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The Effects of Non-Invasive Brain Stimulation on Excitability of the Primary Motor Cortices and Complex Motor Task Learning

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THE EFFECTS OF NON-INVASIVE BRAIN STIMULATION ON EXCITABILITY OF THE PRIMARY MOTOR CORTICES AND COMPLEX MOTOR TASK LEARNING

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ABSTRACT

Transcranial direct current stimulation (tDCS) and transcranial alternating current stimulation (tACS) are promising non-invasive brain stimulation techniques, particularly for clinical applications involving altered or impaired motor function, such as in stroke and Parkinson's disease. Although tDCS and tACS are different modalities, both forms of stimulation operate on the same principle - weak electrical currents are delivered through electrodes placed on the scalp in specific configurations known as "montages". The most commonly used montage for targeting the primary motor cortex (M1) is the M1-SO configuration. This montage leverages the established observation that cortical regions directly beneath the anode generally increase in excitability, while those beneath the cathode generally decrease in excitability. Consequently, the M1-SO montage places the dominant M1 under anodal stimulation, with the cathode positioned on the contralateral supraorbital area. Many publications using the M1-SO montage report enhancements in motor performance, motor learning, skill retention, fatigue resistance, and increases in M1 excitability. However, despite the abundance of data collected using the M1-SO montage over the last 20 years, several important gaps in knowledge have recently emerged. 1) While many studies claim that twenty minutes of anodal stimulation to the dominant M1 produces an increase in cortical excitability, as quantified by MEPs, the changes that occur in the contralateral, non-stimulated M1 during this concurrent tDCS remain unclear. 2) It has been suggested that tACS does not increase cortical excitability as effectively as tDCS. However, due to its unique ability to induce neuronal entrainment, tACS may be more effective at modulating indirectly targeted brain regions, potentially enhancing clinical outcomes. This raises the question of what changes in cortical excitability, as quantified via MEPs, are observed in the contralateral, non-stimulated M1 when

tACS is applied to the dominant M1 concurrently for twenty minutes. 3) While literature suggests that both tDCS and tACS can accelerate motor learning in both healthy and clinical populations, most studies erroneously infer these assumptions based on single-day stimulation protocols and often measure simple, single-joint motor skills as the basis of motor learning. Few publications explore the effects of brain stimulation, especially tACS, over multiple days of stimulation using a complex multi-joint motor skill. Therefore, it is critical to investigate whether multiple days of M1 anodal tACS can enhance motor learning of a complex multi-joint task, such as an overhand throw. In order to address the aforementioned gaps in current literature, the subsequent paragraphs will briefly summarize each chapter of this dissertation.

Chapter 1 provides a comprehensive overview of the history, evolution, mechanisms, theories, and specific applications of non-invasive brain stimulation, and introduces relevant motor learning principles to establish a foundation of essential knowledge. The chapter is intentionally written in a clear, non-technical style to ensure that readers, regardless of their initial understanding of brain stimulation and motor learning, can grasp the technical content presented in chapters two through four.

The study found in chapter 2 aimed to investigate the effect of tDCS applied to the dominant M1 on the excitability of the contralateral non-dominant M1. Utilizing a double-blind, randomized, SHAM-controlled, within-subjects, crossover design, eighteen young adults participated in two experimental sessions (tDCS and SHAM) in a counterbalanced order with a one-week washout period between sessions. Transcranial magnetic stimulation (TMS) was employed to measure the excitability of the contralateral M1 during 20 minutes of anodal tDCS at a current intensity of 1 mA. TMS assessments were conducted in five test blocks (Pre, D5, D10, D15, and Post). The Pre and Post test blocks were administered immediately before and

after tDCS application, while the D5, D10, and D15 blocks were performed at 5, 10, and 15 minutes into the stimulation, respectively. The primary outcome was the 1 mV motor evoked potential (MEP) amplitude. Data were analyzed using a 2 condition (tDCS, SHAM) x 5 test (Pre, D5, D10, D15, Post) within-subjects ANOVA. Results showed no statistically significant main effects for condition ($P = 0.213$) or test ($P = 0.502$), nor was there a significant condition x test interaction ($P = 0.860$). These findings suggest that tDCS does not alter contralateral M1 excitability during or immediately following the stimulation under the tested parameters.

Chapter 3 investigates the impact of tACS on the excitability of the non-dominant right M1 when tACS is applied to the dominant left M1. A double-blind, randomized, SHAM-controlled, within-subjects, crossover design was used. Eighteen young adults participated in both tACS and SHAM conditions on separate days in a counterbalanced order with a one-week washout period between sessions. Transcranial magnetic stimulation (TMS) was employed to measure the excitability of the contralateral right M1 while tACS was administered to the left M1. TMS measurements were taken in five blocks (Pre, D5, D10, D15, and Post) relative to a 20-minute session of tACS (70 Hz, 1 mA). The Pre and Post TMS blocks were conducted immediately before and after tACS, respectively, while the D5, D10, and D15 blocks occurred at 5, 10, and 15 minutes during the stimulation. The primary outcome was the 1 mV motor evoked potential (MEP) amplitude. A 2 condition (tACS, SHAM) x 5 test (Pre, D5, D10, D15, Post) within-subjects ANOVA was performed on the MEP data. Results showed no significant main effect for condition ($P = 0.704$) or for the condition x test interaction ($P = 0.349$). Although there was a significant main effect for test ($P = 0.003$), post hoc analysis revealed that none of the pairwise comparisons were statistically significant. These findings suggest that tACS applied to

the left M1 does not significantly alter the excitability of the contralateral right M1 during or immediately following the stimulation, at least under the parameters used in this study.

Finally, in chapter 4 the primary objective of this study was to evaluate the effect of M1-tACS applied over three consecutive days on the motor learning of a complex overhand throwing task in young adults. Additionally, the study aimed to investigate the impact of M1-tACS on M1 excitability. A double-blind, randomized, SHAM-controlled, between-subjects experimental design was employed. Twenty-four healthy young adults were assigned to either tACS or SHAM groups and participated in three identical experimental sessions. Each session involved blocks of overhand throwing trials with the right dominant arm while tACS was applied to the left M1. Performance was measured by endpoint error. To assess changes in M1 excitability, motor evoked potentials (MEPs) were recorded from the right first dorsal interosseous (FDI) muscle using transcranial magnetic stimulation (TMS). Results showed a significant reduction in endpoint error from pre-test to post-test across the three days of practice ($P = 0.046$), but no significant difference between the tACS and SHAM groups ($P = 0.474$). MEP amplitudes increased from pre-test to post-test ($P = 0.003$), yet these increases did not significantly differ between the groups ($P = 0.409$). Overall, the findings suggest that M1-tACS over multiple days does not enhance motor learning of a complex task more than practice alone (SHAM).

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DEDICATION

This work is dedicated with heartfelt gratitude to my beloved wife, Katie Wilkins, and my two beautiful children, Ezra and Eden.

Katie, your unwavering love, support, and encouragement have been a cornerstone of my journey. Your strength and patience have inspired me every step of the way. To my dear children, Ezra and Eden, you bring matchless joy and purpose to my life. I hope that one day, you will read this work and realize that with determination and perseverance, you can achieve anything you set your minds to.

These past few years have been marked by both triumphs and challenges, testing us as a family in ways we could never have anticipated. Through the highs and lows, we have faced each obstacle together, emerging from the refiner's fire more resilient and united than ever.

May this work serve as a testament to the incredible strength and unity of our family, and may it inspire you to pursue your dreams with dedication and full purpose of heart.

“What matters most is what lasts the longest, and our families are for eternity.”

-M Russell Ballard-

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CHAPTER 1

AN OVERVIEW

A HISTORY OF BRAIN STIMULATION

Benjamin Franklin's 1751 publication, "Experiments and Observations on Electricity," did not mark the genesis of humanity's intrigue with electricity, nor was it the first instance of contemplating its potential effects on human physiology [1]. Ancient Greek and Roman medical practitioners utilized electrical discharges from the Torpedo Fish to alleviate a variety of medical ailments, including headaches, gout, and even prolapsed anus [2]. Nevertheless, the success of Franklin's publication, especially in Europe, seemingly triggered a wave of scientific exploration into the use of electricity within the domains of physics, medicine, anatomy, and physiology. Luigi Galvani, a pioneer in physics, chemistry, and medicine, recognized the inseparable link between electricity and the nervous system of complex living organisms. Although he was unable to fully elucidate or defend his theories, Galvani was convinced that electricity was inherently present within all living animals. The burgeoning curiosity surrounding electricity and animal physiology in the late 1700s and early 1800s led to significant findings in neuroscience and engineering innovations, shaping the landscape of modern technology and science, particularly in the fields of neurophysiology and electrochemistry.

A prominent example of this is Alessandro Volta’s invention of the first stable battery capable of delivering a constant current, which was initially designed to refute Galvani’s widely accepted "animal energy" theory [3]. This invention not only advanced the understanding of electrical principles but also paved the way for further developments in both theoretical and applied sciences. Thus, it is evident that, from the era of the Roman Empire and sporadically

throughout history, particularly during the late 1700s and early 1800s, innovative medical practitioners and scientists have recognized and harnessed the potential of electricity to influence and modulate human physiology.

In 1801, Giovanni Aldini, the understudy and nephew of Luigi Galvani, reportedly treated a 27-year-old farmer in Bologna, Italy, diagnosed with "melancholy madness" (Major Depression) by repeatedly applying electrical stimulation to the patient's skull over several weeks. Despite the considerable pain experienced by the patient, this was one of the earliest documented instances of successful electrical stimulation therapy, with recognizable medical documentation available [4-6]. Seventy-four years later, in 1874, Robert Bartholow, an American physician, conducted the first scientifically recognized experiment involving electrical stimulation of the human motor cortex. By passing an electrical current through the cancerous and ulcerated skull of a woman named Mary Rafferty, Bartholow elicited a visible motor response in the contralateral limbs and neck [7]. Although the ethics of inserting electrical probes into the cortex of a living patient remain highly controversial, the findings from Bartholow's study undeniably laid the groundwork for subsequent research on human motor systems.

The discovery and introduction of transcranial electrical stimulation (TES) by Merton and Morton in 1980 marked a significant resurgence in interest for non-invasive brain stimulation, advancing from the crude devices and methods employed by Aldini and Bartholow nearly a century earlier. TES is a broad categorization of all forms of electrical stimulation, regardless of waveform, stimulation intensity, or electrode location, encompassing techniques such as transcranial direct current stimulation (tDCS), transcranial random noise stimulation (tRNS), and transcranial alternating current stimulation (tACS). Merton and Morton's TES device was unique in delivering a single high-voltage pulse, allowing researchers to penetrate the

skull's thickness and sufficiently stimulate cortical tissue to elicit a twitch in the patients' contralateral limbs [8]. This method enabled the replication of Penfield's famous 1954 motor homunculus in 1988 using non-invasive techniques [9]. However, similar to earlier brain stimulation methods, TES was often reported as painful or unpleasant due to the activation of pain receptors in the scalp by the high-voltage currents necessary to penetrate the skull and elicit measurable motor evoked potentials (MEPs). Prior to Merton and Morton's device, researchers faced challenges in quantifying the effects of electrical stimulation in a consistent and physiologically sound manner. Weaker currents, like those used in tDCS and tACS, applied non-invasively to the scalp, lacked adequate diagnostic tools for accurate measurement of their effects. This changed in 1985 when the Barker research group introduced transcranial magnetic stimulation (TMS), a novel method of delivering a strong enough stimulus to penetrate the skull and elicit a muscular twitch comparable to TES, but virtually pain-free [10]. TMS has since had a profound impact on neuroscience, with thousands of peer-reviewed articles and research projects exploring its use as both a diagnostic tool and therapeutic modality.

TMS

Transcranial magnetic stimulation is quite different from previous forms of non-invasive stimulation. Instead of applying an electric current through electrodes which are placed directly to the scalp or fixed upon exposed portions of the cerebral cortex like those use in more invasive brain stimulation, TMS uses an electromagnetic pulse, or more accurately, an inductive electromagnetic field, which is capable of penetrating the thickness of the skull and reaching the cortical cerebral neurons and interneurons. In order to do this, a brief and high intensity electrical current (400V-3kV & 4kA-20kA) is passed through a loop of conductive material, usually wire, which is encased in a plastic protective case; the looped wire, and the protective case, are often

referred to by researchers as, “the magnetic coil” [11]. When the magnetic coil is placed upon the scalp tangentially, a brief high intensity electrical current is passed through the conductive wires, and a powerful electromagnetic field is produced, usually around 1-2.5 Tesla. Each individual electromagnetic pulse, or discharge, typically lasts less than 1 ms. The electromagnetic field produced by the rapidly changing current being run through the magnetic coil, causes an induction of brain tissue via eddy current, which in turn is thought to penetrate the membranes of nearby neurons, or more specifically the axons of neurons, and results in either an action potential or a subthreshold postsynaptic potential. Because of the current flow and the various configurations of a given magnetic coil, it is imperative that researchers understand which coil configuration is best suited for their desired research and/or application. Generally speaking, single loop round coils are relatively powerful, but lack overall focality. Inversely, the figure 8 or “butterfly” coil configuration, which is commonly used in motor systems research and neuromapping, has the ability to deliver a more focal and predictable field, producing the strongest field directly where the two circular coils intersect [12]. Traditionally, the most commonly used TMS systems are thought to penetrate and subsequently directly modulate neurons that lie about 1-2 cm below the scalp, although new forms of “Deep TMS” are currently being tested and developed, which are capable of penetrating subcortical structures [13]. Indeed, there are many advantages of using TMS over TES for research applications; yet the use of either TES or TMS should be carefully considered due to the advantages and disadvantages each present.

Despite rigorous experimentation for nearly 40 years, relatively little is known about how exactly TMS modulates cortical neurons and the influence this electromagnetic field has on specific structures that comprise individual neurons. However, the common consensus amongst

experts in the field is that TMS likely targets the axons of both excitatory and inhibitory neurons. The propensity of individual axons to fire an action potential in response to TMS depends on the myelination, geometry, and spatial relation to the imposed electric field and the physiological state of the neuron. This is more than likely due to the longer time constant and higher threshold of excitability within the membrane of the soma in comparison to the lower threshold and lower time constant of the membrane within myelinated axons [14-17]. It should be noted that much of this base knowledge was discovered via invasive recordings from rodent motor cortex experiments and other animal models. Yet, much has been recently collaborated in recent studies wherein advanced human head models using morphologically realistic cortical neurons [18]. One major issue associated with non-invasive brain stimulation has always been rooted in the lack of overall understanding of what, where, and how the electrical and electromagnetic stimulus affects cortical neuronal populations. However, with advancements in computational modeling, imaging techniques, and other physiological diagnostic tools, researchers are more successful in elucidating the cellular effects of TMS on cortical neurons.

The most common configurations of TMS include: single pulse, paired-pulse, and repetitive (rTMS). In a single pulse configuration, one single brief pulse is delivered, usually separated by a 3-6 second inter-trial interval. The pulse itself lasts roughly 250-750 ms. It is believed that individual pulses, when given in rapid succession, may inadvertently summate and cause similar effects to that of a paired pulse protocol. Thus, traditional parameters when using single pulse TMS have called for a 4-8 second delay between each stimulatory discharge. Single pulses can be used for diagnostic purposes in order to determine the viability and degree of corticospinal conduction disorders. This is done by recording MEP responses and analyzing the peak amplitude and the latencies therein. Single pulse is also extensively used in order to

quantify changes in cortical excitability in both motor and visual areas of the brain. This is done by measuring the changes in MEPs for the motor system, and the phosphene thresholds in visual systems via plotting shifts in response recruitment curves over multiple TMS intensities [19]. Furthermore, TMS has been extensively used for the purposes of neuro-mapping wherein specific regions of interest, or target areas, are pinpointed in various cortical regions in the brain [20]. For instance, in the primary motor cortex the representation area of muscles can be mapped and changes in representation area can be quantified after interventions such as practice of a highly skilled fine motor task involving the hands.

Paired pulse or double pulse TMS is yet another configuration clinicians and researchers use in order to assess interhemispheric modulatory effects and interregional interactions between two cortical areas, typically within the primary motor cortex. This is accomplished by establishing a resting motor threshold value via single pulse TMS, then delivering both a test stimulus (TS) and a conditioned stimulus (CS), delayed by a specific interstimulus interval (ISI). Typically, a test stimulus is established, ~ +/-10% - +/-30% greater or less than 50% of threshold intensity [21]. The condition response is traditionally set either below or above the previously established threshold intensity. Of primary importance is the latency in which the test stimulus and conditioned stimulus is delivered. At least 5 possible effects have been established based on the interstimulus interval, which are intracortical facilitation, or ICF (10–15 ms ISI), short interval intracortical inhibition, or SICI (1–4 ms ISI) when suprathreshold CS and a subthreshold TS are delivered. Furthermore, long interval intracortical facilitation, or LICF (100 ms, ISI) and long interval intracortical inhibition, or LICI (50–200 ms ISI) are found when a suprathreshold CS is delivered [22].

The final configuration is that of repetitive TMS, or rTMS, which is defined as any combination of more than two pulses delivered with a time interval of 2 s or less. This includes but is not limited to a delivery of short bursts or trains of 3–4 pulses at high frequency, set at 10–20 Hz, with an ISI ~ 50ms, carried out for long periods of total stimulation, sometimes lasting for 30 minutes per session [23]. Currently, rTMS has been FDA approved for the treatment of Major Depressive Disorder (MDD) for those who are unresponsive to traditional SSRI drug therapies [24].

TDCS

As was mentioned in previous paragraphs, tDCS has been utilized in a primitive fashion for centuries and has been in a perpetual state of refinement for the last three decades. More recently, intrigue and experimentation has catapulted tDCS into the forefront of both clinical experimentational research. This is largely due to the affordability of, ease of use, and exceptionally low risk and/or minimal side effects. tDCS is categorized as a form of TES, however, unlike the previously discussed Merton and Morton's high voltage device, tDCS delivers a much lower, less painful current, and is not capable of directly inducing neuronal activity at high enough levels to produce an involuntary muscle twitch. Accordingly, traditionally used tDCS currents are not capable of direct depolarization which leads to the firing of an action potential. Although not originally able to be supported scientifically, developments within technology and extensive work using animal models, show that externally applied electric fields, like those used in tDCS protocols, do in fact have the potential to modulate local neural activity at measurable levels [25,26]. Much of the base knowledge regarding ideal parameters and optimal configurations for effective tDCS modulation stems from the pioneering work of Michael Nitsche and those who helped him co-author several significant papers in the early

2000's. These papers cover a wide range of topics including possible cellular mechanisms which may explain why tDCS currents are capable of depolarizing cortical neurons, how long these changes to cortical excitability last post-stimulation, as well as establishing safe current intensities and stimulation durations when applying these currents on human subjects.

The technical concepts and principles that better explain how neurons are modulated by tDCS will be discussed in subsequent paragraphs. However, a summary of the major findings will be given in order to help provide some context and clarity. tDCS electrical currents are applied by using two or more conductive rubber or metal electrodes which are covered in a conductive medium. These electrodes are then connected to a constant-current stimulator which delivers a stable, but weak direct current, usually ranging from 1–3 mA in human research applications; although most experts agree that 4 mA currents are typically considered safe for human use [27]. When this weak electrical current is delivered, via anode and cathode electrodes, and is fixed upon the scalp, an electrical circuit is created. Traditionally, within most of the foundational publications (unihemispheric stimulation or unilateral), only two electrodes are used, one electrode acts as a "target" electrode, and the other acts as a "reference" electrode; however, it is possible to utilize multiple target and reference electrodes in unique situations such as the case in bihemispheric stimulation and also in high-definition tDCS studies. The location and configuration of how and where the electrodes are placed upon the scalp is often referred to by researchers as a "montage". Various montages have been studied over the years and certain patterns have begun to emerge; as such, a thorough understanding of commonly used montages, the total duration of stimulation, and current intensity is crucial in order to ensure accuracy and produce more predictable outcomes. Generally, the cortical regions beneath and near the anodal electrode (target electrode), both during and immediately after stimulation, produce regional

excitation. Inversely, cathodal stimulation, both during and post stimulation, tends to result in regional inhibition [28-29]. However, current intensity and total time under stimulation play a major role in whether or not anodal stimulation or cathodal stimulation produce net excitatory or inhibitory effects [30]. As such, stimulation duration and current intensity should be carefully considered as the carry over effects of tDCS seem to be dependent upon these parameters. It should be noted that despite the consensus surrounding stimulation parameters and optimal montage configurations, the uniqueness and individuality of each subject receiving stimulation can dramatically affect and/or alter predicted outcomes.

In order to more fully understand the technical aspects of how tDCS can modulate cortical tissue; several pharmaceutical compounds have been implemented and/or combined with tDCS interventions in order to better identify possible cellular mechanisms which could be affected by the electrical currents. For example, a pharmacological study combined tDCS of the motor cortex (M1) with the Na + channel-blocking drug carbamazepine and the N-methyl-D-aspartate (NMDA)-receptor antagonist dextromethorphan [31]. In the no drug condition, anodal tDCS increased cortical excitability by 40%, whereas cathodal tDCS decreased cortical excitability by 60%. However, dextromethorphan suppressed the effects of both anodal and cathodal tDCS, which indicates that NMDA receptors must be involved mediating the effects of tDCS on cortical excitability. In contrast, the administration of carbamazepine eliminated only the anodal tDCS effects by blocking ion channels, whereas the blockade of calcium channels with the drug flunarizine decreased excitability changes induced by anodal tDCS [32].

