at 200 V with a 200 μs pulse width. The current delivered ranged from 5 mA (threshold of detection) to 150 mA (procedural maximum). Rectangular 3.3 x 5.3 cm neurostimulation electrodes (Axelgaard 891200, Fallbrook, California) were placed at Erb's point (cathode) and the acromion (anode). M-wave recruitment curves were obtained individually for the biceps starting from zero at intervals of 10 mA until a plateau in the M-wave amplitude was reached. Five supramaximal stimuli of 120% of the threshold current were delivered to obtain the Mmax for the biceps at rest. M-max was collected to normalize MEPs to account for changes within the muscle, such as muscle fibers length, and sensor positioning [214].

4.2.5 TMS procedures:

TMS was delivered using a 126 mm diameter double cone coil connected to a monophasic stimulator (Magstim BiStim^2, Whitland, United Kingdom). Motor mapping was performed in each session to obtain the location that evoked the largest peak-to-peak MEP in the biceps at the lowest stimulation intensity, the motor hotspot [215]. This location was then marked on a silicone or plastic cap secured to the participant's head; subsequent stimuli were delivered at that location. Resting motor threshold (RMT) was determined as the lowest stimulus intensity able to induce biceps MEPs ≥ 50 μV in at least 5 out of 10 stimuli and expressed at a percentage of the TMS stimulator maximal output (%MSO) [135]. Within each MEP input-output curve session, TMS was administered 48 times, using computerized control, at a range of stimulus intensities from 80%-160% of participants' respective RMT in random order, featuring 5 s inter-stimulus intervals.

4.2.6 Data and Statistical Analysis:

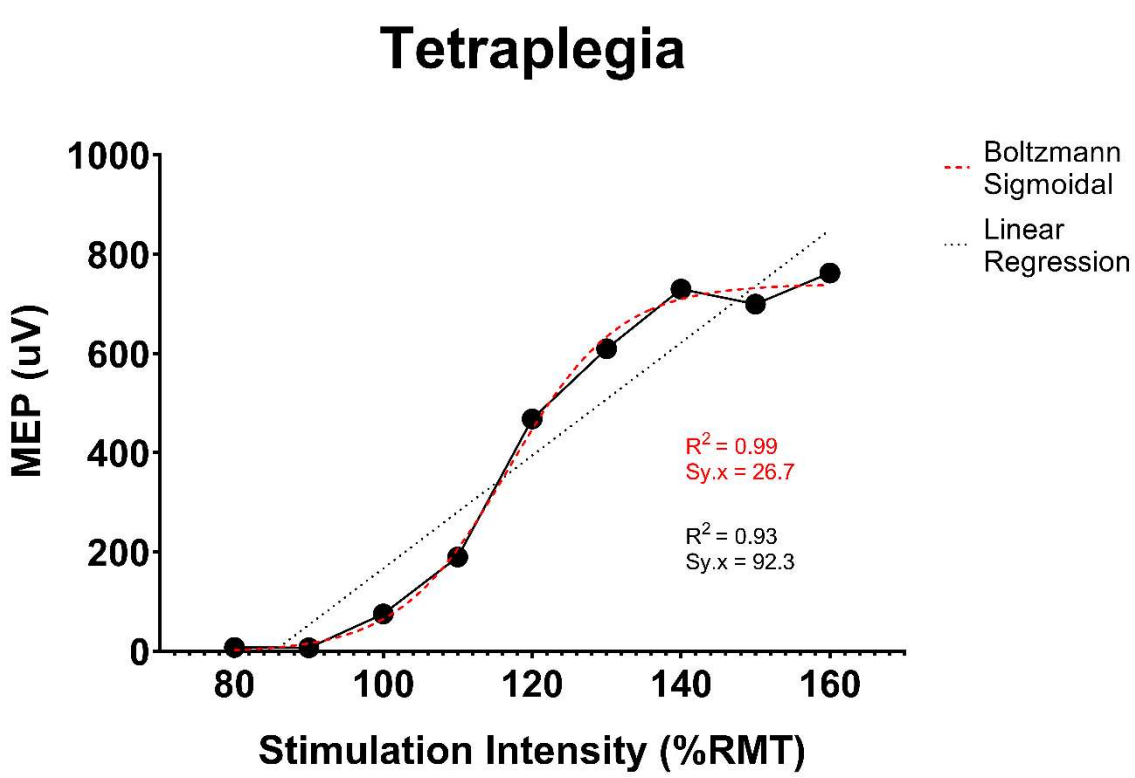
MEP data for each participant per session were analyzed in Matlab using custom-written scripts. To limit the effect of MEP variability, outliers (values beyond three standard deviations from the mean) of each stimulus intensity were excluded from subsequent analyses [216]. Using this process, 15 out of 1456 MEPs were excluded in the nonimpaired group and 10 out of 1144 MEPs were excluded in the SCI group. MEP amplitudes were plotted as a function of stimulus intensity to produce the input-output curve for each session and participant (see Figure 4-1). The input-output curves were analyzed using simple linear regression and Boltzmann sigmoidal in order to extract parameters such as the slope and coefficient of determination (R^2). The Boltzmann function is defined as follows (1):

$$Y = MEP_{min} + \frac{(MEP_{max} - MEP_{min})}{1 + e^{\frac{(V50 - X)}{Slope}}} \quad (1)$$

The variables MEP_{min} and MEP_{max} represent the minimal and maximal MEP size, respectively. V50 represents the stimulus intensity (%MSO) value when the MEP size is half of its maximal size. Statistical analyses were completed in R (R Core Team (2013)) and Prism (GraphPad Software, LLC). Slopes and coefficients of determination (R^2) obtained from the input-output curves of SCI and nonimpaired participants were compared using unpaired t-tests with Welch's correction to account for unequal standard deviations between the SCI and nonimpaired groups. The null hypothesis was that the average slope is not different between the SCI and NI groups. The alternative hypothesis was that the SCI group has higher slopes on average. Inter-session repeatability was determined by calculating intraclass correlation coefficients (ICCs) of the slope parameter [138]. ICCs were calculated using a two-way random model with consistency agreement. ICC values > 0.75 were defined to represent good to excellent agreement between sessions [143].

4.3 Results

A.


Tetraplegia
1000
800
600
400
200
0
MEP (uV)
80
100
120
140
160
Stimulation Intensity (%RMT)
Boltzmann Sigmoidal
Linear Regression
R2 = 0.99
Sy.x = 26.7
R2 = 0.93
Sy.x = 92.3

B.

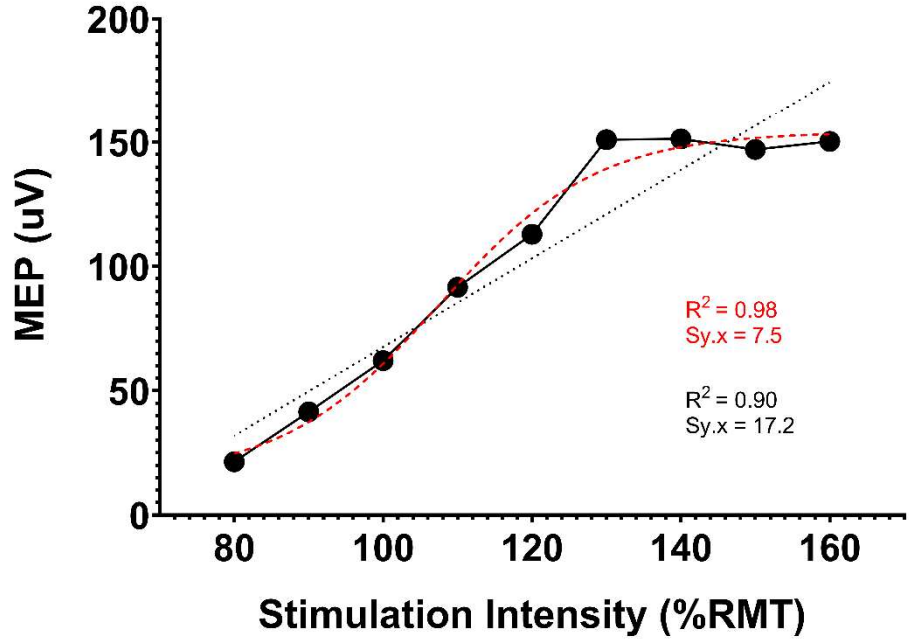

Nonimpaired
200
150
100
50
0
MEP (uV)
80
100
120
140
160
Stimulation Intensity (%RMT)
R2 = 0.98
Sy.x = 7.5
R2 = 0.90
Sy.x = 17.2

Figure 4-1: A. Example of representative individual MEP input-output curve collected in a nonimpaired participant. **B.** Example of representative individual MEP input-output curve collected in a SCI participant. Both curve fitting methods are superimposed onto the discrete MEP data. R^2 is the coefficient of determination that represents accuracy of prediction and Sy.x represents the standard error of estimate.

Input-output slopes computed using linear regression in the SCI group (6.44 ± 6.1) were higher than the slopes computed in the nonimpaired group (1.34 ± 1.32) ($p < 0.001$, $t_{22.5} = 3.8$) (Figure 2, A). No significant differences in RMTs were found between the SCI and nonimpaired group ($p = 0.06$, $t_{48.9} = 1.86$) (Figure 2, B).

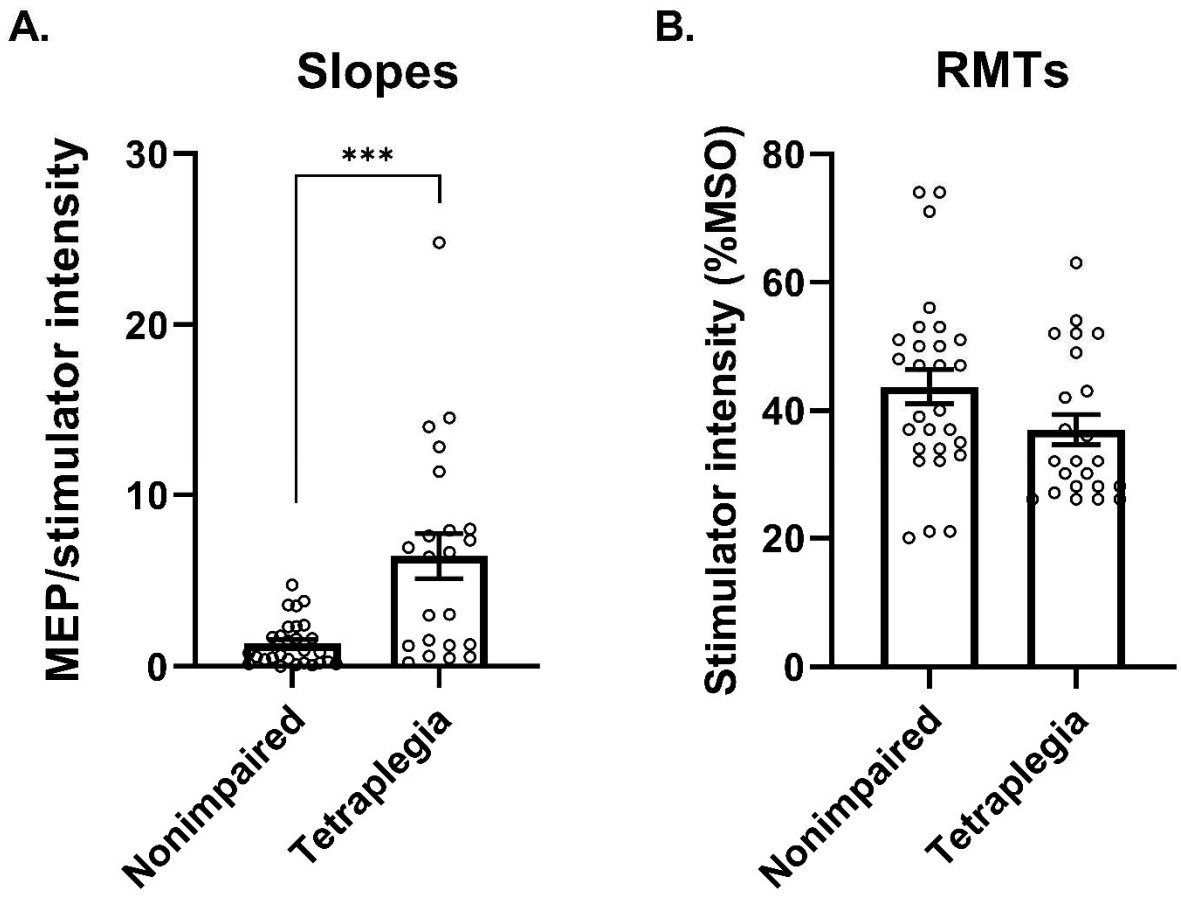


Figure 4-2: A. Slopes obtained from linear regression were significantly greater in the SCI group compared to nonimpaired. **B.** Mean RMTs were not different between groups. Asterisks represent statistical difference ($p < 0.001$) and error bars represent the standard error of the mean (SEM).

Linear regression to fit the MEP input-output curves had higher accuracy of prediction in the SCI group ($R^2 = 0.85$) relative to the nonimpaired group ($R^2 = 0.59$) ($t_{95} = 4.69$, $p < 0.0001$) (Figure 4-3). The Boltzmann sigmoidal function also had higher accuracy of prediction in the SCI group ($R^2 = 0.94$) relative to the nonimpaired group ($R^2 = 0.73$) ($t_{95} = 3.83$, $p < 0.001$) (Figure 4-3). In the SCI group, no differences between curve-fitting methods were found. In the nonimpaired group, accuracy of prediction was greater using the Boltzmann sigmoidal function compared to simple linear regression ($t_{95} = 2.77$, $p < 0.05$). All input-output curves are represented in Figure 4-4 with both curve-fitting methods. Repeatability of the slope extracted from simple linear regression was good in both groups (nonimpaired: ICC(3,k) = 0.83, $p < 0.05$, SCI: ICC(3,k) = 0.78, $p < 0.05$). Average input-output curves per session are represented in Figure 4-5. Comparison of curve-fitting methods and repeatability outcomes are summarized in Table 4-2.

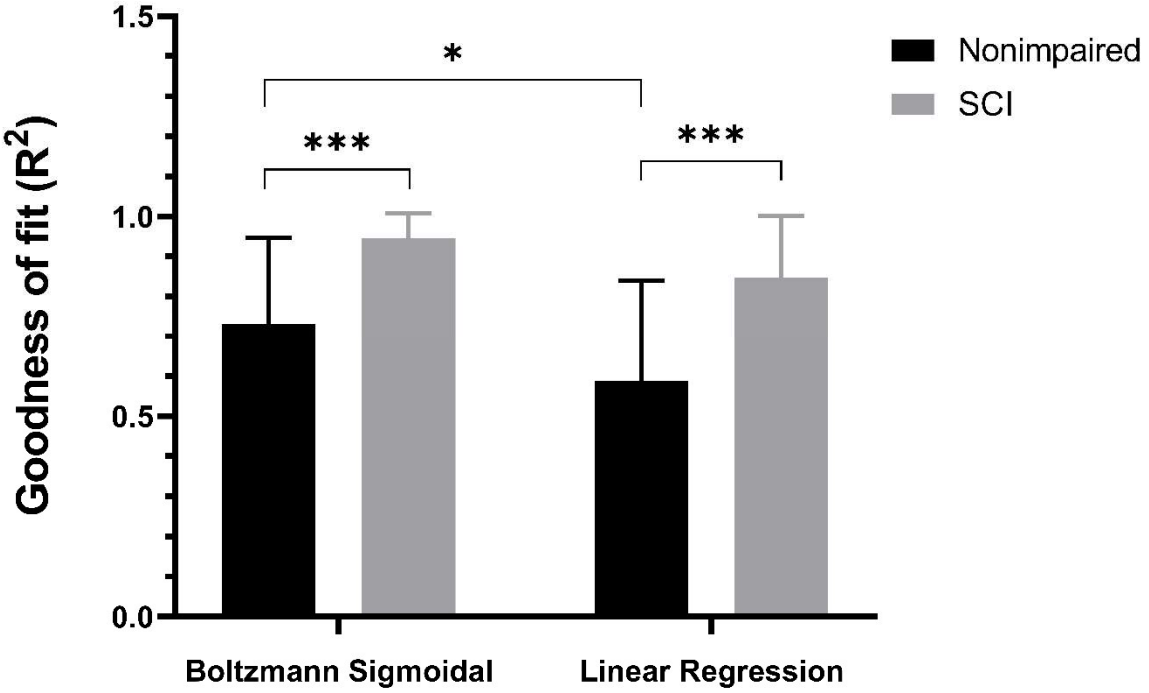


Figure 4-3: Goodness of fit (R^2) representing the accuracy of prediction of each curve-fitting method across groups. Asterisks represent statistical difference (*: $p < 0.05$, ***: $p < 0.001$) and error bars represent the standard error of the mean (SEM).

