Using Stimulation and Repetitive Task Practice to Promote Neuroplasticity Targeted at Improving Hand Function in Individuals with Chronic Tetraplegia.

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USING STIMULATION AND REPETITIVE TASK PRACTICE TO PROMOTE NEUROPLASTICITY TARGETED AT IMPROVING HAND FUNCTION IN INDIVIDUALS CHRONIC TETRAPLEGIA

By Joyce Gomes-Osman

A DISSERTATION

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the requirements for the degree of
Doctor of Philosophy

USING STIMULATION AND REPETITIVE TASK PRACTICE TO PROMOTE
NEUROPLASTICITY TARGETED AT IMPROVING HAND FUNCTION IN
INDIVIDUALS CHRONIC TETRAPLEGIA

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Injury to the cervical segments of the spinal cord causes significant functional deficits to the upper extremities, affecting the performance of daily life activities and quality of life. Functional impairments following a cervical spinal cord injury (SCI) are primarily attributed to the damage to ascending and descending tracts that hinders the flow of information to and from supraspinal centers and the hand muscles, but there is also evidence that post-injury patterns of cortical reorganization can also play an important role. This thesis explored the influence of different approaches used to activate the motor cortex combined with repetitive task practice, and assessed their effects on hand function and corticomotor excitability. In chapter 1, we explored the literature regarding the primary processes (the lesion itself) and secondary processes (cortical reorganization and anatomical changes) that may further contribute to deficits in hand function after tetraplegia. In addition, we explored the evidence regarding the use of stimulation approaches that either target the motor cortex directly (repetitive transcranial magnetic stimulation [rTMS] and transcranial direct current stimulation [tDCS]), or indirectly (transcutaneous electrical nerve stimulation [TENS] and peripherally applied vibration [VIB]) for their effects on hand function and corticomotor excitability and thus, their potential as adjunct tools in neurorehabilitation.
In the study described in chapter 2, we assessed the safety and late effects of a multi-day intervention consisting of a novel electrode montage using tDCS aimed at increasing bi-hemispheric cortical excitability (and thus, targeting the bimanual motor deficits observed after SCI and the cortical activation that exists in bimanual movements) interleaved with bimanual typing task performance in neurologically healthy participants. We found that a multi-day intervention consisting of 5 days of BAC-tDCS was associated with significantly greater gains in bimanual typing performance when compared with sham-tDCS, which was not retained after one week.

In chapter 3, we describe a study where we assessed the late effects of a 3-day intervention of 10 Hz rTMS (a frequency that is associated with increased corticomotor excitability) interleaved with performance of a fine motor task on hand function and cortical excitability in individuals with tetraplegia and neurologically healthy controls. We found that participants who received rTMS made greater improvements in skilled hand function and assessed by the Jebsen-Taylor Hand Function Test and grasp force than participants who received sham-rTMS. The improvement in skilled hand function was not accompanied by changes in pinch grip force or corticomotor excitability.

In Chapter 4, we undertook a study to assess whether there are differences in outcomes of repetitive task practice when combined with each of the clinically accessible approaches to increase corticomotor excitability either directly (tDCS) or indirectly (TENS, VIB). Individuals with tetraplegia participated in this crossover study consisting of one session of each stimulation approach, simultaneously delivered with repetitive task practice. We found transient increases in pinch force in the VIB condition, and increases in skilled hand function measured by the Nine-hole Peg Test with the TENS and tDCS conditions.
In addition, TENS and tDCS seemed to have shared similarities in terms of early increases in corticomotor excitability, whereas VIB seemed to follow a different pattern characterized by late increases in corticomotor excitability. Finally, in chapter 5 we explore discuss the results of these studies and their applicability to upper extremity rehabilitation of individuals with tetraplegia.
Dedication

This thesis is dedicated to the wonderful people who helped me along this journey. First to God, who gave me this wonderful opportunity. I would also like to thank my parents, for being the most beautiful example of a cross between love and teamwork. Dr. Edelle Field-Fote, for taking me into her lab and for showing me strength and sweetness, and that it is possible to be a fantastic editor, mentor, scientist, and still be able to have a family role, hobbies and a life. Brian, my wonderful husband and partner in crime, thank you for being so loving and understanding. My brothers: Hilton who found Edelle and got me my internship at the Neuromotor Rehabilitation Laboratory, I will always be grateful to you. You have always been an example of determination and courage; and Conrado who is simply the wisest, sweetest, and best big brother one could ever have. Thank you to all the people who shared their time with me by agreeing to participate in my research studies, I have learned so much from your lives and perspectives. Thank you to Saumitra Sinha Ray, Deena Cillien, Mohamed Hassan, Rachel Monahan and Anna Crawford, and all the other people who passed by the lab and were part of my life during these years, your input has helped me and your smiles have brightened my days.
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CHAPTER 1: ETIOLOGY OF UPPER EXTREMITY DYSFUNCTION FOLLOWING A CERVICAL SPINAL CORD INJURY- ROLE OF REPETITIVE TASK PRACTICE AND STIMULATION.

Introduction

Prevalence and Significance It is estimated that there are approximately 250,000 individuals living with the consequences of a spinal cord injury in the USA, yielding an annual investment of approximately $7,736 billion in health-care costs. Tetraplegia is the most common manifestation of a spinal cord injury (SCI), affecting approximately 6,000 people each year in the United States alone. Damage to the cervical segments of the spinal cord can result in varying degrees of hand impairments, which severely impact the performance of daily life activities and affect quality of life and participation in society. A systematic review of available evidence revealed that improvement in arm/hand function is the highest health priority among those with tetraplegia, who are injured at a mean age of 20 years old, are otherwise healthy, and have the same life expectancy as individuals who do not have disability. Therefore, it is important to improve the scientific knowledge related to the improvements of upper extremity function after SCI.

Overview In recent years, much knowledge has been gained regarding the etiology of arm and hand function impairments that occur after cervical SCI. The primary injury damages ascending and descending tracts, disrupting the flow of information between the muscles, spinal and supraspinal centers. This creates a local impact that also disrupts normal reflex modulation, which can result in abnormal muscle tone (see for a
review). However, evidence suggests that secondary changes in functional reorganization of the sensorimotor areas that occur post-injury may also contribute to the functional deficits observed. Lastly, recent studies utilizing more sophisticated methods of diagnostic imaging have identified structural changes in the cortex (i.e., atrophy) that after chronic SCI.

**Stimulation Approaches** The combination of stimulation and repetitive task practice has been associated with greater gains in skilled hand function than when either approach was used in isolation, and its effects have been attributed to an increase in corticomotor excitation. There are many clinically available stimulation approaches that enable direct corticomotor activation (using mild electric currents applied transcranially via surface electrodes applied on the scalp or by electromagnetic induction of electric currents via transcranial magnetic stimulation) or indirectly (via electric or vibratory stimuli applied in the periphery). Clinically available approaches offer the advantage of direct clinical application, and comparisons between different types of stimulation combined with training on hand function and cortical excitability in individuals with tetraplegia have not been done before. It is our aim to use activity and stimulation to promote increased corticomotor excitability with the aim of increasing motor output and promoting increases in arm and hand function.

**Motor deficits and early recovery after SCI**

*Primary lesion and classification of functional impairments* Functional deficits after SCI are multifactorial. Immediately after the injury there is a disruption of the cellular homeostasis, and several factors contribute to the initial loss of function such as
local ischemia and vasogenic edema, disruption of the cellular membrane potential, accumulation of calcium and concentration of free radicals, and demyelination surrounding the injury site.\textsuperscript{18,19} There is some functional recovery initially after the injury, which is attributed to a reversal of these initial metabolic processes, and remyelination by oligodendrocytes and Schwann cells.\textsuperscript{20} Following the injury, individuals are classified clinically according to the neurological level determined by the American Spinal Cord Injury Impairment Scale (AIS).\textsuperscript{21} The neurological level can be different from the anatomical level (the spinal segment where the injury actually occurred), and is defined as the most caudal level with preserved sensory and motor function.\textsuperscript{21} The AIS classification is performed with a neurologic exam that includes the assessment of the dermatomes to light touch and pinprick stimuli, and the assessment of the strength of muscles that control ten key body movements, assessed via manual muscle testing. In addition to the neurological level, a descriptor indicates if the injury was motor-complete or incomplete, depending on the absence or presence of sensory and motor function below the neurological level. Motor-complete injuries occur when there is no preservation or function in the S4-S5 sacral segments (AIS A), and the remaining categories are AIS motor-incomplete. Individuals classified as AIS level B have preserved sensory function below the neurological level; individuals classified as AIS C have preserved sensation and limited (less than half of the upper extremity key muscles) ability to move against gravity; individuals classified as AIS level D have preserved ability to move against gravity in more than half of the upper extremity key muscles; finally individuals classified as AIS level E do not have measurable impairments in sensory or motor function. In addition to the factors above, there are also zones of
preservation that are accounted for in the AIS exam but not used to compute the actual score.

*Early physiologic recovery* Individuals with motor-complete injuries are reported to make functional improvements, although this is mostly due to the use of compensatory strategies. Improvement is much greater in individuals with motor-incomplete injuries who can take advantage of spared neurons in the spinal cord to bypass lesioned areas and create new functional circuits between supraspinal areas and muscles. This process results in further improvements in function, and is reported to reach a plateau approximately one year post injury.22,23 In practical terms, when compared to individuals with complete injuries, individuals with incomplete injuries have the possibility of reorganizing the sensorimotor system and reassigning new areas to compensate for the loss of function that occurs in the muscles affected by the injury. In addition, in order to assess the efficacy of rehabilitation approaches, individuals must be tested at least one year post-injury, in order to avoid bias introduced by spontaneous recovery.

**Post-injury reorganization, anatomical changes and late physiologic recovery after SCI**

*Learned non-use and mirror activation in stroke* The functional organization of the cortex is dynamic, and prone to changes depending on differences in experience and environmental demands, imposed by an injury, or rehabilitation strategies.24,25 There is constant competition for cortical representation amongst body parts, with greater cortical area dedicated to body parts that are used more often and/or are activated during the performance of tasks with greater complexity.26 Alternatively, areas that are used less
often can “lose” representation. A relevant example of this concept is learned non-use, a term commonly associated with individuals with stroke, wherein the decreased use of the more affected arm due to the functional impairments (and often frustration) leads to decreased cortical representation of the limb and increased representation of the contralateral limb, leading to further decrements in upper extremity function. Analyses of studies carried out to characterize the cortical activation in persons with stroke found that there is decreased excitability in the lesioned hemisphere and increased excitability in the non-lesioned hemisphere. This alteration in the balance of excitatory and inhibitory influences between hemispheres is thought to be associated with a common clinical finding referred to as “mirror activation”, characterized by bilateral corticomotor activation during the performance of unimanual task. This is likely to reflect activity of muscles that the individual is using to compensate for the loss of function, and has been correlated with the severity of functional deficits in individuals with stroke.

Similarities in cortical excitability after stroke and SCI While the etiologies of motor dysfunction are distinct in individuals with stroke and spinal cord injury, there is evidence that the post-injury movement-related cortical activation is similar after stroke and SCI. Kokotilo et al performed a systematic review of the evidence regarding cortical activation in individuals with SCI during actual or imagined movements. Studies that used functional magnetic resonance imaging, positron emission tomography (both of which measure cerebral blood-flow and neuronal metabolic activity) and electroencephalography and positron emission tomography (which measures cortical electrical signals with electrodes placed on the subject’s scalp) were included in the review. The authors concluded that the cortical activation during actual or imagined
movements after SCI was very similar to what has been reported persons with stroke, with increased bilateral activation of secondary motor areas (such as the premotor area, primary somatosensory area, supplementary motor area, parietal cortex and cingulate motor area) and subcortical areas (thalamus, cerebellum, basal ganglia). They also found evidence that with the progression of physiologic functional recovery this initially widespread activation became more focalized, centered on the primary motor area. Taken together, these results suggest that it is possible to draw from the evidence from individuals with stroke, wherein approaches that are associated with increases in corticomotor excitability have been associated with improvements in upper extremity function.

Posterior shift in the center of gravity Kokotillo also concluded that a posterior shift in the center of gravity related to movement was consistently reported in the literature; this was present in 70 out of 83 participants with cervical SCI in the studies included in the systematic review. The authors suggested that the posterior shift was likely to be associated with a loss of axons in M1 from damage to the corticospinal tract, leading to a greater contribution of neurons from S1 to the corticospinal tract and thus, during movement. There is evidence to support that physiologic functional recovery can be accompanied by a shift of movement-related activation to a more anterior direction, toward the cortical motor area. This is relevant in the context of approaches that emphasize use-dependent activity and increases in corticomotor activity, which may have the potential to parallel (and perhaps surpass, if performed with adequate dose) physiologic patterns of functional recovery.
Transcranial Magnetic Stimulation (TMS) TMS is another method used to assess cortical activity, and has the advantage of doing so in a more focal manner. An electrical current is briefly passed through a magnetic coil placed over the scalp, generating an electromagnetic field that is proportional to the rate of change of the electrical current, resulting in depolarization of the underlying neural tissue. When the coil is placed on the scalp overlying the hand motor area in persons without neurologic impairment, it is possible to record a motor evoked potential (MEP) using recording electrodes placed on the distal muscles. MEPs associated with intrinsic hand muscles can be evoked approximately 20 ms after the electromagnetic pulse, as can be seen on Figure 1.1. The first wave of the compound motor evoked potential (commonly referred to as ‘D-wave’) is thought to reflect direct activation of corticospinal neurons at the axon hillock, and the remaining waves (commonly referred to as ‘I-waves’) reflect indirect or transynaptic activation of corticospinal neurons. Demyelination and damage to the corticospinal tract causes decreases in conduction velocity and has been reported to influence the increases in MEP thresholds and latencies reported after SCI.

Decreased Conduction Speed Davey et al demonstrated that the shape of MEPs obtained from individuals with SCI was altered, when compared to those obtained from neurologically healthy controls. MEPs of individuals with tetraplegia appeared to be more “fragmented,” likely associated with the decreased rate of information flow, and increased summation of transynaptically activated ‘I-waves,’ which are more easily activated in slower-conducting axons. This distortion of the MEP shape was consistent with our observations in most (if not all) participants in the present studies (Figure 1.1). Note the presence of multiple peaks in the MEPs of a subject with tetraplegia when
compared to a control subject. The decreased flow of information through the corticospinal tract is likely to contribute to the decreased ability to activate muscles affected by the injury,\textsuperscript{40} and practical implications of these findings are that approaches increasing cortical excitability may be able to increase motor output by causing a build-up of charge at the upper motoneuron. This is likely to increase the rate of occurrence of action potentials through the corticospinal tract and increase the drive to the lower motoneurons and muscles.

\textit{Alterations in excitability and corticomotor representation} Studies with TMS have provided evidence that muscles affected by the injury have decreased excitability,\textsuperscript{40} which is likely to reflect the deafferentation and deaferentation resulting from the injury, in addition to the \textit{learned non-use}. There is evidence to support that a reduction of movement-related information from acute periods of immobilization and experimentally induced reduction of afferent information, using regional anesthesia, are both associated with decreases in corticomotor excitability of the targeted muscles.\textsuperscript{42,43} In counterpart, muscles rostral to the lesion have increased motor maps measured with TMS within days of a complete cervical SCI.\textsuperscript{44} While the exact relationship between changes in cortical organization and function is not fully elucidated, individuals who exhibit greater functional recovery demonstrate an increased volume of cortical activation in the primary motor cortex (M1), while those who demonstrate less functional recovery maintain a decreased volume of cortical activation.\textsuperscript{45}

\textit{Anatomical changes after SCI} In order to gain further insight into the relationship between hand function, cortical organization changes and anatomical changes, Freund et al\textsuperscript{12} used voxel-based morphometry (which measures grey matter volume changes such as
cortical surface area and thickness) and voxel-based cortical thickness (which measures
cortical thinning) to examine the degenerative changes in white and grey matter volume
in the spinal cord, sensorimotor cortex and subcortical regions of interest within the
corticospinal tract of individuals with complete and incomplete chronic tetraplegia.
Individuals with chronic tetraplegia had a 30% decrease in spinal cord area when
compared with controls, and the extent of atrophy was positively associated with
decreased hand function measured by the Nine-hole Peg Test (NHPT). In addition,
investigators reported a reduction in white matter volume in the corticospinal tract, grey
matter volume reduction in the sensorimotor cortex, and cortical thinning in the
sensorimotor areas. Authors suggested that spinal and corticomotor atrophy could have
been caused by a number of factors, including: retrograde degeneration, decrease in
angiogenesis or alterations in connectivity due to alterations in the shape or morphology
of dendritic spines in M1. The atrophy in the sensory cortex was attributed to reduced
cellular activity, which is a finding consistent with studies in experimental models of SCI
in a primate model. Stimulation can be used to promote adequate levels of cellular
activity of the sensory cortex, and evidence from experimental models suggest that motor
learning is associated with an increased density of dendritic spines in M1, thus possibly
reducing the atrophy in the sensorimotor cortex after SCI.