Taken together, it is clear that despite the relatively weak current involved in tDCS, specific components of cortical neurons, namely axons, are particularly sensitive to both anodal and cathodal stimulation. This is likely due to high concentration of voltage gated sodium,

calcium, and potassium channels specifically in the distal portions of axons. It should also be noted that the soma is also believed to be an area that is also affected by weak tDCS currents; however, according to a recent publication in 2021, researchers suspect the soma has a higher threshold for depolarization [33]. That is to say that the soma membrane is susceptible to modulation via electrical stimulation; however, a more intense electrical current is required. Thus, evidence supports the implication of the axon, over the soma, as the preferential modulation cite for weak electrical currents.

tDCS has a wide range of uses, both in clinical and research applications, as a stand alone intervention and also as a paired modality with other therapeutic interventions. To date, there are thousands of peer reviewed publications, ranging from addictive behavior mitigation to chronic pain management, and even alleviating symptoms of Major Depressive Disorder. However, despite the wide range of documented outcomes in both cognitive and sensory areas of the brain, the most voluminous studied system, as it relates to tDCS, pertains to the primary motor cortex. As such, in later sections, a deep analysis of the current literature involving tDCS and the primary motor cortex will be discussed. However, it suffices to say that a large portion of the publications involving tDCS and the M1 target area, show significant promise in treating, and/or enhancing the motor systems in both healthy and diseased populations.

TACS

Like tDCS, transcranial alternating current stimulation (tACS) is a form of non-invasive TES, which has been exalted to the forefront of both clinical and research neuroscience in recent years because of the potential effects on cognitive, motor, and visual systems. Another reason for this sudden intrigue in tACS is due to the practicality that many devices which are used in

research and clinical applications have the ability to deliver both tDCS and tACS. Furthermore, the placement, or montages, used in tACS are comparable to those used in tDCS; current intensity, duration, and regions of interest are all similar to those traditionally used in direct current stimulation. However, the major difference between tDCS and tACS stems from the waveform, or pulse, in which tACS is delivered.

As the name suggests, alternating current is delivered in either sinus pulses or square pulses which is different from the delivery of direct current stimulation which is held or maintained at a constant state. Although both currents, direct and alternating, do have the potential to depolarize and hyperpolarize cortical neurons as recorded via MEPs, alternating current stimulation has been shown to have a lesser effect on cortical excitation. However, despite the attenuated MEPs in comparison to tDCS, tACS has the unique, but powerful, ability to modulate neurons via neuronal entrainment. That is to say, the intrinsic oscillations frequencies which denote neuronal firing rates within cortical tissue are susceptible to extraneous alternating currents when applied to the scalp. Because of this, relatively weak alternating currents have been shown to promote rhythmic, or synchronizing, neuronal firing rates in groups of neurons both directly and indirectly beneath stimulation sites. Some experts have argued in recent years that the weak current of tACS is unlikely to actually penetrate the thickness of the skull, which is needed to directly influence cortical neurons. Instead, they argue, that it is the sensory neurons embedded within the scalp which are influenced by tACS, which in turn, causes the well documented neuromodulatory effects on neuronal oscillations. Thus, the name, “transcranial” alternating current would be technically or semantically inaccurate; “transcutaneous” is a more correct term [34]. Regardless of what method or mechanisms are responsible for the neuromodulatory effects of tACS, most research teams have found, and

subsequently documented, substantial physiological changes in both human and animal neuronal oscillations. In order to detect these changes in cortical oscillations, researchers use EEG recordings in order to measure and quantify the modulatory effects of tACS. Alternating current frequencies have been tested in both high and low frequencies, ranging from 1 to 5,000 Hz [35]. However, despite the diverse range of frequencies, current intensity, stimulation duration, and montages used, few standards have been established.

As was discussed in previous paragraphs, tACS and its unique properties have the potential to effectively alter, modulate, and otherwise influence neural oscillations. Building upon decades of EEG research, clinicians and researchers alike are increasingly interested in finding ways to utilize tACS in order to improve and enhance patient outcomes. To date, a large number of publications have been conducted using tACS for cognitive, memory, and auditory research with various findings and a broad range of outcomes. One particularly promising area for tACS and clinical applications lies with its potential for helping those who suffer from Major Depressive Disorder [36]. Clinicians recognize that the nature of the alternating currents, due to the common sinus waveform in which the current is delivered, is not too dissimilar to the more powerful, but effective frequency, delivered via rTMS; which is an FDA approved and highly successful intervention for patients suffering from depression.

Unlike tDCS, relatively few papers have been published using tACS in order to discover its effects on human motor systems; in subsequent paragraphs, specific papers will be discussed. Nevertheless, because of the well-documented and widely replicable neuromodulatory effects of alternating currents on both human and animal models, continued exploration of tACS will inevitably increase and thrive in the future for aiding both healthy and diseased populations.

MOTOR LEARNING

Highly trained, highly practiced, and expertly executed movements, especially when carried out by elite level practitioners, have enthralled, entertained, and captivated audiences seemingly since the beginning of time. Evidence of this has been well documented in many ancient societies and carries on today as we cheer on our favorite sports team or nod our heads along to the beat of a skilled musician. Collectively, regardless of nationality or ethnicity, all seem to appreciate the dedication and precision needed to learn, and eventually master specialized human movements. Essentially all animals with an advanced nervous system can generate movement and execute controlled, deliberate locomotion. Yet relatively little is actually known, and subsequently agreed upon, with regards to how the brain plans, refines, and executes movement. As such, the following paragraphs will attempt to detangle and define the major concepts and give brief background on the major motor learning theories.

Motor learning, due to diverse theories and the subsequent limitations therein, is often difficult to accurately and definitively define. However, despite the high level of ambiguity, a general definition can be formulated. Motor learning is an adaptation in which participants are subjected to an external perturbation, which causes systematic errors that are then corrected [37]. In other words, motor learning is the process of acquiring and refining more effective motor patterns and/or strategies in order to improve positive outcomes. Regardless of how one defines motor learning, all definitions seemingly include a form of motor skill acquisition via a series of steps or processes including cognitive processing, which leads to eventual motor execution, and finally retaining those learned movements over time.

Some of the original theories, in their most rudimentary forms, asserted the claim that all movements were simply a series of complex and linked reflexes [38]. It wasn't until Nikolai Bernstein, a Russian neurophysiologist from the nineteenth century, challenged the prevailing reflex theories of movement in his field by introducing the idea of multiple systems collaborating to generate movement. According to Bernstein, to execute a voluntary movement that is both smooth and efficient, one must surmount the problem of, "degrees of freedom". He asserted that when multiple systems interact, there are numerous options for movement; or, "degrees of freedom", which can be used to achieve the same action in a litany of diverse ways. Shortly after Bernstein's work, two main theories emerged and have since dominated the field of motor control and learning. The first theory, known as Motor Program Theory (MPT), suggests that neural storage of motor plans occurs at some point, then are retrieved in order to achieve specific motor goals [39]. However, MPT faces three main issues: the storage problem, the novelty problem, and the problem of motor equivalence. The Generalized Motor Program (GMP) theory proposed by Richard Schmidt addresses some of these issues by suggesting the existence of a generalized motor program, which contains rules for a large class of similar actions, thus minimizing storage needs and accounting for novelty and motor equivalence [40]. The second theory, known as Dynamic Pattern or Dynamical Systems Theory (DST), proposes that movement is an emergent property occurring as the neuromuscular system interacts with the environment in an online adaptation specific to the task at hand. As such, movement is constrained by characteristics unique to the individual, environment, and task [41]. Regardless of which theory is considered more accurate and otherwise applicable by researchers, no single theory has been successful in explaining the totality of human movement. Nevertheless, despite the lack of consensus amongst theories, one of the few commonalities most researchers agree

upon is that the ability to acquire new motor skills, or effectively adapt old ones, lies at the very center of motor learning. Arguably the best way to both quantity and qualify motor learning is by analyzing actual motor performance in at least three specific ways: 1) measuring the rate in which one acquires a new skill or modifies an existing skill, 2) measuring the retention of a skill over a set period of time, and 3) measuring the amount of transferability into other similar tasks [42].

MOTOR SKILL

Motor skill, as defined by *The Oxford Dictionary of Sports Science & Medicine*, is "the product of four different elements: force, velocity, accuracy, and purposefulness…". In other words, a motor skill is essentially the ability to deliberately and consciously maneuver and/or manipulate the body via a series of muscular movements in order to achieve a desirable outcome. The attainment of motor skills involves a process of motor learning [43]. Furthermore, motor skills are traditionally categorized into two main categories: gross motor skills and fine motor skills. Gross motor skills being the most rudimentary of motor skills, such as standing, or sitting upright. Inversely, fine motor skills are those which require a great deal of coordination and/or precision to achieve, like threading a needle or complex tool/object manipulation. In most circumstances, it is advantageous for researchers who specialize in motor behavior, control, or learning to design a study which highlights the acquisition of a specific motor skill via "task" in order to collect meaningful data. Motor tasks, much like motor skills, are often categorized into one of two groups. The most basic tasks are called "simple", and the most difficult are referred to as "complex". An example of a simple task would be something like the tapping of a finger or foot, like those used in some reaction time tests, or the clicking of a computer mouse; essentially any movement that requires the use of a single limb or single joint moving in a single plane of

motion can be categorized as a “simple task” [44,45]. On the opposite side of the spectrum, a complex task is one that requires multiple joints, both large and small muscle groups working synergistically, moving through multiple planes of motion; a baseball swing, a basketball lay-up, sight reading and playing a piece on the piano, or a forehand tennis swing would all be examples of complex motor tasks [46-48]. Because simple tasks are relatively easy to design and are often conducted in a traditional lab setting without major modifications, a disproportionate number of publications using simple tasks have been utilized to extrapolate findings into not only complex motor tasks, but to explain motor learning as a whole. Some argue that this common practice, while well intentioned, is simply an erroneous projection and grossly reduces the sophisticated integration of cognitive, sensory, and motor systems working together in order to complete complex, multi-joint tasks. As such, many recent publications have attempted to highlight the need for more complex task designs in order to elucidate real world applications in which predictions of force and velocity are required in order to have successful motor outcomes [49]. Thus, it is clear to see that selecting the appropriate tasks, whether it is fine, gross, simple, or a complex motor task, is absolutely essential if one wishes to obtain an accurate measurement, and subsequently extrapolate motor skill acquisition, into meaningful benchmarks of motor learning.

MOTOR SKILL ACQUISITION

The acquisition of a new motor skill, as discussed in the previous paragraphs, is an essential portion of what constitutes motor learning. As such, careful consideration must be given as to which measurement best fits the motor skill participants are attempting to acquire [50]. Where overall accuracy is demanded in a given task, such as those commonly used in force matching or any number of throwing tasks, measurements that highlight or emphasize peak velocity or acceleration may not be appropriate. Inversely, in tasks where time is of particular

importance, such as those in certain reaction time tasks, measurements of accuracy may not be as relevant. Thus, it is expedient to discern the appropriate form of measurement, in a specific hierarchy of importance, prior to data collection. In order to correctly formulate a hierarchy of importance it is necessary to understand the ways in which accuracy and variability are measured and defined with relation to errors in performance. Movement accuracy is the degree of correctness of a quantity relative to some standard value or goal; measurement of "error" is the amount of inaccuracy. As such, specifically in tasks where spatial accuracy is particularly relevant, endpoint measurements such as constant error, absolute constant error, and endpoint error are of utmost importance; Endpoint error, which is calculated using constant error, being arguably the best indicator of measured learning in spatial accuracy tasks.

MOTOR SKILL RETENTION

Retention testing, another key component of motor learning, is used to measure the effects of motor learning over the course of time. That is to say that one of the key principles governing motor learning is the ability to perform a learned motor skill after practice or active testing has ceased [51]. Retention testing is often used to highlight the effects of both memory consolidation and decay. Newly acquired motor skills become stabilized through consolidation [52]. In other words, one acquires a novel motor skill via trial and error (practice), those learned movements eventually become stabilized and stored in various, but specific memory areas of the brain, available for retrieval and later use. Retention testing is a way of measuring the effectiveness of this consolidation and retrieval process against the phenomena of decay. Decay is common and even expected in motor learning; it is believed to be caused by the instability of memory consolidation and retrieval which leads to an increase in erroneous, inefficient motor performances after practice has ceased [53]. Through the process of memory consolidation,

retrieval, and then re-consolidation, motor patterns and/or motor skills are executed and are then refined into more effective and efficient performances [54]. Thus, retention testing has become standard practice by those wishing to accurately measure the change in motor learning over time in a meaningful manner.

MOTOR SKILL TRANSFER

Transferability is the last major element of motor learning which is used to measure the adjustability and actual application of motor skill acquisition. Transfer learning is the influence of previous experiences on performing the same skill in a new context or on learning a novel skill. For example, in one classic study published in 1903, a juggling task, wherein the dominant hand was trained, led to increased performance in non-dominate, untrained hand also [55]. Many studies since have shown motor transfer not only between dominant and non-dominant hands, but between arms and feet [56], Limbs and oral movements [57], and nearly every possible configuration, in both ipsilateral and contralateral limbs. However, despite the ability to transfer and adapt previously learned motor skills, it should be noted that “specificity”, as defined by the specificity of practice hypothesis, is more effective than motor transfer alone [58]. Many studies throughout the last century have shown that specific and specialized practice is more effective than relying on untrained, unpracticed motor skill transfer, in order to increase motor learning and performance. Nevertheless, although the exact mechanisms of motor transfer is not fully understood, transferability of motor tasks remains an important component of motor learning measurement.

Simply observing an expert's performance or receiving specific instructions is rarely enough to acquire a novel motor skill. Deliberate and specific practice, repetition, and consistent skill refinement is seemingly imperative to master a given motor skill. As such, practice plays an essential role in successful skill acquisition and overall motor learning. There are multiple measurable effects of practice on motor learning; some of which stems from the ability to quickly and accurately select the appropriate action [59]. The ability to correctly choose the appropriate action is only part of what one learns when applying practice. Of equal importance is the ability to execute those selected actions effectively and efficiently [60-62]. As the ability to accurately select the appropriate motor tasks increases with practice, a positive downstream effect on speed can also be observed. That is to say, practice has the ability to promote incremental improvements on motor performance and can often be quantified by measuring both accuracy and the speed in which motor skills are executed. Furthermore, as practice is increased and both accuracy and speed improves, less cognitive effort is required [63,64]. This well documented phenomena wherein motor skill selection and motor skill execution is correct and quickly applied with less cognitive effort is referred to as, “automaticity” [65]. Thus, it is appropriate to conclude that practice has three specific effects which directly improves overall motor performance: 1) the ability to quickly and accurately select and execute a motor tasks, 2) the ability to lessen the overall cognitive effort required to produce a successful movement; and 3) the ability to select and execute deliberate motor tasks in an seemingly automatic manner thereby displaying masterful, or expert level, motor performance.

One fundamental question that must be considered revolves around the possible cellular mechanisms involved in motor skill acquisition, with special emphasis given to how and why

practice enhances overall motor learning. This concept of changing and/or modifying neuronal behavior is known as synaptic plasticity, which is to say that neurons have the ability to change the way in which they both receive and transmit surrounding neural impulses. The dominant theory surrounding plasticity states that initial synapses are generated relatively imprecisely, and that appropriate synapses are then strengthened by long term potentiation (LTP). Inversely, inappropriate synapses are weakened by long term depression (LTD), and are ultimately eliminated over time and lack of stimulus. In support of this theory, it has been documented that initial glutamatergic synapses innervating a single postsynaptic neuron are often many and weak, and become fewer and stronger with maturation. This developmental refinement of connectivity occurs not only at glutamate synapses, but also at some inhibitory synapses, and at the neuromuscular junction. This synapse strengthening is hypothesized to reflect LTP at a subset of initially weak synapses, while the reduction in synapse number is thought to represent activity dependent, anatomical withdrawal of selected synapses, perhaps following LTD [66]. Thus, evidence suggests that LTP and LTD play an important role in synaptic plasticity thereby directly influencing the ability to learn and acquire motor skills [67]. As such, increased practice and further refinement of specific motor tasks has been shown to fortify applicable neural circuits by either LTP or LTD which traditionally leads to substantial improvements in motor performance.

TDCS ON MOTOR LEARNING

As discussed, it is believed that neuroplasticity is possible due to the neuronal changes brought about by the influences of both LTP and LDP over time. Similarly, tDCS has been shown to effectively manipulate or alter the membrane excitability of neuronal tissue [28]. Thus, it is appropriate to assume that implementation of tDCS, which causes temporary changes in

axonal compartments of neurons, coupled with specified and deliberate practice, could result in increased rates of motor learning.

Currently, there are hundreds of publications in which tDCS was used to enhance motor learning and improve motor performance. Many of these papers show that anodal tDCS over the M1, cerebellum, and dorsolateral prefrontal cortex (DLPFC) regions of the brain at an intensity of 1mA-2mA, lasting ~20 minutes, coupled with online practice/learning over 1-3 days, lead to improvements in overall motor performance. In support of this claim, it is advantageous to review a few of experiments wherein simple tasks or reaction tasks have been measured both with and without tDCS. Using a rapid thumb abduction task, Galea and Celnik were able to show significant improvements over sham conditions using tDCS [68]. In 2009, using a force field-induced motor learning model, researchers were able to show an increase in motor learning, quantified by decreased trajectory error, using 17 minutes of anodal stimulation over the primary motor cortex [69]. Using both a reaction time task and also an explicit sequence learning task, Stagg et al. was able to successfully implement anodal tDCS over the M1 area to increase speed and reaction time [70]. Finally, in one of the most through and compelling tDCS studies completed to date, using a novel simple motor skill over multiple days of practice and both online and offline protocols, Reis et al. were able to show that anodal M1 stimulation improved skill acquisition and 3 month retention [71]. Despite the large number of publications supporting the claim that tDCS enhances motor learning, the majority of the skills or tasks being performed are simple, single joint or single limb movements. Furthermore, an overwhelming majority are completed in a chair or in a seated position, which is rarely the case in most daily activities where motor skills are actually applied. As such, it is safe to state that evidence is currently lacking, or at the very least ambiguous, when attempting to assert the claim that tDCS can

enhance performance in complex multi-joint, dynamic movements. Nevertheless, a handful of studies have reported positive findings using tDCS to increase complex motor tasks. Parma et al. in 2021 was able to enhance performance of a “mini-golf” or putting task using a bi-hemispheric M1 montage [71]. Interestingly, the researchers reported a ceiling effect with tDCS and performance improvements. That is to say that the learner's initial score, if initially performed at a high level, did not noticeably improve from the modulatory effects of tDCS as much as those who performed poorly on the initial baseline test. In a case series investigating tDCS on DLPFC in elite competitive shooting athletes, 3 consecutive days of stimulation and practice did not enhance performance. The researchers speculated that this is more than likely due to the high level of skill the athletes have acquired over years of training. Thus, the effectiveness of tDCS may not be recognized unless further stimulation and practice sessions were implemented [72]. Although complex motor skill tasks are rare, and despite the aforementioned ceiling effect with elite level athletes, one position paper by Buch et al. states that of the reported tDCS studies wherein a single session of stimulation and motor learning were investigated, ~ 72% showed enhancements in motor performance. Furthermore, of those 72%, 82% included some form or variation of M1 stimulation [73]. As such, the M1 montage will inevitably continue to be investigated as many publications have successfully enhanced motor learning by modulating the M1 region in both online and offline paradigms.

TACS ON MOTOR LEARNING

TACS, although similar to tDCS (similar current intensity, density, montages, etc.), seemingly lacks a common consensus and/or standardized “best practices”, agreed upon by most experts within the field. TACS, despite strong evidence which supports its efficacy and potential for neuromodulation via neuronal entrainment, clear and definitive protocols have yet to emerge.

This is primarily due to the diverse range of frequencies in which tACS can be delivered. For example, when using tDCS, most researchers will decide upon and subsequently implement a 1mA or 2mA current configured in one of the traditionally used montages (M1-SO, F7-F8, etc). However, when using alternating current, one must not only decide upon the current intensity and montage configuration, but also the appropriate frequency. Currently, there are many publications, often producing contradictory results, which sufficiently exacerbates the work of establishing a so-called "gold-standard" or "best practices" of tACS. In order to untangle the robust amount of data as it pertains to tACS and motor systems of the brain it is advantageous to group the objective findings into 1) the effects of tACS on cortex excitability, which is done via TMS and MEPs, and 2) the behavioral effects and measurable objective measurements on motor performance. As such, subsequent paragraphs will highlight the major concepts and findings accordingly.

Antal et al published one of the first papers with the goal of exploring the physiological and behavioral effects of tACS in humans using EEG, TMS, and a simple reaction task at a wide variety of frequencies (1, 10, 15, 30 and 45Hz). Unfortunately, the research team chose to use a relatively weak current intensity; thereby producing and recording minimal changes on cortical excitation [74]. Two studies which used longer stimulation times and greater current densities were able to show not only increases in cortical excitability, but also that cortical excitation, which was maintained post stimulation [75]. Furthermore, the same Groppa et al. publication was able to establish that like tDCS, tACS was also polarity specific. That is to say that anodal and cathodal stimulation produces predictable changes in excitation or inhibition like that of direct current stimulation. Feurra et al revealed that short durations of stimulation, lasting only 90 seconds, had excitatory effects exclusively at 20 Hz [76]. Similarly, a paper done by Schutter

and Hortensius showed no increase in MEPs at 10 Hz for 10 minutes but reported substantial excitatory effects when 5 Hz was followed by 20 Hz; both lasting 5 minutes in duration [77]. Interestingly, it appears that high gamma frequencies, 30 Hz - 80+ Hz, are more likely to produce excitatory changes in MEPs. Thus, inferring that high gamma frequencies can in fact increase cortical excitation. In support of this claim, Moliadze et al. published a paper in 2010 which investigated the "ripple range", which is any frequency outside the traditional EEG frequency bands (80 + Hz). In this paper, Moliadze investigated the 80, 140, and 250 Hz frequencies, and concluded that 140Hz produced the largest increase in cortical excitability [78]. Finally, Chaieb et al. experimented with frequency in the 1000, 2000, and 5000 Hz range and subsequently reported significant changes in MEPs; thus concluding that hyper-gamma frequencies are effective at producing positive changes in cortical excitation [35].