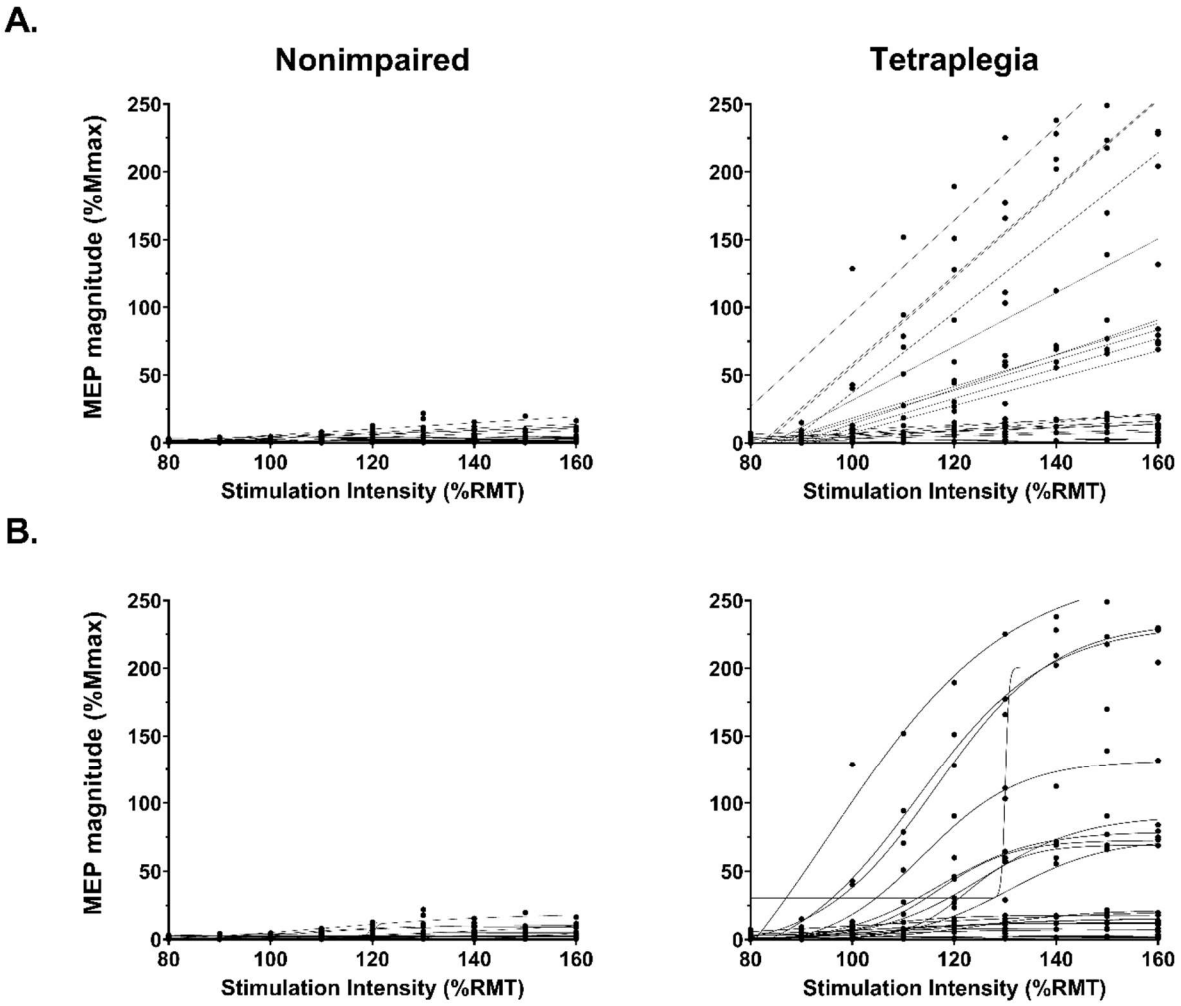


Figure 4-4: A. Simple linear regression was used to fit the MEP data in both groups. **B.** The Boltzmann sigmoidal function was used to fit the MEP data in both groups. Each solid line represents an individual input-output curve for a participant in a given session. Each point represents the mean value of the 5 MEPs collected at a specific TMS intensity for a given individual input-output curve.

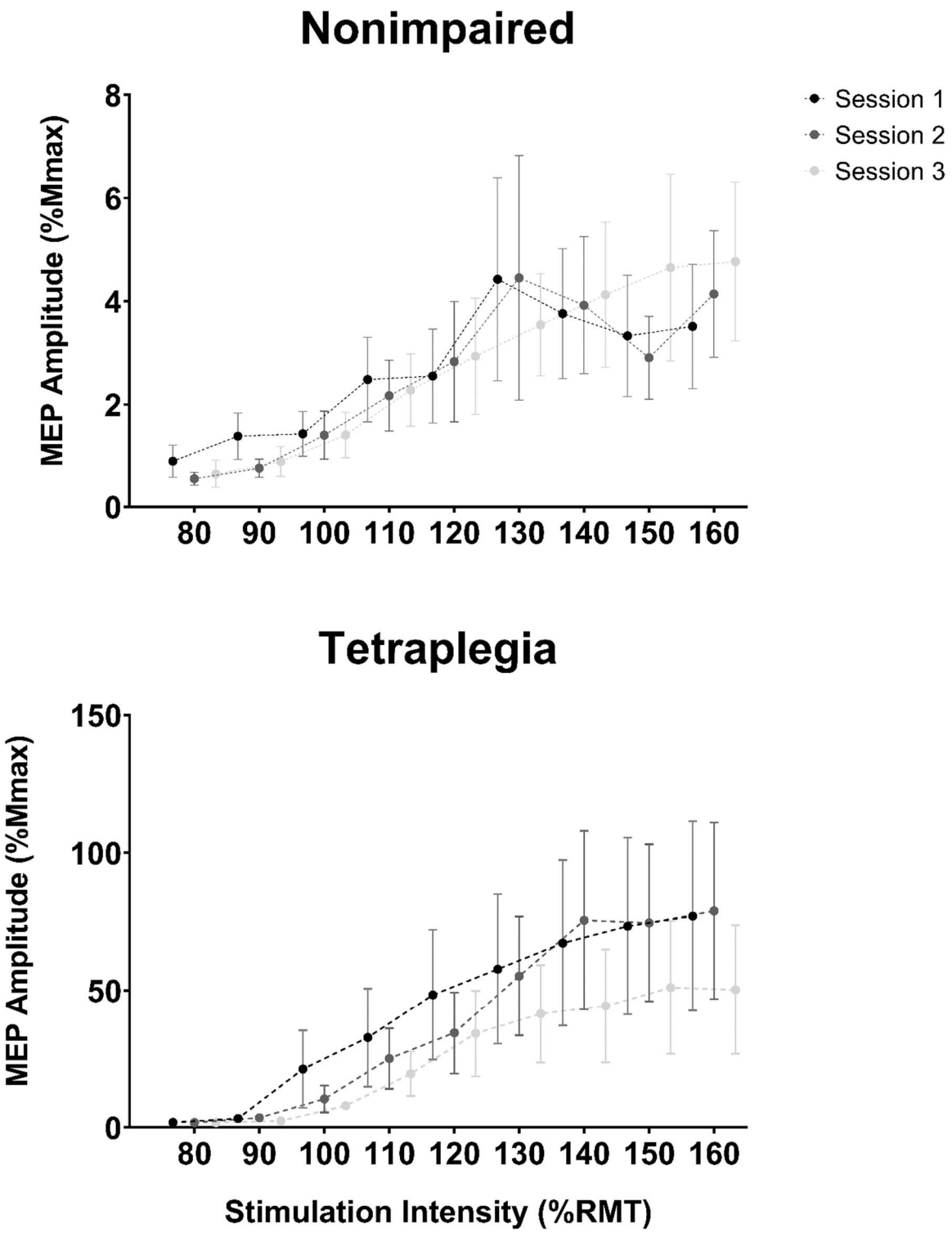

Nonimpaired
MEP Amplitude (%Mmax)
0
2
4
6
8
80
90
100
110
120
130
140
150
160
Session 1
Session 2
Session 3
Tetraplegia
MEP Amplitude (%Mmax)
0
50
100
150
80
90
100
110
120
130
140
150
160
Stimulation Intensity (%RMT)

Figure 4-5: Mean MEP input-output curves represented across three sessions in both groups. MEPs were normalized to Mmax and averaged across participants at each stimulation intensity. Error bars represent the standard error of the mean (SEM).

Table 4-2: Inter-session repeatability and accuracy of prediction of curve fitting methods (mean ± SEM).

Parameters	Nonimpaired	Tetraplegia
Slope ICC (3, k)	0.83	0.78
Sigmoid fit mean R^2	0.73 ± 0.04	0.95 ± 0.05
Linear fit mean R^2	0.71 ± 0.04	0.82 ± 0.05

4.4 Discussion

The primary aim of this study was to collect and compare MEP input-output curves of the biceps brachii from nonimpaired participants and chronic cervical SCI participants. A secondary aim was to investigate curve-fitting methods (linear regression versus Boltzmann sigmoidal function) and their repeatability across days. We first hypothesized that the slopes of input-output curves would be greater in the SCI group compared to nonimpaired. Our first hypothesis was supported: MEP input-output curves of the biceps analyzed with linear regression revealed significantly higher slopes in the chronic SCI group compared to the nonimpaired group. Second, we hypothesized that sigmoidal curve-fitting would result in higher accuracy of prediction compared to linear regression and slopes would be repeatable across days. This was not supported in the SCI group: we found that while using the sigmoid function models the shape of the curve more precisely compared to the linear regression approach, no differences in accuracy of prediction (R^2) were found in the SCI group. Thus, the linear regression approach was found to accurately represent the slope of the modeled data. No significant differences in RMTs were found between groups. Finally, the slopes extracted from the MEP input-output curves were found to be repeatable across three days.

Increased MEP input-output curve slopes of the biceps brachii in the tetraplegia group compared to the nonimpaired group suggests injury-induced corticomotor reorganization following SCI that affects relatively less impaired proximal muscles. This finding is consistent with the outcomes of previous studies focusing on corticospinal tracts innervating more distal muscles [24], [117], [193]. However, it contradicts a previous report in the biceps brachii where no differences were found in the input-output curves of participants with tetraplegia and nonimpaired participants [30]. In the study by Sangari and Perez, TMS was delivered superimposed to voluntary contractions of the biceps (~10% MVC) unlike our protocol (and previously cited reports in distal muscles [24], [117]) where TMS was delivered with the muscle at rest. This is an important difference since responsiveness to TMS greatly increases with voluntary activation of the corticomotor tract, which enhances the membrane conductance of the neurons, placing neurons in a primed state to depolarize [215], [217]. Following SCI, the corticomotor excitability changes as measured by greater slopes compared to nonimpaired controls may only be detectable at rest, especially since the increase in motoneuron excitability during voluntary contractions in SCI individuals is less than that of nonimpaired individuals [194]. Decreased spinal inhibition following SCI may also contribute to the increased slopes when motor pathways are purposefully tested at rest [218]. Greater MEP input-output curve slopes in the SCI group may also indicate expanded cortical motor maps innervating the biceps muscles following SCI. In fact, MEP input-output curves may provide similar results to motor mapping in that an increase in slope correlates to an increased cortical representation of a muscle as measured with motor mapping protocols [66]. The deafferentation of cortical circuits due to SCI leads to long-term reorganization of muscles' cortical representations [3], [5]. Notably but not unexpectedly, this mechanism is not limited to distal muscles and can also be found by probing proximal muscles such as the biceps brachii. Increased slopes may indicate down-regulated activity of excitatory interneurons as reflected by the presence of later I-waves at higher TMS intensities [219]. On the other hand, normal RMTs suggest that the alteration in excitability may be prominently occurring outside of the motor cortex since RMTs are thought to be related to the membrane excitability of a central core of cortical neurons [219]. Thus, the increased input-output curves of the biceps

brachii in tetraplegia are likely due to a combination of changes in spinal inhibition and decreased inhibitory feedback from the brain.

While using approaches such as the sigmoid function may better fit the shape of the MEP input-output curves, simple linear regression yields satisfactory results that are repeatable across days. Similar to a previous study addressing the differences between the Boltzmann sigmoid function method and linear regression in stroke patients, we found no difference in the accuracy of prediction (R^2) between the two methods in the SCI group [213]. Further, despite the challenges of targeting the biceps brachii [121], we were also able to show that MEP input-output curves of the biceps are repeatable across days in SCI patients (ICC(3,k) = 0.78). Previously, test-retest reliability of MEP input-output curves targeting the FDI had been established in nonimpaired participants when using sigmoidal curve fitting [208] and in chronic stroke using linear regression [220]. Repeatability across days is an important requirement to use MEP input-output curves to measure outcomes of neurorehabilitation protocols.

This preliminary study has limitations. First, only ten patients with cervical SCI participated in the study. While they all had C5-C6 level injuries, discrepancies in remaining motor function as reflected by ISNCSCI scores were present among participants. Furthermore, our nonimpaired group tested was on average younger (age 22.7 ± 2.5 versus 39.9 ± 10.6). However, previous research suggests that age may not affect RMTs nor the slope of MEP input-output curves [221]. Finally, five out of ten of our SCI participants were using the drug baclofen (see Table 4-1), known to affect the nervous system at the cortical and spinal level (anti-spasmodic GABA agonist) [222]. While baclofen appears not to affect the amplitude of MEPs (tested in FDI of SCI patients) at rest [183], or even reduce MEP size in nonimpaired lower limb muscles [223], additional research is needed to understand its potential effects on the neuromuscular system when used chronically. Another limitation is the use of manual motor mapping instead of a navigated TMS approach. However, the benefit-cost balance of using navigated TMS systems may be low with respect to its ability to reduce MEP variability [224].

In our cohort, preliminary findings suggest that individuals with tetraplegia following chronic cervical (C5-C6) SCI have increased MEP input-output curves of the biceps as analyzed with linear regression compared to nonimpaired controls. Along with normal RMTs, this indicates injury-induced corticomotor reorganization following SCI. Analyzing MEP input-output curves with linear regression was found to accurately represent the slope of the modeled data in the SCI group, with no differences in accuracy of prediction (R^2) compared to the sigmoid function method. Finally, slopes extracted from the MEP input-output curves were found to be repeatable across three days. Future research could include MEP input-output curves to investigate how motor skill training of impaired muscles may have positive effects on corticomotor excitability. MEP input-output curve protocols are uncomplicated to carry out and may be used as a monitoring tool when combined with motor relearning protocols or corticomotor plasticity inducing techniques, such as repetitive TMS or neuro-feedback training [11], [225]. As corticomotor reorganization may be maladaptive, developing reliable ways to measure it has clinical implications.

5 Chapter 5: Evaluate a Low-cost Navigated Transcranial Magnetic Stimulation System

5.1 Background/Objectives

Transcranial magnetic stimulation (TMS) is a non-invasive form of brain stimulation in which a magnetic field pulse directed via a coil induces an electric current in the brain, which can depolarize neurons. When a TMS pulse is applied to the motor cortex, surface electrodes located on the muscle record the motor evoked potential (MEP). The MEP provides a quantification of cortico-spinal excitability at the time of stimulation. MEP amplitudes often have high variability within and across sessions in the same participant when identical consecutive stimuli are applied [78], [79]. Physiological variability due to state-changes in the nervous system (i.e., fluctuations in excitability of interneurons and motoneurons) are important to capture. MEP variability related to inconsistencies in how the TMS coil is positioned and oriented over the head across trials and sessions is undesired [51], [73]. Changes in coil position and orientation alter the current flow in the brain, thereby affecting the population of neurons being stimulated, which contributes to undesired MEP amplitude variability [72], [73], [79]. Inconsistent positioning of the TMS coil is a potential source of error in TMS studies/outcomes [226]–[228]. Reducing error in replication of coil position and orientation over the TMS hotspot across trials and sessions is important for the use of TMS in clinical neuroscience and rehabilitation.

Neuronavigated TMS systems were developed for various applications [229] and can reduce the error in placing the coil over the hotspot and precisely track the position of the TMS coil during experiments [227] or clinical intervention [230]. Advanced neuronavigation systems can use subject-specific structural and functional magnetic resonance images (MRI) to visualize individual anatomical structures of the brain. TMS coregistered with MRI relates actual scalp locations to virtual cortical surface loci below. The intracranial electric field (E-field) effect on subject-specific cortical anatomical structures is calculated and visualized during TMS delivery [75]. However, advanced neuronavigated TMS systems have important limitations that limit their

use outside of specialist research environments: they are expensive (> $50,000) and add several preparatory steps to a TMS protocol, often including individual MRI scans [76].

Affordable alternatives to neuronavigation that add minimal preparatory steps may improve the repeatability of TMS coil location over the motor hotspot (i.e., spatial consistency of the TMS coil) relative to the conventional, manual method (using cranial landmarks) [77]. A low-cost TMS navigation approach was developed to track the position of the coil relative to the head [77], [231]. Real-time three-dimensional (3D) feedback is provided to the technician to assist in the re-positioning of the coil over the previously determined cortical hotspot [77], [231]. Preliminary evaluation of the low-cost navigated system was completed in four non-disabled participants targeting the first dorsal interosseus (FDI) at rest [77]. Comparing low-cost navigation to the conventional method with limited statistical power, navigation improved accuracy in locating the coil over the FDI hotspot both within and between sessions, as well as improved MEP quality (i.e., greater amplitudes) and consistency. Further research regarding the utility of low-cost navigation is warranted considering the FDI hotspot is easier to target with TMS relative to other muscles or muscle groups [232], [233], and MEP variability depends on the target muscle and its level of voluntary activation [49], [68], [234].

The objective of this study was to determine the effect of a low-cost navigation approach (developed by Rodseth et al., 2017) on spatial consistency of the TMS coil over the biceps brachii motor hotspot, and biceps MEP amplitudes and their variability across a range of voluntary biceps activation and two sessions. A range of voluntary activation levels from 0 to 100% of maximum was assessed because it may be more difficult to maintain coil location during contractions, and MEPs during effort are relevant to neuromuscular function [190]. Participants completed two sessions to elucidate inter-session variability. We hypothesized that relative to the conventional method, low-cost navigated TMS would demonstrate: 1) greater spatial consistency of the TMS coil (i.e., smaller position and orientation errors of the TMS coil with respect to the registered hotspot) over the biceps motor hotspot across voluntary effort levels, 2) smaller intra- and inter-

session biceps MEP variability, and 3) larger MEP amplitudes due to better targeting of the motor hotspot.

5.2 Methods

5.2.1 Experiment Overview:

Ten non-disabled participants (24.5 ± 3.5 years old, five female, five male) were recruited to participate in two sessions during which single pulse TMS was delivered to the biceps hotspot on the motor cortex. To test the effect of low-cost navigation on biceps MEPs, we recorded MEPs each at the following voluntary effort levels: 0, 25, 50, 75, and 100% of the maximum voluntary isometric contraction (MVC) while position and orientation of the coil were tracked in two conditions: (1) navigated and (2) conventional. The "navigated" condition hereafter refers to the condition in which the technician was provided visual feedback (on a monitor) of the position and orientation of the coil relative to the hotspot, and "conventional" refers to the conventional, manual method in which the technician uses cranial landmarks and markings on a cap to position the coil over the hotspot. The order of conditions (navigated or conventional) and voluntary efforts were randomized for each session. To assess repeatability, participants each completed two sessions; sessions were separated by at least one day, no more than a week. A protocol overview is presented in Figure 5-1.