In a follow-up study performed with diffusor tension imaging (a novel non-
invasive technique that can assess cellular microstructure), Freund et al found
degeneration of axons and myelin within M1 and the corticospinal tract after chronic SCI
measured by alterations in fractional anisotropy (which assesses the axonal count and
myelin content), axial diffusivity and radial diffusivity (which assess integrity of axons
and myelin through the water diffusion across axons). There is evidence in experimental models of stroke in rats that when animals engaged in repetitive task practice, there was a significant reduction in the amount of degeneration of cortical tissue.\textsuperscript{52,53} The applicability of these findings to individuals with SCI is that the use of repetitive task practice may decrease the cortical atrophy and thus contribute to improving hand function in SCI.

**Activity-based and stimulation approaches to promote upper extremity improvement after tetraplegia**

*Mechanisms for improvements* A specific electrophysiologic mechanism is proposed. Studies using TMS to assess the corticospinal tract after a cervical SCI have observed increased latencies in MEPs acquired from the upper extremities, suggesting a decrease in the rate of information being sent to the lower motoneurons and muscles.\textsuperscript{40,41} Tseng et al\textsuperscript{54} confirmed this idea in a study of axotomized (i.e., transected) neurons of the corticospinal tracts at different time points post-injury (3 months, nine months, 1 year) using a paradigm that consisted of retrograde labeling and in vitro intracellular current injections. Figure 1.2 refers to is a corticospinal neuron 1 year after axotomy that was stimulated with different electrical current intensities. Note that systematic increases in electrical current were associated with complex excitatory post-synaptic potentials, which led to increases in the number of recorded action potentials. We believe that the mechanisms underlying functional improvements induced by therapeutic approaches including task-specific training and stimulation targeted at increasing cortical excitability of M1 after SCI are due to a “build-up” of charge (similar to what is shown by Tseng et al\textsuperscript{54}), likely to increase the occurrence of action potentials in the corticospinal tract, and
thus increase the output to the lower motoneuron, creating the possibility of increasing the overall output to the muscles.

While there is no direct evidence of this proposed electrophysiologic mechanism in humans after SCI, one study in rats with a thoracic-level transection who participated in stepping training has provided evidence supporting this idea. Rats that were classified as successful responders to the training (indicated by the high number of steps taken in a session) demonstrated an increased propensity of motoneuron firing measured by increased excitatory post-synaptic potentials and decreased afterhyperpolarization. It is widely accepted that locomotor behavior relies heavily on spinal central pattern generators (unlike hand function, which relies heavily on corticospinal function), but there is data to support contribution of the corticospinal tract during walking. Therefore, bringing the motoneurons to threshold is likely to be one main mechanism to enable easier activation of muscles by the central pattern generator (when walking) or by the motor cortex (when performing hand activities).

Somatosensory stimulation Evidence suggests that the sensory cortex has a meaningful role in the excitation of the motor cortex that occurs during movement. Evidence from retrograde labeling of corticomotor neurons that were responsive to afferent input revealed direct cortico-cortical connections from neurons in the primary sensory cortex to M1, in addition to direct projections from the thalamus to the motor cortex. In addition, the sensory cortex is known to contribute to the corticospinal tract. Somatosensory stimulation (SS) consists of the application of submotor threshold electrical stimulation with predefined parameters (10Hz, pulse duration 1 ms, 50% duty cycle) aimed at optimally activating the large diameter sensory fibers. When SS is
applied to the skin overlying the ulnar or median nerves, there is increased blood-oxygenation-level-dependent signals acquired with fMRI\textsuperscript{61} (and thus increased corticomotor activation). Increases in pinch strength and skilled hand function have also been reported in individuals with stroke\textsuperscript{62,63} and SCI\textsuperscript{14}.

**Massed Practice Training in isolation and combined with SS** The studies of Beekhuizen & Field-Fote\textsuperscript{14,64} and Hoffman and Field-Fote\textsuperscript{65} utilized massed-practice training, a task-specific approach similar to constrained-induced therapy,\textsuperscript{66,67} widely used in individuals with stroke. Massed practice training consists of task-specific activities that are practiced with high intensity (usually for 2 hours daily, five days a week for 3-4 weeks), organized in 20 minute blocks where individuals work on activities that require different movement components (grip, grip with rotation, pinch, pinch with rotation, and gross motor).\textsuperscript{14,64,65} Individuals with chronic tetraplegia who participated in a massed-practice training intervention in isolation or combined with somatosensory stimulation (i.e., peripherally applied electrical stimulation at an intensity just below that required to elicit a visible twitch) demonstrated significant changes in skilled hand use.\textsuperscript{64} A follow-up study demonstrated that the combination of repetitive task practice and somatosensory stimulation led to greater gains in skilled hand function than when either approach was used in isolation.\textsuperscript{14} Taken together, these results suggest that that stimulation can have a potent effect on motor control, and repetitive task practice combined with stimulation in the form of somatosensory stimulation may induce complementary effects.

**Disadvantages associated with SS** Despite the positive effects reported in the literature, the direct clinical applicability of somatosensory stimulation is limited. Most clinically available peripheral stimulation devices cannot deliver the appropriate pulse
duration (1000μs) that is likely to be associated with the effects on corticomotor excitability and function. SS also requires at least 45 minutes to induce its effects on corticomotor excitability. In counterpart, most rehabilitation professionals have access to stimulation approaches in their current practice, and it is clinically meaningful to assess the effects of clinically available stimulation approaches on corticomotor excitability and upper extremity function. Stimulation techniques such as transcutaneous electrical nerve stimulation (TENS) and peripherally applied vibration (VIB) are clinically accessible devices that may be associated with increased cortical excitability and improved hand function, and therefore represent an alternative for somatosensory stimulation.

**Indirect approaches to increase corticomotor excitability**

*TENS* Evidence suggests that peripherally applied electrical stimulation, in the form that is often used in pain management, can exert effects on corticomotor excitability. The analgesic effects of TENS are attributed to activation of large-diameter (mechanoreceptor) fibers and inhibition of small-diameter (nociceptor) fiber transmission. Meesen et al assessed the effects of TENS (biphasic symmetrical rectangular pulse-wave at 100 Hz, 250 μs pulse width) in neurologically healthy individuals (daily one-hour sessions over 3 weeks) and found significant enlargement of the corticomotor maps acquired with TMS, while no change was observed in the control group. The activation of large diameter sensory fibers and increases in excitability are two similarities between somatosensory stimulation and TENS, suggesting its potential in influencing hand motor function.
VIB Vibration provides a strong proprioceptive stimulus that activates the primary and secondary muscle spindle afferents,\textsuperscript{70} which increases sensory drive. Preliminary data suggests that a single 2-hour session of local muscle vibration (80 Hz) is associated with transient changes in pinch force in individuals with tetraplegia.\textsuperscript{71} VIB has been associated with synergistic effects when combined with task-specific practice. Marconi et al assessed the effects of a conventional physical therapy intervention in individuals with chronic stroke that was either administered in isolation or preceded by brief periods of VIB (100Hz, 0.2-0.5mm amplitude, in 10 min sessions daily for 3 consecutive days).\textsuperscript{17} Investigators found that the combined approach was associated with greater gains in motor function and increased area of motor maps of vibrated muscles acquired with TMS.\textsuperscript{17}

The frequency selection is also in accordance with earlier studies\textsuperscript{72-75} which have showed that vibration at 80-100 Hz activates the Ia spindle in a one-to-one ratio. Burke et al\textsuperscript{72} also found that the secondary spindle fibers were also responsive to 80Hz vibration. It is likely that the effects of VIB on motor performance are due to the resultant increase in cortical excitability, demonstrated by Marconi et al\textsuperscript{17} and other investigators.\textsuperscript{76-79} While in those studies VIB was delivered to the muscle belly, other studies have found that cortical activity is also increased when vibration at 83Hz is applied on the tendon.\textsuperscript{80,81} While stimulation is aimed at activating the muscle afferents, cutaneous mechanoreceptors are also likely to be activated and process the vibratory input. In specific, the Pacinian Corpuscles can respond to vibration with frequencies in the range of 30-500Hz,\textsuperscript{82} mediated by the rapidly adapting type 2 units. The Aβ sensory fibers have a conduction velocity of 36-72 meters per second.\textsuperscript{82} The average distance between the
index finger and the spinal cord is approximately 0.8 meters, which will yield a delay between impulses of 12 milliseconds.82 As a result of the conduction velocity of the Aβ fibers and the distance between the index finger and the spinal cord, stimuli delivered at a frequency of 80Hz represent the optimal range for the cutaneous processing of the vibratory stimulus.82

**Direct approaches to increase corticomotor excitability**

*Transcranial direct current stimulation (tDCS)* TDCS consists of the use of monophasic currents applied to the scalp to deliver mild electric current (intensity typically set at 1-2mA) to the underlying cortical areas, exerting a neuromodulatory influence.83 There is decreased membrane threshold in the areas underlying the cathode (facilitating the occurrence of action potentials), and increased membrane threshold in the areas underlying the anode (thus hindering the occurrence of action potentials).83 Given the imbalance in intracortical inhibition between cortices reported after stroke,29 tDCS has been associated with a reestablishment of the “interhemispheric competition” described after stroke.84 In practical terms, movement deficits are hindered by the decreased excitability of the lesioned hemisphere, which is further inhibited by the over-active non-lesioned hemisphere. In this context, tDCS (with the anode overlying the lesioned hemisphere in isolation or combined with the anode overlying the non-lesioned hemisphere) has been associated with a “release” from excessive inhibition and increased excitability of the lesioned hemisphere, which has been accompanied by increased motor function.84 In Chapter 2, we assessed an electrode montage utilizing tDCS that would be useful in the context of the bilateral decreases in corticomotor excitability exhibited by individuals with chronic tetraplegia during bimanual training, which has been associated
with a mutual cortical excitation between hemispheres. In Chapter 4, we also compared the effects of unilateral tDCS to other indirect forms of cortical excitation (TENS, VIB).

rTMS Another approach to target the cortex directly consists of the use of repeated pulses of transcranial magnetic stimulation (rTMS), which can be used to promote excitation when using frequencies above 5Hz and inhibition when using frequencies below 1Hz. A meta-analysis concluded that there is sufficient evidence to support the efficacy of rTMS to improve upper extremity in individuals with stroke. Two studies have addressed the effects of rTMS in individuals with SCI, and found mixed results. Belci et al used a paired pulse protocol (100 ms interpulse interval [i.e., 10 Hz] with 2 pulses every 10s, resulting in 720 total pulses applied in doublets) aimed at decreasing intracortical inhibition (which would result in an overall increase in excitation), and found improvements in motor and sensory function, with some effects evident at 3-week follow-up. Kuppuswamy et al used a 5Hz rTMS intervention intended to facilitate corticospinal output and consisted of 5 consecutive days of 5Hz rTMS delivered in 2 s trains separated by 8 s for 15 min (total of 900 pulses per session) performed while the participants were at rest, and reported no effects of rTMS on hand motor performance post-intervention, despite a delayed increase in corticospinal excitability measured by amplitude of active MEPs, with assessments at 3 days and 5 days after the rTMS intervention.

A systematic review of the available literature concluded that it was not possible to identify an optimal set of parameters for increased function in individuals with stroke, but at least in participants without disability, higher increases in cortical excitability
occurred in studies that employed frequencies at or above 10Hz.\textsuperscript{90} Taken together, and
drawing from evidence from individuals with stroke, it is possible that greater effects
may be observed using higher frequencies than employed in previous studies involving
individuals with tetraplegia. In the study described in Chapter 3, we used high-frequency
rTMS at 10 Hz and assessed its effects when administered along with repetitive task
practice in individuals with tetraplegia and neurologically healthy controls. It is possible
that if administered along with repetitive task practice, rTMS can lead to greater effects.

\textbf{Implications for Research}

After tetraplegia, there is extensive reorganization of the cortical activity related
to movement, which may not be ideal for the optimization of function. In the chronic
stage, muscles affected by the injury “lose” cortical representation, and become less
excitable, contributing to the decreased use of the arms and hands. Intense periods of
task-specific training provide a change in the environmental demands of the nervous
system, and are associated with improvements in skilled hand function that are parallel to
increases in corticomotor excitability. We refer to such changes as neuroplasticity.
Evidence suggests that somatosensory stimulation can potentiate the effects of task-
specific training, and its effects are attributed to increased corticomotor excitability.
Other stimulation approaches can be used to modulate corticomotor excitability, either
indirectly (TENS, PAV) or directly (tDCS, rTMS), but the effects of these approaches on
arm and hand function have not been fully investigated. While all clinically available,
these devices may have direct clinical applications if shown to improve corticomotor
excitability and arm and hand function in individuals with tetraplegia.
The research question addressed in Chapter 2 is: Does a tDCS electrode montage with 2 anodes over both M1 and 2 cathodes over the supraorbital area (aimed at increasing cortical excitability bilaterally) influence a bimanual typing task in neurologically healthy participants? Our aims were to:

Aim 2a. Assess delayed changes (measured after the last session) in bimanual typing task performance following a 5-day approach consisting of daily tDCS and bimanual typing task practice;

Aim 2b. Assess delayed changes (measured after the last session) in short-term memory task performance (a function performed by a cortical area contiguous to M1, and possibly affected by the stimulation) following a 5-day approach consisting of daily tDCS and bimanual typing task practice;

Aim3c. Assess retention of changes (measured 7 days after the last session) in bimanual typing task performance and short-term memory.

The research question addressed in Chapter 3 is: Is a 3-day high-frequency rTMS interleaved with repetitive task practice associated with differences in measures of hand function and corticomotor excitability compared with sham-rTMS interleaved with repetitive task practice in individuals with chronic tetraplegia and a separate group of neurologically healthy participants?

Aim 3a. Assess delayed changes (measured the day after the last session) in pinch grip strength and fine motor performance with the JTT separately for the SCI participants and neurologically healthy participants;
Aim 3b. Assess delayed changes (measured the day after the last session) in corticomotor excitability of M1 using the MEP threshold at rest and under slight contraction and recruitment curve separately for the SCI participants and neurologically healthy participants;

Aim 3c. Assess immediate changes in fine motor performance with the NHPT (assessed daily, used for the repetitive task practice) separately for the SCI participants and neurologically healthy participants;

The research question addressed in Chapter 4 is: Is there a difference in outcomes of a single-session of repetitive task practice combined with each of the clinically accessible approaches for directly (tDCS) or indirectly (TENS, VIB) increasing corticomotor excitability in individuals with chronic tetraplegia?

Aim 4a. Assess early changes (measured immediately at post-test) in pinch grip strength, fine motor performance with the NHPT and visuomotor tracking;

Aim 4b. Assess early changes (measured at post-test) in corticomotor excitability of M1 using amplitude of MEPs at 1.2x Motor threshold under slight contraction;

Aim 4c. Assess late changes (measured 30 minutes following post-test) in pinch grip strength and fine motor performance with the NHPT and visuomotor tracking;

Aim 4d. Assess late changes (measured 30 minutes following post-test) in corticomotor excitability of M1 using amplitude of MEPs at 1.2x Motor threshold under slight contraction.
Figure 1.1 MEP recorded from the thenar muscles of neurologically healthy participant (top) and participant with chronic tetraplegia (bottom). The x-axis represents time (in milliseconds) and the y-axis represents intensity of the response (in millivolts). In both instances, MEPs were evoked while participants were at rest and intensity was set at 70% maximum stimulator output. Note the increased latency and presence of multiple peaks in the participant with tetraplegia.
Figure 1.2 Proposed electrophysiologic mechanism to account for changes in function from participation in approaches consisting of repetitive task practice and stimulation. These are recordings from a corticospinal neuron 1 year after axotomy. The x-axis represents time (in milliseconds) and the y-axis represents intensity of the response (in millivolts). Note that as stimulation was increased, it was possible to record increasingly higher numbers of excitatory post-synaptic potentials, which were associated with increased rate of action potentials. We believe that approaches that increase corticomotor excitation and output and can increase the rate of information flow through the corticospinal tract and thus, increase the overall drive to lower motoneurons and muscles.
CHAPTER 2. BIHEMISPHERIC STIMULATION USING TRANSCRANIAL DIRECT CURRENT STIMULATION IMPROVES BIMANUAL TYPING TASK PERFORMANCE.

Transcranial direct current stimulation (tDCS), the application of low-intensity, monophasic electrical current through the scalp via surface electrodes, has neuromodulatory effects, changing the membrane potential (and therefore the activation threshold) of underlying neural structures. Thresholds are reduced in the vicinity of the anodal electrode, facilitating the occurrence of action potentials, thereby promoting excitation. Conversely, thresholds are increased in the vicinity of the cathode electrode, impeding the occurrence of action potentials, thereby promoting inhibition. TDCS is clinically accessible as it is a simple, non-invasive, and painless modality that seems to have value as a means to modulate cortical excitability.