Despite the seemingly lackluster ability to increase MEPs in sub-gamma frequencies, tACS shows atypical promise in terms of enhancing motor learning and motor performance. In 2018, Yamaguchi et al., using a visuomotor isometric force task, were able to effectively reduce errors in overall performance within healthy individuals [79]. The current intensity was 1mA, the regions receiving anodal stimulation were both M1 and cerebellar cortex; the most successful frequency was 70 Hz. Sugata et al., while investigating the effects of various frequencies of tACS on the motor cortex, discovered that 70 Hz produced optimal performance in a visually cued button pressing task [80]. Interesting, in an experiment designed in a go/no-go paradigm wherein force generation and maximal force output were of primary concern; those who received stimulation at 70 Hz performed better than those who received 20 Hz. However, it should be noted that those who received 20 Hz did improve the rate of force generation, which led to greater mean maximal voluntary output [81]. Thus, the data appears to suggest that 70 Hz

frequency, when attempting to increase end performance in a motor task, is ideal. Nevertheless, it should be noted that lower frequencies, specifically 10 and 20 Hz, have been shown to be effective in improving initial motor learning under off-line conditions when applied to the M1 region [82]. Furthermore, Pollok et al., also suggest that an alternating current with a frequency of 10 and 20 Hz was effective at facilitating both motor function and motor learning [83]. Clearly, after investigating the available data, tACS has been shown to be an effective tool in improving motor learning. Unfortunately, due to the lack of consensus and conflicting data points surrounding "optimal" frequencies, isolating and predicting specific outcomes can become challenging for researchers and clinicians alike. As such, future research should focus heavily upon elucidating movement specific, goal specific, and frequency specific parameters for tACS.

As previously mentioned, tDCS has been shown to generally increase cortical excitability as quantified by TMS via MEPs when receiving anodal stimulation; inversely, cortical inhibition is expected when receiving cathodal stimulation in cortical regions directly beneath the electrode. Similarly, albeit to a lesser extent, many tACS studies have shown that high gamma frequencies are also associated with polar specific cortical excitation and inhibition. Furthermore, many experts have reported and subscribed to the idea that there is an inseparable correlation between cortical excitation and positive effects/changes on motor learning. That is to say that the majority of the data points indicate that anodal stimulation to the M1 area of the brain generally promotes positive outcomes in both uni and bimanual motor skill acquisition, retention, and transferability. FMRI data, which is used to indirectly measure changes in cortical excitation via blood oxygenation, supports this notion as well [84]. However, little is known about the changes in the contralateral hemisphere when receiving either tDCS or tACS when quantified by MEP. Because of the data collected from fMRI, many assume that stimulation

indirectly stimulates and activates the contralateral motor areas via transcallosal connectivity. But no known publications at the time of writing have explored contralateral MEPs in real-time during stimulation. In other words, little is known about the changes in cortical excitability while receiving stimulation on the contralateral hemisphere using TMS to record MEPs. Furthermore, given the evidence supporting the claim that alternation currents are capable of global neuronal entrainment, measuring contralateral changes in MEPs should be particularly illuminating for those wishing to quantify and qualify bimanual motor learning. Thus, a logical and pertinent course of action is to explore contralateral cortical excitation and inhibition during tACS stimulation at various time points at a set frequency.

It has been well-documented that when training the dominant hand in a novel motor task, cortical excitation of the corresponding hemisphere is elevated and quantified by MEP recordings, particularly in the early stages of motor skill acquisition. It is also a well-established phenomenon that under unilateral practice or single limb practice, bimanual or contralateral limb motor performance also improves despite the lack of targeted practice. That is to say, when the dominant hand acquires and subsequently learns a novel task through practice, the nondominant hand seemingly receives some residual effects which enhances motor performance. Many publications have attempted to elucidate the reasons behind this well-documented occurrence. FMRI data shows that there is a cooperative exchange of activation across hemispheres which work synergistically to enhance both dominant and nondominant hands [85]. Progressively increasing the complexity of the task through incremental changes in practice sessions, making them more difficult over time, has been shown to further enhance corticospinal excitability and effectiveness of residual gains in nondominant, untrained hand performance [86]. As such, given the effects of tDCS and tACS in enhancing corticospinal excitability, logic would dictate that

applying anodal stimulation on the M1 area of the trained, dominate hand, will result in measurable improvements in untrained, non-dominant hand cortical excitability and motor performance. Unfortunately, despite the wealth of publications addressing motor learning and hand dominance cross-over, little is known about the effects of tDCS directly on untrained, non-dominant hand excitability and performance after stimulation and indirect practice; especially using tACS. Thus, a study designed to investigate the direct and indirect effects of stimulation and practice, quantified by MEPs, on motor learning would help illuminate and progress scientific understanding

Finally, given the complexity and multiple regions of the brain which are involved in any complex motor task, studying the effects of TES within real world applications has been a major stumbling block with the field of neuroscience. As such, many researchers erroneously infer simple motor task data into larger and more complex whole body motor tasks. To date, only a handful of studies have attempted to investigate the effects of tDCS or tACS on actual full body, practical movements. As such, in order to provide applicable data to researchers and clinicians alike, more experiments should be completed attempting to use TES, specifically tACS, in complex motor tasks.

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CHAPTER 2

NON-DOMINANT HEMISPHERE EXCITABLILITY IS UNAFFECTED DURING AND AFTER TRANSCRANIAL DIRECT CURRENT STIMULATION OF THE DOMINANT HEMISPHERE

ABSTRACT

Transcranial direct current stimulation (tDCS) increases primary motor cortex (M1) excitability and improves motor performance when applied unilaterally to the dominant hemisphere. However, the influence of tDCS on contralateral M1 excitability both during and after tDCS application has not been quantified. The purpose was to determine the influence of tDCS applied to the dominant M1 on the excitability of the contralateral non-dominant M1. The study employed a double-blind, randomized, SHAM-controlled, within-subjects, crossover experimental design. Eighteen young adults performed two experimental sessions (tDCS, SHAM) in counterbalanced order separated by a one-week washout. Transcranial magnetic stimulation (TMS) was used to quantify excitability of the contralateral M1 to which anodal tDCS was applied for 20 minutes with a current strength of 1 mA. TMS was delivered in 5 test blocks (Pre, D5, D10, D15, and Post). The Pre and Post TMS test blocks were performed immediately before and after tDCS application, whereas the TMS test blocks performed during tDCS were completed at the 5, 10, and 15-minutes of stimulation time points. The primary outcome measure was the 1 mV motor evoked potential (MEP) amplitude. MEPs were analyzed with a 2 *condition* (tDCS, SHAM) x 5 *test* (Pre, D5, D10, D15, Post) within-subjects ANOVA. The main effect for *condition* ($P = 0.213$), main effect for *test* ($P = 0.502$), and *condition* x *test* interaction ($P = 0.860$) were all not statistically significant. These results indicate that tDCS does

not modulate contralateral M1 excitability during or immediately after application, at least under the current set of common tDCS parameters of stimulation.

INTRODUCTION

Transcranial direct current stimulation (tDCS) is a non-invasive brain stimulation method that involves the application of a low-level electrical field to a specific brain area to modulate cortical excitability and influence motor performance [1-9]. The effects of tDCS are largely determined by the orientation of the electrical field generated by the positions and polarity of the electrode montage [2, 3]. The most widely studied electrode montage involves an electrode pair consisting of an anode and a cathode, which results in current flowing from the cathode to the anode and usually an excitability increase of the targeted brain area underlying the anode (termed anodal tDCS) [1, 6-8]. For example, placement of the anode over the primary motor cortex (M1) and the cathode over the contralateral supraorbital (SO) region (M1-SO montage) usually leads to increased M1 excitability after stimulation ends as measured by increases in motor evoked potential (MEP) amplitude elicited by transcranial magnetic stimulation (TMS) [7, 8, 10, 11]. In addition, other bihemispheric and dual source electrode montages involving stimulation of the left M1 have also demonstrated similar effects on M1 excitability [12-15].

The vast majority of motor system tDCS studies have utilized the SO-M1 electrode montage and anodal tDCS to target the dominant left M1 that primarily controls the contralateral dominant right hand and arm system. Furthermore, a minority of studies have involved the opposite arrangement of anodal tDCS applied to the right M1 to impact motor performance of the contralateral non-dominant left hand and arm system [1, 6, 7, 9, 16]. In both cases, the balance of literature has reported significant M1 excitability increases of about 20-40% and

motor skill improvements of about 10-15% after a single tDCS application of 10-20 minutes with current strengths of 1-2 mA [1, 6, 7, 9, 16-20]. However, it appears that approximately 20-30% of studies have reported no significant effects on these same outcome measures [1, 6, 21]. In addition, the motor skill enhancements observed in these studies have been attained both when tDCS is applied before and during motor task practice. Although it is generally thought that tDCS applied simultaneous with motor task practice is more effective, few studies have directly and systematically compared stimulation timing in the same study using the same participants. Nonetheless, most of the tDCS studies that have observed the greatest increases in motor learning [20, 22, 23] have targeted the dominant left M1 using the SO-M1 electrode montage and the aforementioned stimulation parameters.

Despite extensive research on left dominant M1 excitability and the motor performance of the contralateral right dominant hand controlled primarily by the SO-M1 montage, the effects of this paradigm on the unstimulated right non-dominant M1 and the corresponding left non-dominant hand and arm system remain relatively unknown. This is especially the case in the time period during application of tDCS to the left M1, where it appears that no studies have undertaken concurrent TMS measurements from the right M1. On the other hand, a small number of studies have conducted TMS or brain imaging assessments of right M1 net excitability, activity, or transcallosal effects after delivery of tDCS to left M1. For example, in an early small-scale study, Lang et al. (2004) [24] reported that left M1 tDCS had no effects on right M1 excitability as indicated by motor evoked potential (MEP) amplitudes collected from the left hand immediately and 40 minutes after stimulation. More recently, an extensive study used magnetic resonance spectroscopy (MRS) to measure gamma-aminobutyric acid (GABA) levels in both M1s following tDCS of the left M1. The results revealed a decrease in GABA in

both the stimulated left M1 and the unstimulated right M1, which implied that tDCS of left M1 led to less inhibition and greater excitation in both the left and right M1s [14]. However, TMS measurements were not taken in the study to confirm an overall increase in net excitation of right M1. In another study, anodal tDCS did not influence net contralateral M1 excitability, but did increase the amount of interhemispheric inhibition (IHI) as assessed by paired-pulse TMS exerted onto the contralateral M1, which is mediated by transcallosal pathways [25]. However, these results were obtained by right M1 anodal tDCS application and the effects were measured in the left M1, which may not give the same results expected by the opposite arrangement of left M1 tDCS effects on right M1. In addition, separate previously identified transcallosal pathways that mediate interhemispheric facilitation (IHF) were also not measured in the study. In regard to motor performance, only a few studies have investigated the influence of left M1 tDCS using the SO-M1 montage on the ipsilateral left hand primarily controlled by the right M1. These studies have yielded mixed results as two found no statistically significant effect on left hand motor performance [26, 27], whereas the other found a trend for a decrease [28]. Thus, the effects of left M1 tDCS using the SO-M1 montage on right M1 excitability and motor performance of the corresponding left hand remain unclear even in healthy adults, especially during stimulation.

The lack of direct research on the aforementioned topics is surprising given the promising results of left M1 tDCS for important practical applications such as motor learning, motor rehabilitation, and performance of vital activities of daily living [1]. For example, the viability of tDCS as a long-term intervention with wide-ranging real-world applications would be questionable if improvements elicited in the right hand would be accompanied by left hand performance being simultaneously degraded or unchanged. Furthermore, while the basic view that upper limb muscles are primarily controlled by the contralateral M1 is well-supported, this

does not mean that the ipsilateral M1 has little to no effects on unimanual voluntary movements of the ipsilateral hand that it does not primarily control [29, 30]. In fact, accumulating evidence supports the importance of the ipsilateral (right) M1 for certain aspects of not only voluntary movement production such as movement initiation [31-33], but also motor learning processes in the ipsilateral (right) hand [13, 34-37]. Thus, it is imperative to ultimately understand the effects left M1 tDCS using the M1-SO montage on the unstimulated non-dominant contralateral right M1 in order to elucidate the effects of tDCS on motor skill acquisition of unilateral movements performed by each of the hands. However, a logical first step would be to investigate changes in right M1 excitability in resting conditions without the complicating factors of simultaneous or previous background muscle activation due to muscle contraction in general or specific motor practice.

The purpose was to determine the influence of tDCS applied to the dominant left M1 on the excitability of the unstimulated contralateral non-dominant right M1. In particular, the primary interest was to characterize any changes in right M1 excitability during tDCS of left M1 application using the typical SO-M1 electrode montage, whereas right M1 excitability immediately after tDCS was of secondary interest. This was accomplished by measuring MEP amplitudes in the left first dorsal interosseous (FDI) muscle at rest in response TMS applied to the right M1 in test blocks performed before, during, and after anodal tDCS was delivered to the left M1 for 20 minutes. Based on a prior MRS study [14], it was hypothesized that MEP amplitudes obtained from the right M1 would be greater both during and immediately after left M1 tDCS compared to the SHAM condition. Thus, it was also hypothesized that MEP amplitudes would be increased relative to baseline at timepoints during and immediately after tDCS application, but not in the SHAM condition.

MATERIALS AND METHODS

Eighteen individuals took part in the study (11 males and 7 females; average ± standard deviation age: 24.1 ± 4.0 years). Written informed consent was obtained from all volunteers prior to participation. All participants were strongly right-handed, as confirmed by the Edinburgh Handedness Inventory [38]. Participants were screened to ensure they had no neurological or psychiatric disorders, no uncontrolled medical conditions, and met international non-invasive brain stimulation criteria [39]. The study adhered to the principles of the Declaration of Helsinki and received approval from the Biomedical Institutional Review Board at the University of Nevada, Las Vegas.

The study employed a double-blind, SHAM-controlled randomized, within-subjects, experimental design. The within-subject design was chosen to mitigate the substantial inter-individual differences in the responsiveness to tDCS that are thought to be due to a combination of a range of anatomic, biological, physiological, and genetic factors [40, 41]. In addition, within-subjects designs allow for more statistical power compared with between-subjects designs [42]. The order of presentation of the tDCS and SHAM conditions was assigned to participants using an online application (Research Randomizer; www.randomizer.org) by a member of the research team who did not participate in the collection of data [43, 44]. All participants underwent two experimental sessions that were conducted at the same time of day and spaced 7 days apart [45, 46], which is the most common washout period used in tDCS studies. Both sessions were identical except for the type of stimulation applied (tDCS, SHAM).

Each session lasted approximately 1.25 hours and followed a specific sequence of steps: 1) TMS was applied to the scalp over the left M1 to determine the motor hotspot location of the FDI muscle of the right hand. This was followed by determination of the resting motor threshold (RMT) and the 1 mV MEP stimulation intensity (SI) as a percentage of maximum stimulator output (%MSO) for the right FDI; 2) the tDCS electrode montage was placed over the left M1 FDI motor hotspot location and held in place by a tightly fitting scalp cap. However, the stimulator was not turned on at this time; 3) TMS was applied to the scalp cap over the right M1 to determine the motor hotspot location of the FDI muscle of the left hand. Subsequently, RMT and the 1 mV MEP SI was determined for the left FDI; 4) the Pre TMS test block was performed and MEPs were evoked from the right M1 and collected from the corresponding left FDI; 5) 20 minutes of tDCS or SHAM stimulation was applied to the left M1 while TMS test block were undertaken. Thus, MEPs were evoked from the right M1 and collected from the corresponding left FDI concurrent with the ongoing 20 minutes of stimulation. Data collection for these TMS test blocks started at the 5, 10, 15-minute time points during stimulation (termed D5, D10, and D15); and 6) the Post TMS test block was performed and MEPs were evoked from the right M1 and collected from the corresponding left FDI. Therefore, MEPs attained during the TMS test blocks were always evoked from the right M1 and collected from the corresponding left FDI. Figure 1A illustrates these major experimental steps, with detailed methodological descriptions provided in subsequent sections. Throughout all experimental conditions, the investigators conducting the experiments remained blinded to the stimulation condition applied to participants. Accordingly, the investigator who operated the tDCS device and administered stimulation was not involved in any other experimental procedures [43, 44].

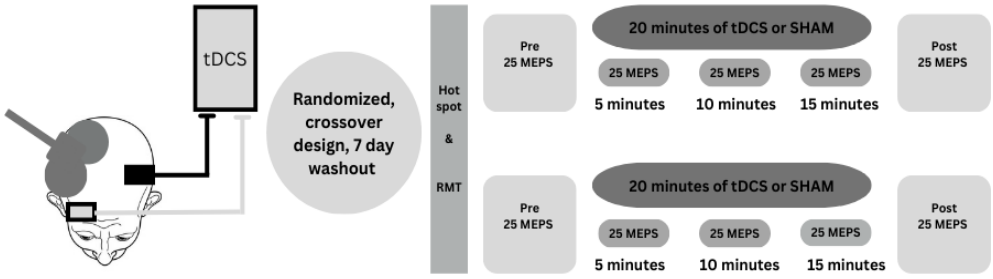

Figure 1 tDCS Study Schematic

Figure 1. Schematic illustration of the study design and major experimental procedures. (**a**) tDCS SO-M1 electrode montage placement over the left M1 and TMS coil placement over the right M1 FDI motor hotspot. (**b**) tDCS or SHAM stimulation was delivered to left M1 for 20 minutes using the standard M1-SO electrode montage while MEPs were evoked from the right M1 and collected from the corresponding left FDI in a total of five TMS test blocks (Pre, D5, D10, D15, Post) performed before (Pre), during (D5, D10, and D15-minute timepoints), and immediately after (Post) stimulation.

TMS was administered utilizing a monophasic Magstim 2002 device coupled to a double 70 mm figure-of-eight remote-controlled coil. The coil was placed in contact with the surface of the scalp and orientated tangentially at an angle of 45 degrees to the sagittal plane with the handle pointed laterally and backwards. This typical arrangement induces a posterior to anterior directed current flow in M1 to elicit MEPs in the contralateral hand. Accordingly, MEPs were evoked in the right and left FDIs using single-pulse TMS of the left and right M1s, respectively. All MEPs were induced the FDI muscle of either the left or right hand was maintained in a state of complete rest.

The electromyographic (EMG) activity of the FDI muscle of each hand was measured using two surface electrodes that were arranged in a belly tendon montage while a ground electrode was placed on the back of the hand. The EMG signals were acquired and recorded employing hardware (1902 amplifiers, micro 1401 data acquisition interface) and software (Signal 5.04) provided by Cambridge Electronic Design (Cambridge, UK). The posture of the participants and experimental arrangement was similar to prior studies when MEPs were elicited from the FDI of the right hand [20, 47]. Analogously, the same set of general experimental conditions were used when MEPs were collected from the left hand in the present study. Briefly, participants were seated upright in a chair with their hand resting palm down on the surface of a small table beside them. The forearm was also resting on the table surface with the wrist in neutral, the elbow flexed to an angle of ~90 degrees, and the shoulder abducted to ~45 degrees. Participants were directed to keep their eyes open and maintain this posture for all TMS measurements as it has been clearly shown that MEP amplitude in hand muscles can vary with changes in shoulder and upper limb position [48, 49]. Importantly, participants were provided with visual feedback of their FDI EMG activity during all TMS testing to make certain that the FDI remained at rest at all times. FDI EMG was displayed online in real-time on a computer monitor located ~0.75 meters in front of the participants [50]. Detailed instructions were given to the participants on how to use the visual feedback to keep the FDI at rest at all times. Finally, one investigator continually monitored the body posture and FDI EMG levels of the participants to ensure that they constantly adhered to these requirements.