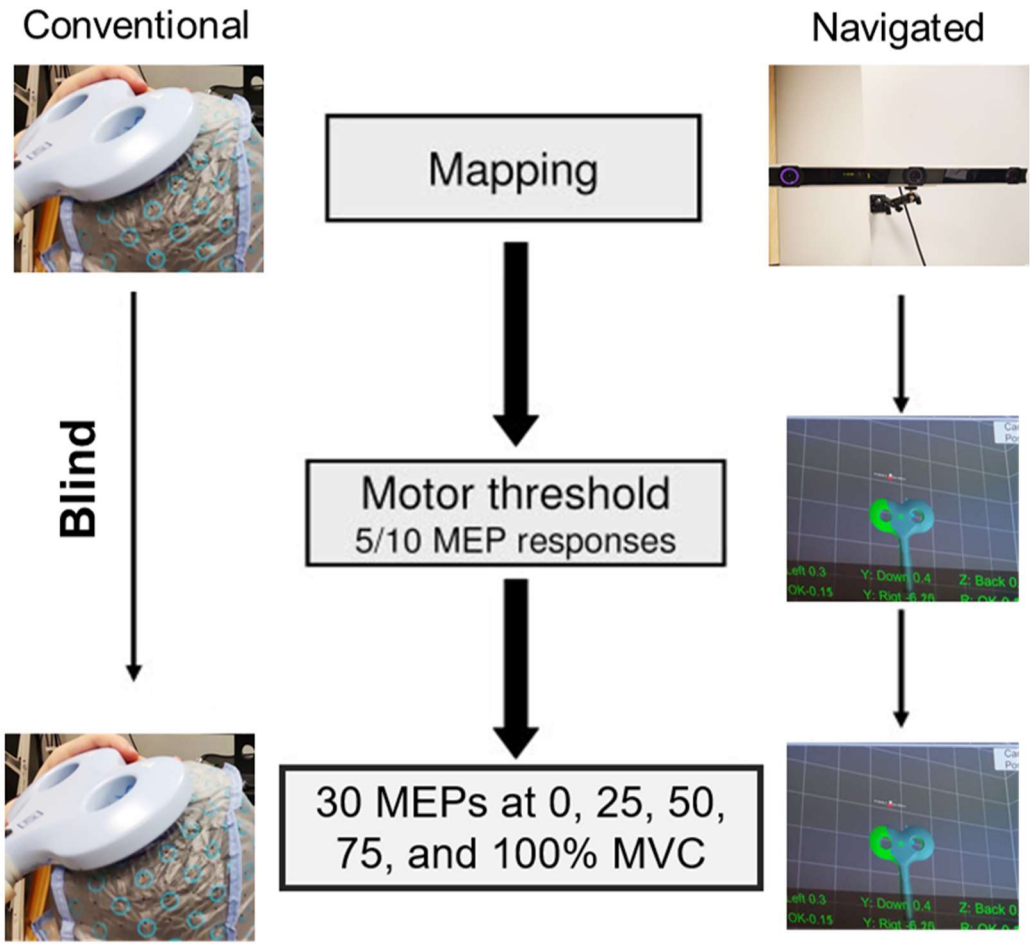


Figure 5-1: Both experimental sessions included navigated and conventional mapping of the motor hotspot. In the conventional condition, navigation feedback was hidden from the technician and markings were made on a cap to record the hotspot location. In the navigated condition, the technician is provided feedback regarding position and orientation of the coil relative to the recorded hotspot. Six MEPs were collected at each effort level (30 MEPs total per block).

5.2.2 Electromyography and Kinetic Data

Surface EMG electrodes (Delsys Trigno Wireless Sensors) were placed over the muscle belly of the biceps brachii. For all trials, the participant's forearm was supported against gravity in an isometric posture via a custom brace attached to a six degree-of-freedom load cell (Model 30E15A4-I40-EF-100L, JR3, Woodland, CA). Kinetic and EMG data were sampled at 2000 Hz using

Spike 2 software (Micro 1401 MkII, Cambridge Electronic Design, Cambridge, UK). EMG signals were amplified and bandpass-filtered at 20-450 Hz prior to A/D conversion.

5.2.3 Maximal Voluntary Isometric Contractions:

At the start of each session (before motor mapping), participants performed three MVCs in elbow flexion for three seconds while receiving real-time visual moment feedback and verbal encouragement (Fig. 2, A). Each maximum effort was separated by at least 90 seconds of rest. The participant's MVC was calculated for each effort as the mean elbow moment maintained over ± 250 ms from the maximal moment achieved. The mean maximum moment of three MVC trials was used for subsequent trials during which participants were asked to generate a voluntary moment to match a percentage of their MVC moment. EMG signals during the MVC trials were also used to normalize MEPs [235]. The greatest root mean squared (RMS) value was computed from the three MVC trials over a ± 250 ms window at the maximum amplitude [236].

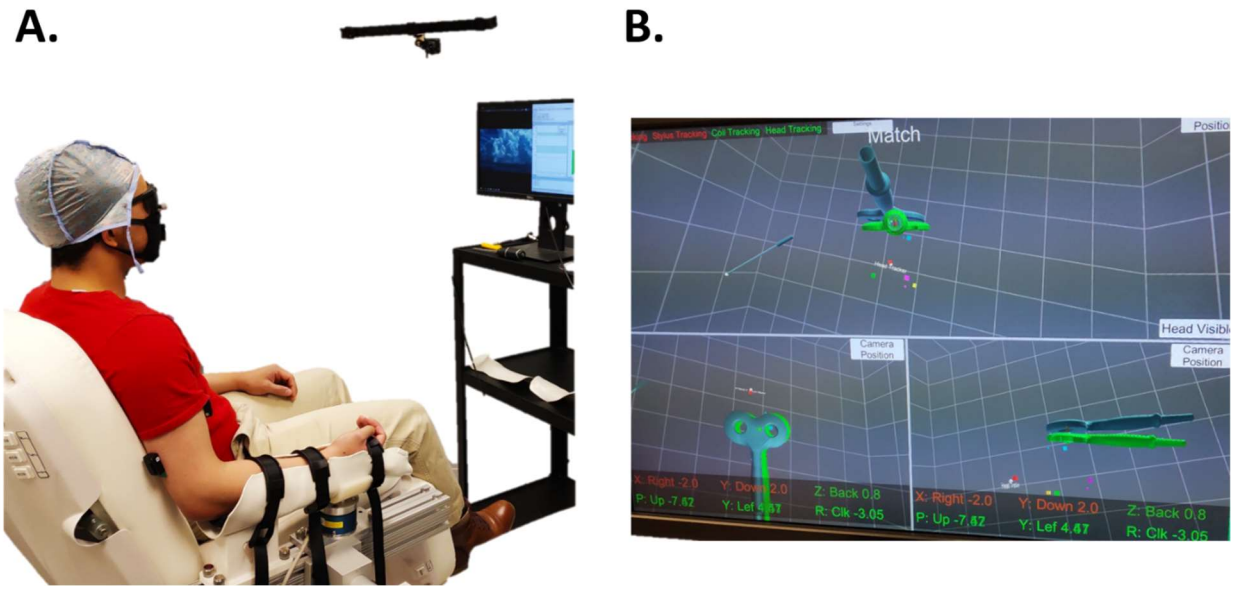


Figure 5-2: A. Participants wore glasses with a reflective marker cluster facing the tracking camera. Elbow moment feedback was provided on a monitor. **B**. Real time visual feedback was presented to the

technician during the navigated condition to match the current (blue) and desired (green) coil position and orientation to target the hotspot.

5.2.4 Low-Cost TMS Navigation:

We implemented a low-cost TMS navigation approach developed by Rodseth et al. within our laboratory; the materials to implement the approach cost ≈ $3200. A motion tracking camera (OptiTrack V120 Trio system, < $3000) was implemented to track the position of reflective markers attached to custom-built accessories including a stylus, coil attachment, calibration tools, and head attachment in Motive 1.8. Real time motion tracking is performed in the motion tracking software where each accessory is registered as a marker cluster. Position and orientation data of each cluster were livestreamed to a computer program. The computer program was based on source code provided by the University of Michigan NeuRRo Lab using Unity's 3D engine [77]. The program was used to capture navigation data during the experiment and provide 3D visual feedback to the technician. Calibration tools and marker clusters were 3D printed from polylactic acid material. Glasses with a triad of reflective markers were used to define the head reference frame. A marker triad attached to the TMS coil defined the coil reference frame.

Motion tracking calibration was performed following the MVCs. After positioning the calibration tools and accessories in front of the camera, Motive 1.8 settings were adjusted to optimize the infrared signal captured by the cameras. Calibration began by placing the calibration stylus in the center of the calibration space to create a virtual object. The calibration stylus was then used to define coil landmarks including the center, front and inner side of the coil. The participants' head geometry was calibrated using the stylus to create landmarks aligned with the nasion, right tragus, left tragus, vertex, and inion.

5.2.5 Transcranial Magnetic Stimulation:

A 70 mm figure-of-eight coil and monophasic stimulator (Magstim, Bistim^2) were used to deliver single pulse TMS to the motor cortex. Motor cortex mapping was performed each session to obtain the hotspot, which is the location that evoked the largest peak-to-peak MEP in the

biceps brachii at the lowest stimulation intensity [237]. The hotspot was recorded and replicated for subsequent stimulation trials using two procedures (in random order): navigated and conventional (Figure 5-1). With the navigated method, after the hotspot was determined, visual feedback from the computer program was subsequently used to match the hotspot. With the conventional method, the hotspot location was marked on a cap secured to the participant's head [238]. Markings were subsequently used to match the hotspot. In both conditions, the position and orientation of the coil relative to the head were saved in the computer program. Resting motor threshold (RMT) was determined at the hotspot as the lowest stimulus intensity able to induce biceps MEPs ≥ 50 μV in at least 5 out of 10 stimuli [237].

5.2.6 Single Pulse TMS Trials:

In each session, participants completed two experimental blocks (one navigated, one conventional) with their arm supported against gravity in an isometric posture with the elbow flexed to 90° (Figure 5-2, A). Each block consisted of a set of 30 isometric elbow flexion moment-matching trials at 0, 25, 50, 75, or 100% MVC presented in randomized order (6 per effort level). A TMS pulse of 120% RMT was delivered when the participant achieved and maintained ± 2.5 percent of the target effort level (i.e., elbow moment) for a sustained 0.5s [239]. The position and orientation of the coil during each stimulus event were recorded for both the navigated and conventional conditions. During trials in the navigation block, the research technician used the position and orientation feedback from the navigation software to position and orient the coil, matching the hotspot previously determined/recorded (Figure 5-2, B). During the trials in the conventional block, the research technician used markings on a cap to position and orient the TMS coil.

5.2.7 Data and Statistical Analysis:

Spatial coil placement consistency was evaluated by the error in matching location and orientation of the coil from the registered motor hotspot during each TMS stimulus event. Position error was recorded in x, y, and z coordinates then represented as the mean Euclidean

distance. Error in orientation was recorded in pitch, yaw, and roll, defined as rotation about the y, z, and x axes of the coil, respectively (Figure 5-3)[240]. Peak to peak amplitudes of MEPs were calculated from the EMG signal using custom-written scripts in MATLAB. Two-way ANOVAs with repeated measures with condition (navigated and conventional) and effort level (0, 25, 50, 75, 100% MVC) as repeated measures were used to compare the mean absolute error in reproducing the coil position and orientation. The null hypothesis was that there are no differences in coil position and orientation error between conventional and navigated conditions. The alternative hypothesis was that navigation decreased coil position and orientation error compared to the conventional condition. A two-way ANOVA with repeated measures with condition (navigated and conventional) and effort level (0, 25, 50, 75, 100% MVC) as repeated measures was also used to compare biceps MEP amplitudes. Bonferroni's multiple comparison tests were used for post-hoc analyses, when applicable. The null hypothesis was that there are no differences in MEP amplitudes between conventional and navigated conditions. The alternative hypothesis was that navigation increased MEPs compared to the conventional condition.

Coefficients of variation (SD/mean × 100) were computed for each participant at each effort level. Coefficients of variation (CV) were then averaged to represent intra-participant and intra-session variability of biceps MEPs. Differences in CV between the navigated and conventional conditions were evaluated using the asymptotic test for the equality of coefficients of variation [241]. To assess repeatability across sessions, intraclass correlation coefficients (ICCs) were calculated for biceps MEPs (two-way random effect model with absolute agreement of measurements, ICC(2,k) formula in R)[242]. ICCs were compared between navigated and conventional conditions using mixed-effect F-statistics [242]. ICC values were interpreted as high (ICC ≥ 0.75), moderate (0.50 ≤ ICC; 0.75), low (0.25 ≤ ICC; 0.50), and very low to none (ICC; 0.25)[143]. All data and statistical analyses were performed in MATLAB (MathWorks, Inc, Natick, MA), R (R Core Team, Vienna, Austria), and Prism (GraphPad Software, La Jolla California USA) with custom-written code. Tests were evaluated at a significance level corresponding to $p < 0.05$.

5.3 Results

Coil orientation errors were lower with navigated TMS relative to conventional (Main effect of navigation: pitch: $F_{(1, 1180)} = 113.0$, $p < 0.0001$, yaw: $F_{(1, 1180)} = 56.06$, $p < 0.0001$, roll: $F_{(1, 1180)} = 45.66$, $p < 0.0001$) at most effort levels (Figure 5-3, B, C, D)**.** Position error differed between the navigated and conventional conditions (Main effect of navigation: $F_{(1, 1180)} = 12.0$, $p < 0.001$) but only for trials at 75% MVC ($t_{(1080)} = 2.651$, $p < 0.05$); there was no difference at the other effort levels (Figure 5-3, A). For the navigated trials, the error in coil position was 0.69 ± 0.1 mm and the orientation errors in pitch, yaw and roll were 1.18° ± 1.2°, 1.99° ± 1.9°, and 1.18° ± 2.2°, respectively (Figure 5-3). For the conventional trials, the error in coil position was 1.2 ± 1 mm, and the orientation errors in pitch, yaw, and roll were 3.7° ± 5.7°, 3.11° ± 3.1°, and 3.8° ± 9.1°, respectively (Figure 5-3).

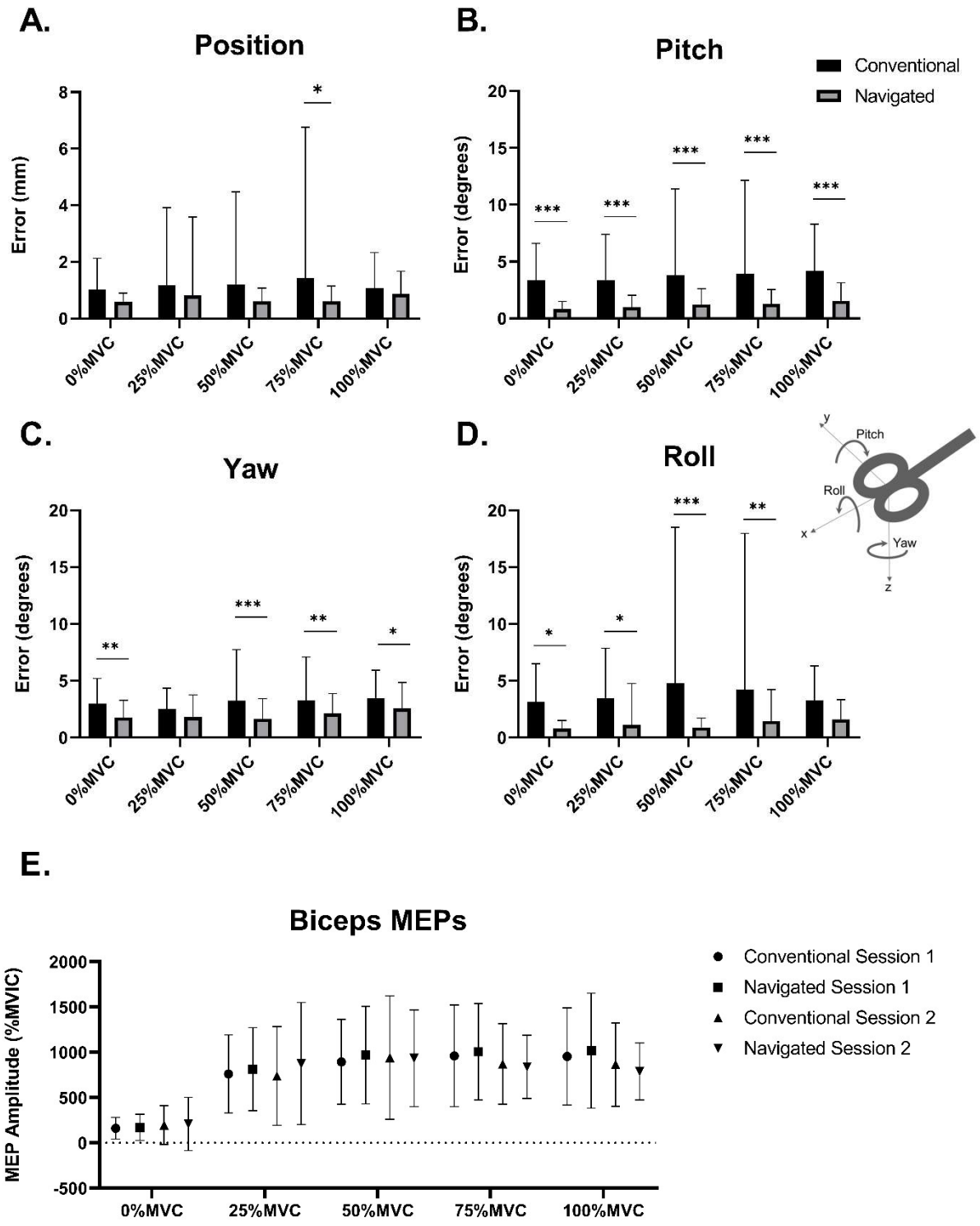


Figure 5-3: Errors (averaged across sessions) in coil position (**A**) and orientation (**B**: pitch, **C**: yaw and **D**: roll) across all voluntary efforts levels. **E.** Normalized biceps MEP amplitudes and MEP variability did not

differ per condition in either session regardless of effort level. Error bars represent ± one standard deviation (SD) and asterisks indicate statistical significance (*: $p < 0.05$, **: $p < 0.01$, ***: $p < 0.001$).

Intra-session variability of normalized biceps MEPs was not different between the navigated and conventional conditions (session 1: $p = 0.15$, session 2: $p = 0.81$) (Table 5-1). Inter-session variability of MEPs was also not different between conditions ($F_{(1, 1188)} = 2.31$, $p = 0.128$) (Table 5-1). Magnitudes of normalized MEPs were also not different across the navigated and conventional conditions ($F_{(1, 1180)} = 1.07$, $p = 0.3$) (Figure 5-3, C). Repeatability of normalized MEP amplitudes was moderate in the navigated and conventional conditions (Table 5-1). Further analyses of coil position and orientation errors revealed no interaction effect of voluntary contraction at any effort level (position: $F_{(4, 1180)} = 0.596$, $p = 0.665$, pitch: $F_{(4, 1180)} = 0.039$, $p = 0.997$, yaw: $F_{(4, 1180)} = 0.956$, $p = 0.430$, roll: $F_{(4, 1180)} = 0.928$, $p = 0.446$). Resting motor thresholds for each session, and all data and supplementary data are available in our shared data repository located here: https://osf.io/bpxhj/.

