Bimanual coordination and spinal cord neuromodulation in neural motor control of upper limbs

by

Behdad Parhizi

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University of Alberta

Abstract

The overall goal of this dissertation was to advance knowledge in the underlying neural mechanisms of motor control of upper limb movement. Arm movements are essential for everyday life. It is not surprising that the neural control of arm movement has been the subject of research investigations for many years. This thesis aimed to address two critical questions currently underexplored: 1) How does the cortical neural correlates of bimanual coordination change when the nature of the movement varies? 2) How does spinal cord stimulation modulate the neural substrates of arm movement across different levels of the central nervous system? Of the wide array of movements that humans are capable of producing, this dissertation examines bimanual coordination. Coordinated movement of the upper limbs is sometimes easy to perform but sometimes requires rigorous training to become adept. Thus, bimanual coordination is regarded as a skilled and complex motor ability that only humans have evolved to possess. Yet, this ability is impaired in persons with neurological conditions. Restoration of upper limb function through neuromodulation has recently attracted attention using transcutaneous spinal cord stimulation (tSCS); however, the neural mechanisms driving improvements are not completely understood.

The first question was addressed by focusing on bimanual coordination to either achieve a common-goal or dual-goals. Goal-conceptualization and the effect of cognitive load during bimanual movements were addressed through kinematic and electrophysiological measures. Results showed that dual-goal reaching significantly increased movement time and error compared to common-goal and unimanual reaching. They also demonstrated increased movement time (except in common-goal) and error with the addition of cognitive load. The

findings in electrophysiological measures exhibited significantly stronger alpha band sensorimotor cortical activation during common-goal movements relative to dual-goal movements, and elevated interhemispheric connectivity in the common-goal task relative to the dual-goal and unimanual tasks. Our findings suggest that goal-conceptualization and complexity are critical factors in defining the behavioral outcome of bimanual movements, and their effect is also reflected in the underlying neural mechanisms.

The second question was addressed in three steps by investigating the effect of cervical tSCS on i) cortical, ii) cortico-spinal, and iii) propriospinal pathways related to the motor control of upper limb movements. First, the effect of cervical tSCS on bimanual and unimanual movements was highlighted. Electroencephalography (EEG) recordings were used as an unconventional approach to explore the modulation of sensorimotor cortical oscillations. Results showed significant synchronization of neural activity in sensorimotor regions, and increased interhemispheric connectivity in the presence of tSCS. This finding points to the suppressive effect of tSCS at the cortical level while allowing tighter communication between hemispheres.

Second, the modulatory effect of cervical tSCS on the circuitry of the cervical segment of the spinal cord projecting to the upper limb muscles and corticospinal connections to this region was assessed. Given that the cervical and lumbar cord are coupled through propriospinal connections, we also sought to determine the effect of lumbar tSCS on the cervical neural networks. No effect of cervical tSCS on either the flexor carpi radialis (FCR) Hoffman-reflex (H-reflex) or motor evoked potentials (MEP) was found; however, lumbar tSCS significantly facilitated the FCR H-reflex. Interestingly, combining lumbar and cervical tSCS significantly facilitated spinal and

corticospinal pathways to the upper limb muscle, as shown by increased FCR H-reflex and MEP. This study highlights the importance of remote spinal segments to the cervical cord facilitated through cervico-lumbar coupling, and how this may be utilized for enhancing the voluntary control of arm movement.

Finally, because cervico-lumbar coupling is bidirectional, the modulatory effect of cervical tSCS on lumbar circuitry was investigated. Affirmative results could mean a potential for engaging cervical spinal cord through tSCS to support voluntary control of the lower limbs. We found that cervical tSCS significantly suppresses the soleus H-reflex. This novel finding demonstrates the potential role activating cervical spinal networks via tSCS to potentiate cervico-lumbar coupling. Collectively, this thesis constitutes a comprehensive study of upper limb movement and its neural correlates at different levels of the central nervous system. Spinal and supraspinal neuromodulatory effects tSCS on neural substrates of upper limb movement were also explored. Findings of this dissertation could pave the way for enhanced and targeted rehabilitation interventions for individuals with neurological conditions.

Dedication

This thesis is dedicated to my wife, mother, and aunt. To my wife, Mahshad Ghasemi, whose love and support has been unwavering. Your steadfast faith in me has been the driving force propelling me forward on this journey. I will be forever grateful for all the sacrifices you made during this process. I will make it my life's mission to make you happy from now on.

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In loving memory of my father, Ahmad Parhizi, who may no longer grace this world with his presence, but whose invaluable life lessons continue to shape my character and inspire me to reach the pinnacle of excellence. Dearest Father, as I stand upon this momentous milestone, I hope your spirit is filled with pride for the person I have become. This thesis is a testament to your enduring love and guidance.

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List of Abbreviations

A&L	Arm and leg
AC	Alternating current
ARAT	Action research arm test
ASR	Artifact Subspace Reconstruction
BB	Bicep brachii
BCI	Brain-computer interface
BF	Biceps femoris
CC	Corpus callosum
CMA	Cingulate motor area
CPG	Central pattern generator
cSCI	Cervical spinal cord injury
DC	Direct current
DTI	Diffusion tensor imaging
ECR	Extensor carpi radialis
EEG	Electroencephalography
EMG	Electromyographic
ERD	Event-related desynchronization
ERS	Event-related synchronization
eSCS	Epidural spinal cord stimulation
FA	Fractional anisotropy
FCR	Flexor carpi radialis

fMRI	Functional magnetic resonance imaging
GABA	γ-amino butyric acid
GMP	Generalized motor program
H-reflex	Hoffman-reflex
IHI	Interhemispheric inhibition
ISMS	Intraspinal microstimulation
ISP	Ipsilateral silent period
ITI	Inter-tap interval
M1	Primary motor cortex
MEG	Magnetoencephalography
MEP	Motor-evoked potential
MEP	Cervicomedullary-evoked potential
MGA	Maximal grasp aperture
Mmax	Maximally evoked motor response
MT	Movement time
MVC	Maximum voluntary contraction
MVF	Maximum voluntary force
NI	Neurologically intact
NMES	Neuromuscular electrical stimulation
PAS	Paired associative stimulation
PMC	Pre-motor cortex
RMSE	Root-mean square error
RT	Reaction time

S1	Primary somatosensory cortex
SCI	Spinal cord injury
SCS	Spinal cord stimulation
SICI	Short-interval cortical inhibition
SMA	Supplementary motor area
SOL	Soleus
SP	Spectral power
STG	Superior temporal gyrus
TA	Tibialis anterior
TB	Tricep brachii
TMS	Transcranial magnetic stimulation
tSCS	Transcutaneous spinal cord stimulation
VGR	Visually-guided reaching
VL	Vastus lateralis
VR	Virtual reality

Chapter 1. Introduction

1.1. Overview

Motor control is integral to all the activities we engage in, from breathing and walking to reaching toward objects. We have a vested interest in improving our understanding of how motor control is organized in different levels of the central nervous system. By doing so, we can design better tools and more effective methods for refined rehabilitation targeted for clinical population that includes spinal cord injury, stroke, and traumatic brain injuries.

Our ability to perform a wide array of upper limb movements such as feeding, tool use, and washing is central to our everyday life. Even when the end goal of a movement is not directly linked to the upper limb movement such as when we are running or walking, movement of the upper limbs are critical for successful execution of such tasks. This extremely important ability of our motor system is often underappreciated and taken as granted, and only appreciated when the ability is lost or impaired such as when the nervous system sustains injuries including spinal cord injury and stroke. For this reason, regaining arm function has been rated by people with tetraplegia as the top priority (Anderson, 2004).

I was personally inspired by the large body of literature and extensive research spanning decades regarding the neural mechanisms of upper limb movements. These research studies boosted our understanding of neural networks involved in the motor control of upper limb movements at different levels of the central nervous system (Scott, 2004). These levels include: 1) cortical: primary motor cortex, pre-motor cortex, supplementary motor area, somatosensory and parietal regions, and etc., 2) subcortical areas such as the basal ganglia, cerebellum and brainstem, and 3) the spinal cord including cervical, lumbar and propriospinal systems. While this increased level of knowledge is beneficial, further work is necessary to address some unanswered questions related to the motor control of the upper limbs. Specifically, two major areas of interest for me requiring more research to bridge the gaps in knowledge are the neural mechanisms underlying bimanual coordination and the effect of neuromodulation of the spinal cord using transcutaneous spinal cord stimulation on bimanual coordination. This doctoral thesis explored these two areas of interest.

To date, research in human motor control of reaching and grasping has been primarily focused on unimanual movements. However, evolution to upright standing has released the upper limbs to perform more complicated movement patterns such as writing, scrolling a web page on the phone, and threading a needle. Of particular interest in this dissertation are the movements that require coordination and synchrony between the upper limbs, referred to as bimanual coordination. Examples of such bimanual actions include opening a jar lid, keyboard typing, driving, and playing the piano. The ubiquitous need for coordination and cooperation of the upper limbs (i.e., bimanual coordination) in daily human activities has been a major drive for investigating bimanual motor control and the effect of bimanual training on clinical populations. Beyond that, bimanual coordination is regarded as a prototype of complex motor behavior that has been a topic for research in cognitive neuroscience. In fact, a cognitive approach towards understanding of bimanual movement remains an active area of investigation (Panzer, Kennedy, Wang, & Shea, 2018). With higher complexity comes the constraint in performing bimanual actions that pose challenges to novice performers. Therefore, the importance of investigating bimanual movement not only lies in its power to advance our understanding of neural motor control, but also the window it provides for studying higher functions(S. P. Swinnen & Gooijers, 2015). In addition, bimanuality offers substantial diversity of tasks available for research experiments, and bimanual tasks are fundamentally and intrinsically different their unimanual counterparts. As a result, bimanual tasks can unveil aspects of motor deficits following neural injuries and brain disorders that otherwise could not been distinguishable by standard unimanual tasks.

With increased interest in bimanual coordination, multiple lines of research have emerged. A common theme in the bimanual movement literature has been to understand how the upper limbs maintain their temporal and spatial coordination. This question arises as a result of a number of inherent constraints that come with bimanual coordination that limit our ability to perform tasks with complex spatiotemporal properties; for example, why are some bimanual movements preferred over others, and what forms the difference between symmetrical and asymmetrical movements? While attempting to answer these questions over the years, researchers have taken another step forward by discovering approaches to overcome such inherent constraints. In addition, bimanual movements are substantially different in their nature and can engage each limb in distinct roles with high degree of flexibility and modularity. For example, symmetrical movements necessitate similar behavior by each limb while asymmetrical movements do not. Also, not all bimanual movements are initiated with similar goals assigned to each limb. Sometimes each limb moves towards its own target while other times both limbs work to achieve a common goal. Finally, a great volume of research has been dedicated to find regions within the central nervous system associated with bimanual movements and how (and to what extent) each region plays a role in the control of this movements (Cardoso de Oliveira, 2002). Taken together, the endeavor to understand this impressive ability of the human motor system has led to fundamental findings in this field: 1) a union of constrains from cognitive to neuromuscular limit performing bimanual coordination tasks, 2) a range of strategies exist to overcome the constraints, 3) motor control of bimanual actions involve contribution from various brain regions, and 4) neural damage to the nervous system adversely affect bimanual coordination. In the first part of this introduction, a comprehensive review of the literature has been conducted to highlight key findings regarding bimanual coordination. We start by understanding the various theoretical frameworks for studying bimanual coordination, followed by research on the limitations in bimanual and ways to overcome them. The focus then shifts to the neural correlates of bimanual coordination to understand which regions within the central nervous system control our bimanual behavior. This will be achieved by reviewing imaging, basic science, and clinical population studies. At the end, a review of the effects of spinal cord injury and stroke on the performance of bimanual movements is presented to provide necessary information about the deficits that follow a damage to the nervous system which will eventually become important in shaping future rehabilitation paradigms. This comprehensive review of research conducted to date on bimanual movements provides a description of the current state of knowledge, but equally as important, allows for accurate and meaningful identification of gaps in the literature that this dissertation aimed to address.

As we read through the first part of the introduction, we will notice that while the literature appears to have made incredible advances in teaching us about bimanual movements/coordination, this field still leaves unaddressed gaps. First, research investigations usually focus on tasks with separate goals for each limb; however, in daily activities bimanual movements where the limbs move together to achieve a common goal are needed to achieve most tasks. This highlights the importance of goal-conceptualization during bimanual movements and underscores the fact that how the end goal is perceived affects the movement. To the best of our knowledge, only two previous studies (Duque et al., 2010; Liao, Whitall, Barton, & McCombe Waller, 2018) investigated how underlying mechanisms of these two goal-conceptualization strategies differ, but the task design in these studies does not mimic real life activities. Moreover, the dynamics of brain connectivity between different cortical regions is likely to be affected by goal-conceptualization. To date, it remains unclear how brain activity in the associated cortical regions and how interhemispheric coupling between these regions might be modulated as a function of bimanual task goal-conceptualization (and how that compares to unimanual movement). Second, there is no consensus in the field about the role of attention, goal-conceptualization, and hemispheric specialization in shaping the kinematic properties of different bimanual movements. This lack of knowledge may raise the following question: what determines movement time and error during bimanual movement? Do bimanual movement kinematics solely rely on hemispheric specialization or do they only depend on goal-conceptualization? Third, usually the issue of complexity is altered in experimental designs by changing the "motor"-related aspect of bimanual complexity and the underlying neural mechanisms are investigated thereafter (Jolien Gooijers et al., 2013); yet, there are novel strategies to modulate the complexity of a bimanual tasks through the addition of cognitive load, which remains uninvestigated. Does cognitive load increase the complexity of bimanual movement and can this possible change in complexity be tracked by neurophysiological measures including cortical activation and connectivity? In chapter two, we will explore how brain dynamics and movement kinematics change across diverse sets of bimanual movements and how they compare to unimanual tasks. Multiple types of unimanual and bimanual tasks that are different in their goal-conceptualization and complexity are used in this chapter. By recording brain activity and movement kinematics of participants during the execution of the tasks, I aimed to address the above identified gaps in knowledge.

Although this dissertation is dedicated to enhancing the basic science behind the motor control of upper limb movements, it should be acknowledged that many scientists are working towards approaches and techniques to improve upper limb function after neural damage. While this application is of immense importance, understanding the neural mechanisms of these techniques and the underlying mechanisms of their effectiveness is equally important. One such technique is now referred to as transcutaneous spinal cord stimulation (tSCS) (Martin, 2021). This neuromodulation approach to reverse the deficit after neural injury (especially spinal cord injury) emerged as a spin off from another popular stimulation method to relieve chronic pain, epidural spinal cord stimulation. In the second part of this introduction, a historical perspective of tSCS will be provided. Then, we pivot to understanding the properties of tSCS, its waveform and current flow to the spinal cord, and mechanisms of neural recruitment. This is then followed by discussing how tSCS alters excitability at cervical and lumbar regions of the spinal cord and whether multisite (lumbar + cervical) stimulation leads to extra excitability in the target segments of the spinal cord. Finally, therapeutic effects of tSCS alone and in combination with previously established rehabilitation approaches will be explored. This comprehensive review of the tSCS literature enlightens us of some of the neglected critical questions, the answers to which are indispensable for advancement of this technique toward meaningful and widespread clinical application.

In 2007, scientists around the world started to apply tSCS to improve the motor function (Karen Minassian et al., 2007). Although shown to be effective in pilot clinical trials (Inanici et al., 2018), the underlying neural mechanisms of this nowadays popular neuromodulation modality are not completely understood. Moreover, the effect of tSCS waveforms that encompass a high frequency component, referred to as “modulated waveform” (Barss, Parhizi, Porter, & Mushahwar, 2022) which is considered the equivalent of “paresthesia-free” stimulation in the chronic pain literature, is still unknown. This novel technique of stimulation is still in early stages, and a lot of work lies ahead about its mechanisms and implementation. There seems to be a lack of agreement about choosing the best parameters, site of stimulation, extent of recovery after use, clinical target population, etc. Part of this disagreement pertains to gaps in our appreciation of the neural substrates of tSCS. The following questions remain debated: 1) how spinal cord neuromodulation using tSCS affects cortical dynamics during the execution of unimanual and bimanual movements? 2) how cervical and multisite tSCS alters the excitability of spinal and corticospinal neural circuitry that play a pivotal role in coordinating upper limb movements? And 3) knowing the existing propriospinal connections between cervical and lumbar segments of the cord which facilitate arm/leg movements, what are the effects of stimulating the cervical region of the spinal cord on the excitability of the remote lumbar region? The answer to the above questions will be explored in chapter 3, 4, and 5, respectively.

The next four chapters of this dissertation will collectively serve an overall goal of understanding novel aspects of the motor control of the upper limbs. Three specific goals are set in this dissertation to address this overall goal: 1) investigate the neurophysiological correlates of bimanual movement, 2) determine the effect of tSCS on cortical mechanisms associated with bimanual motor control, and 3) understand the effect of tSCS on corticospinal and spinal networks related to the upper limbs. The first goal is covered in chapter 2, the second goal in chapter 3, and the third goals in chapters 4, and 5. The key elements of this dissertation are as follows: upper limb motor control, bimanual movements, and tSCS. Neural correlates of each element will be probed throughout the research chapters. Finally, a general discussion will be provided in chapter 6 about the results and findings, and future research opportunities will be proposed.

1.2. Bimanual coordination

1.2.1. Introduction

Many of us have experienced holding our mobile phone with one hand, while lifting a spoon from the plate with the other hand to eat food during the busy hours at work. This is just an example of how bimanual movements are integrated into our daily life. Bimanual movements are an outstanding ability of the human nervous system and our motor repertoire is very rich in orchestrating them. Some tasks require simultaneous actions of the two arms with almost equal contributions such as picking up a relatively large box, while completely dissimilar actions are carried out by the two hands in other tasks such as opening a jar. On one hand, we are well equipped to complete various bimanual actions without prior training or practice, on the other only after several years of practice can we acquire a skilled task. An example of the latter is pianists who skillfully perform complex sequences of musical notes with bilateral activation of multiple fingers, a skilled task that is attained after extensive training. The importance and complexity of bimanual coordination led many scientists to investigate the neural origins of this human motor phenomenon.

Researchers across the world have extensively studied bimanual coordination and the field has recently gained more momentum as it also opened avenues to study higher cognitive and perceptual functions such as task switching and multi-tasking as suggested by Swinnen (S. P. Swinnen & Gooijers, 2015). Multiple theoretical frameworks including generalized motor program (GMP), dynamical systems, information processing (or cross-talk model), muscle synergies, and optimal feedback have laid the foundation for research in bimanual coordination. Here, the focus is on the first three frameworks which have laid the foundation for understanding bimanual movements. These frameworks provide reasoning for the behavioral findings in the bimanual coordination tasks. For example, a major behavioral observation is the existence of an inherent interaction between the two upper limbs that limits our ability to produce some form of bimanual movements while favoring others. Within the GMP perspective, a unified motor plan formulates the complete structure of a movement before the execution stage, and without any feedback during execution (R. A. Schmidt, 1975; Richard A. Schmidt, Zelaznik, Hawkins, Frank, & Quinn Jr, 1979). Instead of storing all the possible movements which is beyond human brain capacity, GMP framework states that the brain controls patterns that form fundamental movements. Each pattern is defined by common features referred to as "invariant features" which are consistent from action to the other. To realize a particular movement, a pattern is retrieved and movement-specific parameters join invariant features. In the context of bimanual movements, a single motor plan also suffices production of multi-limb movements where common and limb-specific parameters of the movement need to be specified: $H(t) = a * F(b * t)$ where a and b are the hand-specific parameters that scale a common motor program F (Cardoso de Oliveira, 2002). A hallmark of bimanual movements is the tendency for spatiotemporal similarities of the left and right limbs. Thus, GMP may explain some of the observed spatiotemporal properties since a single motor program is assigned to both limbs, however cannot account for other behavioral observations. For example, GMP fails to explain the assimilation effect during bimanual movement. In a marked contrast to the GMP model, in the information processing theory, two separate motor plans exist for each limb that govern their associated movement, and limitations arise as a result of cross-talk of the plans dedicated to each side (Cattaert, Semjen, & Summers, 1999; Marteniuk, MacKenzie, & Baba, 1984) (Heuer, Kleinsorge, Spijkers, & Steglich, 2001; Sherwood, 1994; S. P. Swinnen, Young, Walter, &

Serrien, 1991). The neural cross-talk leads to mutual interference which can be overcome by practice or other conceptualization approaches (discussed later in this chapter). This cross-talk occurs at least at two levels of the central nervous system: a lower-level mediated by uncrossed cortico-spinal tract fibers which relay the information of the contralateral side to the ipsilateral side, inducing mutual interference between the two limbs (Cardoso de Oliveira, 2002; S. Swinnen, Walter, & Shapiro, 1988; S. P. Swinnen et al., 1991), and at a higher-level in which callosal fibers connecting the two hemispheres possibly transmitting specified movement parameters (Cardoso de Oliveira, 2002; Heuer et al., 2001). It can be inferred that the modification of the strength of cross-talk makes it possible for us to tackle constraints of bimanual coordination (Cardoso de Oliveira, 2002). In the extreme case, if all the details of the two separate movement plans are shared through neural cross-talk, the information processing model downgrades to the GMP model (Cardoso de Oliveira, 2002). It can also be interpreted that the information processing model is the less intense variant of the GMP model.

Dynamical system theory suggests how coordinative patterns are organized (J. A. Kelso, 1984; Scholz, 1990). A major issue in producing movements is the degrees-of-freedom in our neuro-musculo-skeletal system and how our nervous system handles this problem: only a finite set of movements are generated for a desired action despite the infinite number of possible solutions. In a similar fashion, dynamical system theory chooses the most efficient model and best possible sets of variables to coherently describe the system and measure its performance (Scholz, 1990). The theory identifies the general principles that govern coordination patterns between the two limbs that apply to any observation scale. Dynamical system theory is primarily concerned with biological systems that are comprised of numerous components that self-organize themselves to exhibit a complex behavior (S. P. Swinnen & Gooijers, 2015). The self-organization of the components is ensured by their non-linear dynamical interaction and cooperation (Jirsa, Fuchs, & Kelso, 1998). Such system can be both characterized by order parameters (such as relative phase) and control parameters (such as speed of movement), and identifying these parameters is of paramount importance within this framework (Scholz, 1990). Spontaneous time-dependent pattern changes such as asymmetrical-to-symmetrical transition emerge from an interruption in the stability of the system; thus, order parameters should directly be linked to stability features. While it is not convenient to discover the most appropriate order parameters, many studies have attempted to determine the dynamics of bimanual coordination by exploring phase (ϕ) under various control parameters such as speed and force. The appeal of this approach is in its power to define the intrinsic or default modes of coordination patterns, and therefore the constraints that compromise human motor performance.

Here, we will cover the limitations in performing bimanual movement/coordination and the possible ways to tackle the limitations through a perceptual and cognitive window into the representation of actions. Then, the neural substrates of bimanual movement will be addressed at the cortical/subcortical and network level where the role of corpus callosum and cortical connections becomes prominent. At the end, we will focus on findings related to bimanual coordination after clinical pathologies including stroke and spinal cord injury (SCI), and how rehabilitation strategies might recover this remarkable ability of the human nervous system. A summary will be provided at the end of each section and when related to this dissertation, gaps in the literature will be identified. I will then briefly discuss the contribution of this dissertation to addressing the gaps.

1.2.2. Constraints of coordination: human tendency to execute particular patterns

The human motor control system is very capable of performing bimanual tasks. This is particularly important when we consider that the hands are predominantly used together to complete activities of daily life. The spatial and temporal features in bimanual arm coordination discriminate them from unimanual movements and their lower-limb counterparts during locomotion. For example, unimanual movements are described well by Fitts' law which explains movement time as a function of movement amplitude and target width (speed-accuracy trade-off) (Fitts & Peterson, 1964). However, Kelso and colleagues reported that in bimanual movements, the two hands start and terminate the movement with temporal synchrony when moving to the target with dissimilar difficulties. This is clearly in violation of Fitts' law (J. S. Kelso, Southard, & Goodman, 1979). Such studies embarked years of research on temporal and spatial properties of bimanual movements. While it seems effortless to execute bimanual maneuvers, there are complexities as the two arms effectively interact with cross spatiotemporal relationships.

The nature of concerted manipulation of upper limbs is not without challenges, with multiple degrees of freedom inherent in each limb that enables them to contribute in endless ways to performing a bimanual task. Despite differences in theoretical frameworks discussed in section 1.2.1, they all posit that bimanual coordination depends on the nature of the task. Accordingly, bimanual movements can be divided into categories: a) symmetric vs. asymmetric; b) in-phase, anti-phase vs. out-of-phase; c) rhythmic vs. discrete; d) common-goal vs. dual-goal (S. Kantak, Jax, & Wittenberg, 2017). The distinction between these categories lays the foundation for understanding the constraining factors in coordination between the two limbs. Various constraints imposed upon bimanual coordination have been identified, but they generally fall into one of the following classes: a) muscular; b) spatial; and c) temporal (S. P. Swinnen & Gooijers, 2015). While spatial constraints primarily generalize to discrete tasks, temporal constraints are more related to rhythmic and sequential movements.

Muscular class refers to the constraints with relative timing to activate homologous muscles. The so-called "default" coordination mode engages homologous muscles simultaneously. This preferred activation pattern requires the relative phase of the two limbs to be set at zero, or in-phase pattern ($\phi=0$) (Howard, Ingram, Körding, & Wolpert, 2009). This pattern is suggested to be more stable than the anti-phase pattern where the relative phase between limbs is 180° ($\phi=180$) and the homologous muscles are recruited alternatively (or activation of non-homologous muscles simultaneously). The stability of the latter pattern is reduced with increasing frequency and the original anti-phase mode spontaneously transitions towards in-phase coordination (J. A. Kelso, 1984). In the context of relative phase between the two limbs, in-phase and anti-phase patterns are most commonly employed. The out-of-phase patterns (i.e., $0 < \phi < 180$) are more complicated and require practice to be learned, as seen in instrumentalists. Not all forms of bimanual actions are as simple as activation of homologous muscles on each side; introducing temporal patterns impose additional complexity to this behavior. The timing pattern of unimanual tasks led researcher to investigate temporal properties of bimanual tasks (Elizabeth A. Franz, 2003).

Temporal constraints also impose limitation to performing bimanual movements. These constraints often dominate rhythmic and sequential patterns. Humans show a tendency to perform rhythmic patterns with frequency ratio of 1:1 between the two limbs (S. P. Swinnen & Gooijers, 2015). Take finger tapping for instance, for each tapping of the right finger, there is a tapping of the left finger at the same time. These simple harmonic rhythms along with oscillating patterns in which the frequency of one limb is an integer multiple of the contralateral side (2:1 or 3:1) are more stable and can be performed with ease compared to non-harmonic polyrhythms (frequency ration of 2:3 or 3:8) (Summers, Rosenbaum, Burns, & Ford, 1993). Polyrhythms require precise timing (or phase relationship) between the two hands and can be acquired through practice and musical training. Polyrhythms are more variable in higher-order ratios and tend to be replaced by lower-order ratios when there is a gradual increase in the tapping frequency, even in skilled drummers (Peper, Beek, & van Wieringen, 1995).

Another facet of bimanual coordination is spatial coupling which was initially studied in 1991 by Franz, inspired by research in the temporal domain (Elizabeth A. Franz, 1997; Elizabeth A. Franz, Zelaznik, & McCabe, 1991). When moving in space, disparities of movement amplitude and direction between the two limbs leads to spatial constraints, the third class of constraints. Here, the preferred mode of coordination is moving with equal amplitudes and isodirectionally. Deviating from the preferred mode also compromises the stability of coordination. A very familiar example that any of us might have experienced is during drawing a circle with one hand and drawing a line with the other. Here, unintentional interference between the two limbs arises such that the shape of the line grows towards a curve and circle becomes elliptical (Elizabeth A. Franz, 1997). Converging evidence provides insight into the stability of coordination with regards to spatial constraints, which usually appears while performing discrete tasks. Spatial incongruency in the task, by moving in the horizontal direction with one hand and in the vertical direction with the contralateral one, results in trajectory distortion (spatial deviation in degrees) of both hands (T. D. Lee, Almeida, & Chua, 2002; S. P. Swinnen, Dounskaia, Levin, & Duysens, 2001; S. P. Swinnen et al., 1998), a phenomenon that is not observed in patients with callosotomy, which signifies the role of callosal connections (Elizabeth A. Franz, Eliassen, Ivry, & Gazzaniga, 1996). In fact, patients with callosotomy were able to complete the task of drawing dissimilar shapes without spatial interference. Furthermore, coupling in the amplitude of the movement is also possible and amplitude differences introduce interference (Sherwood, 1994). Different spatial demands between right and left limbs in aiming towards unequal amplitudes leads to an assimilation effect, the limb with the shorter aiming amplitude overshoots and makes longer movements (Marteniuk et al., 1984). Interestingly, it appears that callosal connections are less important in amplitude coupling as patients with callosotomy exhibit more pronounced amplitude-related spatial coupling (Elizabeth A. Franz, 1997, 2003).

Overall, humans are equipped to perform bimanual movements with outstanding capability, but not all the movements are easily performed and require high levels of training to be done with minimal errors. The performance of the bimanual movements depends on various constraints that are imposed including muscular, temporal, and spatial constrains. Over the years, a number of approaches have been suggested and studied to circumvent the constraints in bimanual movements and produce coherent actions instead. The basis of the proposed approaches are a cognitive perspective into bimanual coordination constraints, by essentially relating the constraints to cognitive processing of actions, and not motor interactions.

1.2.3. A cognitive window into bimanual coordination: Constraints revisited

While we observe a strong assimilation between the two hands during the performance of asymmetric short and long aiming movements, it seems simply intuitive to reach and pick a glass closer to us with one hand and pick a tray further away with the other hand without a problem. Could this be an example to argue against the constraints discussed in the previous section? Or this is an example to show that the constraints can be overruled? In an experiment with incongruent aiming between the left and right limbs (short and long distance of aiming), two types of instructions given to the participants resulted in markedly different initiation of movements. In contrast to symbolic cues, target illumination as a form of direct cueing minimizes the reaction time, with no difference between congruent and incongruent conditions (Diedrichsen, Hazeltine, Kennerley, & Ivry, 2001; R. Ivry, Diedrichsen, Spencer, Hazeltine, & Semjen, 2004). Here we can conclude that under the same motor conditions, the manner with which the task is perceived is essential in spatial constraints, supporting the premise that other constraints can be resolved as well. It appears that the spatial constraints stem from representation of action, rather than the action itself, as Ivry and colleagues suggested (R. Ivry et al., 2004). One would expect to see the congruency effect if motor processing was the source of conflict. To scrutinize the reason behind these observations, the emphasis should be placed on the way symbolic and direct cues are encoded. With symbolic cueing, perception of two distinct letters (S for short and L for long) is required for incongruent trials, whereas the letters are identical in congruent trials. On the other hand, perceptual demands of direct cueing remain the same for both congruent and incongruent conditions. Diedrichsen and Ivry even showed there is increased perceptual economy associated with same color targets in movements with different amplitudes relative to the different target colors for symmetric movements (Diedrichsen, Ivry, Hazeltine, Kennerley, & Cohen, 2003).

Here, we clearly observe an effect of perceptual encoding of the cues on spatial interactions during bimanual movements. Ivry proposed that the origins of spatial constraints in bimanual actions have little to do with the motor system, and depend heavily on task representation and movement goal conceptualization. Ivry and colleagues designed an elegant experiment in which participants were instructed to draw a three-sided square, with the open side being on the top for the two hands in the symmetric condition, and the open side facing right and up for the right and left hands, respectively, in the orthogonal condition (R. Ivry et al., 2004). In the symbolic condition, actual movement trajectories were presented, whereas in the direct condition only corners of the trajectory were presented as the end-point goal. With the symbolic condition, conflicting trajectories would be spatially encoded for orthogonal trials, whereas only endpoint locations would be specified to complete each side of the square in the direct condition. This experiment clearly demonstrate that the way action goals are coded and conceptualized have dramatic effect on task performance. Interactions between various conflicting spatial encoding resulting from trajectory representation in the symbolic condition gives rise to bimanual interference, manifested in higher reaction time compared to the direction condition (R. Ivry et al., 2004).

Consistent with this view, temporal constraints can also be overcome by other means including conceptualization methods, binding rules, and visual transformation (S. P. Swinnen & Gooijers, 2015). The purpose of these strategies is to alter an inherently dual task into one integrated action at the perceptual level (Summers et al., 1993; S. P. Swinnen & Wenderoth, 2004). Ivry and colleagues provided an excellent account for characterizing the temporal constraints of bimanual coordination (R. Ivry et al., 2004). They posited that, similar to spatial patterns, conceptualization of the task and goal representation are the most critical elements for stability of the temporal pattern, and ultimately constrain bimanual coordination. As described before, rhythmic repetitive bimanual finger tapping, for example each side tapping at a frequency of 1Hz, is more stable in in-phase or anti-phase mode (i.e., inter-tap interval (ITI) between right and left hand is 0 or 500ms) (Yamanishi, Kawato, & Suzuki, 1980). Deviation from in-phase/anti-phase mode by changing ITI to 200ms or 700ms results in higher variability and performance shifts towards the stable patterns. Semjen and Ivry challenged this study by proposing a unimanual version of the tapping (Semjen & Ivry, 2001). They viewed the finger tapping task from the perspective of tapping of one hand dividing the 1000ms-long tapping of the other hand into two subintervals, thus creating rhythms (rhythm representation hypothesis). For example, the 600ms ITI creates two subintervals of 600ms and 400ms, thus representing a more complex pattern. With this perspective, they reproduced the same results with unimanual finger tapping (Semjen & Ivry, 2001). Even with unimanual tapping, the more complex patterns were less stable and were distorted towards the simpler patterns. Their study clearly demonstrates that we are limited in our ability to represent complex temporal relationship between subintervals, abolishing the two-hand coupling basis for temporal constraints. In 2006, a generalization of the rhythm representation hypothesis was introduced (Spencer, Semjen, Yang, & Ivry, 2006). The event structure hypothesis predicts the stability of temporal coordination based on the complexity of event structure of the temporal pattern. Less stable temporal patterns are composed of more complex event structures. Thus, the temporal goal of the task, reflected in event structure, defines the temporal constraint of rhythmic repetitive movement. This is also correct even for more stable patterns such as in-phase and anti-phase movement which are considered the default mode. As described in 1.2.2, less stable patterns tend to shift toward in-phase and anti-phase default modes but differences exist between the temporal properties of the two. To explain why in-phase movements are more stable than anti-phase, participants were instructed to “say the word 'BA' repeatedly as you move” during both in-phase and anti-phase movements (Spencer et al., 2006). This approach detects the number of salient events formed by the participants’ cognition. Results clearly showed only a single “BA” was vocalized in in-phase, but two during anti-phase. Here, we conclude that movement coordination and task constraints are directly related to the complexity of event structures, as seen in the difference in the number of "BA" produced during in-phase relative to anti-phase movements.

In a remarkable experiment, participants were able to perform a polyrhythm with multi-frequency ratio of 4:3 that is thought to be impossible in un-trained individuals, by a simple visual transformation (Mechsner, Kerzel, Knoblich, & Prinz, 2001). Participants were instructed to circle two visible flags at each side with a ratio of 1:1, by rotating two cranks under the table with a 4:3 ratio that was made possible by a gear system. Thus, iso-frequency in the flags went together with a 4:3 frequency ratio in the cranks (hands). After 20 minutes of practice, participant were able to establish a stable 1:1 pattern through this perceptual transformation (Mechsner et al., 2001). Another strategy is to provide one integrated feedback of the motion of the two limbs instead of separate feedback of each limb. A Lissajous plot was created to serve this goal, which presented the joint motion of the two limbs as a single dot to the participants, enabled them to perform a 1:1 pattern with 90° phase difference (Kovacs, Buchanan, & Shea, 2009). The relatively complex pattern can also be accomplished by simply instructing the participants that the tapping sound is identical to horse galloping. Using the Lissajous plot, study participants were also able to effectively produce 5:3 bimanual coordination only with 10 minutes of practice, and transfer their learning to a 4:3 pattern without further practice (Kovacs, Buchanan, & Shea, 2010).

Overall, we can conclude that both spatial and temporal constraints of bimanual coordination tasks depend heavily on how they are presented to and how they are perceived by the participant. Cognitively transforming task presentation and conceptualization has a dramatic effect on task performance. A cognitive window into bimanual coordination was presented through which constraints can be resolved by various approaches including conceptualization, binding rules, visual cues, and visual transformation. Cognitive and perceptual methods introduced in the literature suggest a minimal role for the human motor system in overcoming bimanual coordination constraints.

1.2.4. Neural substrates of bimanual movements

The supplementary motor area (SMA), the motor region of the mesial wall of the cerebral cortex, has long been considered the prime candidate region involved in bimanual coordination (S. P. Swinnen & Gooijers, 2015). The extensive link between SMA and primary motor cortex (M1) of both hemispheres attests to SMA's critical role in the integration of the two arms. Temporary disruption of SMA through creating a virtual lesion using repetitive transcranial magnetic stimulation (TMS) has been shown to interfere with normal performance of bimanual movement in a task that required opening of a drawer with the left hand and catching a ball with the right hand (Obhi, Haggard, Taylor, & Pascual-Leone, 2002). Taken together, many studies point to the crucial role of SMA in controlling bimanual actions. However, the generation of bimanual actions cannot be exclusively ascribed to SMA or any other single region of the brain (Heitger, Macé, Jastorff, Swinnen, & Orban, 2012). Instead, as evidence suggests, a broad network of brain regions are involved in producing coordinated movement of the two arms including M1, pre-motor cortex (PMC), anterior cingulate cortex, SMA, cerebellum and basal ganglia (S. P. Swinnen, 2002; Wiesendanger & Serrien, 2001). Depending on the demands and requirement of the task, neural recruitment extends beyond the aforementioned regions to include pre-frontal, parietooccipital, and temporal regions.

One framework to understand how bimanual actions engage different brain regions is to compare them with unimanual movements. The evidence from bimanual finger or forearm movements reveal a key finding: the single factor of bimanuality does not require further activation in the basic sensorimotor networks compared to those observed in unimanual movements (Goerres, Samuel, Jenkins, & Brooks, 1998; Immisch, Waldvogel, van Gelderen, & Hallett, 2001; Nair, Purcott, Fuchs, Steinberg, & Kelso, 2003; Toyokura, Muro, Komiya, & Obara, 1999; Tracy et al., 2001; Ullén, Forssberg, & Ehrsson, 2003). For example, in a finger tapping experiment using fMRI, similar activation level was reported over a given somato-motor cortex when comparing bimanual with unimanual movements when the tapping rate was matched between conditions (Jäncke et al., 2000). Interestingly, Koenke and colleagues argued that the experimental paradigms used in the previous studies made comparison between bimanual multi-effector (two-finger) movement with single-effector (one-finger) unimanual movement, and therefore the activation levels were influenced by additional complexity rising from the control of the different number of fingers (Koeneke, Lutz, Wüstenberg, & Jäncke, 2004). They proposed an experiment that matched the number of effectors involved in both unimanual and bimanual conditions, with the unimanual condition requiring tapping of two adjacent fingers. Although they found activation of homologous regions including M1, PMC, SMA and posterior parietal cortex in both unimanual and bimanual conditions, the level of activation was surprisingly stronger for unimanual movement than with bimanual movement, highlighting the importance of task demand and complexity when examining the activation level of neural networks, rather than bimanuality.

Another framework to explore the neural substrates of bimanual movements is to understand how brain activation patterns alter as a function of coordination demands, learning, and age. With respect to coordination demands, as the complexity and difficulty of the movement increase, the activation becomes stronger (Debaere, Wenderoth, Sunaert, Van Hecke, & Swinnen, 2004) and expands into the areas that are not exclusively dedicated to motor function (Debaere et al., 2004). Increments of cycling frequency in rhythmic movement and breaking the symmetry in discrete movements are two common strategies among a multitude of others to change the requirements of bimanual tasks. While cycling speed is considered to be the element of “difficulty” in bimanual coordination, “complexity” of the movements can also be manipulated in a variety of ways including through asymmetric bimanual actions.

With respect to spatial demands, Ivry et al., stressed the importance of a double dissociation model for spatial constraints in asymmetric movements (R. Ivry et al., 2004). They proposed that the variations in behaviour during directly guided versus symbolically cued asymmetric bimanual reaching can be captured by the two visual streams theory (Goodale & Milner, 2018), with the dorsal and ventral stream associated with the type of cue. Visually guided reaching movements rely on dorsal stream and has been shown to be immune to illusions in both unimanual and bimanual movement control (Ozana & Ganel, 2020). In support for the hypothesis that the processing of dorsal streams in each hemisphere is isolated from each other, either one or both targets were displaced at the movement onset during a bimanual reaching task. The results revealed that the performance was unchanged from the unimanual to the bimanual condition. Thus, there is minimal interference when the dorsal stream is involved in the task. In contrast, symbolically-cued movements are linked to the ventral stream which is involved in processing vision for perception (Goodale & Milner, 2018). It is thought that the ventral stream encounters the identification of the two contrasting visual information in the incongruent condition, which poses additional burden to the performance of the bimanual task. This additional step, relative to the direct condition, is then transferred to the motor system which ultimately introduces interference and attenuates performance.

With regards to the temporal aspect of movement, frequency-dependent modulation of activation is observed in SMA, bilateral M1, and subcortical structures (Debaere et al., 2004; Goble et al., 2010). As the "egocentric principle" states (the term used by Swinnen), stability of coordination in symmetrical movements is more robust and easier to maintain in comparison with asymmetrical movements (S. P. Swinnen, Jardin, Meulenbroek, Dounskaia, & Den Brandt, 1997). This behavioral disparity in the coordination leads to recruitment of distinct neural networks. Asymmetric movements require greater neural involvement relative to symmetric movements during response preparation and execution. In an interesting study of bimanual tapping movements by Aramaki and colleagues, it was demonstrated that the neural activity in cerebellum lobule V and PMC is supra-additive to the sum of activity obtained during right and left unimanual movements during asymmetrical tapping, while it is sub-additive in the case of symmetrical tapping (Aramaki, Osu, & Sadato, 2010). Also, changing temporal dynamics of bimanual coordination and producing in- and anti-phase movements are accompanied by recruitment of non-motor regions as well. In-phase movements and anti-phase movements are characterized by additional activations in the cingulate motor area (CMA), cerebellum, inferior parietal gyri, and superior temporal gyri (Immisch et al., 2001; Meyer-Lindenberg, Ziemann, Hajak, Cohen, & Berman Karen, 2002; Ullén et al., 2003). Contrasting polyrhythm finger tapping (3:2 coordination pattern) with the simple 1:1 tapping (i.e., in-phase) exhibit further activations in posterior cerebellum and CMA (Ullén et al., 2003).

Markedly different activation patterns emerge as a result of aging and expertise in bimanual motor coordination. Aging poses challenges in the integrative use of arm and hands in older adults. Over-activation of SMA, inferior parietal cortex, and prefrontal cortex is observed compared to young adults, suggesting that cognition-related regions of the brain are involved in bimanual motor behavior in aging (Goble et al., 2010). Expertise level is a window into understanding how long-term training (and subsequently plasticity) gives rise to efficiency in recruitment of neural substrates. Professional pianists exhibit reduced engagement relative to naïve controls in a variety of brain regions including anterior cingulate cortex, premotor cortex, cerebellum, and basal ganglia (Haslinger et al., 2004; Jäncke et al., 2000). In a similar fashion, skill acquisition through training can be tracked in decreased activation in temperoparietofrontal areas, signifying cost efficiency associated with mastering novel complex skills (Andres et al., 1999; Beets et al., 2015; Puttemans, Wenderoth, & Swinnen, 2005; Rémy, Wenderoth, Lipkens, & Swinnen, 2008; Ronsse et al., 2011; Serrien & Brown, 2003).

Finally, one aspect of coordinated use of upper limbs that is often overlooked is when the intent of a bimanual movement is to achieve a common-goal through cooperative action of the two arms/hand, rather than setting two independent goals for each side (dual-goal movement). While the majority of activities of daily life involves the former, the literature is dominated by research on the latter. Two studies have alluded to the differences in the underlying neural mechanism of common-goal and dual-goal bimanual movement. Using functional magnetic resonance imaging (fMRI), three areas within the right hemisphere appear with stronger activation in common-goal condition: superior temporal gyrus (STG), SMA, and M1 (Duque et al., 2010). Importantly, temporary disruption of STG via TMS transiently impairs bimanual coordination. It can be argued that with the potential role of STG in spatial attention, goal-conceptualization in bimanual coordination alters the attentional demands and has neural consequences. Moreover, disinhibition of both cortical hemispheres is reported in dual-goal bimanual task compared to common-goal, possibly pointing to the mutual activation of both hemispheres (Liao et al., 2018).

To summarize, bimanual coordination encompasses a myriad of movements that are not only inherently different in their behavioral context, but also are driven by distributed neural assemblies. The brain regions involved in bimanual motor behavior are not fixed, but vary depending on internal (aging, learning, expertise) and external (difficulty and complexity) factors. Although these findings were impressive, I targeted two neglected areas as my research questions: 1) while complexity and difficulty of bimanual tasks were manipulated by changing the motor demands, no study to the best of my knowledge has addressed the effect of altering movement complexity by adding a cognitive load during bimanual movement. I sought to investigate how movement kinematics and cortical activation level, primarily in M1 and S1, are influenced by manipulating movement complexity through a novel cognitive load. 2) Goal-conceptualization is another area in bimanual coordination literature that requires further attention and research. Task design in the previous studies does not represent real life movements. Moreover, only bimanual movements were included in the previous research studies and comparison with unimanual movements is missing. In this dissertation, I explored the effect of goal-conceptualization on sensorimotor cortical activation for bimanual movements that mimic movements of daily life, and compared that with unimanual counterpart. Movement kinematics and behavioral outcomes were assessed as well. Chapter two of this dissertation will provide answers to these two gaps.

1.2.5. Corpus callosum and intra- and inter hemispheric connectivity in bimanual actions

The role of the corpus callosum (CC) in interhemispheric transfer of information has been extensively studied, and there is support for both excitatory and inhibitory callosal connections (J. Gooijers & Swinnen, 2014). For example, the absence of the ipsilateral silent period in persons with circumscript surgical lesions of the CC can be attributed to the inhibitory role of callosal connections (Carson, 2005; Reis et al., 2008; Wahl & Ziemann, 2008). On the other hand, a large body of evidence in patients with a split-brain and human agenesis indicate an excitatory role of the CC (Gazzaniga, 2000; Takeuchi, Oouchida, & Izumi, 2012). However, as Gooijers and Swinnen suggested, the role of CC can be described as balancing between these inhibitory and excitatory forces (J. Gooijers & Swinnen, 2014).

Overwhelming evidence supports the idea that bimanual performance improves with maturation of CC (Barral, Debu, & Rival, 2006; Fitzpatrick, Schmidt, & Lockman, 1996; Marion, Kilian, Naramor, & Brown, 2003; S. D. Robertson, 2001), and degrades with deterioration of the CC structure with aging (Bangert, Reuter-Lorenz, Walsh, Schachter, & Seidler, 2010; Desrosiers, Hébert, Bravo, & Rochette, 1999; B. W. Fling & R. D. Seidler, 2012; T. D. Lee, Wishart, & Murdoch, 2002; Marneweck, Loftus, & Hammond, 2011; Serrien, Swinnen, & Stelmach, 2000; Summers, Lewis, & Fujiyama, 2010; Temprado, Vercruysse, Salesse, & Berton, 2010; Wishart, Lee, Murdoch, & Hodges, 2000). As children grow older and maturation of CC structure takes place, performance of bimanual coordination improves over a multitude of tasks (Thompson et al., 2000). Improvements of temporal bimanual demands occur earlier than spatial characteristics, which aligns with the timing of CC development. In particular, the anterior part of the CC which primarily regulates temporal behavior maturates earlier than the posterior parts (de Boer, Peper, & Beek, 2012; Eliassen, Baynes, & Gazzaniga, 1999). In adulthood, inter-individual variation in CC microstructure relates to the performance of bimanual coordination tasks. Fractional anisotropy (FA) metric ranging from 0 to 1 is a common measurement used in diffusion tensor imaging (DTI) studies that represents isotropic/anisotropic diffusion of water molecules and evaluate white matter fiber tracts structure (Beaulieu, 2002), with low values representing unrestricted diffusion in all directions and approaching unity indicates constrained diffusion along the major axis of the white matter tract (occurs in regions with high organization such as CC) and restricted in all other directions (J. Gooijers & Swinnen, 2014; Grieve, Williams, Paul, Clark, & Gordon, 2007). Increased fractional anisotropy (FA) score in the callosal area connecting the supplementary motor areas and caudal cingulate areas of the two hemispheres is associated with improved bimanual coordination performance in asynchronous movements (Johansen-Berg, Della-Maggiore, Behrens, Smith, & Paus, 2007). Fling and Seidler also found that the increased FA value in the CC region connecting primary motor cortices, is correlated with poorer bimanual performance in younger adults, accompanied by greater interhemispheric inhibition (IHI) (B. W. Fling & R. D. Seidler, 2012). The relationship between the microstructure of the CC and bimanual performance has also been studied in advancing age older adults. Older adults have lower FA values in CC than younger adults, and there is abundant evidence for a link between decreased FA values and poor bimanual performance which coincides with macro- and microstructural changes of the CC (Bonzano et al., 2008; Sullivan et al., 2001; Yap et al., 2013). Overall, the CC is maturating and deteriorating over the span of life and its structural changes is reflected in our bimanual performance. The relationship between training induced CC plasticity and CC pathology will be addressed below.

In addition to age-related changes of bimanual coordination following maturation and deterioration of the CC, training-induced plasticity gives rise to enhanced bimanual function, an effect which has been studied especially in complex tasks with musicians. Musicians who experience intensive training dominated by complex multi-finger bimanual action have increased CC size in the anterior half, a finding that is compatible with plastic changes of components of the CC during a maturation period within the first decade of human life (D. J. Lee, Chen, & Schlaug, 2003; Schlaug, Jäncke, Huang, Staiger, & Steinmetz, 1995). DTI analysis showed that experienced musicians have higher FA values than non-musician controls; however, the onset of musical training is also a decisive factor (C. J. Steele, Bailey, Zatorre, & Penhune, 2013). Consistent with developmental changes in the posterior midbody of the CC, musicians with early onset of training have greater FA in this area. This area of the CC provides connections between sensorimotor cortices of the two hemispheres which has unequivocal importance in the successful execution of complex bimanual behavior. Therefore we have compelling evidence that early onset of training, not the duration of training, and practice itself, not genetic factors, promote enduring plastic changes in the brain structure that ultimately nurture successful musicians (Hyde et al., 2009; C. J. Steele et al., 2013).

The study of CC pathologies leading to partial or complete loss of its function as well as the differential impact of these pathologies sheds light on the role of CC in bimanual coordination. These pathologies include agenesis, callosotomy, multiple sclerosis, and traumatic brain injury (J. Gooijers & Swinnen, 2014). It has been argued that bimanual coordination deficits following incomplete or complete sectioning of the CC are tightly associated with the nature of the task. While novel bimanual tasks are impossible to perform after callosotomy, well-learned bimanual actions can be performed with no difficulty (Caillé, Sauerwein, Schiavetto, Villemure, & Lassonde, 2005; Elizabeth A. Franz, Waldie, & Smith, 2000; Sternad, Wei, Diedrichsen, & Ivry, 2007). Moreover, Swinnen proposed another distinction between spatial and temporal coupling in patients with split-brains (J. Gooijers & Swinnen, 2014). Not being disrupted by mutual interference, patients with callosotomy have the ability to draw conflicting spatial trajectories, an outcome that is constrained in persons with intact CC where callosal connections give rise to spatial interference in movements (Elizabeth A. Franz et al., 1996). This result was later replicated, but only in patients in whom the posterior third of the CC was sectioned (Eliassen et al., 1999). We can conclude that the origin of spatial interference during bimanual coordination tasks occur via the posterior area of CC. However, the neural origin of temporal coupling is dissociated from spatial coupling.