To determine the motor hotspot of the FDIs, TMS pulses were given to the presumed area over the scalp corresponding to the hand representation area until the point that evoked the largest MEP in the resting FDI was identified [51]. This point was denoted as the stimulation site

for each M1 and marked with a temporary marker for all subsequent RMT, 1 mV SI, and MEP measurements in the TMS test blocks. The point was marked directly on the scalp for left M1 testing and on tape placed on a scalp cap for right M1 testing (see below). RMT was measured in each FDI at rest using common methodology and was defined as the lowest TMS SI as a %MSO that could produce a MEP with an amplitude greater than 50 microvolts in at least 5 out of 10 consecutive trials. RMT was quantified in the FDI of both hands mainly because some studies [52, 53] have indicated that individuals with lower RMT values may display a larger increase in MEPs following tDCS application. Therefore, if any increases in MEP amplitude were observed due to tDCS, these values could be correlated with the RMT for each participant. In addition, RMT served as a basic baseline and control measure of cortical excitability for each FDI on each of the two experimental days. For the 1 mV MEP SI of the left and right M1s, a great amount of care was taken to identify the TMS SI (%MSO) that would elicit an average MEP of as close to 1 mV as possible for a TMS block of 25 trials. Twenty-five MEP trials per block was chosen as this number has been shown to represent the best trade-off between experimental time efficiency and to obtain valid MEP amplitude averages for blocks of TMS trials [54]. The 1 mV SI determination was done utilizing methodology from two previous studies [20, 47]. Briefly, TMS pulses were initially delivered at ~55 %MSO and this SI was adjusted while MEPs were monitored and quantified online by the Signal software. Once it appeared that MEP amplitudes were as close as possible on average to 1 mV, the Signal software program was reset and the Pre TMS test block was collected. This assured that the Pre TMS test block MEP amplitude was very close to 1 mV in both stimulations performed on the two days. Moreover, this avoided any potential confounds due to presence of substantially different baseline MEP amplitudes before tDCS was applied in the two stimulation conditions. Subsequently, the same 1 mV SI as a

%MSO was used to evoke all MEPs in the following D5, D10, D15, and Post blocks of TMS testing for each participant on a given day. Finally, the inter-trial interval (ITI) between consecutive MEPs was set to 6 seconds for all TMS test blocks.

A NeuroConn DC Stimulator Plus/MR was used to provide anodal tDCS to the dominant left M1. This was accomplished using the standard M1-SO electrode montage and two 5 × 7 cm rubber electrodes that were placed in sponges soaked in saline solution. Accordingly, the anode was placed over the left M1 location corresponding to the previously determined right FDI motor hotspot, whereas the cathode was placed over the contralateral (right) supraorbital region. The anode and cathode were held in these locations by a tightly fitting scalp cap. The typical rubber strap arrangement that normally is placed under the chin and over the anode was not used to hold the anode in place as this would have obviously interfered with the simultaneous TMS measurements. Fortunately, the tight scalp cap was able to hold the anode in position to the same degree as the normal rubber strap arrangement. However, the cathode was further secured using a rubber strap placed around the head in the typical fashion as this did not interfere with the TMS measurements. Overall, this arrangement was successful as although the TMS coil was relatively close to the tDCS electrodes, it did not touch them. tDCS was applied for a duration of 20 minutes at a current strength of 1 mA. The SHAM stimulation condition was administered in accordance with well-established procedures that have been shown to elicit the same sensations to participants as real tDCS, but without eliciting physiological effects [5, 55]. Accordingly, the current was increased over a period of 10 seconds to 1 mA, kept constant at 1 mA for 30 seconds, and decreased down to zero over a period of 10 seconds as in previous studies [20, 56]. Finally, the investigator who operated the tDCS device in the experiments did not participate in

the data collection and the investigators who performed the data collection were blind to the experimental condition as described previously [20, 43]

MEP amplitude was the primary outcome measure, whereas RMT and the 1 mV SI were secondary outcome measures. All MEP and EMG data were collected using a custom Signal 5.04 software (Cambridge Electronic Design, Cambridge, UK) script and further analyzed offline using another custom Signal script. MEPs were quantified and expressed as the peak-to-peak amplitude and the mean of the 25 MEPs [54] in each TMS test block was used for analysis.

RMT and 1 mV SI were analyzed with two separate 2 *condition* (tDCS, SHAM) × 2 *hand* (Left, Right) within-subjects ANOVAs. MEP amplitude was analyzed with a 2 *condition* (tDCS, SHAM) × 5 *test* (Pre, D5, D10, D15, Post) within-subjects ANOVA. Post hoc analyses using Bonferroni adjustment for multiple comparisons were performed if appropriate to identify where significant differences occurred between the TMS test blocks. The significance level was set to $\alpha < 0.05$ for all statistical analyses. The data are presented as means ± standard errors in the figures and means ± standard deviation in the text.

RESULTS

The main effect for *condition* ($P = 0.194$, $\eta_p^2 = 0.097$) and *condition* × *hand* interaction ($P = 0.483$, $\eta_p^2 = 0.019$) were both non-statistically significant for RMT. However, there was a significant main effect for *hand* ($P = 0.049$, $\eta_p^2 = 0.209$), which indicated that RMT was significantly lower in the FDI of the right hand compared to the FDI of the left hand (Figure 2A). For the 1 mV SI, the main effect for *condition* ($P = 0.484$, $\eta_p^2 = 0.029$), main effect for *hand* ($P = 0.235$, $\eta_p^2 = 0.082$), and *condition* × *hand* interaction ($P = 0.667$, $\eta_p^2 = 0.011$) were all non-statistically significant (Figure 2B).

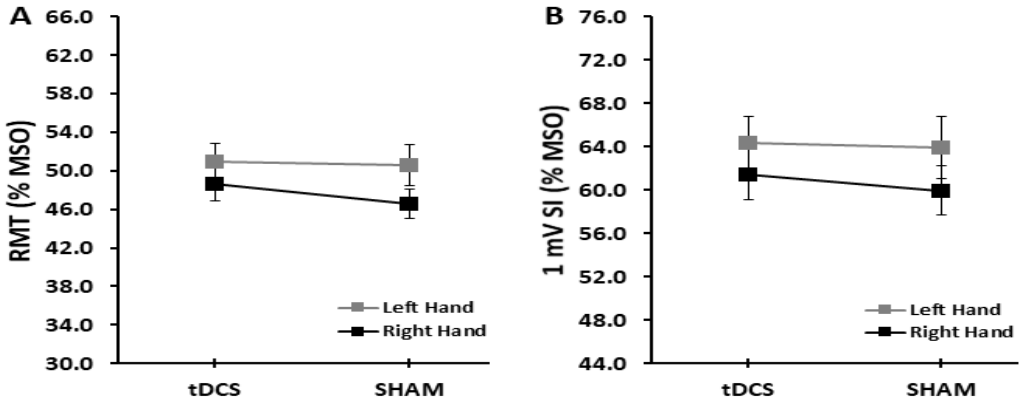

Figure 2 tDCS RMT and 1mV SI

Figure 2. RMT and 1 mV SI. (**A**) RMT for the left and right hands in the tDCS and SHAM conditions; (**B**) 1 mV SI for the left and right hands in the tDCS and SHAM conditions.

For MEP amplitude, the main effect for *condition* ($P = 0.213$, $\eta_p^2 = 0.089$), main effect for *test* ($P = 0.502$, $\eta_p^2 = 0.047$), and *condition* × *test* interaction ($P = 0.860$, $\eta_p^2 = 0.019$) were all non-statistically significant (Figure 3).

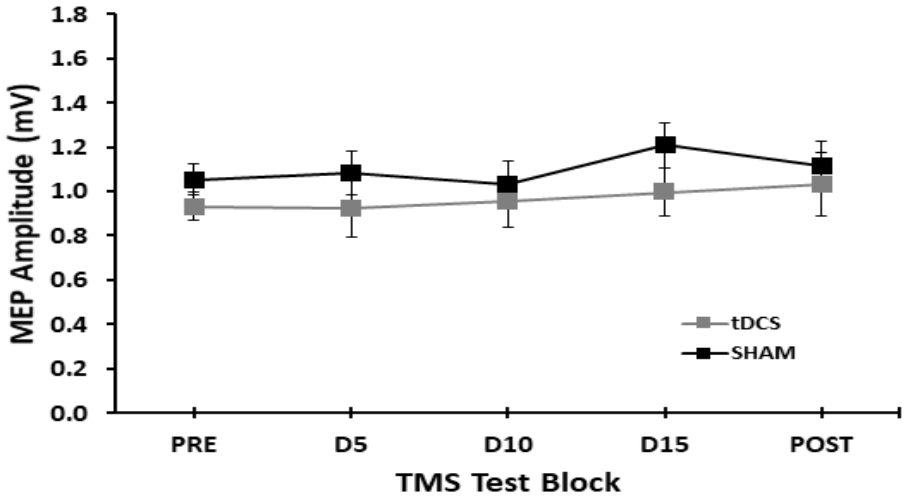

Figure 3 tDCS MEP Amplitude

Figure 3. MEP amplitude for the tDCS and SHAM conditions in the Pre, D5, D10, D15, and Post TMS test blocks. Each data point represents the average of 25 MEPs recorded in each TMS test block.

DISCUSSION

The purpose was to determine the influence of tDCS applied to the dominant M1 on the excitability of the contralateral non-dominant M1. The study yielded two main findings. First, the MEP amplitudes evoked from the right M1 and obtained from the left hand were not statistically different between the tDCS and SHAM conditions at any timepoints during tDCS of the left M1. Second, right M1 MEP amplitude was also similar between the tDCS and SHAM conditions immediately after the 20 minutes of stimulation of the left M1 ended. Taken together, the findings indicate that tDCS does not modulate contralateral M1 excitability during or immediately after application, at least under the current set of common tDCS parameters of stimulation.

Numerous studies have reported that tDCS applied using the SO-M1 electrode montage for 10-20 minutes at 1-2 mA increases left dominant M1 excitability, as assessed by MEPs obtained from the contralateral right dominant hand that it primarily controls [7, 8]. However, a non-trivial minority of studies have found no statistically significant effects of tDCS under those experimental conditions [6]. To our knowledge, the present study was the first to examine the impact of this same set of tDCS parameters on the excitability of the unstimulated, right non-dominant M1 by measuring MEP amplitudes obtained from the corresponding left hand. Most importantly, a novel aspect of the present study was the focus on right M1 excitability during the exact time that tDCS was applied to left M1 as opposed to only undertaking post measurements. Due to obvious technical limitations, TMS measurements cannot be made from the stimulated M1 motor hotspot location during tDCS while the anode electrode is still in place. Accordingly, all tDCS studies that have measured net excitability of the stimulated M1 have performed single-pulse TMS measurements before and after stimulation. In addition, in the one study available to our knowledge that investigated the effects of left M1 tDCS on the contralateral right M1 [24] also performed all TMS measurements before and after stimulation. Furthermore, the assessment of changes in IHI resulting from tDCS applied to M1 also cannot be accomplished during stimulation due to the same aforementioned TMS coil and anode electrode simultaneous location limitations [25]. Overall, the effects of left M1 tDCS on right M1 during tDCS could be functionally important as it is thought that applying tDCS during as opposed to before motor practice is the more effective [1]. Furthermore, several lines of evidence indicate that the ipsilateral M1 plays a significant role in both voluntary movement execution and motor learning of both hands [13, 29, 30, 33, 34, 37].

The primary original hypothesis was that MEP amplitudes obtained from the right M1 would be greater both during and immediately after tDCS was applied to left M1 compared to the SHAM condition. Although scant direct evidence existed and the available studies that were somewhat applicable were mixed [14, 24], this hypothesis was predominantly based on a MRS study that found reduced GABA levels in both the unstimulated right M1 and the stimulated left M1 following tDCS [14]. This would also be generally consistent with prior studies that have reported that reduced GABA levels (increased excitability) are one of the major neurochemical changes that occur in the stimulated M1 following anodal tDCS [3, 57], although other neuromodulators concentrations are also modulated [3]. Contrary to this original hypothesis, the MEP amplitudes evoked from the right M1 and collected from the corresponding left hand were not statistically different between the tDCS and SHAM conditions at any timepoints during or immediately after stimulation of the left M1. In addition, MEP amplitudes collected at all timepoints during and after tDCS application did not differ relative to the Pre TMS test block for either of the two stimulation conditions. Accordingly, MEP amplitudes in the TMS test blocks displayed no statistical changes over time and fluctuated about the average value (block grand average = 1.03 mV) for both stimulation conditions while displaying the well-known variability of MEP measurements (block standard deviation = 0.43 mV; range: 0.26 – 0.59 mV) [54].

These aforementioned results could not have been negatively influenced by differences in control measurements such as baseline RMT and the 1 mV SI values employed for evoking MEPs. This is because these variables were nearly identical and not statistically different between the two stimulation conditions performed on the different days (Figure 2A-B). Although the common observation of a lower RMT for the right compared to the left hand was also found in the current study, the lack of a difference in RMT between stimulation conditions confirms

that this hand difference had no effect on the overall outcomes. Most importantly, the methods used to get the 1 mV SI as a %MSO to elicit an average MEP of ~1 mV for the TMS test blocks was successful. The Pre TMS test block had an average MEP amplitude very close to 1 mV for both the tDCS condition (0.93 ± 0.26 mV) and the SHAM condition (1.05 ± 0.29 mV) with no statistical difference between them. Therefore, there were no potentially confounding effects of a different SI as a %MSO or different MEP amplitudes evoked with this SI in the Pre TMS test blocks. In summary, the results from the control measures indicate that methodological factors or random variations in baseline cortical excitability between the two days could not have significantly influenced the overall MEP amplitude results obtained in the TMS test blocks.

The current findings are seemingly not consistent with the primary study on which the original hypothesis was based [14]. This study was arguably the most relevant as it used MRS to measure GABA and glutamate concentrations in both M1s in response to left M1 tDCS using the SO-M1 montage. The major findings were that GABA levels were decreased compared to baseline in both the stimulated left M1 and the unstimulated right M1 during and following tDCS application, whereas glutamate levels were unchanged. Thus, direct physiological mechanisms were provided that could underlie the possibility that tDCS of left M1 led to less inhibition and therefore greater excitation in both the left and right M1s. However, simultaneous measurements of MEPs were not recorded from either M1 in the study. In contrast, the present study did not include any other physiological measurements to complement the global net assessments of right M1 excitability as indicated of MEP amplitude. Therefore, it could not determine the possible modulation of any intercortical or intracortical pathways or neurochemical concentrations that may have contributed to the lack of MEP changes in right M1. It is possible that GABA concentrations were not changed in the right M1 in the current study, which could possibly

reconcile the results of the two studies. Our findings are also potentially in conflict with the outcomes that would be predicted from current flow modeling studies of the electrical filed produced by the SO-M1 electrode montage. For instance, a review article [58] illustrated (see their Figure 1A) that tDCS of left M1 leads to the generation of an electric field that at least to some degree impacts right M1 and several interconnected contralateral and ipsilateral brain areas that project to it. It is not unreasonable to assume that this should have led to some modulation of right M1 excitability whether up or down. The absence of any modulation of MEP amplitudes in the present study therefore imply that this pattern of current flow must have led no net change in the balance of any inhibitory and excitatory right M1 circuits that it may have impinged upon. Finally, the findings are also generally incongruent with the bulk of the research literature that has reported significant enhancements in excitability following anodal tDCS in the M1 targeted by the stimulation [7, 8], although a significant minority of studies have also reported no significant effects even in these circumstances [6].

In contrast, the current results are consistent with a prior study that had the most similar experimental design of any available study relative to the current one [24]. Although it appears that no existing studies have undertaken concurrent TMS measurements from the right M1 during left M1 tDCS, this small-scale (n = 8) study [24] did perform analogous TMS measurements in right M1 before and after anodal tDCS (1 mA) was delivered to left M1 via the SO-M1 montage for 10 minutes. They reported that left M1 tDCS did not significantly change MEP amplitudes evoked from the right M1 and recorded in the left hand immediately and 40 minutes after stimulation relative to baseline. However, the study did not include a SHAM condition for comparison to the tDCS condition. Nonetheless, the lack of change in MEPs obtained from the left hand after left M1 tDCS are congruent with the current findings. Taken

together, the results of the two studies suggest that left M1 tDCS effects on cortical excitability are confined to the stimulated M1 both during and after stimulation. The findings are also in agreement with a study by Tazoe et. al. (2014) [25] which found that anodal tDCS did not influence net contralateral M1 MEP amplitudes when measured after stimulation. However, paired-pulse TMS measurements indicated that it did increase the magnitude of IHI onto the contralateral M1. This finding is somewhat difficult to interpret based on the MEP results, but likely resulted from the influence of other pathways acting in opposition to IHI leading to no change in net global excitability as indicated by MEP amplitude. It should also be noted that the study involved the opposite experimental arrangement relative to the current study as anodal tDCS was given to right M1 with MEPs and IHI being measured in the left M1. Nevertheless, the outcomes remain generally consistent with the notion that the effects of anodal tDCS may be confined to the stimulated M1.

Indirect findings from the literature also support the current findings. Although the lack of changes in MEPs evoked from the right M1 were unexpected, it should be remembered that at least 20-30% of available tDCS studies that have investigated excitability of the stimulated M1 have reported no significant effects [6-8]. Therefore, it would not be particular surprising that it may be even more difficult to elicit significant excitability enhancements in an unstimulated distant region, despite the strong anatomical interconnections between the two M1s. A demonstration of augmentations in right M1 excitability would have provided rather strong support for the idea that left M1 tDCS may not only improve right hand performance, but also left hand performance. For example, a long-term motor learning study (no tDCS) involving only right hand motor task practice demonstrated that left hand motor skill was also substantially improved [34]. Crucially, this was accompanied by an increase in excitability of the right M1

after several weeks of right hand motor practice. These two results and a number of other studies indicate that the ipsilateral (right) M1 plays an important role in voluntary movement control and motor learning of the right hand [13, 31-37], despite it not being the hand it primarily controls. The current results indicate that left M1 tDCS does not increase right M1 excitability and therefore may not augment motor learning by impacting processes within the ipsilateral right M1. However, this interpretation is highly speculative as no motor skill training was carried out in the current study. Nonetheless, a few studies have investigated the influence of left M1 tDCS on performance of the left hand primarily controlled by the right M1. The findings were extremely mixed with two finding no effects and one finding a trend for a decrease in left hand performance [26-28]. Thus, these studies are in overall broad agreement with the current results as they collectively suggest that the effects of left M1 tDCS using the SO-M1 montage most likely does neither increases or decreases left hand performance, but rather has no discernable effects.

Theoretically, tDCS applied to the left M1 using the SO-M1 montage could impact the right M1 in at least three different general ways, although direct and systematic studies of these specific paths have not been performed. First, the most obvious and probable way would be as a result of the current flow from the cathode over the right supraorbital to the anode over the left M1 that typically leads to the observed net increase in left M1 excitability. Thus, the effects of this current flow on both intracortical neurons in left M1 and neuronal populations responsible for the amount of either IHI or IHF exerted on the right M1 are the most likely candidate mechanisms for any possible modulations of right M1 excitability. Second, this same flow of current could influence right M1 due to possible effects it may have on the dorsolateral prefrontal cortex (DLPFC), supplementary motor complex (SMC) and especially the premotor

cortex (PMC) ipsilateral to the left M1. For example, while left DLPFC does not have direct connections to either right or left M1, paired-pulse TMS studies have shown short-latency inhibitory effects from left DLPFC onto the ipsilateral left M1 [59]. These are likely mediated indirectly through PMC, SMA, or the basal ganglia [59]. In contrast, the left dorsal PMC has dense connections not only to ipsilateral left M1, but also to the contralateral right M1 [60-62]. Thus, right M1 excitability could potentially be elicited by the effects of current flow to the left DLPFC, SMC, and dorsal PMC and their collective ipsilateral connections to left M1. Furthermore, contralateral connections to right M1 could be involved that likely predominately emanate from left dorsal PMC [62]. Third, it is possible as indicated by current flow modeling studies [58] that anodal tDCS of left M1 using the typical SO-M1 electrode montage generates a much more widespread modulation of activity in various brain regions than generally appreciated, even those beyond the previously discussed ipsilateral DLPFC, SMC, and PMC. This oftentimes underappreciated issue is described in a review [58] and illustrated (see their Figure 1) by the simulated electrical field distributions over the right hemisphere. Although the overall electrical field area and strength is lower in the right compared to the left hemisphere, it is shown that even a 1 mA current can clearly influence some portion of the more anterior portions right M1 and to a greater extent areas located further anterior such as the corresponding ipsilateral DLPFC, SMC, and PMC [58].

Despite these three general ways that right M1 tDCS excitability could be modulated by left M1 tDCS applied at a current strength of 1 mA using the SO-M1 montage, the complete absence of changes in right M1 MEP amplitudes in the current study strongly suggests that none of these three individual paths and their underlying potential mechanisms of action exerted any meaningful influence on the right M1. In addition, the same would be true for any potential

combination and relative weighting of these three paths on right M1 excitability. The current study did not include other physiological measures of right M1 activity. Thus, determining the reasons for a lack of left M1 tDCS effects on right M1 was well-beyond the scope of the study. Nonetheless, some potential factors responsible for the results can be briefly speculated upon based on existing literature.

It could be that tDCS simply does not elicit increases in excitability of the magnitude or consistency that many initial studies observed, even in the M1 directly targeted by anodal stimulation [6]. Support for this basic explanation comes from older review article that concluded that the magnitude of tDCS effects [8] on M1 excitability were declining with time as the methodology of these studies advanced. Accordingly, the most recent comprehensive review published on the topic, which uniquely focused on tDCS studies that induced no significant effects on M1 excitability, cited numerous studies that indicated no significant effects [6]. Specifically, the PRISM flow diagram of this study indicated that 92 studies were excluded due to finding significant results while 43 studies and 47 experiments were included that reported no significant tDCS effects on M1 excitability. In particular, a recent comprehensive study with a rigorous design and a large sample size (n = 62) found no effects of anodal tDCS using the SO-M1 montage to target the left M1, although this study used a 2 mA current strength [21]. Another possible reason for the absence of significant effects in the current study is that the sample of participants could have had a relatively large number of tDCS non-responders [53, 63, 64]. While this is a definite possibility, Jonker et al. (2021) [21] also found no evidence for the existence of either responders or non-responders as indicated by a mixed-model cluster analysis. An additional explanation for the lack of significant findings that is typically cited is possible differences in participant characteristics such as age, handedness, and gender distribution [6].

