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Outsmarting the Brain: Augmenting Motor Training with Non-invasive Brain Stimulation in Order to Facilitate Plasticity-Dependent, Functional Improvement within the Motor Cortex

Raymond Joseph Butts

University of South Carolina

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Outsmarting the Brain: Augmenting Motor Training with Non-invasive Brain Stimulation in Order to Facilitate Plasticity-Dependent, Functional Improvement within the Motor Cortex

by

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Submitted in Partial Fulfillment of the Requirements
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2013

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DEDICATION

This PhD dissertation project is dedicated to Jersey and Sydney.
ACKNOWLEDGEMENTS

Thank you to my thesis committee for helping me through the dissertation process. I am especially grateful to my advisor, Dr. Newman-Norlund, for his unwavering kindness, support, and enthusiasm.
ABSTRACT

Brain stimulation techniques capable of optimizing cortical plasticity may provide the key to improved therapeutic techniques and functional outcomes. The primary aim of this dissertation was to examine the potential of motor training (MT) augmented with intermittent theta burst stimulation (iTBS) and anodal transcranial direct current stimulation (a-tDCS). The secondary aim was to investigate whether the training would also be advantageous to older-adults. We hypothesized that right-handed, college-age students exposed to the treatment (n=17) would perform better short-term (directly following MT) and long-term (24 hours and 7 days following MT) on motor-skill retention tests than students receiving sham stimulation (n=14). We also hypothesized that older adults (n=9) exposed to iTBS/a-tDCS enhanced MT would demonstrate greater functional improvements than younger adults (n=16) receiving identical stimulation. iTBS and a-tDCS over the non-dominant motor cortex were used as a primer to, and in conjunction with, 20-minutes of non-dominant, upper extremity MT, respectively. The Jebsen-Taylor Hand Function Test (JTHF) was chosen as the primary outcome measure, while the Pursuit Rotor Tracking Test (PRTT), Purdue Pegboard Test (PPB), and Fitt’s Reciprocal Tapping Test (FRTT) were considered secondary outcome measures. Students receiving iTBS/a-tDCS enhanced MT made significantly greater improvements on the JTHF than the placebo-control group (p = .041). However, differences in improvement between the groups were primarily seen long-term (p=.045). Secondary
outcome measures were not sensitive enough to detect a difference between the groups at any time point. Concerning the overall performance of older vs. younger participants, whose training was augmented by iTBS/a-tDCS, neither group improved more than the other on the JTHF (p = .1801). The older group scored better on the PRTT (p = .016) and the PPB (p = .0036) but not the FRTT. Although there was no short-term performance difference on any outcome measure, older adults made greater functional improvements than younger adults long-term on the PPB (p = .0039), PPB (p = .0008) and JTHF (p = .0384) (7 days post-treatment). Collectively, the results suggest that brain stimulation may be a useful adjunct to MT in healthy, younger and older adults. Brain stimulation may also eventually improve PT outcomes of neurologically-impaired patients.
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<table>
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<th>Abbreviation</th>
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<tbody>
<tr>
<td>APB</td>
<td>Abductor Pollicis Bevis</td>
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<td>AMT</td>
<td>Active Motor Threshold</td>
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<tr>
<td>ANOVA</td>
<td>Analysis of Variance</td>
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<tr>
<td>a-tDCS</td>
<td>Anodal Transcranial Direct Current Stimulation</td>
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<tr>
<td>BDNF</td>
<td>Brain Derived Neurotrophc Factor</td>
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<tr>
<td>CNS</td>
<td>Central Nervous System</td>
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<tr>
<td>c-tDCS</td>
<td>Cathodal Transcranial Direct Current Stimulation</td>
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<tr>
<td>E-LTP</td>
<td>Early Long-term Potentiation</td>
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<td>FDI</td>
<td>First Dorsal Interosseus</td>
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<tr>
<td>FRTT</td>
<td>Fitt’s Reciprocal Tapping Task</td>
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<tr>
<td>GABA</td>
<td>Gamma-aminobutyric Acid</td>
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<tr>
<td>ICI</td>
<td>Intracortical Inhibition</td>
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<tr>
<td>IRB</td>
<td>Institutional Review Board</td>
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<tr>
<td>iTBS</td>
<td>Intermittent Theta Burst Stimulation</td>
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<td>JTHF</td>
<td>Jebsen-Taylor Hand Function Test</td>
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<td>LICI</td>
<td>Long-interval Intracortical Inhibition</td>
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<td>L-LTP</td>
<td>Late Long-term Potentiation</td>
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<tr>
<td>Acronym</td>
<td>Description</td>
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<tr>
<td>M1</td>
<td>Primary Motor Cortex</td>
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<tr>
<td>MEP</td>
<td>Motor Evoked Potential</td>
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<tr>
<td>MT</td>
<td>Motor Training</td>
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<tr>
<td>MVC</td>
<td>Maximal Voluntary Contraction</td>
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<tr>
<td>PPB</td>
<td>Purdue Pegboard Test</td>
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<tr>
<td>PRTT</td>
<td>Pursuit Rotor Tracking Task</td>
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<tr>
<td>PT</td>
<td>Physical Therapy</td>
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<tr>
<td>rTMS</td>
<td>Repetitive Transcranial magnetic Stimulation</td>
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<tr>
<td>tDCS</td>
<td>Transcranial Direct Current Stimulation</td>
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<tr>
<td>SICI</td>
<td>Short-interval Intracortical Inhibition</td>
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<tr>
<td>TMS</td>
<td>Transcranial Magnetic Stimulation</td>
</tr>
<tr>
<td>UE</td>
<td>Upper extremity</td>
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<tr>
<td>USC</td>
<td>University of South Carolina</td>
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CHAPTER I

INTRODUCTION

Of the 795,000 people that will suffer from a stroke in the U.S. this year, two thirds will survive and require rehabilitative services.\textsuperscript{1} Presently, the main focus of rehabilitation is on regaining function in order to optimize quality of life. Despite the best efforts of physical therapists, many patients continue to suffer from limited strength, range of motion and overall decreased coordination of the upper extremity coinciding with the injured hemisphere years after a stroke. While new therapeutic techniques such as constraint-induced movement therapy (CIMT),\textsuperscript{2,3} locomotor training (LT)$^{4,5}$ and intensive mobility training (IMT)$^6$ have improved therapeutic outcomes via principles of forced use and massed practice in both the acute and chronic stroke population, significant stroke-related disabilities often persist. More than 85% of patients that have suffered a stroke have lasting functional impairments,\textsuperscript{2} and approximately 50-60% of survivors continue to require functional assistance to complete activities of daily living after completion of intensive physical therapy.\textsuperscript{7}

As a result, continual investigation into better rehabilitative strategies for the stroke population is necessary. Experiments using fMRI have demonstrated that peripheral motor recovery is accompanied by significant changes within the central nervous system (CNS), suggesting that cortical plasticity plays an important role in the stroke recovery process.\textsuperscript{8} Therefore, techniques that are capable of optimizing cortical plasticity presented in combination with extremity specific training may provide the key
to improved therapeutic techniques and better functional outcomes. In this context, transcranial magnetic stimulation (TMS) and transcranial direct current stimulation (tDCS) are two relatively new technologies that deserve further consideration. However, the efficacy of using a combined TMS/tDCS as a complement to motor training (MT) has yet to be established.

1.1 TERMS AND DEFINITIONS

Transcranial magnetic stimulation (TMS) is one of the first technologies available to safely and noninvasively stimulate specific areas of the cortex from outside the scalp via magnetically induced eddy currents, resulting in a lasting cortical effect. TMS may be applied to the scalp as a single pulse or as a series of pulses known as repetitive transcranial magnetic stimulation (rTMS). High-frequency and low-frequency rTMS have demonstrated the ability to induce cortical excitation and inhibition, respectively, that lasts beyond the stimulation time period. Intermittent theta burst stimulation (iTBS) is a type of rTMS, whereby three TMS pulses at 50 Hz are provided every 200ms (i.e., at 5 Hz) at 80% active motor threshold (AMT). Ten bursts are grouped and repeated every 10 seconds for a total of 20 trains. The AMT is defined as the lowest stimulation intensity able to produce at least 5/10 motor evoked potentials greater than or equal to a 200 µV amplitude (above baseline). For purposes of this study, AMT was measured in the non-dominant abductor pollicis brevis (APB) muscle.

Transcranial direct current stimulation (tDCS) is a second type of non-invasive brain stimulation technology presented via an electrode patch above the region of interest with a reference electrode over a predetermined region of the opposite hemisphere. Cortical areas receiving anodal and cathodal stimulation demonstrate increased or
decreased sensitivity, respectively. Importantly, tDCS does not cause neurons to fire per se (whereas TMS does), but rather, alters the probability that they will fire during the course of normal activity.

1.2 RESEARCH PROBLEM

Understanding the mechanisms behind TMS and tDCS is paramount to their successful implementation in rehabilitation setting. The physiology behind the two brain stimulation technologies suggest that TMS may be more effective as a primer for PT, whereas a-tDCS may be more powerful when used in conjunction with rehabilitative movements. A TMS primer would minimize intracortical inhibition (ICI) and therefore allow glutamatergic neurons within M1 to interact unimpeded during a-tDCS enhanced therapy via E-LTP. Furthermore, a reduction in ICI may facilitate optimal BDNF release and subsequent plasticity within M1 during L-LTP. To our knowledge, this is the first study to attempt MT primed with iTBS and presented in conjunction with a-tDCS.

1.3 STUDY AIMS

The purpose of this study was to examine the potential of MT primed with iTBS and presented in conjunction with a-tDCS. The aims were to:

1) Compare functional outcomes of healthy college-age students following MT enhanced with iTBS/a-tDCS vs. MT enhanced with placebo stimulation.

2) Examine whether older adults would make functional gains comparable to young adults following MT augmented with iTBS/a-tDCS.

3) Speculate whether a combined iTBS/a-tDCS paradigm would benefit the rehabilitation of neurologically impaired patients.
The proposed research questions for this study were as follows:

1) Will non-dominant upper extremity MT primed with iTBS and administered concurrently with a-tDCS result in better short-term outcomes (directly following MT) than MT with placebo stimulation?

- **Primary Question**: Will participants receiving stimulation perform better on the Jebsen-Taylor Hand Function Test (JTHF) than participants receiving placebo stimulation?

- **Secondary Question**: Will participants receiving stimulation perform better on a computer-based, Fitt’s reciprocal tapping task (FRTT), a computer-based, pursuit rotor tracking task (PRTT, and the Purdue Pegboard Test (PPB) than participants receiving placebo stimulation?

2) Will non-dominant upper extremity MT primed with iTBS and administered concurrently with a-tDCS, result in better long-term outcomes (24 hours and 7 days following MT) than MT with placebo stimulation?

- **Primary Question**: Will participants receiving stimulation perform better on the JTHF than participants receiving placebo stimulation?

- **Secondary Question**: Will participants receiving stimulation perform better on the FRTT, PRTT, and PPB than participants receiving placebo stimulation?

3) Will non-dominant upper extremity training of elderly participants primed with iTBS and administered concurrently with a-tDCS result in a greater effect size short-term (directly following MT) than non-dominant upper extremity training of healthy, college aged students primed with iTBS and administered concurrently with a-tDCS?
- **Primary Question:** Will elderly participants receiving cortical stimulation demonstrate a greater effect size on the JTHF pre-post training than college-aged participants receiving cortical stimulation?

- **Secondary Question:** Will elderly participants receiving cortical stimulation demonstrate a greater effect size on the FRTT, PRTT, and PPB than college-aged participants receiving cortical stimulation?

4) Will non-dominant upper extremity training of elderly participants primed with iTBS and administered concurrently with a-tDCS result in a greater effect size long-term (24 hours and 7 days following MT) than non-dominant upper extremity training of healthy, college aged students primed with iTBS and administered concurrently with a-tDCS?

- **Primary Question:** Will elderly participants receiving cortical stimulation demonstrate a greater effect size on the JTHF pre-post training than college-aged participants receiving cortical stimulation?

- **Secondary Question:** Will elderly participants receiving cortical stimulation demonstrate a greater effect size on the FRTT, PRTT, and PPB than college-aged participants receiving cortical stimulation?
WORKS CITED


CHAPTER II

REVIEW OF THE LITERATURE

Of the 795,000 people that will suffer from a stroke in the U.S. this year, two thirds will survive and require rehabilitative services.\(^1\) Presently, the main focus of rehabilitation is on regaining function in order to optimize quality of life. Despite the best efforts of physical therapists, many patients continue to suffer from limited strength, range of motion and overall decreased coordination of the upper extremity coinciding with the injured hemisphere years after a stroke. While new therapeutic techniques such as constraint-induced movement therapy (CIMT),\(^2,3\) locomotor training (LT)\(^4,5\) and intensive mobility training (IMT)\(^6\) have improved therapeutic outcomes via principles of forced use and massed practice in both the acute and chronic stroke population, significant stroke-related disabilities continue to persist. More than 85% of patients that have suffered a stroke have lasting functional impairments,\(^2\) and approximately 50-60% of survivors continue to require functional assistance to complete activities of daily living after completion of intensive physical therapy (PT).\(^7\)

2.1 UPPER EXTREMITY FUNCTION OF OLDER ADULTS

Another population that suffers from loss of function is the elderly. Although not neurologically impaired per se, older individuals gradually lose function throughout life and often seek PT to maintain their level of function and quality of life. Hand function, in particular, is necessary for completion of activities of daily living but tends to decline with age, especially in individuals over the age of 65.\(^8\) As such, hand function has been
correlated with independence and quality of life in older adults. Individuals over the age of 50 have significantly lower manual functional test scores for their dominant hand compared to subjects 30 years younger. Moreover, participants tested with their non-dominant hand demonstrate a reduction in hand function compared to their younger counterparts on the PPB starting at age 40. Grip strength of the non-dominant upper extremity (UE) of elderly women also correlates with signs and symptoms associated with frailty. Pursuit of strategies to maintain optimal motor function of both the dominant and non-dominant hands is therefore advantageous, especially for the elderly. While comprehensive PT has traditionally been the solution for maintaining hand function, decreasing insurance caps and changes in re-imbursement rates may require a change in strategy in the near future.

There are a number of physiologic changes that occur throughout life, resulting in decreased function of the upper extremities. As individuals age, decreased muscle strength and mass combined with loss of bone density are among the most common changes that occur in the hands. Muscle strength gradually decreases from age 30 to 50, with an exaggerated 15% and 30% decrease in the 6th and 8th decade of life, respectively. Ageing individuals gradually lose type I and type II muscle fibers, a result of degenerative motor neurons apoptosis, ventral root axons, and neuromuscular junctions. Studies with surface EMG demonstrate less activation of motor units in agonist muscles combined with greater activation of antagonist muscles via reciprocal inhibition. The agonist-antagonist muscle mismatch likely explains impairments in force production and fine motor control typically observed in this population. Moreover, as muscles atrophy, they are typically replaced with less contractile components such as
adipose tissue and collagen fibers, resulting in less overall elasticity and flexibility.\textsuperscript{15} Peripherally, older adults have less density and distribution of Pacinian corpuscles, Meisner’s corpuscles, and Merkel discs, resulting in impaired spatial acuity, vibration recognition, and tactile sensitivity.\textsuperscript{16} Decreased friction between the skin and handheld objects has also been linked with improper grip force during object manipulation.\textsuperscript{17} Ageing individuals also experience degenerative changes within the central nervous system (CNS). When the motor cortex of an older adult is stimulated with TMS pulses, a greater intensity of stimulation is required to elicit motor evoked potentials equivalent in amplitude to those of a younger person.\textsuperscript{14} This finding implies that the motor cortex and corticospinal tract of older adults are less excitable than those of younger adults. Given that the stimulation of the motor cortex during maximal voluntary contraction is associated with greater torque production, excitability of the nervous system likely plays a role in decreased hand function.\textsuperscript{14,18} Relative to younger adults, the motor cortex of older adults is also less able to encode new motor memories, suggesting that traditional therapy may play a limited role in maintaining function.\textsuperscript{19} Techniques that are capable of optimizing cortical plasticity presented in combination with extremity specific training may therefore help improve therapeutic techniques and facilitate better functional outcomes.

2.2 OVERVIEW OF TRANSCRANIAL MAGNETIC STIMULATION AND TRANSCRANIAL DIRECT CURRENT STIMULATION

As a result, continual investigation into better motor training (MT) strategies is necessary. Experiments using fMRI have demonstrated that peripheral motor recovery is accompanied by significant changes within the central nervous system, suggesting that plasticity plays an important role in the stroke recovery process.\textsuperscript{20} Therefore, techniques
that are capable of optimizing cortical plasticity presented in combination with extremity specific training may provide the key to improved therapeutic techniques and better functional outcomes. In this context, transcranial magnetic stimulation (TMS) and transcranial direct current stimulation (tDCS) are two relatively new technologies that deserve further consideration. However, the efficacy of using TMS and tDCS as a complement to stroke rehabilitation has yet to be established. Moreover, studies that have attempted combined TMS- tDCS / physical therapy strategies are very limited.