It was originally proposed by Franz that the CC does not mediate temporal coupling, but other studies reported the involvement of the anterior area of the CC in for temporal coupling (Eliassen et al., 1999; Kennerley, Diedrichsen, Hazeltine, Semjen, & Ivry, 2002; Ouimet et al., 2010). Surprisingly, properties of temporal coupling remain intact in patients with callosotomy (Elizabeth A. Franz, 2003; Tuller & Kelso, 1989). But it is essential to note that the task that led to this conclusion was bimanual tapping, which constitutes a movement with event structure. This may raise the question of what neural underpinning(s) are responsible for mediating temporal coupling. Strong evidence was provided in favor of temporal coupling mediated by a combination of basal ganglia, cerebellum and thalamus (Elizabeth A. Franz, 2003). With regards to the cerebellum, temporal coupling is controlled by cerebellar commissures. Support for this idea emerged from the study of patients with unilateral cerebellar lesions who showed intact temporal coupling during bimanual tapping while the timing processes were damaged in unimanual movement with increased within-hand variability for the ipsilesional side (E. A. Franz, Ivry, & Helmuth, 1996; R. B. Ivry & Keele, 1989). This so called "bimanual advantage" is a product of integration of two independent times that regulate the tapping of the ipsilateral side. Similar results were re-produced in intact individuals, leading to the proposal that "two hands are better than one" (Helmuth & Ivry, 1996). Among various subcortical structures, intercerebellar coupling is a key-player for the bimanual advantage, as this coupling only exists during simultaneous task execution, and not during unimanual tasks (Pollok, Butz, Gross, & Schnitzler, 2007). Temporal coupling could also occur between both halves of the basal ganglia (Elizabeth A. Franz, 2003) (Kraft et al., 2007). Interestingly, the hands become temporally uncoupled during bimanual circle drawing in patients with split-brains, which constitutes a continuous movement (R. Ivry et al., 2004). This clearly emphasizes that neural substrates of continuous and discontinuous movements are dissociable. Thus, assigning anterior and posterior subdivisions of the CC to temporal and spatial features of bimanual coordination is somewhat inconclusive and there is evidence against this idea. Opposing the dominant view attributing temporal coupling entirely to anterior CC, some studies also noted the involvement of the posterior region of the CC temporal coupling (Eliassen, Baynes, & Gazzaniga, 2000). Overall, the study of CC pathologies serves as an essential window into understanding the neural dynamics of bimanual coordination and how distinct aspects of coordination can be mapped onto

CC substructures. As a structure that serves the function of information transmission across the two hemispheres, virtually all the subregions of the CC along the anterior-posterior gradient are integral to a functioning bimanual coordination, and structural alterations as a result of either training or pathology can modulate the effectiveness of CC function.

While it is essential to pinpoint active regions in bimanual coordination, it seems rational to explore how communication between these key regions facilitated by the CC influences the ability to accomplish bimanual movement, especially that the nature of coordinative action of the upper limbs relies on heavy coupling between the hemispheres (Rueda-Delgado et al., 2014). It is fruitful to assess this connectivity as a function of internal and external factors. Multiple studies suggest that the coordination effort in bimanual movements makes them more arduous that unimanual actions. This is particularly reflected in the interhemispheric interactions. In an EEG study in 2009, it was argued that the successful performance of bimanual movement demands extensive information flow between the hemispheres, and the degradation of performance is due to the lack of sufficient interhemispheric connectivity measured by EEG coherence (Serrien, 2009). In line with this idea, magnetoencephalography (MEG) data corroborated that coordinative output depends strongly on different cortical and subcortical levels within and between both hemispheres, including coupling between cerebellum and contralateral PMC and between both pre-motor areas (Pollok, Südmeyer, Gross, & Schnitzler, 2005).

It is also of interest to examine these interactions when the coordination mode is not default (i.e., anti-phase or asymmetric) and how challenging circumstances affect coupling in cortical and subcortical regions. It is been shown that there is increased functional connectivity in anti-phase, in comparison with in-phase, coordination (Heitger et al., 2013). In parallel to this finding, asymmetric movements are associated with increased inter-hemispheric coherence (Serrien & Brown, 2002). We interpret these finding as the two hemispheres becoming more interactive to cope with additional demands and securing a successful bimanual performance. The important role that hemispheric connections play in bimanual action is further substantiated by the means of dual pulse measurement of interhemispheric inhibition IHI in TMS studies. Individuals with greater IHI exhibit degraded capacity and poorer performance on bimanual assignments (Brett

W. Fling & Rachael D. Seidler, 2012). IHI is also modulated by task, with symmetric movement exhibiting larger IHI than asymmetric ones (Tazoe, Sasada, Sakamoto, & Komiyama, 2013). It may also be the case that IHI is reduced during common-goal movement to serve the required communication between the two arms to achieve their unified goal (Liao et al., 2018). These findings lend support to the fact that suppression of contralateral inhibition (i.e., reduced IHI) subserves bimanual motor control.

In the context of internal factors such as aging and learning, a range of studies underline the significance of interactions within the brain. As the coordination deviates from the default preferred movement mode, such as in-phase or 1:1 frequency, to more challenging scenarios such as polyrhythm, the task is not easily executed and requires training to achieve accurate and stable performance. Training and learning-induced modulation of hemispheric interactions have been studied extensively using both imaging and electrophysiology. A group of participants who learned a complex 90° out-of-phase bimanual coordination pattern showed enhanced functional connectivity coinciding with richer performance, indicating that higher connectivity favors the execution of complex bimanual tasks (M. H. Heitger et al., 2012). Studies conducted using EEG and MEG also support the idea that learning and skill acquisition is accompanied by changes in brain functional coupling. Researchers proposed a training-induced elevation of inter-hemispheric coherence in the initial stage of learning that was facilitated by the CC (Andres et al., 1999). However, this initial increase returned to levels observed in unimanual controls after training (Andres et al., 1999). This might provide us with direction on the reason why patients with lesions to the CC are unable to acquire efficiency in novel bimanual task but are able to execute previously learned tasks (Gerloff & Andres, 2002; Serrien & Brown, 2003). This seminal work was later corroborated by other studies, all pointing to the fact that the initial increase in inter-hemispheric coupling is necessary while learning the coordinative complexity of bimanual task, followed by a downward shift back to baseline when automaticity is accomplished (Puttemans et al., 2005). These task-specific modulations of functional coupling occur in premotor and primary sensorimotor areas (Andres et al., 1999; Geffen, Jones, & Geffen, 1994).

To summarize, the CC facilitates the transfer of information between the two hemispheres and successful performance of bimanual coordination tasks depends heavily on the role that CC plays. Each part of the CC along the anterior-posterior gradient serves characteristics of temporal and spatial aspects of bimanual coordination. As shown in learning and aging studies, interhemispheric connectivity, facilitated by CC, play a major role in coordination between the two arms/hands in bimanual movements. However, the effect of goal-conceptualization on interhemispheric interactions continues to be an active question in the literature, especially when the bimanual task resembles movements of daily life. Two questions should be addressed: does common-goal movement require stronger interhemispheric coupling relative to dual-goal movement? Does the single factor of bimanuality increase interhemispheric coupling when compared to unimanual movements? Chapter 2 of this dissertation endeavors to provide answers to these uninvestigated questions.

1.2.6. Consequence of stroke and spinal cord injury: deficits in bimanual coordination

Like any other motor behavior, bimanual movements are influenced by injuries and diseases of the central nervous system. The ability to produce different patterns of bimanual coordination, from symmetric to asymmetric and from common-goal to dual-goal, is markedly reduced after clinical pathologies such as stroke and SCI. Here, we aim at identifying the aspects of bimanual patterns that are impaired after each condition and the extent to which rehabilitation interventions have been successful in restoring bimanual motor control.

Individuals with unilateral stroke experience reduced use of bimanual actions in their daily lives (Haaland et al., 2012; S. Kantak et al., 2017; Michielsen, Selles, Stam, Ribbers, & Bussmann, 2012). For a long time, clinicians attributed the alterations in using bimanual coordination to the reduced capability of the paretic arm (Sleimen-Malkoun, Temprado, Thefenne, & Berton, 2011). On this basis, it can be assumed that the improvement of the weaker limb performance automatically re-establishes bimanual coordination (S. Kantak et al., 2017). However, evidence suggest that unimanual impairment does not correlate with bimanual coordination deficits (S. Kantak, McGrath, & Zahedi, 2016; C. Lowrey, Jackson, Bagg, Dukelow, & Scott, 2014). Moreover, the reduction in the use of bimanual actions after unilateral stroke was thought to be a consequence of the paretic arm deficits (Beer, Dewald, & Rymer, 2000; Roh, Rymer, & Beer, 2015; Roh, Rymer, Perreault, Yoo, & Beer, 2012). This idea was rejected by Waddle and colleagues who found that improvement in the motor capacity of the paretic arm, evaluated by action research arm test (ARAT), does not translate to improvement of upper limb performance outside of the clinic (Waddell et al., 2016). We may conclude that even in the presence of improvement of the paretic arm performance, recovery of the functional upper limbs activities of daily life including bimanual coordination is out of reach.

Bimanual coordination in stroke patients has been studied extensively in both rhythmic and discrete task using kinematic and kinetic measures. There is accumulating evidence that despite unilateral weakness, individuals with stroke retain some level of temporo-spatial coupling between the two arms for symmetric movements (C. Cunningham, Stoykov, & Walter, 2002; S. Kantak et al., 2017; Lewis & Byblow, 2004; Mudie & Matyas, 2000; Waller, Harris-Love, Liu, & Whitall, 2006), but anti-phase coordination patterns are strongly altered with reduced stability and accuracy (Rose & Winstein, 2013). Two views may prevail. First, activity in the non-paretic arm is eclipsed by the reduced performance of the paretic arm, causing disruption of bimanual coordination (Cohn, 1951). In other words, the non-paretic arm is bound to the frequency limits of the weaker arm; thus, slowing down from its natural frequency to maintain interlimb coupling (S. Kantak et al., 2017). Second, the residual coordination ability after stroke is due to a balanced performance of both arm; a boost up of speed from the paretic arm and decrease of speed from the non-paretic arm (S. Kantak et al., 2017).

In discrete movements, both kinematic and kinetic aspects of the movements have been explored in people experiencing stroke. For example, Harris-Love and co-workers noted a left-right symmetry in peak acceleration and velocity parameters in bimanual reaching, unlike the unimanual task in which paretic versus non-paretic task parameters were significantly different (Harris-Love, Waller, & Whitall, 2005). Supporting the idea of an increase in asymmetric coupling after stroke, Dickstein reported a prolonged movement time for the non-paretic hand in a bimanual elbow-flexion task compared to unimanual elbow-flexion (Dickstein, Hocherman, Amdor, & Pillar, 1993). On the other hand, kinetic analysis revealed asymmetrical (unequal) force contribution in a bimanual isometric force production task, with the weaker arm contributing less compared to the stronger arm (Kang & Cauraugh, 2015). However, this asymmetry was muscle-specific and depended on the nature of the task, whereby force asymmetry was significantly increased in wrist and finger extension task in people with stroke but remained relative symmetric in the gripping task (Kang & Cauraugh, 2015; Lodha, Patten, Coombes, & Cauraugh, 2012). These seemingly contradictory results are also highlighted in common-goal versus dual-goal movements. In 2016, coordination performance of a group of individuals with stroke was assessed during symmetric and asymmetric reaching to a common target versus independent targets in a virtual reality setting (S. Kantak et al., 2016). The results were diametrically different for the two conditions. While the contribution from the paretic and non-paretic limbs was almost equal in the dual-goal task, the non-paretic hand demonstrated greater contribution to the completion of the common-goal task (S. Kantak et al., 2016). Although it is tempting to interpret these findings as inconsistent, it should be noted that the severity of stroke and location of lesion in each of these studies varied, so did the level of motor deficits in study participants with stroke. Moreover, the requirement, complexity, and the nature of the tasks are important factors in interpreting the results of these studies.

Most of the research work discussed earlier was designed for the research laboratory environment and does not conform to the requirement of activities of daily life. Many real life bimanual actions require asymmetric cooperation between the two upper limbs (overwriting the default coordination mode); therefore, it is necessary to quantify bimanual coordination with tasks that represent real life scenarios. For instance, Kantak and colleagues investigated a reach to pick up a box task which is formed by two distinct phases: transport phase that is achieved by bimanual symmetric-parallel reaching followed by cooperative grip and box lifting (S. S. Kantak, Zahedi, & McGrath, 2016). Interestingly, individuals with stroke were efficient in reaching in a symmetric-parallel manner during the transport phase, but demonstrated a deficient coordination pattern during the picking up the box phase manifested in grasping and picking delays. Deficits of bimanual coordination during the second phase were revealed by data from 3D force transducers, and were characterized by multiple non-aligned peaks in the grip force profile of the group with stroke (S. Kantak et al., 2017) (S. S. Kantak et al., 2016). Another example of naturalistic experiments is “open-the drawer press button” task that enforces asymmetric movement of the upper limbs such that one hand pulls the drawer and the other one moves simultaneously in the opposite direction to reach forward for button pressing, a behavior observed in neurologically intact individuals (S. S. Kantak et al., 2016). On the contrary, people with stroke accomplishes the task in a sequential manner, where the paretic hand responsible for button pressing waits until the non-paretic hand finishes the pulling part. The two examples above underscore the fact that emphasis should be placed on such cooperative tasks to better understand the extent to which bimanual actions are deteriorated after stroke. It can be concluded that the ability to execute bimanual tasks that are symmetric and have independent goals is relatively retained, but those with cooperative and asymmetric demands are more affected after stroke.

The primary focus of the rehabilitation research studies after stroke has been largely on improving the functional capacity of the contralesional arm, and only a few studies have explored the benefits of paretic arm rehabilitation on bimanual coordination. Moreover, evidence suggests that only limited, and not total, transfer of learning occurs from unimanual to bimanual skills (and vice versa) within the same limb in individuals with intact neuro-musculo-skeletal system (Hinder, Carroll, & Summers, 2013; Nozaki, Kurtzer, & Scott, 2006). It is tempting to assume that this may also be the case for persons with stroke and hence it is still not clear whether recovery of the paretic arm performance leads to improvement of bimanual coordination. On the other hand, bimanual training protocols have been tested and have been successful in yielding promising impact on unimanual skill, but the effect on bimanual coordination is not determined. For example, bimanual symmetric rehabilitation therapy proved to prime the brain to be more responsive to motor practice, which was reflected in better paretic arm function (Stinear, Barber, Coxon, Fleming, & Byblow, 2008). Bimanual symmetric movements take advantage of the inherent neuromuscular linkage between the two upper limbs, and reduce intracortical inhibition from the contralesional to the ipsilesional M1, while increasing excitability within ipsilesional M1 (Stinear et al., 2008). Despite the fact that the rehabilitation training was bimanual, post-training effects were only assessed on the paretic arm and it remains an open question whether such rehabilitation training is beneficial for remediating bimanual impairments. There is a lack of evidence to strongly recommend bimanual training protocols as the advantageous approach for functional motor improvements (Coupar, Pollock, van Wijck, Morris, & Langhorne, 2010). However, clinical characteristics of stroke among survivors vary substantially and the type/parameters of rehabilitation training should be tailored for each individual. Therefore, contribution of bimanual training to the motor recovery and regaining bimanual coordination after rehabilitation training in people with stroke are both still debated. More research also seems necessary on bimanual training that mimic the asymmetric type of interaction between the upper limbs, and rely less on the inherent neuromechanical coupling in bimanual actions. As suggested by Sleimen-Malkoun and colleagues, symmetry breaking and coupling strength should be targeted on future rehabilitation interventions to promote effective recovery of upper limb function after stroke (Sleimen-Malkoun et al., 2011).

Unlike stroke, there is a dearth of literature on bimanual coordination after cervical spinal cord injury (cSCI). To the best of our knowledge, only two research studies investigated the effects of cSCI on bimanual reach to grasp movements (Britten et al., 2017; Calabro & Perez, 2015). Britten and coworkers recruited participants with acute SCI with lesions at C4-C8 (ASIA B-D), and examined the detrimental effect of their injury on interlimb coordination. Their findings showed that study participants with cSCI had prolonged unimanual reach-to-grasp movement duration compared to the neurologically-intact participants. These effects were worse during the bimanual reach-to-grasp condition (Britten et al., 2017). The authors argued that the prolonged movement time was caused by a longer deceleration phase compared to uninjured participant, likely for the purpose of corrective adjustment in preparation for the grasp phase. Maximal grasp aperture (MGA) occurred earlier in the bimanual condition relative to the unimanual condition in both control and cSCI participants, but individuals with cSCI produced significantly earlier MGA than uninjured individuals (Britten et al., 2017). This strategy could have been utilized by participants with cSCI to compensate for the increased time required to scale the aperture to object size.

Interlimb coordination seems to be relatively retained in participants with cSCI as they terminate movements in a synchronous fashion. Calabro and Perez explored the asymmetries during a bimanual coordination task between the stronger and weaker arms in individuals with cervical injury at the C3-C8 level (Calabro & Perez, 2015). Participants with cSCI struggled to maintain interlimb synchrony during a bimanual reach-to-grasp, and the limb with more impairment caused movement delays of less-impaired limb during the hand opening and closing phase. This effect was exacerbated in individuals with increased interlimb coordination deficits. The study demonstrated that the asymmetric effect of the injury on the arms transfers to asymmetric influences between movements of the arms in such a way that the less impaired arm was slowed by the negative influence from the more impaired arm (Calabro & Perez, 2015). It was previously reported that bimanual mass practice (this involves repetitive practice of a group of tasks that entails both symmetrical and asymmetrical bimanual movement) leads to improvements in clinical outcome measures of bimanual hand function, accompanied by enlargement of the corticomotor map area (L. Hoffman & Field-Fote, 2013; L. R. Hoffman & Field-Fote, 2010). However, there were no significant differences between unimanual and bimanual training outcome. Altogether, a critical message of the above studies is that after cSCI, a relative ability to perform bimanual coordination tasks is still retained, and bimanual movements should be incorporated into the rehabilitation therapy of individuals with SCI. Britten et al. (Britten et al., 2017) suggested a minimal detrimental effect of the more impaired arm on the less impaired arm, which is contradictory to what Calabro and Perez (Calabro & Perez, 2015) found. Moreover, movements in our ever-changing environment are not limited to symmetric actions, further research is necessary with tasks that require breaking symmetry or those that are accomplished with cooperation of the two arms.

In summary, deficits of bimanual coordination after stroke are not uniform and depends on the nature of the task. Two primary inferences can be made: movement of the non-paretic arm is negatively impacted by the paretic arm, and asymmetry of paretic/non-paretic arm contribution to task completion depends on the nature of the task (i.e., common-goal versus dual-goal and symmetric versus asymmetric). This highlights the importance of goal-conceptualization in shaping bimanual movements and recruitment of neural mechanisms. While limited in the number of research articles, it has been shown that bimanual coordination is also impaired after SCI. Prolonged and asymmetric movements are the two major deficits to bimanual coordination after SCI.

1.3. Transcutaneous spinal cord stimulation:[1]

1.3.1. Introduction

Neuromodulation of the spinal cord by means of non-invasive transcutaneous (tSCS) and implanted epidural (eSCS) spinal cord stimulation may improve sensorimotor rehabilitation after spinal cord injury (SCI) (Angeli et al., 2018; Balykin et al., 2017; Harkema et al., 2011; Inanici et al., 2018). However, developing an optimal treatment approach requires taking advantage of the intrinsic ability of the spinal circuits by facilitating preserved sensorimotor pathways that could drive spinal plasticity (Fregni et al., 2015). The influence of spinal cord stimulation (SCS) does not necessarily depend on the nature of the neurological disorder, but on the operational and functional status of residual neural networks (Dimitrijevic, 1988). Epidural SCS has been shown to modulate neuronal circuits in per-sons with motor-complete SCI, including corticospinal, (Grégoire Courtine et al., 2005; Friedli et al., 2015; Rosenzweig et al., 2010) propriospinal (Gregoire Courtine et al., 2008; Gerasimenko et al., 2009), and corticoreticulospinal (Asboth et al., 2018) tracts. The resulting neuroplasticity is thought to improve spinal motor output and volitional movements even in cases of severely reduced supraspinal input, without negatively impacting residual motor function (Formento et al., 2018; Harkema et al., 2011; Herman, He, D'Luzansky, Willis, & Dilli, 2002; Kou et al., 2021; Mayr, Krenn, & Dimitrijevic, 2016; Wagner et al., 2018). Most recently, eSCS applied to the lumbar spinal cord, in conjunction with intensive locomotor training, enabled persons with clinically motor-complete SCI to walk over ground for short distances (Angeli et al., 2018; Gill et al., 2018; Mayr et al., 2016). This demonstrates that dormant neurons that survive the injury may be reengaged with spinal neuromodulation, and can produce stepping-like movements (Angeli, Edgerton, Gerasimenko, & Harkema, 2014; Grégoire Courtine et al., 2009).

While eSCS has important implications for rehabilitation after SCI, its invasive nature, high cost, and limited accessibility are limitations for rapid translation to a broad population. Transcutaneous SCS is a non-invasive, accessible, and cost-effective alternative that is thought to

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[1] A version of this section has been published.
Barss, T. S., Parhizi, B., Porter, J., & Mushahwar, V. K. (2022). Neural Substrates of Transcutaneous Spinal Cord Stimulation: Neuromodulation across Multiple Segments of the Spinal Cord. Journal of Clinical Medicine, 11(3). doi:10.3390/jcm1103063

be a safe assistive technology with important implications for both furthering our understanding of the mechanisms controlling locomotion, and for rehabilitating sensorimotor function after SCI (Y. Gerasimenko et al., 2015; Martin, 2021; Megía García, Serrano-Muñoz, Taylor, Avendaño-Coy, & Gómez-Soriano, 2019). It has been suggested that tSCS of the lumbar spinal cord may activate similar spinal circuitry to eSCS (Danner, Hofstoetter, Ladenbauer, Rattay, & Minassian, 2011; Ursula S. Hofstoetter, Freundl, Binder, & Minassian, 2018; Ladenbauer, Minassian, Hofstoetter, Dimitrijevic, & Rattay, 2010); if accurate, tSCS is likely to enhance functional recovery in a similar manner to eSCS when paired with rehabilitation strategies. This would also allow for the tSCS to build on the foundation of knowledge of the intrinsic circuitry recruited by eSCS. In case studies and small clinical trials, tSCS improved hand and arm function (P. Gad et al., 2018; Inanici, Brighton, Samejima, Hofstetter, & Moritz, 2021; Inanici et al., 2018; Yang Zheng & Hu, 2020), produced locomotor-like stepping (Balykin et al., 2017; Y. P. Gerasimenko et al., 2015), and improved walking function (Y. Gerasimenko et al., 2015; McHugh, Miller, Leech, Salorio, & Martin, 2020; Solopova et al., 2017) in participants with neurological deficits including incomplete and complete SCI, stroke, and cerebral palsy. Evidence suggests that tSCS may also be used as a viable alternative to pharmacological anti-spasticity approaches, altering the excitability of spinal pathways and possibly augmenting pre- and post-synaptic inhibitory mechanisms (S. P. Estes, Iddings, & Field-Fote, 2017; Ursula S. Hofstoetter et al., 2019). Understanding the impact that tSCS has on spinal cord circuitry is vital to ensuring that the stimulation is applied at therapeutically appropriate sites, and that the parameters of stimulation are chosen so as to optimize the desired rehabilitative effects.

It is critical to realize that not all of the studies using tSCS follow the same pattern of stimulation. Transcutaneous SCS patterns including single pulses, trains of pulses, and waveforms with and without carrier frequencies have been used. The present review focuses on the use of alternating current (AC) tSCS, because most studies aimed at improving functional recovery after SCI have used this type of stimulation. Direct current (DC) tSCS also modulates spinal excitability, and may be another promising and novel tool to pair with activity-based interventions (Bettmann et al., 2020; Powell, Carrico, Salyers, Westgate, & Sawaki, 2018); however, this technique is beyond the scope of this review, and requires further research in order to determine the specific mechanisms involved. In this review, the two common patterns of AC tSCS that have been employed to date will be included and discussed in detail. The first pattern, which will be referred to as unmodulated tSCS, does not include a carrier frequency, and is generally composed of rectangular pulses delivered as single individual pulses, or in trains of 1–90 Hz frequency. The second stimulation pattern, which will be referred to as modulated tSCS, includes rectangular pulses with a carrier frequency of 2.5–10 kHz, de-livered at a rate of 5–40 Hz (Taylor et al., 2021). While both patterns have been reported to modulate neural circuitry across the central nervous system and produce functional outcomes, it is unlikely that they share identical mechanisms of action. The fundamental differences between the two patterns will be highlighted throughout this review as the different studies are discussed.

The aims of this review are as follows: first, to identify the parameters and the potential underlying mechanisms that allow tSCS to facilitate ongoing motor output; secondly, to highlight the effects of tSCS on excitability across multiple segments of the spinal cord; thirdly, to address the ability of multiple sites of tSCS to converge and enhance modulation of spinal reflex and corticospinal pathways; and finally, to explore the potential and limitations for engaging cervical and lumbar spinal cord networks through tSCS to enhance the effectiveness of rehabilitation interventions. This review will also underscore the need for further mechanistic work to optimize tSCS parameters that, when paired with targeted rehabilitation strategies, can effectively improve clinical out-comes.

1.3.2. Historical Perspective

The use of electricity for neuromodulation has a storied history, ultimately leading to a variety of therapeutic electrical stimulation techniques that target spinal networks, including tSCS, eSCS, and intraspinal microstimulation (ISMS) (V. K. Mushahwar & Horch, 1997; Vivian K. Mushahwar, Jacobs, Normann, Triolo, & Kleitman, 2007). Epidural SCS initially emerged in the pain literature in 1967 (Shealy, Mortimer, & Reswick, 1967), and is currently most commonly used for the treatment of intractable chronic pain; while originally designed to alleviate pain, it was used in 1971 as a method for facilitating motor control in persons with multiple sclerosis (Cook & Weinstein, 1973), and to reduce spasticity after incomplete SCI (Pinter, Gerstenbrand, & Dimitrijevic, 2000; R. R. Richardson, Cerullo, McLone, Gutierrez, & Lewis, 1979; R. R.

Richardson & McLone, 1978). In 1979, tonic stimulation of dorsal roots of the spinal cord was shown to generate locomotion in low-spinal cats (Andersson, Forssberg, Grillner, & Lindquist, 1978; Grillner & Zangger, 1979). This work then led to initial investigations demonstrating improved step-ping in humans, and providing the potential for this technology to be used as a translational tool to facilitate improved function after neural injury (Carhart, Jiping, Herman, Luzansky, & Willis, 2004; Harkema et al., 2011; He, Jiping, Herman, & Carhart, 2006; Herman et al., 2002).

In humans, eSCS involves implanting electrodes over the dura mater encasing the lumbosacral segments of the spinal cord. Dorsal root fibers are the first to be recruited, with the lowest thresholds, while the ventral root fibers are the least accessible (Rattay, Minassian, & Dimitrijevic, 2000). This recruitment leads to the activation of motor neurons through monosynaptic and poly-synaptic proprioceptive circuits, and increases the overall excitability of the spinal cord, allowing for greater responsiveness of spinal circuits to descending signals and sensory feedback (Formento et al., 2018). Extensive evidence from animal studies has led to the hypothesis that electrically stimulating the human spinal cord through the epidural space can facilitate improvements in motor function.

Transcutaneous SCS was inspired by high-voltage percutaneous electrical stimulation over the lumbosacral spinal column to activate peripheral motor axons (Maertens de Noordhout, Rothwell, Thompson, Day, & Marsden, 1988). In 1997, the generation of locomotor-like activity with the application of tSCS over the lumbar enlargement was demonstrated in individuals with SCI (Shapkova, 2004). It was then suggested that there are low-threshold sites in the posterior structure of the human lumbosacral cord that could be accessed from the surface (Rattay et al., 2000). In 2007, and encouraged by earlier discoveries, Minassian et al. revealed that posterior root afferents can be accessed by tSCS with single pulses (unmodulated), and they reported monosynaptic reflex responses in multiple muscles of the legs (Karen Minassian et al., 2007). Later, it was shown that unmodulated tSCS can enhance voluntary locomotor-like electromyographic (EMG) activity (U. S. Hofstoetter et al., 2013) and modify spasticity in individuals with incomplete SCI (Ursula S. Hofstoetter et al., 2014). In 2015, tSCS was used with a novel waveform that included a carrier frequency (i.e., modulated) to activate spinal networks while reducing the perception of pain associated with the necessarily high stimulus amplitudes (Y. Gerasimenko et al., 2015; Y. P. Gerasimenko et al., 2015). The tSCS parameters were based on a previous finding that a 10 kHz carrier frequency of transcutaneous stimulation reduces the likelihood of activating pain fibers (Ward & Robertson, 1998). Building on these exciting initial investigations, the tSCS literature has incorporated a diverse set of stimulation parameters that are vital to understand, as they may have important implications for improving function in persons experiencing sensorimotor impairments due to neurological conditions.

1.3.3. Properties of transcutaneous spinal cord stimulation (tSCS)

1.3.3.1. Parameters of tSCS

Typically, tSCS is applied through circular adhesive electrodes of 2–3 cm diameter that are placed on the skin overlying the lumbar or cervical segments of the spinal cord (Figure 1.1). Optimal placement of electrodes is dependent on the individual symptoms, desired rehabilitation outcomes, and paired rehabilitation strategies, on a case-by-case basis. When targeting the lower extremities, the most common cathode placement is over the T11–T12 and/or L1–L2 spinous processes, while C6–C7 or C7–T1 is the most common placement for the upper extremities (Taylor et al., 2021). The anode electrodes are placed either over the iliac crests or the anterior superior iliac spine (Taylor et al., 2021).

In addition to electrode placement, it is important to consider the waveform characteristics of the applied current for maximal therapeutic outcomes (Fregni et al., 2015; Kou et al., 2021; Megía García et al., 2019). With unmodulated tSCS, which evolved from the eSCS literature, rectangular mono- or biphasic pulses of 0.4–2 ms duration are typically delivered at a frequency range of 1–90 Hz and stimulation intensity of up to 170 mA (Megía García et al., 2019; Taylor et al., 2021). On the other hand, the novelty of the modulated stimulation pattern comes from its unique waveform, which includes a carrier frequency of up to 10 kHz within a given pulse. Such high-frequency stimulation approaches were originally used to reduce the perception of pain during transcutaneous nerve stimulation (Ward & Robertson, 1998). The waveform in the modulated stimulation pattern generally consists of 0.3–1 ms long rectangular biphasic or monophasic pulses that repeat at a frequency of 5–40 Hz. Each of these pulses encompasses a carrier frequency of 2.5–10 kHz, aimed at suppressing the user's perceived pain and, thus, allowing for greater current amplitudes to be employed. The amplitude of the current for modulated tSCS is similar to that of unmodulated tSCS, and ranges from 30 to 180 mA, depending on the stimulation site and the desired outcome. In neurologically intact participants, the intensity of modulated tSCS (with a 5 kHz carrier frequency) allows for maximal tolerable current amplitudes of 103 mA, while unmodulated tSCS has maximal tolerable amplitudes of 39 mA. However, when considering maximal tolerable stimulation with respect to the stimulation levels needed to evoke motor responses, tSCS with a carrier frequency was no different than unmodulated tSCS in reducing the perception of pain (Manson et al., 2020).

Interestingly, when using an array of electrodes and adjusting the parameters of stimulation—including intensity and location—different patterns of independent and coordinated upper limb motion at both distal and proximal joints have been elicited, showing the potential of tSCS without a carrier frequency to evoke functional movements (Yang Zheng & Hu, 2020). Therefore, the chosen parameters of tSCS can have a meaningful effect on the recruited circuitry and the functional movements that are facilitated or inhibited. Understanding how the applied electrical current is integrated into the spinal circuitry is vital.

1.3.3.2. Current Flow Involved in tSCS

The current flow and electrical potential generated by eSCS and tSCS are markedly different (Ursula S. Hofstoetter et al., 2018; Ladenbauer et al., 2010). With eSCS, 80–90% of the ionic current flows between the active electrodes through the cerebrospinal fluid (Jensen & Brownstone, 2019). In tSCS, the current flow is strongly influenced by the electrical properties of the numerous conductivity boundaries of body tissues (e.g., skin, fat, muscle, and bone), with computer simulations estimating that only ~8% of the overall current flows through the cerebrospinal fluid (Ladenbauer et al., 2010). With the dramatic difference in current flow and the proximity of neural structures to the electrodes between eSCS and tSCS, both the selectivity of spinal circuitry and the required stimulation intensity are dissimilar. Modelling studies suggest that the superficially located large-diameter posterior column fibers with multiple collaterals have a threshold three times higher than that of posterior root fibers (Danner et al., 2011). For both tSCS and eSCS, large-diameter proprioceptive sensory fibers within the posterior rootlets/roots have the lowest thresholds of all neural structures within the vertebral canal (Ursula S. Hofstoetter et al., 2018), making it unlikely that the effects of SCS arise exclusively from dorsal column stimulation (Jensen & Brownstone, 2019). Computer modeling indicates that action potentials generated by tSCS are initiated in the posterior root fibers at their entry into the spinal cord, or along the longitudinal portions of the afferent fiber trajectories, depending on the cathode position (Ladenbauer et al., 2010). Evidence suggests that the reflex nature of unmodulated tSCS exploits the difference in the strength–duration properties of sensory and motor axons; however, future research should be conducted to explore how modulated tSCS generates action potentials in neural tissue (Burke, 2016). At stimulation intensities that result in the recruitment of posterior column axons, co-activation of posterior root fibers of large and small diameters is observed, demonstrating the substantial differences in the thresholds of activation of various components of the spinal cord (Danner et al., 2011). Moreover, increasing stimulation intensity engages spinal interneurons via synaptic projections which, in turn, activate motor neurons (Y. Gerasimenko et al., 2015; Lavrov et al., 2006). These simulation results provide a biophysical explanation for the electrophysiological findings of lower limb muscle responses that are induced by posterior root stimulation (Figure 1.2A). However, it should be noted that these computer simulation studies have all applied unmodulated tSCS (i.e., without carrier frequency), and the results may not necessarily be generalizable to other types of pulses. Understanding the potential unique properties associated with modulated tSCS is vital for implementing tSCS in a manner that optimizes functional recovery after neural injury or disease. Similar simulation studies using high carrier frequencies are necessary in order to extend the knowledge regarding current flow in tSCS.

1.3.3.3. Transcutaneous SCS Carrier Frequency Is Important for Reducing Discomfort, but Its Role in Restoring Motor Function Remains Unclear

The inclusion of a carrier frequency within a given stimulation pulse is used for its ability to disrupt synchronous firing of the high-threshold C-fibers related to pain perception (Wu et al., 2002). Pain management through SCS is based on the gate control theory introduced in 1965 (Jensen & Brownstone, 2019), which proposed that the activation of Aβ mechanoreceptor fibers that synapse onto a range of neurons within the dorsal horn that release inhibitory neurotransmitters—including γ-amino butyric acid (GABA) and adenosine (Ahmed, Yearwood, De Ridder, & Vanneste, 2018)—reduces the activity of nociceptive projection neurons in laminae I and V traveling along the spino-thalamic tract. It has also been proposed that high-frequency stimulation of the spinal cord blocks discomfort by inactivating paresthesia-inducing large-diameter fibers and activating medium–small-diameter fibers that suppress wide-dynamic-range neurons encoding neuropathic pain (Arle, Mei, Carlson, & Shils, 2016). Sub-perception SCS at 1 kHz was more effective for pain relief compared to low-frequency supra-perception stimulation (North, Hong, & Cho, 2016). Moreover, a recent eSCS study suggested that there was no observable difference between 1 kHz and 10 kHz stimulation for the relief of back pain (Chakravarthy, Richter, Christo, Williams, & Guan, 2018). Charge per pulse is lower in high-frequency eSCS in comparison with low-frequency stimulation, while charge per second is higher (Ahmed et al., 2018). While these studies did not use tSCS, and were only aimed at pain management, they can play an important role in explaining the potential mechanisms that reduce dis-comfort in modulated tSCS. Manson et al. have recently shown that the maximal tolerable stimulation intensity is significantly greater during modulated tSCS compared to unmodulated tSCS (Manson et al., 2020); however, the stimulation intensity required to evoke a muscle response (motor threshold) was correspondingly higher with a carrier frequency, leading to no difference in the relative current amplitude required to evoke a motor response (Manson et al., 2020). This study indicated that the addition of a carrier frequency reduces discomfort for a given current amplitude compared to unmodulated tSCS, but does not reduce discomfort when evoking the same motor response.

What is less clear is the impact that the carrier frequency has on the neural circuitry recruited during tSCS, and the specific role it serves to improve functional recovery when paired with rehabilitation strategies. Recently, hand and arm function improved significantly during a single session of cervical tSCS with a 5 kHz carrier frequency applied in individuals with an SCI compared to when a carrier frequency was not included (Benavides et al., 2020). However, limited data are available as to the differences in specific neural substrates recruited by tSCS with and without a carrier frequency. Overall, integrating a carrier frequency may be an important feature of tSCS that not only circumvents pain com-pared to other stimulation profiles, but also promotes effective restoration of function after SCI. Further exploration is required in order to understand whether the carrier frequency is a unique feature necessary for optimizing the use of tSCS for sensorimotor recovery. Incorporating this knowledge into a mechanistic framework for the implementation of tSCS is essential in order to facilitate optimal functional recovery after neurological damage.

1.3.3.4. Mechanisms of tSCS Recruitment

The principal mechanism by which tSCS non-invasively activates inaccessible neuronal networks of the spinal cord likely includes the recruitment of afferent fibers (large–medium) in the posterior root in order to elevate spinal network excitability (Milosevic, Masugi, Sasaki, Sayenko, & Nakazawa, 2019; Sayenko et al., 2018). The excitability of spinal interneuronal networks can be readily modulated (changing the networks' physiological state) without directly producing action potentials (Y. Gerasimenko et al., 2015). The route of stimulation propagation is through the dorsal root afferents, as indicated by the significant inhibition of cervical tSCS responses when using paired stimuli, during passive muscle stretching, and during muscle–tendon vibration (Milosevic et al., 2019). Moreover, it has been suggested that eSCS and modulated tSCS can engage both afferent and efferent path-ways, based on observations of early- and medium-response components of evoked potentials that are partially ascribed to posterior roots/group Ia/group II and motor neurons/anterior roots (Y. Gerasimenko et al., 2015; Sayenko, Angeli, Harkema, Edgerton, & Gerasimenko, 2013). It is proposed that as stimulation intensity is increased, in addition to the Ia afferents, the smaller diameter afferents such as group Ib, larger diameter cutaneous afferents, group II muscle spindle afferents, and even more intraspinal connections and spinal interneurons are recruited through tSCS, similarly to what has been observed in eSCS (Y. Gerasimenko et al., 2015; Lavrov et al., 2006). This, in turn, brings interneurons and motor neurons closer to their firing threshold, making them more likely to respond to limited post-injury descending drive and improving supraspinal control after both modulated and unmodulated tSCS (Y. P. Gerasimenko et al., 2015; Ursula S. Hofstoetter et al., 2018). Both electrophysiological and computer modeling studies to date suggest that unmodulated tSCS excites posterior root fibers similarly to eSCS (Ursula S. Hofstoetter et al., 2018; Karen Minassian et al., 2007).

Recently, a few studies have compared the different effects of modulated and un-modulated tSCS on descending input. Benavides et al. reported that single-site tSCS applied with a 5 kHz carrier frequency at the C5–C6 level facilitated the amplitude of cervicomedullary-evoked potentials (CMEPs), but did not increase the amplitude of the motor-evoked potentials (MEPs) (Benavides et al., 2020); this was accompanied by an increase in the level of short-interval cortical inhibition (SICI). When tSCS was applied without the carrier frequency, both cortically and subcortically driven responses were facilitated. This is similar to our recent investigation, which found that modulated tSCS (33 Hz trains of 1 m long pulses with a 10 kHz carrier frequency) applied over the C3–4 and C6–7 spinous processes in neurologically intact individuals did not alter MEPs assessed in the forearm flexors (Parhizi B, 2021). Moreover, data from a paired associative stimulation (PAS) paradigm involving single pulses of transcranial magnetic stimulation (TMS) and unmodulated tSCS arriving at the same time at spinal motor neurons revealed increases in corticospinal excitability, but facilitation of MEPs following tSCS was less pronounced when tSCS pulses were filled with a carrier frequency (Al'joboori et al., 2021). These studies highlight the fact that in the presence of a carrier frequency, tSCS may be unable to facilitate MEPs. In contrast, it was shown that sub-motor-threshold tSCS without a carrier frequency, applied for a short period of 10 min to the cervical region, did not alter the excitability of the corticospinal and spinal reflex pathways (Sasaki et al., 2021). At first glance, these results seem contradictory; however, the stimulation duration, stimulation amplitude, frequency of stimulation, stimulation waveform (modulated/unmodulated), and target muscles varied across these studies, which may have influenced the neuromodulatory effects of tSCS. By priming neural structures at the level of the spinal cord, unmodulated tSCS modulated spinal reflex excitability and reduced spasticity in a manner similar to that seen with passive cycling movements (S. P. Estes et al., 2017). This suggests that alterations in spinal circuitry—including presynaptic influences—are likely the primary target of tSCS, and play an important role in the recovery of arm and hand function in persons with SCI.

Importantly, dorsal root stimulation is likely not entirely responsible for the effects of tSCS. Group Ia muscle spindle afferent fibres, which travel in the dorsal roots, have a lower threshold of activation compared to the largest cutaneous fibres (Macefield, Gandevia, & Burke, 1989). If the effects of tSCS are only due to the activation of dorsal root afferents, then at low stimulation amplitudes the large-diameter group Ia afferents should be activated, leading to muscle contractions and proprioceptive errors via monosynaptic reflexes (Jensen & Brownstone, 2019). However, cutaneous sensation typically occurs over a large range of stimulus amplitudes that are lower than those required to produce motor responses mediated by purely monosynaptic re-flex pathways, and proprioceptive errors are not a significant occurrence (Rijken, Vonhögen, Duysens, & Keijsers, 2013), making it unlikely that tSCS functions entirely by stimulating dorsal root afferents. Epidural SCS at 1–2 Hz has been shown to activate inhibitory interneurons in laminae I–III, albeit with latencies consistent with trans-synaptic (i.e., indirect) activation (Dubuisson, 1989). Therefore, it is important to consider whether inhibitory neurons in this region are the main or, at least, a contributing mechanism underlying the therapeutic benefit of tSCS; that is, tSCS may re-store inhibition by enhancing dorsal horn GABAergic systems. It has been suggested that islet cells in the substantia gelatinosa require further consideration as prime candidates for the inhibitory effects on pain (Jensen & Brownstone, 2019).

Moreover, while it is widely believed that tSCS depolarizes sensory afferents in the dorsal roots and dorsal horn that transsynaptically recruit motor pools, it remains possible that polysynaptic connections from cutaneous mechanoreceptors in the skin act on both sensory processes and motor pools in the spinal cord. This, in turn, alters the excitability at both the level of the spinal cord—where the stimulation is provided—as well as remote levels of the spinal cord, through propriospinal interneuronal connections. Cutaneous inputs are known to have diffuse input that is specific to the task, phase, and amplitude at which stimulation is delivered (E. P. Zehr et al., 2016; E. Paul Zehr & Stein, 1999). It is therefore plausible that the recruitment of cutaneous mechanoreceptors surrounding the electrodes may contribute to the neuromodulatory effects of tSCS through these polysynaptic connections. The potential role of cutaneous mechanoreceptors in the skin with tSCS remains an important avenue to explore in future work (Beekhuizen & Field-Fote, 2005; Duysens & Pearson, 1976; Vallbo & Hagbarth, 1968).

A potential mechanism by which tSCS improves upon previously developed rehabilitation interventions is potentiation. Guiho et al. observed potentiation of supraspinal evoked responses with both dorsal eSCS and modulated tSCS over the C3–4 and C7–T1 intervertebral spaces in monkeys, but facilitation was stronger with dorsal eSCS (Guiho, Baker, & Jackson, 2021). It is vital to identify the capability of unmodulated tSCS to alter supraspinally driven responses compared to eSCS and modulated tSCS, in order to identify whether unique stimulation parameters are required for individual outcomes. Similarly, PAS with tSCS and TMS induced facilitation of corticospinal excitability for at least 30 minutes after the PAS, which is indicative of long-term potentiation (LTP)-like plasticity in the lower limb region of the primary motor cortex (Kaneko, Sasaki, Masugi, & Nakazawa, 2021). An important component of tSCS is its neuro-modulatory effect on remote segments of the spinal cord, which must be considered during SCI rehabilitation.

Overall, tSCS is a novel technique to modulate the neural circuitry of the spinal cord to help restore functional impairments after SCI. Over the past decade, several research studies focused on understanding the neural substrates activated by tSCS responsible for the observed functional improvements. It has been shown, in both computer modelling and electrophysiological studies, that tSCS engages sensory pathways via medium-to-large diameter fibers of the posterior root (similar to epidural stimulation), hence activating spinal motor pools through transsynaptic connections and producing a response with reflex-like properties. Supra spinal networks are also influenced by tSCS with an inhibitory effect at the cortical level when the 10 kHz carrier frequency is embedded in the stimulation waveform. This review of the literature led me to discover areas that still required investigation. First, modulated stimulation waveforms are sometimes employed with the benefit of reducing stimulation discomfort. But, the underlying neural mechanisms of tSCS differ when a modulated waveform is used relative to an unmodulated waveform. Second, the effect of tSCS on the cortical activation level and interhemispheric connectivity during bimanual and unimanual movements is still unknown. Third, tSCS-induced modulation of cervico-lumbar coupling and corticospinal coupling through ascending/descending spinal connections and corticospinal tract have not been studied before. Fourth, characterization of the effect of multisite (cervical+lumbar) tSCS on neural assemblies across the central nervous system is still missing. In chapter 3, 4, and 5, I aimed to address these unanswered questions. A brief review of the findings of chapter 4 and 5 can be found in 1.3.4 to 1.3.5.

1.3.4. Transcutaneous SCS Alters Excitability across Multiple Levels of the Spinal Cord

Evidence indicates that tSCS alters the excitability of multiple segments of the spinal cord (Al'joboori et al., 2021; Barss, Parhizi, & Mushahwar, 2019). These multi-segmental effects were specifically investigated in our recent work exploring how stimulation alters excitability across multiple levels of the spinal cord in neurologically intact participants, using the setup described in Figure 1.3. We first determined that cervical tSCS suppresses the amplitude of the soleus Hoffmann (H)-reflex by 22.9% (Figure 1.4B), which was similar to the 19.7% reduction produced by rhythmic arm cycling (Figure 1.4C), demonstrating that cervical tSCS alters lumbar excitability (Lavrov et al., 2006). The suppression of H-reflexes evoked in one limb by rhythmic movements of the remote limbs demonstrates coupling between the arms and legs in humans (Ferris, Huang, & Kao, 2006; Hundza & Zehr, 2009; R. Zhou et al., 2018). A bidirectional linkage between the cervical and lumbar segments of the spinal cord exists during rhythmic movements in both quadrupedal mammals and humans (Volker Dietz, 2002; E. Paul Zehr, Hundza, & Vasudevan, 2009), facilitated primarily by propriospinal connections (Ferris et al., 2006; Frigon, Collins, & Zehr, 2004). Therefore, it was hypothesized that a similar reciprocal organization may also be revealed by tSCS applied to the cervical and lumbar networks, suggesting that tonic tSCS activates similar networks to those activated during rhythmic activity of the arms or legs (Frigon, 2017; E. P. Zehr et al., 2016). In contrast to our hypothesis, lumbar tSCS significantly facilitated the amplitude of the H-reflex in the flexor carpi radialis (FCR) by 11.1% relative to no stimulation (Figure 1.4D), as opposed to the expected 13.6% reduction in reflex amplitude during leg cycling (Figure 1.4E) (Parhizi B, 2021). This indicates that separate propriospinal networks are likely responsible for the effects of tSCS and rhythmic cycling.

These results are summarized in Figure 1.4A, as tonic activation of spinal cord net-works via tSCS alters excitability over multiple segments of the spinal cord, and is not bidirectional in its effects. The mechanisms responsible for the disinhibition of the H-reflex results between the upper and lower limbs are unknown. Facilitation of the H-reflex pathway through tSCS may be due to reduced Ia presynaptic inhibition, or to facilitation of the motor pool through activation of posterior root afferents and interneuronal projections (Ursula S. Hofstoetter et al., 2018). It also remains possible that the stimulation of skin itself may be a larger contributing factor in altering the excitability of the spinal cord with tSCS than previously considered (Beekhuizen & Field-

Fote, 2005). Understanding the integration of tSCS across multiple segments of the spinal cord across the range of stimulation parameters is critical in order to determine whether facilitating or inhibiting the circuitry involved is desirable based on the individual, the available technology, and the primary clinical outcome. While single-site tSCS neuromodulates remote segments of the spinal cord, multiple sites of tSCS appear to converge and facilitate the spinal and corticospinal circuitry.

1.3.5. Multiple Sites of tSCS Converge to Facilitate Alterations in Excitability

Further improvements to the reengagement of previously inaccessible networks may be possible using multiple stimulation sites of tSCS. Previous investigations have indicated that unmodulated tSCS delivered at the vertebral level T11 can activate the locomotor circuitry in neurologically intact study participants when their legs are placed in a gravity-neutral position (Gorodnichev et al., 2012). Simultaneous stimulation of cervical, thoracic, and lumbar levels (i.e., C5, T11, and L1, respectively) with a carrier frequency induced coordinated stepping movements with a greater range of motion at multiple joints in five of six neurologically intact participants, compared to stimulation of T11 alone (Gerasimenko et al., 2014). The addition of stimulation at L1 and/or at C5 to stimulation at T11 immediately resulted in enhancing the kinematics and interlimb coordination as well as the EMG patterns in proximal and distal leg muscles. Moreover, paired tSCS at the L2 and S1 segments of the spinal cord resulted in greater potentiation of the evoked response than from either site alone, indicating synergistic effects of multi-segmental pathways (Sayenko et al., 2015). The interactive and synergistic effects indicate multi-segmental convergence of descending, ascending and, most likely, propriospinal influences on the neuronal circuitry during tSCS (Sayenko et al., 2015).