However, this explanation is likely not applicable to the current study as these characteristics were not substantially different in the study relative to previous studies [6-8]. Specifically, the current study involved all strongly-right handed young adults in a tight age range with a gender distribution similar to the average of previous studies. Finally, the absence of effects was likely not due to the tDCS parameters utilized as the most common and efficacious parameters from the literature were employed. This is also apparent based on the data comparisons recent review [6] performed relative to prior reviews on the topic that focused on all available studies whether each study demonstrated significant tDCS effects on M1 excitability or not [7, 8].

Despite the relatively clear and straightforward findings, the present study had a number of limitations that should be acknowledged: 1) the 1 mA current strength may not have been high enough to induce significant changes in excitability in the non-stimulated right M1 [24] to influence any potential mechanisms that could have led to enhanced right M1 excitability. On the other hand, several studies have shown that any increases in M1 excitability resulting from current strengths of 2 mA and above are non-linear [65, 66]. Moreover, they are often not significantly greater than current strengths of 1 mA and can even be lower [65]. Similarly, the study did not involve a condition involving cathodal tDCS of the left M1 performed in an additional session. While it seems doubtful that cathodal tDCS would have elicited an effect in the right M1 if anodal tDCS did not, this would need to be examined directly. Relatedly, the study did not investigate contralateral effects in the opposite direction (anodal tDCS applied to right M1), although once again it seems unlikely that statistically significant effects would have emerged; 2) the present study lacked longer time scale TMS testing blocks (e.g. 5-90 minutes) following the cessation of tDCS that are typically undertaken in tDCS studies of the targeted M1 [6-8]. While this would have been ideal, it seems highly unlikely that these test blocks would

have demonstrated any differing relative results in light of the complete absences of MEP enhancement during and immediately after tDCS; 3) the net right M1 excitability as indicated by MEP amplitude elicited by single-pulse TMS was the only excitability metric acquired and inclusion of paired-pulse TMS measurements such as short-interval intracortical inhibition (SICI) or intracortical facilitation (ICF) were not conducted [66]. These measurements were not undertaken because this study was focused on first determining overall net excitability changes in the right M1 if they were to exist. Additionally, SICI and ICF measurements were not performed due to the inherent time-related complications of having to elicit three types of responses for the requisite 25 trials for each variable (test MEP, condition-test MEPs for SICI and ICF, respectively) with a 6 second ITI; 4) other more involved physiological measures such as EEG or MRS were also not performed as they were not available and may not have even been technically feasible within the context of the current overall experimental paradigm; 5) a motor task was not utilized to concurrently quantify changes in motor skill of the left hand and their possible associations with any changes in right M1 excitability. This will have to be investigated in the future in more complex experimental paradigms; and 6) the sample size was a possible final limitation as this is an issue in many studies in different motor neuroscience related disciplines [67, 68] as well as in most TMS and tDCS studies [6]. This was mitigated to a certain extent with the within-subjects design and the moderately high sample size of 18, which was markedly higher than the average tDCS motor skill study (~13 per group) reported in the tables of an extensive review [1]. The sample size was also comparable (18 versus 19.7) to the average of sample size in 47 experiments included in a recent review of only non-statistically significant studies on tDCS effects on M1 excitability [6]. More importantly, the data provided in that paper indicated that the samples sizes (12.5 and 13.7) of previous reviews on the topic were similar.

This is important because both studies concluded that tDCS significant increased in M1 excitability when all available significant and non-significant studies were included. Finally, the partial eta squared value obtained in the present study was very low ($\eta_p^2 = 0.019$) for the *condition* × *test* interaction for MEP amplitude, likely indicating that an unrealistically high number of participants would have been needed to achieve statistical significance.

Future studies could investigate the interrelated issues of inter-individual differences in response to tDCS and the individualization of tDCS parameters based on the unique characteristics (e.g. anatomy, gender, genetics, hormonal profile, etc.) of a given participant. Furthermore, experimental designs should be more standardized across studies to better determine how a set of tDCS parameters that successfully enhance targeted M1 excitability and motor skill of the contralateral hand and arm system could also influence the unstimulated contralateral M1 and ipsilateral hand. These types of studies may be more valuable in older adult populations or in patients with motor disorders that exhibit degradations in hand dexterity or a differentially affected hemisphere (e.g. stroke, Parkinson's disease) and corresponding hand and arm system. Another avenue of research would be to determine the association, if any, between changes in motor skill and changes in MEPs amplitude in an analogous manner to that of studies involving the M1 targeted with tDCS. For instance, some early studies showed a positive correlation between changes in these variables [69, 70]. Since classic work on motor learning (no tDCS involved) found MEP enhancements in task specific muscles following complex fine motor skill acquisition [71-73], it was initially assumed that even further M1 excitability increases due to tDCS were partly responsible for motor skill improvements that were greater than practice alone. However, a series of more recent investigations [8, 59, 74-76] have shown that concurrent increases in motor skill and MEP amplitudes due to tDCS of M1 are not

significantly correlated. Therefore, it is highly possible that increased MEP amplitude values measured at rest may have limited functional relevance. Nonetheless, all of these correlational studies had limitations and widely varying methodologies, which suggests that further data are needed to before firm conclusions can be drawn on this issue, especially in regard to the unstimulated M1 and corresponding hand and arm system.

CONCLUSIONS

The current study represented a logical first step in the investigation of changes in right M1 excitability during and immediately after tDCS delivered to the left M1. Therefore, the study was performed at rest to first understand the most basic effects of the stimulation without the complicating factors of simultaneous or previous general muscle contractions or specific motor practice. The main findings were that right M1 MEP amplitudes were not statistically different between the tDCS and SHAM conditions at any timepoints during or after tDCS delivered to the left M1. Furthermore, there were no significant increases in right M1 MEP amplitudes in either stimulation condition compared to baseline. Collectively, the findings strongly suggest that tDCS does not modulate contralateral M1 excitability during or immediately after application, at least under the current set of common tDCS parameters of stimulation. Substantially more future research is needed to fully understand and characterize the influence of tDCS on the M1 contralateral to which it is applied. This will be challenging and require comprehensive studies that combine tDCS protocols with multiple physiological measures and a motor skill acquisition paradigm involving one or both hands.

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CHAPTER 3

THE INFLUENCE OF TRANSCRANIAL ALTERNATING CURRENT STIMULATION ON THE EXCITABLIITY OF THE UNSTIMULATED CONTRALATERAL PRIMARY MOTOR CORTEX

ABSTRACT

Transcranial alternating current stimulation (tACS) can enhance primary motor cortex (M1) excitability and improve motor skill when delivered unilaterally to the dominant hemisphere. However, the impact of tACS on contralateral M1 excitability both during and after application has not been studied. The purpose was to examine the effects of tACS delivered to the dominant left M1 on the excitability of the unstimulated contralateral non-dominant right M1. The study implemented a double-blind, randomized, SHAM-controlled, within-subjects, crossover experimental design. Eighteen young adults performed completed a tACS condition and a SHAM condition on two different days in counterbalanced order with a week washout period between days. Transcranial magnetic stimulation (TMS) was utilized to assess excitability of the contralateral right M1 while tACS was delivered to the left M1. TMS was administered in 5 test blocks (termed Pre, D5, D10, D15, and Post) relative to a 20-minute application of tACS (70 Hz, 1 mA current strength). The Pre and Post TMS test blocks were conducted before and immediately after tACS was applied to left M1, whereas the TMS test blocks performed during tACS were completed at time points starting at the 5, 10, and 15-minutes marks of the 20-minute stimulation period. The primary dependent variable was the 1 mV motor evoked potential (MEP) amplitude. MEP data were analyzed with a 2 *condition* (tACS, SHAM) x 5 *test* (Pre, D5, D10,

D15, Post) within-subjects ANOVA. The main effect for *condition* ($P = 0.704$) and *condition* x *test* interaction ($P = 0.349$) were both not statistically significant. There was a significant main effect for *test* ($P = 0.003$), however, post hoc analysis indicated that none of the pairwise comparisons were statistically significant. Overall, the findings indicate that tACS applied to the left M1 does not significantly modulate contralateral right M1 excitability during or immediately after stimulation, at least when utilizing the present tACS parameters.

INTRODUCTION

Transcranial alternating current stimulation (tACS) is a non-invasive brain stimulation method that has been receiving increasing research attention over the past several years [1-7]. tACS is a variation of the much more commonly studied non-invasive brain stimulation technique of transcranial direct current stimulation (tDCS). tDCS involves passing a weak, constant current between a cathode and an anode placed on the scalp [8, 9]. In contrast, tACS involves the application of a sinusoidal waveform at a given frequency via an anode and a cathode placed over two scalp areas to impact a target brain region or two functionally connected regions. Accordingly, tACS can be used to induce entrainment of populations of cortical neurons at the same frequency of oscillation as endogenous oscillations both within and between brain areas [1, 10, 11].

Despite these two basic differences between tDCS and tACS they two techniques have some common features, methodological similarities, and can elicit a few basic overall net effects on physiological and behavioral outcomes relative to human motor performance. For example, two of the basic goals of the application of both techniques are to increase the excitability of the primary motor cortex (M1) and to induce improvements in motor skill acquisition [12-15]. This

is most frequently accomplished in tDCS studies by placing the cathode over the right supraorbital (SO) and the anode over the contralateral left M1, which is termed the SO-M1 electrode montage. Most typically, a current of 1-2 mA in intensity is passed between the electrodes for a duration of 10-20 minutes. These tDCS parameters have shown the ability to increase M1 excitability by approximately 20-40% after stimulation [13, 14, 16, 17] and induce improvements in motor skill of about 10-15% [8, 9, 12-15, 18-20]. In contrast, many fewer studies have investigated the influence of tACS on these outcomes and the parameters of stimulation have arguably been more heterogenous in tACS compared to tDCS studies. Nonetheless, several studies have reported that tACS can also increase M1 excitability [1, 21, 22], although perhaps not to the same degree as tDCS. In addition, a few studies have also demonstrated that t-ACS applied to M1 can significantly enhance fine motor skill in hand muscles. For instance, Sugata et al. (2018) [2] found that 70 Hz tACS delivered to left M1 using the SO-M1 montage and a 1 mA current elicited large improvements in motor skill in a four-finger button pressing task involving the right hand. The magnitude of improvements in that study seemed to indicate that the set of parameters employed may be among the most effective in the literature to date. Overall, the vast majority of M1-tDCS studies along with a few promising M1-tACS studies have shown a clear ability to significantly enhance cortical excitability and motor skill when using these sets of parameters and the SO-M1 electrode montage to target the dominant left M1 that predominately controls the contralateral dominant right hand [12].

In contrast to this large body of research, there is a paucity of studies that have explored the effects of M1-tDCS or M1-tACS on the excitability of unstimulated right non-dominant M1 and the motor performance of the associated left non-dominant hand that it predominately controls. In fact, only one previous study has directly measured the influence of left M1-tDCS on

the net excitability of the right M1 as measured by motor evoked potential (MEP) amplitudes elicited by transcranial magnetic stimulation (TMS). Lang et al. (2004) [23] reported that right M1 excitability was unchanged relative to baseline immediately and 40 minutes left M1-tDCS application. Furthermore, analogous studies exist that have investigated the effects of M1-tACS on right M1 excitability in these experimental circumstances. In addition, no studies have explored right M1 excitability in the crucial time period during the application of tDCS or tACS of left M1. Similarly, only a few tDCS studies have investigated the motor performance related outcomes in response to application of left M1-tDCS using the SO-M1 montage on the ipsilateral left hand primarily controlled by the right M1. The outcomes were contradictory as reported no effects on motor skill of the left hand [24, 25], whereas another other found a trend for a decline in motor skill [26]. In addition, it appears that no analogous tACS studies have been conducted on these interrelated topics. Therefore, the impact of both left M1-tDCS and especially left M1-tACS utilizing on right M1 excitability and motor skill of the left hand remain to be elucidated.

The reasons for scarcity of available research on these topics are difficult to determine given that left M1-tDCS given during task practice has generally been shown to yield superior motor skill outcomes in motor tasks executed with the right hand relative to before practice application [27-29]. Accordingly, it would seem that it would be important to know the effects of the stimulation on the right M1 during that time as it also contributes to the motor skill learning outcomes of the right during and after practice. This is because although the right hand is predominantly controlled by the contralateral left M1, the ipsilateral M1 plays a significant role in the control of movements of the ipsilateral hand that it does not predominantly control [30, 31]. In fact, a large body of evidence provides supports for the meaningful contribution of ipsilateral right M1 to important facets of motor control such as the initiation of movements [32-

34] and ipsilateral right hand motor skill acquisition [35-39]. Therefore, it would be valuable to characterize the influence of left M1-tACS delivered via the most common M1-SO montage on the unstimulated non-dominant contralateral right M1. Theoretically, modulations of right M1 under these conditions could have significant implications for movement control and motor skill learning related processes in both hands. However, a rationale initial exploration of possible modulations of right M1 excitability during and after application of left M1-tACS would need to keep the target muscle at rest to isolate the fundamental effects of stimulation without the influence of concurrent muscle activation or performance of a motor task.

The purpose was to examine the effects of tACS delivered to the dominant left M1 on the excitability of the unstimulated contralateral non-dominant right M1. This was achieved by quantification of MEP amplitudes obtained from the left first dorsal interosseous (FDI) muscle elicited by TMS delivered to the right M1 in test blocks conducted before, during, and after 70 Hz tACS was administered to the left M1 for a duration of 20 minutes. Based on the most relevant previous M1-tDCS and M1-tACS studies [1, 21, 40], it was predicted that MEP amplitudes elicited from the right M1 would be greater during and immediately after application of tACS to the left M1 relative to baseline and compared with the SHAM condition. The presence of modulations of unstimulated contralateral right M1 excitability by left M1-tDCS could have implications for influencing voluntary movement control and motor learning processes in both the left and right hands.

MATERIALS AND METHODS

Eighteen healthy young adults consisting of 10 males and 8 females with an average age of 25.9 ± 5.1 SD years participated in the study. Prior to their involvement, all volunteers

provided written informed consent. Participants were confirmed to be strongly right-handed using the Edinburgh Handedness Inventory [41]. They were screened to ensure the absence of neurological or psychiatric disorders, uncontrolled medical conditions, and compliance with international criteria for non-invasive brain stimulation [42]. The study complied with the Declaration of Helsinki and received approval from the Biomedical Institutional Review Board at the University of Nevada, Las Vegas.

The study implemented a double-blind, SHAM-controlled randomized, within-subjects, experimental design. A within-subjects design was selected to preclude the influence of interindividual variations in physiological, genetic, and anatomical factors on the susceptibility to tDCS [43, 44] while also allowing for greater statistical power compared to between-subjects designs [45]. The presentation order of the two experimental conditions was given to the participants using Research Randomizer (www.randomizer.org) by an investigator not involved in the data collection aspects of the experiments [46, 47]. Each participant completed two experiments at approximately the same time of day and held a week apart [48, 49], which is the typical washout period utilized in the majority of tDCS and tACS studies. Most importantly, the only difference between the two experiments was the type of stimulation (tACS, SHAM) applied.

Each experimental session had a duration of ~1.5 hours and the procedures were performed in the order prescribed: 1) the motor hotspot site, resting motor threshold (RMT), 1 mV MEP stimulation intensity (SI) expressed as a percentage of maximum stimulator output (%MSO) for the right FDI were all determined via TMS applied to the left M1; 2) placement of the tACS electrode montage over the left M1 FDI motor hotspot site; 3) the motor hotspot site, resting motor threshold (RMT), 1 mV MEP stimulation intensity (SI) expressed as a percentage

of maximum stimulator output (%MSO) for the left FDI were all determined via TMS applied to the right M1; 4) Pre TMS test block; 5) tACS or SHAM stimulation was delivered to the left M1 for 20 minutes while three TMS test block were completed during (termed D5, D10, and D15) stimulation. Accordingly, TMS administration began at the 5, 10, 15-minute points in time and lasted for ~ 2.5 minutes; and 6) Post TMS test block. MEPs collected during the TMS test blocks were always elicited from the right M1 and acquired from the corresponding left FDI. This experimental protocol id depicted in Figure 4.

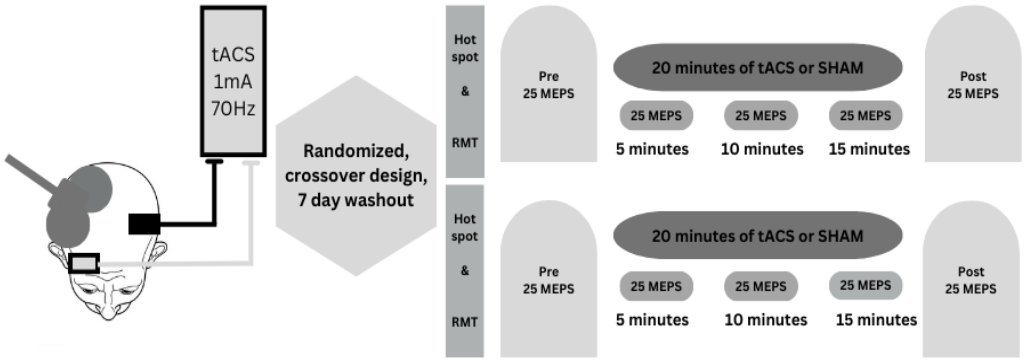

Figure 4 tACS Study Schematic

Figure 4. Schematic of the study design and experimental protocol. (**A**) TMS coil positioning over the right M1 scalp area corresponding to the left FDI motor hotspot and the SO-M1 electrode montage placement for application of tACS over the left M1. (**B**) A total of 20 minutes of tACS or SHAM stimulation was given to left M1 using the M1-SO electrode montage. MEPs were elicited from the right M1 and acquired from the corresponding left FDI in five TMS test blocks (Pre, D5, D10, D15, Post) conducted before (Pre), during (D5, D10, and D15-minute points in time), and immediately after (Post) either tACS or SHAM stimulation.

A monophasic Magstim 2002 unit with a standard double 70 mm figure-of-eight remote-controlled coil was used to deliver single-pulse TMS to the left and right M1s. The TMS coil was placed over the M1 of interest over the surface of the scalp with the handle directed

backward and lateral while being orientated tangential to the scalp at a 45-degree angle relative to the sagittal plane. This coil positioning relative to the M1 hand representation area generates a posterior-to-anterior directed current in the brain to elicit MEPs in the contralateral hand. Surface electromyographic (EMG) activity of the FDI muscle of each hand was collected utilizing two electrodes configured in a belly tendon montage. The ground electrode comprised a thin circular disc and was placed on the back of the hand. EMG activity was collected with Cambridge Electronic Design (Cambridge, UK) hardware (1902 amplifiers, micro 1401 data acquisition interface) and software (Signal 5.04).

TMS administration and EMG signals were collected while participants a constant body and upper limb posture. The experimental arrangement was the same as previous involving TMS of the right FDI [29, 50] and MEPs acquired from the left FDI was accomplished in an analogous manner. In both cases, participants sat in a chair with the upper arm abducted to about 45-degrees with the forearm and elbow placed on the surface of a table located to the side of the body. The wrist was maintained in a neutral position, the elbow was flexed to about a 90-degrees angle, and the palm was placed flat on the table surface. The participants were given strict instructions to keep this posture for all TMS testing procedures as even relatively modest differences in arm position can significantly influence the MEP amplitudes observed in hand muscles [51, 52]. Crucially, participants were directed to keep their eyes open during TMS as closure of the eyes is well-known to significantly diminish MEP amplitude. To make sure that the FDI muscle was kept in a state of complete rest during all TMS testing, real-time visual feedback of FDI EMG was given on a large monitor situated about ~0.75 meters directly in front of the participants [53]. A set of instructions was given to participants on how to utilize the visual feedback provided to keep the FDI in a state of rest throughout the experiment. One

member of the research team had the sole task of constantly monitored the body positioning and FDI EMG activity of participants to further assure that these requirements were met throughout the experiments.

The motor hotspot site, RMT, and 1 mV MEP stimulation intensity (SI) as a %MSO were determined for the right FDI followed by the left FDI. The motor hotspot sites were located by delivering a series of TMS pulses over the scalp of the hand representation area of each M1. This was continued until the point that elicited the greatest MEP amplitude in the corresponding FDI was identified [54]. This point was used as the stimulation site and marked temporary pen for all of the following RMT and 1 mV SI measurements as well as the assessment of MEP amplitudes in the TMS test blocks. More specifically, the point was marked directly on the scalp for left M1 TMS testing, whereas it was marked on tape that was placed on the scalp cap for right M1 testing. RMT was quantified as the lowest TMS SI that could generate a MEP amplitude above 50 microvolts in at least 5 out of 10 successive trials. RMT data was collected for two reasons: 1) a few studies [55, 56] have reported that participants with a higher RMT may exhibit a smaller enhancement of MEPs after tDCS is applied and vice versa. This could also be the case for tACS, which may mean that RMT values could correlate with the percentage increase in MEP amplitude, if it were to occur. Furthermore, RMT also served as a basic control measure and indication of baseline excitability for each FDI in both experiments. For the determination of the 1 mV MEP SI for the left and right M1s, a series of procedures developed in two previous studies was used [29, 50] as they proved very successful in being able to obtain the TMS SI (%MSO) that induced an average MEP of as close to 1 mV as possible for a block TMS trials. This is crucial because a significant difference in the MEP acquired at baseline (Pre TMS block) between the two experimental conditions could represent a potential confound and ideally would

be as close to 1 mV as possible. In brief, this involved applying a series of TMS pulses starting at ~55 %MSO and adjusting the SI while MEPs were simultaneously displayed and quantified online by the data acquisition software. Once MEP amplitudes appeared to be as close to an average value of 1 mV as possible, the data acquisition program was reset and data collection of the Pre TMS test block commenced. This ensured that the MEP amplitude attained in the Pre TMS test block was as close to 1 mV as practically possible in both stimulation conditions performed on the two separate days. The same 1 mV SI as a %MSO was utilized to elicit MEPs in the subsequent D5, D10, D15, and Post TMS testing blocks for each participant on a given day. Lastly, the inter-trial interval (ITI) between successive MEPs was 6 seconds for all TMS test blocks and 25 MEPs were collected per block as this has been shown to be the optimal trade-off between time efficiency and the ability to obtain valid MEP amplitude averages in blocks of TMS trials [57].