TMS is one of the first technologies available to safely and noninvasively stimulate specific areas of the cortex from outside the scalp via magnetically induced eddy currents, resulting in a lasting cortical effect. Both high-frequency and low-frequency repetitive transcranial magnetic stimulation (rTMS) have demonstrated the ability to induce cortical excitation and inhibition, respectively, that lasts beyond the stimulation time period. TMS studies incorporating intermittent theta burst stimulation (iTBS) and repetitive transcranial magnetic stimulation (rTMS) have demonstrated >30 minutes of enhanced motor evoked potentials (MEPs) in target musculature. Moreover, iTBS has been shown to create theta oscillations within the cortex consistent with those associated with learning and memory in the hippocampus. Researchers have also effectively used tDCS to noninvasively stimulate the cortex via a low-intensity, electric current presented continuously on the surface of the scalp. Stimulation is presented via an electrode patch placed directly above the region of interest with a reference electrode over a predetermined non-cranial region. Following as little as 13 minutes of stimulation cortical areas receiving anodal and cathodal stimulation demonstrate up to 90 minutes of increased or decreased sensitivity respectively. Importantly, tDCS does not cause
neurons to fire per se (whereas TMS does), but rather, alters the probability that they will fire during the course of normal activity.

2.3 PROPOSED MECHANISM OF TRANSCRANIAL MAGNETIC STIMULATION

The primary motor cortex is primarily responsible for control of the peripheral musculature and contains both excitatory and inhibitory neurons across its six cortical layers. The cascade of neuronal activity leading up to activation of pyramidal cells, and eventually muscles, has been well studied. Interneurons that produce gamma-aminobutyric acid (GABA) initially inhibit excitatory pyramidal neurons intracortically via GABA-A receptors, a process known as short-interval intracortical inhibition (SIACI). In the healthy brain, SIACI prevents unwanted movement in a muscle-specific manner. Critically, an imbalance in SIACI may exist following a stroke, and can represent a serious obstacle to plasticity dependent recovery. Recent experiments with mice suggest that the stroke-induced imbalance in SIACI may be due to the absence of transporters in the peri-lesional regions that are normally responsible for the re-uptake of GABA. In contrast to GABA-A receptors, GABA-B receptors are activated about 200 ms later than GABA-A receptors and typically function to inhibit GABAergic interneurons, a phenomenon known as long-interval intracortical inhibition (LICI).

Transcranial magnetic stimulation (TMS) pulses presented repetitively and temporally matched with GABA-B receptor channels inflict a period of intracortical disinhibition at approximately 200 ms. For example, intermittent theta burst stimulation (iTBS) involves the delivery of TMS pulse triplets (3 closely spaced pulses) every 200 ms for 3 minutes and results in a theta brain oscillation and up to 30 minutes of enhanced MEPs, peripherally. Interestingly, theta oscillations have been shown to
increase learning and memory via induction of long-term potentiation (LTP) in the hippocampus. Many researchers hypothesize that intracortical disinhibition may allow necessary glutamate release and subsequent activation of postsynaptic NMDA receptors to “kick-start” early long-term potentiation (E-LTP) within motor regions of the brain, thereby enabling motor recovery dependent plasticity to occur unfettered.

2.4 PHYSICAL THERAPY AUGMENTED WITH TRANSCRANIAL MAGNETIC STIMULATION

Utilizing iTBS as a primer to physical therapy may effectively place the ipsilesional motor cortex in an optimal state for relearning motor tasks during CIMT by minimizing GABA-A mediated IACI, thereby resulting in increased plasticity and better functional recovery. While only a few studies have explored this possibility and findings are inconsistent, TMS continues to be a promising adjunct to physical therapy. A recent study found that 1 hour of in-clinic supervised CIMT augmented with rTMS and followed by 5 hours of unsupervised practice at home produced the same motor skill gains as 6 hours of in-clinic supervised CIMT. However, neither group maintained gains 6 months following treatment, and patients subjectively reported a preference for physical therapist-guided training, noting greater use and quality of movement of the affected limb compared to the independent protocol. In a separate study, 10 daily treatments of 20 Hz rTMS and CIMT resulted in improved hand function, but investigators did not report any additive effect from rTMS despite noting enhanced cortical excitability. These latter results remain suspect, as the researchers compared a small and heterogeneous population consisting of patients with both cortical and subcortical strokes.
In contrast, other researchers have used rTMS to stimulate the primary motor cortex (M1) of stroke patients in conjunction with 10 days of traditional physical therapy. This approach resulted in increased MEPs in target muscles and significantly better scores on clinical and neurophysiological tests. Moreover, patients undergoing this type of stimulation maintained greater functional independence 10 days after the completion of treatment relative to those receiving sham stimulation, suggesting that semi-permanent cortical changes had occurred. Similar gains were also noted by patients suffering from chronic hemiplegic stroke following TMS enhanced hand therapy. When rTMS was applied over the hand region of M1 in the lesioned hemisphere prior to therapy, patients experienced larger MEPs in the muscles of the hand along with enhanced motor skill acquisition. In a separate clinical trial, iTBS was also incorporated into physical therapy tailored toward improving function of the paretic hand, resulting in significant improvements in grip-lift kinetics compared to placebo treatment.

2.5 PROPOSED MECHANISM OF TRANSCRANIAL DIRECT CURRENT STIMULATION

Transcranial direct current stimulation (tDCS) may also provide a feasible stimulation strategy post-stroke as an adjunct to physical therapy. Use of tDCS is thought to impact cortical neurons by facilitating ion channels, thereby making the resting membrane potential more conducive to depolarization, especially when paired with active movements such as physical therapy. Recent studies incorporating the GABA antagonist bicuculline reveal that enhanced cortical excitation resulting from tDCS does not result from intracortical disinhibition, as tDCS is likely not powerful enough to unblock NMDA receptors directly. Rather, evidence suggests that cortical
excitation resulting from tDCS may be due to proBDNF release from presynaptic neurons and subsequent TrkB receptor activation at the pre and postsynaptic membrane, events typically associated with late long-term potentiation (L-LTP). Since active postsynaptic TrkB receptors lead to phosphorylation of available glutamate receptors, production of new glutamate receptors, and translation of plasticity dependent proteins, this hypothesis is in line with previous studies demonstrating a correlation between cortical BDNF, motor learning and plasticity of M1.

Studies with animals demonstrate that BDNF is the key mediating factor required for LTP in the motor cortex. Moreover, humans with a BDNF val66met polymorphism have difficulty learning new motor tasks. tDCS-enhanced physical therapy is therefore only successful in the presence of BDNF secretion. In addition, tDCS-enhanced MT is contingent upon simultaneous tDCS and low-frequency, synaptic stimulation provided by physical therapy, presumably because only the combination results in adequate release of BDNF. Surprisingly, a recent animal study found that rats subjected to photothrombic ischemia experienced significantly better functional outcomes when they were treated with BDNF than 5 and 14 days of ipsilateral plaster cast mediated CIMT. Moreover, BDNF treated mice had a greater number of AMPA and NMDA receptors within the peri-lesional region 3 weeks after the stroke. Clearly, this finding contrasts with many investigations of CIMT based physical therapy in human subjects, which have demonstrated increased cortical excitability and expansion post-CIMT along with measureable changes in both gray matter and white matter, all indicative of LTP-mediated plasticity. The fact that physical therapy interventions such as CIMT results in plasticity is therefore irrefutable. However, perhaps plasticity is a graded phenomenon
much like functional improvement. Making this assumption, it is possible that physical therapy alone results in cortical plasticity, which results in suboptimal clinical gains and less than meaningful subjective improvements in function. Perhaps meaningful functional outcomes secondary to PT are only possible under conditions of limited cortical inhibition, during which plasticity dependent neurotransmitters such as BDNF can be released and optimally utilized. In this regard, tDCS may provide an effective adjunct to physical therapy.

2.6 PHYSICAL THERAPY AUGMENTED WITH TRANSCRANIAL DIRECT CURRENT STIMULATION

Like TMS enhanced rehabilitation, studies that have incorporated tDCS into motor recovery protocols have yielded promising results. When used to stimulate the M1 of the right hemisphere of right handed healthy volunteers for 20 minutes, tDCS enhanced therapy resulted in a significant increase in motor performance of the non-dominant upper extremity compared to a sham control. The 9.4% mean motor improvement was not present when the dominant right upper extremity was tested following tDCS of the left M1. However, dominant upper extremity motor improvement following anodal tDCS stimulation of the corresponding M1 was demonstrated in healthy elderly adults, a population known to suffer from age-related loss of motor function. A group of participants receiving tDCS scored on average 7.98% better on the Jebsen-Taylor hand function (JTHF) test with the dominant upper extremity than a similar group that received sham stimulation. Interestingly, the results of these two experiments are in line with studies that have investigated the use of tDCS in stroke rehabilitation. Following tDCS of M1 within the paretic hemisphere, patients scored on average of 11.75% better on the JTHF compared to a sham control group.
Similar improvements were also seen on the Fugl-Meyer standardized assessment in some patients receiving robot-assisted upper extremity training of the paretic limb augmented by tDCS. 7

2.7 THEORETICAL FRAMEWORK

Understanding the mechanisms behind TMS and tDCS is paramount to their successful implementation in rehabilitation setting. The physiology behind the two brain stimulation technologies suggest that TMS may be more effective as a primer for physical therapy, whereas tDCS may be more powerful when used in conjunction with rehabilitative movements. 7 To some extent, this hypothesis is convenient therapeutically, especially when considering the feasibility and external validity of both pieces of equipment. Clinically, tDCS is much more user-friendly as the stimulating electrode can be quickly and easily attached to the scalp, and the battery operated power source can fit comfortably in a patient’s pocket, allowing for more dynamic and functional motor movements. It also follows that that both tDCS and TMS may be most effective cortically if used as complements to one another during physical therapy treatment. Using TMS to prime therapy followed by tDCS in conjunction with therapy may therefore result in optimal motor and functional recovery following stroke.

To our knowledge, no studies have attempted to improve motor function by using TMS to prime M1 followed by tDCS in combination with physical therapy. However, some studies have presented the technology in reverse order, using tDCS to prime therapy and TMS during therapy. 40 Interestingly, studies that have looked at the effect of rTMS enhanced therapy following cathodal tDCS priming demonstrated significant decreases in the amplitude of motor evoked potentials (MEPs) / increased cortical
excitability within M1 compared to anodal tDCS priming. This finding is fairly unexpected, considering the proposed mechanism of anodal tDCS. Anodal tDCS would have been predicted to lower the resting membrane potential of both the pre and post synaptic membranes, allowing the depolarizing effects of TMS to reach E-LTP and L-LTP at a much faster rate. However, the investigators argue that cathodal tDCS may be more effective prior to TMS enhanced therapy because it is able to lower the ceiling threshold of postsynaptic neurons required for LTP induction. Based on the principle of the Bienenstock-Cooper-Munro (BCM) learning, past research has suggested the thresholding is an important aspect of LTP. One study in particular demonstrated that neurons responsible for forelimb movement within M1 of rats did not strengthen their connections via LTP upon completion of a predominately upper extremity reaching training program. However, when lower extremity neurons were activated via the same reaching training with hind limbs, LTP was induced and significantly more synapses were created. The authors argue that LTP was only induced in leg neurons because the ceiling required for LTP is much lower secondary to the unfamiliar movement.

Nevertheless, it seems logical that BCM learning would be less applicable to neurons of M1 within the lesioned hemisphere of stroke patients, which correspond with partially paralyzed muscles. Rather, the threshold required for LTP and motor learning in these neurons would likely already be considerably less secondary to loss of function created by the stroke. The same argument would also apply to neurons responsible for movement of the upper / lower extremity within M1 of the non-dominant hemisphere vs. the dominant hemisphere in healthy patients. Conceivably the motor threshold required for LTP of non-dominant M1 neurons will already be lower simply because of the pre-
existing preference to use the dominant side of the body. In this case, the use of TMS prior to tDCS-enhanced MT would provide 3 distinct advantages. First, the TMS primer would minimize intracortical inhibition and therefore allow glutamatergic neurons within M1 to interact unimpeded during tDCS enhanced therapy via E-LTP. Second, a reduction of intracortical inhibition may facilitate optimal BDNF release and subsequent plasticity within M1 during L-LTP.28 Finally, the purpose of a primer is to stimulate the cortex prior to and independent of a motor activity. If tDCS were used as a primer in place of TMS, it could not be coupled with MT and would therefore lose its ability to initiate L-LTP via the BDNF/TrkB pathway.

Clearly, the role of TMS and tDCS in the induction of E-LTP and L-LTP is complicated, and more research is necessary to determine how to best incorporate the technology into stroke rehabilitation. The purpose of this study is to determine the feasibility and effectiveness of using both TMS and tDCS, in combination with MT, to maximize motor learning. Many studies have established that training with the non-dominant hand provides a reasonable model of hemi-paralysis post stroke.43 In order to optimize safety, sample size and overall power, our studies focused on training of the non-dominant upper extremity of healthy elderly adults and college-age students. Per the proposed mechanism outlined in the literature, TMS was utilized as a primer in order to initiate glutamate dependent E-LTP, and anodal tDCS will be presented in conjunction with MT so as to facilitate BDNF dependent L-LTP.
WORKS CITED


CHAPTER III

METHODOLOGY

This was a randomized, double-blind study comparing the functional improvements made by the non-dominant UE per the Jebsen-Taylor Hand Function Test (JTHF) JTHF, a computer-based pursuit rotor tracking task (PRTT), a computer-based reciprocal tapping task (FRTT), and the Purdue pegboard (PPB). College-age students were randomly assigned to one of two groups: Group 1: iTBS primer followed by a-tDCS enhanced MT. Group 2: iTBS sham primer followed by sham a-tDCS enhanced MT. Although all older adults received MT augmented with real iTBS/a-tDCS, they were told that they could be randomly assigned into either a real-stimulation treatment or sham-stimulation control group so as to maximize internal validity.

In order to establish baseline function, all participants completed a battery of non-dominant UE tasks, which included: JTHF, PRTT, FRTT, and PPB x10 repetitions with the non-dominant UE within 30 hours of beginning formal training. In addition, cortical mapping of the non-dominant UE was individually conducted via single-pulse TMS, as described below, in order to determine active motor threshold.

On the day of treatment, participants performed the JTHF, PRTT, FRTT, and PPB x3 times, and the scores were averaged into pre-test measurements. The treatment consisted of a three-minute iTBS primer followed by 20 minutes of a-tDCS presented in conjunction with MT. The control group received sham stimulation. Immediately following treatment, participants again completed 3 repetitions of the JTHF, and the
scores were averaged as a post-training, “short-term performance” score. Participants were also scored on the JTHF x3 repetitions 24 hours and 7 days post-treatment. Again, the scores were averaged to create a 24-hour and 7-day score for each participant. The 24 hours and 7 day scores were then averaged to create a single “long-term performance” score. (See Figure 1A-C)

3.1 PARTICIPANT RECRUITMENT

Sixteen healthy college-age and 9 older adults who met inclusion / exclusion criteria were recruited via word of mouth and flyers placed in and around high-traffic areas of the university. Inclusion criteria were as follows: 1. Predominantly right-handed, 2. Either age 18-34 (younger group) or > 60 years old (older group), 3. Ability to provide informed written or verbal consent. Handedness was verified via performance testing on the JTHF, PPB, PRTT, and FRTT. Exclusion criteria were summarized on TMS, tDCS, and Neurological Screening Forms (Appendices B-D) approved by the Medical University of South Carolina, Department of Neurology. Participants were excluded from the study if they reported the following: cardiac pacemaker, metal on face / scalp, implanted medical pumps / lines, history of stroke / cortical lesion, history of head injury, history of seizures / epilepsy, history of neurosurgery, pregnancy, electrical / magnetic / mechanical implants, history of migraines, report of taking psychiatric medication known to reduce seizure threshold, and any unstable medical condition. Participants were also excluded if they reported a history of dizziness / vertigo, frequent headaches, tremors, strange movements / bizarre behavior, memory problems, double vision, abnormal muscle weakness, unexplained burning / tingling / numbness, sudden change in sleep patterns, extreme or abnormal fatigue, cognitive limitations, and
unexplained pain in the hands / feet / face. Prior to enrollment in the study, all subjects read and signed a consent form (Appendix E) approved by the University of South Carolina IRB. Participants that satisfied all inclusion / exclusion criteria were randomly assigned to a group (Real or Sham stimulation) Brain stimulation and data collection was performed at the USC Brain Stimulation Laboratory.