Studies of bihemispheric tDCS that have utilized the anode and cathode placed on both M1 for neuromodulation have been prompted by the evidence for decreased excitability of the lesioned cortex and an imbalance in interhemispheric excitability that develops after stroke. In these studies, the anode is placed over the motor cortex of the lesioned hemisphere to promote excitation, while the cathode is placed over the motor cortex of the non-lesioned hemisphere to promote inhibition. This approach is associated with increased hand motor performance in tests of unimanual motor function. The bihemispheric anodal-cathodal stimulation approach is logical as a therapy for persons with stroke given that the goal is to increase excitability of the lesioned cortex.
While unimanual tasks are associated with unilateral cortical excitation, there is bilateral excitation during bimanual movements that require simultaneous use of both hands (referred to as asynchronous tasks)\textsuperscript{85} and also during activities requiring alternating finger movements between hands (referred to as asynchronous tasks) such as keyboard typing and playing the piano. The bilateral cortical activity observed with performance of asynchronous tasks has been found to be greater than that observed during performance of synchronous tasks.\textsuperscript{86} In the study of Haslinger et al,\textsuperscript{86} the task required one finger from each hand to be moving continuously. We used a different task, wherein only one finger from each hand was moving while the fingers from the other hand were maintained in pose of readiness in anticipation of ensuing action, such as when one is typing on a computer.

While the outcomes of studies investigating the use of tDCS in persons with stroke are promising, there are neurologic conditions such as spinal cord injury that result in bilateral impairment of hand function. Consequently it seems useful to design interventions targeting the broad spectrum of hand activities that comprise daily life activities that include bimanual hand use.\textsuperscript{96} Given the evidence for improvements in unimanual typing tasks with anodal tDCS, the next logical step is to assess the influence of tDCS aimed at bihemispheric corticomotor excitation on a bimanual task. While we acknowledge that in the use of tDCS, there is always one anode and one cathode contributing to the effects observed, for didactic purposes we will refer to the electrode montage using tDCS with two anodes placed on both M1 and two cathodes placed on the supraorbital areas as bihemispheric anodal corticomotor tDCS (BAC-tDCS). To our
knowledge no prior studies of this type have been completed, therefore, it is necessary to first assess the effects and the safety of this approach in persons without disability.

To assess the effects of BAC-tDCS on bimanual typing performance and the possible retention of these effects, we adapted a previously published protocol that had shown performance changes in unimanual typing performance after multi-day application of uni-hemispheric corticomotor anodal tDCS. As our testing required participants to perform a bimanual typing (BT) task for 30 seconds before and after application of BAC-tDCS, we anticipated that simply performing the BT task as part of testing would result in practice-related improvements; therefore we compared BAC-tDCS to a sham-tDCS condition to account for these practice effects. Another consideration is that tDCS has relatively low focality and may have the potential to influence cortical areas contiguous to the motor cortex that was the target of our studies. Therefore, we included a secondary outcome measure to assess possible influences of BAC-tDCS on short-term memory (STM), a function subserved by the temporal lobe.

Performance of novel bimanual sequences engages a number of different cortical areas, including primary motor, premotor, and supplementary motor areas. While this provides many possible cortical areas to target with tDCS, we based our hypotheses and study design on previously published studies that demonstrated unimanual performance improvements in sequence tasks in healthy individuals who participated in multi-day tDCS interventions with tDCS applied to the primary motor area (M1). Since BAC-tDCS is a novel electrode montage, we purposefully designed the present study to parallel those prior studies, such that the only novel elements were the use of bilateral anodal stimulation and the use of a bimanual task. We hypothesized that following participation
in the 5-day study both the BAC-tDCS and sham-tDCS groups would exhibit improved bimanual typing performance but that change with BAC-tDCS would be greater. We also hypothesized that bihemispheric anodal corticomotor stimulation would have no effect (either beneficial or adverse) on STM.

Methods

Participants

Healthy volunteers gave written and verbal informed consent to participate in the study, which had been approved by the Human Subjects Research Office of the University Of Miami Miller School Of Medicine, Miami, Florida. Recruitment was performed through email to the university community and through verbal recruitment performed by the investigators. Inclusion criteria were: healthy adults (age 19-65 years). Exclusion criteria were: neurological, orthopedic, or cognitive conditions that would affect performance of the BT task and/or STM task. Participants were enrolled sequentially following initial screening and randomized into the BAC-tDCS experimental group or bihemispheric sham-tDCS control group utilizing a random number list, and no attempt was made to stratify participants with respect to their pre-existing bimanual skills. The randomized assignment order was placed in sequentially numbered envelopes and remained concealed until the time of group assignment.

Procedures

The optimal stimulation sites to activate the right and left corticomotor hand areas (i.e., the “hot spots”) were identified using transcranial magnetic stimulation (Magstim200, Dyfed, UK); detailed methods are described elsewhere. The location of each hot spot
was documented using a coordinate system to define the anterolateral and mediolateral distance from the vertex; the site was located each day for placement of the tDCS anodal electrode. Participants underwent 5 consecutive days of either BAC-tDCS or sham-tDCS. TDCS was delivered by two constant current stimulators (Phoresor, Iomed Inc., Salt Lake City, UT) for which the output of the device was concealed from the participants. Intensity was set at 1mA, according to safety guidelines. Four saline-soaked, disposable iontophoresis electrodes (Optima, Iomed Inc, Salt Lake City, UT) were used for the stimulation: 2 active (anodal) electrodes with an area of 28 cm$^2$ each were placed on the scalp over the hot spot (resulting in a total current density of 35 μA/cm$^2$), and 2 reference electrodes placed on the supraorbital area bilaterally. The area of the inactive electrodes was approximately 37 cm$^2$.

The sham-tDCS protocol used a previously published method that was identical to the BAC-tDCS condition with the exception that, without the knowledge of the subject, the current was reduced to zero after 30 seconds, and was maintained at zero for the remainder of the stimulation period. Published reports substantiate that the stimulation parameters used in this protocol elicit mild tingling/itching only during the initial seconds of stimulation, fading soon after onset. Our approach allowed the groups to be blinded to the stimulation condition. The duration of the BAC-tDCS and the sham-tDCS period was 20 minutes each day; participants were asked to report any adverse effects experienced during/after stimulation.
Experimental Tasks

*Bimanual Typing Task*

Performance on a keyboard sequence task was the primary outcome measure (Figure 2.1). Using this same task, Vines et al.\(^9\) observed improvement in unimanual performance in a study of bihemispheric anodal-cathodal tDCS in persons without disability. We modified the published protocol to a bimanual asymmetrical sequential task with bihemispheric (anodal-anodal) stimulation to measure the effects of BAC-tDCS on bimanual task performance. As in the study of Vines et al.\(^9\) we used a different sequence on each day in order to foster novelty of the task.

At the start of the BT task, participants were asked to rest digits 2-5 of each hand on the respective “asdf” and “hjkl” keys of a standard keyboard, and an 8-letter sequence (generated utilizing custom software; Matlab, Mathworks Inc) was displayed on a computer screen. All sequences consisted in two keystrokes performed with one hand, followed by two keystrokes to be performed with the opposite hand. This pattern was chosen to best mimic the habitual use of a computer keyboard, wherein it is necessary to alternate use of the hands. The participants were instructed to perform this bimanual pattern of 8 sequential keystrokes as accurately and quickly as possible for 30 seconds. Five trials of the same sequence were captured daily prior to stimulation and after stimulation, and at the follow-up assessment one week after the final stimulation session (Figure 2.2). The first two (warm-up) trials of each capture were discarded; the subsequent three trials were analyzed.
Performance on the bimanual typing task was scored as described by Vines et al., wherein points were awarded for correct sequences, and points were deducted for errors. A correctly typed 8-letter sequence (e.g., “as df hj kl”) was awarded 4 points; correctly typing the sequence 4 times during the 30-second capture epoch earned 16 points. An incorrect letter pair or missing space was scored as an error; the total number of errors was deducted from points earned. This method of scoring addresses the speed-accuracy tradeoff, because if an individual increased the typing speed with a proportional increase in the number of errors, this would not result in an increased score. The difference in the number of correct keystrokes between the Day 1 pre-stimulation test (pre-Day1) to the Day5 post-stimulation test (post-Day5) was our primary measure of cumulative change of motor performance. Retention of effects was assessed through comparisons between pre-Day1 and Follow-up scores, as in other studies performed by our group.

STM Task

Our secondary outcome measure was STM to assess possible effects of BAC-tDCS on temporal lobe function. We used a modified version of a published protocol that reported changes in STM following unilateral excitatory tDCS applied over the temporal lobe. Two series of 27 words were presented on a computer screen. Words in the first series all belonged to the same “theme” (e.g., fruits: apple, pear, strawberry, etc.). Each word remained onscreen for 3 seconds. Subsequently, participants viewed a second series of 27 words comprising 9 words from the first series, 9 words from the same theme as the first series but not included in that series, and 9 unrelated words. While viewing the second series, participants were asked to indicate (by checking ‘yes’ or ‘no’) whether the
displayed word had appeared in the first series. The percentage of correctly identified words was calculated. As with the test of bimanual motor function, this test was performed before and immediately after each of the five consecutive daily BAC-tDCS stimulation sessions, and at the follow-up assessment one week later.

Statistical Analysis

Data were inspected for homogeneity of variance and normality of distribution. Outliers, trials in which the number of errors was greater than two standard deviations from the mean of total errors for all trials for that subject, were removed along with the associated pre- or post-test. Baseline equivalence of the two groups was assessed based on pre-Day1 BT scores. The cumulative effects of BAC-tDCS and sham-tDCS on the BT and STM tasks and the possible retention of these effects were examined by comparing the differences in the magnitude of change between the two groups from pre-Day1 to post-Day5.

Data were analyzed using SAS (SAS Institute Inc., Cary, North Carolina) with significance set at $\alpha<0.05$. The primary purpose of the hypothesis testing in this study was to identify between-group differences in mean performance improvement. A recent systematic review and meta-analysis of the literature reported that anodal tDCS (wherein the anode is applied over the motor cortex) is associated with large effect sizes supporting increased hand motor performance in non-disabled individuals. Based on prior evidence related to the cumulative effects of anodal tDCS (Reis et al., 2009; Vines et al., 2008) and based on the known effects of practice, we hypothesized that there would be an
improvement in performance of in the BT task after the 5-day stimulation period in both groups, but that the BAC-tDCS group would exhibit larger magnitude of change. Given our intention to directly address the question of the size of difference between treatments, and because we had declared a hypothesized direction of expected change, the one-tailed, two-sample t-test was deemed the most appropriate statistical approach. Following testing of the primary hypothesis, post-hoc, within-group analyses were carried out using one-tailed, paired-sample t-tests. Confidence intervals (CI) related to 95% upper and lower limits were calculated. The effect size (Cohen’s d) for each of these comparisons was calculated by subtracting the post scores from the pre scores and dividing this mean change score by the baseline standard deviation.

**Results**

Twenty-eight participants (11 men, 18 women) with a mean age of 27 ±7 (mean ± SD) completed the study. Other than itching (reported by both groups in the initial seconds of stimulation) no adverse effects were reported. Data from one subject was excluded from the analysis, as his BT scores met our rigorous criteria to be considered an outlier (see Methods). The groups were equivalent at baseline with respect to their BT and STM scores, as there were no between-groups differences in baseline performance on either task (p=0.7 for the BT, and p=0.1 for the STM scores). The mean pre-Day1 scores for the BT task (BAC-tDCS=28.6±10.13, sham-tDCS=23.7±8.0) and for the STM task (BAC-tDCS=90%, sham-tDCS=92%) were not significantly different between groups. Based on the results of prior studies we anticipated no within-session change in performance,
However for completeness we also assessed the within-session change in scores and identified no between-groups difference (p>0.05) in these comparisons.

Change in BT score was observed in both groups; post-test scores were BAC-tDCS: 48.07±17.5, and sham-tDCS: 36.21±11.51 (Figure 2.3). The within-group change for each group was associated with a large effect size (d=1.9 and 1.5 for BAC-tDCS and sham-tDCS, respectively). However, as the magnitude of change in BT performance from pre-Day1 to post-Day5 was larger for BAC-tDCS (19.4 points; 95%CI:12.82-25.99) compared to sham-tDCS (12.5 points; 95%CI:7.6-17.3).

For the STM task, mean pre-Day1 scores for the BAC-tDCS and sham-tDCS groups were 0.9±0.3 and 0.92±0.11, respectively. Post-Day5 scores for the BAC-tDCS and sham-tDCS groups were 0.93±0.35 and 0.93±0.08, respectively (Figure 2.4). There was no significant between-groups difference (p>0.05) or within-group differences in STM (p=0.38 for each).

There were no differences in retention of BT change in either group. Although the absolute follow-up scores (mean=28.66 for BAC-tDCS and 23.71 for sham-tDCS) were higher than the pre-Day1 scores in both groups, this difference was not significant (p=0.9).

**Discussion**

In the present study we found that a multi-day application of BAC-tDCS in persons without disability was associated with improved performance of a BT task beyond that achieved by practice alone. The improvements in bimanual performance are consistent with findings of improvements in unimanual performance observed in prior multi-day
studies of uni-hemispheric anodal corticomotor tDCS\textsuperscript{98} and bi-hemispheric anodal-cathodal tDCS,\textsuperscript{95} wherein cumulative effects were observed in the absence of within-session differences. These findings are supported by results of neurophysiologic changes in a multi-day study of anodal corticomotor tDCS.\textsuperscript{106} Alonzo et al,\textsuperscript{106} in a study of 12 healthy participants, found that multi-day consecutive application of tDCS was associated with greater increases in amplitude of motor evoke potentials compared to tDCS application on alternate days. Taken together these studies support the conclusion that multi-day application of tDCS is associated with cumulative or “offline” effects that are responsible for the maintenance of an increased state of corticomotor excitability between sessions.\textsuperscript{98,106} Further, our results are in agreement with the conclusions of a recently published meta-analysis of corticomotor anodal tDCS,\textsuperscript{105} which identified a large effect size associated with unimanual motor function improvement in non-disabled persons (d=0.92 [95%CI:−1.02, 2.87], p=0.35). No effects (positive or negative) were observed on STM function, and no adverse effects beyond itching were observed.

This is the first study to report the use of a bihemispheric anodal-anodal tDCS electrode montage over the motor cortex to improve bimanual hand performance. In this study we used a bimanual typing task that required alternating movements between hands. Bimanual movements are associated with bilateral increased cortical excitability.\textsuperscript{85} While asynchronous bimanual tasks have been studied less extensively than synchronous tasks, there is evidence that cortical activity during performance of asynchronous and synchronous tasks is similar. Haslinger et al\textsuperscript{86} compared the blood-oxygen-level-dependent (BOLD) signals during bimanual tasks using functional magnetic resonance imaging and observed greater cortical activation during the alternating task when
compared to a synchronous bimanual task. Although the task performed in the present study required finger movement from one hand while the other hand was actively held in position (i.e., elbow flexion, wrist extension, finger extension) in anticipation of its next movement, a future study could assess similarities in cortical activity between our task and other bilateral tasks that require simultaneous movement between both hands. In addition, a future study could assess if both tasks could be enhanced to a similar degree with BAC-tDCS.

Theoretically, augmenting practice-related excitability with BAC-tDCS enhances excitatory effects beyond those of bimanual practice alone. This is consistent with previous evidence from our lab regarding the relationship between peripheral electrical stimulation and unimanual motor practice. We showed that in persons with tetraplegia, unimanual motor training combined with peripheral electrical stimulation (which, like BAC-tDCS, is intended to increase corticomotor excitability) is associated with greater improvements in skilled hand function than when either modality is administered in isolation.14 We believe this is the reason for the larger magnitude of change in scores from pre-Day1 to post-Day5 in the BAC-tDCS group.

The mean change for the sham-tDCS group (12.5 points) lies in the lower end of the confidence interval for the BAC-tDCS group, and the latter showed a greater magnitude of mean change (19.4 points). Further, some participants in the BAC-tDCS group demonstrated change values nearly 2 times larger than the mean change in the sham group. In terms of clinical interpretation, these results suggest that the magnitude of improvement achieved with physical practice alone was on the lower end of the range improvements achieved when practice was augmented by BAC-tDCS. While effects
were not retained at one week Follow-up, we believe this may be because participants were non-disabled persons whose hand function was close to optimal and therefore had small margin for change. In theory, retention could be greater in persons with bilateral hand impairment engaging in motor training concurrently with stimulation.