A NeuroConn DC Stimulator Plus/MR was utilized to deliver high frequency (70 Hz) tACS for a duration of 20 minutes with a current strength of 1 mA to the left M1. This was conducted using the traditional M1-SO electrode montage that has been used in the majority of tDCS studies and many tACS studies. The two rubber electrodes (5 × 7 cm) that comprised the electrode montage were inserted into two sponged that were in sponges doused in saline solution. The reference electrode was placed above the eyebrow over the right supraorbital region and the target electrode was placed over right FDI motor hotspot site of the left M1. To accomplish the main experimental goal of collecting MEPs from right M1 while tACS was simultaneously applied to left M1, a set of novel procedures were implemented after extensive pilot work. The target and reference electrodes were fixed in place by a close-fitting scalp cap as opposed to usual employment of two separate rubber straps consisting of a headband and a chinstrap.

Specifically, the target electrode was kept in place by the scalp cap only and the typical rubber chin strap was not used for this electrode. This was necessary as this would have obviously interfered with the simultaneous TMS measurements as the strap would have traversed directly over the right M1 motor hotspot site. Overall, the close-fitting scalp cap was able successfully kept the target electrode in place to the same extent as the conventional rubber strap positioning. In contrast, the cathode was able to further secured using the typically applied rubber strap placed around the head in the conventional manner as this rubber strap was not in a position to interfere with the TMS testing. These methods for securing the tACS electrodes was ultimately effective because while the TMS coil was in relatively close proximity to the electrodes, it did not contact them. For the SHAM stimulation condition, the standard [29, 58] ramp up, hold, ramp down protocol was performed. Accordingly, the current was ramped up from zero to 1 mA over 10 seconds, maintained at 1 mA for 30 seconds, and ramped down from 1 mA to zero over 10 seconds as in previous studies. This is the most common SHAM stimulation paradigm used in tDCS and tACS studies and most studies have found that it induces the same sensations on the scalp to participants as real stimulation, but but does not induce any physiological effects [19, 59]. Finally, the research team member responsible for operated the tACS device did not partake in the data collection aspect of the experiments and research team members that conducted the data collection were blind to the stimulation condition as in previous studies [29, 46, 47].

The dependent variables were MEP amplitude, RMT, and 1 mV SI. MEP amplitude was the primary dependent variable, whereas the secondary dependent variables of RMT and 1 mV SI were considered experimental control measures. Signal software (version 5.04) from Cambridge Electronic Design (Cambridge, UK) was used to collect all EMG signals and associated MEP data via custom-written scripts. In addition, offline data analysis of MEP data

was also accomplished using custom-written Signal scripts. MEP amplitude was calculated as the peak-to-peak values and the average of the 25 MEPs [57] collected in each of the TMS test blocks was taken for analysis.

Two separate 2 *condition* (tACS, SHAM) × 2 *hand* (Left, Right) within-subjects ANOVAs were used to analyze the control measures of RMT and 1 mV SI. The primary dependent variable of MEP amplitude was analyzed with a 2 *condition* (tACS, SHAM) × 5 *test* (Pre, D5, D10, D15, Post) within-subjects ANOVA. If appropriate, post hoc analyses were conducted utilizing Bonferroni adjustments for multiple comparisons to determine where any statistically significant differences occurred between the TMS test blocks. The effect sizes were reported as the partial eta squared values. The significance level for the statistical tests was $p < 0.05$. Data were depicted as means ± standard errors in the figures, whereas data referred to in the text were expressed as means ± standard deviations.

RESULTS

The *condition* main effect ($P = 0.221$, $\eta_p^2 = 0.087$), *hand* main effect ($P = 0.110$, $\eta_p^2 = 0.114$), and *condition* × *hand* interaction ($P = 0.857$, $\eta_p^2 = 0.002$) were all not statistically significant (Figure 5A). For the 1 mV SI, the *condition* main effect ($P = 0.409$, $\eta_p^2 = 0.041$), *hand* main effect ($P = 0.821$, $\eta_p^2 = 0.003$), and *condition* × *hand* interaction ($P = 0.700$, $\eta_p^2 = 0.009$) were all not statistically significant (Figure 5B).

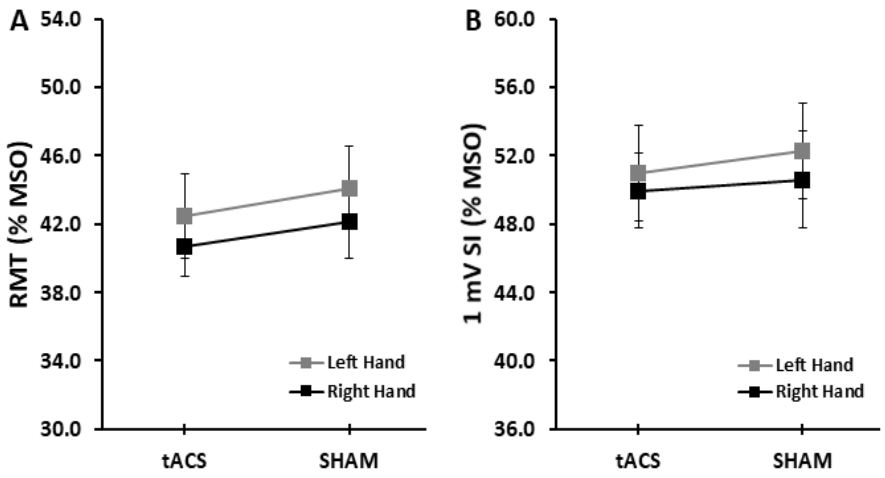

Figure 5 tACS RMT and 1mV SI

Figure 5. RMT and 1 mV SI. (**A**) RMT in the left and right hands for the tACS and SHAM conditions; (**B**) 1 mV SI in the left and right hands for the tDCS and SHAM conditions.

For MEP amplitude, *condition* main effect ($P = 0.704$, $\eta_p^2 = 0.009$) and *condition* × *test* interaction ($P = 0.349$, $\eta_p^2 = 0.061$) were both not statistically significant. In contrast, there was a significant main effect for *test* ($P = 0.003$, $\eta_p^2 = 0.207$) (Figure 6). However, post hoc analyses of the main effect indicated that none of the pairwise comparisons were statistically significant (*P* value range 0.066 - 1.000).

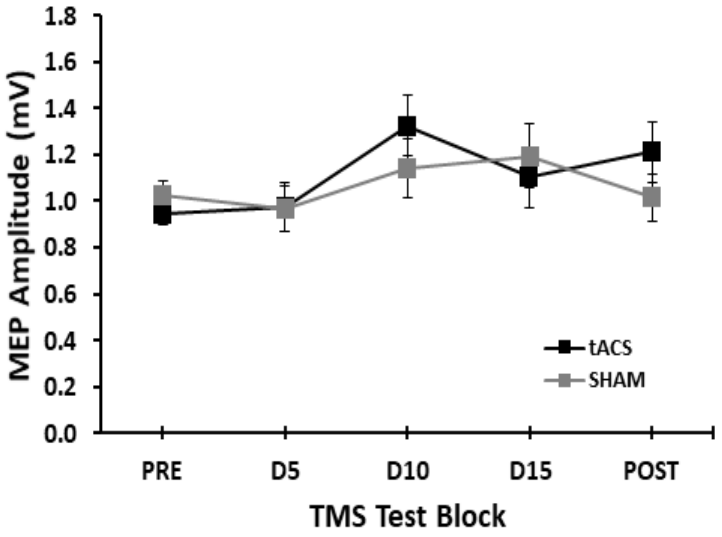

Figure 6 tACS MEP Amplitudes

Figure 6. MEP amplitudes evoked from the right M1 and acquired from the left hand in the tACS and SHAM conditions for the Pre, D5, D10, D15, and Post TMS test blocks.

DISCUSSION

The purpose was to examine the effects of tACS delivered to the dominant left M1 on the excitability of the unstimulated contralateral non-dominant right M1. The main findings were that MEP amplitudes elicited from the right M1 and acquired from the left hand were not significantly different between the tACS and SHAM conditions at any timepoints during or immediately after the stimulation was delivered to the left M1. Overall, the findings indicate that tACS applied to the left M1 does not significantly modulate contralateral right M1 excitability during or immediately after stimulation, at least when the current set of tACS parameters are used.

tACS delivered to the left M1 using the SO-M1 electrode montage usually enhances left dominant M1 excitability, as assessed by MEPs acquired from the contralateral right hand that it

predominantly controls [1, 21, 22]. To the best of our knowledge, this was the first study to investigate the influence of left M1-tACS on the excitability of the unstimulated right non-dominant M1. In particular, the most innovative feature of the study was the quantification of right M1 excitability simultaneous with left M1-tACS administration. Accordingly, there is only a single M1-tDCS study available that directly measured the influence of left M1-tDCS on the excitability of the right M1 [23] and this study only evaluated this issue during stimulation. In addition, no studies exist that have performed analogous assessments of right M1 excitability during or after M1-tACS of the left M1. As a result, all but one study involving either technique have only net excitability of the stimulated left M1 before and after the two types of stimulation were applied. This makes sense based on basic technical constraints related to the fact that MEPs cannot be evoked from the stimulated M1 while that target tACS electrode is over that scalp location during stimulation. However, the current study used a novel arrangement involving the use of a scalp can and he deletion of the typical chinstrap that transverses the head over the right M1 motor hotspot to successfully apply TMS to the right M1 during tACS application to the left M1.

The initial hypothesis proposed that that MEP amplitudes elicited from the right M1 would be higher during and immediately after application of tACS to the left M1 relative to baseline and compared with the SHAM condition. Since no prior tACS studies had investigated these exact topics, this hypothesis was primarily based on the most applicable two tDCS studies available that had explored contralateral right hemisphere responses to left M1-tDCS [23, 40]. One study used MRS to quantify GABA levels in the unstimulated right M1 and the stimulated left M1 [40]. The results revealed that GABA levels declined in both M1s which would imply that both M1 displayed less inhibition and therefore higher excitability following left M1-tDCS.

However, concurrent TMS measures of MEP amplitude were not included in that study to confirm that the net excitability of each both had in fact increased. This could be necessary as other neuromodulator concentrations could have changed due to tDCS and counteracted the inhibitory effects of increased GABA concentrations [9, 61]. In contrast to the original hypothesis, MEPs evoked from the right M1 and acquired from the associated left hand were not significantly different between the tACS and SHAM conditions during or immediately following left M1 stimulation. Furthermore, none of the TMS test blocks performed during or immediately after stimulation exhibited MEP amplitudes that significantly differed relative to the Pre TMS test block, independent of stimulation condition, for either of the two stimulation conditions.

However, the statistical results that led to these overall conclusions were more nuanced than portrayed above. Specifically, while there was no main effect for *condition* or *condition* × *test* interaction. The ANOVA did reveal a significant *test* main effect. However, post hoc analyses using Bonferonni corrections indicated that none of the pairwise comparisons were statistically significant, although the differences between the D10 TMS test block compared to the D5 and the Pre TMS test blocks had *P* values of 0.066 and 0.082, respectively. In addition, visual inspection of Figure 3 and the average MEP values in the tACS and SHAM conditions, indicate that the non-significantly higher MEP amplitudes in the D10 TMS test block were due to a combination of higher MEP amplitudes in both stimulation conditions. Therefore, this pattern of results complicates the interpretation of the results to a certain extent. Since MEP amplitudes were moderately increased in D10 for the tACS, it would be tempting to conclude that left M1 tACS may have had an effect on right M1 excitability that was slightly masked by the unexpected, but non-significant increased MEP values in the D10 test block in the SHAM condition. Despite these statistically nuanced results, we feel that the overall interpretation of no

significant *test* effect due to non-significant post hocs and no significant tACS effects are the correct overall conclusions. Accordingly, the rather high D10 TMS test block MEP values were most likely due to random fluctuations in MEP measurements due to their well-described and notorious inherent variability [57, 59], and not any actual effects due to tACS or SHAM stimulation. Nonetheless, it cannot be completely ruled out that placebo effects could have provided at least some contribution to the enhanced MEPs in both stimulation conditions [59]. Finally, the overall findings could not have been influenced by any potential confounding effects as the control measurements of RMT and the 1 mV SI were not statistically different between the tACS and SHAM conditions. Critically, the 1 mV SI (%MSO) yielded average MEPs very close to the ~1 mV target for the pre TMS test blocks, which eliminated the greatest potential confound of the study.

Our results also potentially conflict with predictions from current flow modeling studies of the electric field generated by the SO-M1 electrode montage. For example, a review article [58] illustrated (see their Figure 1A) that tACS of the left M1 creates an electric field affecting the right M1 and several interconnected contralateral and ipsilateral brain regions. One would expect this to modulate right M1 excitability, either increasing or decreasing it. The absence of any modulation in MEP amplitudes in this study suggests that the current flow pattern did not result in any net change in the balance of inhibitory and excitatory circuits in the right M1. In addition, the absence of significant tACS effects indicates that whatever entrainment of neurons it may have elicited in left M1, did not manifest in meaningful effects on MEPs obtained from the right M1.

In contrast, the current results align with a previous study that had the most similar experimental design to the present one [24]. Although no existing studies have conducted

concurrent TMS measurements from the right M1 during left M1 tACS, [24] performed analogous TMS measurements in the right M1 before and after anodal tACS (1 mA) was applied to the left M1 using the SO-M1 montage. They found that left M1-tACS did not significantly change MEP amplitudes evoked from the right M1 and recorded in the left hand immediately and 40 minutes after stimulation. Thus, the lack of change in MEPs recorded from the left hand after left M1 tACS is consistent with the current findings. Together, these results suggest that left M1 tACS effects on cortical excitability are confined to the stimulated M1 both during and after stimulation.

Indirect evidence from the literature is also consistent with the current findings. Although the absence of changes in MEPs from the right M1 was unexpected, it's important to remember that 20-30% of M1-tDCS studies examining the excitability of the stimulated M1 have report no significant effects [6-8]. Therefore, it is not surprising that enhancing excitability in an unstimulated, distant area might be even more difficult, despite the strong anatomical connections between the two M1s. Evidence of increased excitability in the right M1 would have supported the idea that tACS of the left M1 could enhance performance in both hands. For example, a motor learning study (without tACS) showed that practicing right-hand tasks improved left-hand skills [34]. This improvement was accompanied by increased right M1 excitability after several weeks of right-hand practice. Such findings suggest that the ipsilateral (right) M1 significantly contributes to voluntary movement control and motor learning for the right hand [13, 31-37], even though it primarily controls the left hand. The current study indicates that tACS of the left M1 does not enhance right M1 excitability and may not influence motor learning in the ipsilateral right M1. However, this is speculative since no motor skill training was involved in this study.

tACS delivered to the left M1 using the typical SO-M1 montage could potentially modulate activity in the right M1 in several different ways based on simulated electrical field distributions [62] and known pathways between the involved brain regions: 1) the flow of current from the cathode located over the supraorbital region to the anode located over left M1, which could then reach the right M1 through transcallosal connections; 2) the aforementioned current flow also produces an electrical field over the left premotor cortex (PMC), dorsolateral prefrontal cortex (DLPFC), and supplementary motor complex (SMC) in route from the cathode and anode. In turn, the activation of these areas could also ultimately modulate right M1 activity through their ipsilateral connections [63] to left M1 (DLPFC, SMA, PMC) and the contralateral connections of left dorsal PMC to the right M1[64-66]; and 3) the SO-M1 montage also generates an electrical field in the right hemisphere which is ipsilateral to the cathode. Although the electrical field is lower over the right compared to the left hemisphere it could still modulate activity in the right DLPFC, SMA, PMC, and M1 (see Figure 1 in [62]). However, the exact physiological effects of the current flow in these particular circumstances and relative to right M1 have never been directly investigated.

In light of the three conceivable avenues through which left M1 tACS, administered at 1 mA using the SO-M1 montage, could modulate right M1 excitability, the complete absence of changes in right M1 MEP amplitudes in this study strongly implies that none of these pathways, nor their underlying mechanisms, significantly impacted right M1 function. Moreover, this lack of influence persists regardless of potential combinations or relative strengths of these pathways on right M1 excitability. Since the study did not include additional physiological measures to assess right M1 activity, exploring the reasons for the absence of left M1 tACS effects on right

M1 fell beyond its intended scope. Nonetheless, drawing from existing literature, several potential factors contributing to these results can be cautiously considered.

One plausible explanation for the lack of significant effects observed in this study is that tACS might not consistently induce increases in excitability to the extent observed in earlier studies. This idea gains support from an older M1-tDCS review, which noted a diminishing magnitude of tDCS effects on M1 excitability over time as study methodologies evolved [8]. Furthermore, the most recent comprehensive review, which specifically focused on M1-tDCS studies failing to yield significant effects on M1 excitability, cited 47 experiments that had studies indicating no substantial impact of M1-tDCS [6]. However, there have not been enough tACS studies conducted to date to be able to make the same kind of comparisons and conclusions as the tDCS related ones above. Another conceivable explanation for the absence of significant findings could be the presence of a significant proportion of tACS non-responders among the participants [53, 63, 64] as has been asserted in a series of tDCS studies on the topic. Nevertheless, Jonker et al. (2021) found no indication of either responders or non-responders, as suggested by a mixed-model cluster analysis [21]. These possibilities underscore the need for more research on these topics in tACS studies as there are limits to the inferences that can be drawn from tDCS studies due to the different underlying mechanisms of action by which the two stimulation techniques exert physiological and behavior effects in the motor system.

The current study had a number of limitations that should be briefly addressed: 1) the study only investigated one possible set of tACS parameters of stimulation (e.g. electrode montage, frequency, current strength, stimulation duration, etc). The current parameter set was chosen as they appeared to be the greatest effects on motor skill that were also supported by physiology recordings. Nonetheless, other sets of parameters used in other M1-tACS motor skill

studies [7] could have produced alternative findings; 2) an experimental condition was not included that investigated the opposite arrangement iof tACS delivered to the right M1 on left M1 activity. This could have produced different result due to the numerous intracortical and intercortical differences in organization between the two M1s; 3) the study did not include multiple TMS post-test blocks at various timepoints after M1-tACS administration ended to better characterize any longer-term after effects. However, it seems improbably that this testing would have yielded different findings relative than those obtained in the test block after tACS application; 4) MEP amplitude was the only physiological measurement taken during or after the stimulation. It would have been ideal to have performed paired-pulse TMS assessments of the intracortical neuronal populations that mediate measures such as short-interval intracortical inhibition (SICI), intracortical facilitation (ICF), or short-interval intracortical facilitation [70]; and 5) a motor skill acquisition task was not incorporated, which could have been used to determine if the administration of tACS in these experimental conditions influenced the left hand motor performance and the functional relevance of any modulations of MEP amplitudes evoked form the right M1.

There is substantial room for future research on the influence of tACS on M1 excitability and motor skill as far fewer studies have been done on these topics relative to tDCS. In addition, there is almost an unlimited number of possible combinations of stimulation parameters and electrode montages that could be investigated. One issue that is receiving increasing attention in non-invasive brain stimulation studies is the potential differences in susceptibility to brain stimulation between individuals. Therefore, the parameters of tACS application may have to be customized depending on various anatomic and genetic of an individual. This will not only be challenging but also difficult to reconcile with the common competing viewpoint that non-

invasive brain stimulation study protocols need to be more homogenous [15] to accurately establish the basic effects of a given tACS parameter space on the stimulated and unstimulated M1s and motor output of the two hands. Most importantly, these issues will be even more relevant for studies comprised of individuals with specific movement disorders characterized by a more affected hemisphere and associated hand such as focal hand dystonia, stroke, and Parkinson's disease. An additional line of future inquiry would be the functional relevance of any MEP amplitude modulations on changes in motor skill. Although two early small-scale studies showed an association between increases in MEP amplitudes and increases in motor skill in stroke and Parkinson's disease patients following M1-tDCS [71, 72], more contemporary studies have reported a complete lack or a relationship [14, 63, 73-75]. Furthermore, similar studies have not been performed in response to M1-tACS. Moreover, it is more likely that changes in specific paired-pulse TMS measures such as SICI may be relevant to specific aspects of movement control. For example, SICI is involved in movement initiation [32], muscle force relaxation [76], and motor skill acquisition [77, 78]. Once again there is much more research on the modulation of intracortical inhibitory and facilitatory circuits in response to M1-tDCS compared to tACS. Therefore, future studies could determine the influence of M1-tACS on these pathways in the context of motor skill acquisition in both the stimulated and unstimulated M1 and associated hand and arm system.