Table 5-1: Intra-session and inter-session variability of MEPs

Protocol	Effort Level (%MVC)	Coefficient of Variation (CV)		ICC(2,k)
		Session 1	Session 2	
Conventional	0	26.4%	34.6%	-
	25	21.0%	21.5%	-
	50	9.41%	13.9%	-
	75	12.7%	13.1%	-
	100	16.4%	19.8%	-
	Mean	**17.2%**	**20.6%**	**0.63**
Navigated	0	41.5%	37.6%	-
	25	17.4%	15.2%	-
	50	14.3%	17.2%	-
	75	9.7%	18.1%	-
	100	15.8%	17.7%	-
	Mean	**19.7%**	**21.2%**	**0.61**

5.4 Discussion

We implemented a low-cost navigated TMS approach and evaluated the effect of navigation on intra- and inter-session biceps MEPs (amplitudes and variability) and errors in consistent position and orientation of the TMS coil over the motor hotspot. We hypothesized that relative to the conventional, manual method of coil positioning, low-cost navigated TMS would result in: 1) smaller positional and orientation errors of the TMS coil over the biceps motor hotspot across voluntary effort levels, 2) smaller intra- and inter-session biceps MEP variability, and 3) larger MEP amplitudes. Our first hypothesis was partially supported. The error in positioning was smaller using the navigated TMS system relative to conventional only when MEPs

were superimposed to contractions at 75% MVC. The error in coil orientation was smaller in the navigated condition across all effort levels. Consistent orientation of the coil is particularly difficult to achieve/maintain when using the conventional method, especially with a flat, figure-of-eight coil. In agreement with the perceived difficulty of maintaining coil orientation, the navigated system had a greater impact on the coil orientation compared to position over the motor hotspot. Our second hypothesis was not supported; intra-session and inter-session variability as represented by CV and ICC, respectively, were not different between the conventional and navigated conditions. Our third hypothesis was not supported; the amplitude of biceps MEPs was not affected by the use of the navigated TMS system. Overall, our results suggest that while coil orientation was improved by the use of low-cost navigation, this did not translate to improved biceps MEP quality. Variability in biceps MEPs is likely driven by physiological variability, such as spontaneous fluctuations in corticospinal and segmental motoneuron excitability [79], [243]. The conventional and navigated methods were similarly effective in recording biceps MEPs, although low-cost navigation is advantageous in clinical and research applications in which tracking coil position and orientation is needed or beneficial [72], [244].

Previously, the low-cost system we implemented was tested on the FDI muscle where MEP amplitudes were larger with navigated TMS compared to the conventional method. However, this was only tested in four participants in the resting FDI [77]. In our study, we evaluated the biceps brachii at different levels of voluntary contractions, in ten participants, and did not find an effect of the navigated condition on MEP amplitudes or variability. One possible explanation for our finding is that biceps brachii MEPs may be less sensitive to coil position errors relative to the FDI, especially in a state of activation [234], [245]. Compared to the FDI, the biceps have a smaller cortical representation area and weaker monosynaptic connections which leads to more variable MEPs [121], [233]. TMS delivered during voluntary activation of muscle typically reduces MEP variability because contraction provides the corticospinal tract with a greater degree of organization; contraction increases the membrane conductance of the neurons, placing neuron membrane potentials in a more primed state to depolarize [49], [246], [247]. In

agreement, we found that intra-session variability of biceps brachii MEPs were lower during voluntary contractions (mean CV = 15.8%) compared to at rest (mean CV = 35.1%). The effect of navigated TMS on MEP variability may become negligible when intra-session variability of MEPs is already reduced by muscle activation. Jung et al. assessed a similar navigation system (although not open source) and found that navigated TMS did not reduce MEP variability in the abductor pollicis brevis muscle at rest compared to the conventional method [80]. Our results point to the same conclusion that physiological factors such as ongoing cortical excitability and spinal desynchronization are greater contributors to MEP variability relative to coil location [79], [243].

Low-cost navigation can improve spatial consistency of the TMS coil over the biceps hotspot relative to the conventional method of TMS coil locating. The errors in position consistency we found with the low-cost navigation were similar to previous reports ranging from 1 to 3 mm of optically tracked frameless stereotaxic navigation [248] and MRI guided neuronavigation [227]. Errors in consistency of coil orientation are seldom reported, but the effects of TMS depend strongly on the orientation of the TMS coil [52], [249]. Using the same low-cost navigation system tested here, the system developers reported coil orientation errors of 0.5° ± 0.2° (pitch : 0.4° ± 0.1°; yaw: 0.5° ± 0.2°; roll : 0.6° ± 0.2°) when targeting the FDI at rest, which are lower relative to our orientation errors targeting the biceps at various levels of activation (pitch: 1.18° ± 1.2°, yaw: 1.99° ± 1.9°, roll: 1.18° ± 2.2°)[77]. However, generally, orientation errors were low in both studies.

A limitation of this study is that we did not record MEPs in the FDI or abductor pollicis brevis muscle to enable direct comparison of our results in the biceps brachii. Previous work regarding the effect of low-cost navigation on MEP variability focused on these hand muscles [77], [80]. A potential limitation is that we only assessed one stimulation intensity per participant per session (we did not record MEP recruitment curves). MEP amplitudes and variability depend on stimulation intensity [49]. Thus, stimulation intensity may affect the utility of low-cost navigation targeting the biceps; of note is that preliminary analysis demonstrated stable TMS recruitment curves targeting the FDI at rest [77]. Another potential limitation is that the number

of stimulus events was limited to six per effort level per condition to minimize neuroplastic effects associated with repeated stimuli and fatigue. Further, our sample size was small (n = 10), and we did not investigate the use of a TMS coil stand, which can be used in clinical and research applications to maintain the position of the coil across trials. Finally, the center of gravity of the motor hotspot may shift during voluntary contraction [250]. However, the cortical representation of muscle may increase during activation so our approach to maintain the hotspot as that determined at rest is likely robust [251].

The important new knowledge presented here is that: 1) low-cost navigation can improve coil orientation and track spatial consistency of the TMS coil over the biceps hotspot, 2) voluntary activation of the biceps does not affect spatial consistency of the coil, and 3) biceps MEP variability reflects physiological variability across a range of voluntary efforts, that can be captured equally well with navigated or conventional approaches of coil locating. This low-cost navigated TMS system is a suitable alternative to expensive commercial systems in providing spatial consistency of the TMS coil. Future work should focus on the feasibility of implementing this low-cost navigated TMS approach in clinical settings.

6 Conclusions, Contributions and Future Directions

The focus of this dissertation was the advancement of TMS-based techniques to improve the monitoring of neuromuscular function in individuals with tetraplegia following cervical SCI with considerations for inter-session repeatability and clinical feasibility. Additional new insights on physiological mechanisms may also contribute to the development of better neurorehabilitation approaches in the upper limb. In chapter 1, we began by presenting the scientific background and gaps in knowledge related to this work. In chapters 2 and 3, we focused on investigating a novel approach for directing neurorehabilitation for individuals with SCI through the measurement of VA_{TMS}. Previous approaches to improve the clinical assessment of individuals with SCI have used surface [29], [119] and intramuscular (invasive) EMG [252], dynamometry [253], spinal reflexes [17], [18], rigidly administered protocols of motor tasks [254], or combinations of these. This approach is advantageous because it is quantitative, and the location of neural deficits that are detected can be determined via noninvasive measures while providing simultaneous measurement of muscle force-generating capacity. However, measurement of VA_{TMS} has been limited by technical challenges that limit its interpretation, especially in patient populations, including the difficulty in preferential stimulation of cortical neurons projecting to the target muscle and minimal stimulation of antagonists. Thus, we developed novel methodology to address this challenge.

In chapter 2, we modulated elbow joint posture during the assessment of VA_{TMS} to optimize the biceps/triceps MEP ratio in nonimpaired participants and participants with tetraplegia following cervical SCI (C5-C6). Elbow flexion angle modulation was able to increase the MEP ratio but only in the nonimpaired group and not across the entire range of voluntary efforts used to estimate VA_{TMS}. Thus, we conclude that modulating elbow flexion angle does not improve the MEP ratio and ultimately the assessment of VA_{TMS} in tetraplegia. However, we established that VA_{TMS} and VA_{PNS} in individuals with tetraplegia were repeatable across days, which is an important requirement for future clinical use. In chapter 3, we modulated the stimulation paradigm by using paired pulse TMS to elicit intracortical facilitation and short-

interval intracortical inhibition to optimize the MEP ratio during the assessment of VA_{TMS}. The MEP ratio was modulated in both groups but not across the entire range of voluntary efforts, and did not affect VA_{TMS} estimation compared to single pulse TMS. Thus, modifying TMS stimulation paradigm does not improve the assessment of VA_{TMS} in tetraplegia. However, paired pulse TMS outcomes revealed abnormal patterns of intracortical inhibition in individuals with tetraplegia that may be related to alterations in the activity of GABA-A and GABA-B receptors. Further, our experiments revealed additional challenges in the measurement of VA_{TMS} in tetraplegia. Linearity of the voluntary moment and superimposed twitch relationship, an important requirement for the validity of VA_{TMS} measures, was difficult to achieve in participant with tetraplegia. In fact, linearity was on average lower relative to nonimpaired participants. Increased fatigability and antagonist co-activation in tetraplegia likely contributed to this outcome. So far, measurement and interpretation of VA_{TMS} in tetraplegia remains limited until these challenges can be addressed.

Recent advances in noninvasive brain stimulation technology may be able to address these challenges and improve the measurement of VA in patient populations. An ideal stimulation technique would be one capable of exclusively and fully activating the corticomotor tract innervating the target muscle. One future direction towards this goal is the innovation in TMS coil design. For example, miniaturized TMS coils with focality that can reach up to three times lower compared to standard TMS coils have been recently developed [255], [256]. To date in humans, these new designs have only been tested on peripheral nerves and may not be able to sufficiently activate cortical neurons in the assessment of VA_{TMS}, especially in tetraplegia. Another future direction could investigate alternatives to TMS. For example, transcranial focused ultrasound could be a promising alternative to TMS in the measurement of VA [257], [258]. Relative to TMS, this technique has higher spatial resolution, which may allow more focal stimulation of the motor cortex representation of the target muscle, resulting in less synergist and antagonist muscles recruitment during the assessment of VA. Future investigations are needed to establish the feasibility and efficacy of this technique in the assessment of VA, in nonimpaired and patient populations.

In chapter 4, we collected MEP input-output curves of the biceps in individuals with tetraplegia and nonimpaired. Using this method, we were able to identify, in the proximal upper limb, compensatory reorganization of corticomotor pathways after SCI, similar to what had been observed in more distal upper limb muscles [117]. We also established the repeatability across days of this technique in individuals with tetraplegia. Since MEP input-output curves can predict functional gains in patient populations [71], these outcomes have implications for the monitoring of neuromuscular function after SCI and could contribute to improving the classification standards currently based on manual muscle testing [41], [259]. Future research could focus on using this approach in combination with neuromodulation and motor training protocols in tetraplegia to quantify their effect on corticomotor excitability and better predict rehabilitation outcomes.

In the final chapter, we implemented a low-cost navigated TMS system (< $3000) that uses motion tracking, 3D printed parts and open-source software to monitor coil placement during stimulation. Currently, commercially available navigated TMS systems are expensive (> $20000) and often complicated to use. This affordable alternative was able to improve coil orientation and track spatial consistency of the TMS coil over the biceps hotspot with similar effectiveness compared to commercial systems [244], [223]. Further, since voluntary contraction of the biceps did not affect spatial consistency of the coil, MEP variability reflects physiological variability across a range of voluntary efforts and can be captured equally well with navigated or conventional approaches of coil locating. Future work could focus on the implementation of this system in clinical settings where coil placement tracking and virtual guidance during stimulation may be especially helpful to non-specialized medical practitioners.

7 Publications

7.1 Peer-reviewed Journals

[1] [Pending] **T. Roumengous** and C. L. Peterson, “Assessment of Biceps Voluntary Activation with Transcranial Magnetic Stimulation in Individuals with Tetraplegia” Restorative Neurology and Neuroscience.

[2] [Pending] **T. Roumengous**, B. Thakkar, and C. L. Peterson, “Paired Pulse TMS in the Assessment of Biceps Voluntary Activation in Individuals with Tetraplegia” PLOS One.

[3] [Pending] T. Roumengous, Y. Zeineddine, and C. L. Peterson, “Motor Evoked Potential Input-Output Curves Indicate Neuroplasticity of the Biceps Brachii after Cervical Spinal Cord Injury”. Journal of Clinical Neuroscience.

[4] **T. Roumengous**, A. B. Reutter, and C. L. Peterson, “Effect of low-cost transcranial magnetic stimulation navigation on hotspot targeting and motor evoked potential variability in the biceps brachii” Restorative Neurology and Neuroscience, Oct. 2021, doi: 10.3233/RNN-211207.

[5] C. S. Lynch, **T. Roumengous**, N. Mittal, and C. L. Peterson, “Effects of Stimulus Waveform on Transcranial Magnetic Stimulation Metrics in Proximal and Distal Arm Muscles” Neurophysiologie Clinique.

7.2 Conference Presentations

T. Roumengous, Y. Zeineddine, C. L. Peterson. “Motor Evoked Potential Input-Output Curves Indicate Neuroplasticity after Spinal Cord Injury”. Summer Biomechanics, Bioengineering and Biotransport Conference (SB3C), June 14-18, 2021.

Chris S. Lynch**, T. Roumengous**, C. L. Peterson. “Effect of Stimulus Waveform on Transcranial Magnetic Stimulation Metrics in Proximal and Distal Arm Muscles”. Summer Biomechanics, Bioengineering and Biotransport Conference (SB3C), June 14-18, 2021.

A. Reutter, **T. Roumengous**, C. L. Peterson. “Evaluation of a Low-Cost Navigation Technique for Transcranial Magnetic Stimulation”. Biomedical Engineering Society annual conference, Philadelphia, PA, October 16-19th, 2019.

Y. Zeineddine, **T. Roumengous**, C. L. Peterson. “Motor Evoked Potential Recruitment Curves Indicate Neuroplasticity after Spinal Cord Injury”. Biomedical Engineering Society annual conference, Philadelphia, PA, October 16-19th, 2019.

T. Roumengous, P. A. Howell, C. L. Peterson. "Voluntary Drive Amplifies Effects of Paired-pulse TMS and Arm Posture on Biceps Corticomotor Excitability”. International Biomechanics Society Annual Meeting, Calgary, CANADA, August 4th, 2019.

P. A. Howell, **T. Roumengous**, C. L. Peterson. "Increased Elbow Angle to Improve Measurement of Cortical Voluntary Activation of the Elbow Flexors”. International Biomechanics Society Annual Meeting, Calgary, CANADA, August 4th, 2019.

T. Roumengous, P. A. Howell, C. L. Peterson. “Biceps Voluntary Activation: Method To Calculate Pre-Stimulus Moment Affects Magnitude But Not Reproducibility”. Summer Biomechanics, Bioengineering, and Biotransport Conference, Seven Springs, PA, June 27th, 2019.

T. Roumengous, C. L. Peterson. “Voluntary Drive Increases Detectability of Changes in Corticomotor Excitability”. Presented at the 2019 Virginia Academy of Science Annual Meeting, Norfolk, VA, May 23th, 2019.

P. A. Howell, **T. Roumengous**, C. L. Peterson. "Innovative Methodologies to Reliably Assess Voluntary Activation of the Elbow Flexors”. Central Virginia Society for Neuroscience annual symposium, Richmond, VA, March 24th, 2018.

P. A. Howell, **T. Roumengous**, C. L. Peterson. "Cutaneous Stimulation and Arm Posture to Modulate Biceps Responses to Transcranial Magnetic Stimulation". Biomedical Engineering Society annual conference, Atlanta, GA, October 17-20th, 2018.

8 References

[1] N. Sezer, S. Akkuş, and F. G. Uğurlu, "Chronic complications of spinal cord injury," *World J Orthop*, vol. 6, no. 1, pp. 24–33, Jan. 2015, doi: 10.5312/wjo.v6.i1.24.

[2] K. D. Anderson, J. Fridén, and R. L. Lieber, "Acceptable benefits and risks associated with surgically improving arm function in individuals living with cervical spinal cord injury," *Spinal Cord*, vol. 47, no. 4, pp. 334–338, Apr. 2009, doi: 10.1038/sc.2008.148.

[3] H. Topka, L. G. Cohen, R. A. Cole, and M. Hallett, "Reorganization of corticospinal pathways following spinal cord injury," *Neurology*, vol. 41, no. 8, pp. 1276–1276, Aug. 1991, doi: 10.1212/WNL.41.8.1276.

[4] J. Aguilar *et al.*, "Spinal Cord Injury Immediately Changes the State of the Brain," *J. Neurosci.*, vol. 30, no. 22, pp. 7528–7537, Jun. 2010, doi: 10.1523/JNEUROSCI.0379-10.2010.

[5] L. J. Streletz, J. K. Belevich, S. M. Jones, A. Bhushan, S. H. Shah, and G. J. Herbison, "Transcranial magnetic stimulation: cortical motor maps in acute spinal cord injury," *Brain topography*, vol. 7, no. 3, pp. 245–250, 1995.

[6] H. C. Smith *et al.*, "Corticospinal function studied over time following incomplete spinal cord injury," *Spinal Cord*, vol. 38, no. 5, pp. 292–300, May 2000, doi: 10.1038/sj.sc.3100994.

[7] M. Khorasanizadeh *et al.*, "Neurological recovery following traumatic spinal cord injury: a systematic review and meta-analysis," *J Neurosurg Spine*, pp. 1–17, Feb. 2019, doi: 10.3171/2018.10.SPINE18802.

[8] K. A. Moxon, A. Oliviero, J. Aguilar, and G. Foffani, "Cortical reorganization after spinal cord injury: Always for good?," *Neuroscience*, vol. 283, pp. 78–94, Dec. 2014, doi: 10.1016/j.neuroscience.2014.06.056.

[9] G. Courtine and M. V. Sofroniew, “Spinal cord repair: advances in biology and technology,” *Nat Med*, vol. 25, no. 6, pp. 898–908, Jun. 2019, doi: 10.1038/s41591-019-0475-6.

[10] G. Taccola, D. Sayenko, P. Gad, Y. Gerasimenko, and V. R. Edgerton, “And yet it moves: Recovery of volitional control after spinal cord injury,” *Prog Neurobiol*, vol. 160, pp. 64–81, Jan. 2018, doi: 10.1016/j.pneurobio.2017.10.004.

[11] J. Gomes-Osman and E. C. Field-Fote, “Improvements in hand function in adults with chronic tetraplegia following a multi-day 10Hz rTMS intervention combined with repetitive task practice,” *J Neurol Phys Ther*, vol. 39, no. 1, pp. 23–30, Jan. 2015, doi: 10.1097/NPT.0000000000000062.

[12] A. Jayaraman, C. K. Thompson, W. Z. Rymer, and T. G. Hornby, “Short-term maximal-intensity resistance training increases volitional function and strength in chronic incomplete spinal cord injury: a pilot study,” *J Neurol Phys Ther*, vol. 37, no. 3, pp. 112–117, Sep. 2013, doi: 10.1097/NPT.0b013e31828390a1.

[13] B. J. Hilton and W. Tetzlaff, “A brainstem bypass for spinal cord injury,” *Nat Neurosci*, vol. 21, no. 4, pp. 457–458, Apr. 2018, doi: 10.1038/s41593-018-0099-z.

[14] T. A. Jones *et al.*, “Use-dependent dendritic regrowth is limited after unilateral controlled cortical impact to the forelimb sensorimotor cortex,” *J Neurotrauma*, vol. 29, no. 7, pp. 1455–1468, May 2012, doi: 10.1089/neu.2011.2207.