No significant effects of BAC-tDCS on STM were observed. Boggio et al\textsuperscript{104} found significant improvements in STM in non-disabled participants with unihemispheric anodal tDCS applied over the temporal lobe. TDCS exerts local effects at the stimulated site, but it is not a focal brain stimulation modality.\textsuperscript{107} Our primary intent for testing STM was to identify possible non-motor effects arising from activation of cortical areas contiguous to the motor cortex as part of our assessment of safety associated with BAC-tDCS, as this approach has not previously been studied. There was no detrimental (or beneficial) effect of BAC-tDCS on STM function and no other adverse effects were reported by any subject. These results indicate BAC-tDCS was associated with improvements in performance of a bimanual task, and was safe as applied in this investigation.

A limitation of the present study was that we used only performance-based measures. Therefore, we can only infer that there were changes in neural excitability based on related published evidence.\textsuperscript{91} However, the primary intent of this study was to determine whether further study of BAC-tDCS in persons with disability was warranted based on evidence of effects and safety in non-disabled participants. Secondly, the BT task we used required alternating rather than synchronous use of the both hands, and it is possible that results may not generalize to bimanual tasks requiring simultaneous use of both hands. Lastly, we did not stratify the group randomization on the basis of preexisting
bimanual skills, and pre-existing skill may affect task performance and learning. However, the results of testing for baseline equivalence indicated that there were no differences between the two groups at baseline; therefore we believe it is unlikely that differences in preexisting bimanual skill levels influenced the results of this study.

**Conclusion**

The results of this study showed that a 5-day course of bihemispheric anodal corticomotor tDCS applied over the corticomotor hand area had a positive influence on bimanual skilled hand performance in persons without disability. STM was not affected and no adverse effects were reported. Further work is needed to assess this approach in persons with neurological conditions who have bilateral hand impairment.
Figure 2.1 Experimental setup and electrode placement used during tDCS intervention. The anodes were placed on both corticomotor areas identified with transcranial magnetic stimulation, and cathodes were placed on the supraorbital areas, bilaterally (top). Note also the interface used for the bimanual typing task, and a sample sequence used (bottom).
Figure 2.2 Experimental design. Participants completed a total six of sessions. Measurements of BT and STM tasks were obtained daily prior to and following stimulation (BAC-tDCS or sham-tDCS, 20 minutes) for 5 days, and at follow-up.
Figure 2.3 Change in scores for the assessment of acquisition and retention for the BT task by group (solid line=BAC-tDCS, dashed line=sham-tDCS). Magnitude of change in scores between pre-Day1 and post-Day5 was significantly different between the 2 groups (* refers to a p=0.04, for comparison between pre-Day1 and post-Day5). This was not retained at follow-up (one week after cessation of stimulation and practice).
Figure 2.4 Change in STM scores by group (solid line=BAC-tDCS, dashed line=sham-tDCS). There was no significant between-groups difference.
CHAPTER 3. IMPROVEMENTS IN HAND FUNCTION IN ADULTS WITH CHRONIC TETRAPLEGIA AND HEALTHY ADULTS FOLLOWING A MULTI-DAY 10HZ RTMS INTERVENTION.

In the United States alone, there are approximately 123,000 individuals living with the consequences of incomplete tetraplegia, the most common manifestation of traumatic spinal cord injury (SC). Improvement of arm and hand function is ranked highest of all rehabilitation goals in this population. Prior studies of interventions to improve hand function in persons with tetraplegia have used indirect approaches to increase cortical excitability using somatosensory stimulation combined with repetitive task practice.

Repetitive high-frequency (typically 5Hz or greater) transcranial magnetic stimulation (rTMS) is another approach that can be used to directly target the cortex and promote corticomotor excitation (represented by increased motor evoked potentials [MEPs]). However, the effect of rTMS on hand function and cortical excitability in individuals with SCI is not fully understood. Two prior studies have assessed the use of different stimulation protocols using rTMS to directly activate the cortex to improve hand function in persons with chronic tetraplegia due to SCI, and generated mixed results. Belci et al. used a paired pulse protocol intended to decrease intracortical inhibition, which would result in increased cortical activation to the spared corticospinal neurons. Four participants received a 5-day sham intervention followed by a 5-day real intervention of paired pulse rTMS (100 ms interpulse interval [ie, 10 Hz] with 2 pulses every 10s, resulting in 720 total pulses applied in doublets) to the motor cortex. Investigators reported improvements in motor and sensory function, with some effects evident at 3-week follow-up. A subsequent study in neurologically healthy individuals using the same
protocol found transient changes in intracortical inhibition that did not outlast the stimulation period.11

Kuppuswamy et al12 used a 5Hz rTMS intervention intended to directly facilitate corticospinal output in 15 participants with tetraplegia in a sham-controlled cross-over study. This approach is consistent with the evidence indicating that stroke and SCI share similarities in their decreased movement-related cortical activation of muscles affected by the injury,13 and that high-frequency (typically above 5Hz) rTMS is associated with increases in corticomotor excitability and skilled hand function in persons with stroke.9 The protocol consisted of 5 consecutive days of 5Hz rTMS delivered in 2 s trains separated by 8 s for 15 min (a total of 900 pulses per session) performed while the participants were at rest.12 There was no between-condition difference in the change in hand motor performance, despite a delayed increase in corticospinal excitability measured by amplitude of active MEPs, at 3 days and 5 days after the rTMS intervention.12

Most studies that have employed rTMS to increase cortical excitability in individuals with stroke have used stimulation frequencies of 10Hz14,15 or 20 Hz.16,17 Kim et al16 observed increased skilled hand function and associated increases in cortical excitability in participants with stroke following a single session of 10 Hz rTMS delivered in 40 trains consisting of 2-second pulses, with 30 seconds intertrain intervals (wherein individuals practiced a fine motor task), for a total of 800 pulses. We therefore modeled our study on that of Kim et al,16 hypothesizing that stimulation at higher frequencies than those previously employed in studies of participants with SCI would be associated with
greater corticomotor facilitation and therefore greater improvement in hand function. In addition, the practice of a fine motor task during the intervention would provide an opportunity to direct the increased cortical excitability to a relevant functional task.

Our study design differs from that of Kim et al\textsuperscript{16} as we chose to employ a 3-day intervention that could benefit from cumulative effects. In addition, we included a group of neurologically healthy participants to gain further insight into the effects of this protocol in individuals with no neurologic impairment. We hypothesized that a 3-day approach of high-frequency rTMS interleaved with repetitive task practice (RTP) in persons with tetraplegia and neurologically healthy participants would be associated with greater improvements in hand function and cortical excitability compared with sham-rTMS+RTP, and that improvements would be seen at the domains of both “body functions and structure” and “activity” of the International Classification of Functioning, Disability and Health (ICF) framework.\textsuperscript{18}

**Methods**

**Participants**

Participants in the SCI group were recruited through the research volunteer registry of the Miami Project to Cure Paralysis and neurologically healthy participants were recruited from the University of Miami community via verbal recruitment. To be included participants had to be between the ages of 18-65 years old. Additional inclusion criteria for participants with SCI were: at least one year post-injury, neurological level of injury C7 or above, and sufficient voluntary activation to elicit a visible twitch of the thenar muscles in at least one hand. Exclusion criteria were: history of head injury, history or
family history of seizures, metal elements in the head (e.g., aneurysm clip), and presence of any neurologic, orthopedic, or cognitive condition that would affect the performance on the outcome measures. Participants in the SCI group were advised to not make changes to usual level of physical activity or medication regimen. All participants gave written informed consent to participate in the study, which had been approved by the Human Subjects Research Office of the University of Miami Miller School of Medicine, Miami, FL.

Experimental Design

This double-blind, crossover study was performed over two weeks comprising two conditions: rTMS+RTP and sham-rTMS+RTP. Sample size was estimated based on prior studies of rTMS in persons with SCI in which differences between baseline and follow-up were detected based on 4 participants. A larger number of participants were included to compensate for the high attrition rate (35%) reported in an earlier study. Randomization was performed, based on a random number generator, by a member of the laboratory staff who was not otherwise involved with the study. The investigator involved in the outcomes assessment was blinded to the order in which rTMS+RTP and sham-rTMS+RTP were delivered. Randomization was performed separately for each group (SCI and neurologically healthy participants). We did not perform direct statistical comparisons between the two groups, as we were interested in capturing the effects of the protocol separately in individuals with SCI and participants who have a healthy nervous system.
Upon completing informed consent, participants were randomized to receive rTMS+RTP or sham-rTMS+RTP on week 1, followed by the alternate condition on week 2. On the first and last weekday of each week (Monday and Friday) participants performed the assessments, and on the intervening weekdays (Tuesday, Wednesday and Thursday) participants received rTMS+RTP or sham-rTMS+RTP. The testing order was maintained constant to avoid interference between clinical outcome measures and neurophysiologic outcome measures (and vice-versa), and was as follows: motor threshold, MEP recruitment curves, Jebsen-Taylor hand function test, pinch strength, and grasp strength. Because training effects are known to transfer to the non-trained hand, performance measures were assessed in both hands. For all intervention and testing procedures, participants remained seated in their wheelchairs with shoulders in neutral position and elbows at 90° flexion.

RTMS intervention

According to safety guidelines for TMS studies, participants wore earplugs during the intervention, and completed a subjective symptom and safety assessment prior to and following rTMS or sham-rTMS. RTMS was delivered to the hand motor area of the hemisphere contralateral to the weaker hand using an rTMS stimulator (Magstim Rapid 2; Magstim Co, UK). A total of 800 pulses of rTMS with intensity set at 80% of the biceps RMT were delivered in 2-sec trains of 40 pulses, with an intertrain interval of 30 seconds, during which participants practiced a fine motor task (the Nine-hole Peg Test [NHPT]) in both the rTMS and sham-rTMS phases. Use of the biceps MEP to standardize the rTMS stimulation intensity has also been used by other investigators studying rTMS for persons with SCI. Sham-rTMS was performed using a previously
validated approach that mimics the experience of the real rTMS by using electrical stimulation to create a sensation of stimulation (the surface stimulation electrodes were also in place during the real rTMS so that both conditions were the same from the perspective of the participant). Both interventions (rTMS and sham-rTMS) are illustrated on Figure 3.1.

Neurophysiologic Outcome Measures

Corticomotor excitability was assessed with transcranial magnetic stimulation (TMS). MEPs were recorded from the thenar and biceps muscles via surface electromyography (EMG) using Ag Ag/Cl electrodes (3.2×2.2 cm²). MEPs were elicited under two conditions: 1) while the subject was at rest (resting MEPs), and 2) while the subject performed minimal voluntary contraction of 10 – 15% of their maximum voluntary contraction (active MEPs). For the measurement of active MEPs of the thenar muscles, one electrode was placed at the distal one-third of the thenar eminence, and the other electrode was placed 2 cm caudally; the ground electrode was placed on the styloid process of the ulna. The thenar muscles were chosen because control of the opposing thumb is necessary for prehensile function that comprises many hand activities. In addition, these measures enable comparisons with previous studies. Measurement of MEPs of the biceps muscle was performed to standardize the rTMS/sham-rTMS intensity, with one electrode placed centrally on the muscle belly of the biceps, another electrode placed 2 cm caudally, and the ground electrode placed on the olecranon.

EMG signals were split between two computers, one for data acquisition, storage, and off-line analysis (Signal, CED, UK) and the other for biofeedback of the EMG activity
corresponding to 10-15% of maximum voluntary contraction of the thenar muscles (Spike, CED, UK). For data acquisition, EMG was amplified (x1k), band-pass filtered (10-2kHz) (Grass model P511AC, Grass-Telefactor, USA) and converted from analogue to digital (CED model 1401; CED, UK) at a sampling rate of 2 kHz. EMG activity was captured prior to and following the stimulation artifact, to capture both baseline and post-stimulus activity. TMS was delivered using a figure-of-eight coil (maximum field intensity 2 Tesla; Magstim Rapid 2, Magstim Co, UK) placed tangential to the scalp and in a postero-lateral direction an angle of 45° with the mid-sagital line on the hemisphere contralateral to the weaker hand approximately 5 cm lateral to the vertex. With an initial intensity set at 50% maximum stimulator output, the hotspot was sought and documented using a coordinate system that measured the distance of the point from the vertex. If no MEPs were seen at 50% maximum stimulator output, intensity was incrementally increased until an MEP was recorded.

Motor threshold (MT) was defined as the stimulation intensity wherein it was possible to obtain responses greater than 50 μV peak-to-peak in amplitude either at rest or above baseline EMG during a voluntary contraction of 10-15% of maximum voluntary contraction. The relationship between increases in stimulation intensity and MEP amplitude was assessed using input-output curves. Beginning at the intensity corresponding to 80% of the thenar active MT, five stimuli were delivered at each stimulator intensity with interstimulus interval of 4-6 seconds, in 20% increments, until reaching the maximum stimulator output. To assess changes in cortical excitability, data from the input-output curves was analyzed based on the area under the curve (AUC), which is an alternative to fitting the input-output curve to a sigmoidal function.
Use of the AUC to assess cortical excitability has high reliability, and has both high face validity and concurrent validity compared to the curve-fitting approach.²⁶

Performance Outcome Measures

Change in the Jebsen-Taylor hand function test (JTT) scores²⁷ from pre-intervention (ie, day prior to start of the 3-day rTMS+RTP and sham-rTMS+RTP phases) to post-intervention (ie, the day following the intervention phases) was the primary outcome measure. The JTT is designed to measure skilled use of the hand to assess activity limitations (i.e., ICF activity domain), and has been shown to be sufficiently sensitive to capture training-related change in functional hand use in other studies of persons with tetraplegia.⁵⁻⁸ The time taken to complete the tasks associated with the JTT (eg, turning cards, feeding, and manipulating small, light and heavy objects [as seen on Figure 3.2, top]) was recorded. Individuals have a maximum of 120 seconds per task. As in other studies in individuals with stroke²⁸ and SCI ⁷ we omitted the writing task, and therefore the maximum score would be 720 seconds.

Change in pinch and grasp strength from pre-intervention to post-intervention for each intervention phase was measured to assess body function impairment (i.e., ICF “body functions and structure” domain) using a handheld dynamometer (Microfet4; Hoggan Health Industries, Utah). The average force in 3 maximal voluntary contractions was recorded, as illustrated on Figure 3.2 (bottom). Immediate, within-session effects of rTMS+RTP versus sham-rTMS+RTP on fine motor performance (i.e., ICF “activity” domain) were measured using the NHPT. The NHPT is a measure of fine motor control and has been used previously in rTMS studies targeting the upper extremity in persons
with SCI. For each session, the average number of pegs successfully placed and removed during each inter-train interval was calculated. Figure 3.3 displays a participant performing the NHPT.

Statistical Analysis

Statistical testing was performed using SAS software (SAS Institute Inc., Cary, North Carolina) and built-in functions in Excel 2010 (Microsoft Corporation, USA). We assessed order effects using a published protocol and did not find order effects for Jebsen-Taylor hand function test, pinch strength and grasp strength, motor threshold, recruitment curves and area under the curve, (p>0.05 for all comparisons). Therefore, data from within each condition (rTMS+RTP, sham-rTMS+RTP) regardless of intervention order, were pooled within the two participant groups (SCI, neurologically healthy), and paired t-tests on the difference in score (posttest-pretest) were used to make comparisons between conditions separately for the two groups (SCI participants and neurologically healthy participants). Descriptive statistics, including means, standard deviation and 95% confidence intervals (CI) of the pre-post change for each condition were calculated for all measures. In addition, the median pre-post change was calculated for the JTT. Analyses wherein \( p \leq 0.05 \) were considered to have achieved statistical significance, and analyses wherein \( p > 0.05 \) but \( \leq 0.10 \) were considered to have approached statistical significance.

Order effects were identified in the outcomes related to performance on the NHPT. In addition, evidence suggests that non-invasive brain stimulation may influence the rate of learning rather than the magnitude of the learning effect. Therefore, we assessed within-condition change for each group using trend analysis with simple linear regression.
wherein the independent variable was represented by the training day (1, 2, 3) and the dependent variable was the number of peg transfers successfully completed on each day. Then, comparisons were made between weeks (week 1 and week 2) for each condition (rTMS+RTP and sham-rTMS+RTP) by comparing the slopes of the performance curves for each week.

It has been argued that in the presence of large variability and small sample sizes (such as is true of many intervention studies directed at improving function in clinical populations) it is more useful to interpret the clinical meaningfulness of results based on the effect size\textsuperscript{32,33} and minimal clinically important differences.\textsuperscript{34,35} Therefore we calculated effect sizes using the standardized response mean (SRM) dividing the change in score from pretest to posttest by the standard deviation of the change.\textsuperscript{36} Prior studies have used this measure to assess the size of training effects in persons with spinal cord injury\textsuperscript{37} and as in previous studies, the effect sizes were interpreted as: < 0.2 trivial effect, 0.2 – 0.5 small effect, 0.5 – 0.8 moderate effect, > 0.8 large effect.\textsuperscript{38} Post hoc power analysis and sample size calculations were performed using G-power software (version 3.1.7, Dusseldorf, Germany).