CONCLUSIONS

This study was a rationale initial exploration of possible modulations of right M1 excitability during and after application of left M1-tACS. Consequently, the investigation was conducted while the target muscle was at rest to isolate the effects of the stimulation, free from the influence of concurrent muscle activation or performance of a motor task. The novel findings

produced by the study were that MEP amplitudes elicited from the right M1 and acquired from the left hand were not significantly different between the tACS and SHAM conditions at any timepoints during or immediately after the stimulation was delivered to the left M1. Overall, the findings indicate that tACS applied to the left M1 does not significantly modulate contralateral right M1 excitability during or immediately after stimulation, at least when the current set of tACS parameters are used. Extensive future research is needed to gain a comprehensive understanding of tACS effects on both the stimulated M1 as well as the unstimulated contralateral M1. Such investigations should integrate diverse tACS protocols with multiple physiological measures and motor skill acquisition paradigms involving one or both hands to elucidate to determine the viability of tACS as an intervention to improve motor skill and learning in different populations.

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CHAPTER 4

THE INFLUENCE OF TRANSCRANIAL ALTERNATING CURRENT STIMULATION APPLIED OVER MULTIPLE DAYS ON MOTOR LEARNING OF A COMPLEX MOTOR TASK

ABSTRACT

Transcranial alternating current stimulation delivered to the primary motor cortex (M1) can increase cortical excitability, entrain neuronal firing patterns, and increase motor skill acquisition in simple motor tasks. The primary aim of the study was to assess the impact of M1-tACS applied over three consecutive days of practice on motor learning of a challenging overhand throwing task in young adults. The secondary aim was to examine the influence of M1-tACS on M1 excitability. The study implemented a double-blind, randomized, SHAM-controlled, between-subjects experimental design. A total of 24 healthy young adults were divided into tACS and SHAM groups and performed three identical experimental sessions that comprised blocks of overhand throwing trials of the right dominant arm concurrent with application of tACS to the left M1. Performance in the overhand throwing task was quantified as the endpoint error. Motor evoked potentials (MEPs) were assessed in the right first dorsal interosseus (FDI) muscle with transcranial magnetic stimulation (TMS) to quantify changes in M1 excitability. Endpoint error was significantly decreased in the post-tests compared with the pre-tests when averaged over the three days of practice ($P = 0.046$), but this decrease was not statistically significant between the tACS and SHAM groups ($P = 0.474$). MEP amplitudes increased from the pre-tests to the post-tests ($P = 0.003$), but these increases were also not different between groups ($P = 0.409$). Overall, the main findings indicated that tACS applied to

M1 over multiple days does not enhance motor learning in a complex task to a greater degree than practice alone (SHAM).

INTRODUCTION

Motor learning refers to the relatively permanent improvement of a motor skill through deliberate practice over time [1]. Accordingly, the process of motor learning is characterized by development of the ability to perform a motor skill with increasing levels of precision, movement efficiency, and automaticity [1]. To optimize motor learning, it is not enough to be taught correctly how to perform a motor skill, extensive practice and ideal practice strategies are also needed. Since motor practice is the primary factor responsible for motor learning, substantial research efforts have been made over many decades to determine the best parameters of motor practice to induce motor learning [2]. While these studies have been valuable, the vast majority involved simple motor tasks that were completely novel to the participants and were conducted in laboratory conditions. Therefore, it is not surprising that subsequent research found that many of the practice principles developed in studies involving simple motor tasks are not generalizable to the motor learning [2] and control processes [3, 4] of complex motor tasks. Although many advances have been made in the methods and physiological mechanisms underlying improving motor learning through practice, few modalities exist that can enhance motor learning to a greater degree than progressive well-designed practice regimes alone. This is particularly evident in healthy young adults in difficult motor tasks where ceiling effects often impose an upper limit on motor skill development over both short and relatively longer time periods. Therefore, the development of interventions that can improve motor learning beyond the levels that can be achieved by intensive practice alone would have substantial biomedical significance and practical applications.

Recently, non-invasive brain stimulation methods have been developed that have the potential to enhance motor skill and learning in a variety of populations including older adults [5, 6], individuals with motor disorders [7-9], and healthy young adults [10]. Transcranial direct current stimulation (tDCS) has been the most widely studied non-invasive brain stimulation method and the most practical to implement in both research and real-world applications [11]. tDCS involves passing a weak electric current over a targeted brain region [12], most commonly the primary motor cortex (M1), with the goals of increasing cortical excitability [13], motor skill [14-16], and motor learning [10, 17-21]. Numerous acute studies involving fine control of the hand and arm have demonstrated an increase in motor skill of about 10-15% after one 20-minute session of M1-tDCS [10, 21]. Repeated M1-tDCS application over 3-5 days can sometimes increase performance in young adults by approximately 30% beyond practice alone in relatively simple motor tasks such as isometric pinch grip tasks [17-19]. Nonetheless, there may be further opportunities for improvement by modification of the tDCS parameters (e.g. current strength, duration, electrode montage, stimulation site, number of stimulation sites, timing relative to practice) utilized or by using other methods of non-invasive brain stimulation that are variations of tDCS.

Transcranial alternating current stimulation (tACS) has recently emerged as perhaps the most promising variation of tDCS [22-28]. Although much less research has been done on tACS, research on the technique is increasing rapidly [29] and some initial studies have shown increases in motor skill acquisition similar to tDCS. For example, Sugata et al. (2018) [23] reported that a single session of 70 Hz tACS applied to M1 substantially increased motor skill learning in a twelve-digit motor sequence button pressing task of the right hand compared to SHAM. In addition, these behavioral improvements were accompanied by increased power in the

beta-band as measured by magnetoencephalography (MEG). Similarly, a series of studies by Miyaguchi and colleagues [30-33] all demonstrated increases in motor skill learning, albeit tACS was applied to M1 and the cerebellum (c-tACS) simultaneously. tACS can also increase M1 excitability [22, 34, 35], although perhaps not to the same magnitude as tDCS. However, tACS has a potential advantage over tDCS due to its ability to induce entrainment of large groups of cortical neurons at specified frequencies [22, 36, 37]. This can be accomplished both within a given targeted brain area and between two functionally and anatomically linked brain areas [23, 28, 36, 37]. However, similar to the preponderance of tDCS studies, all tACS related motor skill research has involved a single practice session of relatively simple hand and arm tasks. Furthermore, most studies have not measured changes in M1 excitability and motor skill in the same study. Therefore, it is unknown if tACS can increase motor learning in a complex, multi-joint task involving whole body control and endpoint accuracy, especially in healthy young adults who may have less room for performance improvements compared to older adults or patients with movement disorders.

The primary aim of the study was to assess the impact of M1-tACS applied over three consecutive days of practice on motor learning of a challenging overhand throwing task in young adults. The secondary aim was to examine the influence of M1-tACS on M1 excitability. Based on a series of studies involving M1-tACS [23], and dual site M1-tACS and c-tACS [30-33] in simple motor tasks, it was predicted that tACS application would induce greater enhancements in motor learning in the overhand throwing task compared to SHAM stimulation. Similarly, it was hypothesized that tACS would also increase M1 excitability [22, 34, 35], whereas SHAM stimulation would have no effect. Relatedly, if significant effects of tACS on motor skill learning were to occur relative to SHAM stimulation, it was expected that increases in motor learning

would be positively correlated with increases in M1 excitability, as was been shown in a few early tDCS studies [38, 39]. The overhand throwing task and combination of other task parameters (e.g. small target size, long throwing distance) employed were selected within laboratory space constraints to present a notably complex model motor task to participants for the evaluation of motor learning.

MATERIALS AND METHODS

A total of 24 healthy young adults participated in the study (20 males and 4 females; mean age: 24.9 ± 3.4; 10 men and 2 women in each group). All study participants exhibited right-arm dominance, as indicated by their Edinburgh Handedness Inventory [40] laterality quotient scores. Comprehensive screening ensured the absence of any neurological or psychiatric pathologies, uncontrolled medical comorbidities, or contravention of international transcranial magnetic stimulation (TMS) exclusion criteria [41]. Individuals who were currently actively engaged in recreational, collegiate, or professional throwing disciplines were also ineligible for study inclusion. Prior to enrollment, subjects provided written consent following detailed explanation of the study's objectives and procedures. While participants possessed awareness of the study's overarching goals, they remained blinded to the specific treatment condition assigned to them throughout the experimental process. All experimental procedures were consistent with the ethical guidelines stipulated in the Declaration of Helsinki. Approval for the study was obtained from the Institutional Review Board at the University of Nevada, Las Vegas.

The study implemented a double-blind, SHAM-controlled, randomized, between-subjects experimental design. Overall, the design and methodology nearly identical to two prior studies performed in our laboratory, with the exception that those studies involved tDCS applied to M1

[20] and the cerebellum (c-tDCS) [42], respectively. Participant assignment to either the M1-tACS or SHAM stimulation group was carried out using Research Randomizer (www.randomizer.org) by an investigator not involved in data collection [43, 44]. The SHAM condition served as the control or placebo, utilizing established procedures from previous tDCS and tACS studies. Participants completed three consecutive experimental sessions, each lasting approximately 1.5 - 2 hours, at consistent times of day throughout the entirety of the study. Other than a brief familiarization phase at the start of the first session involving didactic video instruction and throwing demonstrations by investigators, all sessions were identical except for the type of stimulation administered (tACS, SHAM) to each of the two groups. However, the participants themselves did not engage in any familiarization or practice throwing trials before the experimental procedures. Thus, total naivety in this particular overhand throwing task and experimental environment was ensured prior to experimentation. In general, the experimental sessions encompassed the following sequence of steps: 1) pre-test block of overhand throwing trials; 2) TMS testing of M1-tACS effects on M1 excitability; 3), practice blocks of overhand throwing trials concurrent with stimulation 4) post-test block of overhand throwing trials. These steps are described in greater detail in subsequent sections, and a schematic of the experimental protocol is provided in Figure 7. Throughout all experimental conditions, investigators conducting the experiments remained blind to participants' group assignments. Specifically, the investigator responsible for operating the M1-tACS device and applying stimulation was not involved in other experimental procedures [43, 44].

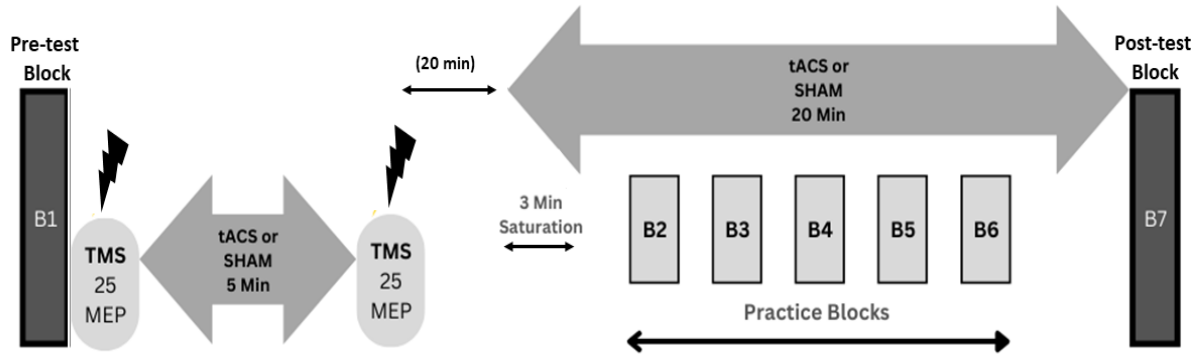

Figure 7 3-Day Study Schematic

Figure 7. Schematic of the experimental protocol of the study. A total of three identical experimental sessions were completed on three consecutive days. A depiction of the one experimental session is shown for illustration. The experimental protocol included a pre-test block of overhand throwing trials, a TMS testing protocol of M1 excitability changes in response to five minutes of tACS, a 20-minute break, five practice blocks of overhand throwing trials performed in association with concurrent tACS application for 20 minutes, and a post-test block of overhand throwing trials

A pre-test block comprising 10 overhand throwing trials was conducted to establish the baseline performance levels for both groups on Day 1, prior to any stimulation application. Similarly, the preliminary evaluations on Days 2-3 followed an identical protocol, serving as baselines uninfluenced by stimulation, but potentially affected by consolidation effects from the preceding day. The selection of 10 trials per block for all preliminary evaluations was based on prior research [20, 42, 45], ensuring adequate baseline data without overly impacting subsequent practice block performance curves. Furthermore, this maintained consistency with the number of trials per block in the practice and post-test phases of the experiments. Finally, the absence of concurrent M1-tACS during the pre-test block facilitated the assessment of the unique contributions of online and offline learning contributions to overall motor learning when combined with the results of the post-test block.

A Magstim 200^2 connected to a double 70mm remote control figure-of-eight coil was utilized to perform single-pulse TMS. The coil was positioned tangentially to the scalp, its handle directed backwards and laterally at a 45-degree angle from the midline, ensuring stimulation over the "motor hot spot" of the first dorsal interosseous (FDI) muscle of the left M1, thereby evoking MEPs in the contralateral FDI muscle of the right hand. The MEP served as an index of M1 excitability, reflecting net corticospinal excitability. EMG activity of the FDI muscle was recorded using surface electrodes arranged in a belly tendon montage, and data was acquired and recorded using Cambridge Electronic Design (CED; Cambridge, UK) hardware (1902 amplifiers, micro 1401 data acquisition interface) and software (Signal 5.04). Participants were positioned with their forearm on a table, wrist in a neutral position, hand prone, elbow flexed to ~90 degrees, and shoulder abducted to ~45 degrees, maintaining consistent posture across recordings [20, 42] to minimize any influences of changing arm position on MEP amplitudes [46]. Continuous monitoring by one investigator ensured the FDI muscle remained at rest during all recordings.

The TMS testing procedures were the same as described in detail in prior tDCS studies [20, 42] and entailed several sequential steps: 1) the FDI hot spot was identified by administering suprathreshold TMS pulses to optimize coil position until the scalp area corresponding to the FDI motor hot spot was identified and marked on a scalp cap [47]; 2) the 1 mV stimulation (%MSO) was determined using the set of methods developed in two previous studies [20, 42]. In brief, a series of TMS pulses were given at a moderate stimulation intensity which was adjusted while MEPs were monitored and monitored online by the Signal software. Once MEP amplitudes were as close as reasonable possible to 1 mV on average, the software was reset; 3), the Pre-TMS test block was performed and 25 MEPs were collected with the previously

determined 1 mV stimulation intensity; 4) 5 minutes of tACS or SHAM stimulation was applied to the left M1; 5) the post-test TMS block was completed as soon as stimulation ended using the same TMS stimulation intensity as before; and 6) a 20-minute rest period (inter-stimulation period) was enforced, ensuring a delay between the end of the 5-minute M1-tACS application and the subsequent beginning of the 20-minute period and associated overhand throwing practice blocks.

This complex paradigm, involving a 5-minute M1-tACS application followed by a 20-minute break, was devised to address methodological concerns surrounding MEP measurement before and after tDCS. Informed by prior research focused on the effects of different tDCS duration protocols on M1 excitability, the paradigm underwent extensive piloting in the laboratory for the prior studies companion studies utilizing M1 and c-tDCS [20, 42]. The paradigm was originally developed with the aim of reconciling three methodological considerations: 1) tDCS applied for 3-5 minutes induces MEP increases lasting 3-5 minutes post-stimulation [48-50]; 2) a 20-30-minute break between tDCS applications sustains MEP increases, while shorter breaks result in inhibition [48, 49]; and 3) tDCS-induced MEP increases can be nullified by muscle contractions, movement, and related activities, potentially rendering post-practice MEP measurement meaningless [51-55]. Thus, the current paradigm was designed to mitigate this limitation while enabling measurement of potential associations between MEP increases and motor learning enhancements [38, 39, 56], under the critical assumption that the second tDCS application had equivalent effects on M1 excitability as the first [48, 49]. Overall, this methodology appeared to work as intended in our aforementioned prior work [20, 42].

The practice blocks were conducted simultaneous with either M1-tACS or SHAM stimulation, encompassing a total practice and stimulation period of 20 minutes (Figure 7). The

practice blocks segment of the study unfolded in several stages. First, the stimulator was activated for 3 minutes while subjects stood quietly before initiating the first block of overhand throwing trials [20, 42, 45]. Second, a sequence of 5 blocks of overhand throwing trials ensued, with each block consisting of 10 overhand throws. These blocks were executed within the remaining 17 minutes of stimulation, with each block requiring approximately 1 to 1.5 minutes to complete, and a 2-minute rest interval was incorporated between blocks. These block parameters and duty cycle of throws were selected in an attempt to optimize the trade-off between performing as many trials as possible while minimizing the potential negative influences of muscle fatigue on muscle activation patterns [57] and motor learning [58]. Finally, the stimulator remained active after the conclusion of the last block of overhand throws, typically lasting 1-2 additional minutes to fulfill the 20-minute stimulation period.

Following the culmination of the practice blocks and the 20-minute stimulation period, participants maintained a stationary stance while the inactive electrode montage persisted on the head. They observed a 5-minute rest interval before engaging in the post-test block, comprising 10 trials. As alluded to above, the execution of the post-test blocks without concurrent M1-tACS facilitated the calculation of both online and offline learning effects on total motor learning.

A NeuroConn DC Stimulator Plus/MR device was employed for the administration of anodal M1-tACS, delivering a current intensity of 1 mA at 70 Hz [23] through a pair of 5 × 7 cm rubber electrodes ensconced within saline-soaked sponges. The anode was positioned directly over the FDI motor “hotspot” of the left primary motor cortex, while the cathode was situated over the contralateral right supraorbital area, which is typically referred to as the M1-SO electrode montage. Securing the anode and cathode in place involved separate rubber elastic straps. As previously noted, M1-tACS was applied for 5 minutes between the TMS pre-test and

post-test blocks, as well as for 20 minutes during the overhand throwing practice blocks, utilizing identical stimulation parameters (70 Hz, 1 mA current strength). During overhand throwing trials, the stimulation device was housed within a small backpack [20, 42, 45], whereas during MEP testing, the stimulator was positioned behind the participant on a table. While alternative M1-tACS parameters exist that may enhance motor learning, the selected combination of M1-tACS frequency, electrode montage, and current intensity were chosen due based on the study of Sugata et. al. (2018) [23]. In that study, not only were large effects on motor leaning due to these tACS parameters observed, but also a functional relation between oscillatory neuronal activity and motor learning as revealed by magnetoencephalography. SHAM stimulation adhered to the established protocol in the field [59] and therefore involved a gradual ramp-up of current over 10 seconds, maintenance at 1 mA at 70 Hz for 30 seconds, and subsequent ramp-down over 10 seconds [60, 61]. This protocol reliably induces similar scalp skin sensations to authentic tDCS and tACS of M1 while eliciting no discernible physiological effects. Notably, participants experience scalp sensations for 1-2 minutes in both conditions before dissipation, rendering them unable to distinguish between real stimulation and SHAM.

The overhand throwing task replicated procedures outlined in a previous single session c-tDCS study [45] and our two previous three-day tDCS studies involving c-tDCS and M1-tDCS [20, 42] that were executed with closely aligned experimental protocols. Participants positioned themselves behind a designated line on the floor, situated 6 meters from a cement wall. Affixed to the wall was a wooden board securely fastened, displaying a laminated poster covered in clear tape. The poster depicted a large overall target area, but with a (1 cm diameter) “bull’s-eye” target at its center. Participants used their dominant right arm to throw a tennis ball in a manner similar to a baseball throw, aiming to achieve precision by targeting the center of the designated

area. Each throw was followed by visual feedback assessment of the ball's endpoint relative to the target center, with participants instructed to minimize error distance on subsequent attempts. An investigator stationed near the participant coated the ball with red chalk both before and midway through each block of 10 trials, marking the final endpoint position upon hitting the target. Subsequently, the same investigator retrieved the ball after rebound and returned it to the participant for the next trial. Each mark, denoted by a trial-numbered circular sticker, was documented by a second investigator stationed near the target area. Following each trial block (interspersed with participant rest intervals), the sticker's x and y endpoint coordinates were measured, recorded, and directly entered into a laptop file by 2-3 investigators working synergistically on those tasks. Finally, the stickers were then removed from the target area between trial blocks, and the process was reiterated for subsequent blocks.

Throughout all trial blocks, the overhand throwing task remained consistent, with participants consistently wearing a snug-fitting backpack housing the tDCS device. Importantly, the tDCS device was activated solely during practice blocks (Figure 7). Therefore, it remaining inactive during the pre- and post-test blocks of overhand throwing trials, but the inert electrode montage was kept on the participant's head. The arrangement of the backpack, stimulator, and associated tACS electrode montage posed no hindrance to task performance [20, 42, 45], ensuring overhand throws were executed under identical experimental conditions and without spatial constraints. Collectively, the combination of the overhand throwing task, diminutive target size, and substantial throwing distance were selected within laboratory constraints to present a notably challenging motor skill.

The unique physiological and biomechanical aspects of overhand throwing that justify it being referred to as a complex motor task have been detailed in depth in a prior publication [20].

In addition, two review articles have described why it is one of the more complex human movements [62, 63]. To briefly summarize here: 1) it is a three-dimensional, unconstrained (many degrees of freedom), multi-joint movement characterized by high joint angular velocities and large amplitudes of movement [62]. Thus, the neural control of the task requires the regulation, exploitation, and prediction of joint interaction torques [64-67]; 2) in general the timing of force pulses is predictive endpoint accuracy [68] and particularly in overhand throwing a large portion of accuracy in the task is dependent on the exact timing of finger forces at release. Specifically, research on skilled and non-skilled throwers has shown that even errors of only 1-2 milliseconds in timing can cause large errors in the endpoint location of the ball relative to the target [69-73]; and 3) the timing of relaxation and activation of not only the throwing hand's finger muscles must be precise, but also many other antagonistic sets of muscles [67, 69]. Collectively, these major factors and several others result in a high speed-accuracy trade-off and formed much of the basis for the selection of the overhand throw as the model motor task for the study.