### 3.2 EXPERIMENTAL BLINDING

A compatible “jump drive” was encoded with either a-iTBS (experimental) or sham-iTBS (control) stimulation and assigned to corresponding college-age participants. The same TMS coil was used during both the treatment and control condition. The coil was flipped based on the instructions encoded on each jump drive, making it impossible for the participants or experimenter (R.B.) to determine whether they were receiving real or sham stimulation. In order to ensure participant blinding with regards to a-tDCS stimulation, electrodes were placed on all participants. Participants were informed beforehand that any cutaneous stimulation on the scalp typically decreases with time secondary to desensitization. The tDCS devices were then turned on until participants confirmed that the stimulation could be felt on the scalp. In the Sham group, the tDCS unit was turned off following 30 seconds of stimulation. A piece of tape was placed over the warning light and selector switches so as to maintain blinding. While the primary investigator responsible for administering stimulation was aware of which group the participants were in, a blinded graduate student unrelated to the study administer all motor assessment testing, thus assuring experimenter blinding.
3.3 MOTOR TRAINING

Motor training focused on the non-dominant UE and consisted of one 20-minute sessions geared toward practicing four primary tasks: the Jebsen-Taylor Hand Function Test (JTHF), a computer-based Fitt’s Reciprocal Tapping Task (FRTT), a computer-based Pursuit Rotor Tracking Task (PRTT), and the Purdue Pegboard Test (PPB). Of the four tasks, the JTHF was chosen as the primary outcome measure because it provided a short but relatively broad measure of hand function. Previous studies have established that it is both valid and reliable, and normative data is readily available for both sexes and various age groups. The JTHF also correlates well with other established standardized assessments such as the Grip strength test, Action Research Arm Test, Nine hold peg test, pinch strength test, and Stroke Impact Scale (Hand domain). The test were set-up and administered according to a pre-established set of instructions. However, the writing portion of the JTHT was not performed as part of the study due to variation in handwriting and subsequent unavoidable complications standardizing among individuals.

Based on Fitt’s Law, the FRTT measured the time required to move a mouse reciprocally between two stationary targets on a computer screen. A previous study demonstrated that the preferred UE performs better than the non-preferred extremity on reciprocal tapping tasks, especially when the size of the target decreased and the distance to the target increased. During the PRTT, participants were asked to keep a visual stimulus within a continuously moving target presented at random velocities by manipulating a computer mouse with the non-dominant hand. Pursuit rotor task are used throughout the literature as a measure of both fine motor control of the fingers and hand. The FRTT and PRTT were created using Presentation software (www.neuobs.com).
Lastly, the PPB was used to measure manipulative dexterity of the hands, as it is both a valid and reliable measure with established normative values.⁶

In order to ensure that participants received equal training, participants were instructed to complete all tasks “as quickly and accurately as possible”. In addition, all tasks were performed consecutively in a randomized but predetermined order for the full length of the treatment time. Also, participants performed all tasks x10 repetitions prior to beginning MT in order to become familiar with each. Previous research suggests that x10 trials of the JTHF are sufficient to reach a stable level of performance among participants.⁷ Moreover, these10 trials allowed investigators to ensure that participants completed the components of JTHF correctly, uniformly, and to standard.

Importantly, all participants completed each of the assessment tests pre-test, post-test, 24-hours post-test, and 7-days post-test, regardless of age. We compared scores of all four measures in the younger group but only found differences in the JTHF. This is likely due to the fact that the other measures were not sensitive enough to differentiate performance in the younger and more skilled subgroup. Alternatively, there may have been no effect on these relatively simple tasks. Therefore, only the JTHF was considered in comparing functional outcomes following MT enhanced with real vs. placebo iTBS/a-tDCS. This was not the case for older adults, as their baseline level of function was less than their younger counterparts. Therefore, all four tests were used to compare functional performance of older and younger participants post treatment.

3.4 MOTOR CORTEX MAPPING AND ACTIVE MOTOR THRESHOLD IDENTIFICATION

Patients were seated in a comfortable MagVenture treatment chair with the non-dominant hand pronated on a soft surface for comfort. The optimal position for the APB
over the scalp (hot spot) was determined using a MagproX100 Magnetic Stimulator, 230V (MagVenture Inc., Atlanta, GA) and a Cool A65 A/P butterfly coil. With the handle oriented backward and the coil 45 degrees in the posterolateral direction, single TMS pulses at a predetermined intensity were directed just anterior of the central sulcus and adjusted in 1-2 cm increments until a “hot spot” was identified. A “hot spot” was identified as the location on the scalp able to generate a visual twitch of the abductor polices brevis (APB) muscle 3/5 times. The “hot spot” was marked on a cloth MagVenture stimulation cap. In addition to the “hot spot,” the center of the nasal bone, right / left external auditory acoustic meatus and occiput were also marked in order to ensure that the “hot spot” was reliably relocated on the day of treatment. Following the identification of the “hot spot,” participants were then be asked to isometrically grip a dynamometer between the proximal interphalangeal joint of the left D2 and the pad of the left D1 at approximately 20% of maximal voluntary contraction (MVC) while the active motor threshold (AMT) of the APB was measured. In order to ensure that patient’s provide a contraction of the APB at 20% MVC, they first practiced distinguishing between MVCs and 20% MVCs with a dynamometer. Verbal feedback was also provided by the primary investigator during the 20% MVC contraction to ensure consistency. The AMT was defined as the lowest stimulation intensity able to produce at least 5/10 MEPs greater than or equal to a 200 µV amplitude (above baseline).

3.5 TRANSCRANIAL MAGNETIC STIMULATION PROCEDURE

iTBS consisted of three TMS pulses at 50 Hz provided every 200ms (i.e., at 5 Hz) at 80% AMT of the target muscle. Ten bursts were grouped and repeated every 10 seconds for a total of 20 trains. This resulted in a total of 600 pulses per participant.
Total stimulation time for the iTBS protocol was 191.84 seconds. iTBS treatment was directed at each participant’s “hot spot” for the APB muscle.

3.6 TRANSCRANIAL DIRECT CURRENT STIMULATION PROCEDURE

Patients underwent either 20 minutes of a-tDCS at 1 mV or sham a-tDCS. A 20-minute duration was chosen so as to be temporally matched with 1 chargeable unit of therapeutic exercise. A previous investigation established that 20 minutes is a safe and effective duration for improving motor performance of the non-dominant UE in healthy, college-age subjects after only one treatment. Cortical anodal stimulation (1 mA) was delivered via a pair of saline-soaked surface sponge electrodes (2.5 x 2.5 cm) and connected to a 9-volt battery-driven, constant current stimulator for 20 minutes (Chattanooga Ionto Iontophoresis System, DJO Global, Vista, California, Salt Lake City, Utah) in conjunction with MT. The stimulating electrode was centered on the “hot spot” for the APB. The “reference” cathodal electrode was placed just below the contralateral motor cortex.
Works Cited


CHAPTER IV

RESULTS

4.1 MANUSCRIPT 1

Facilitation of Motor Skill Acquisition in the Non-Dominant Upper Extremity via
Motor Training Augmented with Intermittent Theta Burst Stimulation and Anodal
Transcranial Direct Current Stimulation\textsuperscript{1}

\textsuperscript{1}Butts RJ, Kolar M, Mettille JR, Newman-Norlund R. To be submitted to Brain Stimulation.
ABSTRACT

Patients that have suffered from a neurologic incident often require physical therapy (PT) to help improve their level of function. Previous investigations suggest that intracortical inhibition (ICI) may limit the speed and extent of their recovery. The purpose of the current study was to examine the therapeutic potential of a non-invasive brain stimulation approach, which combined intermittent theta burst stimulation (iTBS) and anodal transcranial direct current stimulation (a-tDCS) with motor training. This combined approach was designed to reduce ICI, thereby maximizing plasticity. We hypothesized that students exposed to the treatment would perform better on short-term (directly following 20 minutes of motor training) and long-term (24 hours and 7 days following motor training) motor-skill retention tests than students receiving sham stimulation. A total of 27 right-handed, college-age students were randomly assigned to either a treatment (n = 17) or a control group (n = 14). iTBS and a-tDCS over the non-dominant motor cortex were used as a primer to, and in conjunction with, 20 minutes of motor training, respectively. Our primary outcome measure was performance on the Jebsen-Taylor Hand Function Test, and a repeated-measures ANOVA revealed greater overall improvement in the treatment as compared to the control group (p = .041). A repeated-measures ANOVA constrained to either short or long-term improvements demonstrated a significant long-term but not short-term difference between the groups (p=.045). These results suggest that brain stimulation may be a useful adjunct to motor training in healthy participants and may eventually help improve PT outcomes in neurologically impaired patients.
INTRODUCTION

Of the 795,000 people that will suffer from a stroke in the U.S. this year, two thirds will survive and require rehabilitative services. While new therapeutic techniques have improved functional outcomes via principles of forced use and massed practice, significant stroke-related disabilities often persist. More than 85% of patients that have suffered a stroke have lasting functional impairments, and approximately 50-60% of survivors continue to require functional assistance to complete activities of daily living after completion of intensive PT.

Experiments using fMRI have demonstrated that peripheral motor recovery is accompanied by significant changes within the central nervous system, suggesting that plasticity plays an important role in the stroke recovery process. Therefore, techniques capable of optimizing cortical plasticity presented in combination with extremity specific training may provide the key to improved therapeutic techniques and better functional outcomes. In this context, transcranial magnetic stimulation (TMS) and transcranial direct current stimulation (tDCS) may be capable of initiating lasting cortical changes.

Both high-frequency and low-frequency repetitive transcranial magnetic stimulation (rTMS) have demonstrated the ability to induce cortical excitation and inhibition, respectively, that lasts beyond the stimulation time period. TMS studies incorporating iTBS and rTMS have demonstrated >30 minutes of enhanced motor evoked potentials (MEPs) in target musculature. Moreover, iTBS has been shown to create theta oscillations within the cortex consistent with those associated with learning and memory in the hippocampus.
Utilizing iTBS as a primer to PT may effectively place the ipsilesional motor cortex in an optimal state for relearning motor tasks during constraint-induced movement therapy by minimizing IACI mediated by GABA-A receptors, resulting in increased plasticity and better functional recovery.\(^6,7\) While only a few studies have explored this possibility and findings are inconsistent, TMS continues to be a promising adjunct to PT. A recent study comparing 6 hours of in-clinic, supervised CIMT produced equivalent motor skill gains compared to 1 hour of in-clinic, supervised CIMT augmented with rTMS and followed by 5 hours of unsupervised practice at home.\(^8\) In a separate study, rTMS was used to stimulate the motor cortex (M1) of stroke patients in conjunction with 10 days of traditional PT, resulting in increased MEPs in target muscles and significantly better scores on clinical and neurophysiological tests.\(^9\) Patients undergoing this type of stimulation maintained greater functional independence 10 days after the completion of treatment relative to those receiving sham stimulation, suggesting that semi-permanent cortical changes had occurred.\(^9\) Similar gains were also noted by patients suffering from chronic hemiplegic stroke following TMS enhanced hand therapy.\(^10\) When TMS was applied over the hand region of M1 in the lesioned hemisphere prior to therapy, patients experienced larger MEPs,\(^10\) enhanced motor skill acquisition,\(^10\) and greater grip lift kinetics.\(^11\)

In as little as 13 minutes, cortical areas receiving anodal and cathodal tDCS also demonstrate increased or decreased excitability, respectively.\(^3\) Use of a-tDCS is thought to impact cortical neurons by facilitating ion channels, thereby making the resting membrane potential more conducive to depolarization, especially when paired with active movements such as those associated with PT.\(^12\) Like TMS enhanced rehabilitation,
studies that have incorporated a-tDCS into motor recovery protocols have yielded promising results. When used to stimulate the M1 of the right hemisphere of right handed healthy volunteers for 20 minutes, a-tDCS enhanced therapy resulted in 9.4% increase in motor performance of the non-dominant UE compared to a sham control. Dominant upper extremity motor improvement following a-tDCS stimulation of the corresponding M1 was also demonstrated in healthy elderly adults, a population known to suffer from age-related loss of motor function. Participants receiving a-tDCS scored 7.98% better on the Jebsen-Taylor hand function (JTHF) test than those that received sham stimulation. Interestingly, the results of these two experiments are in-line with studies that used a-tDCS to augment stroke rehabilitation. Following a-tDCS of M1 within the paretic hemisphere, patients scored on average of 11.75% better on the JTHF compared to a sham control group. Similar improvements were also seen on the Fugl-Meyer standardized assessment in some patients receiving robot-assisted UE training of the paretic limb augmented with a-tDCS.

Understanding the mechanisms behind TMS and tDCS is paramount to their successful implementation in rehabilitation setting. The physiology behind the two brain stimulation technologies suggest that TMS may be more effective as a primer for PT, whereas a-tDCS may be more powerful when used in conjunction with rehabilitative movements. A TMS primer would minimize intracortical inhibition (ICI) and therefore allow glutamatergic neurons within M1 to interact unimpeded during a-tDCS enhanced therapy via E-LTP. Furthermore, a reduction of ICI may facilitate optimal BDNF release and subsequent plasticity within M1 during L-LTP.
The purpose of this study was to examine the potential of a novel, non-invasive brain stimulation approach, which combined excitatory iTBS and a-tDCS in an advantageous manner. Based on the proposed mechanisms of action of TMS and tDCS, we chose to use iTBS a pre-training primer and administer a-tDCS in conjunction with motor training. We further selected motor training of the non-dominant UE of healthy college-age students as our performance metric, as the non-dominant hand is thought to provide a reasonable model of hemi-paralysis post stroke. We hypothesized that motor training of the non-dominant UE primed with iTBS and administered concurrently with a-tDCS would result in better: short-term outcomes on the JTHF Test directly following motor training, and long-term outcomes (24 hours and 7 days following motor training) than motor training with placebo stimulation.

**METHODS**

This was a randomized, double-blind study comparing the functional improvements made by the non-dominant UE per the JTHF. Following consent and screening, participants were randomly assigned to one of two groups: Group 1: iTBS primer followed by a-tDCS enhanced motor training. Group 2: iTBS sham primer followed by sham a-tDCS enhanced motor training. In order to establish baseline function, all participants completed a battery of non-dominant UE tasks, which included: JTHF, a computer-based pursuit rotor task, a computer-based reciprocal tapping task, and the Purdue pegboard test x10 repetitions with the non-dominant UE within 30 hours of beginning formal training. In addition, cortical mapping of the non-dominant UE was individually conducted via single-pulse TMS, as described below, and active motor threshold was determined.
On the day of treatment, participants performed the JTHF x3 times, and the best two scores were averaged into pre-test measurements. The treatment consisted of a three-minute iTBS primer followed by 20 minutes of a-tDCS presented in conjunction with motor training. The control group received sham stimulation. Immediately following treatment, participants again completed 3 repetitions of the JTHF, and the best two out of the three scores were averaged as a post-training, “short-term performance” score.

Participants were also scored on the JTHF x3 repetitions 24 hours and 7 days post-treatment. Again, the best two scores were averaged to create a 24-hour and 7-day score for each participant. The 24 hours and 7 day scores were then averaged to create a single “long-term performance” score. (See Figure 4.1 A&B)

**Participant Recruitment:**

Twenty-seven healthy participants who met inclusion / exclusion criteria were recruited via word of mouth and flyers placed in and around high traffic areas of the University. Inclusion criteria were as follows: 1. Predominantly right-handed, 2. Age 18-34 years old, 3. Ability to provide informed written or verbal consent. Handedness was verified via performance testing on the JTHF, Purdue pegboard test, computerized pursuit rotor tracking task, and computerized reciprocal tapping task. Exclusion criteria were summarized on TMS, tDCS, and Neurological Screening Forms approved by the Medical University of South Carolina, Department of Neurology. Participants were excluded from the study if they reported the following: cardiac pacemaker, metal on face / scalp, implanted medical pumps / lines, history of stroke / cortical lesion, history of head injury, history of seizures / epilepsy, history of neurosurgery, pregnancy, electrical / magnetic / mechanical implants, history of migraines, report of taking psychiatric
medication known to reduce seizure threshold, and any unstable medical condition. Participants were also excluded if they reported a history of dizziness / vertigo, frequent headaches, tremors, strange movements / bizarre behavior, memory problems, double vision, abnormal muscle weakness, unexplained burning / tingling / numbness, sudden change in sleep patterns, extreme or abnormal fatigue, cognitive limitations, and unexplained pain in the hands / feet / face. Prior to enrollment in the study, all subjects read and signed a consent form approved by the University of South Carolina IRB. Participants that satisfied all inclusion / exclusion criteria were randomly assigned to a group (real or sham stimulation). Brain stimulation and data collection was performed at the USC Brain Stimulation Laboratory.