**Results**

**Participants**

Twenty-three participants, 13 individuals with SCI (3 females, mean age 46.7±12 years) and 10 individuals without disability (4 females, mean age 33±7 years), enrolled in the study. Two individuals with SCI discontinued participation prior to the end of the study. One participant reported needing to tend to a family emergency, and the other participant
did not share a reason. The remaining 11 participants with SCI and 10 neurologically healthy participants completed the study, and were included in the analysis. With the exception of a transient headache (reported by 3 participants), no adverse effects were observed. Descriptive characteristics and baseline characteristics for all outcome measures are given in Table 3.1.

Performance outcome measures

SCI Group

Changes in score from pretest to posttest for each condition for all performance based measures are presented in Table 3.2. The time to complete the JTT was improved in both conditions, and no between-condition differences in the change in JTT time were found. However, the effect size for the improvement in JTT was large for rTMS+RTP (SRM=0.85) while sham-rTMS+RTP was associated with a small effect size (SRM=0.42). Transfer of training effects to the non-trained hand was observed, with improvement in JTT associated with a moderate effect size rTMS+RTP (SRM=0.55), while sham-rTMS+RTP was associated with a small effect size (SRM=0.31) in the non-trained hand. Between-condition comparisons approached significance in the non-trained hand (p=0.06).

The median pre-post changes in JTT time (± lower and upper quartiles) for both rTMS+RTP and sham-rTMS+RTP are illustrated in Figure 3.4 As can be seen on Figure 3.4, the median change in JTT time was greater for participants during rTMS+RTP (21.2) when compared with sham-rTMS+RTP (6.2 seconds). There were no significant between-condition differences in grasp or pinch force in the trained hand or the untrained
hand. However, change in grasp force in the trained hand under the rTMS+RTP condition was associated with a moderate effect size (SRM=0.67), while sham-rTMS+RTP was associated with a small effect size (SRM=0.39). All other performance based and neurophysiologic outcomes were associated with small effect sizes (Table 3.2).

Neurologically Healthy Group

Changes in score from pretest to posttest for all performance based measures in the neurologically healthy group for each condition are presented in Table 3.3. There were no between-condition effects in any performance measures, and all effect sizes for both groups were small.

Neurophysiologic Measures

Pre-post changes in motor threshold at rest and under active condition, and in the AUC are presented for both groups (SCI and neurologically healthy) are presented in Table 3.4. There were no significant between-condition differences. In the SCI group the rTMS+RTP condition was associated with a change in area under the curve that approached a moderate effect size (SRM=0.48). All other effects sizes were small.

Nine-hole peg test

Performance on the NHPT during the inter-train interval improved in both conditions and in both groups over the course of the 3-day intervention during week 1. Regardless of condition or group, the slopes were greater on week 1 compared to week 2 (Table 3.5).
Discussion

We assessed the influence of a 3-day intervention consisting of 10 Hz rTMS applied over the hand motor area interleaved with RTP of a fine motor task during the inter-stimulus intervals in both subjects with SCI and a group of neurologically healthy participants. In participants with SCI, we found a large effect size for improvement in skilled hand function (i.e., “activity” domain) measured by the JTT in the trained hand; moderate effect sizes were found for increase in grasp force in the trained hand and for improvement in JTT in the non-trained hand. Also in the SCI group, change in cortical excitability as measured by the AUC approached a large effect size. No meaningful changes in performance based measures or neurophysiologic measures were found in the neurologically healthy group. Regardless of stimulus condition during week 1, both the SCI and neurologically healthy group had improved performance on the pegboard task that was performed during the intervention that week.

The results indicate that the addition of rTMS to the RTP was associated with an improvement in ability to perform the JTT tasks beyond the improvements associated with RTP alone. The effect size for change in JTT was twice as large with the rTMS+RTP condition (SRM=0.85) compared to the sham-rTMS+RTP condition (SRM=.42). The JTT represents activities such as picking up small objects, handling light and heavy cans, which one would do routinely in daily life. This finding suggests that there is rTMS augments the effects of task-related training. In addition to the effects observed in the trained hand, change in JTT in the non-trained hand was associated with a moderate effect size (SRM=0.55). This finding suggests that the addition of rTMS to RTP resulted in intermanual transfer of training effects. This performance improvement
in the hand contralateral to the hand used in a training intervention has been observed in healthy subjects, however this is the first study to demonstrate intermanual transfer of training effects in individuals with tetraplegia.

Intense task-specific training and peripheral nerve stimulation aimed at increasing corticomotor excitability are both associated with hand function improvements in participants with stroke, and in participants with SCI. Effect sizes calculated from data for a study that had a higher RTP dose than the present study (daily training 2hrs/day 5days/week for 3 weeks) show that improvements in skilled hand use were greater when RTP was combined with peripheral nerve stimulation (SRM=1.11), compared to RTP alone (SRM=0.59). Effect sizes for rTMS interventions have not been reported for persons with SCI, but our results are consistent with the mean effect size of rTMS interventions on motor outcomes in individuals with stroke (effects size=0.55, 95% CI=0.37, 0.72), reported in a recent meta-analysis. Since our rTMS+RTP intervention was only 3 sessions, it is possible that a longer intervention could have led to a greater magnitude of change.

A moderate effect size was found for the change in grasp strength for the trained hand in the rTMS+RTP condition (SRM=0.67), which was higher than sham-rTMS+RTP (SRM=0.31). Our results are in accordance with evidence suggesting that greatest effects are observed in outcome measures that assess the same ICF domain as the training (“activity”, in the present study), but that additional improvement in another ICF domain (“body functions and structure”) is possible when fine motor practice is performed alongside a stimulation approach aimed at increasing corticomotor excitability. We believe that using the biceps brachii as our reference for calculating the stimulator
frequency could have resulted in insufficient excitation in the corticospinal pathway’s projection to the thenar muscles from which we measured the MEPs, which could be responsible for the lack of changes in both pinch force and thenar MEPs in the present study.

The use of rTMS for improving arm and hand function in persons with SCI has led to mixed results. Our study and the two prior studies that assessed the effects of multi-day rTMS approaches in individuals with tetraplegia utilized equivalent daily dosages of stimulation; we used 800 pulses daily while Belci et al\textsuperscript{10} and Kuppuswamy et al\textsuperscript{12} used 720 and 900 pulses daily, respectively. For this reason we believe that differences in outcomes are likely to be attributable to differences in stimulation frequency. Belci et al\textsuperscript{10} used a 10 Hz paired pulse protocol for 5 days, with stimulation parameters intended to reduce intracortical inhibition. The 5-day rTMS intervention was preceded by a 5-day sham-rTMS period that served as a control condition.\textsuperscript{10} They found changes in the silent period, AIS motor and pin-prick scores post-treatment, as well as delayed functional improvement measured by the pegboard test at a follow-up period 3 weeks after the last session.\textsuperscript{10}

While Kuppuswamy et al\textsuperscript{12} have described the study by Belci et al\textsuperscript{10} as a study that incorporated both high and low-frequency rTMS we believe that the findings of Belci et al are attributable to the high-frequency of the paired pulse stimulation (10Hz), as others have shown that rTMS approaches at 0.1Hz does not influence cortical excitability.\textsuperscript{45,46}

The outcomes of classical statistical testing in our study are consistent with those of the study of Kuppuswamy et al\textsuperscript{12} that assessed the effects of a 5-day rTMS intervention (5Hz, 900 pulses applied in 2 sec trains with 8 second inter-stimulus intervals, 15 min daily
sessions) in a sham-controlled crossover study with participants with SCI, and found no statistical between-condition differences in the pre-post change in hand motor performance assessed by the action arm research or corticomotor excitability (resting motor threshold, active motor threshold, silent period). Unfortunately, while Baysian statistics are preferable for identifying the clinical meaningfulness of outcomes and are now commonly used in studies of clinical populations, Kuppuswamy et al did not include sufficient information for estimation of effect sizes, thereby making it difficult to make comparisons between that study and ours difficult.

Our study has several limitations. Statistical power calculations revealed that we did not have sufficient power to detect differences between the two conditions. For the SCI group, the actual power was approximately 24%, and we would have needed at least 90 individuals per group to detect differences between conditions at 80% power. A total sample size of 180 participants is very difficult given the known recruitment challenges in clinical research involving neurological populations. We believe that the lack of effects in the neurologically healthy group was due to a ceiling effect, as individuals with intact nervous systems are likely to be functioning near their maximum capability for performance of routine motor task such as those included in the JTT, therefore they have a smaller capacity for change in response to a brief intervention. Finally, we did not assess the immediate effects of our intervention, as we were interested in after-effects (i.e., retention) which we believe to be of greater value in terms of clinical applicability for neurorehabilitation.
Conclusion

In persons with tetraplegia, 10 Hz rTMS applied over the hand motor area interleaved with RTP of a fine motor task performed during the inter-train intervals was associated with improved skilled hand use and grasp strength. Our results suggest that rTMS may be of value in improving arm and hand function in people with spinal cord injury. Further studies are encouraged to identify the rTMS parameters (frequency, intensity), and the rTMS+RTP duration that are associated with best results.

Acknowledgements and funding source

This work was funded by National Institutes of Health R01HD53854. The authors would like to thank Dr. Alvaro Pasqual-Leone for his contributions to the design of this study and Dr. Kathryn Roach for the assistance with data analysis.
Figure 3.1 Experimental setup for the rTMS session (top) and sham-rTMS (bottom). The direction of the electromagnetic coil was inverted during the sham-rTMS session, so individuals still heard the trigger, but did were not stimulated by the TMS. In addition, participants received a mild electric stimulus which has been reported to adequately mimic the TMS pulse without influencing cortical excitability (see text for details).
Figure 3.2 Participants performing the Jebsen-Taylor Hand Function Test (Top) and Pinch Grip (Bottom).

Flipping Cards     Picking up small objects

Simulated feeding     Stacking checkers     Handling cans

[Images of participants performing various tasks]
Figure 3.3 Participant performing the Nine-hole Peg Test during the inter-train interval. Participants were instructed to pick up the pegs fast and accurately as possible, place them in the 9 holes. If they had successfully placed all pegs, they were instructed to begin immediately returning the pegs to the bowl. The number of pegs successfully manipulated was recorded.
Figure 3.4 Boxplots indicating median pre-post change in JTT time (± lower and upper quartiles) per condition (rTMS=black box) and sham-rTMS (grey box).
Table 3.1 Demographic and baseline characteristics of the participants (SCI Group and Neurologically Healthy Adults).

<table>
<thead>
<tr>
<th>Subject Characteristics</th>
<th>SCI Group</th>
<th>Healthy adults</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Age (years)</em></td>
<td>46.7±12.0</td>
<td>33.7±7</td>
</tr>
<tr>
<td><em>Gender</em></td>
<td>1 female, 10 males</td>
<td>4 females, 6 males</td>
</tr>
<tr>
<td><em>Post-injury (years)</em></td>
<td>6.6±8.2</td>
<td>N/A</td>
</tr>
<tr>
<td><em>Injury Level</em></td>
<td>C6 (median)</td>
<td>N/A</td>
</tr>
<tr>
<td><em>AIS</em></td>
<td>D(6), C(5)</td>
<td>N/A</td>
</tr>
<tr>
<td><em>JTT-t (time)</em></td>
<td>267±216</td>
<td>31±4</td>
</tr>
<tr>
<td><em>JTT-n (time)</em></td>
<td>177±202</td>
<td>29±3</td>
</tr>
<tr>
<td><em>Pinch-t (kg)</em></td>
<td>1.7±1.5</td>
<td>8.6±1.7</td>
</tr>
<tr>
<td><em>Pinch-nt (kg)</em></td>
<td>4.4±2.8</td>
<td>8.7±1.9</td>
</tr>
<tr>
<td>*Grasp-t (kg)</td>
<td>2.3±2.1</td>
<td>29.1±10.4</td>
</tr>
<tr>
<td>*Grasp-nt (kg)</td>
<td>4.8±2.3</td>
<td>34.0±10.8</td>
</tr>
<tr>
<td><em>Resting Motor Threshold</em> (%)MSO</td>
<td>0.62±0.17</td>
<td>0.57±0.12</td>
</tr>
<tr>
<td><em>Active Motor Threshold</em> (%)MSO</td>
<td>0.50±0.14</td>
<td>0.39±0.09</td>
</tr>
<tr>
<td><em>AUC</em></td>
<td>1.3±1.7</td>
<td>4.6±2.6</td>
</tr>
</tbody>
</table>

*AIS=American Spinal Cord Injury Impairment Scale; JTTw=Jebsen-Taylor Hand Function Test, trained hand; JTTs=Jebsen-Taylor Hand Function Test, non-trained hand; Pinch-t=Pinch force, trained hand; Pinch-nt=Pinch force, non-trained hand; Grasp-t=Grasp force, trained hand; Grasp-nt=Grasp force, non-trained hand; MEP=motor evoked potential acquired with transcranial magnetic stimulation (TMS); MSO=maximum stimulator output of TMS device (2Tesla); AUC=area under the curve of input-output curves acquired with transcranial magnetic stimulation.*
Table 3.2. Performance-based outcomes for participants with SCI

<table>
<thead>
<tr>
<th>Outcome Measure</th>
<th>rTMS</th>
<th>Sham-rTMS</th>
<th>Between-condition difference (Paired t-test)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Difference Scores</td>
<td>Effect Size (SRM)</td>
<td>Difference scores</td>
</tr>
<tr>
<td></td>
<td>Mean±SD (95%CI)</td>
<td></td>
<td>Mean±SD (95%CI)</td>
</tr>
<tr>
<td>JTT-t (time)</td>
<td>-45±52 (-80,-9)</td>
<td>0.85</td>
<td>-29±68 (-75,16)</td>
</tr>
<tr>
<td>JTT-nt (time)</td>
<td>-31.3±56 (-69,6)</td>
<td>0.55</td>
<td>-13±41 (-40,14)</td>
</tr>
<tr>
<td>Pinch-t (kg)</td>
<td>0.1±0.5 (-0.1,0.5)</td>
<td>0.38</td>
<td>0.24±0.8 (-0.3,0.7)</td>
</tr>
<tr>
<td>Pinch-nt (kg)</td>
<td>0.2±0.9 (-0.4,0.8)</td>
<td>0.22</td>
<td>-0.05±0.6 (-0.5,0.4)</td>
</tr>
<tr>
<td>Grasp-t (kg)</td>
<td>0.5±0.8 (0.003,1.0)</td>
<td>0.67</td>
<td>0.4±1.1 (-0.3,1.2)</td>
</tr>
<tr>
<td>Grasp-nt (kg)</td>
<td>0.11±1.2 (-0.7,0.9)</td>
<td>0.08</td>
<td>0.2±1.2 (-0.7,0.9)</td>
</tr>
</tbody>
</table>

AIS=American Spinal Cord Injury Impairment Scale; JTTw=Jebsen-Taylor Hand Function Test, trained hand; JTTs=Jebsen-Taylor Hand Function Test, non-trained hand; Pinch-t=Pinch force, trained hand; Pinch-nt=Pinch force, non-trained hand; Grasp-t=Grasp force, trained hand; Grasp-nt=Grasp force, non-trained hand.
Table 3.3 Performance-based outcomes for participants with SCI

<table>
<thead>
<tr>
<th>Outcome Measure</th>
<th>rTMS</th>
<th>Sham-rTMS</th>
<th>Between-condition difference (Paired t-test)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean±SD (95%CI)</td>
<td>Effect Size</td>
<td>Mean±SD (95%CI)</td>
</tr>
<tr>
<td>JTT-t (time)</td>
<td>-0.5±3.4 (-3.2,2.1)</td>
<td>0.16</td>
<td>-0.2±4.7 (-3.9,3.3)</td>
</tr>
<tr>
<td>JTT-nt (time)</td>
<td>-0.3±1.3 (-1.3,0.6)</td>
<td>0.27</td>
<td>-0.07±1.2 (-0.9,0.8)</td>
</tr>
<tr>
<td>Pinch-t (kg)</td>
<td>0.2±2.5 (-1.5,2.1)</td>
<td>0.11</td>
<td>-0.03±2.1 (-1.5,1.5)</td>
</tr>
<tr>
<td>Pinch-nt (kg)</td>
<td>0.2±0.9 (-0.4,0.8)</td>
<td>0.22</td>
<td>-0.05±0.6 (-0.5,0.4)</td>
</tr>
<tr>
<td>Grasp-t (kg)</td>
<td>0.1±6.0 (-4.2,4.4)</td>
<td>0.23</td>
<td>2.3±5.3 (-1.4,6.1)</td>
</tr>
<tr>
<td>Grasp-nt (kg)</td>
<td>1.26±4.2 (-1.7,4.2)</td>
<td>0.29</td>
<td>0.6±2.7 (-1.3,2.6)</td>
</tr>
</tbody>
</table>

AIS=American Spinal Cord Injury Impairment Scale; JTTw=Jebsen-Taylor Hand Function Test, trained hand; JTTs=Jebsen-Taylor Hand Function Test, non-trained hand; Pinch-t=Pinch force, trained hand; Pinch-nt=Pinch force, non-trained hand; Grasp-t=Grasp force, trained hand; Grasp-nt=Grasp force, non-trained hand.
Table 3.4 Neurophysiologic outcomes for participants with SCI and Neurologically Healthy Participants.