[15] A. Turolla, A. Venneri, D. Farina, A. Cagnin, and V. C. K. Cheung, “Rehabilitation Induced Neural Plasticity after Acquired Brain Injury,” *Neural Plast*, vol. 2018, p. 6565418, 2018, doi: 10.1155/2018/6565418.

[16] N. B. Finnerup, “Neuropathic pain and spasticity: intricate consequences of spinal cord injury,” *Spinal Cord*, vol. 55, no. 12, pp. 1046–1050, Dec. 2017, doi: 10.1038/sc.2017.70.

[17] A. K. Thompson, F. R. Pomerantz, and J. R. Wolpaw, “Operant Conditioning of a Spinal Reflex Can Improve Locomotion after Spinal Cord Injury in Humans,” *J Neurosci*, vol. 33, no. 6, pp. 2365–2375, Feb. 2013, doi: 10.1523/JNEUROSCI.3968-12.2013.

[18] R. L. Segal and S. L. Wolf, “Operant conditioning of spinal stretch reflexes in patients with spinal cord injuries,” *Exp Neurol*, vol. 130, no. 2, pp. 202–213, Dec. 1994, doi: 10.1006/exnr.1994.1199.

[19] H. Kumru *et al.*, “Reduction of spasticity with repetitive transcranial magnetic stimulation in patients with spinal cord injury,” *Neurorehabil Neural Repair*, vol. 24, no. 5, pp. 435–441, Jun. 2010, doi: 10.1177/1545968309356095.

[20] R. Nardone *et al.*, “Effects of intermittent theta burst stimulation on spasticity after spinal cord injury,” *Restor Neurol Neurosci*, vol. 35, no. 3, pp. 287–294, 2017, doi: 10.3233/RNN-160701.

[21] C. A. Pelletier and A. L. Hicks, “Muscle characteristics and fatigue properties after spinal cord injury,” *Crit Rev Biomed Eng*, vol. 37, no. 1–2, pp. 139–164, 2009, doi: 10.1615/critrevbiomedeng.v37.i1-2.40.

[22] M. Barat, P. Dehail, and M. de Seze, “Fatigue after spinal cord injury,” *Ann Readapt Med Phys*, vol. 49, no. 6, pp. 277–282, 365–369, Jul. 2006, doi: 10.1016/j.annrmp.2006.04.013.

[23] R. Nardone *et al.*, “Functional brain reorganization after spinal cord injury: Systematic review of animal and human studies,” *Brain Research*, vol. 1504, pp. 58–73, Apr. 2013, doi: 10.1016/j.brainres.2012.12.034.

[24] P. Freund, J. Rothwell, M. Craggs, A. J. Thompson, and S. Bestmann, “Corticomotor representation to a human forearm muscle changes following cervical spinal cord injury,” *Eur J Neurosci*, vol. 34, no. 11, pp. 1839–1846, Dec. 2011, doi: 10.1111/j.1460-9568.2011.07895.x.

[25] N. D. James, S. B. McMahon, E. C. Field-Fote, and E. J. Bradbury, “Neuromodulation in the restoration of function after spinal cord injury,” *The Lancet Neurology*, vol. 17, no. 10, pp. 905–917, Oct. 2018, doi: 10.1016/S1474-4422(18)30287-4.

[26] P. E. Crago *et al.*, “An elbow extension neuroprosthesis for individuals with tetraplegia,” *IEEE Trans Rehabil Eng*, vol. 6, no. 1, pp. 1–6, Mar. 1998, doi: 10.1109/86.662614.

[27] M. A. Robinson, G. J. Barton, A. Lees, and P. Sett, “Analysis of tetraplegic reaching in their 3D workspace following posterior deltoid-triceps tendon transfer,” *Spinal Cord*, vol. 48, no. 8, Art. no. 8, Aug. 2010, doi: 10.1038/sc.2009.193.

[28] J. F. Ditunno, S. L. Stover, M. M. Freed, and J. H. Ahn, “Motor recovery of the upper extremities in traumatic quadriplegia: A multicenter study,” *Archives of Physical Medicine and Rehabilitation*, vol. 73, no. 5, pp. 431–436, May 1992, doi: 10.5555/uri:pii:000399939290030Z.

[29] B. Calancie, M. R. Molano, and J. G. Broton, “EMG for assessing the recovery of voluntary movement after acute spinal cord injury in man,” *Clinical Neurophysiology*, vol. 115, no. 8, pp. 1748–1759, Aug. 2004, doi: 10.1016/j.clinph.2004.03.002.

[30] S. Sangari and M. A. Perez, “Distinct Corticospinal and Reticulospinal Contributions to Voluntary Control of Elbow Flexor and Extensor Muscles in Humans with Tetraplegia,” *J Neurosci*, vol. 40, no. 46, pp. 8831–8841, Nov. 2020, doi: 10.1523/JNEUROSCI.1107-20.2020.

[31] C.-C. Tsao and M. M. Mirbagheri, “Upper limb impairments associated with spasticity in neurological disorders,” *J Neuroeng Rehabil*, vol. 4, p. 45, Nov. 2007, doi: 10.1186/1743-0003-4-45.

[32] C. K. Thomas, R. Bakels, C. S. Klein, and I. Zijdewind, “Human spinal cord injury: motor unit properties and behaviour,” *Acta Physiol (Oxf)*, vol. 210, no. 1, pp. 5–19, Jan. 2014, doi: 10.1111/apha.12153.

[33] S. Cremoux, D. Amarantini, J. Tallet, F. Dal Maso, and E. Berton, “Increased antagonist muscle activity in cervical SCI patients suggests altered reciprocal inhibition during elbow contractions,” *Clin Neurophysiol*, vol. 127, no. 1, pp. 629–634, Jan. 2016, doi: 10.1016/j.clinph.2015.03.016.

[34] A. W. Wiegner, M. M. Wierzbicka, L. Davies, and R. R. Young, “Discharge properties of single motor units in patients with spinal cord injuries,” *Muscle Nerve*, vol. 16, no. 6, pp. 661–671, Jun. 1993, doi: 10.1002/mus.880160613.

[35] S. H. Kozin, L. D’Addesi, R. S. Chafetz, S. Ashworth, and M. J. Mulcahey, “Biceps-to-triceps transfer for elbow extension in persons with tetraplegia,” *J Hand Surg Am*, vol. 35, no. 6, pp. 968–975, Jun. 2010, doi: 10.1016/j.jhsa.2010.03.011.

[36] J. Medina, A. Marcos-García, I. Jiménez, G. Muratore, and J. L. Méndez-Suárez, “Biceps to Triceps Transfer in Tetraplegic Patients: Our Experience and Review of the Literature,” *Hand (N Y)*, vol. 12, no. 1, pp. 85–90, Jan. 2017, doi: 10.1177/1558944716646764.

[37] R. D. Endress and V. R. Hentz, “Biceps-to-triceps transfer technique,” *J Hand Surg Am*, vol. 36, no. 4, pp. 716–721, Apr. 2011, doi: 10.1016/j.jhsa.2011.01.028.

[38] J. Fridén and A. Gohritz, “Tetraplegia Management Update,” *The Journal of Hand Surgery*, vol. 40, no. 12, pp. 2489–2500, Dec. 2015, doi: 10.1016/j.jhsa.2015.06.003.

[39] J. M. Khalifeh *et al.*, “Nerve transfers in the upper extremity following cervical spinal cord injury. Part 1: Systematic review of the literature,” *J Neurosurg Spine*, pp. 1–12, Jul. 2019, doi: 10.3171/2019.4.SPINE19173.

[40] R. T. Lewinson, A. Ganesh, and M. M. C. Yeung, “The Biomechanics of Manual Muscle Testing in the Neuromuscular Exam,” *Canadian Journal of Neurological Sciences*, vol. 45, no. 5, pp. 518–521, Sep. 2018, doi: 10.1017/cjn.2018.53.

[41] B. M. Needham-Shropshire, K. J. Klose, M. E. Tucker, and C. K. Thomas, “Manual muscle test score and force comparisons after cervical spinal cord injury,” *J Spinal Cord Med*, vol. 20, no. 3, pp. 324–330, Jul. 1997, doi: 10.1080/10790268.1997.11719483.

[42] V. Di Lazzaro and U. Ziemann, “The contribution of transcranial magnetic stimulation in the functional evaluation of microcircuits in human motor cortex,” *Front Neural Circuits*, vol. 7, p. 18, 2013, doi: 10.3389/fncir.2013.00018.

[43] Y. Terao and Y. Ugawa, “Basic mechanisms of TMS,” *J Clin Neurophysiol*, vol. 19, no. 4, pp. 322–343, Aug. 2002, doi: 10.1097/00004691-200208000-00006.

[44] A. Auriat, J. Neva, S. Peters, J. Ferris, and L. A Boyd, *A Review of Transcranial Magnetic Stimulation and Multimodal Neuroimaging to Characterize Post-Stroke Neuroplasticity*, vol. 6. 2015. doi: 10.3389/fneur.2015.00226.

[45] W. Klomjai, R. Katz, and A. Lackmy-Vallée, “Basic principles of transcranial magnetic stimulation (TMS) and repetitive TMS (rTMS),” *Ann Phys Rehabil Med*, vol. 58, no. 4, pp. 208–213, Sep. 2015, doi: 10.1016/j.rehab.2015.05.005.

[46] S. Bestmann and J. W. Krakauer, “The uses and interpretations of the motor-evoked potential for understanding behaviour,” *Exp Brain Res*, vol. 233, no. 3, pp. 679–689, Mar. 2015, doi: 10.1007/s00221-014-4183-7.

[47] A. J. Pearce and D. J. Kidgell, “Corticomotor excitability during precision motor tasks,” *J Sci Med Sport*, vol. 12, no. 2, pp. 280–283, Mar. 2009, doi: 10.1016/j.jsams.2007.12.005.

[48] M. A. Perez and L. G. Cohen, “Scaling of motor cortical excitability during unimanual force generation,” *Cortex*, vol. 45, no. 9, pp. 1065–1071, Oct. 2009, doi: 10.1016/j.cortex.2008.12.006.

[49] W. G. Darling, S. L. Wolf, and A. J. Butler, “Variability of motor potentials evoked by transcranial magnetic stimulation depends on muscle activation,” *Exp Brain Res*, vol. 174, no. 2, pp. 376–385, Sep. 2006, doi: 10.1007/s00221-006-0468-9.

[50] A. A. de Goede, E. M. ter Braack, and M. J. A. M. van Putten, “Accurate Coil Positioning is Important for Single and Paired Pulse TMS on the Subject Level,” *Brain Topogr*, vol. 31, no. 6, pp. 917–930, 2018, doi: 10.1007/s10548-018-0655-6.

[51] S. Schmidt, R. Bathe-Peters, R. Fleischmann, M. Rönnefarth, M. Scholz, and S. A. Brandt, “Nonphysiological factors in navigated TMS studies; confounding covariates and valid intracortical estimates,” *Hum Brain Mapp*, vol. 36, no. 1, pp. 40–49, Jan. 2015, doi: 10.1002/hbm.22611.

[52] V. Di Lazzaro, U. Ziemann, and R. N. Lemon, “State of the art: Physiology of transcranial motor cortex stimulation,” *Brain Stimul*, vol. 1, no. 4, pp. 345–362, Oct. 2008, doi: 10.1016/j.brs.2008.07.004.

[53] C. R. Jutzeler *et al.*, “Sensorimotor plasticity after spinal cord injury: a longitudinal and translational study,” *Ann Clin Transl Neurol*, vol. 6, no. 1, pp. 68–82, Dec. 2018, doi: 10.1002/acn3.679.

[54] C. Tscherpel, S. Dern, L. Hensel, U. Ziemann, G. R. Fink, and C. Grefkes, “Brain responsivity provides an individual readout for motor recovery after stroke,” *Brain*, vol. 143, no. 6, pp. 1873–1888, Jun. 2020, doi: 10.1093/brain/awaa127.

[55] C. Stinear, “Prediction of recovery of motor function after stroke,” *Lancet Neurol*, vol. 9, no. 12, pp. 1228–1232, Dec. 2010, doi: 10.1016/S1474-4422(10)70247-7.

[56] H. M. Schambra *et al.*, “Differential Poststroke Motor Recovery in an Arm Versus Hand Muscle in the Absence of Motor Evoked Potentials,” *Neurorehabil Neural Repair*, vol. 33, no. 7, pp. 568–580, Jul. 2019, doi: 10.1177/1545968319850138.

[57] B. Hordacre, R. Ghosh, M. R. Goldsworthy, and M. C. Ridding, “Transcranial Magnetic Stimulation-EEG Biomarkers of Poststroke Upper-Limb Motor Function,” *J Stroke Cerebrovasc Dis*, vol. 28, no. 12, p. 104452, Dec. 2019, doi: 10.1016/j.jstrokecerebrovasdis.2019.104452.

[58] A. Benussi *et al.*, “Classification Accuracy of Transcranial Magnetic Stimulation for the Diagnosis of Neurodegenerative Dementias,” *Ann Neurol*, vol. 87, no. 3, pp. 394–404, Mar. 2020, doi: 10.1002/ana.25677.

[59] M. Kobayashi and A. Pascual-Leone, “Transcranial magnetic stimulation in neurology,” *The Lancet Neurology*, vol. 2, no. 3, pp. 145–156, Mar. 2003, doi: 10.1016/S1474-4422(03)00321-1.

[60] U. Ziemann, “Intracortical inhibition and facilitation in the conventional paired TMS paradigm,” *Electroencephalogr Clin Neurophysiol Suppl*, vol. 51, pp. 127–136, 1999.

[61] U. Ziemann, J. C. Rothwell, and M. C. Ridding, “Interaction between intracortical inhibition and facilitation in human motor cortex.,” *The Journal of Physiology*, vol. 496, no. 3, pp. 873–881, 1996, doi: https://doi.org/10.1113/jphysiol.1996.sp021734.

[62] R. Hanajima *et al.*, “Paired-pulse magnetic stimulation of the human motor cortex: differences among I waves,” *The Journal of Physiology*, vol. 509, no. 2, pp. 607–618, 1998, doi: https://doi.org/10.1111/j.1469-7793.1998.607bn.x.

[63] N. J. Davey *et al.*, “Responses of thenar muscles to transcranial magnetic stimulation of the motor cortex in patients with incomplete spinal cord injury,” *J Neurol Neurosurg Psychiatry*, vol. 65, no. 1, pp. 80–87, Jul. 1998, doi: 10.1136/jnnp.65.1.80.

[64] E. Saturno, C. Bonato, C. Miniussi, V. Lazzaro, and L. Callea, “Motor cortex changes in spinal cord injury: a TMS study,” *Neurological Research*, vol. 30, no. 10, pp. 1084–1085, Dec. 2008, doi: 10.1179/174313208X332968.

[65] H. C. Smith *et al.*, “Modulation of single motor unit discharges using magnetic stimulation of the motor cortex in incomplete spinal cord injury,” *Journal of Neurology, Neurosurgery & Psychiatry*, vol. 68, no. 4, pp. 516–520, Apr. 2000, doi: 10.1136/jnnp.68.4.516.

[66] M. C. Ridding and J. C. Rothwell, “Stimulus/response curves as a method of measuring motor cortical excitability in man,” *Electroencephalography and Clinical Neurophysiology/Electromyography and Motor Control*, vol. 105, no. 5, pp. 340–344, Oct. 1997, doi: 10.1016/S0924-980X(97)00041-6.

[67] B. Boroojerdi, F. Battaglia, W. Muellbacher, and L. G. Cohen, “Mechanisms influencing stimulus-response properties of the human corticospinal system,” *Clinical Neurophysiology*, vol. 112, no. 5, pp. 931–937, May 2001, doi: 10.1016/S1388-2457(01)00523-5.

[68] H. Devanne, B. A. Lavoie, and C. Capaday, “Input-output properties and gain changes in the human corticospinal pathway,” *Exp Brain Res*, vol. 114, no. 2, pp. 329–338, Apr. 1997, doi: 10.1007/pl00005641.

[69] D. R. Roberts *et al.*, “Cerebral Cortex Plasticity After 90 Days of Bed Rest: Data from TMS and fMRI,” *Aviation, Space, and Environmental Medicine*, vol. 81, no. 1, pp. 30–40, Jan. 2010, doi: 10.3357/ASEM.2532.2009.

[70] N. S. Ward *et al.*, “Motor system activation after subcortical stroke depends on corticospinal system integrity,” *Brain*, vol. 129, no. 3, pp. 809–819, Mar. 2006, doi: 10.1093/brain/awl002.

[71] F. Hummel *et al.*, “Effects of non-invasive cortical stimulation on skilled motor function in chronic stroke,” *Brain*, vol. 128, no. 3, pp. 490–499, Mar. 2005, doi: 10.1093/brain/awh369.

[72] U. Herwig, F. Padberg, J. Unger, M. Spitzer, and C. Schönfeldt-Lecuona, “Transcranial magnetic stimulation in therapy studies: examination of the reliability of ‘standard’ coil positioning by neuronavigation,” *Biological Psychiatry*, vol. 50, no. 1, pp. 58–61, Jul. 2001, doi: 10.1016/S0006-3223(01)01153-2.

[73] U. Herwig *et al.*, “The navigation of transcranial magnetic stimulation,” *Psychiatry Research: Neuroimaging*, vol. 108, no. 2, pp. 123–131, Nov. 2001, doi: 10.1016/S0925-4927(01)00121-4.

[74] G. J. Ettinger *et al.*, “Experimentation with a transcranial magnetic stimulation system for functional brain mapping,” p. 10.

[75] J. Ruohonen and J. Karhu, “Navigated transcranial magnetic stimulation,” *Neurophysiologie Clinique/Clinical Neurophysiology*, vol. 40, no. 1, pp. 7–17, Mar. 2010, doi: 10.1016/j.neucli.2010.01.006.

[76] E. Vaghefi, P. Cai, F. Fang, W. D. Byblow, C. M. Stinear, and B. Thompson, “MRI Guided Brain Stimulation without the Use of a Neuronavigation System,” *Biomed Res Int*, vol. 2015, p. 647510, 2015, doi: 10.1155/2015/647510.

[77] J. Rodseth, E. P. Washabaugh, and C. Krishnan, “A novel low-cost approach for navigated transcranial magnetic stimulation,” *Restor Neurol Neurosci*, vol. 35, no. 6, pp. 601–609, 2017, doi: 10.3233/RNN-170751.

[78] G. W. Thickbroom, M. L. Byrnes, and F. L. Mastaglia, “A model of the effect of MEP amplitude variation on the accuracy of TMS mapping,” *Clinical Neurophysiology*, vol. 110, no. 5, pp. 941–943, May 1999, doi: 10.1016/S1388-2457(98)00080-7.