Experimenter Blinding:

A compatible “jump drive” was encoded with either iTBS (experimental) or sham-iTBS (control) stimulation and assigned to corresponding participants. The same TMS coil was used during both the treatment and control condition. The coil was flipped based on the instructions encoded on each jump drive, making it impossible for the participants or experimenter (R.B.) to determine whether they were receiving real or sham stimulation. In order to ensure participant blinding with regards to a-tDCS stimulation, electrodes were placed on all participants. Participants were informed before-hand that any cutaneous stimulation on the scalp typically decreases with time secondary to desensitization. The tDCS devices were then turned on until participants confirmed that the stimulation could be felt on the scalp. In the Sham group, the tDCS unit was turned off following 30 seconds of stimulation. A piece of tape was placed over the warning light and selector switches so as to maintain blinding. While the primary
investigator responsible for administering stimulation was aware of which group the participants were in, a blinded graduate student unrelated to the study administer all motor assessment testing, thus assuring experimenter blinding.

**Motor Training:**

Motor training focused on the non-dominant UE and consisted of one 20-minute session geared toward practicing four primary tasks: the JTHF, a computer-based Fitt’s Reciprocal Tapping Task, a computer-based Pursuit Rotor Tracking Task, and the Purdue Pegboard Test. Of the four tasks, the JTHF was chosen as the primary outcome measure because it provided a short, but relatively broad, measure of hand function. Previous studies have established that it is both valid and reliable, and normative data is readily available for both sexes and various age groups. The JTHF also correlates well with other established standardized assessments such as the Grip strength test, Action Research Arm Test, Nine hold peg test, pinch strength test, and Stroke Impact Scale (Hand domain). The test was set-up and administered according to a pre-established set of instructions. However, the writing portion of the JTHT was not performed as part of the study due to variation in handwriting and subsequent unavoidable complications standardizing among individuals.

In order to ensure that participants received equal training, participants were instructed to complete all tasks “as quickly and accurately as possible”. In addition, all tasks were performed consecutively in a randomized but predetermined order for the full length of the treatment time. Also, participants performed all tasks x10 repetitions prior to beginning motor training in order to become familiar with each. Previous research suggests that x10 trials of the JTHF are sufficient to reach a stable level of performance.
among participants. Moreover, these trials allowed investigators to ensure that participants completed the components of JTHF correctly, uniformly, and to standard.

*Motor Cortex Mapping and Active Motor Threshold Identification:*

Patients were seated in a comfortable MagVenture treatment chair with the non-dominant hand pronated on a soft surface for comfort. The optimal position for the abductor pollicis brevis (APB) muscle over the scalp (hot spot) was determined using a MagproX100 Magnetic Stimulator, 230V (MagVenture Inc., Atlanta, GA) and a Cool A65 A/P butterfly coil. With the handle oriented backward and the coil 45 degrees in the posterolateral direction, single TMS pulses at a predetermined intensity were directed just anterior of the central sulcus and adjusted in 1-2 cm increments until a “hot spot” was identified. A ”hot spot” was identified as the location on the scalp able to generate a visual twitch of the APB 3/5 times. The “hot spot” was marked on a cloth MagVenture stimulation cap. In addition to the “hot spot,” the center of the nasal bone, right / left external auditory acoustic meatus and occiput were also marked in order to ensure that the “hot spot” was reliably relocated on the day of treatment. Following the identification of the “hot spot,” participants were then be asked to isometrically grip a dynamometer between the proximal interphalangeal joint of the left D2 and the pad of the left D1 at approximately 20% of maximal voluntary contraction (MVC) while the active motor threshold (AMT) of the APB was measured. In order to ensure that patient’s provide a contraction of the APB at 20% MVC, they first practiced distinguishing between MVCs and 20% MVCs with a dynamometer. Verbal feedback was also provided by the primary investigator during the 20% MVC contraction to ensure
consistency. The AMT was defined as the lowest stimulation intensity able to produce at least 5/10 MEPs greater than or equal to a 200 µV amplitude (above baseline).

Transcranial Magnetic Stimulation Procedure:

iTBS consisted of three TMS pulses at 50 Hz provided every 200ms (i.e., at 5 Hz) at 80% AMT of the APB. Ten bursts were grouped and repeated every 10 seconds for a total of 20 trains. This resulted in a total of 600 pulses per participant. Total stimulation time for the iTBS protocol was 191.84 seconds. iTBS treatment was directed at each participant’s “hot spot” for APB.

Transcranial Direct Current Stimulation Procedure:

Patients underwent either 20 minutes of a-tDCS at 1 mV or sham a-tDCS. A 20-minute duration was chosen so as to be temporally matched with 1 chargeable unit of therapeutic exercise. A previous investigation established that 20 minutes is a safe and effective duration for improving motor performance of the non-dominant UE in healthy, college-age subjects after only one treatment. Cortical anodal stimulation (1 mA) was delivered via a pair of saline-soaked surface sponge electrodes (2.5x 2.5 cm) and connected to a 9-volt battery-driven, constant current stimulator for 20 minutes (Chattanooga Ionto Iontophoresis System, DJO Global, Vista, California, Salt Lake City, Utah) in conjunction with motor training. The stimulating electrode was centered on the “hot spot” for the APB. The “reference” cathodal electrode was placed just below the contralateral motor cortex.

RESULTS

All subjects tolerated iTBS and a-tDCS well, and no adverse reactions related to the treatment were reported. All participants underwent training prior to the treatment to
achieve a performance plateau. Of the 27 participants in the study, the data of one student was not considered as part of the analysis due to side effects of prescription medication, including apathy, extreme fatigue, and limited attention. Pre and post-test JTHF scores were consistently >3 standard deviations from the other participants, regardless of group.

Plots of scaled residuals were created for all JTHF scores using Statistical Analysis System software (SAS institute Inc. – version 9.2, USA) to ensure homogeneity of variance and normality. A 2x4 repeated measures Analysis of Variance (ANOVA) was performed on total JTHF time to determine the main effect of time (pre-treatment, post-treatment, 24-hour post-treatment, and 7-days post-treatment), condition (iTBS/a-tDCS enhanced motor training and placebo control), and the interaction of time and condition. The 2x4 repeated measured ANOVA showed a non-significant effect for group (F1,24=.03, p=.8733) but a significant effect of time (F1,24=96.31, p<.0001). The interaction between group and time was also statistically significant (F1,24=4.66, p=.0410).

A 2x2 repeated measures ANOVA was further performed to evaluate the main short-term effect of time (pre-treatment, post-treatment) and condition (iTBS/a-tDCS enhanced motor training and placebo control). Despite an 8.3% functional improvement of the iTBS/a-tDCS treatment group, the ANOVA revealed no significant short-term interaction between group and time (pre-test and post-test). The iTBS/a-tDCS treatment group outscored the control group by only 1.6% (.36s). Therefore, the enhanced motor training did not result in greater functional improvements than the sham stimulation. However, a separate 2x2 repeated measures ANOVA evaluating the main long-term
effect of time (pre-treatment, average of 24-hour and 7-days post-treatment) and condition revealed a significant interaction between group and time on JTHF score ($F_{1,24}=4.44$, $p=.0458$) (Figure 4.2 A&B). Long-term, individuals in the iTBS/a-tDCS treatment group improved their JTHF score by 10%, outscoring the control group by 3.3% (.79s). Cohen’s d was further estimated to be .213, suggesting a small effect size of the treatment.

**DISCUSSION**

Multiple studies have demonstrated that the non-dominant UE has relatively less dexterity than the dominant upper extremity, a difference that may be explained by the disproportionate use of the preferred UE and the decreased cortical activation of the nondominant motor cortex. TMS studies further show that the non-dominant motor cortex has increased motor thresholds and decreased MEPs. Altering the excitability of the motor cortex via non-invasive brain stimulation may therefore help augment traditional motor training and improve functional outcomes. In this context, non-invasive brain stimulation may also help to improve outcomes of PT used to improve paretic UE function in patients post-stroke. Indeed, results of previous investigations have demonstrated a 9.4% improvement in non-dominant upper extremity function per JTHF following motor training primed with a-TDCS compared to sham stimulation. Similar studies showed a 9.4% improvement in paretic upper extremity function of patients post-stroke. A short-term improvement of 8.3% following iTBS/a-tDCS augmented motor training in the present study is therefore in-line with previous investigations. However, a .36 second effect represents only a modest functional improvement short-term between the treatment and control group.
Rather, the unique finding of this study was the significant long-term improvement in function \((p=.0458)\) following iTBS/a-tDCS augmented motor training compared to placebo stimulation. This is a logical finding given the physiology of cortical plasticity within M1 and the phases of motor learning. Previous investigations combining paired-associative stimulation with motor training found significant functional improvements long-term but not immediately following treatment.\(^{23}\) While motor training initially resulted in enhanced MEPs and decreased short-interval intracortical inhibition (SICI), the cortical changes disappeared by day 5.\(^{23}\) Investigators therefore speculated that short-term functional improvements occur by increasing the efficacy of existing synaptic connection within M1, while long-term performance is due to cortical reorganization.\(^ {23}\) This theory fits well with animal models that demonstrate cortical synapogenesis and humuncular reorganization following late but not early motor learning.\(^ {24}\) Thus, only long-term motor learning may truly represent plasticity-dependent improvements in function.

To our knowledge, this is one of the first studies to combine a-iTBS primer with a-tDCS in conjunction with motor training. We hypothesize that this stimulation protocol optimizes long-term plasticity dependent functional improvement because it temporally fits with the proposed mechanism of each stimulation device. Although SIACI temporally helps modulate unwanted movement, there is evidence that it blocks long-term potentiation in M1.\(^ {7}\) An imbalance in SIACI may also exist following a stroke, which may be due to faulty transporters in peri-lesional regions normally responsible for the re-uptake of GABA.\(^ {25}\) In this context, iTBS provides two distinct advantages when used as a primer to motor training. First, the 200ms inter-pulse interval helps to
minimize SICI by inhibiting GABAergic interneurons via GABA-b receptors, a phenomenon known as long-interval intracortical inhibition.\textsuperscript{14} Second, many researchers hypothesize that intracortical disinhibition may allow necessary glutamate release and subsequent activation of postsynaptic NMDA receptors to “kick-start” E-LTP within motor regions of the brain, thereby enabling M1 plasticity.\textsuperscript{12}

In contrast, recent studies incorporating the GABA antagonist bicuculline suggest that a-tDCS does not result in intracortical disinhibition, as tDCS is likely not powerful enough to unblock NMDA receptors directly.\textsuperscript{12} Rather, cortical excitation resulting from tDCS may be due to proBDNF release from presynaptic neurons and subsequent TrkB receptor activation at the pre and postsynaptic membrane, events associated with L-LTP.\textsuperscript{12} Activated postsynaptic TrkB receptors lead to phosphorylation of glutamate receptors, production of new glutamate receptors, and translation of plasticity dependent proteins, all required for plasticity in M1.\textsuperscript{26}

Animal studies demonstrate that BDNF is the key mediating factor required for LTP in M1. A recent animal study found that rats subjected to photothrombic ischemia experienced significantly better functional outcomes when treated with BDNF than 5 and 14 days cast mediated CIMT.\textsuperscript{27} Moreover, functional gains correlated with increased AMPA and NMDA receptors within the peri-lesional region 3 weeks post-stroke.\textsuperscript{27} Recent evidence further suggests that a-tDCS-enhanced motor training is contingent upon simultaneous tDCS and low-frequency, synaptic stimulation provided by motor training, presumably because only the combination results in adequate release of BDNF.\textsuperscript{12} The protocol used in this study is therefore optimal, because it presents a-tDCS during motor training.
Perhaps plasticity is a graded phenomenon much like functional improvement, and traditional motor training / PT results in cortical changes associated with suboptimal functional gains. Meaningful functional improvement may only be possible under the following conditions: 1. limited SICI and optimal glutamate release afforded by iTBS mediated E-LTP and 2. BDNF activation of TrkB receptors initiated by a-tDCS mediated L-LTP. In this regard, iTBS/a-tDCS augmented motor training requires further exploration. We recommend more robust studies with larger sample sizes to further investigate the potential of the combined iTBS-a-tDCS protocol. Future studies should also work to correlate short and long-term functional improvements with reliable measures of cortical excitability and depression.
Figure 4.1 (A): Graphical representation of the iTBS/a-tDCS / sham iTBS/a-tDCS enhanced motor training protocol. (B): Graphical depiction of the experimental design comparing functional outcomes of college-age students following MT augmented with real and sham iTBS/a-tDCS. (C): Graphical depiction of the experimental design comparing functional outcomes of older and young participants following motor training augmented with real iTBS/a-tDCS.
Figure 4.2 (A): Graphic representation of the short and long-term pre-post functional improvement on the JTHF test. The dark gray bar represents the pre-post functional improvement of the iTBS/a-tDCS-treatment group, while the light gray bar represents pre-post functional improvement of placebo-control group. (B): Results of the JTHF at each time point (pre-test, post-test, 24-hrs post-test, and 7-days, post-test). The dark gray and light gray line represents the iTBS/a-tDCS treatment group and placebo-control group, respectively. * = significant effect at p< 0.05.
WORKS CITED


MANUSCRIPT 2

Age-Based Differences in the Effects of Non-dominant Upper Extremity Motor Training Augmented with Intermittent Theta Burst Stimulation and Anodal Transcranial Direct Current Stimulation

ABSTRACT

Recent evidence suggests that motor training (MT) augmented with brain stimulation may be advantageous in individuals with a higher learning ceiling. While studies that have combined brain stimulation and rehabilitation post-stroke report promising outcomes, its potential use in a healthy, elderly population has yet to be determined. The purpose of this study was to explore this possibility via iTBS/a-tDCS enhanced MT approach. We hypothesized that older adults exposed to iTBS and a-tDCS would demonstrate greater functional improvements short-term (immediately following MT) and long-term (24 hours / 7 days following MT) than younger adults receiving identical stimulation. The non-dominant M1 of 16 young and 9 older adults were treated with an iTBS primer followed by a-tDCS during MT. Our primary outcome measure was the Jebsen-Taylor Hand Function Test (JTHF), and a 2X4 repeated-measures ANOVA demonstrated that neither group improved more than the other (p = .1801). ANOVAs of secondary outcome measures revealed greater functional improvement in the treatment group on the Pursuit Rotor Tracking Test (PRTT) (p = .016) and the Purdue Pegboard Test (PPB) (p = .0036) but not the Fitt’s Reciprocal Tapping Test (FRTT). A 2X2 repeated measures ANOVA constrained to short-term and long-term performance revealed no short-term performance difference on any of the outcome measures. Regarding long-term improvements, however, older adults made greater improvements than younger adults on the PPB (p = .0039), PRTT (p = .0008) and JTHF (p = .0384) (7 days post-treatment). Brain stimulation may therefore be a useful adjunct to MT for older adults.
INTRODUCTION

Maintaining hand function is an important aspect of ageing, as it has been correlated with independence and quality of life. However, a number of physiologic changes occur throughout life that result in less UE function, to include decreased muscle mass, strength, and contractility. Older adults also have decreased bone density and impaired spatial acuity, vibration recognition, and tactile sensitivity.

Aging individuals also experience degenerative changes within the central nervous system (CNS). When the M1 of an older adult is stimulated with TMS pulses, a greater intensity of stimulation is required to elicit MEPs equivalent in amplitude to those of a younger person. This finding implies that the M1 of older adults is less excitable than those of younger adults. Given that the stimulation of M1 during maximal voluntary contraction is associated with greater torque production, excitability of the CNS likely plays a role in decreased hand function. Relative to younger adults, the M1 of older adults is also less able to encode new motor memories, suggesting that traditional therapy may play a limited role in maintaining function. Techniques that are capable of optimizing cortical plasticity presented in combination with UE specific training may therefore help improve therapeutic techniques and facilitate better functional outcomes. In this context, combining motor training (MT) with transcranial magnetic stimulation (TMS) and anodal transcranial direct current stimulation (a-tDCS), deserve further consideration.

TMS studies incorporating intermittent theta burst stimulation (iTBS) and repetitive transcranial magnetic stimulation (rTMS) have demonstrated >30 minutes of enhanced motor evoked potentials (MEPs) in target musculature. Researchers have used
rTMS to stimulate the motor cortex (M1) of patients post-stroke in conjunction with 10 days of traditional PT, resulting in increased MEPs in target muscles and significantly better scores on clinical and neurophysiological tests. Moreover, patients undergoing this type of stimulation maintained greater functional independence 10 days after the completion of treatment relative to those receiving sham stimulation, suggesting that semi-permanent cortical changes had occurred. Similar gains were also noted by patients suffering from chronic hemiplegic stroke following TMS enhanced hand therapy. Patients have experienced larger MEPs in the muscles of the hand, enhanced motor skill acquisition, and greater grip-lift kinetics compared to placebo treatment.