<table>
<thead>
<tr>
<th>Outcome Measure</th>
<th>rTMS Difference scores</th>
<th>Sham-rTMS Difference scores</th>
<th>Between-condition difference (Paired t-test)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SCI Group Resting Motor Threshold (%MSO)</td>
<td>0.02±0.03 (0.09,0.13)</td>
<td>0.01±0.19 (0.12,0.14)</td>
<td>0.05 0.9</td>
</tr>
<tr>
<td>Active Motor Threshold (%MSO)</td>
<td>-0.04±0.09 (-0.1,0.02)</td>
<td>-0.04±0.1 (-0.1,0.04)</td>
<td>0.34 0.9</td>
</tr>
<tr>
<td>AUC</td>
<td>0.4±0.9 (-0.1,1.1)</td>
<td>0.4±1.5 (-0.6,1.4)</td>
<td>0.26 0.9</td>
</tr>
<tr>
<td>Comparison Group Resting Motor Threshold (%MSO)</td>
<td>-0.002±0.1 (-0.1,0.09)</td>
<td>-0.02±0.1 (-0.1,0.05)</td>
<td>0.20 0.6</td>
</tr>
<tr>
<td>Active Motor Threshold (%MSO)</td>
<td>0.01±0.05 (-0.02,0.4)</td>
<td>0.03±0.1 (-0.1,0.05)</td>
<td>0.26 0.5</td>
</tr>
<tr>
<td>(AUC)</td>
<td>-1.1±3.8 (-3.8,1.6)</td>
<td>-0.2±1.3 (-1.2,3.5)</td>
<td>0.21 0.5</td>
</tr>
</tbody>
</table>

MEP = motor evoked potential acquired with transcranial magnetic stimulation (TMS); MSO = maximum stimulator output of TMS device (2Tesla); AUC = area under the curve of input-output curves acquired with transcranial magnetic stimulation.
Table 3.5 Regression of nine-hole peg test (NHPT) performance by Intervention Day (1,2,3) for participants in both groups (SCI, Neurologically Healthy) who received Stim 1st and Sham 1st. Regardless of condition, individuals demonstrated greater improvement on their first week.

<table>
<thead>
<tr>
<th>Regression of NHPT by Day for SCI Participants</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>rTMS 1st</td>
<td>Sham-rTMS 1st</td>
</tr>
<tr>
<td></td>
<td>Slope</td>
<td>Slope</td>
</tr>
<tr>
<td>Week</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>32.0 ± 15.8, p=0.0685</td>
<td>30.5 ± 21.6, p=0.2815</td>
</tr>
<tr>
<td>2</td>
<td>7.7 ± 16.3, p=0.6478</td>
<td>15.1 ± 10.1, p=0.1789</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Regression of NHPT by Day for Neurologically Healthy Participants</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>rTMS 1st</td>
<td>Sham-rTMS 1st</td>
</tr>
<tr>
<td></td>
<td>Slope</td>
<td>Slope</td>
</tr>
<tr>
<td>Week</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>42.3 ± 15.6, p=0.0301</td>
<td>46.3 ± 19.5, p=0.0371</td>
</tr>
<tr>
<td>2</td>
<td>9.1 ± 13.7, p=0.5271</td>
<td>10.9 ± 14.50, p=0.4681</td>
</tr>
</tbody>
</table>

*NHPT=nine-hole peg test*
Tetraplegia is the most common manifestation of a spinal cord injury (SCI),\textsuperscript{3} affecting approximately 6000 people each year, in the United States alone.\textsuperscript{3} Individuals with tetraplegia have varying degrees of upper extremity impairment, and restoration of hand/arm function is consistently cited as a priority in their rehabilitation goals.\textsuperscript{4-6} In addition to the damage to the ascending and descending tracts resulting from the primary injury, there is evidence of functional reorganization of the sensorimotor cortex\textsuperscript{32} that occurs post-injury. A systematic review of studies that utilized neuro-imaging techniques to characterize movement-related activation after tetraplegia concluded that there is sufficient evidence supporting substantial changes in cortical organization after tetraplegia, such as: increased activation of associated sensorimotor areas during the performance of unimanual tasks, which is thought to reflect compensatory strategies\textsuperscript{32}; and posterior shifts of the center of gravity of movement-related activity, suggesting that the sensory cortex makes a greater contribution to the corticospinal tract in these individuals.\textsuperscript{32} It has been argued that this reorganization represents a form of maladaptive plasticity, perhaps arising from learned non-use that contributes to the impairment of function by limiting the ability of the cortex to drive efferent information down the spared spinal pathways.\textsuperscript{9}

Studies utilizing transcranial magnetic stimulation (TMS) have demonstrated that muscles affected by the injury have decreased corticomotor representation and decreased excitability measured by motor evoked potentials (MEPs).\textsuperscript{40} While the exact relationship
between changes in cortical organization and function is not fully elucidated, individuals who exhibit greater functional recovery demonstrate increased volume of cortical activation in the primary motor cortex (M1), while those who demonstrate less functional recovery maintain the decreased volume of cortical activation. In this context, the use of practice and modulation of cortical excitability via somatosensory input can change the demands of the nervous system, and thus alter cortical excitability and function.

Individuals with chronic tetraplegia who participated in a massed-practice training intervention (i.e., intense repetitive task practice performed for 2 hrs/day, 5 days a week for 3–4 weeks) in isolation or combined with somatosensory stimulation (i.e., peripherally applied electrical stimulation at an intensity just below that required to elicit a visible twitch) demonstrated significant changes in skilled hand use. A follow-up study demonstrated that the combination of repetitive task practice and somatosensory stimulation led to greater gains in skilled hand function than when either approach was used in isolation. In addition, somatosensory stimulation in isolation is associated with significant improvements in pinch grip strength in individuals with tetraplegia and chronic stroke. Taken together, these results suggest that that stimulation can have a potent effect on motor control, and stimulation may augment the effects of repetitive task practice.

The effects of somatosensory stimulation on upper extremity function have been attributed to the current parameters (10Hz, pulse duration 1000 μs, 50% duty cycle). Specifically, the long pulse duration (which is approximately 3 times longer than the longest pulse duration of 300 μs in clinically available devices) has been linked to a
selective activation of large-diameter sensory fibers, and the accompanying excitation of the primary sensory cortex (S1), and primary motor cortex (M1) via cortico-cortical connections between S1 and M1. This is consistent with increases in corticomotor excitability following somatosensory stimulation, measured both by increased amplitude of MEPs acquired with TMS and by blood-oxygen level dependent increases measured by functional magnetic resonance imaging. However, a recent study showed that transcutaneous electrical nerve stimulation (TENS), in the form often used clinically to promote analgesic effects via activation of large-diameter (mechanoreceptor) fiber inhibition of small-diameter (nociceptor) fiber transmission, also exerts effects on corticomotor excitability. Meesen et al assessed the effects of TENS (biphasic symmetrical rectangular pulse-wave at 100 Hz, 250 μs pulse width) in neurologically healthy individuals (daily one-hour sessions over 3 weeks) and found significant enlargement of the corticomotor maps acquired with TMS, while no change was observed in the control group.

Peripherally applied vibration (VIB) is another approach to activate the somatosensory system with the use of a strong proprioceptive stimulus that can increase sensory drive by activating Ia fibers. Preliminary data suggests that a single 2-hour session of local muscle vibration (80 Hz) is associated with transient changes in pinch force in individuals with tetraplegia. Individuals with chronic stroke who participated in a conventional physical therapy intervention preceded by brief periods of VIB with slightly higher frequency (100Hz, 0.2-0.5mm amplitude, in 10 min sessions daily for 3 consecutive days) had greater gains in motor function than individuals who only participated in the conventional physical therapy intervention. In addition, participants
in the combined group (VIB and conventional physical therapy) had increased area of
motor maps of vibrated muscles acquired with TMS. These results suggest that VIB can
also be used to influence corticomotor excitability and motor performance.

While somatosensory electrical stimulation and vibration represent indirect approaches to
activate the motor cortex, transcranial direct current stimulation (tDCS) offers a more
direct approach to increase corticomotor excitability. tDCS uses monophasic currents
applied to the scalp to deliver mild, direct electric current (intensity typically set at 1-
2mA) to the underlying cortical areas, exerting a neuromodulatory influence. There is
decreased membrane threshold in the cortical areas underlying the cathode (facilitating
the occurrence of action potentials), and increased membrane threshold in the areas
underlying the anode (and thus hindering the occurrence of action potentials). tDCS,
with the anode placed on M1 and the cathode placed on the contralateral supraorbital
area, has been associated with increases in corticomotor excitability and hand function in
individuals with chronic stroke (who similarly to individuals with tetraplegia, have
decreased excitability in M1). In addition, gains in motor function have been shown
to be greater when tDCS was paired with repetitive task practice, both in individuals with
stroke and neurologically healthy controls.

In light of the decreased cortical excitability observed after tetraplegia, and increased
effects observed when combining repetitive task practice and somatosensory
stimulation, we undertook a study to assess whether there are differences in outcomes
of repetitive task practice when combined with each of the clinically accessible
approaches for directly (tDCS) or indirectly (TENS, VIB) increasing corticomotor
excitability. To our knowledge, direct comparisons between the effects of these three
approaches combined with repetitive task practice on hand function and corticomotor excitability in individuals with chronic tetraplegia have not been done before. In a prior study wherein individuals with chronic tetraplegia participated in a 3-day intervention consisting of 40 2-sec bouts of non-invasive brain stimulation (repetitive transcranial magnetic stimulation) interleaved with repetitive task practice, we observed modest improvements in skilled hand function on the day following the last session.\textsuperscript{143} We were interested in assessing the early effects (observed during stimulation, also referred to as ‘online effects’) and late effects (observed after stimulation has ceased, also referred to as ‘offline effects’) of clinically accessible approaches for direct and indirect corticomotor activation on outcome measures that addressed different levels of the International Classification of Functioning, Disability and Health (ICF) framework.\textsuperscript{114} We hypothesized that a single session of 30 minutes of tDCS combined with repetitive task practice would be associated with larger early and late effects compared to repetitive task practice combined with either VIB or TENS.

Methods

Participants

Twenty-four participants with SCI were recruited from the research volunteer database of The Miami Project to Cure Paralysis. To be included, participants needed to be between 19-70 years of age, and have had an injury to the cervical spine at C7 or above at least one year prior to enrollment. Additionally, participants needed to have preserved ability to voluntarily produce a visible twitch of the thenar muscles of at least one hand. Exclusion criteria were a history of head injury, family history of seizures or the presence
of metal elements in the cranium (e.g. aneurysm clip), and any other neurologic, orthopedic, or cognitive condition that could affect the performance on the outcome measures. Following screening and informed consent to participate in the study, participants received instructions to maintain their usual level of activity and to not alter their medication regimen. This study was approved by the Human Subjects Research Office of the University of Miami Miller School of Medicine, Miami, FL.

Study Design

We conducted a randomized crossover study consisting of one session of each stimulation condition (tDCS, TENS, VIB) while participants performed a repetitive task practice activity. To reduce the possibility of carryover effects, sessions were separated by 1 week. Because our interest was in the comparative efficacy of the 3 different stimulation approaches, no control condition (i.e., sham stimulation) was included.

Sample sizes were estimated based on a recent crossover study of participants with chronic spinal cord injury in which significant between-condition differences were identified with 19 participants. Target sample size was increased by 20% to compensate for the attrition that was anticipated based on other studies of participants with chronic tetraplegia. Upon obtaining informed consent, participants were randomized to the order of the stimulation conditions. Randomization was performed, based on a random number generator, by a member of the laboratory staff who was not otherwise involved with the study.

A diagram illustrating the study procedures is given in Figure 4.1. During each session, corticomotor and performance-based measurements were performed 3 times. The testing
order was constant for all participants and conditions, to avoid interference between clinical outcome measures and neurophysiologic outcome measures (and vice versa), and was as follows: pinch strength, motor evoked potential threshold and MEP amplitude, Nine-Hole Peg Test (NHPT) and visuomotor tracking task. Participants began with the pre-test measurements, which were followed by 30 minutes of repetitive task practice concurrently with stimulation (TENS, VIB or tDCS). Following this stimulation/practice period, participants performed the early post-test measurements, rested for 30 minutes, and then performed the late post-test measurements. Participants returned 1 and 2 weeks after the first session to repeat the protocol with a different stimulation approach. A washout period of one week was chosen and was deemed appropriate based on evidence from several prior studies that all demonstrated that effects of a single session of vibration,79 motor practice,79 tDCS145 or electrical stimulation137 have not been present after 7 days.

Skilled hand use and strength are elements of hand function that are controlled by the activity in M1,146 and therefore stimulation targeted at increasing the activity of M1 would be associated with improvements in these activities. We included outcome measures that assessed hand function at the “body functions and structures” and “activity” levels of the ICF,114 to gain further insight into the effects of each stimulation approach. Early effects were assessed through changes from pre-intervention to post-intervention, and late effects were assessed through changes from pre-intervention to the late post-test. Testing and training was targeted at the weaker hand.
Study Intervention Procedures

Repetitive task practice

Training was performed simultaneously with stimulation (TENS, VIB, tDCS). As soon as the stimulation was turned on, individuals were instructed to begin performing the hand tasks for a total of 30 minutes. Participants practiced 3 tasks for 10 minutes each, which were selected from previously published massed practice training protocols.\textsuperscript{9,14,64,65} During the first task, participants were instructed to reassemble a puzzle (Mellissa and Doug Inc, USA), piece by piece using their weaker hand to perform the manipulative portion and the stronger hand to stabilize (if necessary). For the second task, a board game (model name Connect Four, Hasbro, USA) was used. Participants were instructed again to use their weaker hands to manipulate the checkers and drop them on the board, which was stabilized by the primary author. The last task consisted in stacking checkers in groups of five (Figure 4.2).

If participants were unable to perform the activities using typical hand kinematics, modifications were made to adjust for the difficulty of the task. For example, during the Connect Four game task minimal assistance was offered to lift the upper limb in a participant with increased spasticity of the proximal musculature. Likewise, if limited hand function prevented participants from stacking checkers in groups of five, then individuals were allowed to stack in groups of 2. All modifications were performed by the primary author, and therefore were consistent for all participants and conditions. Lastly, individuals were allowed to have one minute of rest in-between activities if they indicated feeling tired.
Transcutaneous Electrical Nerve stimulation

TENS (biphasic symmetrical rectangular pulse wave at 100 Hz, 250μs pulse width) was delivered to the volar aspect of the wrist overlying the median nerve utilizing a clinically available portable electrical stimulation unit (TENS 7000, Koalaty Products, Inc., USA) utilizing parameters associated with increased cortical excitability in an earlier study. A photo demonstrating the electrode placement is seen on Figure 4.3. The median nerve was chosen because it innervates the muscles used to perform hand tasks that require fine precision, which are impaired after chronic tetraplegia. As performed in studies that used somatosensory stimulation in individuals with tetraplegia, the optimal site for activation of the median nerve was determined by moving the electrodes to identify the area wherein participants reported the stimulation was best perceived in digits I-III. Intensity was increased until motor threshold was determined, and then set at a level where sensation was described verbally as “strong” and equivalent to at least 5-6/10 described using a verbal analogue scale in the absence of pain or muscle contractions assessed via visual inspection.

Transcranial Direct Current Stimulation

The optimal stimulation site for the corticomotor area controlling the weaker hand was identified using transcranial magnetic stimulation (Magstim200, Dyfed, Wales), detailed methods have been previously described elsewhere. As has been reported in other studies with individuals with tetraplegia, in some instances it was not possible to obtain MEPs from the thenar muscles. In those cases we used the 10-20 electroencephalographic system for electrode placement, as has been previously reported by other investigators in a study. tDCS was delivered by a constant current stimulator
(Phoresor, Iomed Inc., Salt Lake City, UT) with intensity set at 1 mA, according to safety guidelines. Two electrodes (Optima, Iomed Inc, Salt Lake City, UT) were used: the anode was a saline-soaked disposable iontophoresis electrode (with 28 cm$^2$ area), and was placed on the scalp overlying the hotspot of M1 (or alternatively on area C3 or C4, which correspond to either M1$^{141}$); and the cathode was an adhesive electrode (with approximately 37 cm$^2$ area) which was placed on the contralateral supraorbital area (Figure 4.2).$^{138}$ The estimated current density was approximately 46 $\mu$A/cm$^2$.