[79] L. Kiers, D. Cros, K. H. Chiappa, and J. Fang, “Variability of motor potentials evoked by transcranial magnetic stimulation,” *Electroencephalography and Clinical Neurophysiology/Evoked Potentials Section*, vol. 89, no. 6, pp. 415–423, 1993.

[80] N. H. Jung, I. Delvendahl, N. G. Kuhnke, D. Hauschke, S. Stolle, and V. Mall, “Navigated transcranial magnetic stimulation does not decrease the variability of motor-evoked potentials,” *Brain Stimulation*, vol. 3, no. 2, pp. 87–94, Apr. 2010, doi: 10.1016/j.brs.2009.10.003.

[81] G. M. Allen, D. K. McKenzie, and S. C. Gandevia, “Twitch interpolation of the elbow flexor muscles at high forces,” *Muscle & Nerve: Official Journal of the American Association of Electrodiagnostic Medicine*, vol. 21, no. 3, pp. 318–328, 1998.

[82] S. C. Gandevia, R. M. Enoka, A. J. McComas, D. G. Stuart, and C. K. Thomas, “Neurobiology of muscle fatigue,” in *Fatigue*, 1995, pp. 515–525.

[83] S. C. Gandevia, “Spinal and Supraspinal Factors in Human Muscle Fatigue,” *Physiological Reviews*, vol. 81, no. 4, pp. 1725–1789, Jan. 2001, doi: 10.1152/physrev.2001.81.4.1725.

[84] G. M. Allen, S. C. Gandevia, and D. K. McKenzie, “Reliability of measurements of muscle strength and voluntary activation using twitch interpolation,” *Muscle & Nerve*, vol. 18, no. 6, pp. 593–600, 1995, doi: 10.1002/mus.880180605.

[85] G. Hoffmann, M. O. Conrad, D. Qiu, and D. G. Kamper, “Contributions of voluntary activation deficits to hand weakness after stroke,” *Top Stroke Rehabil*, vol. 23, no. 6, pp. 384–392, Dec. 2016, doi: 10.1179/1945511915Y.0000000023.

[86] K.-H. Lin, Y.-C. Chen, J.-J. Luh, C.-H. Wang, and Y.-J. Chang, “H-Reflex, Muscle Voluntary Activation Level, and Fatigue Index of Flexor Carpi Radialis in Individuals With Incomplete Cervical Cord Injury,” *Neurorehabil Neural Repair*, vol. 26, no. 1, pp. 68–75, Jan. 2012, doi: 10.1177/1545968311418785.

[87] S. C. Dongés, C. L. Boswell-Ruys, J. E. Butler, and J. L. Taylor, “The effect of paired corticospinal-motoneuronal stimulation on maximal voluntary elbow flexion in cervical spinal cord injury: an experimental study.,” *Spinal Cord*, May 2019, doi: 10.1038/s41393-019-0291-3.

[88] C. K. Thomas, E. Y. Zaidner, B. Calancie, J. G. Broton, and B. R. Bigland-Ritchie, “Muscle weakness, paralysis, and atrophy after human cervical spinal cord injury,” *Exp. Neurol.*, vol. 148, no. 2, pp. 414–423, Dec. 1997, doi: 10.1006/exnr.1997.6690.

[89] G. Todd, J. L. Taylor, and S. C. Gandevia, “Measurement of voluntary activation of fresh and fatigued human muscles using transcranial magnetic stimulation,” *The Journal of Physiology*, vol. 551, no. 2, pp. 661–671, 2003, doi: 10.1113/jphysiol.2003.044099.

[90] M. Jubeau *et al.*, “Changes in Voluntary Activation Assessed by Transcranial Magnetic Stimulation during Prolonged Cycling Exercise,” *PLOS ONE*, vol. 9, no. 2, p. e89157, Feb. 2014, doi: 10.1371/journal.pone.0089157.

[91] L. Girompaire, B. Morel, and T. Lapole, “Reduced cortical voluntary activation during bilateral knee extension,” *Hum Mov Sci*, vol. 52, pp. 17–23, Apr. 2017, doi: 10.1016/j.humov.2017.01.005.

[92] M. Lee, S. C. Gandevia, and T. J. Carroll, “Short-term strength training does not change cortical voluntary activation,” *Med Sci Sports Exerc*, vol. 41, no. 7, pp. 1452–1460, Jul. 2009, doi: 10.1249/MSS.0b013e3181998837.

[93] M. Lee, S. C. Gandevia, and T. J. Carroll, “Unilateral strength training increases voluntary activation of the opposite untrained limb,” *Clin Neurophysiol*, vol. 120, no. 4, pp. 802–808, Apr. 2009, doi: 10.1016/j.clinph.2009.01.002.

[94] L.-S. Giboin, P. Thumm, R. Bertschinger, and M. Gruber, “Intermittent Theta Burst Over M1 May Increase Peak Power of a Wingate Anaerobic Test and Prevent the Reduction of Voluntary Activation Measured with Transcranial Magnetic Stimulation,” *Front Behav Neurosci*, vol. 10, p. 150, 2016, doi: 10.3389/fnbeh.2016.00150.

[95] V. Rozand, C. W. Sundberg, S. K. Hunter, and A. E. Smith, “Age-related deficits in voluntary activation: a systematic review and meta-analysis,” *Med. Sci. Sports Exerc*, vol. 52, pp. 549–560, 2020.

[96] B. C. Clark, J. L. Taylor, S. L. Hong, T. D. Law, and D. W. Russ, “Weaker Seniors Exhibit Motor Cortex Hypoexcitability and Impairments in Voluntary Activation,” *The Journals of Gerontology: Series A*, vol. 70, no. 9, pp. 1112–1119, Sep. 2015, doi: 10.1093/gerona/glv030.

[97] J. L. Bowden, J. L. Taylor, and P. A. McNulty, “Voluntary Activation is Reduced in Both the More- and Less-Affected Upper Limbs after Unilateral Stroke,” *Front. Neurol.*, vol. 5, 2014, doi: 10.3389/fneur.2014.00239.

[98] K. C. Powers, M. E. Cinelli, and J. M. Kalmar, “Cortical hypoexcitability persists beyond the symptomatic phase of a concussion,” *Brain Injury*, vol. 28, no. 4, pp. 465–471, Apr. 2014, doi: 10.3109/02699052.2014.888759.

[99] M. Kingett, K. Holt, I. K. Niazi, R. W. Nedergaard, M. Lee, and H. Haavik, “Increased Voluntary Activation of the Elbow Flexors Following a Single Session of Spinal Manipulation in a Subclinical Neck Pain Population,” *Brain Sci*, vol. 9, no. 6, Jun. 2019, doi: 10.3390/brainsci9060136.

[100] S. Y. Chiou, Y. F. Shih, L. W. Chou, A. H. McGregor, and P. H. Strutton, “Impaired neural drive in patients with low back pain,” *Eur J Pain*, vol. 18, no. 6, pp. 794–802, Jul. 2014, doi: 10.1002/j.1532-2149.2013.00428.x.

[101] D. Urbach, A. Berth, and F. Awiszus, “Effect of transcranial magnetic stimulation on voluntary activation in patients with quadriceps weakness,” *Muscle Nerve*, vol. 32, no. 2, pp. 164–169, Aug. 2005, doi: 10.1002/mus.20353.

[102] G. Todd, J. L. Taylor, and S. C. Gandevia, “Measurement of voluntary activation based on transcranial magnetic stimulation over the motor cortex,” *J. Appl. Physiol.*, vol. 121, no. 3, pp. 678–686, Sep. 2016, doi: 10.1152/japplphysiol.00293.2016.

[103] J. L. Nuzzo, D. S. Kennedy, H. T. Finn, and J. L. Taylor, “Voluntary activation of knee extensor muscles with transcranial magnetic stimulation,” *J Appl Physiol (1985)*, vol. 130, no. 3, pp. 589–604, Mar. 2021, doi: 10.1152/japplphysiol.00717.2020.

[104] J. Lagan, P. Lang, and P. H. Strutton, “Measurement of voluntary activation of the back muscles using transcranial magnetic stimulation,” *Clin Neurophysiol*, vol. 119, no. 12, pp. 2839–2845, Dec. 2008, doi: 10.1016/j.clinph.2008.09.013.

[105] J. Mira, T. Lapole, R. Souron, L. Messonnier, G. Y. Millet, and T. Rupp, “Cortical voluntary activation testing methodology impacts central fatigue,” *Eur J Appl Physiol*, vol. 117, no. 9, pp. 1845–1857, Sep. 2017, doi: 10.1007/s00421-017-3678-x.

[106] J. Dekerle, P. Ansdell, L. Schäfer, A. Greenhouse-Tucknott, and J. Wrightson, “Methodological issues with the assessment of voluntary activation using transcranial magnetic stimulation in the knee extensors,” *Eur J Appl Physiol*, vol. 119, no. 4, pp. 991–1005, Apr. 2019, doi: 10.1007/s00421-019-04089-7.

[107] D. Bachasson *et al.*, “Transcranial magnetic stimulation intensity affects exercise-induced changes in corticomotoneuronal excitability and inhibition and voluntary activation,” *Neuroscience*, vol. 314, pp. 125–133, Feb. 2016, doi: 10.1016/j.neuroscience.2015.11.056.

[108] R. Souron, A. Farabet, L. Féasson, A. Belli, G. Y. Millet, and T. Lapole, “Eight weeks of local vibration training increases dorsiflexor muscle cortical voluntary activation,” *Journal of Applied Physiology*, vol. 122, no. 6, pp. 1504–1515, Apr. 2017, doi: 10.1152/japplphysiol.00793.2016.

[109] G. Todd, J. L. Taylor, and S. C. Gandevia, “Reproducible measurement of voluntary activation of human elbow flexors with motor cortical stimulation,” *Journal of Applied Physiology*, vol. 97, no. 1, pp. 236–242, Jul. 2004, doi: 10.1152/japplphysiol.01336.2003.

[110] B. K. Barry, Z. A. Riley, M. A. Pascoe, and R. M. Enoka, “A spinal pathway between synergists can modulate activity in human elbow flexor muscles,” *Exp Brain Res*, vol. 190, no. 3, pp. 347–359, Sep. 2008, doi: 10.1007/s00221-008-1479-5.

[111] J. P. M. Mogk, L. M. Rogers, W. M. Murray, E. J. Perreault, and J. W. Stinear, “Corticomotor excitability of arm muscles modulates according to static position and orientation of the upper limb,” *Clinical Neurophysiology*, vol. 125, no. 10, pp. 2046–2054, Oct. 2014, doi: 10.1016/j.clinph.2014.02.007.

[112] J. L. Nuzzo, G. S. Trajano, B. K. Barry, S. C. Gandevia, and J. L. Taylor, “Arm posture-dependent changes in corticospinal excitability are largely spinal in origin,” *Journal of Neurophysiology*, vol. 115, no. 4, pp. 2076–2082, Feb. 2016, doi: 10.1152/jn.00885.2015.

[113] S. C. Dongés, J. L. Taylor, and J. L. Nuzzo, “Elbow angle modulates corticospinal excitability to the resting biceps brachii at both spinal and supraspinal levels,” *Exp Physiol*, vol. 104, no. 4, pp. 546–555, 2019, doi: 10.1113/EP087472.

[114] S. C. Schwerin, J. Yao, and J. P. A. Dewald, “Using paired pulse TMS to facilitate contralateral and ipsilateral MEPs in upper extremity muscles of chronic hemiparetic stroke patients,” *J Neurosci Methods*, vol. 195, no. 2, pp. 151–160, Feb. 2011, doi: 10.1016/j.jneumeth.2010.11.021.

[115] R. Nardone *et al.*, “Spinal cord injury affects I-wave facilitation in human motor cortex,” *Brain Res Bull*, vol. 116, pp. 93–97, Jul. 2015, doi: 10.1016/j.brainresbull.2015.06.006.

[116] E. W. J. Cadigan, B. W. Collins, D. T. G. Philpott, G. Kippenhuck, M. Brenton, and D. C. Button, “Maximal Voluntary Activation of the Elbow Flexors Is under Predicted by Transcranial Magnetic Stimulation Compared to Motor Point Stimulation Prior to and Following Muscle Fatigue,” *Front. Physiol.*, vol. 8, 2017, doi: 10.3389/fphys.2017.00707.

[117] R. Nardone *et al.*, “Assessment of corticospinal excitability after traumatic spinal cord injury using MEP recruitment curves: a preliminary TMS study,” *Spinal Cord*, vol. 53, no. 7, Art. no. 7, Jul. 2015, doi: 10.1038/sc.2015.12.

[118] P. Menon, M. C. Kiernan, and S. Vucic, “Cortical excitability varies across different muscles,” *Journal of Neurophysiology*, vol. 120, no. 3, pp. 1397–1403, Sep. 2018, doi: 10.1152/jn.00148.2018.

[119] B. Calancie, N. Alexeeva, J. G. Broton, S. Suys, A. Hall, and K. J. Klose, “Distribution and Latency of Muscle Responses to Transcranial Magnetic Stimulation of Motor Cortex After Spinal

Cord Injury in Humans," *Journal of Neurotrauma*, vol. 16, no. 1, pp. 49–67, Jan. 1999, doi: 10.1089/neu.1999.16.49.

[120] H. J. Fassett, C. V. Turco, J. El-Sayes, and A. J. Nelson, "Alterations in Motor Cortical Representation of Muscles Following Incomplete Spinal Cord Injury in Humans," *Brain Sci*, vol. 8, no. 12, Dec. 2018, doi: 10.3390/brainsci8120225.

[121] V. Sankarasubramanian *et al.*, "Reproducibility of transcranial magnetic stimulation metrics in the study of proximal upper limb muscles," *J Electromyogr Kinesiol*, vol. 25, no. 5, pp. 754–764, Oct. 2015, doi: 10.1016/j.jelekin.2015.05.006.

[122] S. C. Gandevia, G. M. Allen, and D. K. McKenzie, "Central fatigue. Critical issues, quantification and practical implications," *Adv. Exp. Med. Biol.*, vol. 384, pp. 281–294, 1995.

[123] S. K. Hunter, G. Todd, J. E. Butler, S. C. Gandevia, and J. L. Taylor, "Recovery from supraspinal fatigue is slowed in old adults after fatiguing maximal isometric contractions," *Journal of Applied Physiology*, vol. 105, no. 4, pp. 1199–1209, 2008.

[124] L. R. P. Garmirian, A. M. Acosta, N. M. Hill, and J. P. A. Dewald, "Estimating Voluntary Activation Of The Elbow And Wrist Muscles In Chronic Hemiparetic Stroke Using Twitch Interpolation Methodology," in *2018 40th Annual International Conference of the IEEE Engineering in Medicine and Biology Society (EMBC)*, Jul. 2018, pp. 2244–2247. doi: 10.1109/EMBC.2018.8512791.

[125] C. L. Peterson, M. S. Bednar, A. M. Bryden, M. W. Keith, E. J. Perreault, and W. M. Murray, "Voluntary activation of biceps-to-triceps and deltoid-to-triceps transfers in quadriplegia," *PLOS ONE*, vol. 12, no. 3, p. e0171141, Mar. 2017, doi: 10.1371/journal.pone.0171141.

[126] R. D. Herbert and S. C. Gandevia, "Twitch interpolation in human muscles: mechanisms and implications for measurement of voluntary activation," *Journal of Neurophysiology*, vol. 82, no. 5, pp. 2271–2283, 1999.

[127] J. Dekerle, P. Ansdell, L. Schäfer, A. Greenhouse-Tucknott, and J. Wrightson, "Methodological issues with the assessment of voluntary activation using transcranial magnetic stimulation in the knee extensors," *European journal of applied physiology*, vol. 119, no. 4, pp. 991–1005, 2019.

[128] M. Oudega and M. A. Perez, "Corticospinal reorganization after spinal cord injury," *The Journal of Physiology*, vol. 590, no. 16, pp. 3647–3663, 2012, doi: 10.1113/jphysiol.2012.233189.

[129] G. Todd, J. L. Taylor, and S. C. Gandevia, "Measurement of voluntary activation of fresh and fatigued human muscles using transcranial magnetic stimulation," *The Journal of Physiology*, vol. 551, no. 2, pp. 661–671, 2003, doi: 10.1113/jphysiol.2003.044099.

[130] E. W. J. Cadigan, B. W. Collins, D. T. G. Philpott, G. Kippenhuck, M. Brenton, and D. C. Button, "Maximal voluntary activation of the elbow flexors is under predicted by transcranial magnetic stimulation compared to motor point stimulation prior to and following muscle fatigue," *Frontiers in Physiology*, vol. 8, no. SEP, pp. 1–11, 2017, doi: 10.3389/fphys.2017.00707.

[131] G. Wu *et al.*, "ISB recommendation on definitions of joint coordinate systems of various joints for the reporting of human joint motion—Part II: shoulder, elbow, wrist and hand," *Journal of biomechanics*, vol. 38, no. 5, pp. 981–992, 2005.

[132] S. J. Aboodarda, D. B. Copithorne, G. E. P. Pearcey, D. C. Button, and K. E. Power, "Changes in supraspinal and spinal excitability of the biceps brachii following brief, non-fatiguing submaximal contractions of the elbow flexors in resistance-trained males," *Neurosci. Lett.*, vol. 607, pp. 66–71, Oct. 2015, doi: 10.1016/j.neulet.2015.09.028.

[133] M. Schecklmann, M. Schmaußer, F. Klinger, P. M. Kreuzer, L. Krenkel, and B. Langguth, "Resting motor threshold and magnetic field output of the figure-of-8 and the double-cone coil," *Scientific Reports*, vol. 10, no. 1, Art. no. 1, Feb. 2020, doi: 10.1038/s41598-020-58034-2.

[134] M. Oliveri *et al.*, “Left frontal transcranial magnetic stimulation reduces contralesional extinction in patients with unilateral right brain damage,” *Brain*, vol. 122, no. 9, pp. 1731–1739, 1999.

[135] P. M. Rossini *et al.*, “Non-invasive electrical and magnetic stimulation of the brain, spinal cord and roots: basic principles and procedures for routine clinical application. Report of an IFCN committee,” *Electroencephalography and Clinical Neurophysiology*, vol. 91, no. 2, pp. 79–92, Aug. 1994, doi: 10.1016/0013-4694(94)90029-9.

[136] A. Frigon, T. J. Carroll, K. E. Jones, E. P. Zehr, and D. F. Collins, “Ankle position and voluntary contraction alter maximal M waves in soleus and tibialis anterior,” *Muscle & Nerve*, vol. 35, no. 6, pp. 756–766, 2007, doi: 10.1002/mus.20747.

[137] A. Lackmy and V. Marchand-Pauvert, “The estimation of short intra-cortical inhibition depends on the proportion of spinal motoneurones activated by corticospinal inputs,” *Clinical Neurophysiology*, vol. 121, no. 4, pp. 612–621, Apr. 2010, doi: 10.1016/j.clinph.2009.12.011.