Following stimulation, for as little as 13 minutes, cortical areas receiving a-tDCS demonstrate up to 90 minutes of increased sensitivity. Use of a-tDCS is thought to impact cortical neurons by making the resting membrane potential more conducive to depolarization, especially when paired with active movements such as MT. Like TMS enhanced rehabilitation, studies that have incorporated a-tDCS into MT protocols have yielded promising results. When used to stimulate the M1 of the non-dominant hemisphere of right-handed healthy volunteers for 20 minutes, a-tDCS enhanced MT resulted in a significant increase in motor performance of the left UE compared to a sham control. Similar results have also been published in patients post-stroke.

The efficacy of using TMS and a-tDCS to augment MT in the elderly population is not well-established. Some investigators have demonstrated that aged-cortices are 15% less responsive to TMS, which may be due to decreased cortical cells / synapses, volume of cortical gray matter, and spinal neurons. In contrast, a group of elderly adults receiving a-tDCS scored on average 7.98% better on the JTHF with the dominant UE
than a similar group that received sham stimulation.\textsuperscript{17} In another study, elderly individuals made functional improvements exceeding 24 hours following a-tDCS enhanced MT.\textsuperscript{18} To our knowledge, no studies have investigated the additive use of TMS and a-tDCS with MT in an elderly population.

Understanding the mechanisms behind TMS and tDCS is paramount to their successful implementation in a rehabilitation setting. The purpose of this study was to determine the efficacy of using TMS and a-tDCS with MT in order to optimize MT in an elderly population. A population of healthy, older adults underwent iTBS/a-tDCS enhanced MT, and outcomes were compared to a group of college-aged adults receiving the same treatment. Based on the proposed mechanisms of action of TMS and tDCS, we chose to use iTBS as a pre-training primer in order to initiate glutamate dependent early LTP (E-LTP) and administer a-tDCS in conjunction with MT so as to facilitate BDNF dependent late LTP (L-LTP).\textsuperscript{13}

We hypothesized that non-dominant UE MT of healthy, older adults primed with iTBS and administered concurrently with a-tDCS would result in better short-term outcomes (directly following MT) than those achieved by healthy, college-age adults that received the same training. Specifically, we predicted that older participants would i) demonstrate greater pre-post functional improvement on the Jebson Taylor Hand Function Test JTHF (primary outcome measure) than younger participants and ii) demonstrate greater pre-post functional improvement than younger participants on the following secondary outcome measures: a) computerized version of the Fitt’s Reciprocal Tapping Task (FRTT), b) computerized version of the Pursuit Rotor Tracking Task (PRTT), and c) the Purdue Pegboard Test (PPB). We further hypothesized that our
training protocol would lead to better long-term outcomes (24 hours and 7 days following MT) on primary and secondary outcome measures in older adults as compared to younger adults receiving the same training.

**METHODS**

This was a randomized, double-blind study comparing the functional improvements made by the non-dominant UE per the JTHF, FRTT, PRTT, and PPB. Although all participants received an iTBS primer followed by a-tDCS enhanced MT, both young and old participants were told that they could be randomly assigned into either a real-stimulation treatment or sham-stimulation control group. In order to establish baseline function, all participants completed the JTHF, FRTT, PRTT, and PPB x10 repetitions with the non-dominant UE within 30 hours of beginning formal training. In addition, cortical mapping of the non-dominant UE was individually conducted via single-pulse TMS, as described below, in order to determine active motor threshold.

On the day of treatment, participants performed the JTHF x3 times, and the scores were averaged into pre-test measurements. The treatment consisted of a three-minute iTBS primer followed by 20 minutes of a-tDCS presented in conjunction with MT. Immediately following treatment, participants again completed 3 repetitions of the JTHF, and the scores were averaged as a post-training, “short-term performance” score. Participants were also scored on the JTHF x3 repetitions 24 hours and 7 days post-treatment. Again, the scores were averaged to create a 24-hour and 7-day score for each participant. The 24 hours and 7 day scores were then averaged to create a single “long-term performance” score. (See Figure 4.3 A&C)

*Participant Recruitment:*
Sixteen healthy college-age and 9 older adults who met inclusion / exclusion criteria were recruited via word of mouth and flyers placed in and around high-traffic areas of the university. Inclusion criteria were as follows: 1. Predominantly right-handed, 2. Either age 18-34 (younger group) or > 60 years old (older group), 3. Ability to provide informed written or verbal consent. Handedness was verified via performance testing on the JTHF, PPB, PRTT, and FRTT. Exclusion criteria were summarized on TMS, tDSCS, and Neurological Screening Forms approved by the Medical University of South Carolina, Department of Neurology. Prior to enrollment in the study, all subjects read and signed a consent form approved by the University of South Carolina (USC) IRB.

**Experimenter Blinding:**

Although none of the participants received sham stimulation, all participants were told that they could be randomly assigned to receive sham-TMS instead of real-TMS. Since the same coil was capable of providing either real or sham stimulation, participants were unable to determine the type of stimulation they were receiving. In order to ensure blinding during a-tDSCS stimulation, participants were told to expect desensitization of the scalp, regardless of group. tDSCS devices were turned on until participants confirmed that the stimulation could be felt on the scalp, and a piece of tape was placed over the warning light and selector switches. A graduate student blinded to group administered all motor assessment testing, thus assuring experimenter blinding.

**Motor Training:**

MT focused on the non-dominant UE and consisted of one, 20-minute sessions geared toward practicing four primary tasks: JTHF, FRTT, PRTT, and PPB. Of the four
tasks, the JTHF was chosen as the primary outcome measure because it provided a short but relatively broad measure of hand function. Previous studies have established that it is both valid and reliable. The writing portion of the JTHT was not performed as part of the study due to variation in handwriting and subsequent complications standardizing among individuals.

Based on Fitt’s Law, the FRTT measured the time required to move a mouse reciprocally between two stationary targets on a computer screen. A previous study demonstrated that the preferred UE performs better than the non-preferred extremity on reciprocal tapping tasks, especially when the size of the target decreased and the distance to the target increased. During the PRTT, participants were asked to keep a visual stimulus within a continuously moving target presented at random velocities by manipulating a computer mouse with the non-dominant hand. Pursuit rotor task are used throughout the literature as a measure of both fine motor control of the fingers and hand. The FRTT and PRTT were created using Presentation software (www.neuobs.com). Lastly, the PPB was used to measure manipulative dexterity of the hands, as it is both a valid and reliable measure with established normative values.

In order to ensure that participants received equal training, they were instructed to complete all tasks “as quickly and accurately as possible”. In addition, all tasks were performed consecutively in a randomized but predetermined order for the full length of the treatment time.

Motor Cortex Mapping and Active Motor Threshold Identification:

Patients were seated in a MagVenture treatment chair with the non-dominant hand pronated on a soft surface. The optimal position for the abductor pollicis brevis (APB)
muscle over the scalp (hot spot) was determined using a MagproX100 Magnetic Stimulator, 230V (MagVenture Inc., Atlanta, GA) and a Cool A65 A/P butterfly coil. With the handle oriented backward and the coil 45 degrees in the posterolateral direction, single TMS pulses at a predetermined intensity were directed just anterior of the central sulcus and adjusted in 1-2 cm increments until a “hot spot” was identified. A ”hot spot” was identified as the location on the scalp able to generate a visual twitch of the APB 3/5 times. The “hot spot” was marked on a cloth MagVenture stimulation cap. In addition, the center of the nasal bone, right / left external auditory acoustic meatus and occiput were marked in order to ensure that the “hot spot” was reliably relocated on the day of treatment. Following the identification of the “hot spot,” participants were asked to isometrically grip a dynamometer between the proximal interphalangeal joint of the left D2 and the pad of the left D1 at approximately 20% of maximal voluntary contraction (MVC) while the active motor threshold (AMT) of the APB was measured. Verbal feedback was provided during the 20% MVC contraction to ensure consistency. The AMT was defined as the lowest stimulation intensity able to produce at least 5/10 MEPs greater than or equal to a 200 µV amplitude (above baseline).

Transcranial Magnetic Stimulation Procedure:

iTBS consisted of three TMS pulses at 50 Hz provided every 200ms (i.e., at 5 Hz) at 80% AMT of the APB. Ten bursts of 3 triplets (30 pulses) were grouped according to trains and repeated every 10 seconds. Participants in the treatment group received a total of 20 trains, totaling 600 pulses. Total stimulation time was 191.84 seconds. iTBS treatment was directed at each participant’s “hot spot” for APB.

Transcranial direct Current Stimulation Procedure:
Patients underwent either 20 minutes of a-tDCS at 1 mV or sham a-tDCS. A previous investigation has also established that 20 minutes is a safe and effective duration for improving motor performance of the non-dominant UE in healthy, college-age subjects after only one treatment.14 Cortical anodal stimulation (1 mA) was delivered via a pair of saline-soaked surface sponge electrodes (2.5 x 2.5 cm) and connected to a 9-volt battery-driven, constant current stimulator for 20 minutes (Chattanooga Ionto Iontophoresis System, DJO Global, Vista, California, Salt Lake City, Utah) in conjunction with MT. The stimulating electrode was centered on the “hot spot” for the APB. The “reference” cathodal electrode was placed just below the contralateral M1.

RESULTS

All subjects tolerated iTBS and a-tDCS well, and no adverse reactions were reported. All participants underwent training prior to the treatment to achieve a performance plateau. As in younger adults,14 ten trials were adequate for older adults to reach a performance plateau on the JTHF, PPB, FRTT, and PRTT. (See Figure 4.4, 1A-D) Of the 14 younger participants and 9 older participants in the study, the data of one older adult was not considered as part of the analysis due to significant hand osteoarthritis. Given that the JTHF test is the only valid and reliable measure that has been used within the context of a brain stimulation study, all variables were independently modeled, and adjusting for multiple comparisons was not considered.

Overall Statistical Model:

Plots of scaled residuals were created using Statistical Analysis System software (SAS institute Inc. – version 9.2, USA) for all primary and secondary variables to ensure homogeneity of variance and normality. A 2x4 repeated measures Analysis of Variance
(ANOVA) was performed on the JTHF, PPB, FRTT, and PRTT in order to evaluate the main effect of time (pre-treatment, post-treatment, 24-hour post-treatment, and 7-days post-treatment), condition (old age and young age), and the interaction between time and condition. The 2x4 repeated measured ANOVA performed on total JTHF time revealed a significant effect of time ($F_{1,22}=100.14$, $p<.0001$) and group ($F_{1,22}=22.22$, $p=.0001$) but was non-significant for the interaction term ($F_{1,22}=1.93$, $p=.1801$). The same test, performed on the PRTT scores revealed a significant effect of group ($F_{1,22}=40.69$, $p<.0001$), time ($F_{1,22}=14.41$, $p=.0011$), and group x time interaction ($F_{1,22}=6.92$, $p=.0160$). PPB scores also showed a significant effect of group, ($F_{1,22}=76.77$, $p<.0001$), time ($F_{1,22}=10.88$, $p=.0036$) and a significant group x time interaction ($F_{1,22}=10.87$, $p=.0036$). FRTT scores showed a significant effect of group ($F_{1,22}=61.51$, $p<.0001$), a non-significant effect of time, ($F_{1,22}=3.48$, $p=.0769$) and a non-significant group x time interaction ($F_{1,22}=0.00$, $p=.9730$). Post-hoc comparisons revealed a 17% and 7.2% functional improvement pre-post treatment of older individuals on the PRTT and PPB, respectively. In contrast, the younger group improved pre-post treatment by only .05% on the PRTT and 2.9% on the PPB.

We also conducted two 2x2 ANOVAs in order to evaluate the main effects of time and condition for both short-term (pre-treatment score vs. immediate post treatment score) and long-term (pre-treatment vs. average of 24hr and 7-days post treatment stores). Older adults did not experience greater functional improvement than younger adults on any test immediately following iTBS/tDCS enhanced MT. With regards to the long-term 2X2 ANOVA, there was no significant interaction between group and time (pre-test and average of 24 hour-post test and 7 days post-test) on FRTT score ($F_{1,22}=.01$ $p=.9153$).
However, older adults improved more than younger adults on the PRTT (F\(_{1,22}=15.59, p=.0008\)) and PPB (F\(_{1,22}=10.64, p=.0039\)). Long-term, older adults bettered their PRTT and PPB score by 17.5% and 8.2%, respectively. Older adults demonstrated an 11.6% (12.46 pixels) greater improvement on the PRTT and a 5.6% (.58 pegs) greater improvement on the PPB than their younger counterparts. In addition, a post-hoc 2X2 ANOVA only considering 7-days post-test data demonstrated a significant long-term interaction on the JTHF (F\(_{1,22}=4.91, p = .0384\)). At 7 days post-treatment, older adults improved their JTHF score by 11.9%, a 1.2% (1.48s.) greater improvement than the younger group. (See Figure 4.4, 2A-2D & 3A-3D)

**DISCUSSION**

Previous research indicates that MT augmented with iTBS/a-tDCS results in better motor performance than placebo stimulation in healthy college-age students.\(^{19}\) The present study demonstrates the efficacy of iTBS/a-tDCS enhanced MT in an elderly population. Regardless of participant and grader blinding, short-term improvements in JTHF performance of both groups following iTBS/a-tDCS enhanced MT were similar to those published in previous work incorporating MT and brain stimulation technology.\(^{9,10,14,15,17}\) In the short-term, iTBS/a-tDCS augmented MT was, therefore, at least as beneficial as MT combined with TMS and tDCS alone for young and old age-groups.

Because of age-related loss of motor function, we expected older individuals to achieve better functional gains following MT augmented with iTBS/a-tDCS than younger individuals. A repeated-measures, 2X4 ANOVAs revealed that this was the case for the PRTT and PPB but not the JTHF and the FRTT. Notably, performance on the FRTT was
not significantly different at any time point. We believe this may be due to poor standardization of task instructions regarding whether participants should stress speed, accuracy, or both. A post-hoc analysis further revealed that neither group improved more than the other on any test short-term. Older individuals may therefore benefit from MT augmented with iTBS and a-tDCS equally as much as younger participants in the short-term. In contrast, the participants in the older group achieved significantly greater functional improvement long-term on the PRTT, PPB, and JTHF. This unique finding suggests that older individuals may enjoy greater long-term benefits from iTBS/a-TDCS enhanced MT than younger individuals.

The difference in long-term learning effects may be best explained by the physiological mechanisms underlying LTP. Studies of LTP in the hippocampus clearly distinguish between early and late LTP. While E-LTP occurs in the first 4-6 hours following MT and does not result in mRNA synthesis, L-LTP occurs after 6 hours and results in the formation of new proteins. As a result, E-LTP works to strengthen existing synapses and lasts from minutes to hours, while L-LTP results in new synapse production, lasting from days to months. This effect seems to also apply to plasticity in M1. Motor learning was assessed via a pursuit rotor task 45 minutes and 7 days after either excitatory or inhibitory paired-associative stimulation (PAS) of the median nerve and contralateral M1. Although neither group improved more than the other 45-minutes post-PAS, as structural changes within M1 had not yet occurred, the group receiving excitatory PAS made greater functional improvements than the group receiving inhibitory PAS 7-days post-stimulation. Such structural changes within M1 have been demonstrated in animal models. While rats trained to perform a skilled reaching task
performed better than rats practicing unskilled reaching at 3, 7, and 10 days, only skilled reaching rats had significantly greater synapses per neuron and a larger forelimb representation.\textsuperscript{27} Moreover, plastic changes did not occur until after day 7.\textsuperscript{27} In another experiment designed to train rats on a forelimb reaching task, performance improvements made within the first 6 days of training were independent of signal-to-noise ratio of M1 neuronal spiking.\textsuperscript{28} In contrast, the firing pattern of M1 neurons after day 6 better correlated with muscle recruitment patterns, indicative of synapogenesis associated with L-LTP.\textsuperscript{28} In the present study, both groups had the same potential for synapse strengthening associated with E-LTP. The fact that the older-group made greater functional improvements than the younger group at long-term follow-up suggests that plasticity associated with L-LTP is preserved and possibly exaggerated in older adults.

One explanation for this finding may be the difference in the learning ceiling of the older and younger participants prior to training. Older individuals arguably have less motor function and subsequently a higher motor learning ceiling. Previous research suggests that thresholding is an important aspect of LTP.\textsuperscript{29,30} Studies that have looked at the effect of rTMS enhanced therapy following an inhibitory, cathodal tDCS (c-tDCS) primer demonstrated significant decreases in MEPs and increases cortical excitability within M1 compared to a-tDCS priming.\textsuperscript{29,30} The investigators argued that c-tDCS was more effective prior to TMS enhanced MT because of its ability to alter the threshold required for LTP induction.\textsuperscript{29,30} In another study, neurons responsible for forelimb movement in rats within M1 did not strengthen their connections via LTP upon completion of a predominately UE reaching training program.\textsuperscript{31} However, significantly more synapses were created following hind limb reaching because the movement was
unfamiliar and the learning ceiling was higher.\textsuperscript{31} iTBS/a-tDCS enhanced MT may therefore have a greater potential to improve function in older individuals simply because they are beginning training with less function. This raises the tantalizing possibility that patients may also benefit from such treatment post-stroke.