Peripherally applied vibration

We selected the VIB parameters to best model those used by Marconi et al.$^{17}$ In that study, investigators demonstrated that conventional physical therapy preceded by brief periods of continuous vibration (performed for 3 days, 10 minutes/session at 100 Hz, 2-3 mm displacement) was associated with greater gains in hand function and enlargement of corticomotor representation than conventional physical therapy alone. The vibration device (CEN, USA) used in the present study delivered a similar frequency (80 Hz, continuous), which is common in the devices that are widely available for clinical use, and which has also been associated with increases in corticomotor excitability.$^{147}$ The vibrating device was placed on the volar aspect of the distal wrist over the distal tendon of the flexor carpi radialis$^{17}$ using elastic wrap and adhesive tape. This site was selected as it is associated with perceived vibration in the thenar muscles, which likely arises from the influence of vibration on the proximal insertion of the thenar muscles, but uses a placement that will not interfere with performance of hand-related activities (as would be the case if the vibrator was placed directly on the thenar muscles. We attached the device
so that it made optimum contact without causing discomfort or altering wrist range of motion that could interfere with the repetitive task practice activities (Figure 4.4).

Study Assessment Procedures

Performance Outcome Measures

Changes in pinch and grasp strength for each phase were measured to assess body function impairment (i.e., ICF body structure and function level) using a handheld dynamometer (Microfet4; Hoggan Health Industries, Utah). Participants remained seated in their wheelchairs with the shoulder in neutral position, elbow at 90° flexion and the forearm rested on a table. The average force produced in 3 maximal voluntary contractions was recorded. Change in the NHPT was the primary outcome measure. The NHPT is a measure of fine motor control and has been used to assess activity limitations (i.e., ICF activity level) in studies that targeted improved of upper extremity function in persons with SCI. For each session, a video record was made of the task performance, which included 3 trials of 30 seconds per phase. Participants were instructed to pick up pegs as fast and accurately as possible and place them in the holes until filled, and without pausing, continue placing them back into the bowl using the weaker hand. In this context, the minimum score is zero and reflects poor performance (individual was not able to successfully pinch and release one peg), and high scores indicate better performance. While neither participants nor the primary author were blinded to study procedures, objectivity was maintained during the NHPT as the scoring was performed based on the video recording by an assistant not otherwise involved in the study, who
timed each trial, counted the number of pegs successfully placed and removed, and calculated an average.

Visuomotor tracking is a function considered to be under direct corticomotor control that in theory would reflect specific changes in corticomotor excitability\textsuperscript{149} and was used to assess the ability to control graded force production. Evidence suggests that individuals with SCI have deficits in muscle recruitment patterns\textsuperscript{150} that are likely to be associated with their deficits in force production.\textsuperscript{151} The visuomotor tracking task was custom designed using a piezoresistive force transducer (model FSR, 426) connected to the analogue port of a micro-controller for conversion of the signal from analogue to digital (Arduino, Italy). The signal was displayed on a laptop containing a custom Labview application (National Instruments, USA). For each participant, the device was calibrated to their maximum voluntary contractions (MVC) based on the average of three attempts.

The main software interface of the visuomotor tracking task was a line generated based on the pinch force which rose and fell depending on the amount of force the participant produced. The target force was indicated by a sinusoidal wave, and participants were encouraged to track the target wave as accurately as possible by controlling the force of their pinch grasp. Feedback of the desired trace and the actual trace were both available to the participant. The minimum and maximum amplitudes of the sinusoidal wave (y-axis) were adjusted to 10% and 20% of the MVC for each participant (Figure 4.5). Two familiarization trials were performed, and the following three trials (30 seconds each) were used for the analysis. The scoring was performed according to other studies that have utilized a visuomotor tracking task,\textsuperscript{152} by comparing the participant’s input to the desired force using root mean square error, calculated with the following formula,
wherein $x_i$ the participant’s input while is $y_i$ is the desired input generated by the program. The variable $n$ is the number of data points received by the program in the 30 second interval. With this scoring system, a decrease in score indicates improvement.

$$\sqrt{\frac{\sum_{i=1}^{n} (x_i - y_i)^2}{n}}$$

Neurophysiologic Outcome Measures

Corticomotor excitability was assessed with TMS. During the neurophysiologic assessment, participants sat comfortably with the shoulder in neutral position, elbow at $90^\circ$ flexion, and the forearm in neutral position. MEPs were recorded for the thenar muscle of the weaker hand via surface electromyography (EMG) using two Ag Ag/Cl electrodes ($3.2 \times 2.2$ cm$^2$): one electrode was placed at the distal one-third of the thenar eminence and the other electrode was placed 2 cm caudally; the ground electrode was placed on the styloid process of the ulna. MEPs were recorded while the subject performed minimal voluntary contraction of 10-15 percent of their maximum voluntary contraction (active MEPs)$^{118}$

Two computers were used during the data acquisition: one computer was used to store data for off-line analysis (Signal version 5.0, CED, UK), and another for biofeedback display of the EMG activity during the acquisition of active MEPs. For the data acquisition, EMG was amplified (x1000), band-pass filtered (10-2kHz; Grass model P511AC, Grass-Telefactor, USA), and converted from analogue to digital (CED model 1401; CED, UK) at a sampling rate of 2 kHz. EMG activity was captured 20 ms prior to and following the stimulation artifact (20 millisecond period in each case), in order to
capture both baseline and post-stimulus activity. TMS was delivered using a figure of eight coil (maximum field intensity 2 Tesla; Magstim Rapid 2, Magstim Co, UK) placed tangential to the scalp and in a postero-lateral direction in an angle of 45° with the mid-sagittal line. The biofeedback interface (Spike, CED, UK) was calibrated using the rectified and smoothed EMG signal corresponding to the average intensity (in millivolts) of 3 maximum voluntary contractions. Cursors were placed to provide a target corresponding to EMG amplitude of 10-15%, which was presented to the participant on-screen. While the primary author assessed the MEP amplitude in real time, an assistant triggered the TMS only when participant’s EMG activity was within the targets established and displayed in the biofeedback computer. This ensured that every TMS pulse was given while the nervous system was facilitated to the same extent in all participants.

The TMS coil was placed on the hemisphere contralateral to the weaker hand with initial intensity set at 50% maximum stimulator output (MSO). Beginning at the hand motor area, estimated to be located approximately 5 cm lateral to the vertex, pulses were delivered until the area was identified where the highest MEPs in amplitude could be recorded with the same stimulation intensity, also referred to as the “hot spot”. The hot spot location and the position of the coil were marked on the participant’s scalp using a colored marker, and were documented using a coordinate system that measured the distance of this point from the vertex. Motor threshold (MT) was defined as the stimulation intensity wherein it was possible to obtain 5-7/10 responses having peak-to-peak amplitude greater than 50 µV above baseline during a minimal voluntary contraction. Following MT determination, ten responses were recorded with intensity
set at 1.2xMT (i.e., 20% higher than the MT intensity). The amplitude of 10 averaged MEPs at 1.2 times MT was used to assess corticomotor excitability. In order to normalize the MEP amplitude data across participants, MEP values were calculated as a proportion of the maximum M-wave (i.e. maximum response evoked from the muscle with electrical stimulation) of the median nerve. To assess M-wave amplitude, electrodes were placed in the cubital fossa with the cathode positioned caudal to the anode. Single pulses of 1000 μsec pulse duration were applied in increasing intensities until no further increases in amplitude of the response was observed.

Statistical Analysis

Statistical testing was performed using SAS software (version 9.2, SAS Institute Inc., Cary, North Carolina) and built-in functions in Excel 2010 (Microsoft Corporation, USA). Our hypothesis testing focused on comparisons between the 3 stimulation conditions (TENS, VIB, tDCS), all performed while participants were engaged in a repetitive task practice protocol. Preliminary analyses were carried out to assess order effects. For this purpose, independent t-tests on the sum of group means for subjects who received all three conditions (TENS, VIB, tDCS) in all possible different combinations of orders were compared. No order effects were identified for the NHPT, pinch force or visuomotor task (p>0.05 for all comparisons), we are aware that we are very likely to be underpowered to detect order effects, and this may have contributed to this finding. Due to the pilot nature of the study and the fact that we did not see order effects in our analysis, we decided to treat conditions separately in the analysis, neglecting the order effects. Early effects were calculated by subtracting the scores at early posttest from pretest and late effects were calculated by subtracting the scores at late posttest from
pretest for all outcome measures. For the NHPT, pinch strength and MEP measurements, improvements are represented by increased change scores and for the VM task, improvements are represented by decreased change scores. We chose to analyze the early effects (i.e., changes from pretest to early posttest) and the late effects (i.e., changes from pretest to late posttest) using separate one way repeated measures ANOVA on the relative changes over time for each performance measure, as done in other crossover studies with similar design. When appropriate, post hoc tests were carried out using paired t-tests. Descriptive statistics including means, standard deviations, and 95% confidence intervals (CI) were calculated for the early and late pre-post changes for all measures. Analyses wherein $p \leq 0.05$ were considered to have achieved statistical significance and analyses wherein $p > 0.05$ but $\leq 0.10$ were considered to have approached statistical significance. Because the hypotheses were pre-specified, no adjustments were made to the $P$ values reported.

Due to the small number of participants from whom it was possible obtain MEP measurements (TENS=11; VIB=9, tDCS=7), we opted not to perform statistical analyses, as negligible value can be obtained from between-condition comparisons in such a small sample. Instead, we used paired t-tests to assess changes in corticomotor activity from pretest to posttest (early effects) and from pretest to late posttest. These changes are also of clinical interest, as they enable the assessment of early changes in cortical excitability and short-term retention of these changes. Although not assessed in the present study, there is evidence to suggest that the changes in corticomotor excitability associated with electrical stimulation and tDCS occur in the absence of changes in F-waves, and are therefore unlikely to be attributable to changes in the
excitability of spinal circuits. F-waves have been reported to be both, unchanged and
decreased after VIB.

Effect sizes were calculated for all outcome measures. The Cohen’s d\textsuperscript{126} were computed by dividing the change from pre-test to early post-test (early effects) or from pre-test to late post-test assessment (late effects) by the standard deviation of the pre-test values. Effect sizes were interpreted based on published values: <0.2 trivial effect, 0.2-0.5 small effect, 0.5-0.8 moderate effect, >0.8 large effect.\textsuperscript{126} In the presence of a small sample size (as is common in clinical research studies involving participants with neurologic conditions), the effect size is a better estimate of clinical meaningfulness than the p-values.

Results

Twenty-four participants completed the study (3 females, mean age 43.7±10.8). Three subjects did not complete all sessions, and were lost to follow-up. One individual participated in the training, but was not able to perform any of the outcome measures; this participant was treated as an outlier and was excluded from the overall analysis. Twenty individuals completed all stimulation and assessment sessions. Demographic information is given in Table 4.1.

Performance measures

Scores for all performance measures at pretest are presented in Table 4.2. Note the similarity of values for all pretest measures in all three consitions (TENS, VIB and tDCS). In table 4.3, early effects (post-pretest) and late effects (late post-pretest) for performance measures can be seen for all conditions. Between-condition comparisons for
the change in pinch force approached significance (p=0.07). Post hoc test revealed that pinch force increases were significantly greater when participants received VIB compared with TENS (p=0.03), and no differences were found in the comparisons between tDCS and either VIB or TENS (p=0.4 and p=0.1, respectively). The effect sizes for early changes were small for all conditions (Cohen’s d TENS=0.0, VIB=0.21, tDCS=0.13). Improvements in pinch force were not present at late post-test, as between-condition comparisons failed to reach significance (p=0.14). Late changes in pinch force were associated with small effect sizes in for TENS, VIB and tDCS (d=0.07, 0.23 and 0.29, respectively).

At early post-test, TENS was associated with the greatest improvement in the successful manipulation of pegs (mean change=1.8±1.8), when compared with tDCS (mean change=1.1±2.1) and VIB (mean change=0.6±1.8). Between-condition differences in change approached significance (p=0.08). Post hoc tests revealed significant differences between TENS and VIB (p=0.06) and TENS and tDCS (p=0.02). No differences were found between VIB and tDCS (p=0.4). Effect sizes for early changes were moderate for TENS (d=0.59), and small for VIB (d=0.21) and tDCS (d=0.13). Late changes followed a similar pattern, and between-condition differences in change achieved significance (p=0.04) (mean change TENS= 2.0±2.5, tDCS=1.8±2.5, VIB=0.3±1.6). Post-hoc tests revealed significant differences between TENS and VIB (p=0.002) and tDCS and VIB (p=0.01). No differences were found between TENS and tDCS (p=0.6). The effect sizes associated with late changes were moderate for TENS (d=0.65) and tDCS (d=0.52), and small for VIB (d=0.07).
There were no between-group differences in visuomotor tracking performance during the early or late post-test (p=0.99 and p=0.49, respectively). Effect sizes for all conditions were small during the early post-test (Cohen’s d TENS=0.22, VIB=0.45, tDCS=0.07). At late post-test tDCS was associated with greater improvement in VM performance, as evidenced by a moderate effect size (d=0.51). TENS and VIB demonstrated small effect sizes (d=0.18 and d=0.21, respectively).

Corticomotor Excitability Measures

Two participants could not tolerate the procedure for determining the maximum motor response (M-max), which was intended to normalize the MEP to differences in neuromuscular properties. Evidence suggests that increases in alterations in corticomotor excitability associated with electrical stimulation and vibration occur in the absence of changes in M-waves. In addition, we believe that by using EMG biofeedback and monitoring to ensure that pulses were given only when participants were at their “target” level (10-15%MVC) ensured a consistent level of baseline excitability. Therefore, we report the non-normalized MEP amplitudes, which were available for all participants (Table 4.2 and Figure 4.7).

Changes in corticomotor excitability in the different conditions were characterized by different patterns, as illustrated in Figure 1. Early increases in corticomotor excitability were observed in all conditions, but achieved significance after TENS (p=0.003), and approached significance after tDCS (p=0.07). VIB was not associated with an early modulation in corticomotor excitability (p=0.6). At the late post-test assessment, corticomotor excitability returned to pre-test levels in the TENS and tDCS condition
(p=0.8 and p=0.9, respectively) but continued to increase in the VIB condition, achieving significance (p=0.006).

**Discussion**

In the present study, we demonstrated that TENS, VIB and tDCS influence different aspects of hand function when administered in combination with repetitive task practice. In addition, these changes appear to be associated with distinct temporal patterns of modulation of corticomotor excitability. Early gains in pinch force were greater in the VIB group, but were not present at late post-test. Early gains in fine motor performance measured by the NHPT were greater with TENS. At late post-test, increased NHPT performance was seen with TENS. There was no between-condition difference in the change in visuomotor tracking ability, but greatest improvements were seen with tDCS at late post-test. Early increases in corticomotor excitability were observed with TENS and tDCS, but were not retained 30 minutes later. VIB was associated only with increases in corticomotor excitability during the late assessment.

We identified a transient increase in pinch force (i.e., ICF body structure and function level) when repetitive task practice was paired with VIB. Increased pinch force following a single-session of vibration delivered at 80Hz has been found in one study with neurologically healthy controls and one pilot study with individuals with tetraplegia. We speculate that our findings might be associated with increased activation of the spinal circuits involved in the stretch reflex, both reported to be associated with the activation of mostly primary, but also secondary muscle spindle afferents by the VIB stimulus. Although we did not measure excitability of the spinal circuits in the present
study, this idea is supported at least partially by the lack of early increases in
corticomotor excitability in the individuals in whom we were able to measure MEPs in
the VIB condition (n=9), allowing the inference that increases in force associated with
VIB may not be accompanied by changes in corticomotor excitability. The increase in
force in the VIB condition was associated with a small effect size (d=0.21) and was not
present at late post-test, and therefore it is less relevant for clinical practice. We believe
that greater increases in strength would have been found if participants had engaged in
strength training instead of repetitive task practice, or if the number of sessions (i.e.,
dose) had been greater.