[138] P. E. Shrout and J. L. Fleiss, “Intraclass correlations: uses in assessing rater reliability.,” *Psychological bulletin*, vol. 86, no. 2, p. 420, 1979.

[139] K. Mitsuhashi, K. Seki, C. Akamatsu, and Y. Handa, “Modulation of Excitability in the Cerebral Cortex Projecting to Upper Extremity Muscles by Rotational Positioning of the Forearm,” *Tohoku J. Exp. Med.*, vol. 212, no. 3, pp. 221–228, 2007, doi: 10.1620/tjem.212.221.

[140] V. D. Lazzaro *et al.*, “Effects of voluntary contraction on descending volleys evoked by transcranial stimulation in conscious humans,” *The Journal of Physiology*, vol. 508, no. 2, pp. 625–633, 1998, doi: 10.1111/j.1469-7793.1998.625bq.x.

[141] C. A. Pelletier and A. L. Hicks, “Muscle fatigue characteristics in paralyzed muscle after spinal cord injury,” *Spinal Cord*, vol. 49, no. 1, pp. 125–130, Jan. 2011, doi: 10.1038/sc.2010.62.

[142] M. F. Levin, J. M. Solomon, A. Shah, A. K. Blanchette, and A. G. Feldman, “Activation of elbow extensors during passive stretch of flexors in patients with post-stroke spasticity,” *Clinical Neurophysiology*, vol. 129, no. 10, pp. 2065–2074, Oct. 2018, doi: 10.1016/j.clinph.2018.07.007.

[143] T. K. Koo and M. Y. Li, “A Guideline of Selecting and Reporting Intraclass Correlation Coefficients for Reliability Research,” *J Chiropr Med*, vol. 15, no. 2, pp. 155–163, Jun. 2016, doi: 10.1016/j.jcm.2016.02.012.

[144] D. V. Cicchetti, “Guidelines, criteria, and rules of thumb for evaluating normed and standardized assessment instruments in psychology,” *Psychological Assessment*, vol. 6, no. 4, pp. 284–290, 1994, doi: 10.1037/1040-3590.6.4.284.

[145] M. Lee, S. C. Gandevia, and T. J. Carroll, “Cortical voluntary activation can be reliably measured in human wrist extensors using transcranial magnetic stimulation,” *Clinical Neurophysiology*, vol. 119, no. 5, pp. 1130–1138, 2008.

[146] S. K. Sidhu, D. J. Bentley, and T. J. Carroll, “Cortical voluntary activation of the human knee extensors can be reliably estimated using transcranial magnetic stimulation,” *Muscle & nerve*, vol. 39, no. 2, pp. 186–196, 2009.

[147] G. M. Allen, S. C. Gandevia, and D. K. McKenzie, “Reliability of measurements of muscle strength and voluntary activation using twitch interpolation,” *Muscle & nerve*, vol. 18, no. 6, pp. 593–600, 1995.

[148] C. K. Thomas, M. E. Tucker, and B. Bigland-Ritchie, “Voluntary Muscle Weakness and Co-Activation After Chronic Cervical Spinal Cord Injury,” *Journal of Neurotrauma*, vol. 15, no. 2, pp. 149–161, Feb. 1998, doi: 10.1089/neu.1998.15.149.

[149] H. van Duinen, S. C. Gandevia, and J. L. Taylor, “Voluntary activation of the different compartments of the flexor digitorum profundus,” *J. Neurophysiol.*, vol. 104, no. 6, pp. 3213–3221, Dec. 2010, doi: 10.1152/jn.00470.2010.

[150] M. Lee and T. J. Carroll, “The amplitude of Mmax in human wrist flexors varies during different muscle contractions despite constant posture,” *Journal of neuroscience methods*, vol. 149, no. 2, pp. 95–100, 2005.

[151] C. Crone, L. L. Johnsen, H. Hultborn, and G. B. Orsnes, “Amplitude of the maximum motor response (Mmax) in human muscles typically decreases during the course of an experiment,” *Exp Brain Res*, vol. 124, no. 2, pp. 265–270, Jan. 1999.

[152] M. Pensini and A. Martin, “Effect of voluntary contraction intensity on the H-reflex and V-wave responses,” *Neuroscience Letters*, vol. 367, no. 3, pp. 369–374, Sep. 2004, doi: 10.1016/j.neulet.2004.06.037.

[153] S. C. Gandevia, G. M. Allen, and D. K. McKenzie, “Central fatigue: Critical issues, quantification and practical implications,” *Advances in Experimental Medicine and Biology*, vol. 384. Springer New York LLC, pp. 281–294, 1995. doi: 10.1007/978-1-4899-1016-5_22.

[154] G. M. Allen, S. C. Gandevia, and D. K. McKenzie, “Reliability of measurements of muscle strength and voluntary activation using twitch interpolation,” *Muscle & Nerve*, vol. 18, no. 6, pp. 593–600, 1995, doi: 10.1002/mus.880180605.

[155] N. K. Vøllestad, “Measurement of human muscle fatigue,” *Journal of Neuroscience Methods*, vol. 74, no. 2, pp. 219–227, 1997, doi: 10.1016/S0165-0270(97)02251-6.

[156] S. K. Hunter, J. E. Butler, G. Todd, S. C. Gandevia, and J. L. Taylor, “Supraspinal fatigue does not explain the sex difference in muscle fatigue of maximal contractions.,” *Journal of applied physiology (Bethesda, Md. : 1985)*, vol. 101, no. 4, pp. 1036–1044, Oct. 2006, doi: 10.1152/japplphysiol.00103.2006.

[157] M. Hallett, “Transcranial Magnetic Stimulation: A Primer,” *Neuron*, vol. 55, no. 2, pp. 187–199, 2007, doi: 10.1016/j.neuron.2007.06.026.

[158] M. Kobayashi and A. Pascual-Leone, “Transcranial magnetic stimulation in neurology.,” *The Lancet. Neurology*, vol. 2, no. 3, pp. 145–156, Mar. 2003, doi: 10.1016/s1474-4422(03)00321-1.

[159] G. Todd, J. L. Taylor, and S. C. Gandevia, “Reproducible measurement of voluntary activation of human elbow flexors with motor cortical stimulation.,” *Journal of applied physiology (Bethesda, Md. : 1985)*, vol. 97, no. 1, pp. 236–242, 2004, doi: 10.1152/japplphysiol.01336.2003.

[160] G. Todd, J. L. Taylor, and S. C. Gandevia, “Measurement of voluntary activation based on transcranial magnetic stimulation over the motor cortex,” *Journal of Applied Physiology*, vol. 121, no. 3, pp. 678–686, 2016, doi: 10.1152/japplphysiol.00293.2016.

[161] J. L. Smith, P. G. Martin, S. C. Gandevia, and J. L. Taylor, “Sustained contraction at very low forces produces prominent supraspinal fatigue in human elbow flexor muscles,” *Journal of Applied Physiology*, vol. 103, no. 2, pp. 560–568, 2007, doi: 10.1152/japplphysiol.00220.2007.

[162] J. D. P. Ansdell, L. S. A. Greenhouse, and T. J. Wrightson, “Methodological issues with the assessment of voluntary activation using transcranial magnetic stimulation in the knee extensors,” *European Journal of Applied Physiology*, vol. 119, no. 4, pp. 991–1005, 2019, doi: 10.1007/s00421-019-04089-7.

[163] J. Mira, T. Lapole, R. Souron, L. Messonnier, G. Y. Millet, and T. Rupp, “Cortical voluntary activation testing methodology impacts central fatigue,” *European Journal of Applied Physiology*, vol. 117, no. 9, pp. 1845–1857, 2017, doi: 10.1007/s00421-017-3678-x.

[164] S. Kotan, S. Kojima, S. Miyaguchi, K. Sugawara, and H. Onishi, “Depression of corticomotor excitability after muscle fatigue induced by electrical stimulation and voluntary contraction,” vol. 9, no. June, pp. 1–7, 2015, doi: 10.3389/fnhum.2015.00363.

[165] G. Todd, J. E. Butler, S. C. Gandevia, and J. L. Taylor, “Decreased input to the motor cortex increases motor cortical excitability,” *Clinical Neurophysiology*, vol. 117, no. 11, pp. 2496–2503, 2006, doi: 10.1016/j.clinph.2006.07.303.

[166] C. K. Thomas, E. Y. Zaidner, B. Calancie, J. G. Broton, and B. R. Bigland-Ritchie, “Muscle weakness, paralysis, and atrophy after human cervical spinal cord injury,” *Exp Neurol*, vol. 148, no. 0014-4886 (Print), pp. 414–423, 1997, doi: 10.1006/exnr.1997.6690.

[167] J. P. Brasil-Neto, L. G. Cohen, M. Panizza, J. Nilsson, B. J. Roth, and M. Hallett, “Optimal focal transcranial magnetic activation of the human motor cortex: effects of coil orientation, shape of the induced current pulse, and stimulus intensity,” *J Clin Neurophysiol*, vol. 9, no. 1, pp. 132–136, Jan. 1992.

[168] J. Nielsen and Y. Kagamihara, “The regulation of presynaptic inhibition during co-contraction of antagonistic muscles in man,” *The Journal of Physiology*, vol. 464, no. 1, pp. 575–593, 1993, doi: https://doi.org/10.1113/jphysiol.1993.sp019652.

[169] S. H. N. L. H. L. O. D. Christensen and N. T. P. J. B. Nielsen, “Coupling of antagonistic ankle muscles during co-contraction in humans,” *Experimental brain research*, vol. 146, pp. 282–292, 2002, doi: 10.1007/s00221-002-1152-3.

[170] T. M. Kesar *et al.*, “Agonist-Antagonist Coactivation Enhances Corticomotor Excitability of Ankle Muscles,” vol. 2019, 2019, doi: 10.1155/2019/5190671.

[171] A. Rotenberg, J. C. Horvath, and A. Pascual-Leone, *Transcranial Magnetic Stimulation, Neuromethods, vol. 89*. 2014. doi: 10.1007 /978-1-4939-0879-0.

[172] Z. Deng, “Electromagnetic Field Modeling of Transcranial Electric and Magnetic Stimulation :,” 2013.

[173] C. A. Angeli, V. R. Edgerton, Y. P. Gerasimenko, S. J. Harkema, and A. F. Way, “Altering spinal cord excitability enables voluntary movements after chronic complete paralysis in humans,” pp. 1394–1409, 2014, doi: 10.1093/brain/awu038.

[174] R. Nardone *et al.*, “Spinal cord injury affects I-wave facilitation in human motor cortex,” *Brain Research Bulletin*, vol. 116, pp. 93–97, 2015, doi: 10.1016/j.brainresbull.2015.06.006.

[175] M. Oudega, M. A. Perez, M. Oudega, and M. A. Perez, “Corticospinal reorganization after spinal cord injury,” *J Physiol J Physiol J Physiol*, vol. 59016, no. 59016, pp. 3647–3663, 2012, doi: 10.1113/jphysiol.2012.233189.

[176] C. K. Thomas, M. E. Tucker, and B. R. Bigland-Ritchie, “Voluntary muscle weakness and co-activation after chronic cervical spinal cord injury.,” *Journal of neurotrauma*, vol. 15, no. 2, pp. 149–161, 1998, doi: 10.1089/neu.1998.15.149.

[177] S. C. Schwerin, J. Yao, and J. P. A. Dewald, “Using paired pulse TMS to facilitate contralateral and ipsilateral MEPs in upper extremity muscles of chronic hemiparetic stroke patients,” *Journal of Neuroscience Methods*, vol. 195, no. 2, pp. 151–160, 2011, doi: 10.1016/j.jneumeth.2010.11.021.

[178] B. Corwell *et al.*, “Intracortical Inhibition and Facilitation in Different Representations of the Human Motor Cortex,” *Journal of Neurophysiology*, pp. 2870–2881, 1998.

[179] A. Vahabzadeh-Hagh, “Paired-Pulse Transcranial Magnetic Stimulation (TMS) Protocols,” in *Transcranial Magnetic Stimulation*, A. Rotenberg, J. C. Horvath, and A. Pascual-Leone, Eds. New York, NY: Springer New York, 2014, pp. 117–127. doi: 10.1007/978-1-4939-0879-0_6.

[180] T. Kujirai *et al.*, “Corticocortical inhibition in human motor cortex,” *J Physiol*, vol. 471, pp. 501–519, Nov. 1993, doi: 10.1113/jphysiol.1993.sp019912.

[181] H. Nakamura, H. Kitagawa, Y. Kawaguchi, and H. Tsuji, “Intracortical facilitation and inhibition after transcranial magnetic stimulation in conscious humans.,” *J Physiol*, vol. 498, no. Pt 3, pp. 817–823, Feb. 1997.

[182] M. C. Ridding, J. L. Taylor, and J. C. Rothwell, “The effect of voluntary contraction on cortico-cortical inhibition in human motor cortex.,” *The Journal of Physiology*, vol. 487, no. 2, pp. 541–548, 1995, doi: 10.1113/jphysiol.1995.sp020898.

[183] M. D. Barry, K. L. Bunday, R. Chen, and M. A. Perez, “Selective Effects of Baclofen on Use-Dependent Modulation of GABAB Inhibition after Tetraplegia,” *Journal of Neuroscience*, vol. 33, no. 31, pp. 12898–12907, Jul. 2013, doi: 10.1523/JNEUROSCI.1552-13.2013.

[184] L. Sciavicco and B. Siciliano, “Kinematics,” in *Modelling and Control of Robot Manipulators*, London: Springer London, 2000, pp. 21–77. doi: 10.1007/978-1-4471-0449-0_2.

[185] P. M. Rossini *et al.*, “Applications of magnetic cortical stimulation. The International Federation of Clinical Neurophysiology.,” *Electroencephalography and clinical neurophysiology. Supplement*, vol. 52, pp. 171–185, 1999.

[186] C. B. Ah Sen, H. J. Fassett, J. El-Sayes, C. V Turco, M. M. Hameer, and A. J. Nelson, “Active and resting motor threshold are efficiently obtained with adaptive threshold hunting.,” *PloS one*, vol. 12, no. 10, p. e0186007, 2017, doi: 10.1371/journal.pone.0186007.

[187] S. K. Hunter, C. J. McNeil, J. E. Butler, S. C. Gandevia, and J. L. Taylor, “Short-interval cortical inhibition and intracortical facilitation during submaximal voluntary contractions changes with fatigue,” *Exp Brain Res*, vol. 234, no. 9, pp. 2541–2551, Sep. 2016, doi: 10.1007/s00221-016-4658-9.

[188] A. H. Herring, “Applied Longitudinal Analysis, 2nd Edition, by Garrett M. Fitzmaurice, Nan M. Laird, and James H. Ware, John Wiley & Sons, 2011,” *Journal of Biopharmaceutical Statistics*, vol. 23, no. 4, pp. 940–941, Jul. 2013, doi: 10.1080/10543406.2013.789817.

[189] P. G. Martin, S. C. Gandevia, and J. L. Taylor, “Output of Human Motoneuron Pools to Corticospinal Inputs During Voluntary Contractions,” *Journal of Neurophysiology*, vol. 95, no. 6, pp. 3512–3518, Jun. 2006, doi: 10.1152/jn.01230.2005.

[190] V. Di Lazzaro *et al.*, “Effects of voluntary contraction on descending volleys evoked by transcranial stimulation in conscious humans,” *J Physiol*, vol. 508 (Pt 2), pp. 625–633, Apr. 1998, doi: 10.1111/j.1469-7793.1998.625bq.x.

[191] T. V. Ilić, F. Meintzschel, U. Cleff, D. Ruge, K. R. Kessler, and U. Ziemann, “Short-interval paired-pulse inhibition and facilitation of human motor cortex: the dimension of stimulus intensity,” *J Physiol*, vol. 545, no. Pt 1, pp. 153–167, Nov. 2002, doi: 10.1113/jphysiol.2002.030122.

[192] R. Hanajima *et al.*, “Paired-pulse magnetic stimulation of the human motor cortex: differences among I waves,” *J Physiol*, vol. 509 (Pt 2), pp. 607–618, Jun. 1998, doi: 10.1111/j.1469-7793.1998.607bn.x.

[193] U. Ziemann, B. Corwell, and L. G. Cohen, “Modulation of plasticity in human motor cortex after forearm ischemic nerve block,” *J Neurosci*, vol. 18, no. 3, pp. 1115–1123, Feb. 1998.

[194] R. Vastano and M. A. Perez, “Changes in motoneuron excitability during voluntary muscle activity in humans with spinal cord injury,” *J Neurophysiol*, vol. 123, no. 2, pp. 454–461, Feb. 2020, doi: 10.1152/jn.00367.2019.

[195] R. M. Grumbles and C. K. Thomas, “Motoneuron Death after Human Spinal Cord Injury,” *J Neurotrauma*, vol. 34, no. 3, pp. 581–590, Feb. 2017, doi: 10.1089/neu.2015.4374.

[196] J. M. D’Amico, E. G. Condliffe, K. J. B. Martins, D. J. Bennett, and M. A. Gorassini, “Corrigendum: Recovery of neuronal and network excitability after spinal cord injury and implications for spasticity,” *Frontiers in Integrative Neuroscience*, vol. 8, p. 49, 2014, doi: 10.3389/fnint.2014.00049.

[197] F. D. Roy, E. T. Zewdie, and M. A. Gorassini, “Short-interval intracortical inhibition with incomplete spinal cord injury,” *Clin Neurophysiol*, vol. 122, no. 7, pp. 1387–1395, Jul. 2011, doi: 10.1016/j.clinph.2010.11.020.

[198] S. Sangari and M. A. Perez, “Imbalanced Corticospinal and Reticulospinal Contributions to Spasticity in Humans with Spinal Cord Injury,” *J Neurosci*, vol. 39, no. 40, pp. 7872–7881, Oct. 2019, doi: 10.1523/JNEUROSCI.1106-19.2019.

[199] F. D. Roy, E. T. Zewdie, and M. A. Gorassini, “Short-interval intracortical inhibition with incomplete spinal cord injury,” *Clinical Neurophysiology*, vol. 122, no. 7, pp. 1387–1395, 2011, doi: 10.1016/j.clinph.2010.11.020.

[200] J. A. Norton, D. J. Bennett, M. E. Knash, K. C. Murray, and M. A. Gorassini, “Changes in sensory-evoked synaptic activation of motoneurons after spinal cord injury in man,” *Brain*, vol. 131, no. Pt 6, pp. 1478–1491, Jun. 2008, doi: 10.1093/brain/awn050.

[201] P. Boulenguez *et al.*, “Down-regulation of the potassium-chloride cotransporter KCC2 contributes to spasticity after spinal cord injury,” *Nat Med*, vol. 16, no. 3, pp. 302–307, Mar. 2010, doi: 10.1038/nm.2107.