The fact that the older individuals experienced greater functional gains long-term in comparison to younger individuals is somewhat surprising in light of numerous research studies available in the literature that demonstrate an age-dependent reduction in motor learning and associated M1 plasticity.\textsuperscript{32,33} Investigations of elderly subjects report increased short (SICI) and long-interval intracortical inhibition (LICI) along with decreased cortical facilitation at rest, which is likely mediated by increased GABAergic interneuron activity.\textsuperscript{34} While some studies also describe a reduction of glutamate within the cerebral cortex of elderly,\textsuperscript{35,36} in vivo and in vitro animal models reveal no change of glutamate release with age.\textsuperscript{34} Rather, a decreased density of NMDA channels on post-synaptic membranes has been demonstrated.\textsuperscript{34} In this context, an iTBS-primer may lend itself particularly well to M1 plasticity in older individuals. The 200ms inter-pulse interval likely helps minimize SICI by inhibiting GABAergic interneurons via GABA-b receptors. The subsequent period of intracortical disinhibition may facilitate glutamate release to “kick-start” E-LTP and up-regulate NMDA receptors on post-synaptic neurons.

Our finding of greater functional improvement of the older-age group following iTBS/a-tDCS may also be related to the dynamics of brain derived neurotrophic factor (BDNF). Although a recent study demonstrated no association between BDNF genotype,\textsuperscript{37} cortical plasticity and motor performance in older adults, performance was measured directly following 30 minutes of exercise. Since BDNF has been associated
with L-LTP, a short-term functional measurement likely does not account for the effects of BDNF in M1. Notably, many studies demonstrate that BDNF is a key mediating factor required for LTP in M1. A recent animal study found that rats subjected to photothrombic ischemia experienced significantly better functional outcomes when treated with BDNF than 5 and 14 days cast mediated CIMT. Moreover, functional gains correlated with increased AMPA and NMDA receptors within the peri-lesional region 3 weeks post-stroke. As such, a-tDCS may lend itself well to plasticity within M1. Recent studies suggest that tDCS is likely not powerful enough to unblock NMDA receptors directly. Rather, cortical excitation resulting from tDCS may be due to proBDNF release from presynaptic neurons and subsequent TrkB receptor activation at the pre and postsynaptic membrane.

The results of this study suggest that while MT augmented with iTBS and a-tDCS may be useful for individuals of all ages, the benefits of the treatment may be particularly pronounced in elderly individuals. This benefit may be due to lower levels of function at baseline. In this context, MT augmented with iTBS/a-tDCS may also be useful in the recovery of neurologically impaired patients. We recommend more robust studies with larger sample sizes to further investigate the potential of the combined iTBS-a-tDCS protocol. Future studies should also work to correlate short and long-term functional improvements with reliable measures of cortical excitability and depression.
Figure 4.3 (A): Graphical representation of the iTBS/a-tDCS / sham iTBS/a-tDCS enhanced motor training protocol. (B): Graphical depiction of the experimental design comparing functional outcomes of college-age students following MT augmented with real and sham iTBS/a-tDCS. (C): Graphical depiction of the experimental design comparing functional outcomes of older and young participants following motor training augmented with real iTBS/a-tDCS.
Figure 4.4 (1A-1D): Plateau of older adults on the PRTT (1A), PPB (1B), JTHF (1C), and FRTT (1D) per 10 trials of each task. (2A-2D): Results of the 4 functional tasks at each time point (pre-test, post-test, 24-hrs post-test, and 7-days, post-test). The dark gray and light gray line represents the group of older adults and younger adults pre and post iTBS/a-tDCS enhanced MT, respectively. (3A-3D): Graphic representation of the short and long-term pre-post functional improvement on all 4 tasks. The dark gray bar represents the pre-post functional improvement of the old-age group, while the light gray bar represents pre-post functional improvement of the young-age group. * = significant effect at p< 0.05.
WORKS CITED


5.1 DISCUSSION

As the United States continues to struggle with a budget crisis, rising health care costs and dwindling reimbursement rates plague post-stroke healthcare providers. However, the demand for PT services continues to rise. Since 2000, there has been a 15% increase in the number of elderly adults over the age of 65 living in the U.S., and a 36% increase is expected by 2020. To date there are also over 7 million people that have suffered a stroke in the U.S., and two thirds of them continue to report a physical disability. Continual investigation of better and more efficient ways to conduct MT and rehabilitation is, therefore, absolutely essential.

Motor recovery following a neurologic incident such as a stroke is accompanied by significant changes within the CNS. Presently, physical therapists attempt to facilitate motor recovery by maximizing the stimulation of paretic musculature peripherally in an effort to initiate cortical plasticity centrally. There are many studies available in the literature that demonstrate that the principles of forced use and massed practice afforded by techniques such as constraint-induced movement therapy (CIMT), locomotor training (LT) and intensive mobility training (IMT) result in significant therapeutic outcomes. The fact remains, however, that for many neurologically impaired patients, significant disabilities persist. More than 85% of patients that have suffered a
stroke have lasting functional impairments,7 and approximately 50-60% of survivors continue to require functional assistance to complete activities of daily living after completion of intensive PT.8 Given the imbalance of cortical inhibition that is present post-stroke, it is plausible that the CNS improperly limits itself from achieving full motor recovery. Perhaps the use of TMS and tDCS to directly alter cortical inhibition centrally in combination with MT, provided by traditional PT to stimulate paretic musculature peripherally, would result in better functional outcomes. The primary purpose behind this PhD dissertation was to explore this possibility.

Thus, we examined the feasibility and effectiveness of a combined CNS/PNS approach to enhance motor learning. Previous literature examining the physiological underpinnings of enhanced plasticity elicited by our two brain stimulation technologies of choice suggest that TMS may be more effective as a primer for PT, whereas a-tDCS may be more powerful when used in conjunction with rehabilitative movements.9 A TMS primer would minimize ICI and therefore allow glutamatergic neurons within M1 to interact unimpeded during a-tDCS enhanced therapy via E-LTP.10 Furthermore, a reduction of ICI may facilitate optimal BDNF release and subsequent plasticity within M1 during L-LTP.9

Data analysis revealed that 20 minutes of MT presented in conjunction with a-tDCS and primed with iTBS resulted in significantly greater functional gains than MT augmented with placebo stimulation. Moreover, a 9.4% improvement in function seemed to be consistent with previous studies that combined MT with either TMS or a-tDCS.11,12 Notably, significant motor improvements were seen 24 hours and 7 days post-MT but were not observed directly following treatment. This finding fits well with our present
understanding of LTP. Within the first 4-6 hours after MT, existing synapses within M1 are likely stimulated and strengthened via E-LTP.\textsuperscript{13,14} However, long-term functional changes require recruitment of intracortical and corticospinal connections along with synaptogenesis, events typically associated with L-LTP. In contrast to E-LTP, L-LTP begins approximately six hours post-MT and continues five to seven days until completion.\textsuperscript{13,14}

The present thesis project further found that older adults benefitted from MT augmented with iTBS/a-tDCS just as much as their younger counterparts in the short-term. Moreover, the older group outperformed the younger group relative to baseline at long-term follow-up, which suggests that plasticity associated with L-LTP is preserved and possibly exaggerated in older adults. This finding is somewhat surprising in light of numerous research studies reporting age-dependent reductions in motor learning and associated M1 plasticity.\textsuperscript{15,16} We feel that this may be because older adults have a lower level of baseline function and, subsequently, a higher learning ceiling than younger adults.\textsuperscript{17,18} Simply put, this leaves greater room for improvement.\textsuperscript{19,20}

As is the case with patient’s post-stroke, the lower baseline function of older adults may be explained by deficiencies within the CNS. Older adults have been shown to have greater ICI and subsequently less NMDA receptors on post-synaptic membranes.\textsuperscript{21} In this context, an iTBS-primer lends itself particularly well to M1 plasticity in older individuals. The 200ms inter-pulse interval likely helps minimize SICI by inhibiting GABAergic interneurons via GABA-b receptors.\textsuperscript{22} The subsequent period of intracortical disinhibition may facilitate glutamate release to “kick-start” E-LTP and up-regulate NMDA receptors on post-synaptic neurons. tDCS may further facilitate
plasticity dependent functional gains via the release of BDNF and subsequent initiation of L-LTP.

5.2 LIMITATIONS

The primary limitation associated with this study was a small sample size. TMS and tDCS are two relatively new technologies. Prospective study participants were understandably hesitant and anxious at the thought of having their brain stimulated. Recruiting older adults was particularly problematic due to medications, implantable devices, and past medical histories. We recognize that a small sample size results in limited power and raises questions about external validity. Future studies will need to be considerably more robust in order to fully investigate the potential of a MT protocol enhanced with iTBS and a-tDCS motor training protocol.

This investigation primarily measured changes in functional performance via four standardized assessment tests: the JTHF, FRTT, PRTT, and PPB. However, more careful consideration should have been given to standardization of task instructions. This problem was particularly evident on the FRTT, as some participants were unsure whether to focus on task speed, accuracy, or both. While all four tests were successfully used to measure functional improvements in older adults, only the JTHF was sensitive enough to detect changes in younger participants. Hopefully, future studies will investigate MT enhanced with iTBS/a-tDCS primarily in functionally impaired populations. However, future studies that use college-age students as participants should carefully consider the motor task being trained and tested. Previous studies suggest that optimal plasticity results from motor tasks that are physically challenging and functionally meaningful to
participants. Future studies must also carefully consider the difficulty and ‘coolness’ of the tasks.

We have repeatedly made the argument that MT augmented with TMS and a-tDCS results in optimal functional improvement because it is able to temporally initiate E-LTP and L-LTP, respectively. We also suggest that MT and iTBS/a-tDCS results in long-term but not short-term changes in function, as only at later time points has sufficient time passed to allow both E-LTP and L-LTP to occur. Importantly, we therefore only infer that cortical plasticity has occurred. We did not measure changes in cortical excitability resulting from the brain stimulation nor did we quantify changes in motor map representation. We also did not measure ICI, glutamate release, or NMDA receptor activation associated with E-LTP or BDNF release and synaptogenesis associated with L-LTP. An interdisciplinary approach should be adopted in the future in order to assess the relationship of each of these important variables to motor learning.

The use of healthy, college-age students and older-adults to investigate the feasibility and effectiveness of MT augmented with iTBS/tDCS was advantageous, as it allowed us to minimize the safety risks associated with non-invasive brain stimulation while achieving adequate sample size and power. Applying iTBS and a-tDCS to rehabilitation protocols post-stroke will require careful consideration. Patients that have suffered a stroke have altered levels of cortical excitability, which may increase their risk of having a seizure during TMS. Special consideration must also be given to the site of brain stimulation post-stroke. Magnetic resonance imaging may be useful in identifying lesions and guiding the optimal location of TMS and tDCS. In this regard, high definition tDCS may also be advantageous, as it provides more powerful and focused
stimulation of cortical areas.\textsuperscript{26} Given that cortical plasticity typically occurs in the direction of least ICI,\textsuperscript{27} TMS and tDCS to specific brain sites with respect to the lesion may be able to guide and subsequently optimize plasticity dependent motor recovery.

5.3 CONCLUSIONS

Perhaps traditional MT / PT generates limited cortical plasticity, resulting in suboptimal functional gains. Meaningful functional improvement may only be possible under the following conditions: 1. limited ICI and optimal glutamate release afforded by iTBS mediated E-LTP and 2. BDNF activation of TrkB receptors initiated by a-tDCS mediated L-LTP. In this regard, iTBS/a-tDCS augmented motor training holds great promise for enhancing motor learning in healthy and impaired populations. While MT augmented with iTBS and a-tDCS may be useful for individuals of all ages, the benefits of the treatment may be particularly pronounced in elderly individuals. Since the benefit may be due to lower levels of function at baseline, MT augmented with iTBS/a-tDCS may also be useful in the recovery of neurologically impaired patients. We recommend more robust studies with larger sample sizes to further investigate the potential of the combined iTBS-a-tDCS protocol. Future studies should also work to correlate short and long-term functional improvements with reliable measures of cortical plasticity.
WORKS CITED


REFERENCES


McHughen SA, Cramer SC. The BDNF val(66)met polymorphism is not related to motor function or short-term cortical plasticity in elderly subjects. *Brain Res.* 2013 Feb 7;1495:1-10.


Rossi S, Hallett M, Rossini PM, Pascual-Leone A; Safety of TMS Consensus Group. Safety, ethical considerations, and application guidelines for the use of


Wolf SL. Revisiting constraint-induced movement therapy: are we too smitten with the mitten? Is all nonuse "learned"? and other quandaries. *Phys Ther*. 2007 Sep;87(9):1212-23.


APPENDIX A: BRAIN STIMULATION STUDY ADVERTISEMENT

Stimulating the Brain to Achieve Optimal Motor Performance

You may qualify for a research study designed to safely stimulate the brain in order to achieve optimal motor performance. This study uses transcranial magnetic stimulation (TMS) and transcranial direct current stimulation (tDCS) to alter the excitability and overall plasticity of specific brain areas in order to overcome the plateau phase of motor training and optimize motor performance. You will undergo 4 daily training sessions (9-18 minutes in duration) with the nondominant upper extremity that will incorporate either TMS, TDCS, or both TMS and TDCS. Motor performance will be measured prior to and immediately following the 4 days of motor training. In order to evaluate long-term motor improvements, performance of participants will also be measured 24 hours and 7 days following completion of training. The study will require participation in 8 total sessions that will last approximately 45 minutes each. You will be compensated $100.00 ($12.50 per session) for your time.

To qualify you must
- Be between 18 and 30 years old and be right handed and speak English
- Have no recent/present neurological symptoms
- No history of seizures
- Have not had previous brain surgery
- Do not have a sensitive scalp

Contact Dr. Roger D. Newman-Norlund via phone at 1-803-777-7176 or via e-mail at rnorlund@mailbox.sc.edu for more information.
APPENDIX B: TMS SAFETY SCREENING FORM

TMS Screening Form
Study Subject ID#________
Name of TMS Subject ________________________________

Your head will be exposed to a strong magnetic pulse. To maximize safety, please answer the questions below. Please do not hesitate to ask any questions you may have regarding below. Do you have, or have you ever had, any of the following? If Yes, please explain on back

Y/N 1. Metallic hardware on the scalp

Y/N 2. Cardiac pacemaker

Y/N 3. Implanted medication pumps, intracardiac line, or central venous catheter

Y/N 4. History of cortical stroke or other cortical lesion such as brain tumor

Y/N 5. Prior diagnosis of seizure or epilepsy

Y/N 6. Previous brain neurosurgery

Y/N 7. Any chance you are pregnant?
   Date of last menstrual period: _________

Y/N 8. Any electrical, mechanical, or magnetic implants?

Y/N 9. Migraine headaches – if yes, are they controlled?

Y/N 10. List current medications on back of form (we are interested in medicines that affect seizure threshold such as tricyclic antidepressants and neuroleptics)

Y/N 11. Unstable medical conditions

Y/N 12. Any body or clothing metal above your shoulders? If so, please remove.

Y/N 13. Any metal on your body (i.e. watch or jewelry, hair holders or pins, eye glasses, body piercings, wallet, keys)? If so, please remove.

I have read /understand all questions in this document. My signature below indicates that I have accurately and completely answered all questions in this document.

Signature of TMS Subject: ________________________________ Date: ______
Signature of investigator: ________________________________ Date: ______
**APPENDIX C: TDCS SAFETY SCREENING FORM**

**tDCS Safety Screening Form**

Study Subject ID#________

*For safety reasons, it is important that you answer all of the following questions carefully. Please ask if you have any questions.*

<table>
<thead>
<tr>
<th></th>
<th>Yes</th>
<th>No</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Have you ever had an adverse reaction to tDCS?</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2. Have you ever had a seizure?</td>
<td></td>
<td></td>
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<tr>
<td>3. Have you ever had a head injury (including neurosurgery)?</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4. Have you ever had any illness that caused brain injury?</td>
<td></td>
<td></td>
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<tr>
<td>5. Have you ever had any other brain-related condition?</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6. Have you ever been diagnosed with a neurological or psychiatric disorder?</td>
<td></td>
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</tr>
<tr>
<td>7. Do you have any metal in your head (outside of the mouth) such as shrapnel, surgical clips, or fragments from welding or metalwork?</td>
<td></td>
<td></td>
</tr>
<tr>
<td>8. Do you have a sensitive scalp (is your skin very dry, or do you use products designed for people with a sensitive scalp)?</td>
<td></td>
<td></td>
</tr>
<tr>
<td>9. If any item above was marked ‘yes’, please provide a comment here:</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

10. Please list all medications you are currently taking:

The possible hazards of tDCS have been explained to me, and I understand that I can withdraw at this point for any reason, and that I do not have to disclose the reason to the experimenter. Your signature below indicates that you understand this screening form and attest to its accuracy.