Interestingly, multisite (i.e., combined) modulated tSCS in both the cervical and lumbar segments of the spinal cord led to a convergence in the upper limbs (FCR muscle) that significantly increased H-reflex and MEP amplitude, by 19.6% (Figure 1.5B) and 19.7% (Figure 1.5C), respectively. Cervical tSCS alone did not increase H-reflex or MEP amplitude in the FCR, but both were significantly facilitated with the addition of lumbar tSCS. This indicates that tSCS alters excitability across multiple segments of the spinal cord, and converges to facilitate both spinal and corticospinal transmission, as demonstrated in Figure 1.5A. The facilitation of MEPs in the FCR by combined cervical and lumbar tSCS could be due to reinforced projection of ascending propriospinal and corticospinal axons onto cervical spinal motor neurons (John C Rothwell, 1994). Therefore, the activation of proprioceptive in-puts at both the cervical and lumbar spinal cord by tSCS, which synapse on cervical motor neurons, may be a major contributor to the facilitation of H-reflexes and MEPs to the FCR muscle. An important consideration with the potential use of multisite tSCS is the role that spasticity plays in the rehabilitation strategy; facilitating H-reflexes in muscles that have significant spasticity could compound the effect. Further study is required for understanding the effects of multisite tSCS in individuals living with an SCI, as well as its effects on spasticity both within a session and after training.

Interestingly, in neurologically intact study participants, modulated tSCS was unable to alter the excitability of either H-reflexes or MEPs when combined with either arm or leg cycling, regardless of whether single-site or multisite tSCS was applied (Al'joboori et al., 2021; Barss et al., 2019). This indicates that in neurologically intact individuals where interlimb coordination and the corticospinal tract are intact, the effects of arm or leg cycling on cervicolumbar coupling and corticospinal drive were not impacted significantly by the tSCS intensity used. Therefore, it will be a vital next step to determine the role that multisite tSCS has on interlimb connectivity after SCI. The potential impact of using multisite tSCS as a strategy to neuromodulate the spinal circuitry has significant implications in furthering our understanding of the mechanisms controlling posture and locomotion, and for regaining significant sensorimotor function even after neural injury.

1.3.6. Is There a Role for tSCS to Facilitate Cervicolumbar Coupling to Improve Walking?

Since single-site modulated tSCS alters excitability at remote segments of the spinal cord, and multisite modulated tSCS shows a significant convergence effect, it is possible that tSCS may influence coupling between the arms and legs after SCI. The coordination between the legs and arms is an inherent feature of locomotor neural networks (Guiho et al., 2021), with coupling between the cervical (arms) and lumbar (legs) spinal networks (cervicolumbar coupling) well demonstrated in both animals and humans (Juvin, Le Gal, Simmers, & Morin, 2012; Juvin, Simmers, & Morin, 2005; Yamaguchi, 1986). Oscillatory movements are governed by separate locomotor centers known as central pattern generators (CPGs), which are located in the cervical and lumbar spinal cord segments (Frigon, 2017; E. P. Zehr et al., 2016). Similarly to quadrupedal mammals, a bidirectional linkage between the cervical and lumbar segments of the spinal cord during rhythmic movements is present in humans (Volker Dietz, 2002; E. Paul Zehr et al., 2009), facilitated primarily by propriospinal connections (Ferris et al., 2006; Frigon et al., 2004).

Engaging these connections with simultaneous arm and leg (A&L) cycling training improves walking capacity after both chronic incomplete SCI (Rui Zhou et al., 2018) and stroke (Klarner et al., 2016a; Klarner et al., 2016b). Highlighting the importance of these interlimb connections, arms-only cycling has also been shown to improve overground walking function after stroke (Kaupp et al., 2017). A&L cycling often capitalizes on the incompleteness of the injury to the spinal cord, even in cases where the injury is clinically classified as complete. The effect of neuromodulation is maximized when accompanied by a residual intact descending/ascending input. While the beneficial effects of rehabilitation strategies such as arm and leg cycling on cervicolumbar coupling after incomplete SCI and stroke have been outlined previously, little is known about severe cases when the injury to the spinal cord is clinically complete. Pairing tSCS with A&L cycling may allow for similar improvements in interlimb connections after complete SCI or multiple sclerosis. However, the impact of tSCS on propriospinal connectivity has yet to be investigated. Enhancing cervicolumbar connectivity by pairing A&L cycling with tSCS to improve mobility outcomes also remains a vital avenue for future research.

1.3.7. Trunk Stability Improvements with tSCS

While direct evidence of tSCS influencing remote segments of the spinal cord is limited, enhancing trunk stability—which is often an overlooked component—may provide indirect evidence of the influence of tSCS. Postural stability via regulation of trunk function is an integral part of locomotor control and a key element of the kinematic chain for reaching movements (Cetisli Korkmaz, Can Akman, Kilavuz Oren, & Bir, 2018; J. V. G. Robertson & Roby-Brami, 2011). Modulation of lumbosacral networks via modulated tSCS has enabled individuals with various levels of SCI to stand without assistance from a therapist; more importantly, individuals showed improved postural control after repeated sessions of training, as demonstrated by an increased range of the center of pressure excursion during self-initiated body weight displacement (Milosevic et al., 2019). As argued by the authors, biphasic pulses were perceived similarly to the sensation caused by monophasic pulses; however, biphasic stimulation could not enable unassisted standing, and was ineffective in producing motor output in the lower extremities, even at higher stimulation intensities (Milosevic et al., 2019). Although critical, this observation is limited to one specific task of the lower extremity using only a modulated waveform; thus, future investigation is necessary in order to compare the effects and the underlying mechanisms of monophasic and biphasic tSCS paradigms, in an effort to uncover the best stimulation paradigm for improving functional outcomes. Moreover, modulated tSCS applied to the lumbar region increased the level of activity in the trunk muscles, adjusted the abnormal sitting posture, and extended the limits of multidirectional seated displacement, overall enhancing postural control (Rath et al., 2018). The ability of lumbar tSCS to improve muscle activity in the trunk and postural control provides indirect evidence for tSCS inducing meaningful effects across multiple segments within the spinal cord. While further investigation is necessary in order to determine the specific pathways responsible for improved postural control, there is an additional incentive to pair tSCS with rehabilitation interventions in order to improve functions that are often overlooked in research and rehabilitation interventions.

1.3.8. Previously Developed Rehabilitative Approaches Are Enhanced through tSCS

Understanding the role of tSCS across multiple converging segments of the spinal cord is an important consideration when designing optimal rehabilitation interventions. The use of tSCS in conjunction with functional training appears imperative for optimizing functional recovery after SCI (P. Gad et al., 2017; P. Gad et al., 2018; Kou et al., 2021; McHugh et al., 2020). When tSCS (either modulated or un-modulated) and training are combined, functional changes emerge more rapidly and to a greater degree than with either method alone, making these strategies vital to one another's success (P. Gad et al., 2018; McHugh et al., 2020). Importantly, participants with SCI previously considered to be at maximal functional capacity following walking-based therapy were able to gain significant improvements in the 6 min and 10 m walking tests after incorporating unmodulated tSCS into a paired tSCS- and walking-based therapy intervention (McHugh et al., 2020). Likewise, unmodulated tSCS as an adjunct to locomotor training was shown to improve walking out-comes in individuals with subacute motor-incomplete SCI (S. Estes, Zarkou, Hope, Suri, & Field-Fote, 2021). Furthermore, pairing modulated tSCS with walking using an exoskeleton can improve lower limb coordination (P. Gad et al., 2017). Positive synergistic effects of tSCS neuromodulation and previously successful rehabilitation strategies are a promising avenue for increasing what is currently possible for recovery after neurotrauma. This may be enhanced by further understanding of the unique properties of tSCS, and how it may modulate spinal circuitry differentially based on stimulation parameters and waveforms, muscles of interest, and desired functional outcomes.

1.3.9. Conclusions and Future Directions

In general, tSCS appears to be a safe approach for modulating the excitability of neural networks of the spinal cord. This emerging neuromodulatory technique has been shown to promote short- and long-term restoration of upper extremities function (Inanici et al., 2021). While promising, our understanding of the underlying neural mechanisms of tSCS is still at early stages. Efforts for investigating tSCS neural correlates have been focused on tasks related to walking, but especial emphasis should be placed on arm movement tasks. Moreover, it is critical to explore the remote effect of tSCS on cortical neural networks responsible for the motor control of arm movements. As described in 1.2, bimanual coordination is a cardinal feature of our motor system and is an important aspect of daily life, yet the effect of tSCS on bimanual coordination performance and its neural substrates is still unknown and needs to be addressed. Furthermore, it has been demonstrates that the activation of spinal cord networks with tSCS alters excitability over multiple segments of the spinal cord (Barss et al., 2019; Parhizi B, 2021). This finding may implicate modulation of arm movements through activation of the lumbar spinal circuitry. All in all, acquiring the knowledge in the aforementioned areas will enrich our understanding of the motor control of arm movement. Rehabilitation intervention methods will also benefit from this extended comprehension which consequently translates to enhanced quality of life for people with neural injury and diseases.

1.4. Thesis objectives

Even small steps toward expanding the boundaries of knowledge about human motor control is essential as normal movement (either for upper lower limbs), and quality of our lives are intertwined. In addition, to helping individuals experiencing neurological conditions that limit their movement and deteriorate their quality of life, comprehensive knowledge of human motor system and the way it works is necessary as it leads to targeted and efficacious training paradigms to regain motor function. With all the advances that the field of upper limb motor control has made to date, more work has yet to be done to reach functional recovery. Two major areas were the focus of this dissertation: motor control of bimanual movements and how neuromodulation of the spinal cord modulates pathways and circuitry involved in coordinating upper limb movements.

One spectacular feature of the human motor system is the ability to generate bimanual movement where the two upper limbs are used together. In order to better understand this ability, a number of fundamental unknowns should be addressed. One such issue is how goal-conceptualization affects movement kinematics and brain dynamics during the execution of bimanual movements. Therefore, the primary focus of chapter 2 was to characterize the differences in kinematics, brain activation, and interhemispheric connectivity between common-goal and dual-goal bimanual movements which are rooted in distinct goal-conceptualization strategies. We designed novel tasks that bear close similarity to real life activities and recorded brain activity using electroencephalography (EEG) and movement kinematics using an upper limb robotic exoskeleton called, KINARM. We hypothesized that a decline in sensorimotor activation exists during common-goal movement concomitant with an increase in interhemispheric connectivity relative to dual-goal movement. We also hypothesized that dual-goal movements decrease movement time and accuracy compared to common-goal movements. Multiple factors contributed to the reasoning behind the above hypotheses including: hemispheric specialization, attentional demands, and the role of goal-conceptualization in interhemispheric inhibition. A secondary purpose of this study was to examine whether a cognitive load that manipulates the complexity of movement affects our outcome measures in bimanual movement. Manipulation of complexity is usually accomplished by changing the “motor” features of the movement. Here we used a cognitive approach to do so and hypothesized that the cognitive load increases sensorimotor activation, requires stronger interhemispheric connectivity, elevates movement time but degrades movement accuracy.

In chapter 3, we pivoted to investigating the role of tSCS in modulating sensorimotor cortical oscillations during the execution of both unimanual and bimanual reaching movement. It is necessary to determine the site of effects of tSCS before applying it as a rehabilitation intervention. This will eventually lead development of future methodologies and training designs with improved expected motor outcome for individuals with SCI. To do so, we evaluated sensorimotor activation, interhemispheric connectivity, and behavioral outcomes in the presence of cervical tSCS and compared it with when tSCS was absent. This work was critical since the common techniques used to evaluate the effects of tSCS usually involve electrophysiological measures such as Hoffmann reflex, motor evoked potentials induced by transcranial magnetic stimulation, and electromyography. By using EEG, brain dynamics can be captured non-invasively and the cortical effect of spinal cord neuromodulation can be explored. It was hypothesized that tSCS, although applied at the spinal cord level, will have remote effect on sensorimotor cortical oscillations in the form of either synchronizing or desynchronizing oscillations (i.e., reduction and elevation in activation) and increases interhemispheric connectivity. Since this study was conducted on individuals with no history of neurological conditions, their nervous system was fully functional and we further hypothesized that tSCS does not affect movement kinematic parameters either in negative or positive direction. This study shed light, for the first time, on cortical effects of tSCS on both unimanual and bimanual movements which can have crucial implications for the application of spinal cord stimulation for population with damage to their cortex such as stroke and traumatic brain injury.

The focus then shifted towards changes caused by tSCS to corticospinal and spinal neural mechanisms involved in motor control of arm movements in chapters 4 and 5. It is unknown whether tSCS input to spinal cord circuitry may alter corticospinal transmission responsible for producing upper limb movements. Moreover, there are extensive connections between leg-related segments of the spinal cord (lumbar enlargement) and arm segments (cervical enlargement). This connection is referred to as cervicolumbar coupling. Strengthening the cervicolumbar coupling has been shown to be influential in improving motor function after neural injury. But how and to what extent tSCS modulates this coupling remain basic neuroscience questions. To answer these questions, the power of electrophysiological measures such as the H-reflex and motor evoked potentials (MEPs) was utilized. It was hypothesized that 1) cervical tSCS facilitates flexor carpi radialis (FCR) MEP and H-reflex while suppressing the soleus H-reflex; 2) lumbar tSCS suppresses the FCR H-reflex. This work highlighted how the arm/hand region of the spinal cord influences and is influenced by the leg-related region.

1.5. Figures

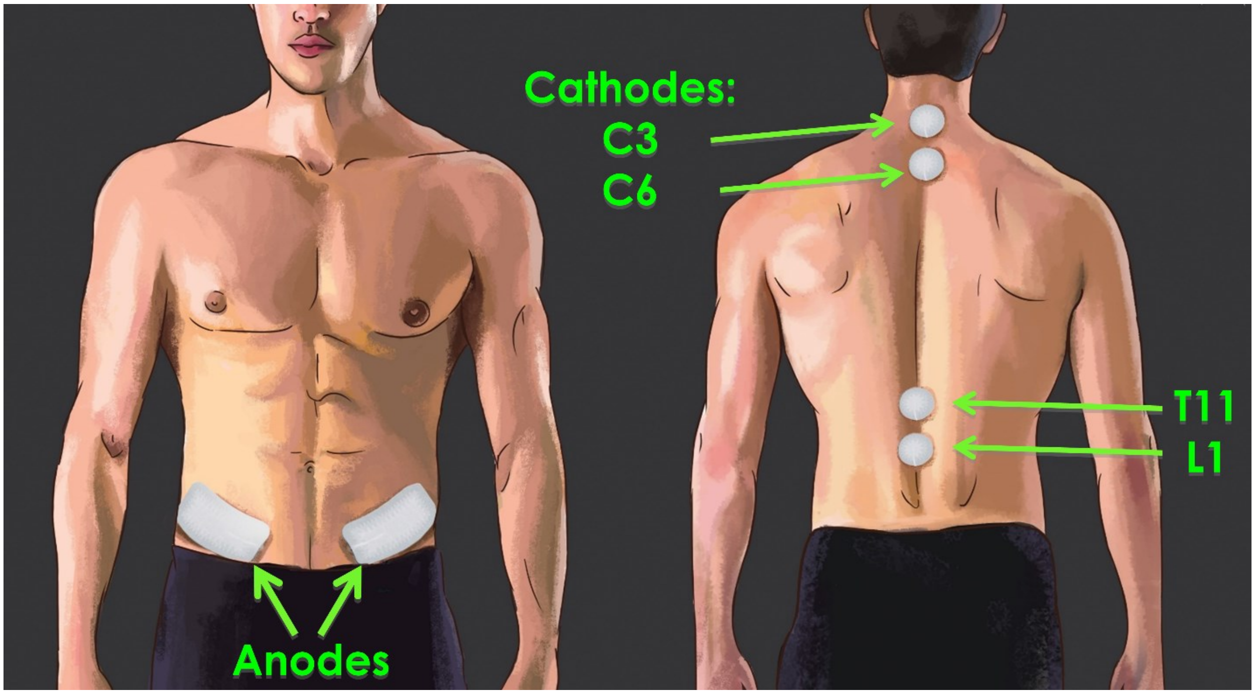


Figure 1.1. Typical tSCS electrode placement: Transcutaneous SCS is commonly delivered via two 2.5 cm round cathodic electrodes placed over the C3–4 and C6–7 (cervical) or T11 and L1 (lumbar) spinous processes. Two 5 × 10 cm rectangular anodic electrodes are placed bilaterally over the iliac crests.

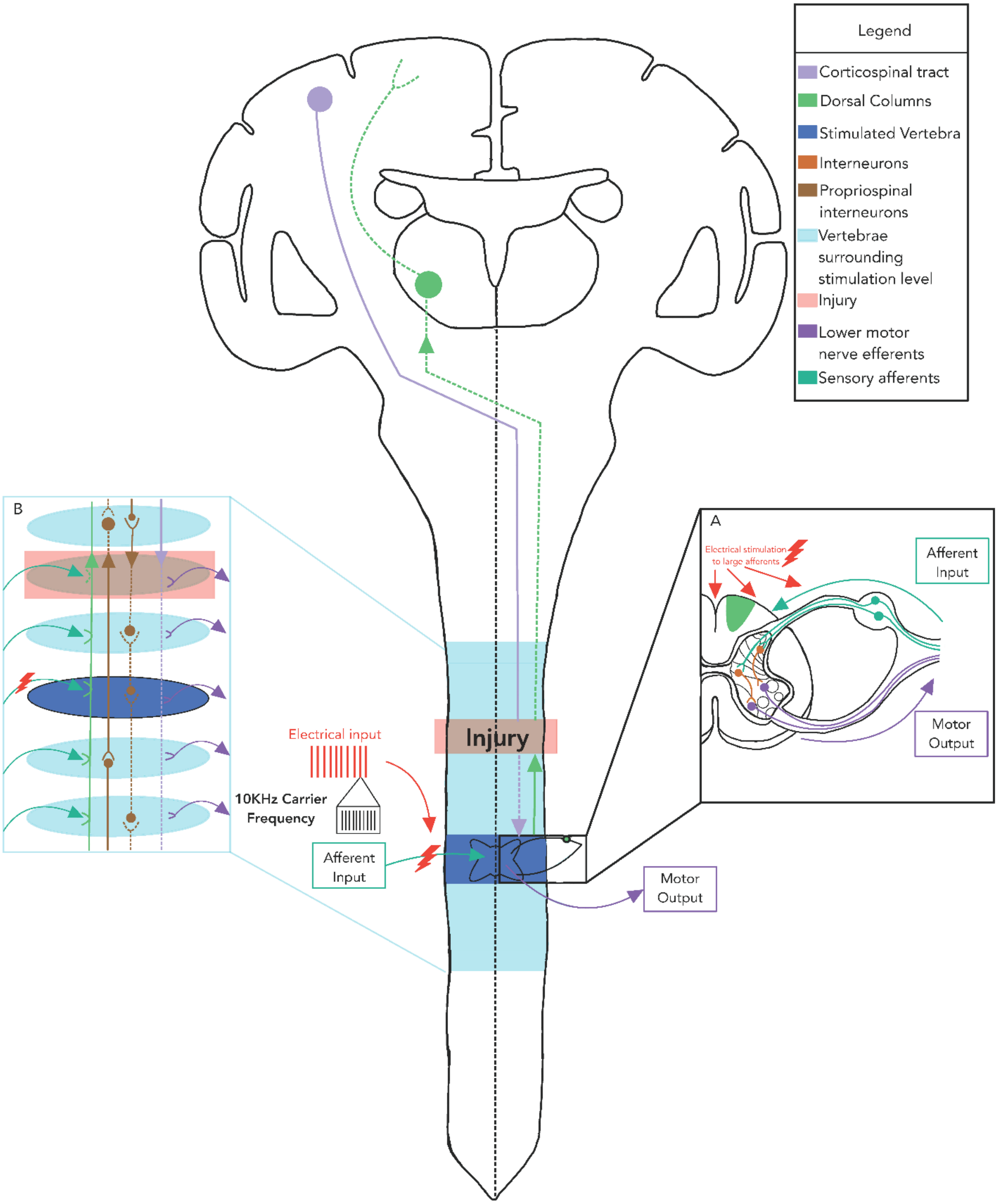


Figure 1.2. Schematic of networks within the spinal cord that are potentially altered with tSCS: The main figure highlights the ability of tSCS to modulate ongoing motor output through dorsal root afferents that trans-synaptically facilitate motor output by bringing previously inaccessible

motor units closer to their threshold, allowing them to contribute to the execution of a desired task. (**A**) Large-diameter afferents are likely activated and synapse on several types of interneurons that facilitate ongoing motor output. (**B**) Among these interneurons are propriospinal interneurons, which transmit this input to multiple segments of the spinal cord in order to alter excitability and impact ongoing motor output throughout the cord. Solid lines indicate that transmission remains intact to the point of injury to the spinal cord, while dashed lines indicate that transmission is impaired, and may be facilitated by tSCS. Typically, tSCS is applied in single unmodulated or modulated monophasic or biphasic pulses or trains of pulses.

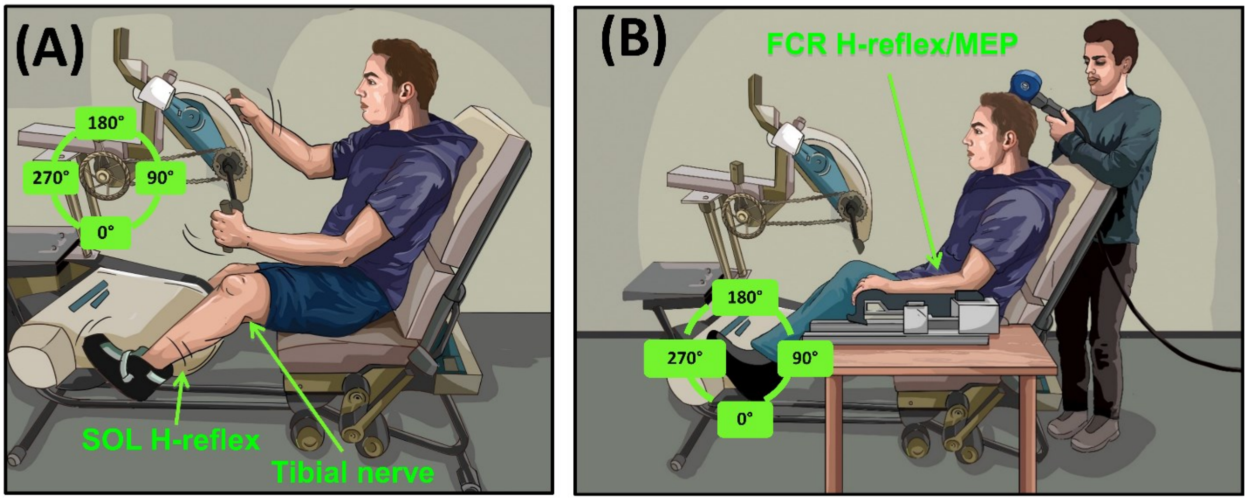


Figure 1.3. Experimental setup for investigating the effect of modulated tSCS on cervicolumbar connectivity and corticospinal facilitation: (**A**) Hoffmann (H-) reflexes were evoked during tSCS via stimulation of the tibial nerve and recorded in the soleus (SOL) muscle. The left leg was held static in an extended position, and stimulation to evoke the H-reflex was delivered with either the left arm held at 0° or during arm cycling. (**B**) H-reflexes were evoked during tSCS via stimulation of the median nerve and recorded in the flexor carpi radialis (FCR) muscle, while motor evoked potentials (MEPs) were evoked in the contralateral motor cortex and recorded in the FCR muscle, either with the legs held static, or during leg cycling. Responses were evoked during a consistent background contraction of ≈5–10% peak muscle activity at the same position, regardless of condition.

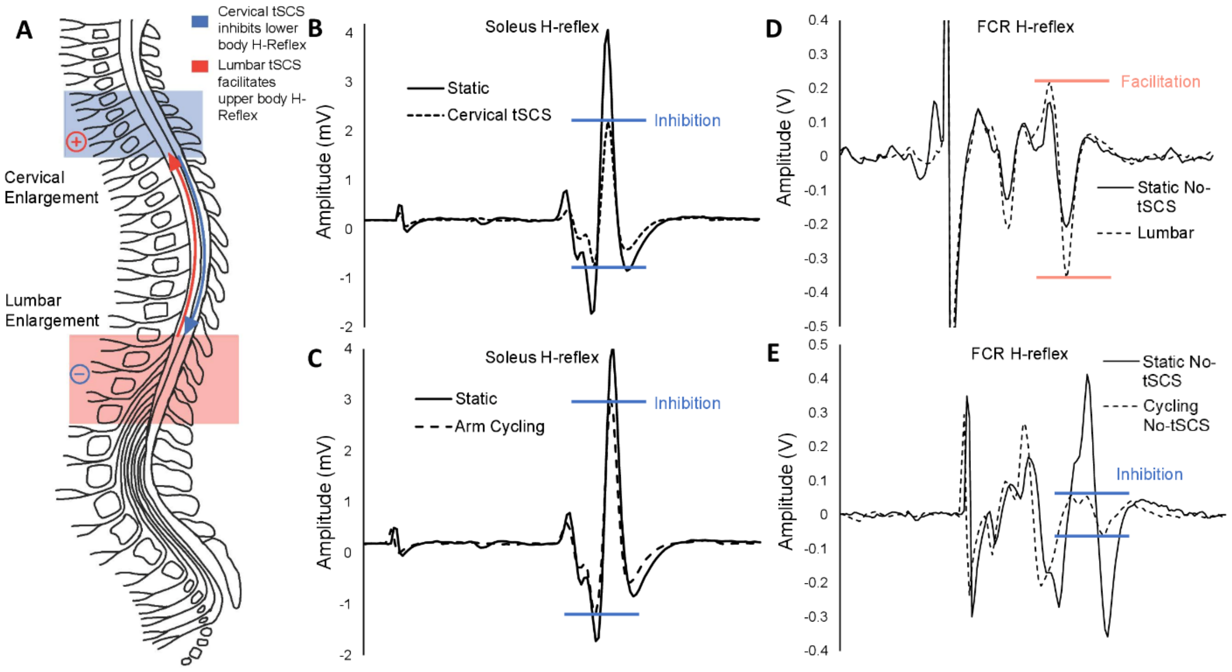


Figure 1.4. Effects of tSCS on interlimb connectivity are not similar to those of cycling in terms of reciprocal organization: (**A**) The schematic highlights common spinal segments activated by tSCS, including the cervical (blue) and lumbar (pink) enlargements. The blue arrow indicates that tonic cervical tSCS inhibits lumbar excitability, while the red arrow indicates that lumbar tSCS facilitates cervical excitability in neurologically intact individuals. (**B**) Spinal reflex excitability as assessed by the H-reflex in the soleus muscle is significantly inhibited in the presence of cervical tSCS (Barss et al., 2019). (**C**) Spinal reflex excitability is similarly reduced in the lower limbs during arm cycling, which is a known condition for altering interlimb connectivity via presynaptic mechanisms (de Ruiter, Hundza, & Zehr, 2010; Hundza & Zehr, 2009). (**D**) Conversely, spinal reflex excitability as assessed by the H-reflex in the FCR muscle is significantly facilitated in the presence of lumbar tSCS (Al'joboori et al., 2021). (**E**) Leg cycling continues to inhibit spinal reflex excitability in the upper limbs. Panels (**B**–**D**) adapted from published data in (Al'joboori et al., 2021; Barss et al., 2019).

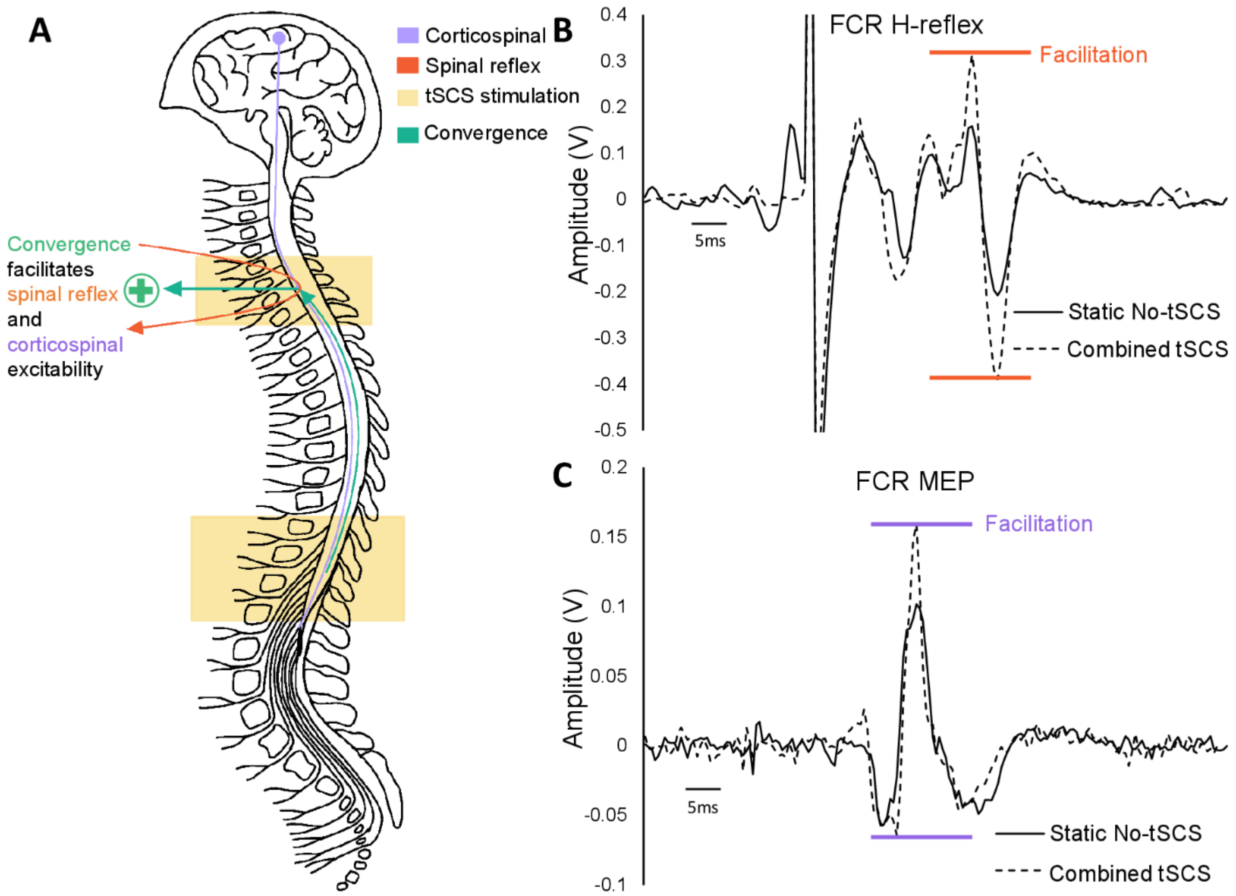


Figure 1.5. Convergence across multiple spinal segments facilitates spinal and corticospinal excitability: (**A**) The schematic highlights that simultaneous cervical and lumbar tSCS (yellow) significantly facilitates cervical spinal reflex and corticospinal excitability. (**B**) Spinal reflex excitability as assessed by the H-reflex in the flexor carpi radialis (FCR) muscle is significantly facilitated in the presence of combined cervical and lumbar tSCS. (**C**) Similarly, corticospinal excitability as assessed by MEPs in the FCR elicited from the contralateral motor cortex was also significantly facilitated by combined cervical and lumbar tSCS. Panels (**B**,**C**) adapted from published data in (Parhizi B, 2021).

Chapter 2. The role of goal conceptualization in the neural motor control of bimanual movements

2.1. Introduction

Bimanual movements are utilized extensively in our daily life and the study of the neural underpinnings of this remarkable ability of the human motor system has attracted researchers for so many years. A major area of focus has been to determine the factors that influence the level of activation within each cortical region and the extent to which regions are recruited. These factors include transitioning from unimanual to bimanual movements (Nair et al., 2003; Ullén et al., 2003), complexity of movements(Debaere et al., 2004), and the end goal of bimanual movements (Liao et al., 2018). For so many years, the supplementary motor area (SMA) has been considered as the most important region for coordinating integrative movements of the upper limbs (S. P. Swinnen & Gooijers, 2015). However, it has been shown that a collection of brain regions is engaged in successfully producing bimanual movements. This broad network entails the primary motor cortex (M1), pre-motor cortex (PMC), anterior cingulate cortex, SMA, cerebellum and basal ganglia, pre-frontal, parietooccipital, and temporal regions, depending on task requirements (S. P. Swinnen, 2002; Wiesendanger & Serrien, 2001). It has been shown that the factor of bimanuality does not require stronger activation in the basic sensorimotor network compared to that observed in unimanual movements (Immisch et al., 2001; Tracy et al., 2001). However, with increasing coordination demands, not only does the activation become stronger in the basic sensorimotor areas, but it also propagates beyond the motor-related regions (Aramaki et al., 2010; Meyer-Lindenberg et al., 2002; Ullén et al., 2003).

Goal conceptualization is another important aspect in bimanual movements that has long been neglected in the literature, but has implications for the bimanual actions we perform daily. During bimanual actions, we either move our arms independently by setting separate goals for each, or move them cooperatively to achieve a common goal. The critical distinction between these two types is the “bimanual cross talk” that is observed when the actions of the left and right limbs are guided by separate goals. For example, when one hand draws a line and the other a circle, bimanual interference results in the shapes becoming curved and elliptical, respectively (Elizabeth A. Franz, 1997). But when we hold a jar with one hand while opening the lid with the opposite hand, the interference is eliminated as both hands strive for a common goal. While the literature is rich with regards to dual-goal bimanual movements, only a few studies have provided perspective on the neural substrates of common-goal bimanual movements. Using functional magnetic resonance imaging (fMRI), Duque et al. demonstrated that three areas in the right hemisphere have stronger activation during a common-goal versus a dual-goal condition (Duque et al., 2010). These areas are the superior temporal gyrus (STG), SMA, and M1. Importantly, only temporary disruption of STG via transcranial magnetic stimulation (TMS) temporarily disrupts the coordinative action during common-goal task. It can be argued that with the potential role of STG in spatial attention, goal-conceptualization in bimanual coordination alters the attentional demands, which has neural consequences.

While many electrophysiological and imaging studies have focused on the movement complexity or effect of learning in bimanual movements (Gross et al., 2005; Mayville, Jantzen, Fuchs, Steinberg, & Kelso, 2002), goal-conceptualization has been overlooked. In particular, the cortical neural correlates of common-goal bimanual movements remain unclear. In addition, the alterations in motor control of goal-directed arm movements with increasing cognitive demands (as opposed to increasing motor demands commonly addressed in the literature) is still a matter of ambiguity. To the best of our knowledge, no studies have yet investigated the underlying neural mechanisms of bimanual movements with a comprehensive set of movement types that entails unimanual, bimanual dual-goal, and bimanual common-goal with and without cognitive load. This arrangement of movements allows us to investigate how I) unimanual to bimanual transition, II) goal-conceptualization, and III) movement complexity affect motor control mechanisms of bimanual movements. Given the alterations in the power of cortical oscillations and interhemispheric connectivity as a function of the nature of bimanual action (Serrien, 2008), we would expect that a change in the movement goal and demand to be reflected in the modulation of sensorimotor cortical oscillations as well as interhemispheric connectivity in the form of spectral power and coherence, respectively.

To this effect, we recruited neurologically intact (NI) participants to perform 3 types of elbow extension goal-directed arm reaching movements using a KINARM exoskeleton facilitated by a virtual reality (VR) display. The movements were: 1) unimanual visually-guided reaching (VGR); 2) dual-goal bimanual VGR; and 3) common-goal bimanual VGR. A reverse version of these three movements was also performed by the participants, adding a cognitive load in an effort to boost the complexity of the movement in a novel way. In the reverse version, the visual feedback moves in the reverse direction of the actual movement, making the reaching movements more complex. Cortical activity was recorded via electroencephalography (EEG) electrodes while the participants performed the reaching movements. Alpha and Beta frequency component are the most remarkable characteristics of EEG sensorimotor signals during movement imagery and execution (Neuper, Scherer, Reiner, & Pfurtscheller, 2005; Rueda-Delgado et al., 2014; Stancák & Pfurtscheller, 1995). Thus, alpha (8-12 Hz) and beta band (13-30Hz) cortical activity associated with sensorimotor processes were computed using spectral power. Interhemispheric connectivity between the right and left M1 and primary somatosensory cortex (S1) was evaluated as well. We hypothesized that dual-goal movement increases all the kinematic outcome measures including reaction time, movement time and movement error relative to unimanual and common-goal movements. Similarly, moving in the reverse direction would increase the kinematic measures. We hypothesized that common-goal bimanual movement decreases sensorimotor cortical activity relative to dual-goal movements, but elevates the level of interhemispheric connectivity. Additionally, we hypothesized that moving in the reverse direction requires increased cortical activity and interhemispheric coherence in line with the augmented complexity of the movement.

2.2. Materials and Methods

2.2.1. Participants: Fourteen (14) study participants aged 20 to 35 years, 3 female and 11 male, with no history of neurological conditions completed experiment 1. All participants were right-handed based on self-report, and their vision was normal or corrected to normal with contact lenses or glasses. Participants signed an informed consent form to participate in the study, which was approved by the University of Alberta Human Research Ethics Committee, and conducted in accordance with the Declaration of Helsinki (1964). All participants were verbally instructed about the experimental procedures.

2.2.2. Experimental setup: Reaching movements were performed using the bilateral robotic exoskeleton, KINARM (BKIN Technologies Ltd, Kingston, ON, Canada,) (Figure 2.1A). This exoskeleton allows arm movements in the horizontal plane around the shoulder and elbow joints in the direction of both flexion and extension. The device also provides gravitational support of the arms, forearms and hands. Each arm segment (arm, forearm plus hand) is supported by a plastic arm trough attached to an adjustable 4-bar linkage. The experimenter calibrated each segment of the KINARM exoskeleton in order to fit and support the left and right arm of each participant. The device is equipped with a VR system located in front of the participant that displays the tasks in the same plane as the arm movements. The experimental tasks in the study were unassisted by the experimenter, and the device did not provide any assistance for the completion of the tasks. Participants were seated in the KINARM chair with shoulders abducted ~ 80° and horizontally abducted ~45°, and the elbow in ~90° of flexion. These anatomical positions were adjusted for some participants to ensure that they remain comfortable throughout the experiment and to guarantee that both arms can reach their final locations. Instructions provided to the participants were to reach from the starting target, the "home position," to the final target as quickly and as accurately as possible.

Participants were instructed to perform three movements facilitated by the KINARM (Figure 2.1B): 1) unimanual VGR where they were instructed to move their right hand to a virtual peripheral target on the top right corner of a home position (elbow extension movement); 2) dual-goal bimanual VGR in which each arm performed similar center-out reaching movements to the peripheral targets simultaneously; 3) common-goal bimanual reaching movements where the participants moved a ball placed on a horizontal bar to a peripheral target through cooperative movement of the two arms, each holding one end of the bar. The common-goal task was designed such that the ball could roll to the sides of the bar if the orientation of the bar deviates from horizontal, thus participants needed to maintain the horizontal orientation of the bar to keep the ball at the center of the bar. Additionally, a reverse version of the three tasks was performed where visual feedback was provided 180 degrees opposite to the movement direction. Thus, the physical movement of the right arm was still in the direction of elbow extension (i.e., up and right) but the target for the visual feedback was dislocated to the left and down (Figure 2.1C). Participants were exposed to a single trial of each task during the KINARM calibration and adjustment phase. Overall, during the data collection phase, each participant performed six tasks that were randomly ordered for participants 8 to 14 using simple randomization. Participants 1 to 7 were engaged in a motor learning study in conjunction with the current study and as a result, task randomization was not possible due to the requirements of the motor learning tasks. Since no difference was found in the results of the two groups of participants, data from both groups were combined.

For each of the movements, participants were directed to move from a home circular position (1.0 cm radius) to a peripheral target located (1.0 cm radius) on the upper right corner (10.0 cm to the right and 10 cm to the top), in the direction of right arm elbow extension. Participants started each movement by holding the tip of their index finger in the home position for 750 ms while the home position was red. Once the home position's color turned green and the peripheral target appeared on the VR display in red, the participant moved to the peripheral target and held the tip of their index finger for another 750 ms until the target turned green and the home position reappeared, at which time the participant returned back to the home position and waited for the next repetition. The wait period between repetitions was 1750 ms and there were a total of 20 repetitions for each movement. All the movements were performed against 5-10% of tricep brachii (TB) maximum voluntary contraction (MVC). To obtain MVC, participants performed three trials of isometric maximal voluntary elbow extension. The KINARM exoskeleton was then programmed to produce a force in the direction opposite to the movement direction equivalent to 5-10% elbow extension MVC. This was to ensure similar voluntary cortical drive throughout all experimental tasks.

2.2.3. Electroencephalography: EEG data were recorded from 64 channels using Brain Vision Recorder (Brain Products, Gilching, Germany) according to the international 10-20 system (Kilicarslan, Prasad, Grossman, & Contreras-Vidal, 2013). Data were recorded and sampled at 1000 Hz. Electrode AFz was used as the ground and electrode TP10 was used as the reference during online data collection. During offline processing, data were re-referenced to the average of electrodes TP9 and TP10. Electrode impedances were consistently monitored during the experiment and were always kept below 5 kΩ. Participants were instructed to minimise their extraneous movements including eye blinks and head movements to guarantee high quality recordings. EEG data were band-pass filtered between 0.1 and 100 Hz using a Butterworth filter and notch filtered at 60 Hz. Artifact Subspace Reconstruction (ASR) was used as a pre-processing data cleaning technique (Mullen et al., 2013) (K. Nathan & Contreras-Vidal, 2016).

ASR is very effective at removing transient, high-amplitude noise from sources such as eye-blink and EMG bursts. ASR is available as a MATLAB plugin and automatically identifies clean regions of EEG to remove subspaces that deviate from baseline. We used a sliding window of 500 ms and a threshold of 5 for standard deviations cutoff, without channel rejection.

2.2.4. Quantification of movement kinematics: We included three kinematic measures in this study: reaction time (RT), movement time (MT), and root-mean square error (RMSE) (Desrochers, Brunfeldt, & Kagerer, 2020). The method introduced by Coderre and colleagues was used to calculate movement onset and offset, which were then used to compute RT and MT (Coderre et al., 2010). RT was calculated as the time interval between peripheral target illumination and movement onset. MT was the time interval between movement onset and movement offset. Movement error was based on RMSE which measured the straightness of the movement. RMSE measured the displacement between the real hand coordinates and the closest point on the straight line between the home position and peripheral target (perpendicular distance).

2.2.5. Computing sensorimotor spectral power and coherence: Spectral power of EEG and coherence between EEG channels were determined for each task. Each of the 20 repetitions of the different tasks was first extracted from continuous EEG recording based on movement onset and offset. Then all 20 repetitions were concatenated. Spectral power was calculated over 1024-point FFT segments with zero over-lap using the following formula (Johnson, Wheaton, & Shinohara, 2011):

$$P_x(f) = \frac{1}{n}\sum_{i=1}^{n} C_i(f) * \ C_i^*(f)$$

where $P_x(f)$ shows the spectral power for EEG channel x and $C_i(f)$ is the fourier transform of data segment i of EEG channel x. We computed the alpha and beta band spectral power during movement execution, and the average of power within each band was calculated. Spectral power analysis was completed for the electrodes over the left M1 (C3 electrode) and S1 (CP3).

Coherence was then calculated with the following formula (Halliday et al., 1995):

$$Coh_{xy}(f) = \frac{\left|P_{xy}(f)\right|^2}{P_{xx}(f) * P_{yy}(f)}$$

where $P_{xy}(f)$ is the cross spectral power of EEG signal x and signal y, and $P_{xx}(f)$ and $P_{yy}(f)$ are the spectral power of EEG signal x and y, respectively. Coherence provides a real value between 0 and 1 that explains the amount of coupling between two signals. Confidence interval at $\alpha=0.95$ quantile of the coherence is defined as $cl = 1 - (1-\alpha)^{1/(L-1)}$ where L is the number of segments (Rosenberg, Amjad, Breeze, Brillinger, & Halliday, 1989). Coherence was completed between the right and left M1 (C3-C4 electrodes) and S1 (CP3-CP4 electrodes). Only coherence values above the cl threshold were accepted.

2.2.6. Statistical analysis: RT, MT, RMSE, spectral power, and coherence were compared across different experimental tasks using multi-factor repeated-measure ANOVA. The main effects were compared for kinematic measures, spectral power, and coherence with a 3×2 ANOVA. This was followed by the simple main effect of task (unimanual, bimanual common-goal, and bimanual dual-goal) held in the forward and reverse directions. Significant effects were followed by pairwise comparisons, and multiple comparisons were corrected by Bonferroni's adjustment. Moreover, a priori test, independent of ANOVA, was conducted using T-Test to compare the effect of movement direction (forward and reverse) for different tasks. This test was performed based on the importance of movement complexity during bimanual movements outlined in the literature. It has been shown that movement complexity is a determining factor in activation and recruitment of cortical neural networks (Jäncke et al., 2000), and the reverse movement (relative to forward movement) in our study alters the cognitive complexity of the tasks. Descriptive statistics are shown as mean ± standard error, unless otherwise stated. Statistical significance was set for $p \leq 0.05$. All statistical analyses were performed with SPSS Statistics (IBM, Chicago, IL, United States).

2.3. Results

2.3.1. Movement kinematics: An example of the raw traces from the right arm for all six movement conditions from one participant is shown in Figure 2.2. For MT, a 3×2 rmANOVA indicated a significant main effect of task [$F_{(2,26)}=22.699$, $p < 0.001$], movement direction [$F_{(1,13)}=31.784$, $p < 0.001$], and interaction (task * movement direction) [$F_{(2,26)}=12.937$, $p < 0.001$](Figure 2.3A). The simple main effect of task in both the forward and reverse direction was significant (forward: [$F_{(2,26)}=7.663$, $p = 0.002$], reverse: [$F_{(2,26)}=19.550$, $p < 0.001$]). Post-hoc pairwise comparison revealed that in the forward direction, unimanual movement had significantly lower MT compared to both common-goal (p=0.02) and dual-goal (p=0.016) bimanual movements. There was no significant difference between MT of common-goal and dual-goal bimanual movement in the forward direction (p=1.00). In the reverse direction, MT for dual-goal bimanual movement was significantly higher relative to unimanual (p=0.002) and common-goal bimanual (p=0.001) movement. A comparison of forward and reverse reaching was then conducted within each task (i.e., unimanual, bimanual common-goal, and bimanual dual-goal) using a T-Test. Relative to the forward direction, reverse bimanual common-goal movement did not significantly affect MT (p=0.313). However, moving in the reverse direction significantly increased MT compared to forward direction in unimanual (p=0.003) and bimanual dual-goal movement ($p < 0.001$).

Movement accuracy was measured by the deviation from the straight line between home and target positions for all movements (Figure 2.2). Analysis of RMSE using a 3×2 rmANOVA revealed a significant main effect of task [$F_{(1.407,18.290)}=43.373$, $p < 0.001$], movement direction [$F_{(1,13)}=20.831$, $p = 0.001$], and interaction (task * movement direction) [$F_{(2,26)}=12.054$, $p < 0.001$](Figure 2.3B). The simple main effect of task was significant under both forward [$F_{(2,26)}=15.518$, $p < 0.001$] and reverse [$F_{(1.380,17.938)}=33.247$, $p < 0.001$] directions. A post-hoc pairwise comparison showed that in the forward direction, dual-goal bimanual movement led to a significantly higher RMSE than unimanual ($p = 0.003$) and bimanual common-goal movement ($p < 0.001$), but RMSE of unimanual movement was not different than common-goal bimanual movement ($p = 1.000$). In the reverse direction, dual-goal bimanual movement resulted in a significant increase in RMSE compared to unimanual ($p < 0.001$) and bimanual common-goal ($p < 0.001$) movements, but there was no difference between unimanual and bimanual common-goal movements ($p = 0.492$). RMSE of forward versus reverse direction were also compared within each task using a T-Test. Moving in the reverse direction caused a significant increase of

RMSE for unimanual ($p = 0.011$), bimanual common-goal ($p = 0.010$), and bimanual dual-goal ($p < 0.001$) movements relative to their forward counterparts.

RT analysis demonstrated a significant main effect of task [$F_{(2,26)}=20.714, p < 0.001$] and movement direction [$F_{(1,13)}=15.604, p = 0.002$]. Additionally, there was a significant effect of interaction of task × movement direction [$F_{(2,26)}=9.937, p = 0.001$](Figure 2.3C). This main effect pointed to significantly lower RT for bimanual common-goal movement compared to unimanual ($p=0.003$) and bimanual dual-goal ($p < 0.001$) movements. The simple main effect of task only indicated significance under the reverse direction [$F_{(2,26)}=20.231, p < 0.001$]. Post-hoc pairwise comparisons showed that reverse bimanual common-goal movement had significantly lower RT relative to both reverse bimanual dual-goal ($p < 0.001$) and reverse unimanual ($p = 0.007$) movement. RT was also lower for reverse unimanual compared to reverse bimanual dual-goal movement ($p = 0.047$). Moreover, comparison was also made for the effect of movement direction on RT within each task using a T-Test. RT increased by moving in the reverse direction in bimanual dual-goal ($p < 0.001$) and unimanual movement ($p = 0.013$) relative to forward direction, but remained unchanged in bimanual common-goal movement ($p = 0.470$).

2.3.2. Sensorimotor spectral power and coherence: We calculated spectral power during each movement in beta and alpha bands to investigate the effect of task and direction on cortical oscillations (Figure 2.4A-B). No significant main effect of task and movement direction was detected in the beta band spectral power from electrode C3 (task: [$F_{(1.345,17.485)}=1.282, p = 0.288$], movement direction: [$F_{(1,13)}=2.256, p = 0.157$], interaction: [$F_{(1.340,17.421)}=0.372, p = 0.612$]). Similarly, no significant main effect of task and movement direction was detected in the beta band spectral power from electrode CP3 (task: [$F_{(2,26)}=0.583, p = 0.565$], movement direction: [$F_{(1,13)}=2.625, p = 0.129$], interaction: [$F_{(1.217,15.822)}=0.458, p = 0.546$]). This indicated that all movement types tended to incur a similar pattern of cortical activation (Figure 2.5A-B). Nonetheless, we detected a decrease of spectral power (i.e., desynchronization) for reverse movements regardless of the task (i.e., bimanual common-goal, bimanual dual-goal, and unimanual) in the beta band, but this did not approach significant level ($p > 0.05$).

Importantly, we found a significant main effect of task in the alpha band spectral power over both C3 [$F_{(2,26)}$=4.458, p = 0.022] and CP3 [$F_{(2,26)}$=4.297, p = 0.024] electrodes (not significant for direction and interaction) (Figure 2.6A-B). This main effect of task revealed that spectral power was significantly lower in common-goal movement than that of dual-goal movement over both C3 (p = 0.017) and CP3 (p = 0.006) electrodes.

Figure 2.7 shows time-frequency coherence using wavelet from a representative participant. Our rmANOVA analysis of C3-C4 coherence only indicated a significant main effect of task [$F_{(2,26)}$=10.269, p = 0.001], but not for movement direction [$F_{(1,13)}$=1.428, p = 0.254] or interaction [$F_{(2,26)}$=0.288, p = 0.752] in the beta band. According to this main effect, we found that bimanual common-goal movement significantly increased C3-C4 coherence (Figure 2.8A) compared to bimanual dual-goal (p = 0.001) and unimanual (p = 0.03) movement. We also found a similar result for the main effect of task in the coherence between CP3 and CP4 electrodes (Figure 2.8B) [$F_{(2,26)}$=9.890, p = 0.001] which points to stronger coupling between left and right S1 in the beta band when performing bimanual common-goal movement compared to unimanual (p = 0.014) and bimanual dual-goal (p = 0.008). In the alpha band, C3-C4 coherence analysis revealed a similar main effect of task [$F_{(2,26)}$=5.823, p = 0.008] (but not for direction and interaction) (Figure 2.9A). Similarly, common-goal bimanual movement showed a significantly higher connectivity relative to dual-goal (p = 0.03) and unimanual movements (p = 0.028). Coherence in the alpha band for CP3-CP4 electrodes nearly reached significance [$F_{(2,26)}$=3.258, p = 0.055] (Figure 2.9B).