Greatest improvement in NHPT performance (i.e., ICF activity level) was seen with
TENS, followed by tDCS, and VIB was not associated with changes in NHPT
performance. In the present study, performance improvements in skilled hand function
following one session of TENS while performing repetitive task practice were
comparable to those reported with one session of somatosensory electrical stimulation in
individuals with chronic stroke. In that study, skilled hand performance was measured
with the Jebsen-Taylor Hand Function Test (also a measure of ICF activity level), highly
correlated with the NHPT in individuals with chronic stroke, whom have similar
deficits in corticomotor excitability and hand function, and exhibit similar degree of
improvement when participating in interventions consisting of motor training and
stimulation. Effect sizes could not be calculated from the information provided in the
article by Conforto et al, but the percent improvement after both somatosensory
stimulation and training reported by Conforto et al (approximately 28%) was comparable
to the improvement we found in the TENS condition (34%). The similarity between the
effects associated with TENS (short pulse duration) in the present study, and with somatosensory electrical stimulation (long pulse duration) in prior studies suggests that pulse duration may not be a critical variable. These findings are consistent with the evidence supporting that these two approaches activate the same large diameter afferents\textsuperscript{60,157} that can in turn excite M1 via cortico-cortico connections between S1 and M1.\textsuperscript{57}

One difference between the study discussed above by Conforto et al\textsuperscript{63} and the current study, is that our participants received stimulation while engaged in repetitive task practice, while in the study of Conforto et al participants performed motor training after stimulation had ceased. It is not clear if one approach leads to better results, but there is evidence to suggest that they may be similar.\textsuperscript{158} Studies indicate that the application of a protocol that can increase the integrated postsynaptic response of corticomotor neurons (such as somatosensory stimulation or TENS, which have been associated with transient disinhibition of the motor cortex) can increase the effects of repetitive task practice (and thus, lead to greater gains in motor function) if stimulation is administered prior to or during repetitive task practice.\textsuperscript{158} This increase in the integrated postsynaptic response is associated with a removal of a voltage-sensitive magnesium block from NMDA receptors during depolarization,\textsuperscript{159} and a concurrent increase in the responsiveness to training mediated by increased intracellular calcium levels.\textsuperscript{158} While the corticomotor excitability results from the present study must be interpreted with caution, we found an early increase in corticomotor excitability in the TENS condition, which paralleled the NHPT improvements. Taken together, these results suggest that the concomitant application of TENS with repetitive task practice may be preferable to somatosensory stimulation prior
to repetitive task practice, as similar results can be achieved with a more efficient use of
time.

One could argue that the training alone could have been responsible for the effects
observed. However, we found a moderate effect size for the improved performance in the
NHPT associated with the TENS condition (d=0.59), during the early post-test. In
addition, moderate effects size for the improvement in NHPT was found at late post-test
for both TENS and tDCS (d=0.65 and d=0.52, respectively). While we did not assess
training alone in the present study, in an earlier study with participants recruited using the
same inclusion criteria as the present study, we found the effect sizes associated the pre-
post changes in fine motor function assessed with the JTT following 3 days of fine motor
practice alone to be smaller than the effects reported for TENS and tDCS (SRM=0.42).

There was no between-condition difference in the change in visuomotor tracking ability,
which can be explained by a number of factors. It is possible that modulation of small
forces is not impaired in individuals with tetraplegia. Lindberg et al152 found that
performance in a VM task calibrated in the range of 10-20%MVC (as in the present
study) was not different between individuals with stroke and neurologically healthy
controls. We found a moderate effect size for the improvement in visuomotor tracking
with the tDCS condition at late post-test (d=0.51). This finding is consistent with the
literature demonstrating that tDCS exerts online effects (observed during stimulation) and
offline effects (observed after stimulation has ceased).98

The time course of cortical excitability increases appeared to be different between the
three conditions. TENS and tDCS were associated with early increases in cortical
excitability, which were not present 30 minutes after post-test. Alternatively, corticomotor excitability was unchanged with VIB during the early assessment, but showed a late increase. This difference in the time course of excitability between the conditions can be explained by a number of factors. Given that the dose of VIB (10 min) was smaller than the TENS and tDCS (both administered for 30 minutes), it is likely that the lack of excitability increase at post-test could be due to due a difference in dose. In addition, vibration of the distal tendon of the flexor carpi radialis could have been insufficient to stimulate the thenar muscles, which is consistent with the evidence demonstrating that the increased corticomotor excitability following VIB is specific to the vibrated muscle. However, we found increased excitability at late post-test. It is also possible that VIB opens a delayed window of opportunity wherein there is corticomotor excitability. Marconi et al found late increases in cortical excitability measured 1 hour after VIB (100Hz, 0.2-0.5mm amplitude, in 10 min sessions daily for 3 consecutive days). In addition, participants who received VIB made greater improvements in a standard physical therapy intervention that was initiated approximately 30 minutes after vibration had ceased. While studies characterizing the time course and mechanisms underlying corticomotor excitability after VIB are encouraged, the clinical importance of these findings is that it may be best to engage in skilled motor practice after vibration has ceased, to take advantage of the available increase in corticomotor excitability to make improvements in skilled hand use.

This study has several limitations. Firstly, the small sample size results in limited power to detect differences. The lack of a control group who only participated in repetitive task practice introduces potential bias. However, it was our interest to assess the comparative
efficacy of TENS, VIB and tDCS, which can then lead to the subsequent design of costly, larger clinical trials. In addition, VIB was only applied for 10 continuous minutes, while tDCS and TENS were applied for 30 minutes. Data from our lab and others suggest brief periods of vibration, ranging from 3 minutes to 15 minutes (continuously) can influence the nervous system. While we realize that this may result in a lower dose than the other stimulation techniques, our interest was in assessing the most promising parameters that could be translated into direct clinical application. Pilot data suggested limited application would be safer.

Conclusion

In summary, the results of the present study suggest that a single-session of VIB while performing repetitive task practice is associated with a transient increase in pinch force. TENS was associated with increased in fine motor function with duration of at least 30 minutes, suggesting that it can be a potential tool to improve fine motor function during repetitive task practice in persons with chronic tetraplegia. Greater increase in fine motor function with tDCS was seen at late post-test. Given that somatosensory stimulation has been shown to lead to greater gains in skilled hand function in studies with greater doses of task-specific practice (2hrs per day, 5 days per week for 3 weeks), the results of the present study suggest that TENS and tDCS, which are easily accessible devices to physical therapists and patients, can exert similar effects on motor function. In addition, TENS and tDCS may share similarities in early increases in corticomotor excitability, whereas VIB seems to follow a different pattern characterized by late increases in corticomotor excitability. However, studies performing assessments between the three conditions are encouraged to further examine this idea.
Acknowledgements and Funding Source

This work was funded by National Institutes of Health NINDS NS083064. The authors would like to thank Dr. Jorge Bohorquez and Daniel Gonzalez for designing and building the visuomotor tracking task.
Figure 4.1 Experimental Setup. Participants completed a total three of sessions, one week apart. Measurements of pinch force, cortical excitability (amplitude of motor evoked potential), fine motor performance (Nine-Hole Peg Test; NHPT), and visuomotor tracking at pre-test, following 30 minutes of repetitive task practice with either TENS, tDCS or VIB (early post-test) and after a 30 minute rest period (late post-test).
Figure 4.2 Participant performing repetitive task practice on a day that he was randomized to the tDCS condition (Note the electrodes placed on his forehead and M1). Each task was performed for 10 minutes.
Figure 4.3 Example of electrode placement during the TENS condition.
Figure 4.4 Example of electrode placement during the VIB condition.
Figure 4.5 Interface of the software used for visuomotor tracking. The curve was calibrated for each individual. The x-axis is time (in seconds) and the y-axis is force (in Newtons). The peak was set at the 20% and the valley was set at 10% of the force corresponding to the maximum voluntary activation during pinch. The white line is the target, and the red line corresponds to the output exerted by the participant.
Figure 4.6 Example of spontaneous potentials recorded during recording of MEPs from the thenar muscles. The x-axis represents time (in milliseconds) and the y-axis represents intensity of the response (in millivolts). Note the electromyographic activity when the participant is at rest, approximately 20 milliseconds after the stimulus artifact (the appropriate latency and amplitude for MEPs from the thenar muscles)(top). In the subsequent frame, a waveform of similar shape and amplitude is measured prior to the stimulus, indicating that this activity is not related with the electromagnetic induction or voluntary activity (bottom).
Figure 4.7 Temporal pattern of corticomotor excitability associated with VIB (in blue), TENS (in green) and tDCS (in red), all administered while participants were engaged in repetitive task practice for 30 minutes. Note that tDCS and TENS followed a similar pattern characterized by increased MEP amplitude at early post-test, which returned to pre-test levels during the late post-test. VIB did not alter excitability at post-test, but led to a delayed increase in excitability measured at late post-test.
Table 4.1. Demographic characteristics of the participants included in the study.

<table>
<thead>
<tr>
<th>Subject Characteristics (SCI group)</th>
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<tbody>
<tr>
<td>Age (years)</td>
<td>43.7±10.8 (min=25; max=59)</td>
</tr>
<tr>
<td>Post-injury (years)</td>
<td>7.3±6.9 (min=1; max=28)</td>
</tr>
<tr>
<td>Sex</td>
<td>3 females, 21 males</td>
</tr>
<tr>
<td>Injury Level</td>
<td>C6 (median)</td>
</tr>
<tr>
<td>AIS</td>
<td>D (median)</td>
</tr>
</tbody>
</table>

*AIS=American Spinal Cord Injury Impairment Scale*
Table 4.2. Values for all performance outcome measures (pinch force, Nine-hole Peg Test, Visuomotor tracking task and motor evoked potentials (MEPs) for individuals at pre-test for all conditions (TENS, VIB and tDCS). Note that values were equivalent at baseline.

<table>
<thead>
<tr>
<th></th>
<th>Pre-test values</th>
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<tbody>
<tr>
<td></td>
<td>TENS</td>
<td>VIB</td>
<td>tDCS</td>
<td></td>
</tr>
<tr>
<td><strong>Pinch force</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(kg) <strong>N=20</strong></td>
<td>3.0±1.9</td>
<td>2.7±1.9</td>
<td>2.8±2.0</td>
<td></td>
</tr>
<tr>
<td>(2.0,4.0)</td>
<td>(1.8,3.7)</td>
<td>(1.8,3.8)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>NHPT</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(average pegs) <strong>N=20</strong></td>
<td>4.9±3.1</td>
<td>5.3±4.2</td>
<td>4.7±3.4</td>
<td></td>
</tr>
<tr>
<td>(3.0,6.1)</td>
<td>(3.4,8.1)</td>
<td>(3.0,6.3)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>VM</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(error) <strong>N=20</strong></td>
<td>0.05±0.02</td>
<td>0.04±0.01</td>
<td>0.05±0.03</td>
<td></td>
</tr>
<tr>
<td>(0.03,0.06)</td>
<td>(0.03,0.04)</td>
<td>(0.03,0.06)</td>
<td></td>
<td></td>
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<tr>
<td><strong>MEPs</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(mV) <strong>N=7 tDCS N=9 VIB N=11 TENS</strong></td>
<td>0.16±0.01</td>
<td>0.16±0.01</td>
<td>0.17±0.01</td>
<td></td>
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<tr>
<td>(0.15,0.16)</td>
<td>(0.15,0.16)</td>
<td>(0.16,0.17)</td>
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</tbody>
</table>
Table 4.3. Early and late effects (change scores) for performance-based outcomes for participants by condition (TENS, VIB and tDCS). Note: Early effects were calculated by subtracting the scores at early posttest from pretest and late effects were calculated by subtracting the scores at late posttest from pretest for all outcome measures. † Refers to values 0.05≤0.1 (see Statistical Analysis for details). * Refers to groups that were associated with significant differences (p<0.05) in the planned post hoc comparisons performed with paired t-tests.

<table>
<thead>
<tr>
<th></th>
<th>Early effects (Early posttest-pretest)</th>
<th>Late effects (Late posttest-pretest)</th>
<th>Early effects (Main effect of Condition)</th>
<th>Late effects (Condition)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean±SD (95%CI)</td>
<td>Mean±SD (95%CI)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Effect size</td>
<td>Effect size</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TENS</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NHPT (average pegs)</td>
<td>1.8±1.8 (1.0, 2.7)</td>
<td>1.1±2.0 (0.1, 2.1)</td>
<td></td>
<td>1.8±2.5 (0.6, 3.0)</td>
</tr>
<tr>
<td>N=20</td>
<td>0.59</td>
<td>0.32</td>
<td></td>
<td>0.08†</td>
</tr>
<tr>
<td>Pinch force (kg)</td>
<td>-0.01±0.3 (-0.2, 0.1)</td>
<td>0.2±0.7 (-0.07, 0.6)</td>
<td></td>
<td>0.6±1.0 (0.1, 1.0)</td>
</tr>
<tr>
<td>N=20</td>
<td>0.0</td>
<td>0.13</td>
<td></td>
<td>0.07†</td>
</tr>
<tr>
<td>VM (error) N=20</td>
<td>-0.006±0.01 (-0.01, 0.003)</td>
<td>-0.009±0.01 (-0.01, -0.001)</td>
<td></td>
<td>0.99</td>
</tr>
<tr>
<td>MEPs (mV) N=7 tDCS, N=9 VIB, N=11 TENS</td>
<td>0.20±0.02 (0.18, 0.21)</td>
<td>0.20±0.06 (0.14, 0.25)</td>
<td></td>
<td>0.17±0.05 (0.12, 0.21)</td>
</tr>
</tbody>
</table>

0NHPT=nine-hole peg test; kg=kilogram; VM=visuomotor tracking task; MEP=motor evoked
CHAPTER 5. IMPLICATIONS FOR NEUROREHABILITATION

Evidence suggests that there are secondary post-injury changes after SCI, including decreased corticomotor excitability and atrophy in the sensorimotor cortices and corticospinal tracts, all of which can contribute to the movement deficits resulting from the primary injury. There is also evidence to support that the use of stimulation aimed at increasing corticomotor excitability can lead to greater gains in skilled hand function when paired with repetitive task practice. In chapter two, found that the a 5-day approach consisting of tDCS using an electrode montage aimed at increasing corticomotor excitability (BAC-tDCS) interleaved with repetitive task practice led to greater increases in the performance of a bimanual typing task than repetitive task practice alone in a group of neurologically healthy participants. Our results add to the available evidence regarding the relationship between stimulation aimed at increasing corticomotor excitability and unimanual motor practice, suggesting that during bimanual repetitive task practice, it is likely that participants can also take advantage of the increased excitability to make further improvements in function. We encourage studies examining this idea in participants with chronic tetraplegia. The clinical implication of these findings is that it may be of value to use BAC-tDCS while performing bimanual upper extremity training with individuals with bimanual functional deficits, including tetraplegia.

In Chapter 3 we found that in participants with SCI only (and not in a separate group of neurologically healthy participants), a 3-day application of 10 Hz rTMS applied over the hand motor area with a fine motor task performed during the inter-stimulus intervals, was associated with a delayed improvement in fine motor function measured by the JTT and grasp force. The improvement in JTT with rTMS was greater than the improvements
made with sham-rTMS (and repetitive task practice). This is the first study to
demonstrate intermanual transfer of skilled learning (assessed with the JTT) from the
trained hand to the non-trained hand in individuals with tetraplegia. In addition, increased
hand function measured by the JTT was greater than the changes made while participants
were receiving sham-rTMS. These findings are consistent with the previous evidence that
demonstrated greater gains in function when using peripheral stimulation aimed at
increasing corticomotor excitability combined with repetitive task practice,\textsuperscript{14,64} and
suggest that cortical stimulation using indirect stimulation that engages the afferent
sensory fibers\textsuperscript{60,157} and sensory-to-motor connections between S1 and M1\textsuperscript{57} and direct
cortical stimulation using rTMS are equally valuable approaches to augment repetitive
task practice.

In Chapter 4, we demonstrated that TENS, VIB and tDCS (all clinically available
devices) influence different aspects of hand function when administered in combination
with repetitive task practice, and appeared to be associated with distinct temporal patterns
of modulation of corticomotor excitability. VIB was associated with transient increases in
pinch force, which is consistent with an earlier pilot study.\textsuperscript{71} Early gains in fine motor
performance measured by the NHPT were greater in the TENS condition, and were
retained for at least 30 minutes. Late gains in fine motor function were seen both with
TENS and tDCS. Late increases in visuomotor tracking performance were greater with
tDCS. Given that somatosensory stimulation has been associated with greater gains in
skilled hand function in studies with greater doses of task-specific practice (2hrs per day,
5 days per week for 3 weeks),\textsuperscript{9,14,64,65} the results of the present study suggest that TENS
and tDCS, both easily accessible devices to physical therapists and patients, can exert a
similar effects on motor function and may be useful in augmenting repetitive task practice. Further studies are encouraged to examine the effects of TENS and tDCS on multi-day repetitive task approaches in individuals with tetraplegia. TENS and tDCS may share similarities in early increases in corticomotor excitability, which were not retained 30 minutes later. VIB seems to follow a different pattern characterized by late increases in corticomotor excitability.

**Conclusions**

We found that: 1) in neurologically healthy participants, a 5-day approach combining tDCS (using an electrode montage aimed at increasing corticomotor excitability bilaterally, [i.e., BAC-tDCS]) and repetitive practice was associated with greater improvements in bimanual typing performance than those observed with repetitive task practice alone; 2) in individuals with chronic tetraplegia, a 3-day approach of 10 Hz rTMS (aimed at increasing corticomotor excitability) interleaved with repetitive task practice was associated with greater improvements in fine motor function and grasp force compared with sham-rTMS and repetitive practice; 3) a single-session of TENS combined with 30 minutes of repetitive task practice is associated with greater improvements in fine motor function measured immediately after training and late increases in fine motor function are seen both with tDCS and TENS; 4) a single-session of VIB combined with 30 minutes of repetitive task practice is associated with greater improvements in pinch force than repetitive practice combined with TENS or tDCS, and this improvement is not retained.
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