[202] M. N. McDonnell, Y. Orekhov, and U. Ziemann, “The role of GABA(B) receptors in intracortical inhibition in the human motor cortex,” *Exp Brain Res*, vol. 173, no. 1, pp. 86–93, Aug. 2006, doi: 10.1007/s00221-006-0365-2.

[203] D. Romaus-Sanjurjo, S. M. Valle-Maroto, A. Barreiro-Iglesias, B. Fernández-López, and M. C. Rodicio, “Anatomical recovery of the GABAergic system after a complete spinal cord injury in lampreys,” *Neuropharmacology*, vol. 131, pp. 389–402, Mar. 2018, doi: 10.1016/j.neuropharm.2018.01.006.

[204] C. S.-Y. Lin, V. G. Macefield, M. Elam, B. Gunnar Wallin, S. Engel, and M. C. Kiernan, “Axonal changes in spinal cord injured patients distal to the site of injury,” *Brain*, vol. 130, no. 4, pp. 985–994, 2007.

[205] H. Van De Meent, A. J. Hosman, J. Hendriks, M. Zwarts, and M. Schubert, “Severe Degeneration of Peripheral Motor Axons After Spinal Cord Injury: A European Multicenter Study in 345 Patients,” *Neurorehabil Neural Repair*, vol. 24, no. 7, pp. 657–665, Sep. 2010, doi: 10.1177/1545968310368534.

[206] A. Maruyama, K. Matsunaga, N. Tanaka, and J. C. Rothwell, “Muscle fatigue decreases short-interval intracortical inhibition after exhaustive intermittent tasks,” *Clin Neurophysiol*, vol. 117, no. 4, pp. 864–870, Apr. 2006, doi: 10.1016/j.clinph.2005.12.019.

[207] M. A. J. Van den Bos, P. Menon, J. Howells, N. Geevasinga, M. C. Kiernan, and S. Vucic, “Physiological Processes Underlying Short Interval Intracortical Facilitation in the Human Motor Cortex,” *Frontiers in Neuroscience*, vol. 12, p. 240, 2018, doi: 10.3389/fnins.2018.00240.

[208] S. N. Kukke, R. W. Paine, C. Chao, A. C. de Campos, and M. Hallett, “Efficient and reliable characterization of the corticospinal system using transcranial magnetic stimulation,” *J Clin Neurophysiol*, vol. 31, no. 3, pp. 246–252, Jun. 2014, doi: 10.1097/WNP.0000000000000057.

[209] M. Suzuki *et al.*, “Reciprocal changes in input–output curves of motor evoked potentials while learning motor skills,” *Brain Research*, vol. 1473, pp. 114–123, Sep. 2012, doi: 10.1016/j.brainres.2012.07.043.

[210] L. Christiansen, M. N. Larsen, M. J. Grey, J. B. Nielsen, and J. Lundbye-Jensen, “Long-term progressive motor skill training enhances corticospinal excitability for the ipsilateral hemisphere and motor performance of the untrained hand,” *Eur J Neurosci*, vol. 45, no. 12, pp. 1490–1500, Jun. 2017, doi: 10.1111/ejn.13409.

[211] M. A. Hunt, J. R. Zabukovec, S. Peters, C. L. Pollock, M. A. Linsdell, and L. A. Boyd, “Reduced Quadriceps Motor-Evoked Potentials in an Individual with Unilateral Knee Osteoarthritis: A Case Report,” *Case Reports in Rheumatology*, vol. 2011, p. e537420, Sep. 2011, doi: 10.1155/2011/537420.

[212] V. Di Lazzaro *et al.*, “The physiological basis of the effects of intermittent theta burst stimulation of the human motor cortex,” *J Physiol*, vol. 586, no. 16, pp. 3871–3879, Aug. 2008, doi: 10.1113/jphysiol.2008.152736.

[213] C. L. Massie and M. P. Malcolm, “Considerations for Stimulus–Response Curves in Stroke: An Investigation Comparing Collection and Analysis Methods,” *International Journal of Neuroscience*, vol. 123, no. 3, pp. 175–183, Jan. 2013, doi: 10.3109/00207454.2012.738734.

[214] J. L. Taylor and S. C. Gandevia, “Noninvasive stimulation of the human corticospinal tract,” *Journal of Applied Physiology*, vol. 96, no. 4, pp. 1496–1503, Apr. 2004, doi: 10.1152/japplphysiol.01116.2003.

[215] P. M. Rossini *et al.*, “Non-invasive electrical and magnetic stimulation of the brain, spinal cord, roots and peripheral nerves: Basic principles and procedures for routine clinical and research application. An updated report from an I.F.C.N. Committee,” *Clin Neurophysiol*, vol. 126, no. 6, pp. 1071–1107, Jun. 2015, doi: 10.1016/j.clinph.2015.02.001.

[216] L. Labruna, F. Lebon, J. Duque, P.-A. Klein, C. Cazares, and R. B. Ivry, “Generic inhibition of the selected movement and constrained inhibition of nonselected movements during response preparation,” *J Cogn Neurosci*, vol. 26, no. 2, pp. 269–278, Feb. 2014, doi: 10.1162/jocn_a_00492.

[217] B. L. Day *et al.*, “Electric and magnetic stimulation of human motor cortex: surface EMG and single motor unit responses,” *J Physiol*, vol. 412, pp. 449–473, May 1989, doi: 10.1113/jphysiol.1989.sp017626.

[218] J. M. D’Amico, E. G. Condliffe, K. J. B. Martins, D. J. Bennett, and M. A. Gorassini, “Recovery of neuronal and network excitability after spinal cord injury and implications for spasticity,” *Frontiers in Integrative Neuroscience*, vol. 8, p. 36, 2014, doi: 10.3389/fnint.2014.00036.

[219] U. Ziemann, “Chapter 32 - Pharmaco-transcranial magnetic stimulation studies of motor excitability,” in *Handbook of Clinical Neurology*, vol. 116, A. M. Lozano and M. Hallett, Eds. Elsevier, 2013, pp. 387–397. doi: 10.1016/B978-0-444-53497-2.00032-2.

[220] H. Liu and S. S. Y. Au-Yeung, “Reliability of transcranial magnetic stimulation induced corticomotor excitability measurements for a hand muscle in healthy and chronic stroke subjects,” *Journal of the Neurological Sciences*, vol. 341, no. 1, pp. 105–109, Jun. 2014, doi: 10.1016/j.jns.2014.04.012.

[221] J. B. Pitcher, K. M. Ogston, and T. S. Miles, “Age and sex differences in human motor cortex input–output characteristics,” *The Journal of Physiology*, vol. 546, no. 2, pp. 605–613, 2003, doi: 10.1113/jphysiol.2002.029454.

[222] I. Stetkarova and M. Kofler, “Differential effect of baclofen on cortical and spinal inhibitory circuits,” *Clin Neurophysiol*, vol. 124, no. 2, pp. 339–345, Feb. 2013, doi: 10.1016/j.clinph.2012.07.005.

[223] M. Willerslev-Olsen, J. Lundbye-Jensen, T. H. Petersen, and J. B. Nielsen, “The effect of baclofen and diazepam on motor skill acquisition in healthy subjects,” *Exp Brain Res*, vol. 213, no. 4, p. 465, Jul. 2011, doi: 10.1007/s00221-011-2798-5.

[224] N. H. Jung, I. Delvendahl, N. G. Kuhnke, D. Hauschke, S. Stolle, and V. Mall, “Navigated transcranial magnetic stimulation does not decrease the variability of motor-evoked potentials,” *Brain stimulation*, vol. 3, no. 2, pp. 87–94, 2010.

[225] N. Takeuchi, T. Tada, M. Toshima, T. Chuma, Y. Matsuo, and K. Ikoma, “Inhibition of the Unaffected Motor Cortex by 1 Hz Repetitive Transcranial Magnetic Stimulation Enhances Motor Performance and Training Effect of the Paretic Hand in Patients with Chronic Stroke,” Apr. 2008. https://www.ingentaconnect.com/content/mjl/sreh/2008/00000040/00000004/art00009# (accessed Apr. 01, 2019).

[226] A. B. Conforto, W. J. Z'Graggen, A. S. Kohl, K. M. Rösler, and A. Kaelin-Lang, "Impact of coil position and electrophysiological monitoring on determination of motor thresholds to transcranial magnetic stimulation," *Clin Neurophysiol*, vol. 115, no. 4, pp. 812–819, Apr. 2004, doi: 10.1016/j.clinph.2003.11.010.

[227] P. Julkunen *et al.*, "Comparison of navigated and non-navigated transcranial magnetic stimulation for motor cortex mapping, motor threshold and motor evoked potentials," *NeuroImage*, vol. 44, no. 3, pp. 790–795, Feb. 2009, doi: 10.1016/j.neuroimage.2008.09.040.

[228] K. R. Mills, S. J. Boniface, and M. Schubert, "Magnetic brain stimulation with a double coil: the importance of coil orientation," *Electroencephalography and Clinical Neurophysiology/Evoked Potentials Section*, vol. 85, no. 1, pp. 17–21, Feb. 1992, doi: 10.1016/0168-5597(92)90096-T.

[229] G. J. Ettinger *et al.*, "Experimentation with a transcranial magnetic stimulation system for functional brain mapping," *Med Image Anal*, vol. 2, no. 2, pp. 133–142, Jun. 1998, doi: 10.1016/s1361-8415(98)80008-x.

[230] C. Schönfeldt-Lecuona, J.-P. Lefaucheur, L. Cardenas Morales, R. C. Wolf, T. Kammer, and U. Herwig, "The value of neuronavigated RTMS for the treatment of depression," *Neurophysiologie clinique = Clinical neurophysiology*, vol. 40, pp. 37–43, Mar. 2010, doi: 10.1016/j.neucli.2009.06.004.

[231] E. P. Washabaugh and C. Krishnan, "A low-cost system for coil tracking during transcranial magnetic stimulation," *Restorative Neurology and Neuroscience*, vol. 34, no. 2, pp. 337–346, Jan. 2016, doi: 10.3233/RNN-150609.

[232] M. P. Malcolm, W. J. Triggs, K. E. Light, O. Shechtman, G. Khandekar, and L. J. Gonzalez Rothi, "Reliability of motor cortex transcranial magnetic stimulation in four muscle representations," *Clin Neurophysiol*, vol. 117, no. 5, pp. 1037–1046, May 2006, doi: 10.1016/j.clinph.2006.02.005.

[233] A. A. van Kuijk, L. C. Anker, J. W. Pasman, J. C. M. Hendriks, G. van Elswijk, and A. C. H. Geurts, “Stimulus–response characteristics of motor evoked potentials and silent periods in proximal and distal upper-extremity muscles,” *Journal of Electromyography and Kinesiology*, vol. 19, no. 4, pp. 574–583, Aug. 2009, doi: 10.1016/j.jelekin.2008.02.006.

[234] G. Kamen, “Reliability of motor-evoked potentials during resting and active contraction conditions,” *Med Sci Sports Exerc*, vol. 36, no. 9, pp. 1574–1579, Sep. 2004, doi: 10.1249/01.mss.0000139804.02576.6a.

[235] M. Halaki and K. Ginn, *Normalization of EMG Signals: To Normalize or Not to Normalize and What to Normalize to?* IntechOpen, 2012. doi: 10.5772/49957.

[236] C. Schwartz *et al.*, “Normalizing shoulder EMG: An optimal set of maximum isometric voluntary contraction tests considering reproducibility,” *Journal of Electromyography and Kinesiology*, vol. 37, pp. 1–8, Dec. 2017, doi: 10.1016/j.jelekin.2017.08.005.

[237] P. M. Rossini *et al.*, “Applications of magnetic cortical stimulation. The International Federation of Clinical Neurophysiology,” *Electroencephalogr Clin Neurophysiol Suppl*, vol. 52, pp. 171–185, 1999.

[238] M. Oliveri *et al.*, “Interhemispheric asymmetries in the perception of unimanual and bimanual cutaneous stimuli: A study using transcranial magnetic stimulation,” *Brain*, vol. 122, no. 9, pp. 1721–1729, Sep. 1999, doi: 10.1093/brain/122.9.1721.

[239] F. Hashemirad, M. Zoghi, P. B. Fitzgerald, and S. Jaberzadeh, “Reliability of Motor Evoked Potentials Induced by Transcranial Magnetic Stimulation: The Effects of Initial Motor Evoked Potentials Removal,” *Basic Clin Neurosci*, vol. 8, no. 1, pp. 43–50, Jan. 2017, doi: 10.15412/J.BCN.03080106.

[240] L. Claudino, S. J. Hussain, E. R. Buch, and L. G. Cohen, “Offline coil position denoising enhances detection of TMS effects,” *bioRxiv*, p. 256081, Jul. 2018, doi: 10.1101/256081.

[241] C. J. Feltz and G. E. Miller, “An asymptotic test for the equality of coefficients of variation from k populations,” *Stat Med*, vol. 15, no. 6, pp. 646–658, Mar. 1996, doi: 10.1002/(sici)1097-0258(19960330)15:6<647::aid-sim184>3.0.co;2-p.

[242] K. O. McGraw and S. P. Wong, “Forming inferences about some intraclass correlation coefficients,” *Psychological Methods*, vol. 1, no. 1, pp. 30–46, 1996, doi: 10.1037/1082-989X.1.1.30.

[243] S. Schmidt, R. M. Cichy, A. Kraft, J. Brocke, K. Irlbacher, and S. A. Brandt, “An initial transient-state and reliable measures of corticospinal excitability in TMS studies,” *Clinical Neurophysiology*, vol. 120, no. 5, pp. 987–993, May 2009, doi: 10.1016/j.clinph.2009.02.164.

[244] P. B. Fitzgerald *et al.*, “A study of the effectiveness of high-frequency left prefrontal cortex transcranial magnetic stimulation in major depression in patients who have not responded to right-sided stimulation,” *Psychiatry Res*, vol. 169, no. 1, pp. 12–15, Aug. 2009, doi: 10.1016/j.psychres.2008.06.017.

[245] J. C. Rothwell, P. D. Thompson, B. L. Day, S. Boyd, and C. D. Marsden, “Stimulation of the human motor cortex through the scalp,” *Experimental Physiology*, vol. 76, no. 2, pp. 159–200, 1991, doi: https://doi.org/10.1113/expphysiol.1991.sp003485.

[246] P. M. Rossini *et al.*, “Non-invasive electrical and magnetic stimulation of the brain, spinal cord, roots and peripheral nerves: Basic principles and procedures for routine clinical and research application: An updated report from an I.F.C.N. Committee,” *Clinical Neurophysiology*, vol. 126, no. 6. Elsevier Ireland Ltd, pp. 1071–1107, Jun. 01, 2015. doi: 10.1016/j.clinph.2015.02.001.

[247] B. L. Day *et al.*, “Electric and magnetic stimulation of human motor cortex: surface EMG and single motor unit responses.,” *The Journal of Physiology*, vol. 412, no. 1, pp. 449–473, May 1989, doi: 10.1113/jphysiol.1989.sp017626.

[248] C. Schönfeldt-Lecuona, A. Thielscher, R. W. Freudenmann, M. Kron, M. Spitzer, and U. Herwig, “Accuracy of stereotaxic positioning of transcranial magnetic stimulation,” *Brain Topography*, vol. 17, no. 4, Art. no. 4, 2005, doi: 10.1007/s10548-005-6033-1.

[249] J. Reijonen, L. Säisänen, M. Könönen, A. Mohammadi, and P. Julkunen, “The effect of coil placement and orientation on the assessment of focal excitability in motor mapping with navigated transcranial magnetic stimulation,” *J Neurosci Methods*, vol. 331, p. 108521, Feb. 2020, doi: 10.1016/j.jneumeth.2019.108521.

[250] Y. Jono *et al.*, “The effect of tonic contraction of the finger muscle on the motor cortical representation of the contracting adjacent muscle,” *Somatosens Mot Res*, vol. 32, no. 2, pp. 114–121, 2015, doi: 10.3109/08990220.2014.994738.

[251] C. V. Turco, H. J. Fassett, M. B. Locke, J. El-Sayes, and A. J. Nelson, “Parallel modulation of interhemispheric inhibition and the size of a cortical hand muscle representation during active contraction,” *J Neurophysiol*, vol. 122, no. 1, pp. 368–377, Jul. 2019, doi: 10.1152/jn.00030.2019.

[252] M. K. Jung *et al.*, “Intramuscular EMG-driven Musculoskeletal Modelling: Towards Implanted Muscle Interfacing in Spinal Cord Injury Patients,” *IEEE Trans Biomed Eng*, vol. PP, Jun. 2021, doi: 10.1109/TBME.2021.3087137.

[253] L. Noreau and J. Vachon, “Comparison of three methods to assess muscular strength in individuals with spinal cord injury,” *Spinal Cord*, vol. 36, no. 10, pp. 716–723, Oct. 1998, doi: 10.1038/sj.sc.3100646.

[254] C. Morawietz and F. Moffat, “Effects of locomotor training after incomplete spinal cord injury: a systematic review,” *Arch Phys Med Rehabil*, vol. 94, no. 11, pp. 2297–2308, Nov. 2013, doi: 10.1016/j.apmr.2013.06.023.

[255] M. Colella, M. Liberti, F. Apollonio, and G. Bonmassar, “A Miniaturized Ultra-Focal Magnetic Stimulator and Its Preliminary Application to the Peripheral Nervous System,” in *Brain*

and Human Body Modeling 2020: Computational Human Models Presented at EMBC 2019 and the BRAIN Initiative® 2019 Meeting, S. N. Makarov, G. M. Noetscher, and A. Nummenmaa, Eds. Cham (CH): Springer, 2021. Accessed: Oct. 20, 2021. [Online]. Available: http://www.ncbi.nlm.nih.gov/books/NBK562094/

[256] H. Tischler *et al.*, "Mini-coil for magnetic stimulation in the behaving primate," *J Neurosci Methods*, vol. 194, no. 2, pp. 242–251, Jan. 2011, doi: 10.1016/j.jneumeth.2010.10.015.

[257] Y. Tufail *et al.*, "Transcranial Pulsed Ultrasound Stimulates Intact Brain Circuits," *Neuron*, vol. 66, no. 5, pp. 681–694, Jun. 2010, doi: 10.1016/j.neuron.2010.05.008.

[258] L. di Biase, E. Falato, and V. Di Lazzaro, "Transcranial Focused Ultrasound (tFUS) and Transcranial Unfocused Ultrasound (tUS) Neuromodulation: From Theoretical Principles to Stimulation Practices," *Frontiers in Neurology*, vol. 10, p. 549, 2019, doi: 10.3389/fneur.2019.00549.

[259] S. C. Kirshblum *et al.*, "International standards for neurological classification of spinal cord injury (revised 2011)," *J Spinal Cord Med*, vol. 34, no. 6, pp. 535–546, Nov. 2011, doi: 10.1179/204577211X13207446293695.