2.4. Discussion

In this study, we examined the neural correlates of integrative actions of the two arms during the performance of bimanual movements constrained by 1) different goal conceptualizations (common goal and dual-goal), and 2) manipulation of movement complexity by adding a cognitive load to the movement. Our findings indicate that moving in the reverse direction, which increases the complexity of the reaching movements, leads to increased MT (except in common-goal) and alters the straightness of the reaching movement (measured by RMSE) in all three movement types. Moreover, dual-goal reaching significantly increased RMSE compared to common-goal and unimanual reaching, and had the highest MT in the reverse direction. As shown in other studies (Jäncke et al., 2000), bimanual movements do not require further activation in the sensorimotor region of the cortex relative to unimanual movements in beta band. Counter to our hypothesis that dual-goal bimanual movement would require stronger sensorimotor cortical activation compared to common-goal, we found reduced activity in the dual-goal task in alpha band. Interestingly, common-goal movements had significantly higher interhemispheric connectivity between right and left M1 and S1 compared to dual-goal and unimanual movements, consistent to our hypothesis. Furthermore, we hypothesized that manipulating task demand by reverse moving leads to suppression of spectral power (i.e., elevated cortical activity), but we did not find alteration of left M1 and S1 activation compared to forward movement.

Among the most important and probably least understood aspect of bimanual coordination is the issue of goal-conceptualization. So far, particular attention has been given to movements with independent goals where the task is often performed with symmetric engagement of the arms. Yet the activities of daily life require overriding the default bimanual mode (i.e., symmetrical bimanual movements) by accomplishing a common-goal through asymmetric actions of the two arms. To the best of our knowledge, this study is the first to compare kinematic and electrophysiological measures in common-goal and dual-goal bimanual movements vs. unimanual movements. Moreover, the effect of task demand manipulation was addressed in this study. Unlike other studies with finger movements (Serrien, 2008; Yan Zheng, Kanosue, & Muraoka, 2021), isometric force production(Liao et al., 2018), or particular focus on dual-goal tasks (Grefkes, Eickhoff, Nowak, Dafotakis, & Fink, 2008; Perez, Butler, & Taylor, 2013; S. P. Swinnen, 2002), our task design not only entailed dynamic movement around the elbow joint, but also encompassed three reaching movements with distinct conceptualization requirements. Therefore, our tasks closely mimicked scenarios of real life movement. For instance, the common-goal movement design in the present study was very similar to extending the arms to pick up a tray, and the dual-goal movement represented reaching to pick objects at two different locations on the table.

2.4.1. Behavioural assessment of bimanual movements: Previous studies examined the kinematics of bimanual movements in comparison with unimanual movements. Asai and colleagues suggested that movement accuracy is best for bimanual movement relative to unimanual movement when no visual feedback is provided in a VGR task where participants had to move a mouse cursor from a center point to targets on the circumference of a circle in a straight line (Asai, Sugimori, & Tanno, 2010). They also demonstrated that unimanual movements had the shortest RT while bimanual movements had the worst RT. They attributed these results to hemispheric specialization within each hemisphere and the notion that each hemisphere is superior in some aspects of motor control. While the right hemisphere is specialized in spatial features of the movement thus enhancing the accuracy of left limb movements, the left hemisphere has the advantage for temporal processing of the movement resulting in producing more rapid movements of the right limb (Lenhard & Hoffmann, 2007). This traditional view of hemispheric dichotomy goes hand in hand with another view on hemispheric motor control. It is established that the preferred hand is equipped with closed-loop, or feedback control, of the movements, but the non-preferred hand is adept at open-loop, or feedforward control (Mieschke, Elliott, Helsen, Carson, & Coull, 2001). As a result, the right is capable of producing quicker responses needing less planning, but this compromises the accuracy of the computation. However, in the presence of visual feedback, the accuracy advantage of the left hand is compensated for by the close-loop proficiency of the right hand (Sainburg & Kalakanis, 2000). Moreover, it has been suggested that internal models for bimanual movement yield more accurate motor planning under the assumption that two distinct internal models govern the two limbs (Asai et al., 2010; Sainburg & Kalakanis, 2000; Wolpert & Kawato, 1998). This means that although the dual computation of internal models requires more time to complete (i.e., increased RT), more accurate predictions (i.e., decreased error) are produced.

In the present study, movement error was the highest for the dual-goal movement compared to the unimanual and common-goal movements in the forward direction. In line with Asai et al, movement error was not different between unimanual and common-goal movements (Asai et al., 2010). With visual feedback, which was the case in our study, right-hand unimanual and common-goal bimanual movements were similar with respect to movement accuracy. It is reasonable to assume that visual feedback provides both movement types the same information during path traversal to remain on the straight line between the home and target positions, eliminating the advantage of dual modelling suggested for bimanual movements. It is noteworthy that the error in our study was defined as the perpendicular distance between the position of the hand and the straight line connecting the home and target positions, as opposed to the endpoint error used previously. However, the higher RMSE for the dual-goal bimanual movement cannot be justified by hemispheric advantages. A critical concept in bimanual movement has been temporal and spatial interference (Cardoso de Oliveira, 2002; Elizabeth A. Franz, 1997), especially during asymmetrical tasks. In dual-goal movements where separate goals should be conceptualized for each limb, the contralateral motor plan interferes with the ipsilateral motor plan (Desrochers et al., 2020), leading to neural cross talk which increases RMSE. Common-goal movements require a markedly different conceptualization strategy in which the two limbs work towards one single goal. It has been shown that conceptualization approaches can be deployed to cancel the effect of neural cross talk in bimanual coordination tasks (Kovacs et al., 2010; S. P. Swinnen & Gooijers, 2015). In fact, Ivry and colleagues proposed that the spatial constraint of bimanual coordination can be directly ascribed to how the task is presented and conceptualized by the participant, downplaying the role of the motor system (R. Ivry et al., 2004). In our study, it is likely that a competition for common neural processes arises during the dual-goal task between the independent goals of each hand, leading to spatial interference which in turn degrades accuracy. We also investigated MT to further understand the behavioral discrepancies between reaching movements. Unlike the previous work that reported no difference between unimanual and bimanual reaching movements (Asai et al., 2010), we found that MT was lower for unimanual reaching relative to both types of bimanual reaching movements in the forward direction. Opposing our hypothesis, we found no disparity in RT between the three tasks in the forward direction. This comes in contrast to previous findings (Asai et al., 2010); however, visual feedback was present in our study which may have provided equal motor planning time across the three movement types.

We factored in the role of goal conceptualization and hypothesized that the dual-goal movement will take longer to complete than the common-goal movement. Contrary to our hypothesis, MT was not different between common-goal and dual-goal movements (in the forward direction). The attentional demands in execution of bimanual movements are decisive for MT. A shift of attention in a bimanual aiming task causes a marked increase in MT (Riek, Tresilian, Mon-

Williams, Coppard, & Carson, 2003). Here, we expected that there would be multiple shifts of attention between the two limbs in the dual-goal task leading to a “hover phase” that increases MT. However, this can be overruled by manipulating the attentional demands and minimization of the shifts of attention. We believe that the close proximity of the separate targets in the dual-goal task, which minimized the shift of attention, is the reason behind the similar MT to the common-goal task. An alternative strategy may have been utilized related to speed-accuracy trade off (Asai et al., 2010). Participants may have adopted a strategy to complete the dual-goal task in a shorter time at the cost of accuracy.

In this study, we used a novel strategy to increase movement complexity. The task was designed such that the motion of the visual feedback was reversed (C. R. Lowrey, Dukelow, Bagg, Ritsma, & Scott, 2022). This was very similar to anti-saccade tasks where participants are asked to look in the opposite direction when a target appears (Hallett, 1978). To successfully perform this task, one needs to override the pre-developed internal models of the reaching movement and inhibit the tendency to move to the displayed spatial target (Bells et al., 2020; Munoz & Everling, 2004). This consequently adds a cognitive load. Thus, we hypothesized that moving in the reverse direction has detrimental consequences on the behavioral outcomes of the tasks. In accordance to our hypothesis that moving in the reverse direction would increase RMSE, RT, and MT, we found that RMSE was higher for reverse relative to forward movement in all three movement types. Also, MT and RT were negatively affected by the reverse movement load compared to the forward movement in the unimanual and dual-goal tasks, but not in the common-goal task. It is unclear why movement complexity did not affect the kinematics of common-goal movements; however, it is reasonable to suggest that the combination of two internal models involved in the control of each limb (Sainburg & Kalakanis, 2000) and dual computation involved in the common-goal task may have improved movement duration as well as preparation time reflected in the RT. Finally, we found that MT and RMSE were highest for reverse dual-goal movements in comparison with reverse unimanual and reverse common-goal movements. The reverse dual-goal VGR was the most challenging task, a fact that was also verbally attested to by the participants. The neural cross-talk and goal-conceptualization disadvantages of the dual-goal task are confounded by the cognitive load of moving the visual feedback in the opposite direction, thus resulting in the dramatic increase of MT and RMES.

2.4.2. Neural correlates of bimanual movements: Multiple research studies have investigated the neural correlates of bimanual movements (Goble et al., 2010; Obhi et al., 2002; S. P. Swinnen & Gooijers, 2015; Wiesendanger & Serrien, 2001). Several cortical and subcortical areas are involved in coordinative movement of the two limbs, and multiple factors have been studied including complexity (Debaere et al., 2004), learning (Beets et al., 2015) (Haslinger et al., 2004), coordination mode (Aramaki et al., 2010), goal-conceptualization (Duque et al., 2010), that impact the recruitment of neural resources. One framework to understand how bimanual actions engage different brain regions is to compare them with unimanual movement. The evidence from bimanual finger or forearm movement reveals a key finding: the single factor of bimanuality does not require further activation in the basic sensorimotor network compared to those observed in unimanual movements. For example, in a finger tapping experiment using fMRI, similar activation levels were seen over a given somato-motor cortex when comparing bimanual with unimanual finger tapping movements when the tapping rate was matched between conditions (Jäncke et al., 2000). Consistent with previous findings, we found no difference in the left motor and somatosensory cortical activation between unimanual and bimanual movements in the alpha and beta bands. However, common-goal bimanual movements had increased cortical activity (decreased spectral power) over the M1 and S1 regions relative to dual-goal movements. Alpha band desynchronization has been implicated during motor execution and motor imagery tasks (Ramos-Murguialday & Birbaumer, 2015; Yeom, Kim, & Chung, 2020). Alpha band activity is also important in intention detection and EEG-based motor discrimination studies (Bai et al., 2007; Pfurtscheller, Neuper, Flotzinger, & Pregenzer, 1997). Our data suggest stronger activation of M1 and S1 cortical regions during common-goal movement, which highlights the importance of goal-conceptualization in the activation level of motor and sensory cortical regions. This information can be utilized for designing EEG-based bimanual rehabilitation interventions in the future. In addition, modulation of alpha band activity correlates with attentional changes (Magosso, De Crescenzio, Ricci, Piastra, & Ursino, 2019). This points to a fundamental difference between execution of tasks with distinct conceptualization strategies. Consistent with the findings of Duque and colleagues (Duque et al., 2010), our study shows that common-goal movements demand greater visual attention compared to the dual-goal task.

Another framework to explore the neural substrates of bimanual movements is to understand how brain activation patterns change as a function of coordination demands. As the complexity and difficulty of movement increase, the activation becomes stronger (Rueda-Delgado et al., 2014; Ullén et al., 2003). We hypothesized that reverse reaching is more demanding and requires stronger activation in the cortical sensorimotor regions. Counter to our hypothesis, the cortical activation level measured by spectral power was not significantly different between reverse and forward movements in all three tasks, but we observed a trend of reduction in the spectral power for the reverse movement relative to forward indicating desynchronization of neuronal activity (i.e., enhanced activation level in the primary sensorimotor cortex). Asymmetric movements, which are considered to be more complex, require greater neural involvement relative to symmetric movements during execution of the tasks. It was previously demonstrated that the neural activity in pre-motor cortex is supra-additive to the sum of activity obtained in right and left unimanual movement during asymmetric movements, while it is sub-additive in the case of symmetric movements (Aramaki et al., 2010). In a similar manner to asymmetric task that increases complexity, we expected to observe a significant desynchronization (decrease of power) of neural population associated with M1 and S1 during the reverse reaching task.

Previous electrophysiological studies reported suppression of power with increasing task demand (Gross et al., 2005; Pollok et al., 2007). However, the importance of SMA involvement should be considered with alteration of task complexity, as virtual lesions of this region using repetitive TMS (rTMS) alter the coordination pattern (Obhi et al., 2002). It would have been favorable if we were able to provide insight from the activity of SMA, but this was not possible with EEG recordings because SMA is located deeply in the interhemispheric sulcus. Another explanation that we did not find a change of power with increasing task complexity relates to the cognitive nature of the reverse movement. In the reverse reaching movement, the arm moves away from the target while the visual feedback, which represent the mirror motion of the arm, moves toward the virtual target. Therefore, reverse movement requires cognitive control to override the tendency to move the arm toward the target (C. R. Lowrey et al., 2022), in order to guide the visual feedback to the target location. This ability is impaired with individuals with a history of cognitive impairment such as concussion or Alzheimer’s disease (Brown, Dalecki, Hughes, Macpherson, & Sergio, 2015; Hawkins, Goyal, & Sergio, 2015). It may be the case that the reverse reaching task activated cortical and subcortical areas that are associated with cognitive processing, including frontoparietal networks, basal ganglia and cerebellum (C. R. Lowrey et al., 2022).

Interestingly, our coherence analysis showed a stronger coupling between the left and right M1 and S1 during the execution of bimanual common-goal task (in both the alpha and beta bands). To the best of our knowledge, this is the first study comparing modulation of coherence between bimanual common-goal and bimanual dual-goal movements. Previously, the dynamics of interhemispheric interactions during bimanual movements were investigated by assessing the effect of dual-pulse TMS on interhemispheric inhibition (IHI) or short single-pulse TMS on the ipsilateral silent period (ISP). Perez et Al. reported stronger ISP during the contraction of homologous muscles relative to non-homologous muscles, suggesting stronger interhemispheric inhibition during actions with independent goals (Perez et al., 2013). In line with this finding, Tazoe et al. reported larger IHI during symmetrical tasks relative to asymmetrical tasks (Tazoe et al., 2013). It is plausible to assume that the increased IHI (accompanied by reduced short interval cortical inhibition (SICI)) blocks the interference during the dual-goal task to enable each arm to accomplish its respective goals (Liao et al., 2018). The opposite occurs during common-goal tasks where the reduced IHI facilitates stronger communication between hemispheres such that the two arms achieve their common-goal (Liao et al., 2018). In agreement with this idea, our coherence analysis showed a stronger coupling between the two hemispheres during the common-goal task. We speculate that there is a need for tighter communication between the hemispheres such that the two arms not only work towards their united goal, but also cooperate to hold the bar between them in a horizontal alignment such that the ball on the bar does not roll to the sides. An alternative explanation originates from a study pinpointing the activation of STG during common-goal movements, and not during dual-goal movements (Duque et al., 2010). It has been shown that the STG is involved in orchestrating spatial attention (Gharabaghi, Fruhmann Berger, Tatagiba, & Karnath, 2006; Karnath, Ferber, & Himmelbach, 2001), and the role of the STG in common-goal movements may be to continuously monitor the location of each hand to produce a coordinated movement (Duque et al., 2010). This monitoring of the spatial location of each hand raises the need for information sharing between the hemispheres if the purpose is to achieve a united goal. The common-goal movement in our study necessitates information sharing (i.e., stronger connectivity) for two reasons: holding the bar horizontally and reaching towards a united goal, thus requiring elevated interhemispheric connectivity.

Restoration of bimanual arm function after bimanual training is still a matter of uncertainty in the SCI and stroke populations. Studies in 2010 and 2013 found that improvement of bimanual hand function is greater in a group of participants with chronic tetraplegia that received bimanual training relative to the group that received unimanual training (L. Hoffman & Field-Fote, 2013; L. R. Hoffman & Field-Fote, 2010). A Cochrane review did not find sufficient evidence to recommend simultaneous bimanual training (defined as completion of a motor activity at the same time by both upper limbs independently, example: lifting two cups) as the superior option compared to unimanual training (example: picking up coins or grasping blocks) after stroke (Coupar et al., 2010). Importantly, other studies only unilaterally (i.e., only in one arm) evaluated the outcome of bimanual training. We believe this lack of clear evidence is related to the gap in our understanding of the nature of bimanual movements. As our study suggests, the way the end goal of bimanual movements is conceptualized has dramatic consequences on both behavioral and electrophysiological outcomes. Our study guides future clinical research in two important ways. First, before planning for any rehabilitation training, clear identification of what aspect(s) of bimanual movements is impacted by the neural injury is needed. We believe that there could be a difference between how common-goal versus dual-goal movements are performed after a neural insult. Second, bimanual rehabilitation training should be primarily focused on the more impaired bimanual movement type. Daily life activities encompass both common- and dual-goal movements and it is therefore critical to include both types in the rehabilitation regime, an approach that has not been utilized so far in clinical studies.

2.5. Limitation and future direction

One limitation of this study is the lack of a left-hand unimanual task. This would have allowed us to better understand the role of hemispheric specialization (specifically, the right hemisphere's spatial accuracy advantage) and how it may have contributed to the differences we observed with goal-conceptualization. Also, measurement of eye-movements using an eye tracking system would have allowed us to investigate the role of attention in bimanual coordination. This is especially important since the two bimanual movements chosen for this study are fundamentally different with regards to attentional demands. Dual-goal movement possibly requires shift of attention between the spatial locations of the two arms, even though this can be small in our study because the space between the arms is not wide. However, visual attention is possibly placed on the ball on the bar during the common-goal task and we may assume the attention is not divided. But it is also possible that there were instances of attentional shift towards the two sides of the bar to ensure the bar remains horizontal during the execution of the task. Finally, neuroimaging techniques, such as fMRI, may add valuable insight into the modulation of cortical and even subcortical activity, which is not accessible with EEG recordings, with high spatial resolution. EEG lacks spatial accuracy and electrodes do not represent cortical sources. This highlights the importance of fMRI-based assessments of cortical activity with highest spatial resolution. Moreover, two regions of interest including SMA and cerebellum cannot be investigated using EEG. These two regions have been implicated in bimanual coordination, but how their activity is modulated with different goal-conceptualization and reverse reaching movements is still an open question. Future work would also benefit from investigations of the effect of neural injury or disease such as SCI, multiple sclerosis or Parkinson’s disease on bimanual movements, and how kinematic and electrophysiological measures differ between dual-goal and common-goal movements in these groups. Accurate identification and measurement of bimanual deficits is essential after neural damage. Depending on the injury site and its severity, the bimanual coordination ability can be impacted in distinct ways. There might be a difference between how much common-goal versus dual-goal maneuvers are retained after injury. Quantifying such differences enables targeted rehabilitation with improved outcomes. Rehabilitation training using bimanual common-goal tasks in addition to unimanual and dual-goal tasks could lead to new, possibly more effective, rehabilitation training paradigms.

2.6. Figures

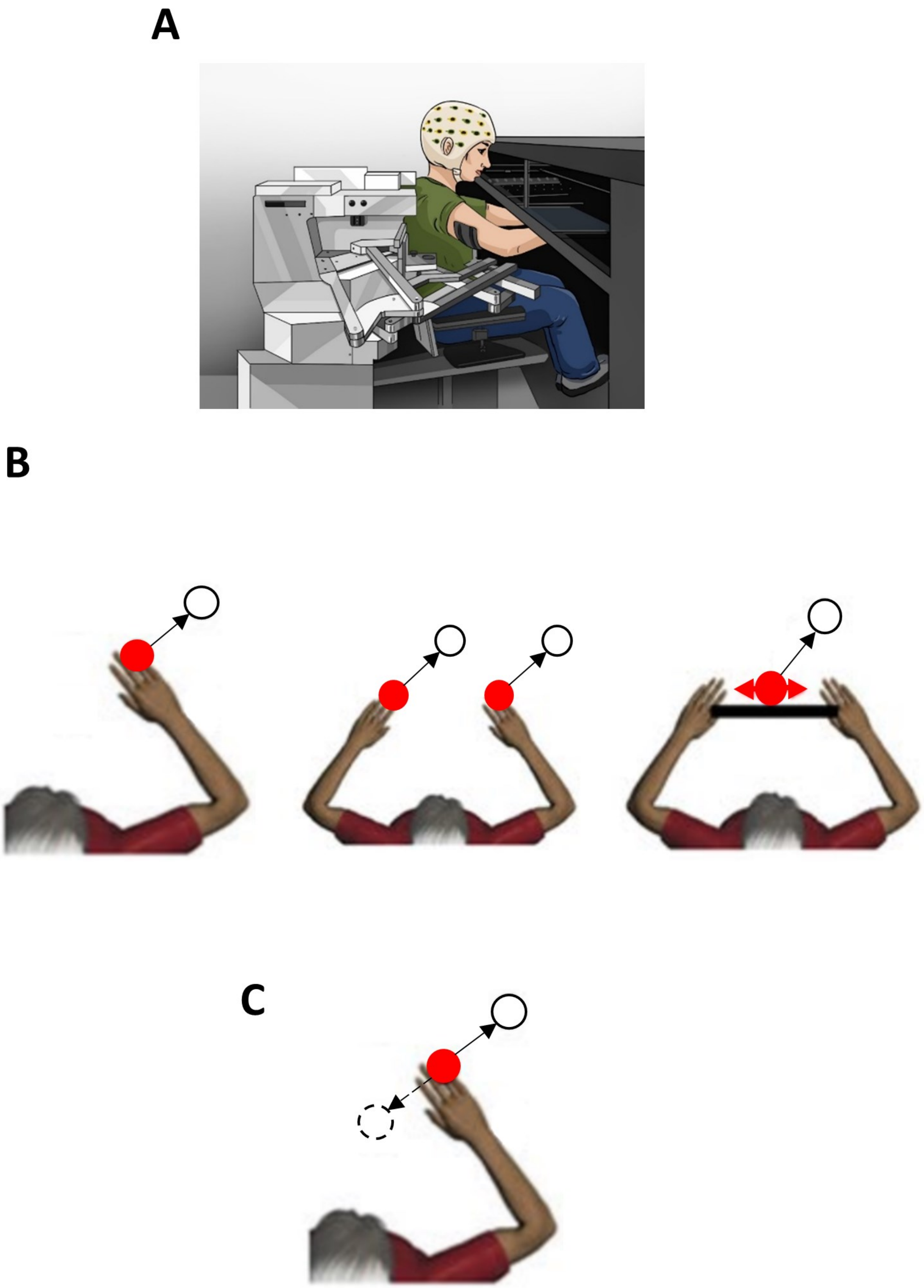


Figure 2.1. Experimental design. **(A)** Illustration of KINARM exoskeleton robotic platform and experimental setup. A virtual reality display was used for the visually guided reaching tasks. **(B)** Representation of visual tasks performed without the cognitive load, top left - unimanual; top right - bimanual dual-goal; and bottom - bimanual common-goal. All the movements started from a home position (red circle) and ended on a target (white circle). **(C)** Representation of the task with cognitive load (i.e., reverse reaching). The solid line shows the real movement of the arm starting from the home position. While moving in the direction of elbow extension, the visual feedback of the movement (red circle, currently located at the home position) moves in the reverse direction to the target (dashed circle) as illustrated by the dashed line.

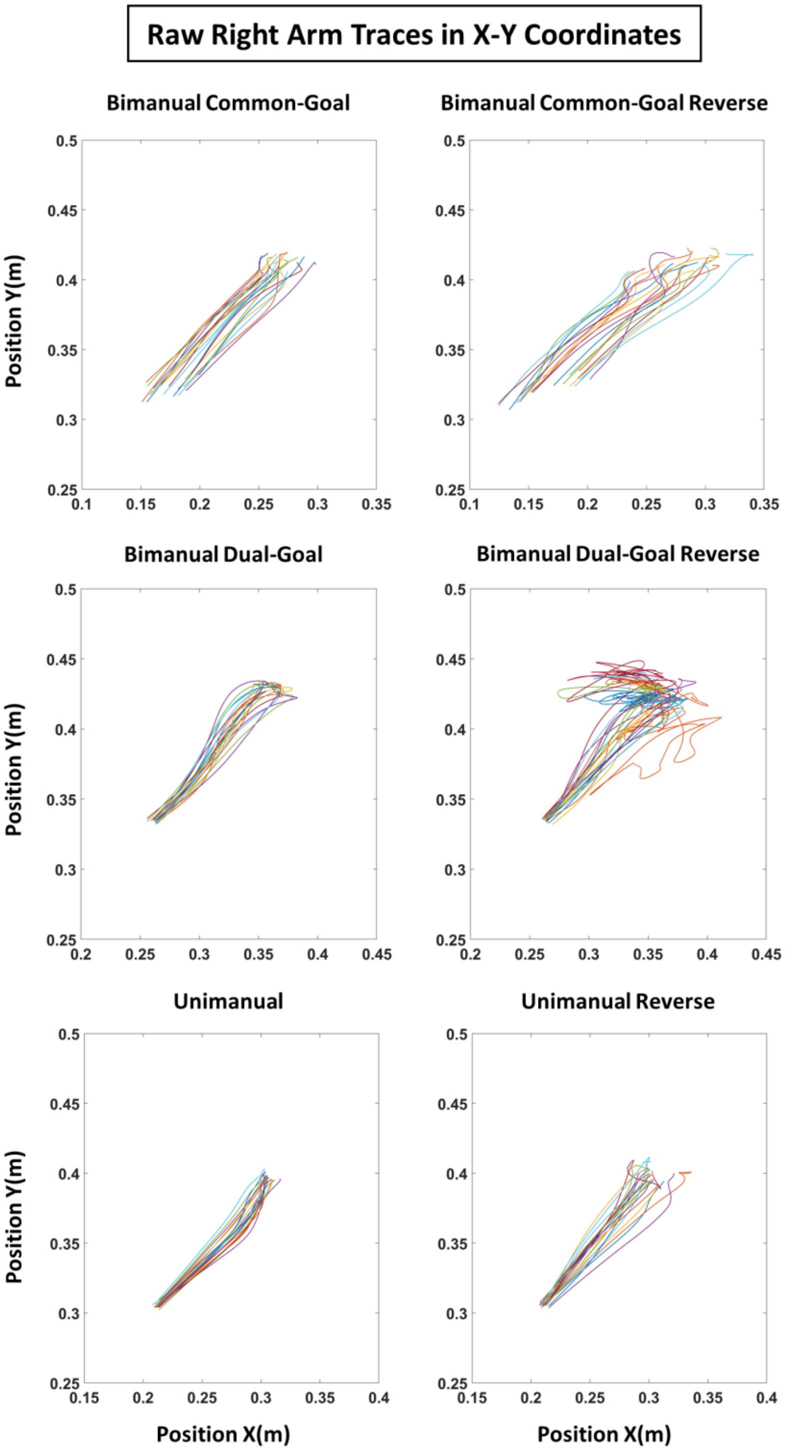


Figure 2.2. Representative raw movement traces of the right arm in x-y coordinates from a single participant. Each color is a single reaching movement, and each plot illustrates an overall

of 20 repetitions per task. The left column is movement in the forward direction, and the right column shows reverse movements.

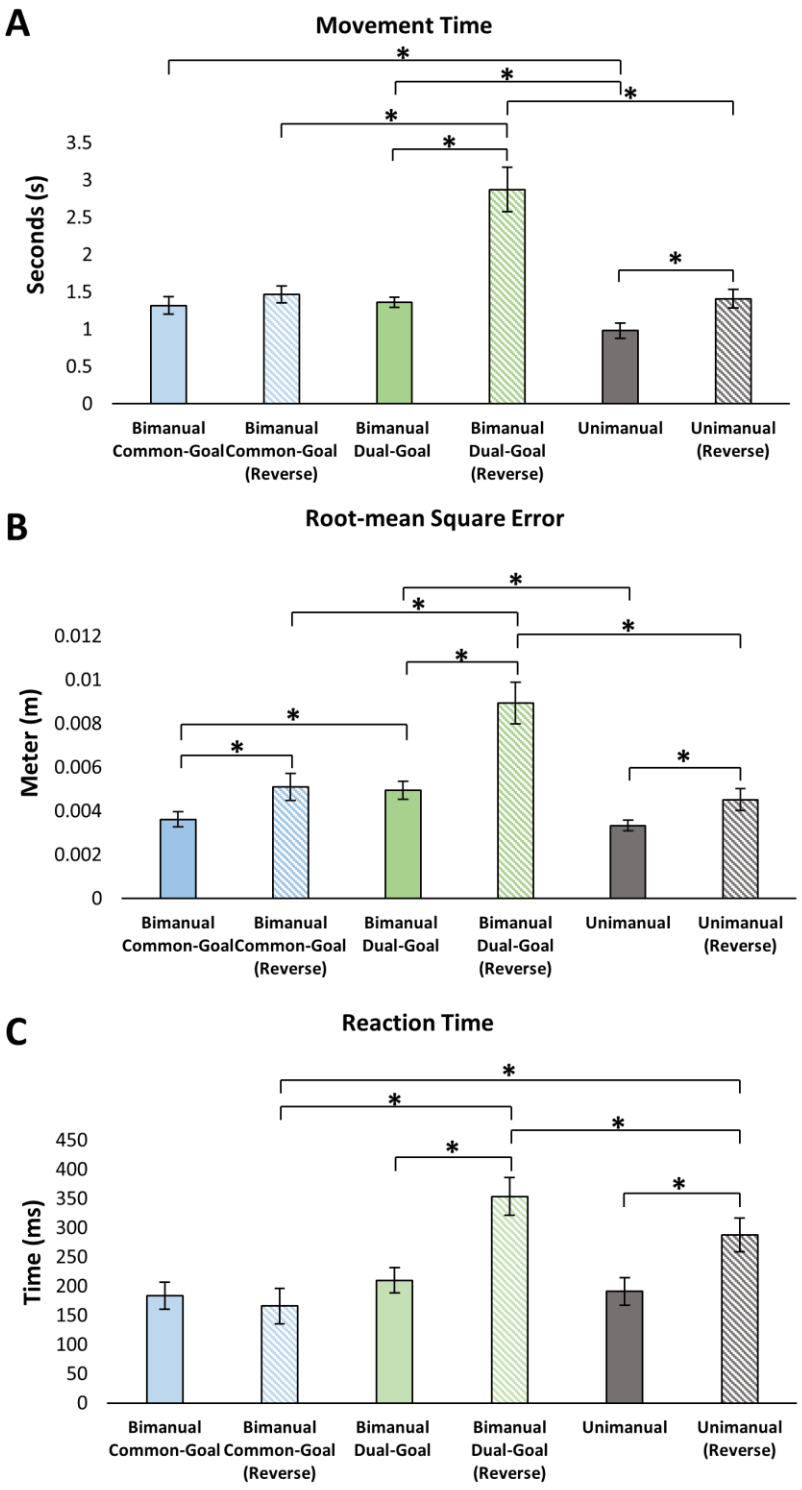


Figure 2.3. Relationship between movement kinematics, task, and movement direction. Values are Mean ± SE. **(A)** Movement time (MT) in seconds during the execution of the six tasks. In

forward direction, unimanual movement had the lowest MT. In the reverse direction, dual-goal movement had the highest MT. Reverse direction increased MT relative to forward in unimanual and bimanual dual-goal movements. **(B)** Movement error (RMSE) in meter as a measure of straightness. Dual-goal movement had the highest RMSE in both forward and reverse direction. Moving in the reverse direction increased the RMSE relative to forward in all three movement types. **(C)** Reaction time (RT) in millisecond. In the reverse direction, RT was higher during bimanual dual-goal movement relative to bimanual common-goal and unimanual. Reverse movement elevated RT compared to forward in unimanual and bimanual dual-goal movements.

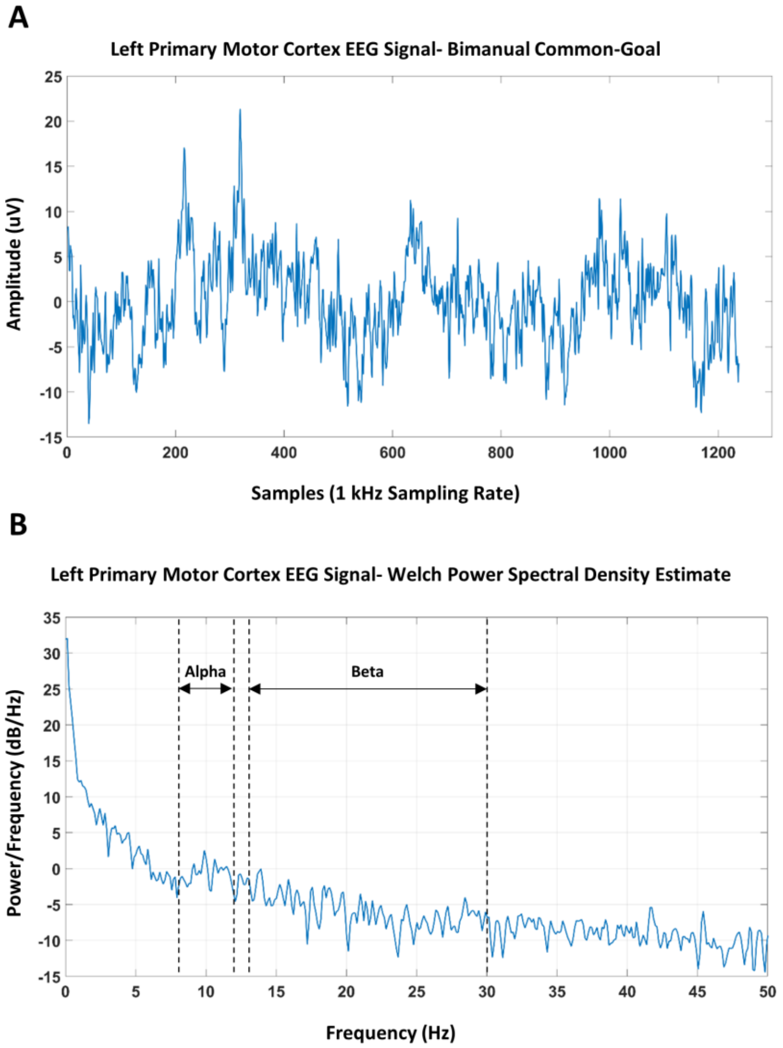


Figure 2.4. Raw EEG signal**. (A)** Representative raw EEG data from a single participant during the execution of bimanual common-goal task. EEG was recorded from the C3 electrode. **(B)** Power spectral density estimate of the EEG data in A.

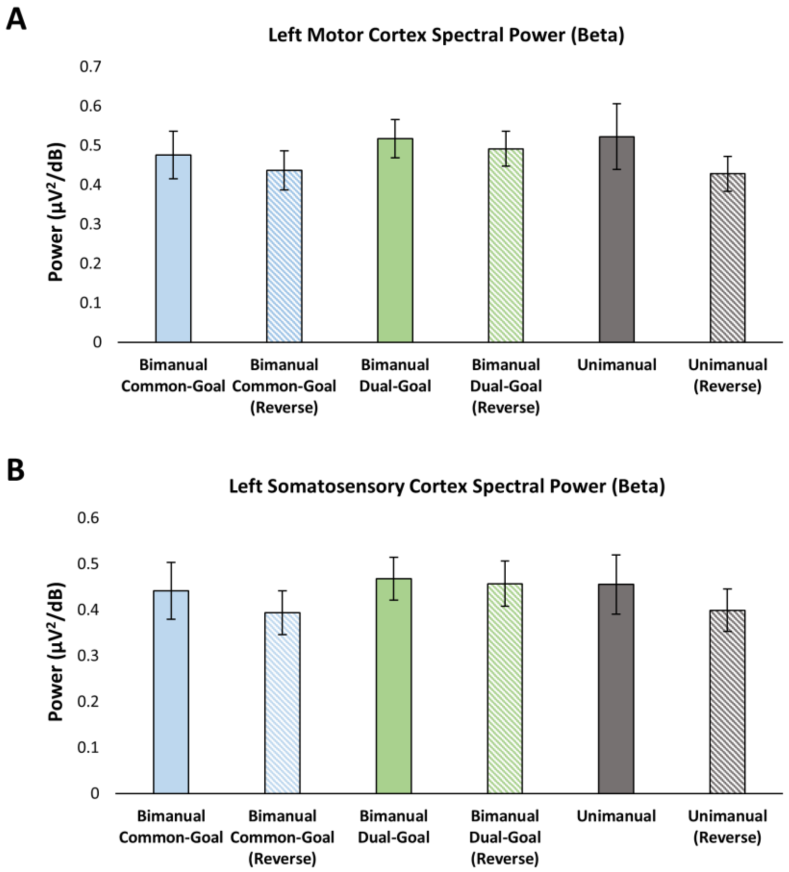


Figure 2.5. Beta band spectral power (SP) during the execution of the six movement tasks. **(A)** SP from C3 electrode, **(B)** SP from CP3 electrode. Cortical activity in the beta band was similar across all tasks, and contrary to our hypothesis, increasing task demand by adding a cognitive load did not modulate SP.

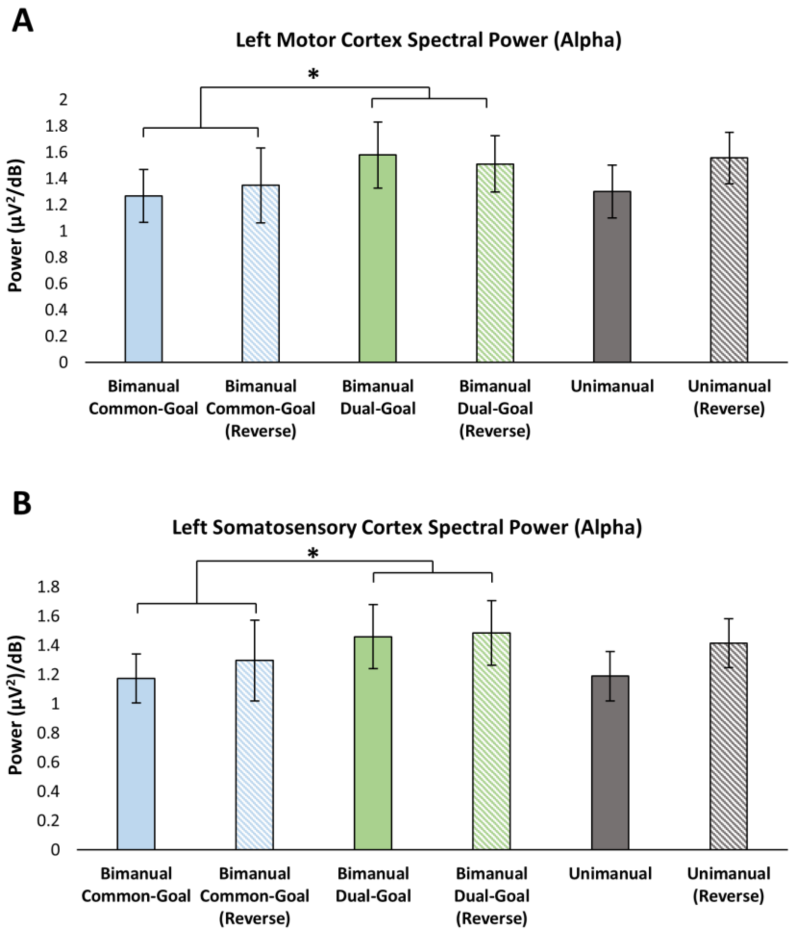


Figure 2.6. Alpha band spectral power (SP) during the execution of the six movement tasks. **(A)** SP from C3 electrode, **(B)** SP from CP3 electrode. Cortical activity was not different between bimanual and unimanual movements, but common-goal movements had decreased power relative to dual-goal movements. Contrary to our hypothesis, increasing task demand by adding a cognitive load did not modulate SP.

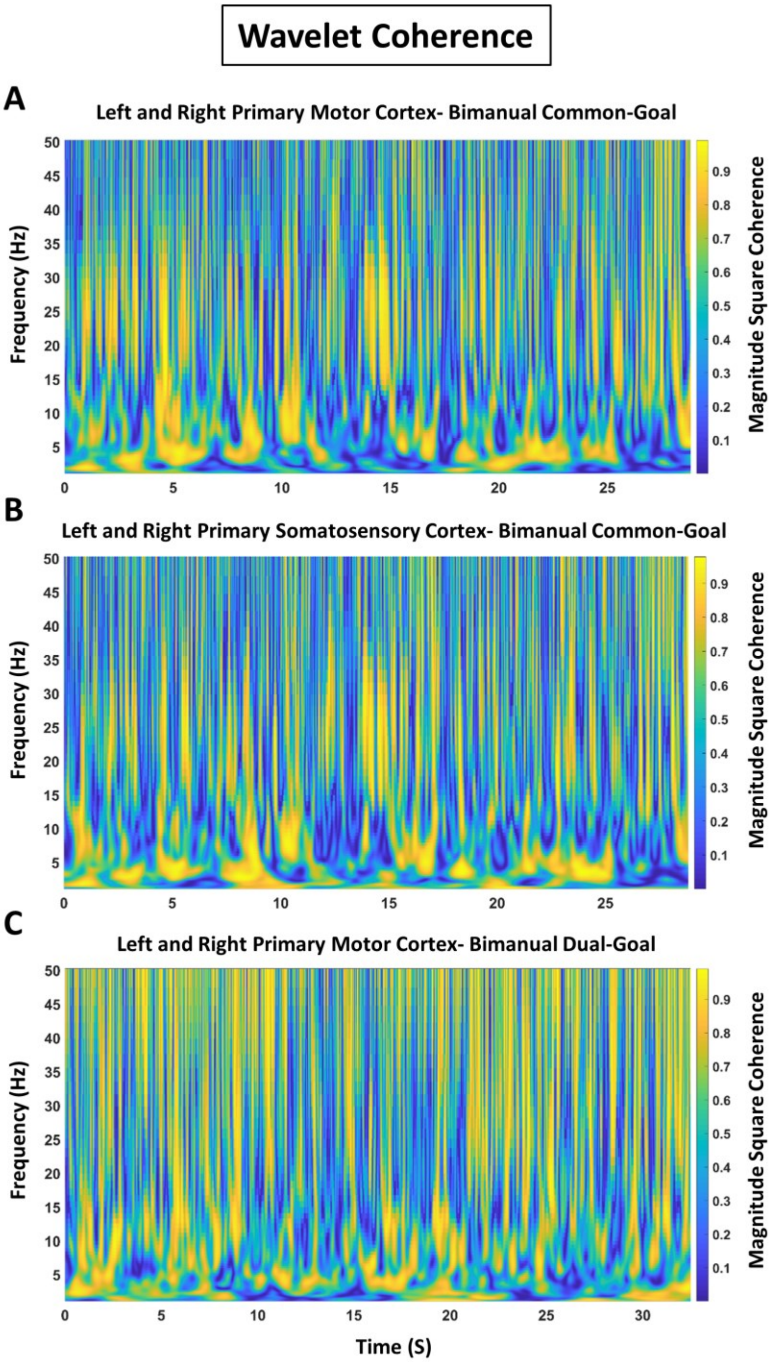


Figure 2.7. Time-frequency analysis of coherence using wavelet method from a single participant between **(A)** left and right M1 and **(B)** left and right S1 during the execution of bimanual common-goal movement, and between **(C)** left and right M1 in the dual-goal task.

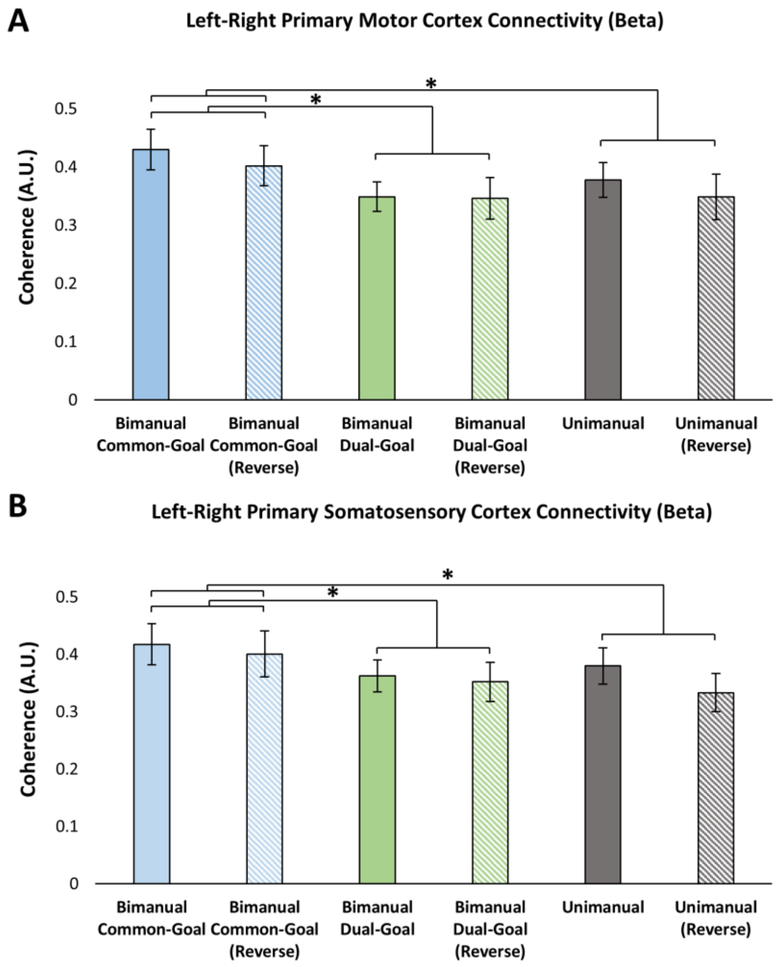


Figure 2.8. Interhemispheric connectivity in beta band measured by coherence between **(A)** left and right primary motor cortex (M1), and **(B)** left and right primary somatosensory cortex in beta band (S1). Interhemispheric connectivity was elevated in common-goal bimanual movement relative to unimanual and dual-goal bimanual movement.

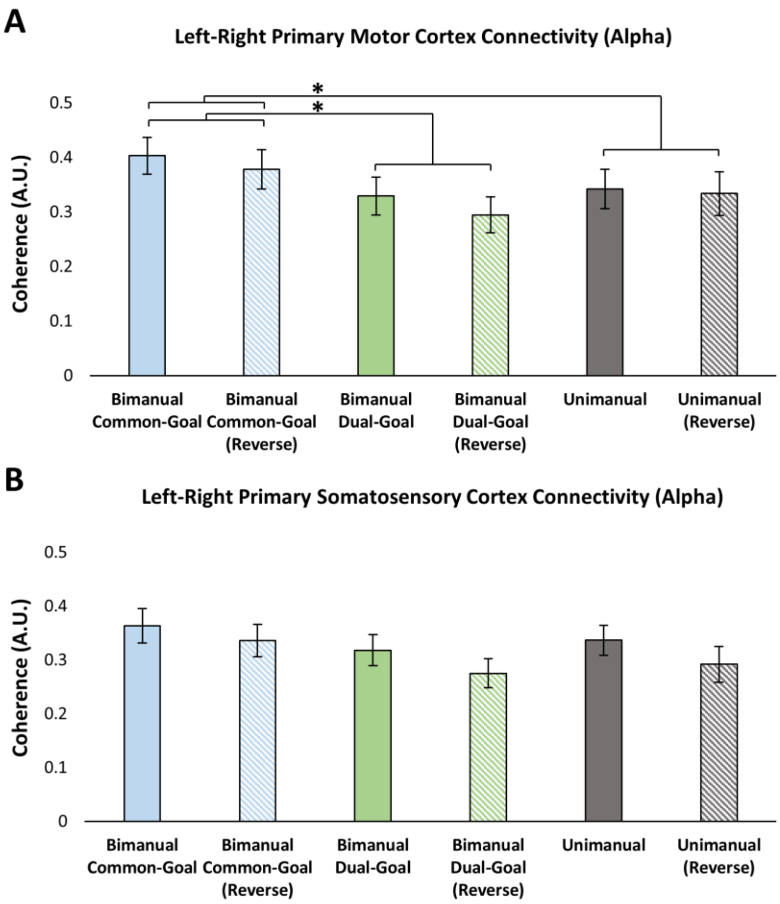


Figure 2.9. Interhemispheric connectivity in alpha band measured by coherence between **(A)** left and right primary motor cortex (M1), and **(B)** left and right primary somatosensory cortex (S1). Interhemispheric connectivity was elevated in common-goal bimanual movement relative to unimanual and dual-goal bimanual movements over the two hemispheric motor regions, but not for the somatosensory regions (although the main effect was close to the significance threshold with $p = 0.055$).

Chapter 3: Bimanual coordination and spinal cord neuromodulation: how neural substrates of bimanual movements are altered by transcutaneous spinal cord stimulation

3.1. Introduction

One impressive capability of the human motor system is coordinative movement of the hands to accomplish a task. A large variety of functional activities require some degree of coordination and collaboration between the two hands. Yet, many of these daily activities are impaired by neurological conditions such as spinal cord injury (SCI). For instance, cervical SCI results in prolonged movement time and lower peak velocity in a unimanual reach-to-grasp task compared to non-injured individuals. The same kinematic measures are exacerbated during a bimanual version of the reach-to-grasp task (Britten et al., 2017). Restoration of the ability to use the hands in a coordinated manner can substantially improve independence in the performance of daily activities after SCI.

Transcutaneous spinal cord stimulation (tSCS) has emerged as a non-invasive neuromodulatory technique that has the potential to reverse sensory and motor loss after SCI (Y. Gerasimenko et al., 2015; Gerasimenko et al., 2014; U. S. Hofstoetter et al., 2013; Inanici et al., 2018; Rath et al., 2018). Cervical tSCS promoted both immediate and long-term improvement in hand and arm function (P. Gad et al., 2018; Inanici et al., 2021; Inanici et al., 2018). However, motor gains in the upper limbs after tSCS are commonly reported by clinical tools that evaluate each limb's function in isolation of the other using unimanual tasks (Inanici et al., 2018). Moreover, the underlying mechanisms of cervical tSCS are investigated using static tasks that only record muscle responses unilaterally (Milosevic et al., 2019) (Sasaki et al., 2021). While this technique has produced promising results, and may become a versatile clinical tool, the utility of tSCS as a means for improving bimanual motor performance is not yet known. To address the therapeutic effect of tSCS on bimanual actions, it is necessary to first identify how tSCS modulates bimanual motor performance and the activity of brain areas subserving it.

To date, a number of studies have investigated the underlying neural substrates of tSCS along the spinal neuroaxis (Barss et al., 2019; Danner et al., 2011; Milosevic et al., 2019; Karen Minassian et al., 2007; Sasaki et al., 2021). Both computer modelling and electrophysiological studies have provided substantial evidence that tSCS primarily recruits large-to-medium afferent fibers in the posterior root and dorsal horn of various spinal segments (Danner et al., 2011). Moreover, a combination of neural structures are activated with increasing stimulation intensity, including group Ib afferents, group II muscle spindle afferents, and spinal interneurons (Barss et al., 2022; Y. Gerasimenko et al., 2015). Importantly, these effects are not restricted to the site of stimulation, and propagate to remote segments of the spinal cord (Parhizi B, 2021). We explored the spinal multi-segmental effect of tSCS in two recent studies in neurologically intact individuals. We demonstrated that cervical tSCS suppresses the H-reflex in the soleus muscle of the leg while lumbar tSCS facilitates H-reflex in the flexor carpi radialis (FCR) muscle of the arm(Barss et al., 2019; Parhizi B, 2021).