<table>
<thead>
<tr>
<th>Volunteer's signature</th>
<th>Researcher's signature</th>
<th>Date</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
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</tbody>
</table>


**APPENDIX D: NEUROLOGICAL SCREENING FORM**

**Neurological Symptom Checklist**
Study Subject ID#________

For safety reasons, it is important that you answer all the following questions carefully. Please ask if you have any questions.

<table>
<thead>
<tr>
<th>Check All That Apply</th>
<th>Yes</th>
<th>No</th>
<th>Details</th>
</tr>
</thead>
<tbody>
<tr>
<td>Do you experience frequent dizziness or vertigo?</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Do you experience frequent headaches?</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Do you experience tremors?</td>
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<td></td>
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<tr>
<td>Are you prone to strange movements or bizarre behavior?</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Do you experience memory loss or problems?</td>
<td></td>
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<tr>
<td>Have you recently experienced double vision change or loss of vision?</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Have you experience abnormal muscle weakness?</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Do you experience burning, tingling or numbness?</td>
<td></td>
<td></td>
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<tr>
<td>Have you noticed any sudden change in your sleep patterns?</td>
<td></td>
<td></td>
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<tr>
<td>Do you experience extreme fatigue or become fatigued easily?</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Do you experience staring or twitching spells?</td>
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<tr>
<td>Are you experience difficulty of slowness understanding what other s say to you?</td>
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<td></td>
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<tr>
<td>Do you experience any unexplained pain in your hands, feet or face?</td>
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</tbody>
</table>

When the form has been checked through with you by a member of staff, please sign below to confirm that you have read and understood all the questions.

<table>
<thead>
<tr>
<th>Volunteer's signature</th>
<th>Researcher's signature</th>
<th>Date</th>
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APPENDIX E: INFORMED CONSENT FORM

CONSENT FORM
Raymond J. Butts, PT DPT MS, PhD candidate
The Enhancement of Motor Training of the Non-dominant Hand via TMS and tDCS

1) Introduction
You are being asked to volunteer for a research study because you are a healthy, right-handed adult. Please read the following paragraphs carefully. If you have any questions or concerns regarding participation in this study, you are encouraged to raise these concerns with the investigators. The research is sponsored by the Department of Exercise Science at the University of South Carolina. The investigator in charge of this study is Raymond J. Butts.

Purpose of Study
The purpose of this study is to determine how two forms of brain stimulation, transcranial magnetic stimulation (TMS) and transcranial direct current stimulation (tDCS), may help to enhance motor training of the left hand. We will use TMS and tDCS to pass a weak electrical current into the area of your brain responsible for active movement. This current will increase nerve activity or the likelihood of nerve activity under the location we are stimulating. In the current study, we are interested in whether motor training of the non-dominant hand combined with TMS and tDCS will result in greater functional improvement than training combined with placebo stimulation. As such, one half of participants will be randomly selected to receive TMS and tDCS stimulation while the other half will receive placebo stimulation. Participants will not be able to determine which stimulation they are receiving. Training will consist of one, 20-minute session made up of exercises that will simulate typical activities of daily living. Motor improvements will be recorded immediately following, 24 hours, and 7 days after completion of motor training. The data obtained through your participation may help to find better ways of helping patients that have suffered from a stroke regain function.

Eligibility to Participate
Approximately 32 healthy adults will participate in the current study. You must meet the following criteria: 1) be right handed 2) be able to provide informed written or verbal consent and 3) be from 18-34 or >65 years old. Only participants who clearly understand the research and are able to indicate consent to participate can be enrolled.
in this study. You must pass a TMS and tDCS Safety Screening along with a general Neurological Symptoms Screening in order to participate in this experiment. The following criteria will be used to exclude participants from the study: history of brain surgery, sensitive scalp (i.e. very dry skin requiring the use of moisturizing products), history of seizures, or history of taking medication to prevent / treat seizures (e.g. Ritalin, Adderall, Buproprion or Theophylline).

2) **Description of Study Procedures**
   If you agree to be in this study, the following will happen:
   1. If you qualify and agree to participate, you will take part in 4 separate appointments according to the following schedule. (See chart below for a sample schedule)

   **Appointment 1:**
   You are presently completing session 1, whereby all necessary paperwork will be filled out to complete this study. After completion of all screening forms, you will receive a schedule with dates and times of sessions 2-4.

   **Appointment 2:**
   Seven days prior to beginning motor training, participants will meet on the second floor of the Discovery I building with Raymond Butts. Ray will provide instruction on all exercises that will be included in motor training. Participants will be asked to practice the exercises X10 times each during this session. In addition, TMS will be used to map a specific region of the brain that is responsible for moving the index finger. This region will provide the target for TMS and tDCS during motor training with the left hand. TMS will also be used to measure the baseline excitability of the part of your brain responsible for moving your index finger.

   **Appointment 3:**
   The third session will take place on a Monday and within 7 days of Session 2. During Session 3, participants will use their left hand to complete 3 pre-assessment tests (X3 times each). Afterward, participants will receive 3 minutes of TMS followed by 20 minutes of tDCS in conjunction with motor training. After motor training, participants will complete the same 3 assessment tests (X3 times each). TMS will again be used to measure the excitability of the part of your brain responsible for moving your index finger.

   **Appointment 4:**
   Participants will return 24 hours after completion of motor training to complete the same 3 assessment tests (X3 times each)

   **Appointment 5:**
   Participants will return 7 days after completion of motor training to complete the same 3 assessment tests (X3 times each)

   See Figure E.1 for an example appointment schedule for participants in this study:
3) Statement of Health Risks

**tDCS:**
Transcranial direct current stimulation involves the application of weak electric currents (generated by a single 9-volt battery) to change the firing rates of neurons under the scalp. The actual current entering your brain during tDCS is very small. tDCS has been used safely in hundreds of experimental studies. tDCS is safe to use when in accordance with appropriate safety guidelines, which the investigators will strictly follow during this study. Please be aware that the application of tDCS may cause you some temporary discomfort. You may notice some mild tingling where the electrode is placed on your scalp. It is also possible that you may feel some fatigue after treatment or some itching under the site where the electrode was placed on your scalp. There is a small chance you will experience a headache, nausea, or insomnia (1%). It is not absolutely known that these are the only risks associated with tDCS. Thus, it may be possible that there are unknown risks associated with the application of tDCS.

The effects of tDCS are temporary, and it is not known to cause any permanent effects, either beneficial or harmful. Please report any adverse effects you may experience during tDCS stimulation to the experimenter so that they can monitor these symptoms.

**TMS:**
Like tDCS, stimulation with TMS may also result in a minor headache or discomfort at the site of stimulation. An additional risk of TMS is seizures, which are thought to be caused by group of nerves that become hyper-synchronized. According to the 2008 TMS consensus group, the risk of seizures with repetitive TMS is VERY low. Out of 3000 studies published within the last 10 years, only 17 have resulted in seizures, 12 of which occurred following parameters that exceeded clinical safety guidelines. The current study is accordance with these guidelines. Of the 4 studies that met the clinical safety guidelines, all participants suffered from additional neurological impairments. All TMS seizures have occurred under close observation. All seizures have stopped spontaneously with no long-term adverse effects. Importantly, no one has ever developed a recurring seizure disorder (epilepsy) after a TMS-induced seizure.

4) Participant Injury

In the unlikely event that you are injured as a result of your participation in this study, the research staff will assist you in obtaining appropriate medical treatment. However, you will be responsible for any costs associated with medical treatment.

5a) Benefits of Participation

There is no prediction that participants will directly benefit from participation in this experiment. Although greater motor function with the left hand may result from TMS / tDCS stimulation and training, the effects may be short-term.
5b) **Participant Compensation**
Participants will be compensated for their participation at the rate of $50 for the entire experiment. In the event that you should wish to discontinue your participation, which you may do at any time, you will be paid for the time you have already invested in the experiment (rounded up to the nearest half-hour).

6) **Data Confidentiality and Participant Identification**
Your name will not be used in any publication that may result from this study. The USC Office of Research Compliance may request access to this form to ensure procedures designed to protect research participants are being properly followed. Your data may also be shared with other researchers around the world or with a publicly available data archive. In such cases, every reasonable effort will be made to remove identifiers from the data that would indicate any connection to you (e.g. the removal of your name, address, etc.). Any information that is obtained in connection with this study and that could identify you will remain confidential and will not be released or disclosed without your further consent, except as specifically required by law.

7) **Expiration Date on the Viability of the Collected Data**
Data concerning your age, gender, handedness, task performance, etc. will be collected. All data gathered from this study will be maintained by the principle investigator for three-years or as required by journal, federal or state regulation.

8) **Voluntary Withdrawal**
Participation in this study is voluntary. You are free to withdraw your consent and discontinue participation in the study at any time throughout the study without negative consequences to your relationship with the University of South Carolina.

9) **Involuntary Withdrawal**
You may be removed from the study if you do not adhere to the study guidelines outlined above (e.g. failure to show up for assigned appointments). In the case of involuntary removal from the study you will be paid for all work completed to that point in the study (rounded up to the nearest half-hour).

10) **Investigator Contact Information**
This research is being conducted by faculty and researchers of the University of South Carolina. For further information about this study, you may contact:

Raymond Butts, PhD candidate (Graduate Student)
Department of Exercise Science
Phone Number: (803) 422-3954
Email Address: buttsraymond@yahoo.com

If you have any questions regarding your rights as a research participant, contact Mr. Thomas Coggins, Director, Office of Research Compliance, University of South Carolina, Columbia, SC 29208, tcoggins@mailbox.sc.edu, Phone: (803) 777-7095.
11) **Participant Signatures**

I have read this informed consent form and have been given a chance to ask questions about this research study. These questions have been answered to my satisfaction. I agree to participate in this study. I have received (or will receive) a copy of this form for my own records.

Participant ___________________________________________

Date _____/______/______

Investigator ___________________________________________

Date _____/_____/_______

For IRB Staff Use Only
University of South Carolina
IRB Number: Pro00007355
Date Approved 8/29/2012
Version Valid Until: 8/28/2013

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Figure E.1: Example appointment schedule for study participants.
APPENDIX F: PILOT WORK

Methods:

Approach (Overview):
This pilot was a randomized study comparing the motor improvements and functional gains made by the non-dominant upper extremity according to four primary measures: 1. The Purdue Peg Test (PPB), 2. The Jebsen-Taylor Hand Function test (JTHF), and 3. A computer based Fitt’s reciprocal Tapping Task (FRTT) and 4. A computer-based pursuit-rotor tracking task (PRTT). Following consent, participant screening, and a handedness evaluation via the Edinburgh Handedness Inventory (Appendix A), participants were randomly assigned to one of two groups (See Figure1 below): Group 1: iTBS primer followed by motor training. Group 2: TDCS primer followed by motor training. In order to establish baseline performance and allow for familiarization, all participants completed each of the assessment tests listed above X10 times with the nondominant upper extremity within 7 days of beginning motor training. They also completed each test once with the dominant upper extremity to confirm handedness. During the same session, cortical mapping of the non-dominant upper extremity was individually performed via single pulse TMS, as described below. In addition, the resting motor threshold and baseline cortical excitability was determined. On the day of treatment, participants performed each of the four assessment tests X3 times, and the scores were averaged into baseline measurements. Treatment consisted of 4 consecutive days of training, whereby participants trained on each of the four tests during 9 minute sessions primed with the cortical stimulation characteristic of their randomly assigned group. Immediately following the last training session, participants again performed the three assessment tests, and the scores were averaged into a post-training evaluation. In order to evaluate the after-effects of the training, participants were also scored on the four tests 24 hours and 7 days following the completion of the four training sessions.

Participants:
10 healthy participants who meet the above requirements were recruited from the University of South Carolina. Participants were primarily recruited via word of mouth and informational presentations of TMS and TDCS. Participants were not recruited from classes whereby Dr. Newman-Norlund was the primary instructor so as to prevent conflict of interest.
Prior to enrollment in the study, all potential subjects met with Raymond Butts on the second floor of the Discovery I building for a discussion of the study and the requirements for participation. During this meeting, participants were asked to read and sign a written consent form. Participants were also informed verbally and in writing via the consent form that that they may quit the study at any time if they choose to do so.
Inclusion and Exclusion Criteria:
Participants were carefully screened according to inclusion and exclusion criteria. Inclusion criteria included 1. Right handed according to Edinburgh Handedness Inventory (Appendix A), 2. Age 18-34 years old, 3. Ability to provide informed written or verbal consent. Only participants who clearly understood the research and were able to indicate consent to participate were enrolled in the study. All participants had full, functional use of both upper extremities. Participants were cognitively able to follow instructions required to complete all physical / functional assessments along with motor treatments. Participants also completed tDCS and TMS screening forms to ensure they met specific inclusion / exclusion criteria associated with each individual piece of equipment (Appendix C and D). In addition, participants with recent / present neurological symptoms were identified and excluded from the study via the Neurological Symptom Checklist found in Appendix E. One participant was excluded from the study secondary to general anxiety of the protocol and reoccurring migraine headaches.

Participants that satisfied all inclusion / exclusion criteria were assigned a number between 1-10. Each number was written on a slip of paper and then placed in a bowl. Slips of paper were selected out of the bowl one at a time. Odd number selections were assigned to the TMS stimulation group, and even number slips were assigned to the tDCS stimulation group. Each participant was issued a detailed schedule corresponding with 8 appointment times. The goal of the 1st appointment was to screen patients and provide information about the study. During appointment 2, participants underwent cortical mapping, and the region corresponding with the first dorsal interosseous (FDI) muscle of the nondominant M1 was identified. In addition, baseline cortical excitability of the hand region of motor cortex was measured and participants completed familiarization training of all assessment. Appointment 3 was the first official day of training. On this day, participants completed baseline testing followed by the first 9-minute training session in accordance with their assigned group. Appointments 4 and 5 took place 24 and 48 hours after appointment 3, respectively, and consisted of the same cortical stimulation and 9 minute training session. During appointment 6, participants completed the final 9 minute training session followed by post-training testing. In addition, cortical excitability of the hand region of the motor cortex was again measured. Appointments 7 and 8 occurred 24 hours and 7 days after the last day of training, respectively, and primarily included post-training testing. See Figure F.1 for a graphical representation of the study layout.

Motor Training
Motor training focused on the non-dominant upper extremity and consisted of 4, 9-minute sessions geared toward practicing four primary assessments: JTHF, PPB, PRTT, and FRTT. In order to ensure that participants trained equally on all three tests, the tests were performed consecutively but in a predetermined, randomized order for the full length of the treatment time. In order to randomize the tasks, all possible order combination were written on 3X5 cards prior to motor training. The cards were shuffled, and participants were asked to select a card and perform the motor tasks in the order written on the card.
Motor Cortex Mapping and Active Motor Threshold Identification:
Participants were seated in a comfortable, MagVenture treatment chair with the non-dominant hand pronated on a soft surface for comfort. The optimal position for the FDI over the scalp (hot spot) was determined using a MagproX100 Magnetic Stimulator, 230V (MagVenture Inc., Atlanta, GA) and a Cool A65 A/P butterfly coil. With the handle oriented backward and the coil 45 degrees in the posterolateral direction, single TMS pulses at a predetermined intensity were directed just anterior of the central sulcus and adjusted in 1-2 cm increments until a “hot spot” was identified. The ”hot spot” was identified as the location on the scalp able to generate a visual twitch of the FDI 3/5 times. The investigator then adjusted the intensity of stimulation until the resting motor threshold (RMT) was identified at the location of the “hot spot.” The RMT was identified as the lowest intensity of stimulation able to generate a visual twitch of the FDI 5/10 times. The RMT was then verified via the TMS Motor Assessment Threshold Tool, a computer driven algorithm created by Friedemann Awiszus and Jeffrey Borckardt (medical University of South Carolina). Single pulse TMS were then presented X30 repetitions with a 5 second inter-space interval at a stimulus intensity of 120% of the RMT while MEPs were recorded by the Magstar Computer Program (Germany). The 30 evoked MEPS were then averaged, and the baseline motor evoked potential (bMEP) was calculated.

Measuring Cortical Excitability:
Immediately following the 4th and final day of motor training, the average post-training motor evoked potential (pMEP) was calculated by averaging 30 MEPs of the FDI resulting from single pulse stimulation of the “hot spot” at an intensity of 120% RMT. The pMEP was directly compared to the bMEP in order to determine cortical excitation / inhibition resulting from stimulation and motor training.