While numerous other studies have investigated the mechanisms by which tSCS recruits neural structures at the spinal cord level, only a few studies alluded to the cortical effects of tSCS. Benavides et al. reported that cervical tSCS with 5 kHz carrier frequency does not modulate the amplitude of motor evoked potentials (MEPs) in proximal and distal arm muscles (Benavides et al., 2020). In the absence of the carrier frequency, the MEP amplitude increased, suggesting a cortical inhibitory effect when the tSCS waveform is modulated with 5 kHz carrier frequency. Similarly, in our own work, we found that the amplitude of MEPs in the FCR muscle remain unchanged during tSCS when applied with a 10 kHz carrier frequency over the C3–4 and C6–7 spinous processes (Parhizi B, 2021). Although the majority of studies use MEPs as a measure of cortical excitability, further knowledge can be captured by cortical oscillations recorded by electroencephalography (EEG). Both measures reflect motor cortical excitability, but the excitability is likely driven by different neural processes. Thus, cortical oscillations are an alternative approach to understanding the physiological effects of tSCS, especially that cortical regions (along with their activation patterns) are prominent to explore the processes underlying bimanual movements. A recent study reported that tSCS did not have a consistent effect on sensorimotor cortical oscillations among study participants, but those who received the highest intensities of stimulation showed cortical inhibition (McGeady, Alam, Zheng, & Vučković, 2022).

Most research studies aiming to investigate the effects of tSCS have focused on conditions where the upper limbs are static (either at rest or exerting some levels of isometric muscle contraction), and responses are recorded unilaterally by utilizing measures such as MEPs and H-reflexes

(Benavides et al., 2020; Sasaki et al., 2021). However, the question of how tSCS induces modulation of sensorimotor cortical oscillations during bimanual movements is yet to be addressed. In the present study, we assessed the effect of cervical tSCS on sensorimotor cortical regions using EEG during the execution of dynamic unimanual and bimanual movements. Two scenarios were expected in the presence of tSCS: cortical excitation as a result of an increase in sensory afferent volleys, or cortical inhibition as a result of inhibition of nociceptive input and as a consequence of the presence of a carrier frequency in the tSCS waveform (Benavides et al., 2020; Fürst, 1999; Insausti-Delgado, López-Larraz, Omedes, & Ramos-Murguialday, 2021; Sharon, Fahoum, & Nir, 2021). We hypothesized that 1) the kinematics of the reaching movements are improved when cervical tSCS is administered, 2) tSCS induces event-related synchronization (ERS) in cortical oscillations indicative of cortical inhibition, and 3) tSCS elevates the level of interhemispheric connectivity during the execution of movements.

To address these hypotheses, we recruited neurologically intact (NI) participants to perform three types of goal-directed arm reaching movements using a KINARM exoskeleton facilitated by a virtual reality (VR) display. These movements were: 1) unimanual visually-guided reaching (VGR); 2) dual-goal bimanual VGR; and 3) common-goal bimanual VGR. The same three tasks were repeated in the presence of cervical tSCS applied over the C3-4 and C6-7 (cervical) spinous processes. Movement kinematics were tested by measuring reaction time (RT), movement time (MT) and movement error during the execution of the movements. Cortical activity was recorded via EEG electrodes while the participants performed the reaching movements. Alpha (8-12Hz) and beta (13-30Hz) band cortical activity associated with sensorimotor processes was computed using spectral power. Interhemispheric connectivity between the right and left primary motor (M1) and somatosensory (S1) cortices were also evaluated.

3.2. Materials and Methods

Participants: Twelve (12) participants were recruited aged 19 to 36 years. All participants were right-handed on the basis of self-report, had normal or corrected vision through the use of contact lenses or glasses, and had no history of neurological conditions. Participants signed a written consent form to participate in the experimental protocol which was approved by the

University of Alberta Human Research Ethics Committee, and conducted in accordance with the Declaration of Helsinki (1964). All participants were verbally instructed about the experimental procedures.

Experimental design: Participants were seated in front of a KINARM exoskeleton to perform the VGR movements (BKIN Technologies Ltd, Kingston, ON, Canada) (Figure 3.1A). The KINARM exoskeleton allows the performance of movements around the elbow and shoulder joints in the horizontal plane. The participants were able to interact with a virtual reality screen that projected the task environment, and the participants' limbs were supported against gravity by the exoskeleton. The segments of the KINARM were adjusted to accommodate each participants' limb geometry, and the arm, forearm and hand were supported by troughs attached to adjustable 4-bar linkages. Participants received no assistance from the robot while completing the experimental tasks.

Participants performed three movements facilitated by the exoskeleton (Figure 3.1B): 1) unimanual VGR where they were instructed to reach with their right arm to a virtual peripheral target on the top right corner from a home position; 2) dual-goal bimanual VGR where each arm separately but simultaneously performed home-to-target reaching movements to two peripheral targets on the top-right corner of the home position; and 3) common-goal bimanual VGR where participants moved a ball on top of a horizontal bar connecting both their hands to a peripheral target through cooperative movement of the two arms, each holding one end of the bar. The ball could roll to the sides of the bar if the orientation of the bar deviated from horizontal, thus participants were instructed to maintain the horizontal orientation of the bar throughout the movement. The movement was rejected if the ball fell off of the bar. The tasks were randomly presented to the participants using simple randomization, and each reaching task was repeated 20 times. Study participants were instructed to move as quickly and as accurately as possible from the home position to the final target. During the KINARM calibration and adjustment steps, participants were exposed to one trial of each task.

Participants started each movement from a home position where their index finger was aligned with a 1.0 cm radius circle and moved to a peripheral target of the same size positioned on the upper right corner (10.0 cm to the right and 10 cm to the top) extending their elbow. Before the movement began, participants held the tip of their index finger on the home position for 750ms while it was colored red. The color of the home position turned green as the go signal, and the peripheral target appeared on the VR display in red. Once the participant reached the target, they were required to hold their index finger for another 750ms until the target turned green. At this moment, the home position reappeared and the participant returned back to the home position and waited for 1750 ms for the next repetition of the movement to start. In the bimanual dual-goal task, the same procedure applied except that each arm performed the reaching movement to the two separate targets simultaneously. In the case of the bimanual common-goal task, the movement started from a home position between the two hands located in the middle of the horizontal bar. The participant moved from the home position to the target positioned on the upper right corner. To ensure similar voluntary cortical drive across all experimental tasks, all movements were performed against a load equivalent to 5-10% of the tricep brachii (TB) maximum voluntary contraction (MVC). To obtain MVC, participants performed three trials of isometric maximal voluntary elbow extension. The KINARM exoskeleton was then programmed to produce a force in the direction opposite to the movement direction and equivalent to 5-10% elbow extension MVC. To compare the effects of cervical tSCS on sensorimotor cortical oscillations and movement kinematics, all tasks were repeated twice, once with and once without tSCS.

Transcutaneous spinal cord stimulation: Biphasic cervical tSCS was delivered by two constant current stimulators each having one output channel (DS8R, Digitimer, Hertfordshire, UK). Two cathode electrodes, 3.2 cm diameter (Axelgaard Manufacturing Co., Ltd., United States), were placed midline at C3-4 and C6-7 spinous processes, and two 5 × 7 cm rectangular electrodes were placed bilaterally over the iliac crest as anodes (Figure 3.2A). We used a modulated waveform (Barss et al., 2022) consisting of bursts of ten 100µs-long biphasic square pulses (enveloped in a 1ms pulse) repeated at a frequency of 40 Hz (Figure 3.2B).

We used evoked potentials to determine the stimulation intensity (Benavides et al., 2020). Single 1ms-long biphasic pulses were delivered to both cathodes simultaneously and the stimulation intensity was defined as the minimum amplitude required to evoke potentials in electromyographic (EMG) recordings from the bicep brachii (BB) muscle that were 50µV peak-to-peak amplitude above background muscle activity in 5 out of 10 trials. This intensity was then used for the continuous stimulation. Participants reported a strong buzzing or vibration-like sensation at cathodic sites as well as tolerable discomfort associated with neck muscle contraction or skin irritation. Stimulation was turned on a few seconds prior to the initiation of a movement task and turned off immediately after the completion of 20 repetitions. Therefore, for each task tSCS remained on for about 3-4 minutes including the time prior to the initiation of data collection for each movement task.

Quantification of movement kinematics: RT and MT were calculated using a method introduced by Coderre and colleagues (Coderre et al., 2010) that is based on identification of movement onset and offset. Accordingly, RT was defined as the time interval between the appearance of the peripheral target and the onset of movement. MT was the time interval between movement onset and offset. We also measured movement root mean square error (RMSE) to evaluate the straightness of the participants' movement. Ideally, each reaching movement should be on the straight line between the home position and peripheral target. RMSE measured the deviation between the real hand coordinates and the closest point (perpendicular distance) to the ideal line.

Electroencephalography: All EEG recordings were obtained using a 64 channels Brain Vision Recorder (Brain Products, Gilching, Germany) according to the international 10-20 system (Kilicarslan et al., 2013). Data were recorded and sampled at 1000 Hz. During EEG recordings, the AFz and TP10 electrodes were used as ground and reference, respectively. All data were then re-referenced to the average of electrodes TP9 and TP10 during offline processing. Electrodes impedance was kept below 5 kΩ and was repeatedly checked throughout the experiment. Since EEG data are prone to unwanted electrophysiological noise, we instructed the participants to sit still and minimize eye blinks and neck movement to ensure high quality recordings.

EEG data were pre-processed using a band-pass filter between 0.1 to 200 Hz Butterworth and notch filtered at 60 Hz. One complication in EEG recordings with nearby surface stimulation is the high-amplitude artifacts associated with stimulation. A recent study showed that EEG recordings are feasible in the presence of tSCS and artifacts were only manifested at the frequency of stimulation in the spectral density analysis (McGeady, Vučković, Zheng, & Alam, 2021); therefore, tSCS posed no detrimental effects on the EEG frequency domain analyses in this study. Given that the artifact occurred every 25ms (stimulation at 40Hz), no artifact removal techniques were used, such as artifact subspace reconstruction (ASR), to avoid discarding meaningful EEG information. This approach was successfully used in a previous study (McGeady et al., 2022).

Computing spectral power and coherence: Since the data were continuously recorded during each task, we first split the data into 20 repetitions and then concatenated them. The concatenated data were then used to compute spectral power and coherence between EEG channels for each task. Spectral power was calculated over 1024-point FFT segments with zero over-lap using the following formula (Johnson et al., 2011):

$$P_x(f) = \frac{1}{n}\sum_{i=1}^{n} C_i(f) * C_i^*(f)$$

where $P_x(f)$ is the spectral power for EEG channel x and $C_i(f)$ is the fourier transform of data segment i of EEG channel x. Alpha (8-12 Hz) and beta (13-30 Hz) band information for the electrodes over the left M1 (C3 electrode) and S1 (CP3) were included in this study, and the average of power within each band was calculated.

Coherence was then calculated with the following formula (Halliday et al., 1995):

$$Coh_{xy}(f) = \frac{\left|P_{xy}(f)\right|^2}{P_{xx}(f) * P_{yy}(f)}$$

where $P_{xy}(f)$ is the cross spectral power of EEG signal x and signal y, and $P_{xx}(f)$ and $P_{yy}(f)$ are the spectral power of EEG signal x and y, respectively. Coherence is a scalar value ranging between 0 and 1 and describes the strength of coupling between two signals. Confidence interval at $\alpha=0.95$ quantile of the coherence is measured by $cl = 1 - (1-\alpha)^{1/(L-1)}$ where L is the number of segments (Rosenberg et al., 1989). Right and left interhemispheric coherence was computed for M1 (C3-C4 electrodes) and S1 (CP3-CP4 electrodes) cortical regions in the alpha and beta bands. Only coherence values above the cl threshold were accepted.

Stimulation artifact removal: It was previously suggested that the effect of stimulation on frequency domain analyses is contained at the stimulation frequency, manifested as an obvious transient high-amplitude peak in the spectral power of the EEG signal (McGeady et al., 2021). As suggested by (McGeady et al., 2021), notch filtering in the frequency domain and superposition of moving averages in the time domain are the optimal approaches to eliminate the contamination of frequency bands of interest such as alpha and beta caused by the stimulation artifact. Here, we introduce an alternative approach to remove the stimulation artifact from the EEG time series data and as a consequence, reducing the possible detrimental effect of stimulation artifact on the spectral power especially those spreading into the alpha and beta bands.

In this study, stimulation frequency was set at 40Hz and therefore stimulation artifacts should ideally be seen in the EEG time series every 25ms, each with a duration of 1ms. However, the stimulation artifacts are captured by the EEG data persist for 7-11ms. Our approach to remove these artifact consisted of multiple steps: 1) the time series data were inspected to find the high amplitude peaks produced by the stimulation artifacts, 2) the first and the last point of the stimulation artifact waveform spanning around the peak point in step 1 were found (stimulation artifacts varied slightly in duration for different participants), 3) the average of the EEG data between two consecutive stimulation artifacts (i.e., the last data point of artifact i and the first point of artifact i+1) was calculated, and 4) the stimulation artifact (i.e., artifact i) waveform (from the first to the last data point detected in step 2) was replaced by the average value calculated in step 3. Through this approach, the stimulation artifact data points were effectively replaced by the average value of the succeeding EEG data points; therefore, suppressing the negative contribution of stimulation artifacts to the spectral power of the nearby bands.

Statistical analysis: We used a paired samples T-Test to compare the means of RT, MT, movement error, spectral power, and coherence between tasks without and with cervical tSCS (No-tSCS and tSCS) for each movement type. This statistical design allowed us to solely compare the effect tSCS on each task. To directly investigate the effect of tSCS intensity on experimental tasks relative to when tSCS was not provided, the normalized amplitude of event-related desynchronization (ERD) or ERS were correlated with stimulation intensity using

Pearson's correlation. Normalized ERD/ERS was calculated using min-max normalization method ($x-x_{min}/x_{max}-x_{min}$). Descriptive statistics are shown as mean ± standard error, unless otherwise stated. Statistical significance was set for $p \leq 0.05$. All statistical analyses were performed with SPSS Statistics (IBM, Chicago, IL, United States).

3.3. Results

Movement kinematics: Figure 3.3 illustrates right arm traces of a reaching movement with and without tSCS from a representative participant. In partial agreement with our hypothesis, tSCS applied to the cervical spinal cord significantly decreased movement error (RMSE) relative to when tSCS was off during the bimanual common-goal task ($p = 0.010$) (Figure 3.4C). In addition, MT was faster with tSCS during the bimanual common-goal movement and approached significance ($p = 0.072$) (Figure 3.4B). In a partial contradiction to our hypothesis, cervical tSCS had no effect on other movement kinematic measures. Cervical tSCS had no effect on RT for any of the tasks [common-goal ($p = 0.217$), dual-goal: ($p = 0.458$), unimanual: ($p = 0.702$)] (Figure 3.4A), and tSCS had no significant effect on MT for the bimanual dual-goal or unimanual movements [dual-goal: ($p = 0.238$), unimanual: ($p = 0.457$)] (Figure 3.4B). Movement error (RMSE) was not affected by tSCS for the bimanual dual-goal and unimanual movements [dual-goal: ($p = 0.992$), unimanual: ($p = 0.468$)] (Figure 3.4C).

Sensorimotor spectral power and coherence after artifact removal: Figure 3.5A and 3.5B depict examples of single trial raw EEG signal in the absence and presence of tSCS, respectively. An example of the welch power spectral density estimate before and after artifact removal from the C3 electrode during the execution of the common-goal task from one representative participant is provided in Figure 3.5D-F. These figures show the power of artifact removal technique in correcting the transient abnormal peaks in the EEG power spectrum.

In the alpha band, cervical tSCS yielded a significant increase of spectral power over the C3 electrode during dual-goal bimanual ($p = 0.033$) and unimanual movements ($p = 0.005$), but not during common-goal bimanual movement ($p = 0.144$) (Figure 3.6A). We also observed a significant increase in spectral power when stimulation was present in the alpha band over the

CP3 electrode during the unimanual task, but not during the common-goal and dual-goal bimanual movements [common-goal ($p = 0.376$), dual-goal: ($p = 0.207$), unimanual: ($p = 0.005$)] (Figure 3.6B). Significant increases in spectral power in the beta band in the presence of tSCS (relative to No-tSCS condition) was found over the C3 electrode during the execution of the common-goal bimanual ($p = 0.001$) and unimanual ($p < 0.001$) movements (Figure 3.6C). Similarly, cervical tSCS led to an increase in the beta band spectral power over the CP3 electrode ([common-goal ($p = 0.028$), unimanual: ($p = 0.001$)] (Figure 3.6D). There were no significant differences in C3 ($p = 0.097$) and CP3 ($p = 0.837$) beta band spectral power in the dual-goal task when tSCS was delivered compared to when it was absent.

In the alpha band, a significant increase in C3-C4 coherence was found during the unimanual task when tSCS was present ($p = 0.043$), but not during common-goal and dual-goal bimanual tasks [common-goal ($p = 0.825$), dual-goal: ($p = 0.922$)] (Figure 3.7A). CP3-CP4 coherence in the alpha band was not affected by the application of cervical tSCS relative to when tSCS was not present for all movement tasks [common-goal ($p = 0.812$), dual-goal: ($p = 0.629$), unimanual: ($p = 0.285$)] (Figure 3.7B). In the beta band, coherence between C3 and C4 electrodes was not significantly different between tSCS and No-tSCS conditions regardless of the task [common-goal ($p = 0.225$), dual-goal: ($p = 0.149$), unimanual: ($p = 0.473$)] (Figure 3.7C). Similarly, no difference in beta band coherence between CP3 and CP4 electrodes was found between tSCS and No-tSCS conditions [common-goal ($p = 0.804$), dual-goal: ($p = 0.641$), unimanual: ($p = 0.725$)] (Figure 3.7D).

3.4. Discussion

In the current study, we examined the modulation of the cortical mechanisms involved in unimanual and bimanual tasks in the presence of cervical tSCS. The choice of studying the M1 and S1 sensorimotor cortical regions was based on the pivotal role of these areas in modulating bimanual performance (Pixa & Pollok, 2018). We found that beta cortical oscillations associated with left sensorimotor regions were significantly modulated by tSCS during the execution of both unimanual and bimanual common-goal movements, pointing to the increase in synchronous neural firing in M1 and S1 induced by tSCS. In the alpha band however, we observed ERS of sensorimotor cortical activity only during unimanual movement. Our finding demonstrated that there is no significant modulation of interhemispheric connectivity between left and right M1 and S1 when cervical tSCS was applied. Furthermore, our study revealed that cervical tSCS improved performance during the bimanual common-goal task as characterized by MT and RMSE, but had no effect on movement kinematics during the execution of bimanual dual-goal and unimanual tasks. To the best of our knowledge, this is the first study to investigate the neural correlates of three behaviorally distinct unimanual and bimanual tasks under the influence of cervical tSCS using EEG measures. Up until now, knowledge about the effect of tSCS on cortical networks underlying bimanual motor control as well as cortical neurophysiological mechanisms of tSCS was very limited.

A number of studies over the past few years demonstrated that tSCS may be effective in improving sensorimotor function after SCI (P. Gad et al., 2018; Inanici et al., 2018; Rath et al., 2018). These studies used metrics such as spinally evoked potentials, MEPs (Milosevic et al., 2019), cervicomedullary evoked potentials (CMEP) (Benavides et al., 2020), and H-reflexes (Barss et al., 2019; Parhizi B, 2021) to investigate the underlying mechanisms of this electrical stimulation neuromodulatory technique. Only one previous study provided information regarding the effect of tSCS on cortical oscillations captured by EEG. McGeady et al. reported that 10 minutes of cervical tSCS is not sufficient to produce significant modulation of sensorimotor brain rhythm, but this finding was not consistent among all participants and relied on the intensity of stimulation (McGeady et al., 2022). Participants who received the highest doses of stimulation had suppression of cortical activity (10% ERS), implying that stimulation intensity is a critical factor at cortical level. In line with this view, a crucial finding in our work was a significant suppression of sensorimotor cortical activity in some of the performed tasks. Nonetheless, there are two important differences between our procedure for electrode placement and determining stimulus intensity and the work by McGeady and colleagues. First, we placed two adhesive cathodic electrodes midline at C3-4 and C6-C7 (Parhizi B, 2021), instead of single electrode at C5-6 as was the case in the McGeady et al. study (McGeady et al., 2022). Second, instead of subjectively setting the current intensity by asking the participants about their maximum tolerance level, we followed the procedure outlined in Benavides at al., in which stimulation intensity was determined based on the threshold that induces spinally evoked potentials (Benavides et al., 2020). In addition, we used a stimulation frequency of 40 Hz instead of the 30Hz used in McGeady et al. (McGeady et al., 2022).

This suppression of cortical activity is not surprising in this study. We previously reported unchanged MEPs in the presence of cervical tSCS with 10 kHz modulation (Parhizi B, 2021). However, a recent study determined that tSCS with 5 kHz carrier frequency facilitated the amplitude of CMEPs but did not modulate the amplitude of MEPs (Benavides et al., 2020), and suggested that tSCS activates cortical inhibitory networks projecting to corticospinal neurons. Interestingly, the facilitation of MEPs in Benavides et al. only happened when the carrier frequency was removed from the stimulation waveform, suggesting that the carrier frequency contributed to the inhibitory mechanism (Benavides et al., 2020). This effect was further substantiated by an increase in the level of short-interval intracortical inhibition (SICI) only when tSCS was applied with 5 kHz carrier frequency (Benavides et al., 2020). It has been suggested that the modulation of SICI is mediated by intracortical GABA inhibitory networks (Di Lazzaro et al., 2000). Therefore, with the presence of 10 kHz carrier frequency in our study, it is rational to contemplate that a similar inhibitory intracortical mechanism is responsible for the suppression of motor and sensory cortical activity (i.e., ERS) found in this study.

Alternatively, the ERS may be a consequence of exposure to discomfort caused by stimulation (López-Larraz, Ray, Birbaumer, & Ramos-Murguialday, 2019; Peng, Hu, Zhang, & Hu, 2014). Participants in our study verbally reported a strong fluttering or vibration sensation at the cathodic sites. The amplitude of stimulation for each participant was also close to maximal tolerance, at this level the participant could not tolerate the stimulation for more than 3-4 minutes (the duration of the task). Maximal tolerance with tSCS applied laterally across the spinous process between lumbar L1 and L2 vertebrae was shown to be more than 50% lower than the stimulation level required to elicit spinally evoked potentials (Manson et al., 2020). The stimulation amplitude in our study was set similarly (i.e., at the level that induces spinally evoked potentials), which would have caused experience of discomfort. Moreover, discomfort and painful sensations are associated with reduced ERD during movement (Tan, Oswald, & Kuner, 2021). Thus, discomfort experienced by the participants may have contributed to the suppressive effect on cortical activity observed in this study.

Parallels can be drawn from neuromuscular electrical stimulation (NMES) studies. Modulation of brain activation induced by NMES has been reported previously (Schürholz et al., 2012; Smith, Alon, Roys, & Gullapalli, 2003). For example, NMES of wrist extensor induced stimulation intensity dependent modulation of sensorimotor cortical activity, with above motor threshold intensities producing cortical facilitation and below motor threshold intensities causing cortical inhibition (Insausti-Delgado et al., 2021). Importantly, motor threshold level in the NMES study is defined as the intensity that induces finger twitches, and this level proprioceptive receptor as well as cutaneous mechanoreceptors are activated (Bergquist et al., 2011; Golaszewski et al., 2012). With below motor threshold however, only cutaneous mechanoreceptors are activated (Insausti-Delgado et al., 2021). The procedure to determine the amplitude of stimulation ensured that all the participants in our study received stimulation at the level that elicits spinally evoked potentials. At this level, posterior root afferent are recruited (Barss et al., 2022; Oh et al., 2022). Thus, we may conclude that tSCS through recruitment of posterior root afferents should produce the same facilitation of ERD observed in the NMES study, however we found the opposite. This effect may be due to exposure to high-intensity stimulation and activation of intracortical inhibitory network which could have interfered with the conduction of sensory information (Benavides et al., 2020; McGeady et al., 2022).

We did not find significant modulation of interhemispheric connectivity in cortical sensorimotor regions. Our results suggest a trend towards increased beta band interhemispheric connectivity between left and right M1when tSCS was delivered relative to when tSCS was off across all movement conditions, but the opposite of this trend was seen between the left and right S1 (i.e., decrease of interhemispheric connectivity). No particular trend was observed in the alpha band interhemispheric connectivity results. A recent study suggested both a decrease (in areas associated with direct motor control) and an increase (in areas of motor planning) of functional connectivity in the presence of lumbar tSCS during tonic and rhythmic muscle contraction of the lower limbs (A. G. Steele, Manson, Horner, Sayenko, & Contreras-Vidal, 2022). However, this effect was only observed at the level of cortical sources, and was absent for the EEG electrode-based analysis (A. G. Steele et al., 2022). Similarly, NMES has been shown to strengthen interhemispheric functional connectivity between cortical sensorimotor regions (Guo et al., 2022). Modulation of interhemispheric inhibition may explain increased/decreased functional connectivity between sensorimotor regions (D. A. Cunningham et al., 2019; Guo et al., 2022). The current investigation is unable to identify specific neural pathways or regions responsible for changes in the level of interhemispheric connectivity. Future investigation is needed using measures such as fMRI-based functional connectivity and TMS-based IHI to further explore tSCS-induced modulation of interhemispheric connectivity. Moreover, as suggested by Steele et al. (A. G. Steele et al., 2022), connectivity analysis is more accurate when performed at cortical source levels as opposed to when sensor-based information is employed.

Stimulation applied to regions near EEG electrodes is considered a major source of artifacts in the data and complicates the interpretation of the results. To alleviate the effect of stimulation artifacts in the EEG recordings, two approaches including artifact removal and inter-stimulus data extraction have been suggested previously (Kohli & Casson, 2019). The limitation of these approaches is the exclusion of brain data in the analysis during stimulation. To overcome this challenge, a recent study suggested that EEG during tSCS "bares statistically similar characteristics to that of normal EEG" if the frequency band of interest does not overlap with stimulation frequency (McGeady et al., 2022; McGeady et al., 2021). Therefore, no artifact removal techniques were thought to be necessary, but notch filtering was recommended in the frequency domain. In this study, we followed the procedure outlined in (McGeady et al., 2022), which only involves applying a band-pass filter between 0.1 to 200 Hz. Additionally, we suppressed the tSCS-induced contamination of EEG data in the time domain by replacing artifacts with an average of clean EEG signal. This additional step was necessary because we observed the signs of stimulation artifact spreading beyond its frequency to nearby frequency bands (i.e., alpha and beta) in the spectral power analysis. This was evident as an abnormal brief peak near 20Hz in Figure 3.5D. The artifact removal approach led to a substantial reduction in both alpha and beta tSCS-induced sensorimotor ERS relative to when only band-pass filtering was applied. This reduction of ERS demonstrates that retaining the simulation artifact in the data comes at the cost of exaggerated ERS and hence misinterpretation of the results. On the other hand, artifact removal leads to deprivation of results from brain signals during the stimulation period. In this study, pulse duration was set to 1ms but the stimulation artifact recorded by EEG persists for approximately 7-11ms. In other words, ~28%-45% of the EEG data were replaced with an average of the clean EEG when the artifact removal approach was used. Thus, these two approaches create a trade-off between the possibility of inaccurate frequency domain results and data loss.

We speculate that there are two underlying reasons contributing to the discrepancy between our view of handling stimulation artifact and what was suggested in McGeady et al. (McGeady et al., 2022; McGeady et al., 2021). First, stimulation frequency was 30Hz in the McGeady et al. study (McGeady et al., 2022; McGeady et al., 2021) compared to 40Hz in this study. This means that the inter-stimulus interval was wider in the previous study allowing for ~25% higher amount of clean and useful data for frequency-domain analyses. Second, delivering stimulation through two cervical electrodes may have caused the pronounced stimulation artifact in the time series EEG data, which led to having only ~55%-72% clean EEG signals between successive stimulation pulses. It is perhaps the case that with lower stimulation frequencies, tSCS presents no threat to frequency domain analyses as suggested by McGeady et al. (McGeady et al., 2021), but artifact removal is required at higher frequencies. Future research is necessary to explore whether EEG recordings are feasible with tSCS at different stimulation frequencies, especially to assess how artifacts affect spectral power in frequency bands of interest such as alpha, beta, and gamma. Moreover, a change in the interhemispheric connectivity occurred after applying artifact removal. Particularly after artifact removal, beta band CP3-CP4 interhemispheric connectivity reversed to a decrease in the connectivity level when tSCS was present. This supports the idea that contaminated EEG with stimulation artifact can provide misleading representations in the frequency domain (A. G. Steele et al., 2022). Thus, we believe that artifact removal is still necessary at least for higher stimulation frequencies such as 40Hz.

Importantly, our results suggest that tSCS improves MT and RMSE of bimanual common-goal movements in participants with no history of neural injury or disease, but is ineffective in improving bimanual dual-goal and unimanual tasks. It has been previously shown than tSCS primarily activates afferent fibers of the dorsal roots and dorsal horn of the spinal cord (Barss et al., 2022; Y. Gerasimenko et al., 2015). Through monosynaptic and oligosynaptic connections from sensory afferents, spinal α-motoneurons are recruited (de Freitas et al., 2021; Ursula S. Hofstoetter et al., 2018). We speculate that there is an increase in the transmission of proprioceptive information that enhanced the performance of the bimanual common-goal task. Successful performance of common-goal reaching movements requires extensive coordination between the two arms and constant sharing of spatial location between the two arms (Duque et al., 2010). Therefore, it is plausible that the increased proprioceptive input during cervical tSCS contributed to improved kinematics particularly during bimanual common-goal movements.

Although improvements in unimanual hand and arm function were previously reported with tSCS after SCI (Inanici et al., 2018), the reason improvements in kinematic performance were not seen during the unimanual and dual-goal tasks in this study is likely because the participants in our study were neurologically intact. Nonetheless, our current findings critically highlight the potential of tSCS in promoting recovery of bimanual movements after neurological conditions. If tSCS is capable of improving movement accuracy and movement time in participants with no history of neural injuries/diseases, it is possible that kinematic outcomes can be improved for participants with SCI or stroke. We posit that this behavioral improvement can be achieved through hybrid rehabilitation training that consists of bimanual coordination tasks and tSCS. Moreover, our findings highlight the importance of comprehensively and accurately assessing bimanual impairments and quantifying bimanual performance in SCI/stroke. Stroke survivors exhibit varying performance levels when engaged in different bimanual movements (S. Kantak et al., 2017). Elucidating what aspect of bimanual movements is primarily targeted by tSCS in participants with neurological conditions is a question for future studies.

If the kinematic performance of common-goal movements is improved with tSCS in people with SCI or stroke, analogous to what the present study found, our results could serve to inform the optimal bimanual rehabilitation training design. Our task design offers a precise and sensitive measure for kinematic analyses of arm and hand function before and after rehabilitation training. At this time we cannot make a conclusion about the link between cortical synchronization and potential behavioral improvement caused by tSCS when tested in clinical population. The key is to track the changes in the level of cortical ERS (or ERD) during the course of a tSCS-based bimanual rehabilitation training paradigm and correlate it with behavioral improvements. Since our study suggests sensorimotor cortical inhibition when tSCS is applied with a modulated waveform (i.e., 10 kHz waveform), a non-modulated tSCS for a clinical population maybe preferred. A previous study suggested stronger corticospinal excitability after the application of tSCS for 20 minutes only when the kHz modulation was removed (Benavides et al., 2020). We may conclude that a non-modulated tSCS waveform that does not cause cortical inhibition and leads to stronger corticospinal excitability is more beneficial for improving upper limb function after neural injury.

3.5. Study Limitations

This study has three primary limitations. First, while we tested the effects of tSCS on movement performance and cortical activity during stimulation, short- or long-term effects on movement performance in the absence of stimulation were not evaluated. For example, the effect of 20 minutes of tSCS on motor/cervicomedullary evoked potentials was tested only after the stimulation was turned off (Benavides et al., 2020). It is important to determine whether the stimulation exerts its influence only during the administration period or it provides short and/or long-lasting effects after it is switched off. Second, measures such as IHI or SICI were not tested; such measures can provide valuable knowledge regarding intracortical inhibitory and excitatory interactions and circuits. Since the underlying neurophysiological mechanisms of IHI and SICI are known (J. C. Rothwell, Day, Thompson, & Kujirai, 2009), these methods directly inform us of intra-hemispheric and interhemispheric connections. They can also serve a comparative measure to corroborate the results obtained by EEG connectivity analysis.

The study was conducted in neurologically intact participants; future studies in persons with neural injury or disease such as SCI, stroke or multiple sclerosis would unravel the effects of these neurological conditions on cortical activity and kinematics of bimanual arm movements and the potential benefits of tSCS. Multiple studies involving clinical populations reported marked improvement of function after tSCS-based rehabilitation (Benavides et al., 2020; Y. Gerasimenko et al., 2015; Inanici et al., 2021; Sayenko et al., 2018), with one study noting long-term benefits (Inanici et al., 2018). A ceiling effect imposes a limit on the recruitment of additional fibers in neurologically intact participants where the nervous system is being utilized to its fullest extent (McGeady et al., 2022). When the nervous system is fully functional due to the absence of neural injury or disease, modulation of outcome electrophysiological and kinematic measures may not be overtly present with tSCS. Thus, assuming the effectiveness of tSCS, it is reasonable to anticipate observing the most pronounced effects in clinical populations.

Future work should also investigate the effects of tSCS at different frequencies (such as 30 Hz) allowing for wider inter-stimulus intervals and using EEG source identification methods to accurately localize cortical regions of interest along with their corresponding activation level and connectivity to other sources. At 40Hz, the stimulation artifacts crept into the spectral power density and contaminated the frequency bands of interest. The 1ms tSCS pulses in the time domain resulted in 7 to 11ms artifacts in the EEG recording. Therefore, as opposed to what was suggested in McGeady et al. (McGeady et al., 2021), stimulation is a threat to both time and frequency domain analyses and artifact cleaning/removal measure should be implemented.

3.6. Figures

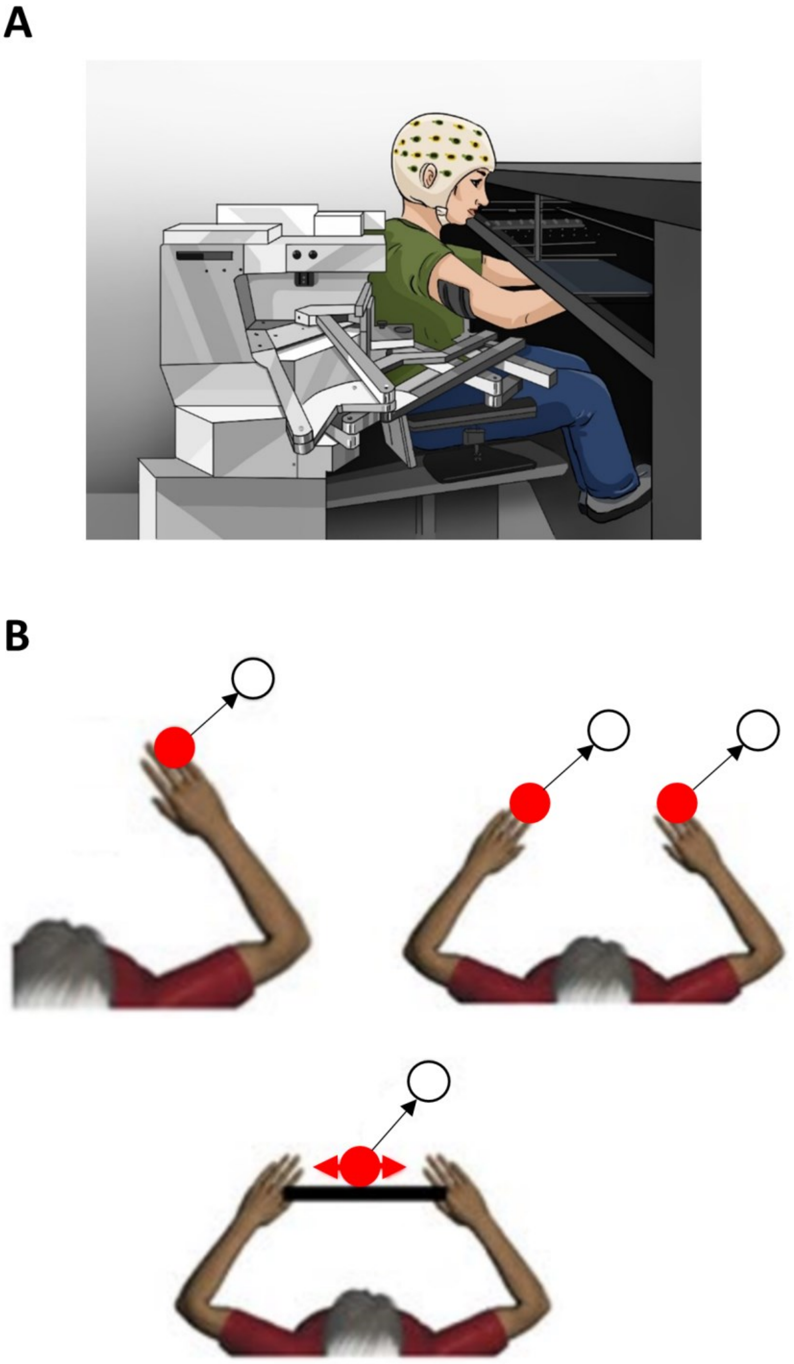


Figure 3.1. Experimental design. **(A)** Illustration of the KINARM exoskeleton robotic platform and experimental setup. Participants performed visually-guided reaching tasks guided by a virtual reality display. **(B)** Representation of the visually-guided tasks: top left – unimanual movement, top right – bimanual dual-goal movement, and bottom – bimanual common-goal movement. All the movements started from a home position (red circle) and ended on a target (white circle).

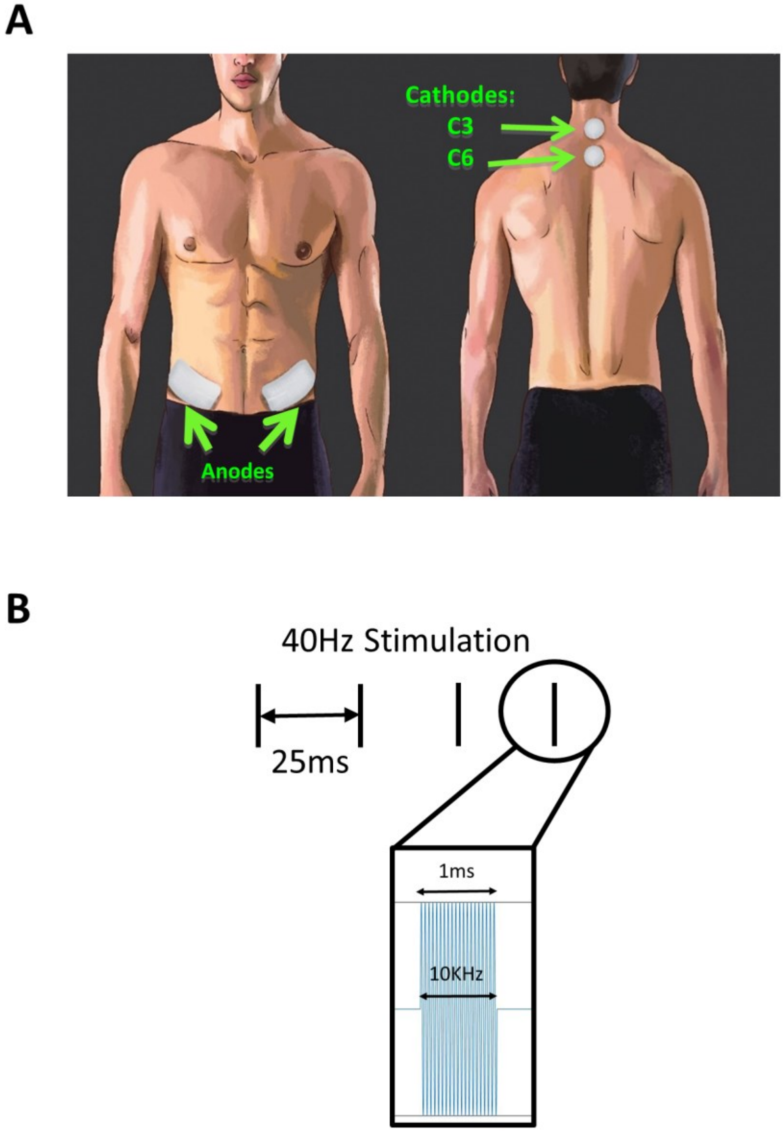


Figure 3.2. Transcutaneous spinal cord stimulation. **(A)** Cervical tSCS were delivered through cathodic electrodes placed midline at C3-4 and C5-6 spinous processes. Two anodic electrodes were placed bilaterally over the iliac crests. **(B)** Stimulation waveform: 1ms long pulses with a carrier frequency of 10 kHz are delivered at 40Hz.

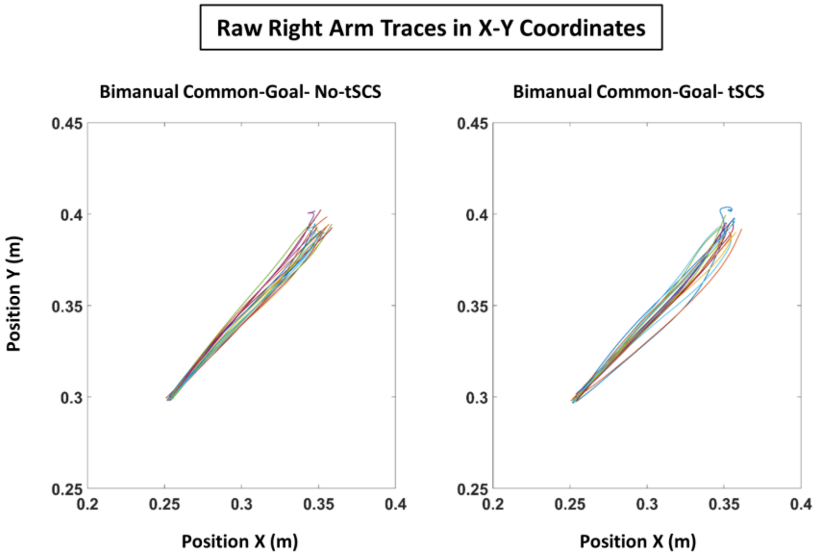


Figure 3.3. Right arm raw movement traces during the execution of bimanual common-goal task in X-Y coordinates **(A)** when tSCS was off, and **(B)** when tSCS was applied to the cervical spinal cord from a representative participant. Each color is a single reaching movement, and each plot illustrates an overall of 20 repetitions per task.

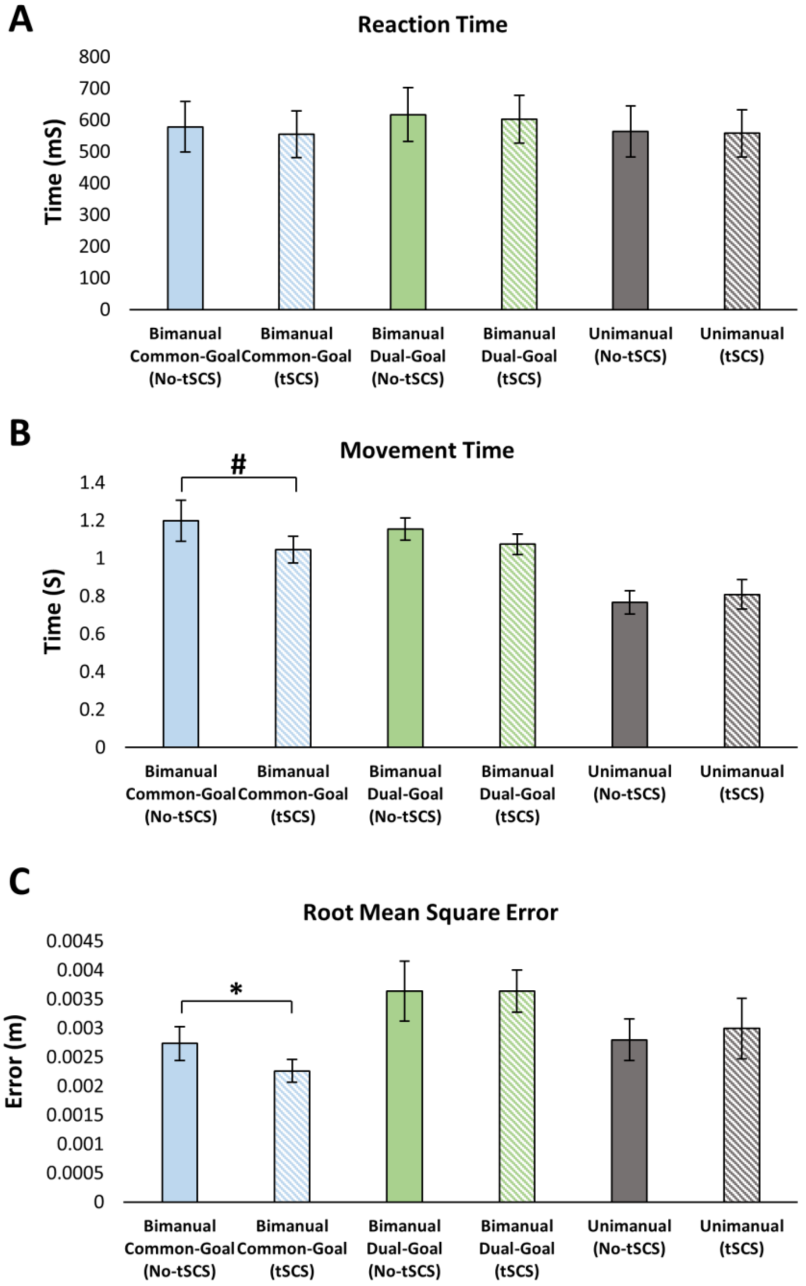
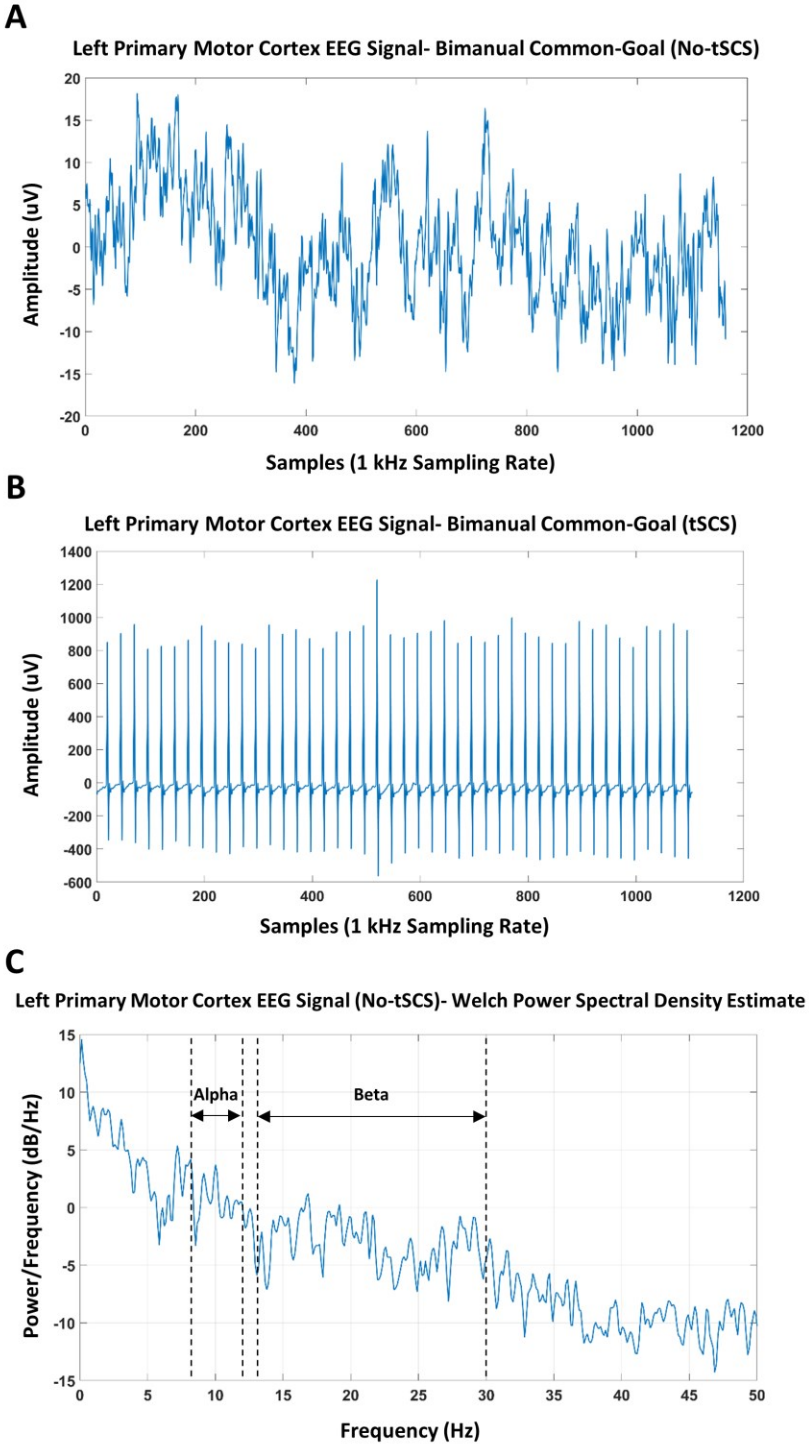


Figure 3.4. Relationship between movement kinematics and stimulation. Values are Mean ± SE. Cervical tSCS did not significantly alter **(A)** reaction time, **(B)** movement time, and **(C)** movement error relative to the no tSCS condition. (**P*<0.05; #*P*<0.1).


A
Left Primary Motor Cortex EEG Signal- Bimanual Common-Goal (No-tSCS)
Amplitude (uV)
20
15
10
5
0
-5
-10
-15
-20
0
200
400
600
800
1000
1200
Samples (1 kHz Sampling Rate)
B
Left Primary Motor Cortex EEG Signal- Bimanual Common-Goal (tSCS)
Amplitude (uV)
1400
1200
1000
800
600
400
200
0
-200
-400
-600
0
200
400
600
800
1000
1200
Samples (1 kHz Sampling Rate)
C
Left Primary Motor Cortex EEG Signal (No-tSCS)- Welch Power Spectral Density Estimate
Power/Frequency (dB/Hz)
15
10
5
0
-5
-10
-15
Alpha
Beta
0
5
10
15
20
25
30
35
40
45
50
Frequency (Hz)

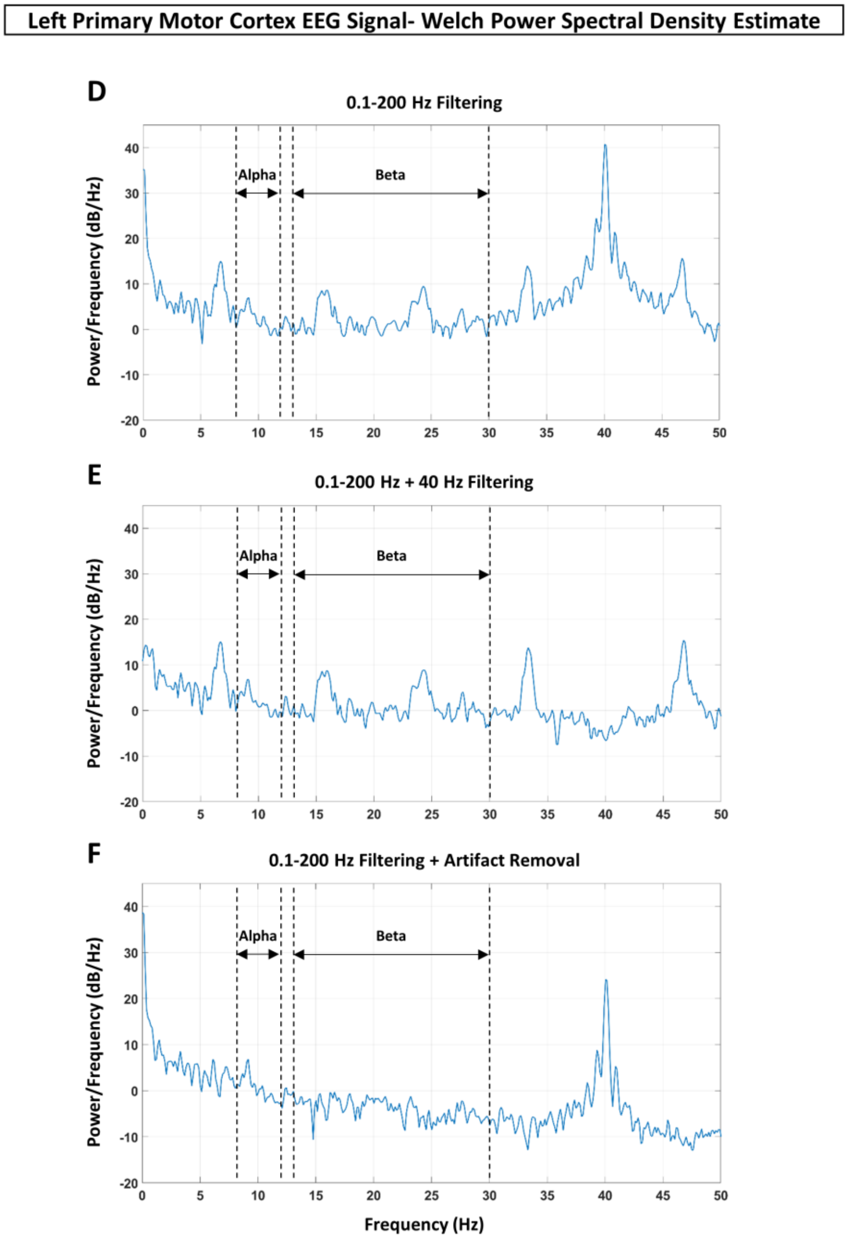
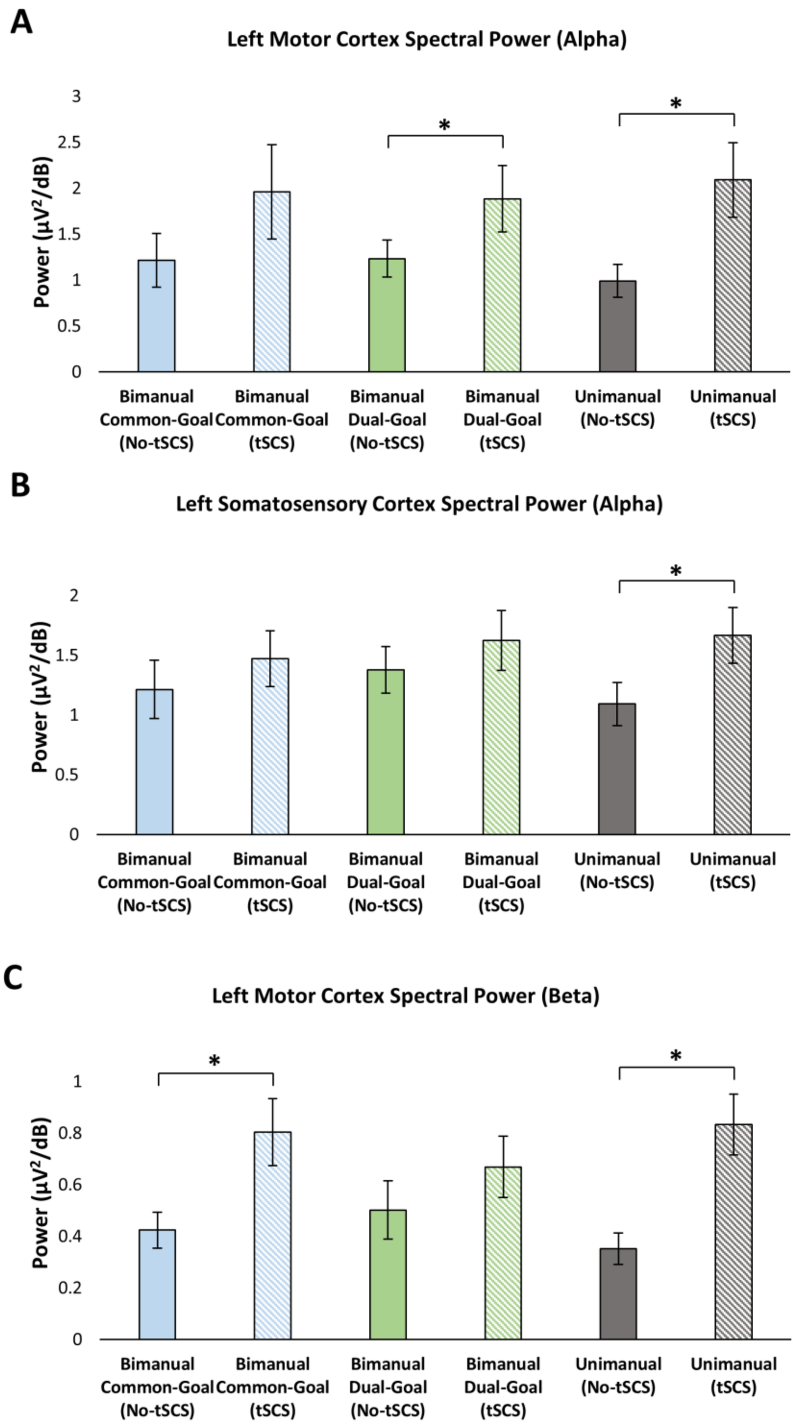


Figure 3.5. Raw EEG signal and power spectral density with different filtering approaches and artifact removal from a representative participant. Single trial EEG signal recorded from the left primary motor cortex during the execution of bimanual common-goal task (A) without tSCS, (B) in the presence of tSCS. (C) Power spectrum of the concatenated EEG signal (a single trial of it is shown in A) without tSCS. (D) Representative welch power spectral density estimate of the EEG signal recorded from left primary motor cortex with only basic band-pass filtering of 0.1-200 Hz. (E) Power spectral density of the same EEG signal with the addition of 40 Hz notch

filtering. (F) Power spectral density of the artifact-free EEG signal. A general reduction of spectral power is observed in both the alpha and beta bands when the transient high-amplitude peaks are eliminated.


A Left Motor Cortex Spectral Power (Alpha)

B Left Somatosensory Cortex Spectral Power (Alpha)

C Left Motor Cortex Spectral Power (Beta)

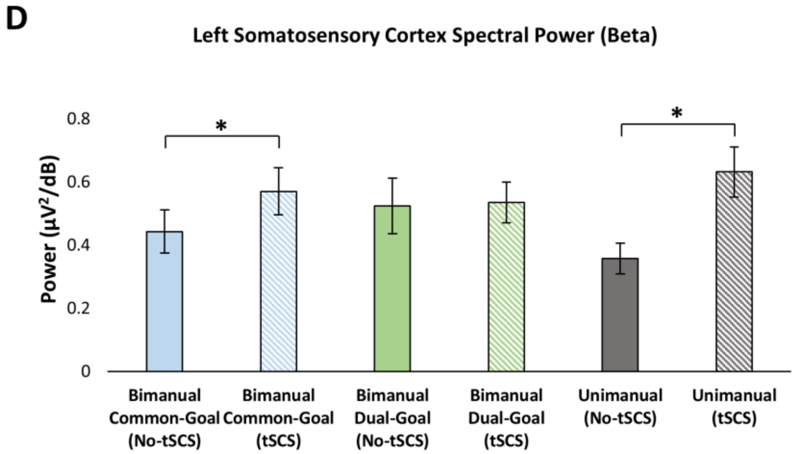
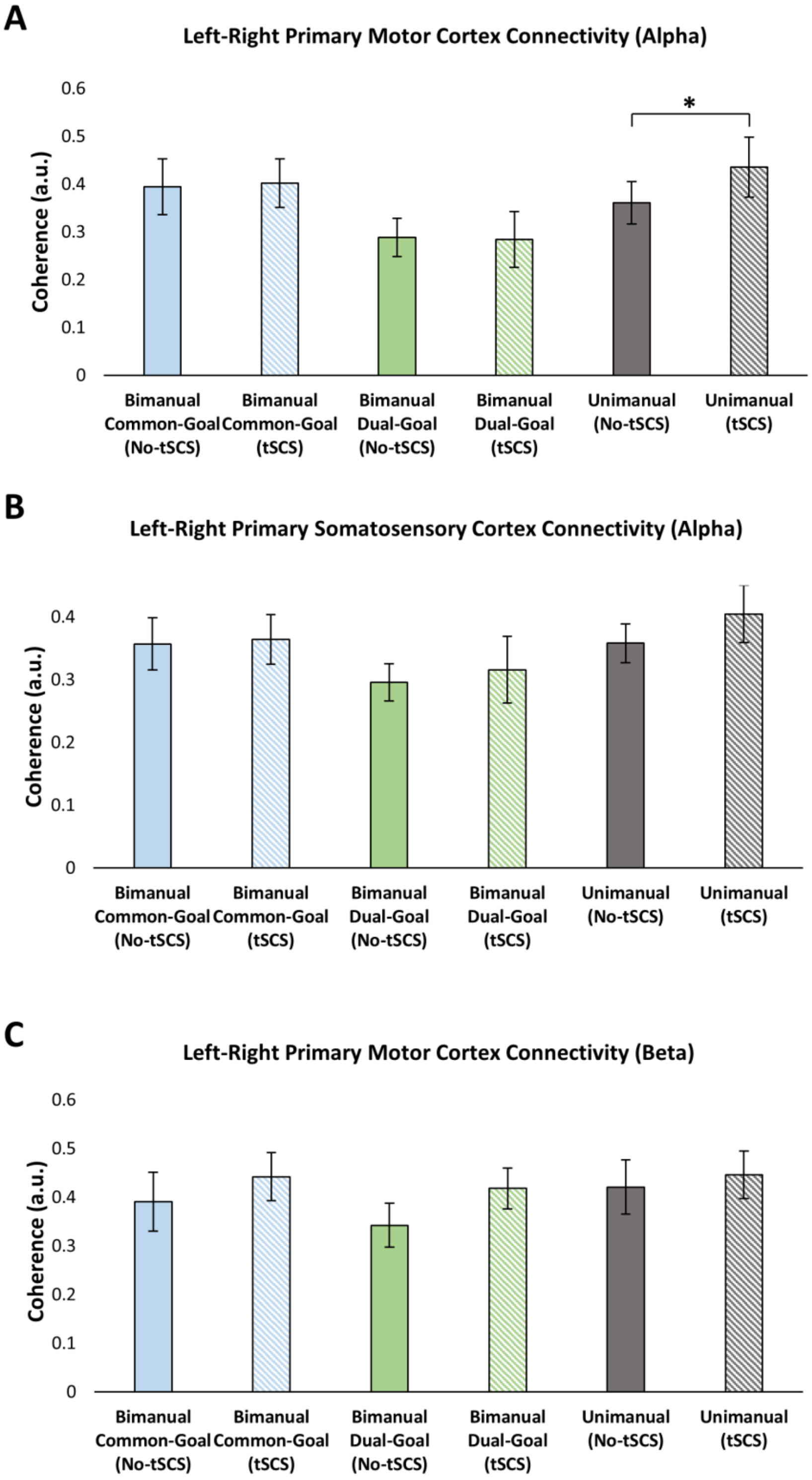


Figure 3.6. Alpha and beta band spectral power analysis. Alpha (A-B) and beta band (C-D) spectral power during the execution of the three movement tasks with and without tSCS. An augmentation in spectral power is seen in the alpha band during both unimanual and dual-goal movements in the presence of tSCS. Additionally, elevated spectral power is observed in the beta band for unimanual movement, as well as in the beta band during common-goal movement.


A
Left-Right Primary Motor Cortex Connectivity (Alpha)
Coherence (a.u.)
0.6
0.5
0.4
0.3
0.2
0.1
0
*
Bimanual Common-Goal (No-tSCS)
Bimanual Common-Goal (tSCS)
Bimanual Dual-Goal (No-tSCS)
Bimanual Dual-Goal (tSCS)
Unimanual (No-tSCS)
Unimanual (tSCS)
B
Left-Right Primary Somatosensory Cortex Connectivity (Alpha)
Coherence (a.u.)
0.4
0.3
0.2
0.1
0
Bimanual Common-Goal (No-tSCS)
Bimanual Common-Goal (tSCS)
Bimanual Dual-Goal (No-tSCS)
Bimanual Dual-Goal (tSCS)
Unimanual (No-tSCS)
Unimanual (tSCS)
C
Left-Right Primary Motor Cortex Connectivity (Beta)
Coherence (a.u.)
0.6
0.5
0.4
0.3
0.2
0.1
0
Bimanual Common-Goal (No-tSCS)
Bimanual Common-Goal (tSCS)
Bimanual Dual-Goal (No-tSCS)
Bimanual Dual-Goal (tSCS)
Unimanual (No-tSCS)
Unimanual (tSCS)

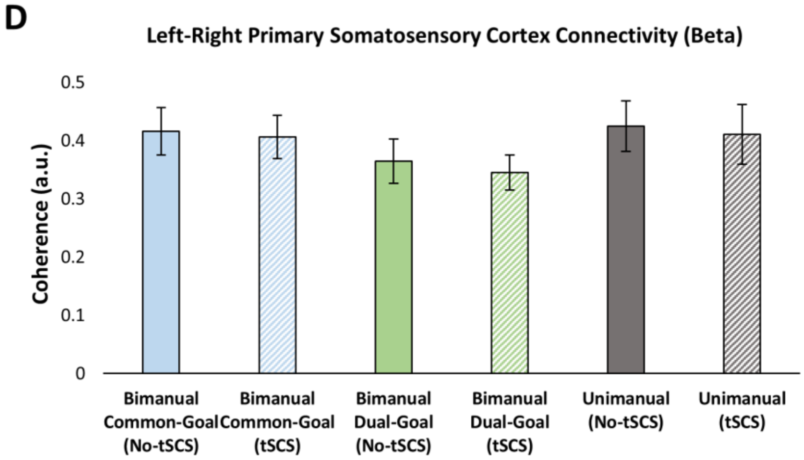


Figure 3.7. Interhemispheric connectivity with artifact removal applied between left and right **(A, C)** primary motor cortex, and **(B, D)** primary somatosensory cortex. The alterations in interhemispheric connectivity do not exhibit consistency across frequency bands and tasks. A general elevation of interhemispheric connectivity is evident in the alpha band. However, a reduction in interhemispheric coupling is observed in the beta band, specifically over the somatosensory cortex.

Chapter 4. Simultaneous cervical and lumbar spinal cord stimulation induces facilitation of both spinal and corticospinal circuitry in humans[2]

*Behdad Parhizi[1,2,3], *Trevor S. Barss[1,2,3], Vivian K. Mushahwar[1,2,3]

[1] Neuroscience and Mental Health Institute, University of Alberta, AB, Canada
[2] Division of Physical Medicine and Rehabilitation, Department of Medicine, University of Alberta, Edmonton, AB, Canada
[3] Sensory Motor Adaptive Rehabilitation Technology (SMART) Network, University of Alberta, Edmonton, AB, Canada

* Indicates shared first authorship

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[2] A version of this chapter has been published.
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4.1. Introduction

The recent surge of investigations in modulating the circuitry of the spinal cord by means of non-invasive transcutaneous spinal cord stimulation (tSCS) suggests that this approach has the potential to facilitate improved sensorimotor rehabilitation (Balykin et al., 2017; Inanici et al., 2018). Applying tSCS at either the cervical or lumbar level of the spinal cord has been shown to enhance upper and lower limb motor function and mitigate spasticity in persons with spinal cord injury (SCI) (Y. Gerasimenko et al., 2015; Ursula S. Hofstoetter et al., 2019; Inanici et al., 2018; Sayenko et al., 2018). Epidural spinal cord stimulation (eSCS) and tSCS may activate similar neural structures, and computer modeling and evoked electrophysiological responses suggest the likely involvement of primary afferent fibers of the posterior root in evoking motor outputs (Danner et al., 2011; Ursula S. Hofstoetter et al., 2018; Ladenbauer et al., 2010). eSCS improves spinal motor output and volitional movements even in cases of severely reduced supraspinal input (Carhart et al., 2004; Harkema et al., 2011; Herman et al., 2002; Mayr et al., 2016; Wagner et al., 2018). Most recently, eSCS applied to the lumbar spinal cord, in conjunction with intensive locomotor training, allowed persons with clinically complete SCI to walk over ground for short distances (Angeli et al., 2018; Gill et al., 2018). This demonstrated that dormant neurons that survived the injury may be reengaged with spinal neuromodulation and produce stepping-like movements (Angeli et al., 2014; Grégoire Courtine et al., 2009).

The coordination between the legs and arms is an inherent feature of locomotor neural networks (E. P. Zehr et al., 2016) with coupling between cervical (arms) and lumbar (legs) spinal networks (cervico-lumbar coupling) well demonstrated in both animals and humans (Juvin et al., 2005; Yamaguchi, 1986). Oscillatory movements are governed by separate locomotor centers known as central pattern generators (CPG) located in the cervical and lumbar spinal cord segments (Frigon, 2017; E. P. Zehr et al., 2016). In mammalian quadrupedal locomotion, coordinated rhythmic movements of the forelimbs and hindlimbs are mediated primarily by inter-CPG connections (Ballion, Morin, & Viala, 2001; Gordon, Dunbar, Vanneste, & Whelan, 2008; Juvin et al., 2005). In animal models, the hindlimbs can modulate neural networks associated with the forelimbs, and vice versa (Ballion et al., 2001). Similarly, to quadrupedal mammals, a bidirectional linkage between the cervical and lumbar segments of the spinal cord during rhythmic movements is present in humans (Volker Dietz, 2002; E. Paul Zehr et al., 2009), facilitated primarily by propriospinal connections (Ferris et al., 2006; Frigon et al., 2004).

Coupling between the arms and legs in humans has been demonstrated by the suppression of H-reflexes evoked in one limb by rhythmic movements of the remote limbs (Ferris et al., 2006; Hundza & Zehr, 2009; R. Zhou et al., 2018). Moreover, engaging cervico-lumbar connections with simultaneous arm and leg (A&L) cycling training has been shown to improve walking after both chronic incomplete SCI (Rui Zhou et al., 2018) and stroke (Klarner et al., 2016a; Klarner et al., 2016b) Strikingly, the addition of the arms in A&L cycling training appears to transcend gait-specific training strategies including treadmill and over-ground locomotor training by doubling the magnitude of improvements in walking parameters (Rui Zhou et al., 2018). Highlighting the importance of these interlimb connections, arms-only cycling has also been shown to improve over ground walking function after stroke (Kaupp et al., 2017). The substantially larger functional improvements experienced after A&L training relative to gait-specific training are therefore at least partially rooted in the reengagement of cervico-lumbar connections.

Corticospinal projections to spinal motor neurons are generally facilitated during cycling in neurologically intact individuals. However, this facilitation was not present during arm cycling for individuals with incomplete SCI prior to A&L cycling training (Zhou, Alvarado, Kim, Chong, & Mushahwar, 2017). Excitingly, 12 weeks of A&L cycling training reengaged these connections by significantly increasing the amplitude of the motor evoked potential (MEP) in the tibialis anterior muscle compared to baseline levels prior to the intervention (Zhou et al., 2017). Furthermore, disruptions in cervico-lumbar connectivity, which are noted after both incomplete SCI and chronic stroke, can be reduced by the simultaneous A&L cycling paradigm (Klarner et al., 2016b; R. Zhou et al., 2018). Presynaptic inhibition of Ia afferent terminals (pre-motorneuronal level) is thought to exert such an effect (Frigon et al., 2004; Gossard, 1996; Nakajima et al., 2013). Therefore, the coupling of cervical and lumbar networks, mediated by both ascending and descending propriospinal connections, is vital to interlimb coordination and the restoration of walking after neural injury (Laliberte, Goltash, Lalonde, & Bui, 2019).

Enhancing cervico-lumbar connectivity by pairing A&L cycling with tSCS may further improve mobility outcomes (Pradarelli, Samejima, Inanici, & Moritz, 2020). Recently, we showed that cervical tSCS significantly suppresses the soleus H-reflex (the remote limb) (Barss et al., 2019), similarly to the effect of arm cycling on the soleus H-reflex. Benavides and colleagues showed that after 20 minutes of tSCS, the amplitude of subcortical motor evoked potential (i.e., cervicomedullary evoked potentials or CMEP) increased, but not the amplitude of MEPs (Benavides et al., 2020). They determined that tSCS causes an increase in intracortical inhibition (measured by paired stimuli conditioning) that restricted the cortical MEPs. The results imply that the effect of tSCS varies between cortical and spinal networks, inhibiting the former and facilitating the latter. Moreover, ulnar nerve stimulation has been shown to potentiate spinally-evoked motor responses (evoked by single pulse tSCS at a level between the lower thoracic and upper lumbar) across multiple muscles of the lower limb in both neurologically intact and spinal cord injured individuals (both complete and incomplete) (D. A. Atkinson et al., 2020). This signifies that conditioning of descending interlimb projections to lumbosacral motor pools occurs at least in part by similar networks that are activated with tSCS. Moreover, paired tSCS at the L2 and S1 segments of the spinal cord resulted in potentiation of the evoked response from either site alone, indicating synergistic effects of multi-segmental pathways (Sayenko et al., 2015). Preliminary reports in abstract form have shown that combined cervical and lumbar tSCS may improve locomotor function, sensation, and bladder function in a single participant when combined with intensive physical therapy (Pradarelli et al., 2020). However, little information is known about how cervical or lumbar tSCS influences the excitability of corticospinal and spinal networks in the upper limb, and how it alters interlimb coupling. It also remains unknown if synergistic effects of multi-segmental tSCS occur between the cervical and lumbar segments of the spinal cord. This lack of information limits the translational ability of tSCS and highlights several key issues that need to be addressed prior to the appropriate implementation of tSCS into rehabilitation strategies.

The primary purpose of this study was to determine the extent to which cervical spinal and corticospinal circuitry can be modulated by either cervical or lumbar tSCS during a static task. Secondarily, this study aimed to establish whether combined cervical and lumbar tSCS further facilitates neuromodulation of spinal and corticospinal circuitry compared to either site alone. Finally, this study aimed to determine whether tSCS influences cervico-lumbar connectivity and corticospinal excitability during a rhythmic task (leg cycling). Based on previous work, we hypothesized that tonic activation of the lumbar spinal cord through tSCS would significantly inhibit the activity of cervical networks but increase the excitability of the corticospinal tract. We also hypothesized that simultaneous stimulation of cervical and lumbar networks would further enhance the effects seen with either site alone. Addressing these hypotheses will shed light on how the neural control of interlimb coordination may be most effectively facilitated by tSCS.

4.2. Methods

Experimental design

Building on our previous work (Barss et al., 2019), this project aimed to determine the effect of cervical, lumbar, or combined tSCS on spinal (H-reflex) and corticospinal (MEP) excitability during a static or cycling task. The neuromodulatory effects of tSCS were assessed in neurologically intact study participants who were seated in a recumbent leg cycling system. H-reflexes and MEPs were assessed in the flexor carpi radialis (FCR) muscle of the left arm during 2 tasks (static and leg cycling) and 4 conditions: (1) No tSCS, (2) tSCS applied to the cervical enlargement (Cervical); (3) tSCS applied to the lumbar enlargement (Lumbar); and (4) simultaneous cervical and lumbar tSCS (Combined) (Figure 4.1). Thus, separate trials assessed H-reflex and MEP excitability during eight conditions for each study participant (16 conditions total).

Experimental setup

Participants were seated in a custom-adapted leg cycling ergometer (ERGYS 2, Therapeutic Alliances, Fairborn OH) with a fixed back support and movable seat to accommodate for participants' height (Figure 4.2A). The left and right side of each leg crank were linked with 180-degree phase difference. The torso was restrained using a seat belt, and the experimental arm (left) was secured in a fixed pronated position using straps into a secure brace, embedded with a force sensor (Neurolog, Hertfordshire, UK). The left arm was chosen to be comparable to previous investigations from the same laboratory (Zhou et al., 2017; R. Zhou et al., 2018). The effect of leg cycling on upper limb reflex responses between the right and left limbs, and the relative difference between experimental conditions is expected to be similar regardless of which arm is used (Nakajima et al., 2013; E. Paul Zehr, Klimstra, Johnson, & Carroll, 2007). Participants were instructed to maintain the same position throughout the experiment and to place the non-experimental arm on the right armrest of the chair. The 180° position of the left leg (i.e., 12 o'clock) was chosen as the phase of the leg during which both H-reflexes and MEPs are evoked for both the leg static and cycling conditions. This placement of the leg was chosen based on previous studies indicating that the 180° leg position produces both peak muscle activity and the largest inhibition of the FCR H-reflex (Zhou et al., 2017; R. Zhou et al., 2018). Therefore, the positions of the left and right leg were held constant at 180° and 0°, respectively, during static trials (Figure 4.2A). During the cycling trials, participants performed counter-clockwise rhythmic leg cycling (viewed from the left) loaded with a resistance equivalent to 50% of the ergometer's maximal resistance at a constant frequency of ≈1 Hz (~60 rpm) (P. E. Zehr, 2002). Online visual feedback of cycling speed was provided on a monitor in front of them.

Participants

Fourteen (14) neurologically-intact participants completed the H-reflex (3 female, 11 male) and MEP (4 female, 10 male) assessments, with 11 completing both protocols. Because 3 individuals were excluded from MEP assessment due to possible contraindications to transcranial magnetic stimulation (TMS), 3 additional participants were recruited to complete only the MEP portion of the protocol. Participants signed an informed written consent form prior to their participation in the study. The study protocol was approved by the University of Alberta Human Research Ethics Committee, and conducted in accordance with the Declaration of Helsinki (1964). All participants were verbally instructed about the experimental procedures and completed a safety questionnaire about the use of TMS.

Transcutaneous spinal cord stimulation (tSCS)

Transcutaneous stimulation of the spinal cord was delivered by a constant current stimulator (NEOSTIM-5, Cosyma Ltd., Moscow, Russia) through two adhesive 2.5 cm round cathodic electrodes (Axelgaard Manufacturing Co., Ltd., USA) placed midline at C3-4 and C6-7, and T11 and L1 spinous processes for activating the cervical and lumbar regions of the cord, respectively (Figure 4.2B). Two 5 × 10 cm rectangular electrodes were placed bilaterally over the iliac crests as anodes (Figure 4.2B) for the cervical tSCS while two additional anode electrodes were placed laterally for the lumbar tSCS (Figure 4.2C). In total, four anodic electrodes corresponding to four cathodic electrodes were used to ensure that the cervical and lumbar channels were isolated during combined stimulation. The tSCS waveform consisted of 1 ms-long trains of 10 kHz biphasic square pulses repeated at a frequency of 30 Hz (Figure 4.3A).

To identify maximal intensity, tSCS amplitude was increased in 1 to 5mA increments to the point when the participants reported their tolerance capacity. At this intensity, participants felt a strong buzzing, fluttering, or vibration sensation at the cathodic site. However, the sensation was free from pain with little to no sensation at anodic sites. There were no evoked motor responses in the arm or leg due to tSCS in the current work. This approach to identifying the maximal tolerable intensity was used to ensure relative similarity in stimulation intensity between individuals compared to the threshold intensity used in our previous investigation (Barss et al., 2019).

The intensity of tSCS across all participants for H-reflex assessment was 50.4±10.7 mA at the cervical level and 41.3±11 mA at the lumbar level across all conditions. The tSCS intensity for MEP assessment was 51.7±10.5 mA at the cervical level and 42.2±11.3 mA at the lumbar level across all conditions. Table 4.1 provides the tSCS amplitudes for all participants at the cervical and lumbar sites. Stimulation was initiated 30 seconds to 1 minute prior to each condition, remained on during the course of each condition and was turned off immediately after the recording of H-reflexes/MEPs was completed. Recordings of H-reflexes and MEPs lasted about 2-3 minutes. Therefore, including the time prior to data collection, tSCS remained on for 3-4 minutes during each condition. The stimulation was turned off between conditions and a break of 2-3 minutes was given to reduce fatigue or summation effects of tSCS.

Hoffmann (H-) reflex

The FCR H-reflex was evoked by stimulating the median nerve near the cubital fossa using bipolar electrodes with square wave pulses (1 ms-long). The electrical stimulation was delivered using a constant current stimulator (Digitimer model DS7A, Medtel, NSW, Australia) with 5-8 seconds of inter-stimulation interval. A minimum of 3 seconds between each random stimulation is recommended for evoking H-reflexes to avoid post-activation depression (Rossi-Durand, Jones, Adams, & Bawa, 1999; P. E. Zehr, 2002). For each trial during the static condition, participants held a consistent low-level contraction of FCR muscle between 5 and 10% of their maximum voluntary force (MVF). Maximum voluntary force (MVF) was defined as the highest isometric force recorded during maximum voluntary contractions (MVCs). To acquire the MVF, each participant completed three trials of maximal voluntary wrist flexion while the force was measured with a force transducer. MVF was then used as a reference to set a target for background contraction. The equivalent of 5-10% of the measured MVF was displayed on an oscilloscope for visual feedback to maintain the same level of contraction throughout all conditions of the experiment. This was done to ensure similar descending drive to spinal motoneurons throughout all experimental trials, and the choice of target force in this range was based on each individual's comfort to track and maintain the chosen level of background contraction throughout the experiment. During cycling conditions, a position sensor tracked the left leg rotational angle. Stimulation for H-reflex assessment was delivered at the 180° position (with reference to the left leg) (Figure 4.2A) every 5-8 revolutions. A total of 10 stimuli were delivered for each experimental condition.

To evoke consistent H-reflexes, a recruitment curve was first constructed to determine both the ascending and descending limb of the H-reflex amplitude curve, including the maximal H-reflex amplitude (H_{max}). This was followed by finding the stimulation intensity that elicited a reflex that was approximately 70% of H_{max} on the ascending limb. In this range of intensity, motor responses (M-wave) co-occur with the H-reflex, which were needed as a guide for maintaining similar stimulation conditions across trials. The amplitude of maximal motor responses (M_{max}) was recorded by averaging three supra-maximal stimulation trials where the amplitude of M-wave no longer increased, indicating that all motor axons are recruited (Pierrot-Deseilligny & Mazevet, 2000). M_{max} was used to normalize H-reflex values across all trials to allow for comparison between individuals. For the remainder of the experiment, the stimulation intensity was set to maintain a consistent small, but measurable M-wave amplitude (~10% of M_{max}) across trials to minimize antidromic effects (Figure 4.3B). The amplitude of the M-wave was monitored throughout the experiments and the stimulation amplitude was adjusted when necessary to ensure consistency in the evoked M-wave. Examples of FCR H-reflex responses (10 sweeps) from an individual participant are provided in Figure 4.4A-C and Figures 4.5A,B.

Motor Evoked Potentials

To assess excitability of the corticospinal tract, TMS was applied to the contralateral motor cortex (single-pulse, monophasic) using a double cone coil to elicit motor evoked MEPs in the FCR muscle (Magstim2002, Magstim, Whitland, UK). To find the optimal coil position, stimulation was provided at multiple locations over the primary motor region of the forearm. The location that consistently produced the largest FCR MEP was then marked and maintained across all MEP trials. The same experimenter held the coil throughout the trials and care was taken to align the coil position with the marker. Each participant held a background contraction between 5 and 10% MVC, and an MEP recruitment curve was established by increasing the TMS intensity in increments of 5% maximal stimulator output, from a level where a minimal response was elicited to a level where the MEP amplitude reached its maximum and no longer increased in magnitude with increasing stimulation (MEP_{max}). At each stimulus amplitude, two repetitions of the stimulus were delivered, and peak-to-peak amplitude as well as times of onset and offset were determined. The TMS intensity that generated ~60% of MEP_{max} was chosen for comparison across experimental conditions so both facilitation and inhibition of corticospinal projections would be possible (Figure 4.3C). Examples of FCR MEP responses (10 sweeps) from an individual participant are provided in Figure 4.4D-F and Figures 4.5C,D.

A control assessment of MEP amplitude was repeated three times during the experimental protocol to ensure that cortical excitability or coil placement had not changed throughout the experimental session. These assessments occurred before the first task, between the first and second task, and after all trials were completed to verify the ~60% value MEP amplitude was maintained over time. Ten TMS pulses were delivered for each of the 8 experimental conditions.

Electromyography

Muscle activity of four muscles in the left arm was recorded via electromyography (EMG) during each trial: FCR, extensor carpi radialis (ECR), biceps brachii (BB) and triceps brachii (TB). Muscle activity was recorded from surface Ag-AgCl electrodes placed on the muscle belly and recorded at a sampling rate of 2000Hz using a CED 1401 analog to digital conversion board and Spike 2 associated software (Cambridge Electronic Design, Cambridge, UK). All EMG signals were amplified 1000x during data collection and band-pass filtered from 30 to 1000 Hz. The EMG signals were used to record H-reflexes and MEPs from the FCR muscle. EMG from the other three muscle groups was recorded to ensure that homonymous and heteronomous muscle activity remained constant and did not affect H-reflexes and MEPs of the FCR muscle.

Data analysis

The peak-to-peak amplitude of M-wave, H-reflex, and MEP, as well as baseline activity of the FCR muscle, were analyzed in a window of 400 ms (staring 100ms pre-stimulus, ending 300ms post-stimulus) using an off-line custom-written MATLAB script (Matlab, Nantick, MA, USA). A window of 100ms pre-stimulus (-100ms to 0ms relative to stimulus onset) was selected to calculate the baseline FCR and ECR EMG activity averaged over ten sweeps for each experimental condition. To obtain the value of pre-stimulus muscular contraction, the mean of the signal in this 100ms window was calculated and subtracted from the whole trace to remove any offset in the signal. The pre-stimulus background activity was then rectified and calculated as the mean activity in the 100ms window. The peak-to-peak amplitude of post-stimulus H-reflex, M-wave, and MEP were calculated by averaging ten sweeps per condition (Figure 4.3B,C). The average values were then normalized to the value of M_{max} for H-reflex measurements and to the value of MEP_{max} for MEP measurements, obtained in a separate trial immediately before the initiation of the testing conditions. The post-stimulus window of analysis for each evoked response was selected based on visual inspection.

Statistical analysis

The amplitude of FCR H-reflexes, M-waves, and MEPs along with FCR/ECR pre-stimulus baseline activity were compared across different experimental conditions using repeated-measure ANOVA (rmANOVA). During the static task, the effects of condition (No tSCS, Cervical, Lumbar, and Combined) were compared for H-reflex, MEP, M-wave and baseline muscle activity with a 1 x 4 ANOVA. To determine directly the influence each tSCS condition had relative to when tSCS was not provided, FCR H-reflex and MEP data were compared as the percent change in amplitude relative to the No tSCS condition using a 1 x 3 ANOVA (% change Lumbar vs. Cervical vs. Combined). Similarly, during the cycling task, the effects of condition (Static No-tSCS, Cycle No-tSCS, Cycle Cervical, Cycle Lumbar, and Cycle Combined) were compared for H-reflex, MEP, M-wave, baseline muscle activity, and cycling cadence with a 1 x 5 ANOVA. The percent change in H-reflex and MEP modulation from Static No-tSCS to Cycle No-tSCS is used in the literature as a measure of interlimb connectivity (R. Zhou et al., 2018). Therefore, to assess potential influences of tSCS on interlimb connectivity, FCR H-reflex and MEP data were compared as the percent change relative to no-tSCS using a 1 x 4 ANOVA (% change Cycle No-tSCS vs. Cycle Cervical vs. Cycle Lumbar vs. Cycle Combined). Significant effects were followed by pairwise comparisons corrected by Tukey's HSD adjustment for multiple comparisons. Differences with $p \leq 0.05$ were accepted as statistically significant. Descriptive statistics are shown as mean ± standard error, unless otherwise stated. All statistical analyses were performed with SPSS Statistics 20 (IBM, Chicago, IL).

4.3. Results

A two-way ANOVA showed a significant main effect for tSCS intensity across all conditions ($F_{(3,52)}=3.428$, $p=0.023$). Post-hoc analysis indicated a significant difference in tSCS intensity between the two sites ($p=0.002$), but no significant difference between intensities used during MEP and H-reflex assessment ($p>0.05$). During the leg cycling task, participants aimed to maintain a 1Hz (60 rpm) cadence. Across all trials, the average actual cycling cadence for participants during the H-reflex assessment was 59.25±1.54 (mean ± SD) and 59.42±1.04 during MEP assessment, and the cadence was not different across conditions ($p>0.05$).

Baseline muscle activity and evoked motor responses (M-wave) across conditions

The same level of background EMG was maintained across all conditions throughout the experiment. Moreover, baseline FCR and ECR muscle activity (normalized to M_{max} and MEP_{max}) was maintained across tasks as there were no significant differences across all static and cycling conditions during both H-reflex and MEP assessments (Figure 4.6A,B and Figure 4.8A,B). During the static task a 1 x 4 ANOVA indicated no difference in FCR [$F_{(3,42)}=0.35$, $p=0.786$] or ECR [$F_{(3,42)}=0.625$, $p=0.603$] baseline muscle activity between H-reflex conditions (Figure 4.6A). Across all static MEP assessment conditions, there was no difference in baseline FCR [$F_{(3,42)}=0.807$, $p=0.497$] or ECR [$F_{(3,42)}=0.589$, $p=0.626$] muscle activity (Figure 4.8A). During cycling trials, a 1 x 5 ANOVA revealed no difference in pre-stimulus FCR [$F_{(4,52)}=0.508$,

$p=0.730$] or ECR [$F_{(4,52)}=0.833$, $p=0.510$] muscle activity during H-reflex assessment (Figure 4.6B). There was also no difference in FCR [$F_{(4,52)}=0.124$, $p=0.973$] or ECR [$F_{(4,52)}=0.321$, $p=0.862$] muscle activity during MEP cycling assessment (Figure 4.8B).

During H-reflex assessment, there was no difference in FCR M-wave amplitude between conditions (static: [$F_{(3,42)}=0.112$, $P=0.953$]) (Figure 4.6C). Also, no significant difference was found in M-wave amplitude during H-reflex cycling assessment across all conditions [$F_{(4,52)}=0.640$, $p=0.637$] (Figure 4.6D). Thus, the descending input to the motor pool and effects of reciprocal inhibition from the antagonist muscle group were similar across all tasks and conditions. Moreover, a similar direct motor response during FCR H-reflex assessments was maintained irrespective of task and condition.

Effect of tSCS on H-reflex excitability

During the static tasks, a 1 x 4 rmANOVA indicated a significant main effect of condition on H-reflex amplitude [$F_{(3,42)}=6.79$, $p<0.001$]. Post-hoc pairwise comparisons revealed a significant facilitation of FCR H-reflex during Combined tSCS (29.8% M_{max}; $p<0.001$; d=0.34) compared to No-tSCS (24.2% Mmax) (Figure 4.7A). H-reflex amplitude during Combined tSCS was also significantly greater than Cervical (26.2% M_{max}; $p=0.041$) but not different than Lumbar tSCS ($p>0.05$) (Figure 4.7A). Lumbar tSCS approached statistical significance (29.1 % M_{max}; $p=0.0596$; d=0.22) compared to No-tSCS (24.8% M_{max}). Cervical and lumbar tSCS was not significantly different from No-tSCS ($p>0.05$). A 1 x 3 rmANOVA indicated a significant main effect of condition on % change in H-reflex amplitude [$F_{(2,28)}=7.09$, $p=0.004$]. The percent increase in H-reflex amplitude relative to No-tSCS was significantly larger for Combined tSCS (19.6 % increase) than for Cervical (6.9 % increase; $p=0.003$; d=0.89) and approached significance compared to Lumbar tSCS (11.1% increase; $p=0.053$; d=0.59) (Figure 4.7C).

During the cycling task, a 1 x 5 rmANOVA indicated a significant main effect of condition on H-reflex amplitude [$F_{(4,52)}=3.80$, $p=0.009$]. All cycling conditions significantly suppressed the amplitude of the H-reflex compared to the legs static No-tSCS condition, including cycling with No-tSCS (13.6% decrease; $p=0.024$; d=0.32), cycling with Lumbar (13.6% decrease; $p=0.035$; d=030.), cycling with Cervical (14.3% decrease; $p=0.015$; d=0.33), and cycling with Combined

(11.8% decrease, $p=0.042$; d=0.30) tSCS (Figure 4.7B). However, there was no difference in the percent reduction in H-reflex amplitude between any of the cycling conditions relative to the legs static No-tSCS condition ($p>0.05$) (Figure 4.7D) indicating that tSCS likely did not influence interlimb connectivity.

Effect of tSCS on MEP excitability

Average baseline EMG activity in FCR remained constant across all leg static (Figure 4.8A) and leg cycling (Figure 4.8B) conditions. As well, static No-tSCS MEP amplitude did not change from the beginning to the middle to the end of the experiment. Together this demonstrates that the background corticospinal drive was consistent throughout the experiment and corticospinal excitability was similar when assessed at multiple timepoints under the same conditions. During the legs static task, there was a significant main effect of condition on MEP amplitude revealed by a 1 x 4 ANOVA [$F_{(3,42)}=3.28$, $p=0.031$]. Post-hoc pairwise comparisons showed that Combined tSCS significantly facilitated MEP amplitude relative to Lumbar ($p=0.047$; d=0.66) and No-tSCS ($p=0.047$; d=0.76) (Figure 4.8C). Lumbar and Cervical tSCS alone did not significantly alter the amplitude of the MEP relative to No-tSCS ($p>0.05$) and Combined tSCS was not significantly different than Cervical tSCS ($p>0.05$). There was a significant main effect of the percent increase in MEP amplitude from No-tSCS [$F_{(2,26)}=3.39$, $p=0.049$] (Figure 4.8E). Combined tSCS (19.8% increase) facilitated an increase in MEP amplitude that approached significance compared to Lumbar tSCS (1.8% increase $p=0.056$; d=0.68). Combined was not different than Cervical tSCS (4.9% increase; $p>0.05$; d=0.63) and there was no difference between Lumbar and Cervical tSCS ($p>0.05$).

Figures 4.8D,F summarize the changes in corticospinal excitability while the legs were cycling. All cycling conditions increased the amplitude of MEPs relative to the static, No-tSCS condition; however, there were no significant differences in MEP amplitude between conditions [$F_{(4,52)}=3.80$, 1.579, $p=0.194$] (Figure 4.8D). Relative to the legs static, No-tSCS condition, corticospinal excitability was facilitated during cycling without tSCS (18.6% increase), as well as during cycling with Lumbar (11.6% increase), Cervical (10.0% increase), and Combined tSCS (20.6% increase) (Figure 4.8F).

4.4. Discussion

Overview

Although tSCS provides functional improvements in the upper and lower limbs in people with a SCI, there is a continued lack of knowledge regarding the neuromodulation in sensorimotor circuitry that occurs with its use. The present results demonstrate that tSCS can alter spinal reflex and corticospinal excitability in neurologically intact individuals, observed as changes in the amplitude of the FCR H-reflex and MEP. In our previous work, cervical tSCS significantly suppressed the activity of lumbar networks in a manner similar to the effect produced by rhythmic arm cycling. Thus, we hypothesized that lumbar tSCS would suppress the FCR H-reflex as well, signifying bidirectional tSCS effects on the cervico-lumbar networks. We also expected that with simultaneous cervical and lumbar tSCS, these neuromodulatory effects on the H-reflexes may be cancelled out. Moreover, based on the findings in Benavides et al (Benavides et al., 2020), we expected that cervical tSCS would suppress the corticospinal drive to the FCR muscle, and that combined cervical and lumbar tSCS would produce an even larger suppression.

Contrary to our hypothesis, during the legs static task, lumbar tSCS facilitated the FCR H-reflex amplitude by 11.1% (relative to No tSCS), while cervical tSCS altered the FCR H-reflex amplitude by 6.8%. Interestingly, combined cervical and lumbar tSCS significantly enhanced the facilitation of the FCR H-reflex (by 19.6%) compared to either site alone. Moreover, while neither cervical nor lumbar tSCS altered MEP amplitude alone (+4.9% and 1.8% relative to static tSCS), combined tSCS significantly increased MEP amplitude by 19.7% compared to No tSCS.

Leg cycling alone significantly suppressed the FCR H-reflex relative to legs static, No-tSCS (by 13.6%) while facilitating MEP amplitude by 18.6%. tSCS was unable to further alter H-reflex or MEP excitability in any condition. This indicates that in neurologically intact individuals where interlimb coordination and corticospinal tract are intact, the effect of leg cycling on cervico-lumbar coupling and corticospinal drive was unable to be impacted significantly with the intensity of tSCS used. This study demonstrates, for the first time, that tonic activation of spinal cord networks through multiple sites of tSCS provides a facilitation of both spinal reflex and corticospinal pathways.

During all trials, participants maintained a consistent baseline muscle contraction in the FCR muscle across all tasks and conditions (Figure 4.6A-B and Figure 4.8A-B) to ensure that changes in voluntary contraction did not influence the amplitude of H-reflex (Matthews, 1986). Moreover, the amplitude of the direct motor response (M-wave), which is a neural signature of the amount of recruited efferent axons (Brooke et al., 1997; V. Dietz, Faist, & Pierrot-Deseilligny, 1990), was carefully maintained across tasks and conditions for during H-reflex assessments (Figure 4.6C-D). Disynaptic reciprocal inhibition can also influence the amplitude of H-reflex (Morita, Crone, Christenhuis, Petersen, & Nielsen, 2001; Petersen, Morita, & Nielsen, 1999; P. E. Zehr, 2002), making it important that there were no significant differences in ECR baseline muscle activity occurred across conditions or tasks in the current investigation Furthermore, background FCR muscle activity remained unchanged across all MEP measurements (Figure 4.8), which shows that the motorneuron excitability was held constant. Thus, it is unlikely that the level of descending drive, the number of recruited axons, or reciprocal inhibition underlie the modulation seen in this study in H-reflexes and MEPs in the forearm with tSCS.

tSCS alters excitability of remote segments of the spinal cord

Results from the current investigation highlight that tSCS can alter excitability across multiple segments of the spinal cord. Importantly, multi-site (i.e., Combined) tSCS led to a 19.6% increase in H-reflex amplitude, while Lumbar tSCS increased the amplitude of FCR H-reflex relative to No-tSCS by 11.1% with the legs static (Figure 4.7C).

It may be possible that non-invasive spinal stimulation activates the spinal motor pools by increasing sensory inputs through Ia afferents (Sayenko et al., 2018). Our previous investigation determined that tonic activation of the cervical region through tSCS suppresses the amplitude of the soleus H-reflex (Barss et al., 2019) to a similar extent as that produced by rhythmic arm cycling (R. Zhou et al., 2018), indicating that tSCS may also engage propriospinal interneuronal connections exerting effects on multiple segments of the spinal cord. Therefore, it was tempting to assume this suppressive effect would be bidirectional: tonic activation of the lumbar networks of the spinal cord by tSCS would reduce the amplitude of the H-reflex in FCR similarly to the suppressive effect rhythmic leg cycling has on the FCR H-reflex. While the present findings indicate that tSCS alters excitability across multiple segments of the spinal cord, the resulting facilitation in H-reflex amplitude with lumbar tSCS and suppression with leg cycling suggest that separate networks are responsible for the effects. The current investigation is unable to identify specific pathways or sites responsible for the disinhibition of the FCR H-reflex. However, facilitation of the H-reflex pathway through tSCS may be due to reduced Ia presynaptic inhibition or facilitation of the motor pool through activation of posterior root afferents and interneuronal projections (Ursula S. Hofstoetter et al., 2018). With the current methodology, it also remains possible that the stimulation of skin itself may alter cutaneous afferent transmission, altering the excitability of the spinal cord. The potential role of the skin with tSCS remains an important avenue to explore in future work (Beekhuizen & Field-Fote, 2005).

tSCS does not alter H-reflex amplitude during rhythmic leg cycling

It is well established that rhythmic movements of the arm or leg modulate spinal reflex excitability of the remote limb (Palomino, Hundza, & Zehr, 2011), and these reciprocal neural connections are damaged after SCI (R. Zhou et al., 2018) and stroke (Klarner et al., 2016b). However, spared neural connections are viable to be retrained by a rhythmic A&L cycling intervention to restore intersegmental linkages (Klarner et al., 2016b). Previously, our group assessed the benefits of actively engaging the arms rhythmically with the legs in a rehabilitation paradigm. Twelve weeks of simultaneous A&L cycling improved cervico-lumbar coupling, exemplified by the restoration of a significant reduction in the soleus H-reflex amplitude during dynamic arm cycling in study volunteers with incomplete SCI (R. Zhou et al., 2018).

Evidence suggests that modulation of H-reflex amplitude in the FCR muscle during rhythmic leg cycling (Nakajima et al., 2013) and in the soleus muscle during rhythmic arm cycling (Frigon et al., 2004) likely arise from elevated presynaptic inhibition of Ia afferent terminals. Thus, locomotor circuits of the cervical and lumbar spinal cord responsible for generating rhythmic movements act on presynaptic interneurons, which at least in part, reduces the transmission from Ia afferents onto spinal motoneurons. Interlimb coupling is composed of long descending and ascending propriospinal interneurons (Volker Dietz, 2002; Frigon, 2017) mediating coordination of the locomotor control centers of the upper and lower extremities, and play a role in gating the excitability of reflex pathways (Volker Dietz, 2002; Huang & Ferris, 2009). The lack of soleus H-reflex suppression during arm cycling after stroke and incomplete SCI is attributed to disruption or abolition of propriospinal networks (Barzi & Zehr, 2008; R. Zhou et al., 2018). While A&L cycling training has been shown to reengage these connections, it remains unknown whether tSCS can actively engage previously inaccessible networks to potentially incite further Hebbian plasticity and improve outcomes.

As expected, we found that leg cycling significantly suppresses the amplitude of the H-reflex relative to the leg static, No tSCS condition (Figure 4.7B), a finding verified by various investigations (Nakajima et al., 2013; E. P. Zehr et al., 2016; R. Zhou et al., 2018). Strengthened presynaptic inhibition projecting to Ia cervical afferent terminals is likely the primary contributor to this suppression, although reciprocal and recurrent inhibition may also contribute to the effect (Nielsen, Petersen, & Crone, 1995; Petersen et al., 1999; Shefner, Berman, Sarkarati, & Young, 1992). Here, cycling trials with tSCS (Cervical, Lumbar, and Combined) did not alter excitability beyond what was produced by cycling alone (Figure 4.7B). The percent decline in H-reflex amplitude relative to the legs static, No-tSCS condition was similar among all cycling trials, suggesting the suppressive effect of cycling in a neurologically intact population may be too strong; thus, overriding the impact caused by tonic activation of the spinal cord for all cycling conditions with tSCS. It remains vital for future investigations to determine if tSCS can influence interlimb coupling after neural injury when cervico-lumbar connectivity is impaired.