Transcranial Magnetic Stimulation Procedure:
The following rTMS protocol was used in this pilot study: 2200 biphasic, posterior-anterior directed pulses at 5 Hz intensity broken down into 11 trains of 200 pulses with a 200 millisecond interpulse interval. Pulses were given at 90% RMT of the first dorsal interosseus muscle of the non-dominant hand. A 10 second inter-train interval was also provided so as to minimize decay between trains. The coil was positioned such that it is centered on the “hot spot” for the FDI as described above. Pulses were given with the handle pointing backwards such that the coil is approximately 45 degrees in the posterolateral direction. The 540 second (9 minutes) protocol was advantageous because it was temporally comparable to the tDCS protocol described below. Moreover, it optimized the number and frequency of stimuli presented to the cortex while staying within previously established safety guidelines for TMS within M1.

Transcranial direct Current Stimulation Procedure:
Cortical anodal stimulation (1 mA) was delivered via a pair of saline-soaked surface sponge electrodes (5 x 7 cm) and connected to a 9-volt battery-driven, constant current stimulator for 9 minutes (Chattanooga Ionto Iontophoresis System, DJO Global, Vista, California, Salt Lake City, Utah). The stimulating anode electrode was centered on the
“hot spot” for the FDI as described above. The “reference” cathodal electrode was placed over the contralateral orbita.

**Results:**
Please note that only 10 participants completed the pilot study. As a result, only general trends may be extracted from the data, as the n-value is not large enough to discuss statistical significance.

**Dominant vs. Nondominant Upper extremity:**
Prior to familiarization training, all participants completed the Edinburgh Handedness Inventory to ensure that they were right hand dominant. In addition, participants performed each of the 4 motor test with each hand. Per the charts below, participant performance on each test was in accordance with findings of the Edinburgh Handedness Inventory. As expected, tracking errors were greater with the left hand than the right hand on the PRTT at baseline. Speed and accuracy were greater for the right hand on the FRTT and PPB. The average JTHF composite score was also less with the left hand compared to the right. Error bars indicate that differences in left and right hand performance are greater than one standard deviation above / below mean scores. See Figure F.2 (A-D).

Determining whether patients are right or left hand dominant is crucial to the outcome of this study. Therefore, we plan to continue to assess participants via the Edinburgh Handedness Inventory and motor assessment testing to ensure right hand dominance prior to beginning the study. In order to maintain consistency, left hand dominant and ambidextrous participants will not be included as part of this investigation.

**Jebsen-Taylor Hand Function Test:**
The graphs represented in Figure F.3 (A-G) represent a comparison of average scores between the TMS and TDCS group for each subcomponent of the JTHF. The data represented in the graphs has been adjusted by removing outliers, defined as data 2X the standard deviation of the JTHF subcomponent scores across all 10 participants. Standard deviations were calculated separately for each time point in which measurements were taken in order to ensure that values were deviations in measured data and not changes in performance. As the graphs demonstrate, there is a general trend toward better performance on moving checkers and light / heavy cans with the left hand immediately following, 24 hours post, and 7 days post-motor training primed with TMS. In contrast, participants primed with tDCS appear to have performed better on writing and feeding tasks at all time points following treatment. No observable trend exists for either group following page turning. Moreover, error bars demonstrate that difference in performance between the groups did not exceed one standard deviation above/below the mean for any subcomponent of the JTHF.

Per the aforementioned hypotheses, we would have expected the participants in the TMS group to perform better than those in the TDCS group. However, this trend held for only half of the JTHF subcomponent tests, considered independently. In order to analyze the results of the JTHF as a whole instead of subcomponent tests, an adjusted, composite
JTHF score was also calculated for each participant at each time point (pretest, post-test, 24 hours post-test, and 7 days post-test) via the following steps:

1. All outlier data was removed. Outliers were defined as data 2X the standard deviation of the JTHF subcomponent scores across all 10 participants. Standard deviations were calculated separately for each time point in which measurements were taken in order to ensure that values were deviations in measured data and not changes in motor performance.

2. For each time point in which measurements were taken, scores were then divided by the lowest score across both groups. Therefore, participants with the best score for a given subcomponent test received a 1.0 (100%). The purpose of creating adjusted scores was to ensure that one subcomponent test was not weighted more heavily than another. For example, if the completions times were simply added together, the handwriting portion of the JTHF would have been most heavily weighted, as that score consistently required the most amount of time to complete.

3. Adjusted scores on each subcomponent test were then averaged across participants in each group and at each time point in which assessments were measured (pretest, post-test, 24 hours post-test, and 7 days post-test). The average, adjusted scores were then added together into one overall JTHF score across each time point, such that the maximum score a group could achieve was 7.0 points. In order for a group to achieve a score of 7.0, every participant in that group would have had to score equally and the time would have to be faster than all the participants in the other group.

Graph 5A represents adjusted, composite JTHF scores for each group pre-treatment, post-treatment, 24-hours post-treatment, and 7-days post-treatment. As described above, a greater adjusted score indicates a faster average completion time of the JTHF test and therefore is representative of better functional performance. As the graph portrays, there is a trend toward slightly better performance of the TMS group at each time point following completion of treatment with the exception of 7-days post-treatment. The difference is most dramatic at 24-hours post-treatment. This finding is in line with our original hypotheses, as we would have expected motor training primed with TMS to result in better functional improvement as TMS is powerful enough to initiate E-LTP in the absence of simultaneous motor training based on the proposed physiological mechanism. In contrast, this is likely not the case for tDCS when used as a primer to motor training. Interestingly, the biggest difference between the TMS and TDSC group occurred at 24 hours, as this is likely an appropriate length of time for LTP to take place and cortical changes to occur. In contrast, the adjusted scores at 7 days post-training are slightly better for the tDCS group than the TMS group. This finding is unexpected and may suggest that the long-term outcome of motor training primed with TMS and tDCS may not significantly differ from one another. Alternatively, the findings may suggest that the stimulation resulting from TMS and TDCS may both be enough for some cortical changes to occur long-term and that neither one is superior. Please note that these are
simply observations based on trends, as the n-value in the pilot study was not enough to
draw significant conclusions.
Notably, however, the y-axis of Figure F.4 (A) has been adjusted so as to magnify the
differences in adjusted composite score between the groups. Figure F.4 (B) compares the
same adjusted composite scores with a minimum y-axis value = 0 and a maximum y-axis
value = 7. These y-axis values were chosen because they represent the minimum and
maximum possible adjusted composite JTHF score, respectively. As F.4 (B) highlights,
there is little to no true difference in adjusted composite score between the TMS and
tDCS group at any time point post motor training. Error bars further reveal that
differences in adjusted composite score do not exceed one standard deviation. The pilot
study therefore suggests that neither the TMS nor TDCS group achieved greater
functional improvements with the nondominant upper extremity.

**Purdue Peg Test (PPB), Computer based Fitt’s reciprocal Tapping Task (FRTT),
and Computer-based pursuit-rotor tracking task (PRTT):**
The following graphs represent a comparison of average scores between the TMS and
TDCS group for the PPB, FRTT, and PRTT. The data represented in the graphs has been
adjusted by removing outliers, defined as data 2X the standard deviation of scores across
all 10 participants. Standard deviations were calculated separately for each test and time
point in which measurements were taken in order to ensure that values were deviations in
measured data and not changes in performance.

Per the graphs in Figure F.5 (A-C), there is a small general trend toward better
performance on the FRTT and PRTT for participants primed with TMS at each time point
post-treatment, with the exception of 7 days post-treatment. Like the JTHF test, these
finding are in accordance with our original hypothesis that the motor training group timed
with TMS would enjoy greater functional improvements with the left hand based on the
proposed physiological mechanism of TMS. However, error bars demonstrate that
difference in performance on the FRTT and PRTT between the groups never exceeded
one standard deviation above/below the mean. There was no difference in performance
between the two groups on the Purdue Peg test, indicating that the test may not be
sensitive enough for the population being tested. Also similar to the JTHF test, the
findings of the FRTT, FRTT, and PPB suggest that there may be little to no long-term
effect between motor training primed with TMS and TDCS.

Taken together, the results of this pilot study suggest that there may be a difference in the
physiological mechanisms by which TMS and tDCS affects the brain. The JTHF, FRTT,
and PRTT tests suggest that TMS may result in slightly greater improvements when used
as a primer because it has the ability to depolarize neurons and initiate E-LTP. In
contrast, tDCS may be less effective used as a primer because of its inability to
depolarize neurons and initiate L-LTP in the absence of simultaneous motor training.
Moreover, the pilot study suggests that the JTHF, FRTT, and PRTT may be appropriate
tests with adequate sensitivity to demonstrate the change in performance between the two
groups. Neither motor training enhanced with TMS nor TDCS seemed to be superior to
the other long-term.
These conclusions must be cautiously considered, however, as the differences measured in this pilot are very small and may therefore not be meaningful, even with a greater sample size. Since the goal of this study is to eventually improve rehabilitation post-stroke, functional change that is meaningful to both the clinician and the patient must be carefully considered. With this in mind, we plan to adjust the methodology of the pilot study to compare motor training that is primed by and presented in conjunction with TMS and tDCS, respectively, with a placebo control group. We hypothesize that TMS and TDCS temporally presented in accordance with their proposed physiological mechanisms will result in significantly greater motor improvement in the nondominant upper extremity of healthy college students than placebo stimulation. Future studies will hopefully compare TMS-tDCS enhanced motor training with tradition physical therapy and test its feasibility in the stroke population.

**Measurement of Cortical Excitability:**
Cortical excitability was measured via 30 single TMS pulses spaced 4.5-5.5 seconds apart over the “hot spot” of the motor cortex corresponding with the FDI region of the homunculus. MEP amplitudes corresponding with the FDI muscle in response to 30 single, TMS pulses presented at 120% of RMT were averaged prior to motor familiarization training and termed the bMEP. MEP amplitudes in response to 30, single TMS pulses of equal intensity following motor training were also averaged as the pMEP. All motor evoked potentials were measured via surface EMG and recorded by iWORX-ix228s 10-channel data acquisition technology. For each participant, the % change in excitability was calculated by taking the (pMEP-bMEP)/pMEP. The % change in cortical excitability was then averaged across all participants in each group. The results are summarized Figure F.6:

Figure F.7 suggests that the tDCS group experienced a 71.34% average increase in cortical excitability following motor training primed with tDCS, while the TMS group experienced only 5.5% average increase in excitability following motor training primed with TMS. This finding is opposite of what we would have expected, based on the proposed mechanisms of TMS / tDCS and the results of the motor assessment testing described above. We hypothesized increased cortical excitability following TMS primed motor training, corresponding with improved performance on motor assessments. However, a closer look at the data reveals huge variability in the measurements taken within and between participants. This is likely due to significant noise experienced during EMG recording. The iWORX company was contacted in an effort to troubleshoot the system. However, it was determined that the software was not compatible with TMS. Following the pilot study, official MagStim compatible EMG software was ordered, installed, and tested. The MagStim compatible system clearly produces cleaner and more reliable EMG signals, as demonstrated by the graph in Figure F.7. This is a graph of 5 MEPs evoked from single pulse stimulation, using the methodology outlined in the pilot study.

**Additional Discussion:**
Per the aforementioned hypotheses, we would have expected participants that received motor training primed with TMS to perform better than those receiving motor training
primed with anodal tDCS. While the TMS group performed slightly better than the tDCS group immediately following and 24 hour-post treatment, neither group was superior. Moreover, there was no difference between the groups at 7-days post-treatment. To improve the study, we plan to make the following changes to the methodology:

- While TMS and tDCS likely have unique effects on the cortex via their proposed physiological mechanisms, both types of stimulation seem to result in cortical enhancement and motor improvement. Clinically, it may be more useful to determine if their combined effects result in significantly better motor improvements than a placebo control. In other words, a more appropriate question within the context of patient treatment may be to determine whether motor training primed with TMS and presented in conjunction with anodal tDCS results in better motor outcomes than task-matched motor training alone. The future direction of this study will focus on answering this question using the nondominant upper extremity of healthy, university students with the hope that future investigations will have similar success in the post-stroke population.

- The PPB is measured by the number of pegs retrieved, transported and placed in a hole by the left hand. Per the standardized instructions, pegs are counted only after all steps have been accomplished, and 0 points are awarded for anything less. While this test may be appropriate for distinguishing the general function of manual laborers, it is likely not sensitive enough to consistently distinguish between performance of healthy college age students without any history of functional impairments. As such, the PPB will not be utilized in the formal portion of this study.

- To improve the study, we plan to drop the handwriting subcomponent of the JTHF. From the pilot study, we saw large variability in handwriting styles and subjective interpretation of “legibility” of handwriting. Although instructions were standardized, many participants chose to sacrifice quality of writing for speed. Discontinuing the handwriting test is in accordance with previously published research using the JTHF test. While page turning resulted in little to no difference between the TMS and tDCS group, we will continue to use the page turning subcomponent, as there is no reason to believe that it is not more or less functionally relevant than the other subcomponent tests.

- Recent studies suggest that the excitatory effects of rTMS and iTBS do not significantly differ from one another. Both result in ~60 minutes of enhanced cortical activity within M1. In the pilot study, rTMS was used instead of iTBS so as to maintain a temporally matched primer with the tDCS group. However, the protocol has been updated so as to compare the ability of TMS and tDCS to augment motor training compared to a placebo control. Therefore, it is no longer necessary that TMS and tDCS be temporally matched. iTBS is advantageous for this study because it allows for decreased intensity and duration of stimulation. The iTBS protocol is therefore likely more comfortable for participants and more feasible when used in the context of a clinical setting. The iTBS protocol has been established by previous investigations and will be modeled in this study.
Specifically, iTBS will consist of three TMS pulses at 50 Hz provided every 200ms (i.e., at 5 Hz) at 80% AMT of the FDI. Ten bursts will be grouped and repeated every 10 seconds for a total of 20 trains of 600 pulses. Total stimulation time will be 191.84 seconds.15

- In accordance with previous studies on iTBS, the AMT will be used in place of the RMT to determine the intensity of stimulation.15 Generally, the AMT is less intense than the RMT, as it is measured within the context of a muscle that is already voluntarily contracting. Therefore, the AMT is safer than the RMT when presented repetitively.82

- In the pilot study, 9 minutes of motor training was chosen so as to facilitate future studies comparing a temporally matched TMS primed motor training with motor training presented in conjunction with tDCS. In the present investigation, TMS and tDCS will both be used to augment motor training, and they no longer need to be temporally matched. As a result, 20 minutes of motor training was chosen in order to model a previous study that demonstrated increased motor improvement following 20 minutes of motor training primed with anodal tDCS compared to placebo stimulation.25 A duration of 20 minutes is also more representative of a typical physical therapy treatment session.

- A protocol with multiple TMS/tDCS enhanced motor training sessions is ideal because it typifies a physical therapy plan of care. Moreover, multiple sessions would likely result in greater motor improvements. To our knowledge, however, a combined TMS / tDCS enhanced motor training approach has never been attempted. As a result, it is necessary to first demonstrate motor improvements following a single treatment session. A single treatment session also simplifies the organizational aspect of the investigation, which may facilitate an increased sample size and power. Notably, one treatment session has been enough to demonstrate a significant difference in motor outcomes following motor training primed with tDCS and motor training primed with sham stimulation.25

- In order to increase the internal validity of the study, both patients and graders will be blinded to patient group. Only the primary investigator will have knowledge of the patients within each group so as to set-up and administer proper cortical stimulation (TMS / tDCS or sham).
Figure F.1 (A): Graphical representation of the pilot study treatment groups. (B): Graphical representation of the experimental design used in the pilot study.
Figure F.2 (A-D): A comparison of performance between the left hand (red bar) and right hand (blue bar) prior to treatment. Graph A-D depicts performance on PRTT, PTT, FRTT and JTHF, respectively.
Figure F.3 (A-G): Graphs of each subcomponent test of the JTHF after removal of outlier data. Red lines represent the average TMS group score, while blue lines represent the average tDCS group score at each time point measured (pretest, post-test, 24 hours post-test, and 7 days post-test).
Figure F.4 (A): JTHF adjusted, composite score after removal of outlier data (y-axis scale from 5.0-5.8). Red lines represent the average TMS group score, while blue lines represent the average tDCS group score at each time point measured (pretest, post-test, 24 hours post-test, and 7 days post-test). (B): JTHF adjusted, composite score after removal of outlier data (y-axis scale from 0.0-7.0).
Figure F.5 (A-C): Graph of scores on the FRTT, PRTT and PPB after removal of outlier data. Red lines represent the average TMS group score, while blue lines represent the average tDCS group score at each time point measured (pretest, post-test, 24 hours post-test, and 7 days post-test).
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<tr>
<td><strong>Average % Change in Cortical Excitability</strong></td>
<td><strong>5.55%</strong></td>
<td><strong>71.34%</strong></td>
</tr>
</tbody>
</table>

**Figure F.6:** Average % change in cortical excitability of the homuncular region of the motor cortex corresponding with the non-dominant FDI.
Figure F.7: 5 example MEPs of the non-dominant FDI resulting from single TMS pulses at 120% of RMT. Example MEPS recorded with Magstim compatible EMG software.