Combined tSCS provides a non-linear facilitation of MEP amplitude

A crucial finding of this project was the effect of tSCS on the excitability of the corticospinal tract as tested by MEPs produced with TMS. Simultaneous tSCS at the lumbar and cervical sites (Combined) significantly increased corticospinal transmission to the FCR muscle compared to static No-tSCS and Lumbar tSCS conditions (Figure 4.8C). This provides novel evidence that multiple sites of tSCS converge to facilitate corticospinal transmission (19.7%) to a greater extent than lumbar and cervical tSCS alone (6.7%). This increase in the amplitude of MEPs could be due to reinforced projection of corticospinal axons onto spinal motoneurons (J. Rothwell et al., 1994). Therefore, proprioceptive inputs generated by tSCS delivered to spinal motor neurons may be the main contributor to the facilitation of MEPs to the FCR muscle. Recently, a study determined that single site tSCS applied with a 5 kHz carrier frequency at the C5-C6 level facilitated the amplitude of CMEPs, but did not increase the amplitude of the MEPs (Benavides et al., 2020). This was accompanied by an increase in the level of short-interval cortical inhibition (SICI). While this suggests that alterations in spinal circuitry are likely the target of tSCS for facilitating corticospinal excitability, it is important to note that those results occurred after 20 minutes of tSCS and were assessed over a long duration compared to the results of the current investigation.

tSCS does not alter corticospinal excitability during rhythmic leg cycling

Across all cycling trials, with or without tSCS, there was a general facilitation of MEPs relative to the static condition, but with no significant differences between conditions. Both cortical and spinal mechanisms are involved in modulating corticospinal excitability during rhythmic movements of the legs (Zhou et al., 2017). Propriospinal neurons that link the cervical and lumbar locomotor networks, transmit locomotor commands from supraspinal locomotor regions (Cowley, Zaporozhets, & Schmidt, 2008) (Laliberte et al., 2019); thus, corticospinal commands propagating along these propriospinal connections can possibly be modulated, and may have partially played a role in the facilitation of FCR MEPs. Additionally, owing to the overlap of the representations of the arm and leg muscle representations in the pre-motor and supplementary motor areas of the human cortex that project to primary motor cortex, modulation of forearm corticospinal excitability occurs during cyclical ankle movements enhancing hand-foot coordination (Byblow et al., 2007). Furthermore, voluntary rhythmic activity of the foot causes fluctuations in the activity of cortical regions projecting to the forearm muscles along with full activation of the foot-associated cortical area (Baldissera, Borroni, Cavallari, & Cerri, 2002). Hence, intracortical connections and changes in intracortical excitability may contribute to the increased FCR MEP amplitude during leg cycling.

Mechanisms involved in transcutaneous spinal cord stimulation

Neuromodulation of spinal circuitry through the use of tSCS improved functions such as increased pinch and hand grip force, strength and dexterity of the upper extremity, stepping, standing, posture, mitigation of spasticity below the level of injury, and regulation of blood pressure (Balykin et al., 2017; P. Gad et al., 2018; Y. Gerasimenko et al., 2015; Gerasimenko et al., 2014; Inanici et al., 2018; Sayenko et al., 2018). While tSCS can facilitate motor retraining, the neural mechanisms and pathways responsible for the effect have yet to be comprehensively identified. The principal mechanism by which tSCS non-invasively activates inaccessible neuronal networks of the spinal cord likely includes recruitment of afferent fibers (large-to-medium) of the posterior root (Sayenko et al., 2018). It has also been suggested that tSCS may share similar physiological principles to eSCS, although tSCS targets a broader network within the spinal cord (P. Gad et al., 2018). In a comparative study, EMG characteristics of evoked responses elicited by tSCS and eSCS in multiple leg muscles including rectus femoris, biceps femoris, and tibialis anterior were analyzed. Both tSCS and eSCS produced reflex-based responses as manifested in post-activation depression of responses. This shared nature was ascribed to similarities in latency, peak-to-peak amplitudes, and waveform of the evoked EMG responses (Ursula S. Hofstoetter et al., 2018). In addition, computer simulation of the posterior root fibers demonstrated that tSCS initiated action potentials in those fibers at their entry into the spinal cord or at their exit from the spinal canal, replicating the effect of eSCS (Ladenbauer et al., 2010). Although the activation of posterior root afferents has been identified as a contributing mechanism of tSCS, other pathways are likely involved such as activation of interneuronal circuits via synaptic projections (K. Minassian et al., 2007) or enhances in the efficacy of cortico-motoneuronal synapses (Inanici et al., 2018).

An important feature of the present study is the implementation of 10 kHz burst carrier frequency. Previous investigations have highlighted that for distal muscles, the occurrence probability of antidromic collisions is very high for afferent firing rates of 30 impulses per second (Imp/s) at 30Hz tSCS (Formento et al., 2018). This could lead to a lack of proprioceptive information being available during locomotor training if incorporated and could limit the clinical relevance. However, including burst stimulation is thought to mitigate the cancellation of proprioceptive information enabling greater control of motoneuron activity (Schu et al., 2014) and has become an important component in many applications of tSCS (Benavides et al., 2020; Inanici et al., 2018; Pradarelli et al., 2020). Our observations help to elucidate contributing mechanisms involved in the use of tSCS which may facilitate its targeted use to reengage previously inaccessible circuitry to improve motor function after neurological injury.

4.5. Limitations and future directions

Prior to the widespread clinical use of tSCS, vital steps remain to be addressed including a detailed understanding of the circuitry being recruited and its influence on excitability both the neurologically intact and impaired nervous system (e.g., SCI, stroke, MS, etc.). It remains critical to evaluate whether tSCS can alter cervico-lumbar connectivity during cycling in clinical populations in which these connections are impaired. Importantly, our stimulation intensity in a neurologically intact population is much less than generally used in a clinical setting. Our average stimulation intensity for cervical and lumbar tSCS were 51 mA and 43 mA, whereas recent investigations after SCI have used stimulation intensities in the range of 80-120 mA for the same sites of stimulation. Thus, it will be vital for future experiments to determine if higher amplitudes of stimulation delivered to a spinal cord with impaired cervico-lumbar connectivity provides larger H-reflex and corticospinal effects compared to those in the current investigation. Moreover, the current investigation chose tSCS intensities based on the participants' subjective maximal tolerable intensity without pain. It is common practice for many other groups to base their tSCS intensity on evoked motor thresholds which makes comparison between studies possible. Nonetheless, while the lack of evoked potentials limits direct comparison to other studies, the comparison between conditions in the current study remains valid. Combining tSCS with A&L cycling for people with SCI may facilitate improved motor outcomes such as walking, standing and balance. Further exploration into how best to incorporate tSCS into rehabilitation, while carefully considering other neuromodulation techniques including invasive stimulation, implantable technologies, exoskeletons, and assistive devices (bionic gloves, functional electrical stimulation, walkers, etc.) will be necessary considerations as the field continues to move forward at an increasingly rapid pace.

4.6. Figures

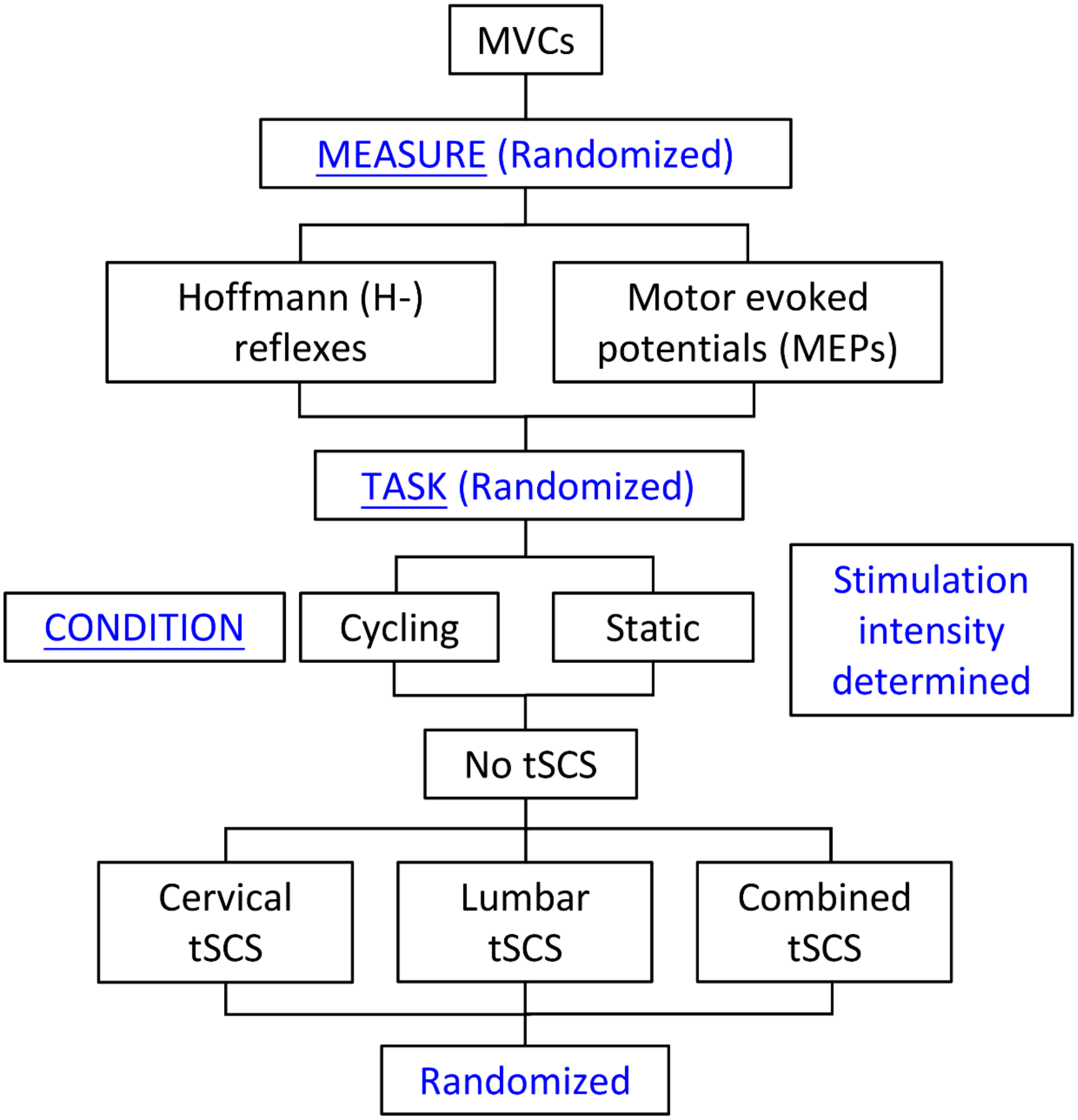

Figure 4.1. Study Design. Maximal voluntary contractions were collected and amplitudes of cervical and lumbar tSCS were determined at the beginning of each experiment. This was followed by random selection of the Hoffmann (H-) reflex or motor evoked potential (MEP)

measures. The cycling or static tasks were then randomly selected. For each of these tasks, the No-tSCS condition was tested first, followed by random selection of the remaining conditions (Cervical tSCS, Lumbar tSCS, and Combined tSCS). The No tSCS condition was completed first in order to obtain the stimulation amplitudes to the median nerve and TMS needed to evoke the H-reflex and MEP, respectively, to be used across all conditions.

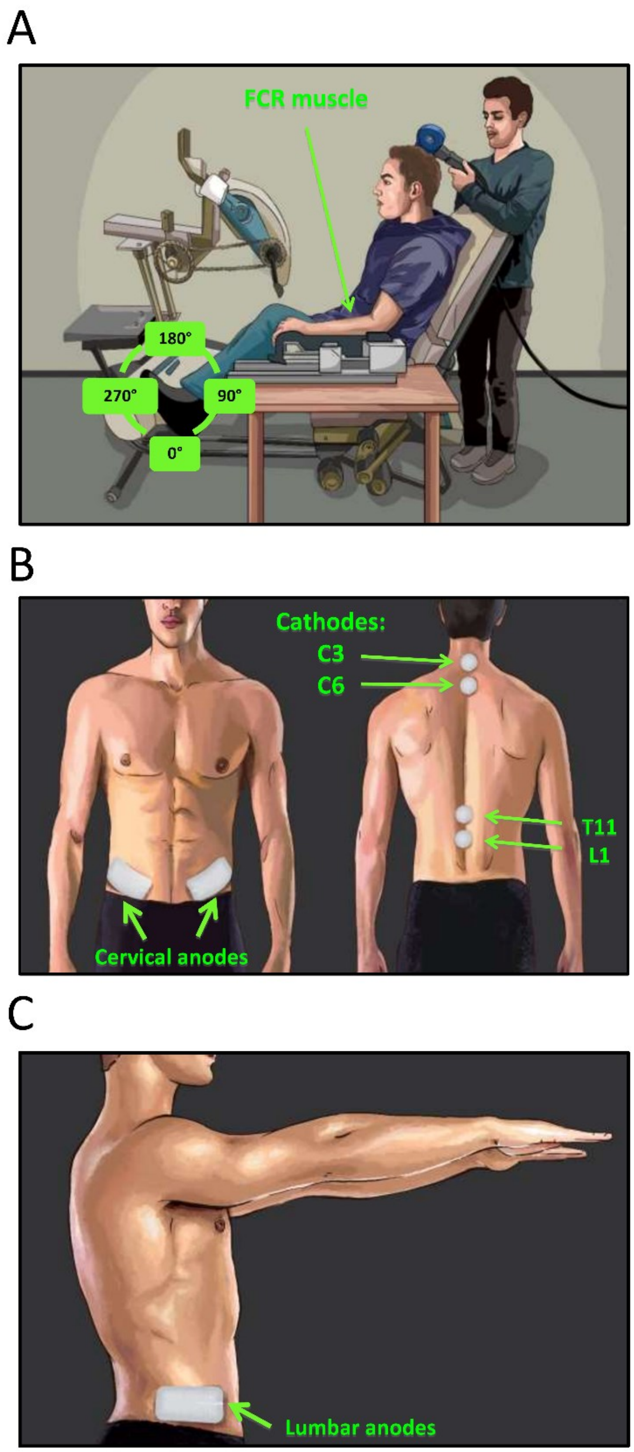

Figure 4.2. Experimental Setup and tSCS electrode placement. A: H-reflexes were evoked via stimulation of the median nerve and recorded in the FCR muscle during a consistent baseline contraction of 5-10% maximum voluntary force (MVF). MEPs were evoked via transcranial

magnetic stimulation of the motor cortex and recorded in the FCR muscle during consistent baseline contraction of 5-10% MVF. B: tSCS was delivered via two 2.5 cm round cathodic electrodes placed midline at C3-4 and C6-7 (cervical) or T11 and L1 (lumbar) spinous processes. Two rectangular anodic electrodes were placed bilaterally over the iliac crests, C: in addition, two extra anode electrodes were place laterally beside the first two to accommodate simultaneous cervical and lumbar tSCS condition.

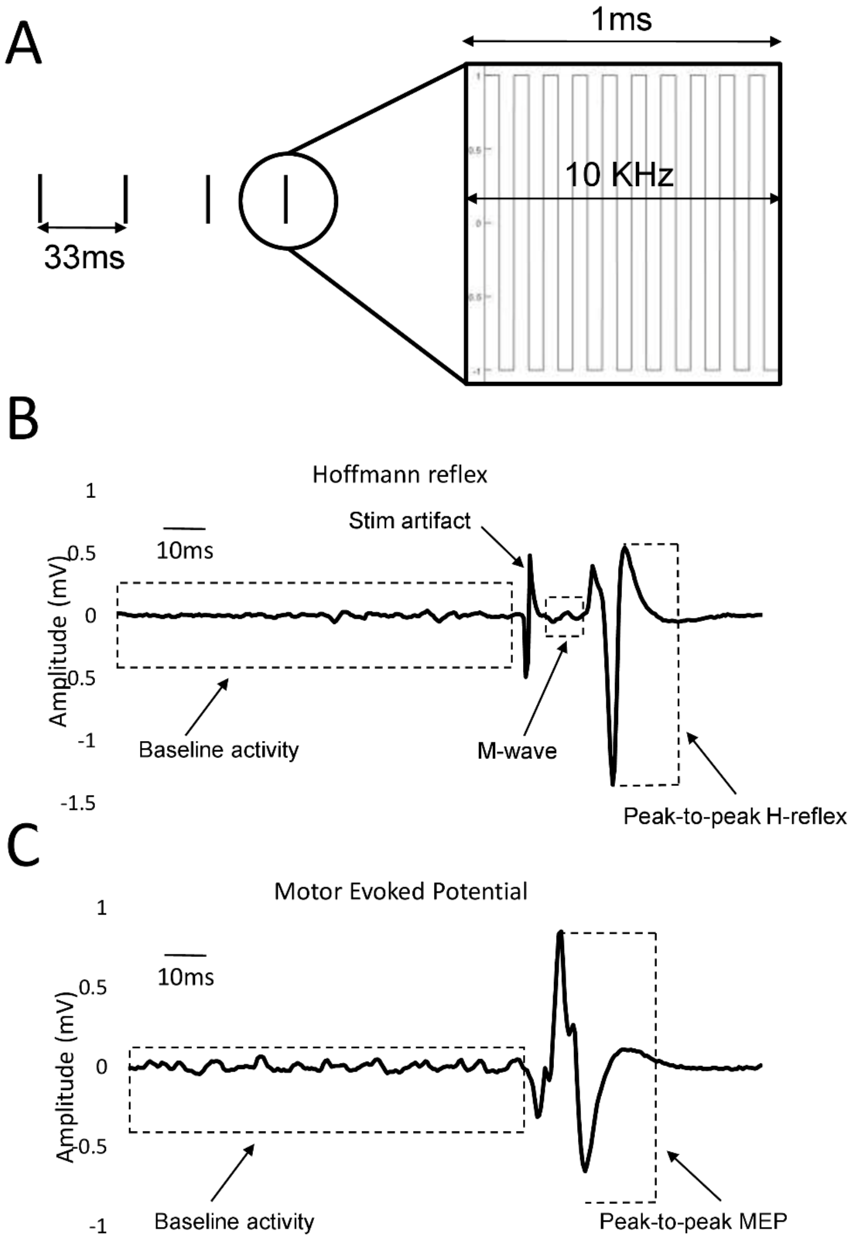

Figure 4.3. Stimulation parameters and waveforms. A: Stimulation pattern: Envelops of 1ms-long burst of 10 kHz square-wave biphasic pulses carried at the rate of 30 Hz. B: Representative example of individual evoked FCR H-reflex trace that also encompasses M-wave, stimulation artifact and baseline activity. Different components are measured in a 400-ms window (starting at 100 ms pre-stimulus to 300 ms post-stimulus). Peak-to-peak H-reflex amplitude used in data

analysis is shown with dashed lines. C: Single example of recorded FCR MEP trace with pre-stimulus baseline activity. Peak-to-peak MEP amplitude used in data analysis is shown with dashed lines.

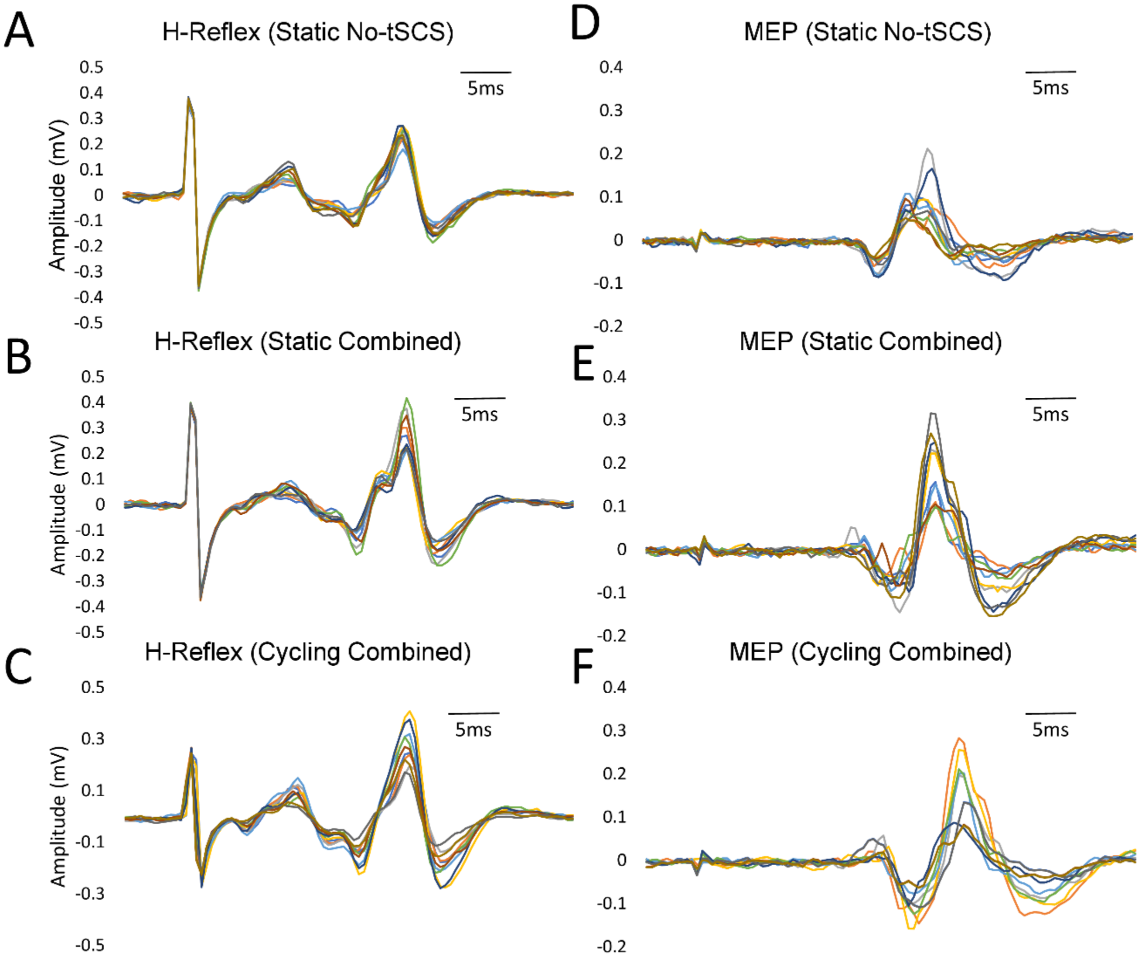

Figure 4.4. Variability within individual participants. Examples of FCR H-reflex and MEP responses (10 sweeps) from an individual participant within a condition are provided. (A) Static H-reflexes evoked with No-tSCS. (B) Static MEPs evoked with No-tSCS. (C) Static H-reflexes evoked with Combined tSCS. (D) Static MEPs evoked with Combined tSCS. (E) Cycling H-reflexes evoked with Combined tSCS. (F) Cycling MEPs evoked with Combined tSCS.

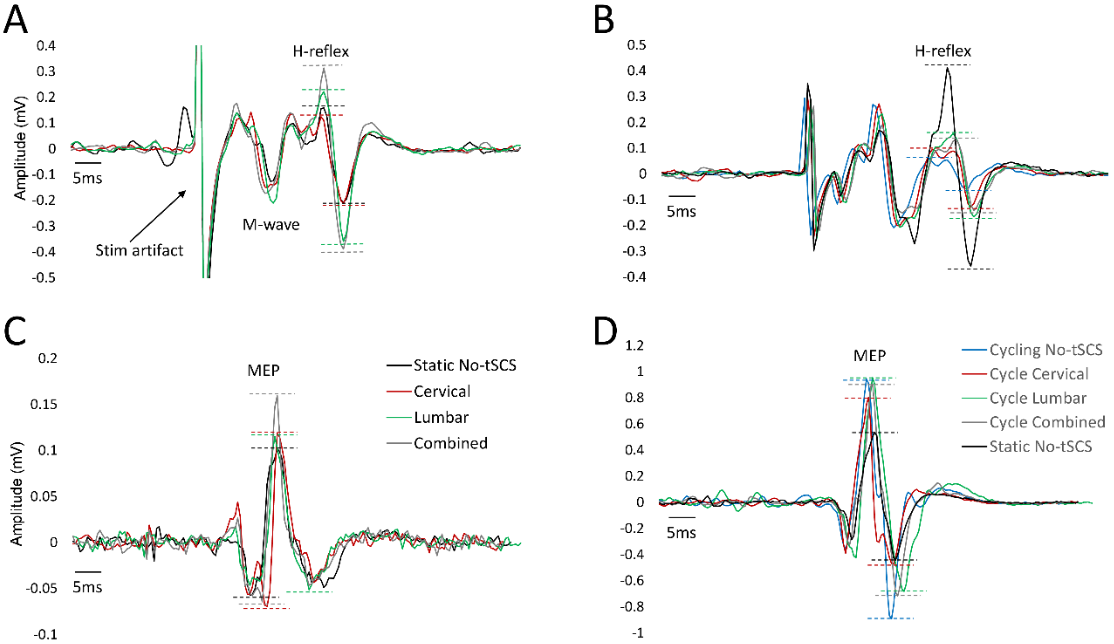

Figure 4.5. Typical FCR H-reflex and MEP traces across conditions. An example of FCR H-reflex and MEP responses from one participant across all conditions. (A) Changes in H-reflex amplitude with Cervical, Lumbar and Combined tSCS compared to No-tSCS while the legs were held static. (B) Suppression of H-reflex amplitude across all cycling conditions compared to legs static, No-tSCS. (C) Changes in MEP amplitude with Cervical, Lumbar and Combined tSCS compared to No-tSCS while the legs were held static. (D) Increase in MEP amplitude across all cycling conditions compared to legs static, No-tSCS.

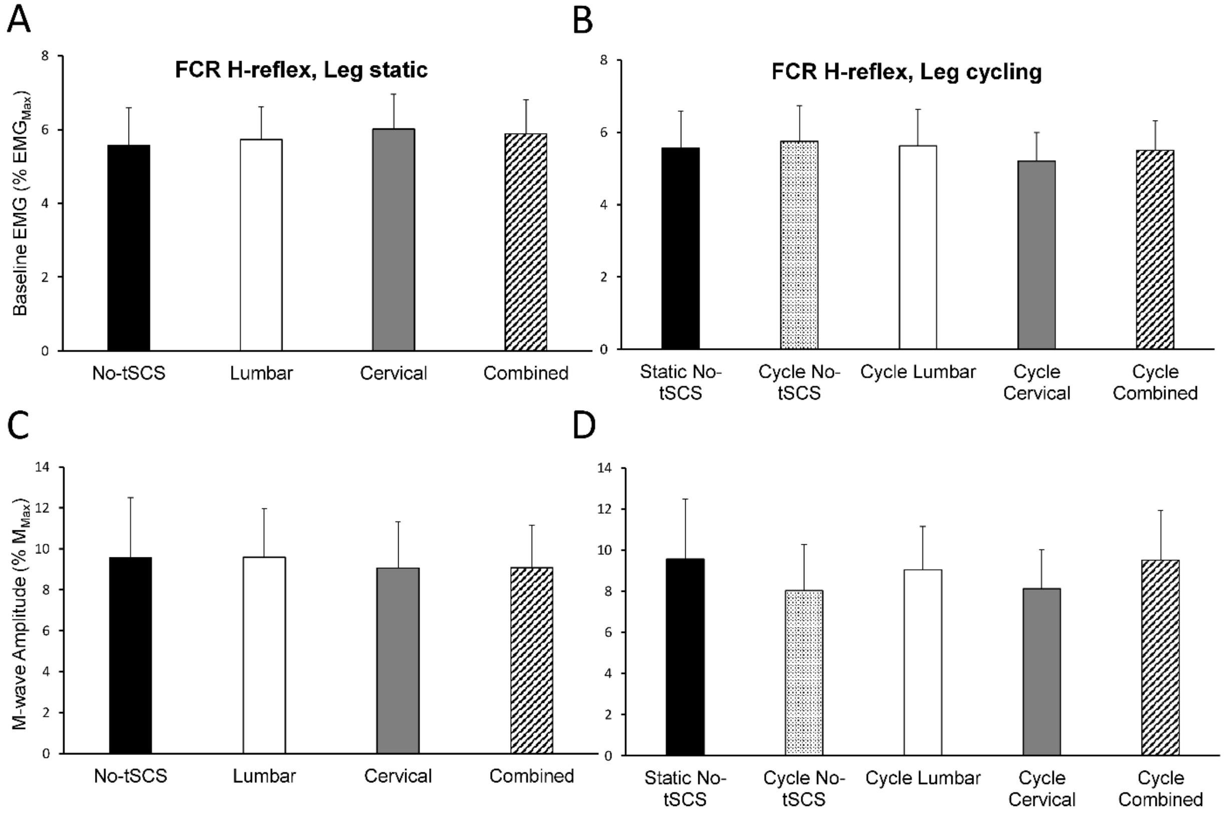

Figure 4.6. Baseline FCR muscle activity and M-wave amplitude during H-reflex assessments. The average baseline activity in the FCR muscle during H-reflex assessment while the legs were (A) static and (B) cycling was similar indicating that a consistent contraction was held across all experimental conditions. The average peak-to-peak amplitude of the FCR M-wave was similar across all (C) static and (D) cycling conditions indicating that a similar direct motor response was evoked by the median nerve stimulus across all experimental conditions. Values are Mean ± SE.

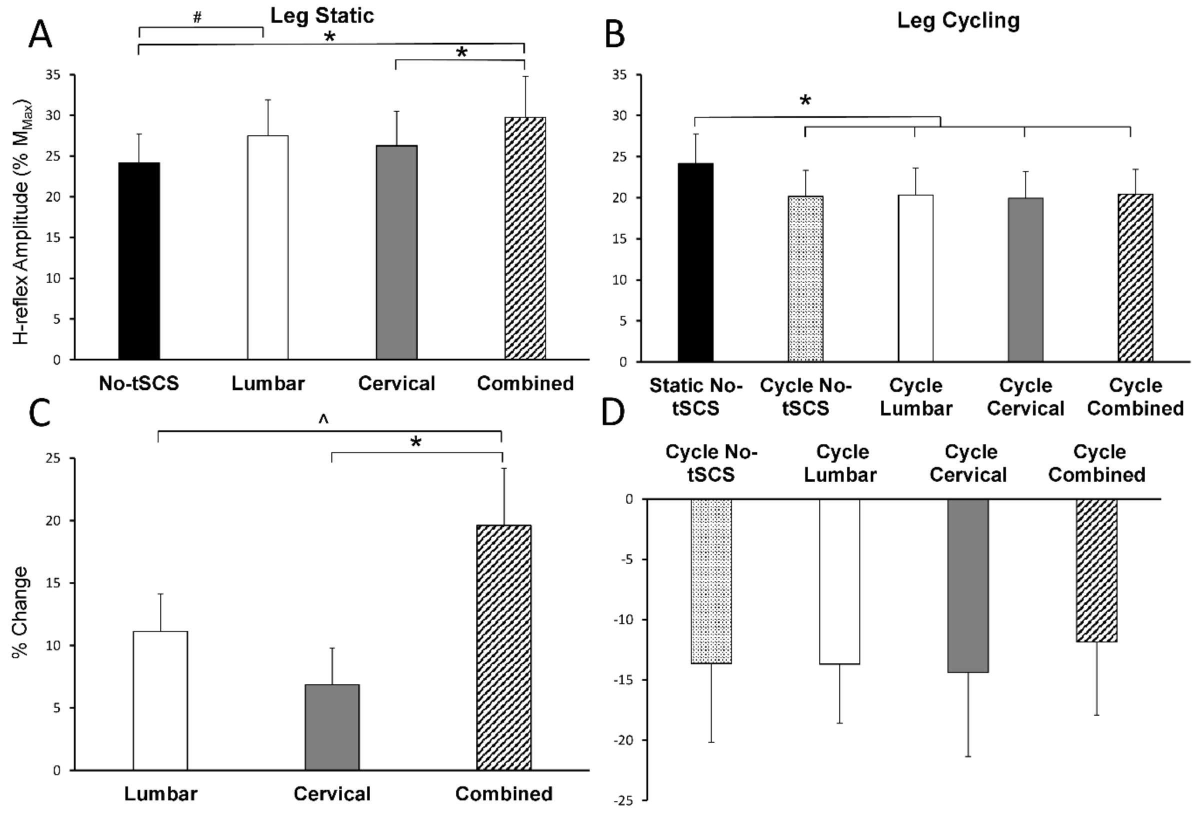

Figure 4.7. Effect of tSCS on FCR H-reflex amplitude while the legs were static or cycling. (A) Average H-reflex peak-to-peak amplitude during the application of tSCS while the legs were held static. Combined tSCS increased the amplitude of the H-reflex significantly more than No-tSCS and Cervical tSCS. Lumbar tSCS significantly increased the amplitude of the H-reflex compared to No-tSCS. (B) Average peak-to-peak H-reflex amplitude during the application of tSCS while the legs were cycling. There was a significant reduction in H-reflex amplitude for all cycling conditions regardless of tSCS site compared to the legs static, No-tSCS condition. (C) Percent increase in H-reflex amplitude during Lumbar, Cervical and Combined tSCS while the legs were static relative to No-tSCS. The % increase in H-reflex amplitude with Combined tSCS was significantly larger than that with Lumbar or Cervical tSCS. (D) Percent decline in H-reflex amplitude during all tSCS conditions while the legs were cycling relative to the legs static, No-tSCS condition. * $p<0.05$. $^{\#}p=0.059$. ^ $p=0.053$. Values are Mean ± SE.

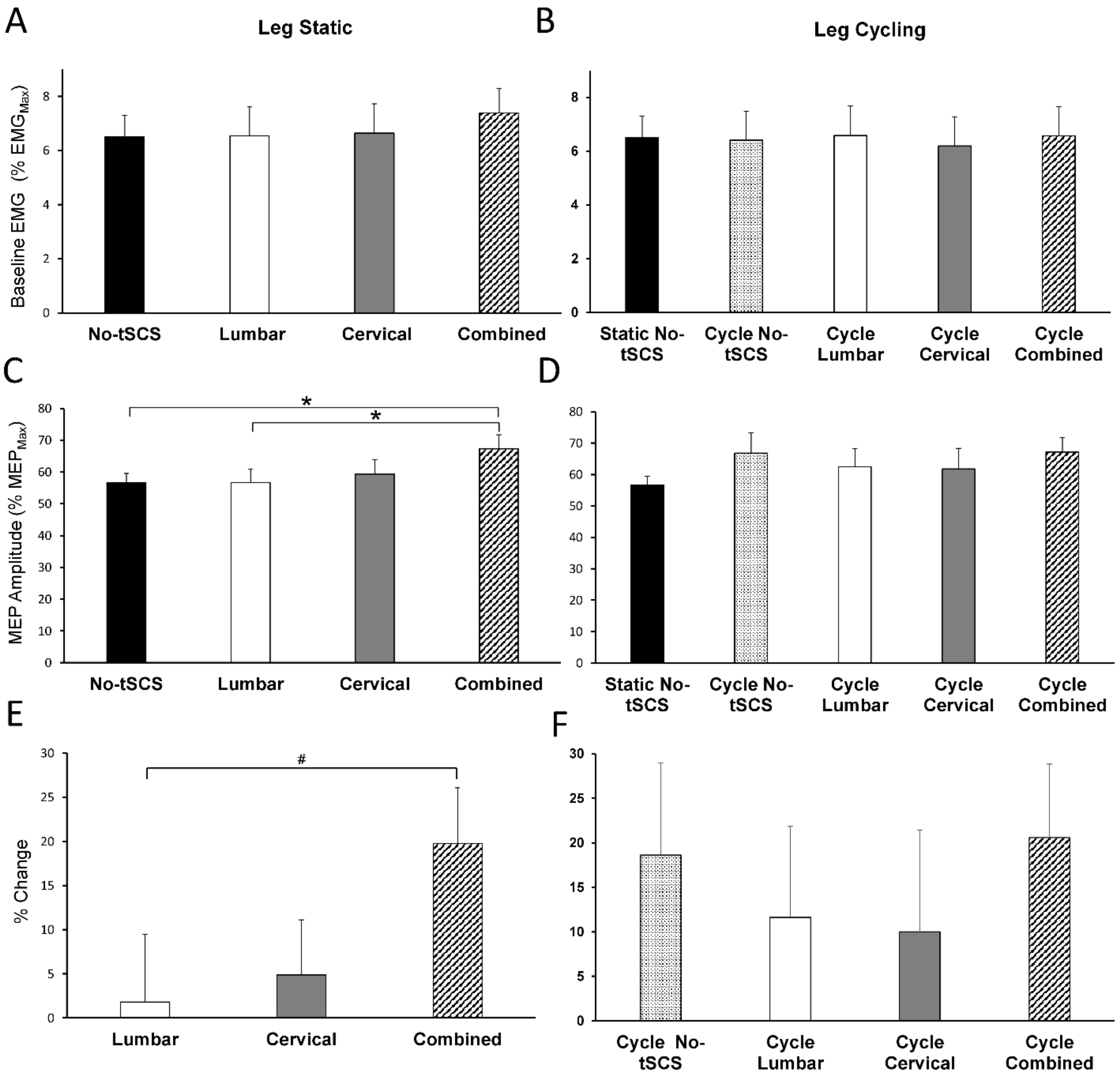

Figure 4.8. Effect of tSCS on FCR MEP amplitude while the legs were static or cycling. Average amplitude of baseline activity during MEP assessments while the legs were (A) static and (B) cycling was similar across all conditions. (C) Average MEP peak-to-peak amplitude during tSCS while the legs were held static. Combined tSCS significantly increased the amplitude of the MEPs relative to No-tSCS and Lumbar tSCS. (D) Average MEP amplitude during tSCS while the legs were cycling. There was no significant difference in the amplitude of MEPs compared to the static, No-tSCS condition for all cycling conditions. (E) Percent increase

in MEP amplitude during Lumbar, Cervical and Combined tSCS relative to the legs static, No-tSCS condition. The % increase in MEP amplitude with Combined tSCS was significantly larger than that with Lumbar tSCS. (F) Percent increase in MEP amplitude during leg cycling relative to the legs static, No-tSCS condition. * $p<0.05$. $^{\#}$ $p=0.056$. Values are Mean ± SE.

Hoffmann reflex (H-reflex)						
Amplitude (mA)	***tSCS amplitude for each participant (n=14)***					
	Cervical	P1 = 65	P2 = 42	P3 = 37	P4 = 50	P5 = 55
		P6 = 50	P7 = 35	P8 = 44	P9 = 65	P10 = 48
		P11 = 70	P12 = 40	P13 = 50	P14 = 55	***Mean: 50.43*** ***SD: 10.72***
	Lumbar	P1 = 48	P2 = 40	P3 = 29	P4 = 32	P5 = 44
		P6 = 38	P7 = 27	P8 = 30	P9 = 65	P10 = 33
		P11 = 55	P12 = 40	P13 = 50	P14 = 48	***Mean: 41.36*** ***SD: 11.01***
Motor evoked potential (MEP)						
Amplitude (mA)	***tSCS amplitude for each participant (n=14)***					
	Cervical	P1 = 65	P2 = 42	P3 = 37	P4 = 50	P5 = 55
		P6= 50	P7 = 35	P8 = 44	P9 = 65	P10 = 48
		P11 = 70	P12 = 50	P13 = 60	P14 = 53	***Mean: 51.71*** ***SD: 10.53***
	Lumbar	P1 = 48	P2 = 40	P3 = 29	P4 = 32	P5 = 44
		P6 = 38	P7 = 27	P8 = 30	P9 = 65	P10 = 33
		P11 = 55	P12 = 50	P13 = 52	P14 = 48	***Mean: 42.21*** ***SD: 11:36***

Table 4.1. Summary of tSCS Amplitude across Study Participants

Chapter 5: Transcutaneous spinal cord stimulation of the cervical cord modulates lumbar networks[3]

Trevor S. Barss[1,2,3], Behdad Parhizi[1,2,3], Vivian K. Mushahwar[1,2,3]

[1] Neuroscience and Mental Health Institute, University of Alberta, AB, Canada

[2] Division of Physical Medicine and Rehabilitation, Department of Medicine, University of Alberta, Edmonton, AB, Canada

[3] Sensory Motor Adaptive Rehabilitation Technology (SMART) Network, University of Alberta, Edmonton, AB, Canada

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[3] A version of this chapter has been published.
Barss, T. S., Parhizi, B., & Mushahwar, V. K. (2019). Transcutaneous spinal cord stimulation of the cervical cord modulates lumbar networks. Journal of Neurophysiology, 123(1), 158-166. doi:10.1152/jn.00433.2019

5.1. Introduction

Neuromodulation of spinal circuitry via transcutaneous spinal cord stimulation (tSCS) provides a non-invasive and safe assistive technology to both further our understanding of mechanisms controlling locomotion and improve rehabilitation after neurological impairment (Y. Gerasimenko et al., 2015; Phillips et al., 2017). Locomotor behavior is controlled by specific neural circuits called central pattern generators located in the cervical and lumbosacral spinal cord (E. P. Zehr et al., 2016). Cervicolumbar coupling has been demonstrated in animal experiments as early as the 1970s and 80s (Yamaguchi, 1986), with clear functional interconnections between the locomotor networks for the forelimbs and hindlimbs, providing interlimb coordination (Juvin et al., 2005). These connections have been demonstrated via the generalized suppression of spinal reflexes in the legs during rhythmic movements of the arms in neurologically intact individuals (E. Paul Zehr, Balter, et al., 2007) or after stroke (Barzi & Zehr, 2008). This modulation suggested an intersegmental linkage between the cervical and lumbar cord through propriospinal connections (P. W. Nathan & Smith, 1959).

Locomotor-related neuronal circuits in the spinal cord have a high level of automaticity; that is, they can produce a stepping-like movement pattern and rhythmic electromyographic (EMG) activity in the absence of supraspinal and/or peripheral afferent inputs (E. P. Zehr et al., 2016). Applying epidural spinal cord stimulation (eSCS) to the cervical cord has been shown to modulate spinal circuitry and improve volitional hand control and grip strength in individuals with a chronic SCI (Lu et al., 2016). Recently, eSCS applied during intense locomotor training has been shown to neuromodulate locomotor-related neuronal circuits in patients with motor-complete spinal-cord injury (SCI) facilitating postural and locomotor adjustments (Angeli et al., 2018). Importantly, tSCS of both the cervical and lumbar spinal cord appear to neuromodulate similar circuity as modulated by epidural stimulation (Ursula S. Hofstoetter et al., 2018). Specifically, epidural and transcutaneous electrical stimulation of the spinal cord may activate afferent input which in turn activates spinal interneuronal networks that control efferent output (Mayr et al., 2016).

The ability to walk is often lost after SCI, leading to multiple secondary complications that reduce quality of life and increase healthcare costs (A. Richardson, Samaranayaka, Sullivan, & Derrett, 2021). Current rehabilitation interventions focus on restoring leg movements through intensive training on a treadmill or a robotic device but ignore engaging the arms (Wernig & Müller, 1992). It has been recently demonstrated that active engagement of the arms coordinately with the legs in an arm and leg (A&L) cycling paradigm significantly improves over-ground walking in people with both incomplete SCI (Rui Zhou et al., 2018) and chronic stroke (Klarner et al., 2016a) compared to their baseline levels. Importantly, the improvements were significantly larger than those obtained by legs-only cycling (Rui Zhou et al., 2018). Moreover, while legs-only cycling resulted in increases in walking speed and distance that were similar to those reported using gait-specific interventions such as bodyweight-supported treadmill training, A&L cycling training doubled the increases in walking speed and distance (Rui Zhou et al., 2018). These larger increases were at least in part due to improvements in corticospinal drive (Zhou et al., 2017) and modulation of cervico-lumbar connectivity after chronic SCI (R. Zhou et al., 2018) and stroke (Klarner et al., 2016a). Cervical and lumbar circuits are coupled through long propriospinal neurons which gate the excitability of reflex pathways to facilitate general arm and leg coordination (Huang & Ferris, 2009). Rhythmic arm cycling suppresses the amplitude of H-reflex in the soleus differentially depending on the arm position which is likely mediated by reinforced presynaptic inhibition in Ia lumbar afferent terminals (R. Zhou et al., 2018). Enhanced cervico-lumbar connectively was identified by the restoration of H-reflex (R. Zhou et al., 2018) or stretch reflex suppression in the legs (soleus) (Klarner et al., 2016a) during arm cycling. Suppression of the soleus H-reflex during arm cycling has been consistently identified in neurologically intact individuals and is used as a test of interlimb connectivity (E. Paul Zehr, Balter, et al., 2007). These results highlight the importance of engaging cervical locomotor networks in the recovery of walking regardless of neurological deficit. Actively engaging the arms in rehabilitation paradigms for the improvement of walking substantially regulates the excitability of the lumbar spinal networks.

However, what is unclear is the extent to which lumbar spinal networks can be engaged via tSCS applied to remote segments of the spinal cord. If lumbar networks can be modulated via cervical or lumbar tSCS in a fashion similar to the inclusion of the arms in the rehabilitation of walking, tSCS may play a vital role in future rehabilitation paradigms. Therefore, the primary purpose of this investigation was to investigate the effect of non-invasive tSCS on cervico-lumbar connectivity. A secondary purpose was to determine differences in the strength of interlimb coupling depending on whether tSCS was applied to the cervical or lumbar regions of the spinal cord. This was accomplished by determining the modulation of the H-reflex in the soleus muscle during tSCS while the arms were either held in a static position or were rhythmically cycling in neurologically intact (NI) volunteers. It was hypothesized that the H-reflex would be suppressed during cervical tSCS and during arm cycling, with additional suppression when cervical tSCS and arm cycling were combined.

5.2. Methods

Participants

Thirteen neurologically intact participants (4 female; 9 male) completed all experimental conditions. Participants were informed of the experimental procedures and signed a written consent form. Protocols used in the experiments were approved by the University of Alberta Human Research Ethics Committee and performed according to the Declaration of Helsinki (1964).

Experimental procedures

To establish whether tSCS produced measurable changes in cervico-lumbar connectivity, Hoffmann (H-) reflexes were assessed in the left soleus muscle via electrical stimulation to the tibial nerve. The excitability of the H-reflex was examined under 6 different conditions: 1) arms held in a static position (‘STATIC’) without tSCS; 2) arms static with tSCS applied to the lumbar region of the spinal cord (‘sLUMBAR’); 3) arms static with tSCS applied to the cervical cord (‘sCERVICAL’); 4) arms cycling without tSCS (‘CYCLE’); 5) arms cycling with tSCS applied to the lumbar cord (‘cLUMBAR’); and 6) arms cycling with tSCS applied to the cervical cord (‘cCERVICAL’). The order of experimental conditions was randomized between participants to limit the impact of order effects on the amplitude of the soleus H-reflex.

Participants sat in a custom-adapted arm and leg cycling ergometer (THERA-vital; Medica Medizintechnik, Hochdorf, Germany; and ERGYS 2, Therapeutic Alliances, Fairborn OH) with fixed back support, and the feet were strapped to foot pedals. The arm and leg compartments of the ergometer were not mechanically linked. However, the arm cranks (left and right) and foot pedals (left and right) were coupled and positioned 180° out of phase relative to each other. The legs were fixed in the cycle ergometer to restrict movement and maintain the same joint angles throughout the experiment (Figure 5.1). The left leg was held in extension at the 270° position while the right leg was held in flexion at the 90° position throughout the experiment (Figure 5.1A).

During the arm static condition, participants were asked to grip the ergometer handle with the left arm held at the 0° position and the right arm at the 180° position. Unloaded rhythmic left and right arm cycling (no resistance) was performed at a constant frequency of ≈1 Hz (~60 rpm) (E. Paul Zehr, Collins, Frigon, & Hoogenboom, 2003). Visual feedback related to cycling speed was continuously provided to ensure consistency of speed throughout the testing.

Soleus Hoffmann (H-) reflex

The H-reflex was evoked by electrically stimulating the tibial nerve and recording EMG activity in the soleus (SOL) muscle (Figure 5.1A). Bipolar surface electrodes were placed over the tibial nerve in the popliteal fossa on the left leg as in previous studies (R. Zhou et al., 2018). Square wave pulses (1 ms-long) were delivered with a constant current stimulator (Digitimer model DS7A, Medtel, NSW, Australia) every 5 - 8 s while the arms were static and every 10 rotations during arm cycling (Figure 5.2). During cycling, a position sensor was used to trigger the stimulation at a position of the left arm that matched the position held during the static task. Note that when evoking H-reflexes, it is recommended that the stimulus pulses are delivered at random intervals apart, with 3 seconds being the smallest interval between pulses (P. E. Zehr, 2002). During cycling, stimulation was triggered based on left arm position sensors and was therefore unable to be randomized. Ten stimuli were delivered during each testing condition. The recording EMG electrodes were placed on the surface of the skin in a bipolar configuration, oriented longitudinally along the predicted fibre direction of the SOL, tibialis anterior (TA), vastus lateralis (VL) and biceps femoris (BF) in accordance with SENIAM procedures (Hermens, Freriks, Disselhorst-Klug, & Rau, 2000).

H-reflex amplitude was evaluated at the stimulation intensity required to match the motor response (M-wave) across all conditions. Stimulation intensity was adjusted as needed to maintain a consistent small but measurable M-wave amplitude across conditions to minimize antidromic effects (Figure 5.2B). Initial M-wave amplitude was determined by finding an intensity which produced an H-reflex amplitude of approximately 70% of maximal H-reflex amplitude (Hmax). Care was taken to ensure that the evoked H-reflex was on the ascending limb of the recruitment curve, thus allowing it to be either suppressed or facilitated during the various experimental conditions. To provide a position-matched control between arm static and cycling conditions, SOL H-reflexes were always evoked midway between extension and flexion (i.e., when the left handle of the arm crank crossed the 0° position) during cycling (Figure 5.1A). At this position, arm cycling has been previously shown to suppress the amplitude of the ipsilateral SOL H-reflex significantly (R. Zhou et al., 2018), regardless of which arm was used as the reference (Frigon et al., 2004). The same leg position was maintained across all experimental conditions with participants sustaining a low-level contraction (~10% of maximal voluntary contraction, MVC) of the left SOL. Visual feedback of the rectified and filtered SOL EMG signal was displayed on an oscilloscope to assist in maintaining the consistent contraction. Maximally evoked motor responses (Mmax) were collected by applying 3 stimuli at supramaximal intensity to ensure all available motor units were being recruited.

Transcutaneous spinal cord stimulation (tSCS)

Non-invasive, transcutaneous electrical stimulation (NeuroRecovery Technologies Inc., San Juan Capistrano, CA, USA) was delivered to the cervical or lumbar region of the spinal cord during half of the experimental conditions. The stimulation waveform consisted of 1 ms-long trains of 10 kHz biphasic square pulses repeated at a frequency of 30 Hz (Figure 5.2A) (Inanici et al., 2018). Stimulation was delivered via two 2.5 cm round cathodic electrodes (Axelgaard Manufacturing Co., Ltd., USA) placed midline at C3-4 and C6-7 and T11 and L1 spinous processes, for the cervical and lumbar locations, respectively. Two 5 × 10 cm rectangular anodic electrodes were placed bilaterally over the iliac crests as anodes (Figure 5.1B).

Threshold stimulation intensity was set as the intensity at which individuals first felt sustained sensation at the anode electrodes. At this intensity, the sensation was strong but tolerable at each of the cathode sites. Perceived sensation threshold was chosen as evoked motor responses in leg EMG were not present with tSCS in the current investigation. Since the effects of tSCS stimulation amplitude on SOL H-reflex amplitude were previously unknown, we tested both threshold and maximal tolerable stimulation intensities at the cervical and lumbar sites in 11 of the 13 participants. The effect of tSCS intensity on the amplitude of the soleus H-reflex was compared to that during tSCS at threshold intensity.

Data analysis

All EMG signals were amplified by 1000x and band-pass filtered from 30 to 1000 Hz. The signals were then digitized at a sampling rate of 2000 Hz using a CED 1401 analog to digital conversion board and Spike 2 associated software (Cambridge Electronic Design, Cambridge, UK). The signals were analyzed off-line using custom-written software (Matlab, Nantick, MA, USA). A window of SOL EMG activity including 100 ms pre-stimulus and 300 ms post-stimulus was analyzed for all stimuli within each condition.

The background (pre-stimulus) SOL EMG activity was calculated as the mean activity over the 100 ms before the stimulus and averaged across stimuli. The peak-to-peak amplitudes of the post-stimulus evoked H-reflex and M-wave were calculated and averaged over the 10 stimuli per condition. Mmax was calculated from the average of the 3 supramaximal M-waves.

Statistical analysis

All statistical analyses were performed using SPSS Statistics 20 (IBM, Chicago, IL). To compare the effects on the amplitude of the SOL H-reflex when the arms maintained a static position, a 1 x 3 factor ANOVA was applied, with STATIC, sLUMBAR and sCERVICAL as the factors. The percent reduction in SOL H-reflex amplitude was compared using a paired sample t-test (% decline sLUMBAR vs. % decline sCERVICAL). To compare the effects during arm cycling, 1 x 4 ANOVA was used with STATIC, CYCLE, cLUMBAR and cCERVICAL as the factors. The percent reduction in H-reflex amplitude was compared using a 1 x 3 ANOVA (% decline CYCLE vs. % decline cLUMBAR vs. % decline cCERVICAL). A 2 x 2 ANOVA compared whether the stimulation intensity (threshold vs. maximal) was different between the sites of stimulation (lumbar vs cervical). Paired samples t-tests assessed the effect of stimulation intensity on H-reflex amplitude at both the cervical and lumbar locations. When significant main effects were detected, pairwise comparisons were used. Significance was accepted at $p < 0.05$. All values are expressed as mean ± standard deviation except in the figures where standard error is used for clarity of display.

5.3. Results

Across all participants, threshold tSCS intensity was 35.4±8.3 mA and 39.6±8.4 mA for lumbar and cervical stimulation, respectively, during both arms static and cycling conditions. The maximal intensity (in 11 of the 13 participants tested) during arms cycling was 40.7±9.1 mA and 47.1±10.7 mA for lumbar and cervical stimulation, respectively. Results from a 2 x 2 ANOVA shows significant main effects of both site ($F_{(1,10)} = 15.232$, $p = 0.003$) and intensity ($F_{(1,10)} = 55.326$, $p < 0.001$) with no significant interaction ($F_{(1,10)} = 0.226$, $p = 0.624$). This indicates that stimulation amplitude was significantly higher at the cervical site compared to the lumbar site regardless of stimulation intensity and that the maximal intensity was significantly higher than the threshold intensity regardless of site of stimulation.

Background muscle activity and evoked SOL motor response (M-wave) across condition

Representative examples of SOL H-reflex from one participant across all experimental conditions are shown in Figure 5.3. Similar background muscle activity and M-wave amplitude were obtained during both static and arm cycling conditions. During static trials, a 1 x 3 (STATIC, sLUMBAR, sCERVICAL) ANOVA indicated no difference in either SOL ($F_{(2,24)} = 0.224$, $p = 0.801$) or TA ($F_{(2,24)} = 0.423$, $p = 0.660$) background muscle activity between conditions across all participants (Fig. 5.4A). There was also no difference in the evoked M-wave between conditions ($F_{(2,24)} = 0.369$, $p = 0.695$) (Fig. 5.4B). Similarly, during cycling trials, a 1 x 4 (STATIC, CYCLE, cLUMBAR, cCERVICAL) ANOVA indicated no difference in background muscle activity between conditions across all participants for either SOL ($F_{(3,36)} = 0.287$ $p = 0.834$) or TA ($F_{(3,36)} = 0.197$, $p = 0.898$) (Fig. 5.4C). Moreover, there was no difference in the evoked M-wave between conditions ($F_{(3,36)} = 0.162$, $p = 0.921$) (Fig. 5.4D).

Therefore, the level of background activation in both the agonist and antagonist muscle as well as the intensity of tibial nerve activation by the electrical stimuli were held constant under all testing conditions.

Effect of static cervical and lumbar tSCS on SOL H-reflex excitability

An example from one participant of SOL H-reflex suppression by cervical tSCS while the arms were held static is shown in Figure 5.3A. Figure 5.3B provides an example of the suppression of H-reflex peak-to-peak amplitude with arm cycling alone as well as with lumbar or cervical tSCS. During the arm static trials, a 1 x 3 (Condition) ANOVA indicated a significant main effect of condition on H-reflex amplitude ($F_{(2,24)} = 4.186$, $p = 0.028$). Post-hoc pairwise comparisons showed that cervical tSCS significantly reduced the H-reflex amplitude compared to STATIC (35.4 ± 17.3 % M_{max} vs. 44.6 ± 17.5 % M_{max}, $p = 0.011$) (Figure 5.5A). Interestingly, lumbar tSCS did not have a measurable effect on SOL H-reflex amplitude: the amplitude of the H-reflex during lumbar tSCS was not significantly different from that during the STATIC condition (42.1 ± 21.4 % M_{max} vs. 44.6 ± 17.5 % M_{max}, $p = 0.449$). The amplitude of the H-reflex during lumbar tSCS was also not significantly different from that during cervical tSCS (42.1 ± 21.4 vs. 35.4 ± 17.3 % M_{max}, $p = 0.089$). Figure 5.5B highlights a significantly greater percent reduction in H-reflex amplitude with cervical tSCS (-22.9 ± 25.9 %) compared to lumbar tSCS (-3.8 ± 25.2%; $p = 0.01$) as determined by a paired sample t-test.

Effect of cycling with simultaneous cervical or lumbar tSCS on SOL H-reflex excitability

During cycling trials, a 1 x 4 (Condition) ANOVA indicated a significant main effect of condition on H-reflex amplitude ($F_{(3,36)} = 3.824$, $p = 0.018$). Post-hoc pairwise comparisons revealed a significant reduction in H-reflex amplitude during CYCLE relative to STATIC (38.1 ± 16.0 % M_{max} vs. 46.3 ± 17.4 % M_{max}, $p = 0.025$), cLUMBAR relative to STATIC (39.4 ± 21.1 % M_{max} vs. 46.3 ± 17.4 % M_{max}, $p = 0.040$) and cCERVICAL relative to STATIC (37.9 ± 20.4 % M_{max} vs. 46.3 ± 17.4 % M_{max}, $p = 0.015$) (Figure 5.5C). There were no significant differences in H-reflex amplitude between CYCLE, cLUMBAR or cCERVICAL ($p > 0.05$). There was also no significant main effect ($F_{(2,24)} = 0.307$, $p = 0.738$) of condition on percent reduction in H-reflex amplitude between CYCLING (-19.2 ± 24.1 %), cLUMBAR (-19.4 ± 29.6 %), or cCERVICAL (-22.9 ± 29.9 %) (Figure 5.5D). Paired sample t-tests revealed that tSCS at threshold and maximal stimulation levels during cycling reduced H-reflex amplitude similarly when applied to the cCERVICAL (40.4 ± 19.3 vs. 39.4 ± 20.1, $p > 0.05$) or cLUMBAR stimulation sites (42.2±20.3 vs. 43.6 ± 19.1). This demonstrated that there was no additional effect of the higher intensity tSCS relative to that obtained at the threshold stimulation levels.

5.4. Discussion

The goals of this project were twofold: 1) Investigate cervico-lumbar connectivity during tSCS; and 2) evaluate differences in interlimb coupling when tSCS was applied to either the cervical or lumbar region of the spinal cord. Interestingly, there was no significant modulation of the lumbar networks (changes in the soleus H-reflex) when tSCS was applied to the lumbar region of the spinal cord. However, tonic activation of the cervical spinal cord through tSCS significantly modulated the activity of the lumbar networks. The modulation induced in the lumbar networks by tonic cervical tSCS was similar to that produced by arm cycling. The combination of arms cycling with cervical tSCS or lumbar tSCS did not result in additional suppression of the soleus H-reflex beyond that obtained with arms cycling alone or cervical tSCS alone. To the best of our knowledge, this is the first study to assess the modulatory effects of tSCS on interlimb coupling.

Background muscle activity and stimulation amplitude did not impact interlimb coupling

Although background EMG activity and stimulus amplitude influence muscle afferent reflexes, pre-stimulus EMG activity in both the agonist and antagonist muscle as well as the amplitude of the M-wave were the same across the static and cycling conditions (Figure 5.4). This indicates that factors such as descending drive, reciprocal inhibition, or the number of recruited axons did not influence the suppression of H-reflexes during arm cycling and cervical tSCS. Therefore, inter-limb effects influencing presynaptic inhibition of Ia afferents are the likely mechanism for modulating the lumbar networks (E. P. Zehr et al., 2016).

Reduced H-reflex amplitude with rhythmic arm cycling

As expected and similar to previous investigations, SOL H-reflex amplitude was significantly suppressed by 19.2 % when the arms were cycling (without tSCS) relative to arms static (without tSCS) (R. Zhou et al., 2018). This suppression is primarily mediated by reinforced presynaptic inhibition in Ia lumbar afferent terminals (E. P. Zehr et al., 2016), although reciprocal inhibition may contribute as well (Dragert & Zehr, 2013). The cervical and lumbar circuits which gate the excitability of reflex pathways to facilitate general arm and leg coordination, are coupled through long propriospinal neurons (Frigon, 2017). Engagement of these networks is improved after neural injury with both arm only (Kaupp et al., 2017) and A&L cycling (Rui Zhou et al., 2018), highlighting their importance in functional restoration.

Cervical tSCS modulates H-reflex amplitude similarly to rhythmic arm cycling

While activation of cervical locomotor networks via arm cycling consistently modulates cervico-lumbar connectivity, it has remained unclear if similar networks can be engaged via tSCS in order to produce modulatory effects on lumbar excitability. Interestingly, tSCS applied to the cervical cord with arms static significantly suppressed the soleus H-reflex (-22.9%) while tSCS applied to the lumbar cord had no effect on the soleus H-reflex (-3.8%). While the current results do not allow for mechanistic claims in terms of pathways or sites of inhibition, the amplitude of suppression is similar between arm cycling (-19.2%) and cervical tSCS (-22.9%) compared to static, indicating the potential for activation of similar networks (Y. P. Gerasimenko et al., 2015). It may be possible that cervical tSCS can shift spinal networks both within and between segments of the spinal cord into a physiological state that enables greater access of supraspinal control to lumbar sensory-motor networks (Ursula S. Hofstoetter et al., 2018). Stimulation of the skin itself may also contribute to altered neural excitability between or within segments of the spinal cord (E. P. Zehr et al., 2016). However, the degree to which tSCS activates the sensory afferent system in the periphery, at the level of the dorsal roots, and/or via the spinal grey matter is currently unknown.

Previous studies have shown that rhythmic arm cycling supresses H-reflex amplitude in muscles of the leg, and is likely mediated by reinforced presynaptic inhibition in Ia lumbar afferent terminals (Nakajima et al., 2013). The cervical and lumbar circuits, coupled through long propriospinal neurons, gate the excitability of reflex pathways to facilitate general arm and leg coordination (Huang & Ferris, 2009). These cervico-lumbar connections during rhythmic movements have been shown to be either weakened or abolished after stroke (Barzi & Zehr, 2008) or spinal cord injury (R. Zhou et al., 2018). Excitingly, repeated activation of these circuits via arm and leg cycling training has reengaged these propriospinal connections and enhanced interlimb coordination (R. Zhou et al., 2018). It is possible that cervical tSCS recruits similar networks within the spinal cord influencing propriospinal connections between cervical and lumbar segments of the spinal cord. Determining whether similar networks that modulate pre-synaptic inhibition at the Ia terminals are activated with both arm cycling and tSCS will be an important next step in determining its utility as a rehabilitation tool.

While the current results indicate that lumbar tSCS did not impact SOL H-reflex amplitude, lumbar tSCS may have different effects on the SOL H-reflex when tested under different experimental conditions. For example, it is well established that H-reflex excitability is influenced by factors which include muscle length, proprioceptive feedback and degree of postural stability (P. E. Zehr, 2002). Moreover, it has recently been reported that lumbar tSCS-evoked responses in the muscles of the lower limb are altered when moving from a supine to a prone to a standing position (Danner et al., 2016). Therefore, while the current investigation does not provide evidence of lumbar tSCS influencing SOL H-reflex excitability, it may have effects during different tasks and in different muscles.

Similar mechanisms of epidural and transcutaneous spinal cord stimulation

A recent electrophysiological study identified that both epidural and tSCS activate similar primary afferent fibers within multiple posterior roots (Ursula S. Hofstoetter et al., 2018). The study directly compared characteristics of short-latency EMG responses in multiple leg muscles to both stimulation techniques in ten individuals with SCI. Post-activation depression of responses evoked by paired pulses applied either epidurally or transcutaneously confirmed the reflex nature of the responses. The muscle responses to both techniques had the same latencies, EMG peak-to-peak amplitudes, and waveforms (Ursula S. Hofstoetter et al., 2018). The most likely direct mechanism of stimulation occurs via tonic activation of dorsal root afferent fibers which elevates the excitability of spinal networks. This in turn brings interneurons and motor neurons closer to motor threshold; thus making them more likely to respond to limited residual post-injury descending drive (Y. P. Gerasimenko et al., 2015). During tSCS, spinal circuits are engaged through activation of afferent fibers of the posterior root neurons, providing input to spinal interneuronal networks that control efferent output to the muscles (Mayr et al., 2016).

These results provide a mechanistic framework for the implementation of tSCS as a spinal neuroprosthetic system to improve function after neurological disorders.

Cervical tSCS improves upper extremity function

Cervical epidural stimulation has previously been shown to neuromodulate the corresponding level of the spinal cord to improve function after a spinal cord injury, both in the acute and chronic stages post-injury (Lu et al., 2016). However, cervical tSCS has been proposed as a method to recruit previously non-functional sensory-motor networks within the cervical spinal cord to enable and amplify voluntary control of the hand. Recently, a case study proposed that cervical tSCS improves upper extremity rehabilitation after an incomplete (C3 AIS D) cervical SCI (Inanici et al., 2018). Combined tSCS and physical therapy training improved strength, pinch force and dexterity, and the study participant was able to resume self-feeding for the first time since his injury. The improvements persisted throughout the training, but more importantly, throughout a three-month follow-up period (Inanici et al., 2018). Importantly, similar improvements with both acute and chronic cervical tSCS have been shown across a group of participants (AIS B, n=3. AIS C, n=5) with severe paralysis (P. Gad et al., 2018). Not only did hand function improve within a single session of cervical tSCS, but 4 weeks of training simultaneously with tSCS improved handgrip forces by over 300% (stimulation on) and 200% (stimulation off). Excitingly, tSCS appears to result in similar improvements as epidural stimulation, without the need for implanted electrodes (Inanici et al., 2018). Moreover, the present study demonstrates, for the first time, that cervical also modulates the lumbar networks. Enhancing cervico-lumbar connectivity with tSCS may provide additional benefits if paired with previously successful exercise interventions aimed at improving interlimb coordination and walking after neural injury (Rui Zhou et al., 2018).

Lumbar tSCS activates locomotor circuitry

Previously, tSCS at vertebral level T11 has been shown to activate locomotor circuitry in neurologically-intact subjects when their legs were placed in a gravity-neutral position (Gorodnichev et al., 2012). More recently, (Balykin et al., 2017) evaluated tSCS at the T11-T12 site on locomotor function of individuals with stroke or spinal cord injury. Single pulse stimulation showed both monosynaptic and polysynaptic reflexes within muscles of the legs while trains of stimulation caused patients with paresis to move their legs involuntarily in a locomotor-like fashion, providing evidence of increased excitability of the lumbar spinal neural structures in clinically meaningful populations (Balykin et al., 2017). Interestingly, simultaneous tSCS of cervical, thoracic, and lumbar levels (C5, T11, L1) induced coordinated stepping movements with a greater range of motion at multiple joints in five of six neurologically-intact subjects compared to T11 alone (Gerasimenko et al., 2014). The authors highlighted the synergistic and interactive effects of multi-site tSCS, suggesting multi-segmental convergence of descending and ascending influences on the spinal neuronal circuitries associated with locomotor activity (Sayenko et al., 2015). It is likely that this also includes the propriospinal connections responsible for the suppression of H-reflexes during arm cycling (R. Zhou et al., 2018).

5.5. Future directions

Ongoing work focuses on the modulatory effects on the upper limbs of tSCS when applied to the cervical and lumbar regions during leg cycling. These effects will then be explored in neurologically impaired populations in whom this assistive technology is likely to have its most dramatic effects. Previously, a case study has shown that tSCS paired with over ground stepping in an exoskeleton produces promising synergistic effects after a motor and sensory complete spinal cord injury (P. N. Gad et al., 2015). Ultimately, pairing tSCS with previously successful training paradigms to determine the potential for enhancing interlimb coordination and walking function after neurotrauma will be of vital importance (R. Zhou et al., 2018).

5.6. Figures

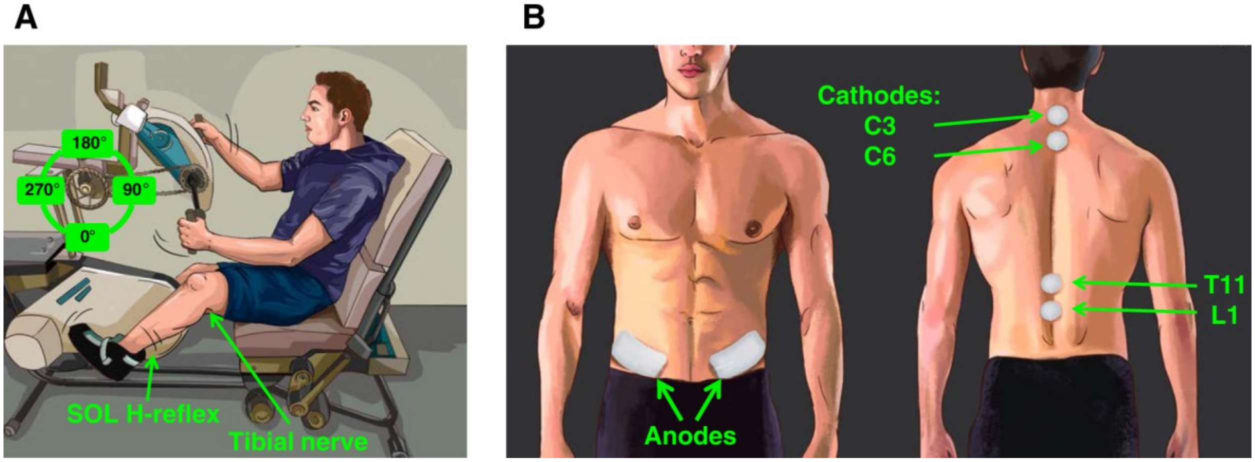

Figure 5.1. Experimental Setup and tSCS electrode placement. (A) H-reflexes were evoked via stimulation of the tibial nerve and recorded in the soleus muscle during a consistent background contraction of ≈5% peak muscle activity. The left leg was held static in an extended position, and stimulation to evoke the H-reflex was delivered with the left arm at 0°. (B) tSCS was delivered via two 2.5 cm round cathodic electrodes placed midline at C3-4 and C6-7 (cervical) or T11 and L1 (lumbar) spinous processes. Two 5 × 10 cm rectangular anodic electrodes were placed bilaterally over the iliac crests.

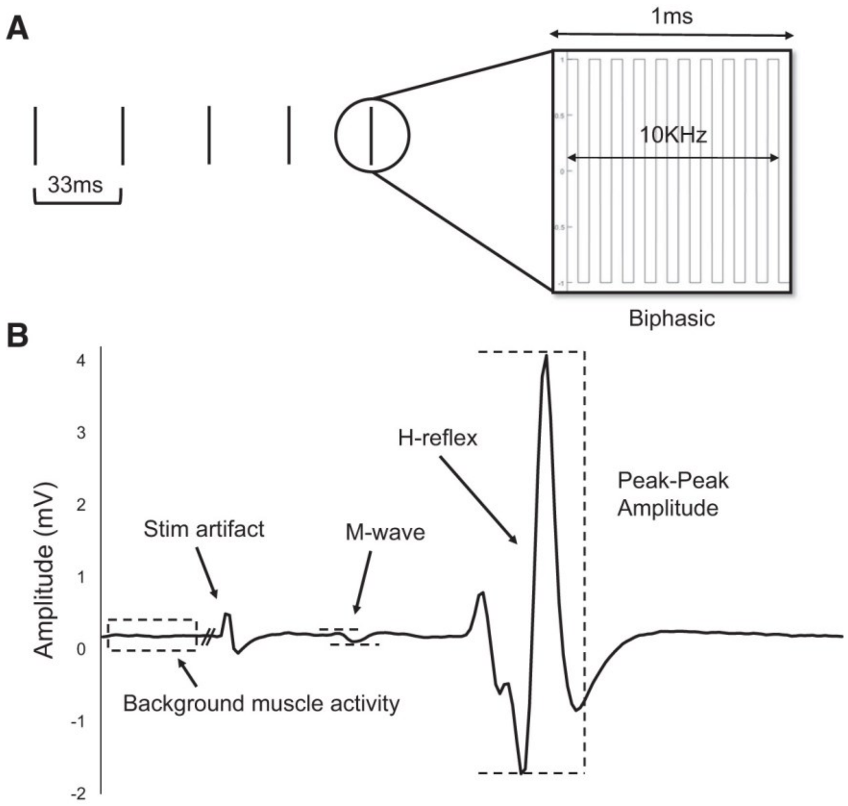

Figure 5.2. Stimulation Parameters. (A) Pattern of tSCS, 1ms-long bursts of 10 kHz square wave biphasic pulses were repeated every 33ms (i.e., at a rate of 30 Hz). (B) Example of a raw soleus H-reflex trace obtained without tSCS while the arms were static. Peak-to-Peak amplitude of the H-reflex and M-wave used for analysis is indicated by the dashed lines. Background muscle activity was calculated as the mean activity over the 100 ms before the stimulus and averaged across stimuli as shown by the dashed box.

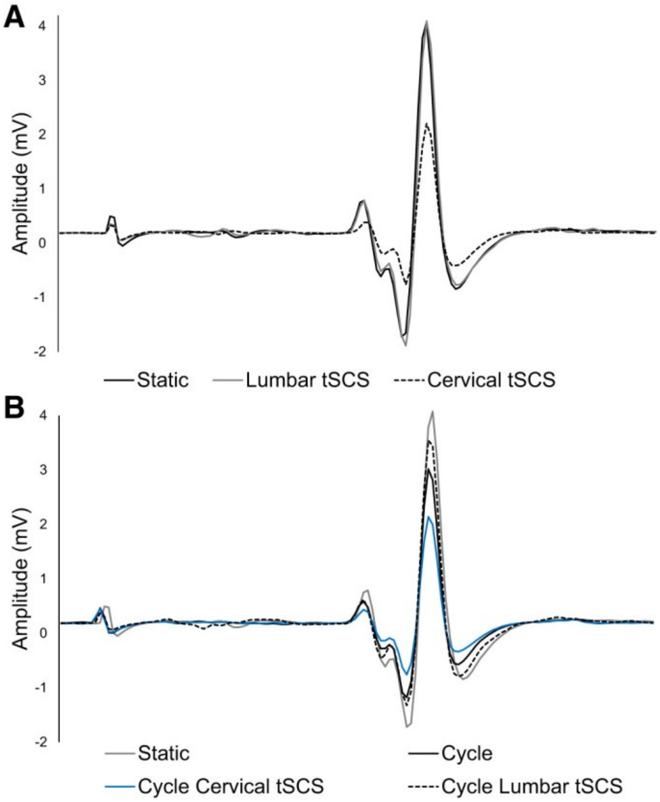

Figure 5.3. Examples of H-reflex responses from one participant across all conditions. (A) Reduction in H-reflex amplitude with cervical tSCS compared to lumbar tSCS while the arms were static. (B) All three cycling conditions exhibited a reduction in H-reflex amplitude compared to arms static.

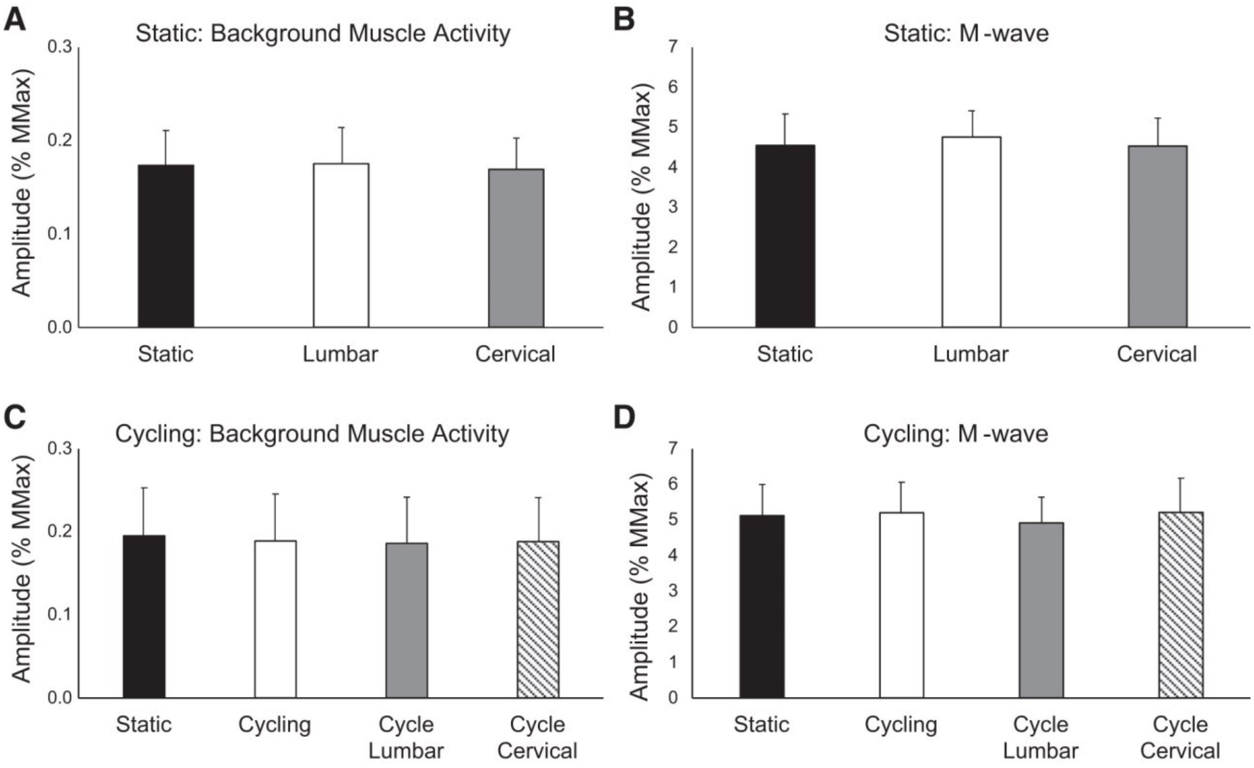

Figure 5.4. Background muscle activity and M-wave amplitudes during H-reflex assessment. The average background activity in the soleus muscle while the arms were (A) static or (C) cycling was similar, indicating a consistent contraction was held across testing conditions. Average amplitude of the M-wave (direct activation of motor fibers) during (B) arm static or (D) arm cycling, demonstrating that a consistent level of stimulation was used to evoke the H-reflex across testing conditions. Values are Mean ± SE.

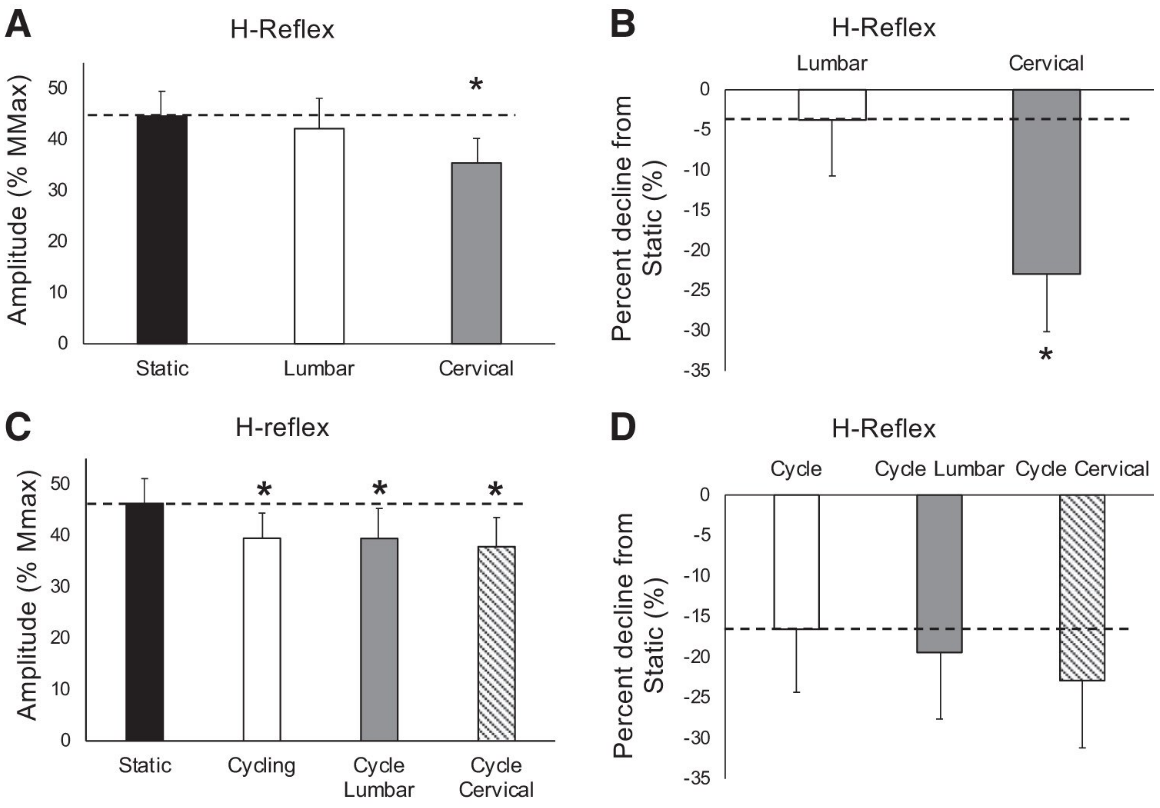

Figure 5.5. Effect of tSCS on soleus H-reflex amplitude while the arms were static (top row) or cycling (bottom row). (A) Average H-reflex amplitude while the arms were held static and during the application of lumbar tSCS or cervical tSCS. Cervical tSCS significantly reduced the amplitude of the H-reflex while the arms were held static. (B) The % decline in H-reflex amplitude during lumbar or cervical tSCS relative to the arms static condition. (C) Average H-reflex amplitude while the arms were cycling and during the application of lumbar tSCS or cervical tSCS while cycling. There was a significant reduction in H-reflex amplitude compared to static for all cycling conditions regardless of tSCS stimulation site. (D) The % decline in H-reflex amplitude during arm cycling and lumbar or cervical tSCS applied during arm cycling relative to the arms static condition. There was a similar % decline in H-reflex amplitude across all cycling conditions compared to static. * Significant reduction in H-reflex amplitude compared to static. $p<0.05$. Values are Mean ± SE.

Chapter 6. General discussion and future directions

Human upper limb movement is very sophisticated and years of research have been invested into learning about how the central nervous system organizes our motor actions. While we take natural movement for granted, neural injuries and diseases have devastating consequences on upper limb movement. The long-term objective of the work in this thesis is to design an effective rehabilitation intervention for improving cooperative bimanual movements after spinal cord injury (SCI). As a first step towards achieving this objective, the goals of my PhD thesis were to: 1) gain a fundamental understanding of the motor control of upper limb movements, especially common-goal bimanual movements; and 2) to investigate the effect of the non-invasive spinal cord neuromodulation approach, transcutaneous spinal cord stimulation (tSCS), on sensorimotor processes associated with the control of arm movements. The first goal signifies the fact that bimanual movements are ultimately diverse in their nature and the human motor system is capable of producing a wide variety of bimanual actions, each requiring involvement of distinct neural mechanisms (R. Ivry et al., 2004; S. P. Swinnen & Gooijers, 2015). Specifically, a full understanding of the neural correlates of bimanual common-goal movements is needed, considering the everyday use of common-goal movements in our life. The second goal of my thesis serves as an intermediate step before clinical implementation of tSCS for improving sensorimotor rehabilitation. A number of previous studies reported the effectiveness of tSCS in improving both upper and lower limb function after spinal cord neurotrauma (Barss et al., 2022; Y. Gerasimenko et al., 2015; Sayenko et al., 2018). In this thesis, I wanted to understand the neural substrates of tSCS driving the functional improvement observed in other research studies. To serve this purpose, the neuromodulatory effect of tSCS at different levels of the central nervous system including on cortical, cortico-spinal, spinal, and propriospinal circuitry was thoroughly investigated.

I focused on bimanual coordination which requires integration of the left and right limbs to achieve a purpose. Bimanual coordination is a consequence of the human's integrative motor, perceptual, and cognitive ability which ultimately serves various goals. Also, as Swinnen mentioned, "bimanual skills form an entry point for the study of higher cognitive functions in perception and action, even including executive functions" (S. P. Swinnen & Gooijers, 2015). It was on this basis that I chose to investigate some unanswered questions regarding bimanual coordination. Chapter two of this dissertation addressed questions associated with goal-conceptualization and task complexity. The results demonstrated a distinction in behavioral and neural correlates between bimanual common-goal and dual-goal movements. Increased movement time (in the presence of a cognitive load) and error was observed during the dual-goal movement relative to the common-goal movement. The differences in behavioral outcome of dual-goal versus common-goal movement are rooted in how action goals are conceptualized. Attentional demands and spatial interference (caused by neural cross talk) deteriorate movement kinematics during dual-goal movement. On the other hand, due to a different conceptualization strategy during common-goal movement, the effect of spatial interference is canceled. Sensorimotor cortical activation in the alpha band increased during the common-goal task compared to the dual-goal task. Execution of the common-goal movement was accompanied by elevated interhemispheric connectivity. This emphasizes that the two hemispheres are in a state of stronger coupling and tighter communication to facilitate the purpose of reaching toward a unified goal. Moreover, the addition of cognitive load increased movement time (except in the common-goal condition) and movement error, compared to the movements without a cognitive load, but had no significant effect on electrophysiological outcomes. While there was a trend without statistical significance in favor of stronger cortical activation for the task with cognitive load, I speculate that the neural correlates of the higher task demand with the addition of cognitive load can be tracked in other cortical and subcortical regions such frontal cortex, parietal cortex, supplementary motor area (SMA) and cerebellum.

Previous studies of bimanual coordination have been limited by experimental designs that did not allow for exploration of multiple aspects of coordination, and the interplay of them. For example, the issues of movement complexity (Jäncke et al., 2000), attentional demands (Riek et al., 2003), goal-conceptualization (Liao et al., 2018), and lateralized contribution (Asai et al., 2010) in bimanual coordination have been addressed individually and not in relation to the other aspects. This project highlights the fact that the interplay of multiple factors forms the outcome of a bimanual action, and consequently the activation of distinct neural mechanisms. The outcomes of the work in chapter 2 suggested that goal-conceptualization and movement complexity in bimanual coordination are two critical factors in determining movement kinematics, cortical sensorimotor activation, and interhemispheric connectivity. As Ivry and colleagues suggested, “bimanual coordination and interference depends critically on how these actions are represented on a cognitive level" (R. Ivry et al., 2004). Additionally, while divided attention during dual-goal movements leads to negative impact on movement kinematics, the increased attention contributes to successful performance of common-goal movement (Duque et al., 2010). Lastly, it has been suggested that kinematics of bimanual movements benefit from the combination of two internal models of the two limbs (i.e., hemispheric specialization) (Asai et al., 2010). However, findings of this project suggest that the idea of hemispheric specializations and their contribution to bimanual movements should only be interpreted in the context of goal-conceptualization; the two hemispheric specializations are employed for improved movement kinematics only during common-goal, and not during dual-goal tasks. Collectively, the knowledge gained from this project comprehensively informs the motor control of upper limbs during bimanual coordination with different end-goals and complexity.

One of the complications after neural damage to the nervous system is deficits in generating unimanual and bimanual arm movements. The use of transcutaneous spinal cord stimulation has led to measurable functional gains, but a comprehensive understanding of the neural basis of these functional improvements remains unclear. Chapters 3, 4, and 5 focused on addressing some of the mechanisms of action of tSCS. Previously, EMG-based approaches such motor evoked potentials (MEPs), cervicomedullary evoked potentials (CMEP), spinally evoked potentials, and H-reflexes were used to study neural structures recruited by tSCS (Benavides et al., 2020; Y. Gerasimenko et al., 2015; Milosevic et al., 2019; Parhizi B, 2021). As a neuromodulatory modality applied at the spinal cord, past research studies were mostly motivated by understanding the possible neural structure activated at the spinal cord level. Chapter 3 examined the alteration in the sensorimotor "cortical" activation during unimanual and bimanual coordination induced by cervical tSCS. This study deployed EEG recordings, and showed a suppressive effect of tSCS on both the alpha and beta band sensorimotor cortical activation. It should be noted that suppression of cortical activity occurred in the presence of a 10 kHz carrier frequency embedded in each stimulation pulse. This result was in line with previous findings suggesting a suppressive effect of tSCS when applied with a carrier frequency (Benavides et al., 2020) (McGeady et al., 2022). While the question of whether the suppressive effect is advantageous for improving upper limb function or not needs to be addressed in future studies, it was interesting to see a decrease in movement time and error during a bimanual common-goal task in the presence of cervical tSCS. The results also showed an increase of sensorimotor interhemispheric connectivity. These findings illustrated the remote effects of cervical tSCS on cortical networks and is promising for improving movement kinematics. This knowledge can be translated to the clinical to maximize the benefits of rehabilitation interventions in the future. In this study, I strived to capture changes in tSCS-driven cortical neural dynamics during unimanual and bimanual movements, but the lack of two additional measures is the limitation of this study. Short-interval intracortical inhibition (SICI) and interhemispheric inhibition (IHI) are two transcranial magnetic stimulation (TMS)-based techniques that inform motor cortical physiology and intracortical excitatory and inhibitory interactions. The use of SICI and IHI could have provided additional knowledge about cortical activity and interhemispheric connections in the primary motor and somatosensory regions. I also did not test the effect of tSCS on cortical mechanisms without a 10 kHz carrier and with different stimulation frequencies such 30 and 25 Hz. These two additions should be investigated in the future studies.

Although the first two projects in chapter 2 and 3 were conducted in the context of basic neuroscience research, the clinical implications of the studies are consequential. Bimanual rehabilitation training and tSCS are two interventions used for treatment of deficits after neurological conditions such as stroke and SCI (Inanici et al., 2018; Sleimen-Malkoun et al., 2011). Nonetheless, it is still debated whether changing the rehabilitation interventions from unimanual to bimanual training can promote improved recovery of bimanual upper limbs function or not (S. Kantak et al., 2017). Part of the reason can be attributed to the lack of knowledge in correctly identifying the nature of bimanual deficits. In this regard, chapter two proposes experimental tasks that not only mimic real world movements, but also help in the identification of deficits after neural damage and injury to the nervous system. These tasks take into account both issues of "coupling" and "symmetry breaking" suggested by Sleimen-Malkoun and colleagues as critical factors for designing more efficient bimanual trainings (Sleimen-Malkoun et al., 2011). "In the quest for evidence and guidelines concerning the appropriate use and settings of BMT [bimanual movement training] protocols, "coupling" and "symmetry-breaking" are very promising concepts to guide researchers (randomized controlled research studies) and therapists (individualized rehabilitation protocols)" (Sleimen-Malkoun et al., 2011). Moreover, it has been established that tSCS promotes the recovery of the upper limb function after SCI (Inanici et al., 2021). While everyday life is intertwined with bimanual movements, only lateralized effects of tSCS on upper limb function have been previously addressed. Findings of chapter 3, for the first time, report the potential of tSCS in improving the performance of bimanual common-goal movements, supported by tighter communication between hemispheres reflecting increased connectivity.

At the spinal cord level, tSCS is thought to act by activating sensory afferents at the site of stimulation. With the literature mostly concerned with the local neurocircuitry activated by tSCS, I wanted to explore how tSCS influences other networks within the central nervous system. I hypothesized that tSCS engages neural networks beyond the site of stimulation including propriospinal and corticospinal connections. This area has been overlooked in the past and is of paramount importance for motor control and restoration of arm movement. Chapters 4 and 5 investigated this hypothesis. H-reflexes and MEPs were used as probes to answer two questions: 1) does cervical and/or lumbar tSCS modulate the level of coupling between these two segments of the spinal cord? 2) does cervical tSCS change the amplitude of corticospinal transmission to this segment? These two questions were inspired by the idea of interlimb coupling (E. P. Zehr et al., 2016). Cervico-lumbar coupling mediated by propriospinal connections facilitates the task of locomotion. The reciprocal connections between cervical and lumbar segments of the spinal cord are damaged after SCI (Rui Zhou et al., 2018). Excitingly, cervico-lumbar coupling can be improved with arm and leg cycling (R. Zhou et al., 2018). Moreover, rhythmic input to the cervical/lumbar segment through cycling modulates the excitability of the remote lumbar/cervical region of the spinal cord (R. Zhou et al., 2018). Therefore, it was hypothesized that similar to rhythmic input, segmental tonic activation of the spinal cord via tSCS can modulate spinal and corticospinal excitability of the remote segment by presumably acting on propriospinal connections. The Results in chapter 4 demonstrated that a combination of tonic electrical stimulation inputs delivered at both lumbar and cervical sites facilitates the corticospinal pathways to the cervical cord. The same effect was not observed during single site tSCS, consistent with findings of a previous study (Benavides et al., 2020). This novel finding demonstrates, for the first time, that multisite stimulation of the spinal cord converges to modulate corticospinal projections to the upper limbs with a facilitatory effect. This novel finding may raise the idea of “more is better,” meaning that multisite tSCS can promote functional benefits beyond what can be achieved with single site stimulation. The effect of multisite stimulation is now an active area of research that is still at its initial stages (Darryn A. Atkinson et al., 2022; Moshonkina et al., 2021; Samejima et al., 2022).

As shown in chapter 4, tonic tSCS input to the lumbar spinal cord facilitates the H-reflex pathway of the flexor carpi radialis muscle by modulating the remote cervical segment of the spinal cord. Chapter 5 demonstrated that the effects of tSCS on the cervico-lumbar coupling is bidirectional, but the modulation is in opposite directions. Instead of facilitating the soleus muscle H-reflex pathway, tonic tSCS delivered at the cervical level suppresses the H-reflex amplitude. With the suppression of the soleus H-reflex by tonic activation of the cervical level, one might assume that tSCS acts on similar propriospinal interneurons activated by rhythmic arm cycling since arm cycling also suppresses the amplitude of the soleus H-reflex (chapter 5). However, lumbar tSCS produced facilitation of the cervical H-reflex instead of the suppressive effect seen by rhythmic leg cycling (chapter 4). Therefore, tSCS alters excitability in a bidirectional but non-reciprocal manner. Findings of chapters 4 and 5 support the engagement of interlimb coupling networks via tSCS and the possibility to modulate cervical and lumbar motor output via tonic activation of the remote segment. Thus, spinal segmental neural circuits can be influenced through interlimb arm and leg connections. These findings were later supported by Atkinson et at. (Darryn A. Atkinson et al., 2022).

Taken together, the results in chapters 4 and 5 demonstrate that tSCS neuromodulates pathways related to the control of upper limb movements not only at the site of stimulation, but also engages propriospinal and corticospinal connections. In other words, spinal sensorimotor networks related to the control of the upper limbs can be modulated by activation of lumbar networks related to the control of the lower limbs. Also, intersegmental effects brought by segmental activation of the cervical or lumbar spinal cord marks the potential of tSCS in neuromodulating the motor control of both the upper and lower extremities. Beyond arm and hand function, the results are promising for enhancing locomotor capacity in individuals with neural injuries, parallel to the walking improvements seen with arm and leg cycling (Rui Zhou et al., 2018). Both chapters 4 and 5 are not without limitations. One limitation is the lack of inclusion of persons with neurological conditions as study participants, especially persons with

SCI. It remains important to assess the outcome measures on a cohort of participants with SCI where not only the cervical or lumbar networks are damaged, but also the cervico-lumbar coupling is impaired. This allows for exploring the potential of tSCS in restoring the intersegmental connections and improving cortico-spinal transmission. Moreover, chapters 4 and 5 cannot elaborate on the role of cutaneous input on the neuromodulatory effects of tSCS. Furthermore, only a modulated waveform consists of a 10 kHz carrier frequency was used. As previously reported (Benavides et al., 2020), the underlying neural mechanisms of tSCS with and without carrier frequency are different. Here, a comparison of the effects when tSCS is administered with the modulated (i.e., with 10 kHz) versus unmodulated (i.e., without 10 kHz) waveform is lacking.

Overall, this dissertation unraveled some of the fundamental questions that are important for the development of future therapeutic approaches. There are three active elements throughout this thesis: upper limb motor control, bimanual coordination, and transcutaneous spinal cord stimulation. Each chapter of the thesis incorporated one or more of these elements. The studies in this dissertation provide compelling evidence regarding 1) the neural markers of the role of goal-conceptualization and movement complexity during bimanual movements (chapter 2), and 2) the multi-level impact of tSCS across the central nervous system on pathways related to the motor control of upper limbs (chapters 3,4, and 5). The importance of the results lies in the unwinding of the neural mechanisms underlying bimanual arm movements and how non-invasive stimulation of the spinal cord may potentially be used for enhancing upper limb movements. These findings are crucial for advancing future electrophysiological and clinical research studies by informing a) the correct behavioral and neurophysiological nature of bimanual coordination which can guide accurate identification and restoration of deficits after neural injuries and diseases; and b) the influence of tSCS at different regions of the central nervous system which can guide targeted therapeutic interventions.

This dissertation inspires a number of future investigations that could benefit the field of systems and rehabilitation neuroscience. The following research activities are proposed:

A) A comprehensive comparison between the neural correlates of bimanual movements found by non-invasive brain recordings and neuroimaging (e.g., fMRI) to more invasive methods (e.g., electrocorticograms): In this thesis, I used EEG to record and measure cortical activity during execution of upper limb movements. EEG offers a non-invasive measurement of electrical activity of the brain with high temporal resolution. But the spatial resolution of EEG recordings is limited by the volume conduction issue. Findings of this thesis were sensor-based which did not allow for accurate mapping of cortical regions and access to deeper and subcortical areas. The important role of SMA (Obhi et al., 2002) and cerebellum (Tracy et al., 2001) in bimanual coordination has been previously addressed, especially during movement with higher complexity. fMRI-based recording of cortical and subcortical activity offers high spatial resolution which can accurately reveal the contribution of most important regions during bimanual coordination task, especially those that are not accessible with EEG recordings.

B) The effect of SCI on bimanual movement with different goal-conceptualization strategies and multi-levels of complexity: Numerous studies have previously investigated the impact of stroke on bimanual coordination (S. Kantak et al., 2017). In line with the findings of this thesis that emphasizes the importance of goal-conceptualization in bimanual movements, deficits in bimanual coordination in individuals with stroke depends on how the task goal is conceptualized (S. Kantak et al., 2016). In contrast, the literature of bimanual coordination after SCI is very limited. Bilateral movement asymmetries have been indicated after SCI (Britten et al., 2017). Future studies should characterize how SCI influences different aspects of coordination. Next steps should include accurate identification of deficits with task design that takes into account the issues of goal-conceptualization and task complexity.

C) The impact of bimanual rehabilitation training on elevating the functional state of the upper limbs compared to conventional unimanual training: the effectiveness of bimanual interventions is still unknown in the field. Previous findings concluded that bimanual training is equally effective as unimanual interventions after SCI and stroke (Coupar et al., 2010) (L. R. Hoffman & Field-Fote, 2010). However, bimanual maneuvers can be of different natures and training protocols should be tailored to the specific bimanual deficits. I propose that future investigations should compare the effect of common-goal versus dual-goal bimanual training after neurological impairments on the recovery of bimanual coordination. With asymmetric deficits observed in previous studies, it is also important to assess both lateralized and bilateral improvements after bimanual rehabilitation interventions.

D) The impact of a hybrid rehabilitation paradigm that incorporates both bimanual training and tSCS on the recovery of the upper limb function compared to conventional approaches: The use of hybrid strategies to augment the outcome of rehabilitation training is gaining momentum. For example, combination of exoskeleton training with lumbar tSCS is capable of producing stronger lower limb muscle activation (Y. Gerasimenko et al., 2015). For upper limb movement, a combination of cervical tSCS and activity based physical therapy led to both immediate and long-term recovery of upper extremity function following SCI (Inanici et al., 2018). Although promising, previous training activities are mostly unimanual and do not represent some of the real-world actions. Bimanual training combined with cervical tSCS should be included in future studies. An interesting addition may be to replace conventional activity based physical therapy with exoskeleton-assisted training paradigms. KINARM is an example of an upper limb exoskeleton that has the ability to produce bimanual movements with the capability of adjusting the amount of assistance provided to the user.

E) Design of brain-computer interface (BCI)-based bimanual training and the utility of BCI-based approaches in improving upper limb movements compared to conventional methods: With the recent advances in the field of BCI, widespread use of BCI-based rehabilitation interventions is foreseeable in the near future. Currently, researchers have shown the feasibility and safety of BCI-based functional electrical stimulation as a novel method of rehabilitation with promising upper limb functional outcomes (Jovanovic et al., 2021). This approach, however, is limited by its EEG-based switch-on design which triggers a set of stimulation parameters that result in a pre-defined movement. In the future, the intention of the user can be accurately decoded from the brain recording using state-of-the-art artificial intelligence methods which increases the automaticity of the approach and reduce the reliance on the therapist. Such design can then be compared with conventional training paradigms to measure the potential efficacy of BCI-based training for the recovery of bimanual upper limbs motor function.

F) Effect of arm and leg cycling training combined with tSCS on interlimb networks after SCI: An important future step is to investigate how arm and leg cycling combined with tSCS can influence interlimb coupling relative to arm and leg cycling alone. In this thesis, the ability of tSCS to modulate interlimb coupling networks in neurologically intact individuals was demonstrated. Moreover, a previous study showed that impaired interlimb coupling can be improved with arm and leg cycling training (R. Zhou et al., 2018). I anticipate that the effect of arm and leg cycling will be strengthened with the addition of tSCS leading to increased restoration of interlimb coupling.

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