The primary outcome measure was the endpoint error in the overhand throwing task, while the secondary outcome measure was the MEP amplitude. The endpoint error was determined following methodologies established and briefly described in prior research [20, 42, 47] while detailed procedures for endpoint error calculation in goal-directed tasks can be found in Poston et al. (2013) [74]. The Pythagorean Theorem was used to calculate the shortest distance between the target center's coordinates and the final coordinates of the ball's endpoint. A custom Microsoft Excel program was utilized to compute the endpoint error for each trial, based on the ball's endpoint coordinates. The average endpoint error across the 10 overhand throwing trials within each trial block was considered as the final endpoint error value for analysis. In

contrast, MEP data were subjected to analysis using a custom script in Signal software (Cambridge Electronic Design, Cambridge, UK). MEP size was determined as the peak-to-peak amplitude for each MEP, and the average of the 25 MEPs within each TMS test block was used for analysis [75].

All statistical analyses were conducted using IBM SPSS Version 28.0.1.0. The analysis of endpoint error followed a methodology primarily based on Cantarero et al.'s (2015) [76] three-day c-tDCS study, while also being very similar to our previous work [20, 42, 45]. These procedures involve a series of sequential statistical analyses that allow for the mathematical determination of the unique contributions of online (within-session) and offline (between-session) effects to the overall total learning effects exhibited from the beginning to the end of multi-day studies involving brain stimulation interventions.

Accordingly, the endpoint error analysis proceeded through three steps. First, endpoint error obtained solely from the test blocks underwent a 2 *group* (tADCS, SHAM) × 3 *day* (1, 2, 3) × 2 *test* (pre-test, post-test) three-way mixed ANOVA with the factor *group* being between-subjects and the factors *day* and *test* being within-subjects. This analysis utilized data only from test blocks since no stimulation was applied during these blocks, facilitating comparison with the results of Cantarero et al.'s (2015) [76] as well as those of other three-day tDCS studies by other researchers [17-19]. Second, all of the endpoint error data from each day (test and practice blocks) was analyzed with a two-way mixed ANOVA: 2 *group* (tACS, SHAM) × 3 *day* (1, 2, 3) with the factor *group* being between-subjects and the factor *day* being within-subjects. Accordingly, this analysis used the average endpoint error value across all seven blocks (2 test blocks and 5 practice blocks) performed each day to provide a comprehensive representation of overall daily performance variations, considering the task's difficulty. Relatedly, this was done to

complement the first analysis since our previous work had shown that performance in the test blocks can sometimes differ rather substantially from some of the practice blocks. In addition, this analysis encompassed all block performed on a given day both with (practice blocks) and without (test blocks) stimulation, unlike the first analysis. Third, online, offline, and total learning effects were compared between groups using a series of three separate unpaired two-tailed *t*-tests. This was accomplished using data from specific combinations of the test blocks according to the formulas provided by Cantarero and colleagues [76].

For MEP amplitude, the data underwent a 2 *group* (tACS, SHAM) × 3 *day* (1, 2, 3) × 2 *test* (pre-test, post-test) three-way mixed ANOVA with the factor *group* being between-subjects and the factors *day* and *test* being within-subjects. In addition, similar to two previous studies with the same design [20, 42], it was planned to perform bivariate linear regression analyses to examine the relationship between changes in MEP amplitudes and changes in endpoint error if they were to occur. This would be done for each group between the pre-test and post-test blocks (change in MEP vs change in endpoint error) for each of the three days, but only for participants who exhibited an increase in both variables on given day. In contrast to those studies [20, 42], ultimately these analyses could not be done in the present study because there were not enough participants in either group who demonstrated both an increase in MEP and an increase in endpoint accuracy (lower endpoint error) within any of the three days. Thus, these analyzes were not appropriate based on the outcomes in the present study and provide further support for recent studies that have shown no correlation between increases in MEP amplitude and increases in motor skill learning [20, 42, 51, 56, 77], in contrast to a few small-scale early studies in patient populations [38, 39].

Bonferroni adjustments were applied for post hoc comparisons, where necessary, to identify significant differences. Effect sizes are reported as the partial eta squared for ANOVAs and Cohens *d* for the *t*-tests. The significance level was set at $\alpha < 0.05$ for all analyses and means ± standard errors are depicted in the figures, whereas means ± standard deviations are reported in descriptions in the text.

RESULTS

The 2 *group* (tACS, SHAM) × 3 *day* (1, 2, 3) × 2 *test* (pre-test, post-test) three-way mixed ANOVA revealed that the main effect for *group* ($P = 0.741$, $\eta_p^2 = 0.005$) and main effect for *day* ($P = 0.433$, $\eta_p^2 = 0.037$) were both non-statistically significant. However, there was a significant main effect for *test* ($P = 0.046$, $\eta_p^2 = 0.169$), which indicated that endpoint error was significantly lower in the post-tests compared with the pre-tests when averaged over the three day of practice. In contrast, the *day* × *group* interaction ($P = 0.307$, $\eta_p^2 = 0.052$), *test* × *group* interaction ($P = 0.474$, $\eta_p^2 = 0.024$), *day* × *test* interaction ($P = 0.307$, $\eta_p^2 = 0.052$), and *day* × *test* × *group* interaction ($P = 0.844$, $\eta_p^2 = 0.08$) were all non-statistically significant (Figure 8).

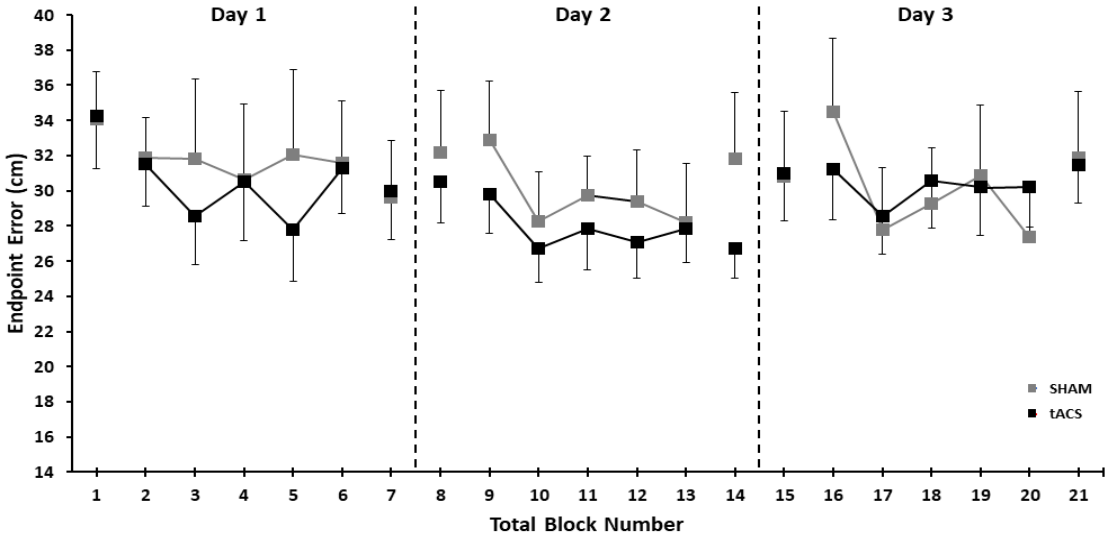

Figure 8 3-Day Endpoint Error by Block

Figure 8. Endpoint error in the overhand throwing task for the tACS and SHAM groups as a function of block number for all of the three days of practice. Over the entire course of practice, there were no significant differences in endpoint error between the tACS and the SHAM groups *(P* = 0.741). Thus, only demonstrated small non-significant improvements in throwing performance from the beginning to the end of practice (no total learning effect). However, endpoint error was significantly decreased in the post-test blocks compared with the pre-test blocks when averaged over all three days of practice (*test* main effect: *P* = 0.046; online effects), but this decrease was not significant between the tACS and SHAM groups (*group* main effect: *P* = 0.474).

The *2 group* (tACS, SHAM) × 3 *day* (1, 2, 3) mixed ANOVA revealed that the main effect for *group* ($P = 0.730$, $\eta_p^2 = 0.006$), main effect for *day* ($P = 0.275$, $\eta_p^2 = 0.057$), and *day* × *group* interaction ($P = 0.600$, $\eta_p^2 = 0.023$) were all non-statistically significant (Figure 9A).

The unpaired two-tailed *t*-tests revealed that the online effects ($P = 0.474$, $d = 0.297$), offline effects ($P = 0.419$, $d = 0.336$), and total learning effects ($P = 0.880$, $d = 0.062$) were all statistically non-significant between the tACS and SHAM groups (Figure 9B).

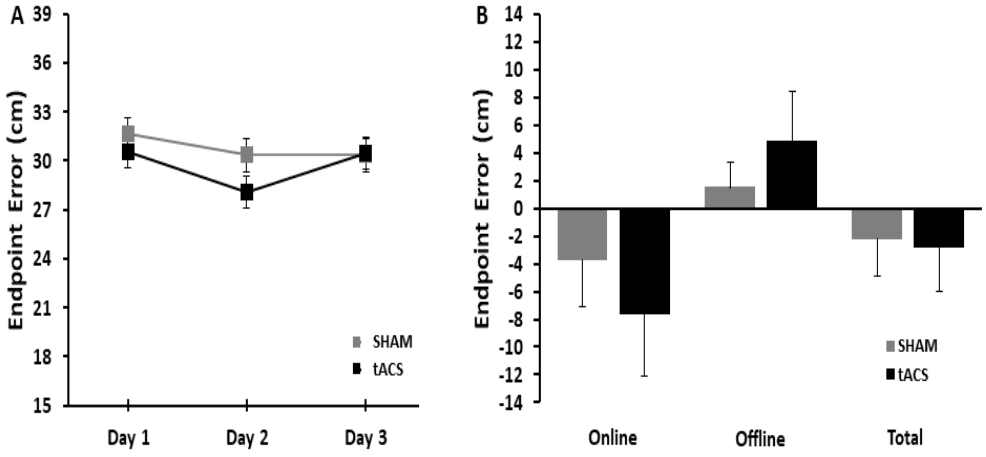

Figure 9 3-Day Endpoint Error

Figure 9. Endpoint error in the tACS and SHAM groups. (**A)** Endpoint error was not significantly different between the tACS and the SHAM group across the three days of practice (*group* main effect: $P = 0.730$). Participants in both groups only demonstrated small improvements in overhand throwing performance over the course of the three days. (B) Online ($P = 0.474$), offline ($P = 0.419$), and total learning effects ($P = 0.880$) were similar between the tACS and the SHAM group. The gains in online learning (reduced endpoint error) during the practice sessions were partially mitigated by offline losses (increased endpoint error) between days, which resulted in only small non-significant improvements in total learning at the end of the three days of practice in both groups.

The 2 *group* (tACS, SHAM) × 3 *day* (1, 2, 3) × 2 *test* (pre-test, post-test) three-way mixed ANOVA revealed that the main effect for *group* ($P = 0.297$, $\eta_p^2 = 0.049$) and main effect for *day* ($P = 0.291$, $\eta_p^2 = 0.054$) were both non-statistically significant. There was a significant main effect for *test* ($P = 0.003$, $\eta_p^2 = 0.337$), which indicated that MEP amplitude was significantly greater in the post-tests compared with the pre-tests. However, the *day* × *group*

interaction ($P = 0.924$, $\eta_p^2 = 0.004$), *test* × *group* interaction ($P = 0.409$, $\eta_p^2 = 0.031$), *day* × *test* interaction ($P = 0.148$, $\eta_p^2 = 0.089$), and *day* × *test* × *group* interaction ($P = 0.813$, $\eta_p^2 = 0.09$) were all non-statistically significant (Figure 10).

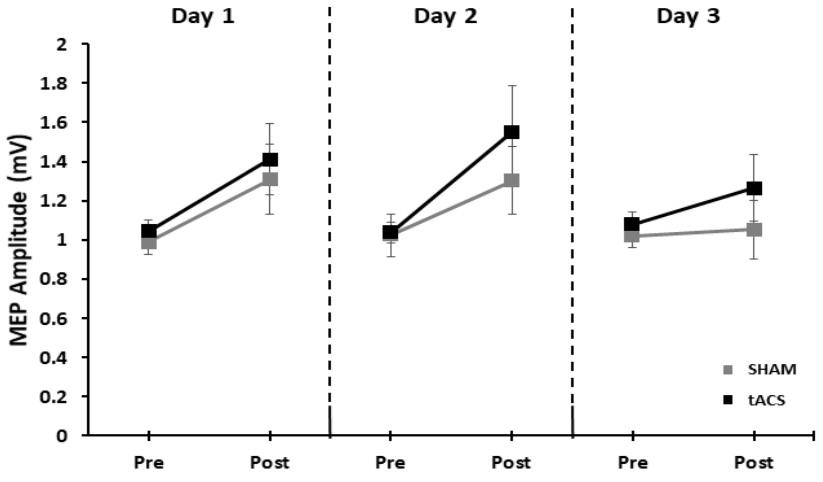

Figure 10 3-Day MEP Amplitude

Figure 10. MEP amplitude for the three days in the pre-tests and post-tests in the tACS and SHAM groups. MEP amplitude was significantly increased between the pre-test and post-tests when averaged over the three days (*test* main effect; $P = 0.003$), but this increase was not significantly different between the tACS and SHAM groups (*test* × *group* interaction; $P = 0.409$).

DISCUSSION

The primary aim of the study was to assess the impact of M1-tACS applied over three consecutive days of practice on motor learning of a challenging overhand throwing task in young adults. The secondary aim was to examine the influence of M1-tACS on M1 excitability. The study yielded five key findings: 1) the total learning effects exhibited in the overhand throwing

task over the three days of practice was not significantly different between the tACS and SHAM groups; 2) both the tACS and SHAM groups displayed only small non-significant increases in total learning effects over the three days of practice; 3) endpoint error significantly declined from the pre-tests to the post-tests during practice when averaged over the three days, but these online (within-session) effects were not statistically different between the tACS and SHAM groups; 4) the contribution of offline effects (between-sessions) to total learning was not significant and did not differ between the tACS and SHAM groups; and 5) M1 excitability as assessed by MEP amplitudes evoked during TMS testing increased from the pre-tests to the post-tests, but these increases were also not different between the tACS and SHAM groups and were not related to motor skill learning. Collectively, these findings indicate that tACS does not improve online, offline, or total motor learning to a greater extent than practice alone in a complex motor task.

Motor skill acquisition is a relatively transient increase in motor performance during and shortly after a practice session, whereas motor learning is a relatively more permanent improvement in movement accuracy realized through longer-time periods and extended practice [1]. It is thought that a minimum of 24 hours [1, 78] must pass from the end of practice until assessment in a retention test for changes in movement accuracy to be defined as motor learning. In addition, many of the physiological mechanisms contributing to skill acquisition and retention during the development of motor learning can be different and dependent on the characteristics of the motor task [1, 78]. Although a number of brain regions contribute to motor skill acquisition and learning [79], decades of research have suggested the M1 plays a major and perhaps dominant role in these processes [1, 80-82]. Therefore, interventions that could impact physiological processes within M1 that underlie motor learning substantial biomedical and practical significance. Accordingly, a large amount of research over the last decade and a half

has indicated that the currently tDCS delivered to M1 before or after motor practice significantly improvements in motor skill. However, the vast majority of this research has involved relatively simple motor tasks that were novel to the participants and practiced in a single stimulation session [10].

Recently, a small but increasing number of studies have also applied tACS to M1 based on the idea that it may possess several features as well as advantages to tDCS that could be relevant to motor learning [23, 28, 36, 37]. However, they have also all involved simple motor tasks performed in association with a single stimulation session [30-33]. To the best of our knowledge, the present study was the first to investigate the influence of M1-tACS over multiple days in a complex motor task. The original central hypothesis was that tACS application would elicit greater enhancements in motor learning in the overhand throwing task compared to SHAM stimulation. Contrary to this prediction, the total learning exhibited in the overhand throwing task from the initial pre-test block on Day 1 to the final post-test block on Day 3 was not significantly different between the tACS and SHAM groups. Specifically, both the tACS and the SHAM groups demonstrated relatively small improvements in overhand throwing accuracy as indicated by reductions in endpoint error of only approximately -6.5% and -8.2%, respectively. This general overall pattern of results was the same if endpoint error was quantified over all the practice blocks completed each day or just in the pre- and post-test blocks. Overall, these findings are mixed relative to the most relevant and applicable previous studies on tACS and motor skill learning.

The present total learning results are not consistent with the findings of Sugata et al. (2018) [23] who reported that a single application of 70 Hz tACS applied with the SO-M1 electrode montage at a current strength of 1 mA elicited substantial motor skill enhancements

compared to SHAM. The motor task involved a sequence comprising 12 button presses by the four fingers of the right hand, which could be viewed as a relatively simple motor task. A strength of the study was that MEG data were collected in the same study and indicated that increased beta-band power accompanied the observed behavioral improvements. Based on these strong and convincing results, this set of tACS parameters were selected for utilization in the current study. Therefore, it is somewhat difficult to reconcile the disparate findings between the two studies. However, that study did apply tACS before task practice and for only 10 minutes, whereas the current study used a 20-minute stimulation duration and the more common tactic of stimulation application during practice. This was done to allow as many practice trials of the overhand throwing task as possible without inducing fatigue. In addition, our previous single day c-tDCS study [45] and a three-day M1-tDCS overhand throwing study [20] both improved motor learning with stimulation applied during practice. Overall, it seems that the differences in the complexity of the motor tasks used in the two studies is the most probable explanation for the conflicting findings. The results obtained here are also in opposition to a series of tACS studies conducted by Miyaguchi and colleagues [30-33] that all demonstrated significant motor skill enhancements in a visuomotor tracking task of the index finger, although these studies applied tACS to M1 and the cerebellum simultaneously. Similarly, Pollok et al. (2015) [28] found that 20 Hz tACS of M1 enhanced a four-digit motor sequence task performed with the right hand. Overall, the most relevant available studies involving M1-tACS or M-tACS in combination with the c-tACS, have provided evidence that these stimulation protocols acutely increase motor skill in hand tasks. The reasons for the conflicting findings between these studies and the current study are unclear, but are likely due to the motor task involved and some differences in the tACS parameters utilized. In contrast, the number of stimulation sessions performed seems much less

likely to have played a role as multi-day stimulation would be predicted to be better able to display positive effects if they exist [17-20, 76].

The one statistically significant finding in the current study was that endpoint error declined from the pre-tests to the post-tests during practice when averaged over the three days, which would reflect online (within-session) effects due to practice. However, this decline in endpoint error was not statistically different between the tACS and SHAM groups. Accordingly, independent of group, task practice led to a small (-5.9%) within-session decrease in endpoint error. Interestingly, these online learning effects were primarily the result of the reductions of endpoint error during practice on Day 1 (-12.7%) and Day 2 (-6.7%) as Day 3 even exhibited an increase (2.5%) between the pre-and post-tests. Although these differences across days did not reach significance (*day* × *test* interaction: ($P = 0.307$), the pattern of results of the mean changes in endpoint error indicate overhand throwing accuracy may have reached a short-term asymptote by the end of the second day of practice. This suggests that in this complex overhand throwing task it may take dedicated and sustained practice over longer-term time scales of perhaps a few weeks for considerable motor skill improvements to be realized [1], at least for the current participant sample. Despite the presence of significant online effects, there was no evidence of significant offline (between-session) effects in either group. Accordingly, tACS application did not lead to significant (positive) between-session effects as would need to be indicated by lower endpoint error in the pre-test block on Days 2 and 3 compared with the post-test block on Days 1 and 2, respectively. Beneficial between-session effects of tACS could have also been demonstrated if the loss of gains in endpoint performance between days was at least less compared with the SHAM group (sometimes referred to as a warm-up decrement) [1, 19], but this pattern of results did not occur. Therefore, both groups displayed an equal increase in

endpoint error between-sessions as quantified by offline effects. Although this increase was not of a magnitude to reach statistical significance, it was great enough to negate much of the positive within-session online effects of practice. Consequently, the combined online and offline effects lead to the small insignificant improvements in total learning effects exhibited by the two groups over the entirety of the three experimental sessions. Based on some prior research and assertions [1, 19] as well as common real-world observations, the warm-up decrement observed in the present study was likely to have been due to the complexity of the overhand throwing task.

Taken together, the results for the online effects, offline effects, and total learning effects were unexpected and contrary to the initial hypotheses. Furthermore, the current findings are mixed relative to the most relevant and applicable previous studies from our laboratory and other research groups. More precisely, those that have involved the application of various non-invasive brain stimulation techniques to M1 or the cerebellum either in simple or complex motor tasks over multiple days. Accordingly, the rationale and experimental design was primarily based on three of our prior studies using the same overhand throwing task [20, 42, 45] and four studies by Reis and colleagues [17-19, 76] that involved 3-5 days of practice a sequential visual isometric pinch grip task (SVIPT) of the index finger and thumb. The current findings are not consistent with our previous 3-day M1-tDCS overhand throwing study that used the same experimental protocol. Specifically, the M1-tDCS group exhibited a significant improvement in total learning compared to the SHAM group who displayed minimal total learning. Interestingly, this significant difference was due to a combination of greater online and offline effects in the M1-tDCS group. Interestingly, the M1-tDCS group in that study achieved a 22% improvement in total learning (-22% endpoint error) compared to -8.2% in the tACS group in the current study, whereas the SHAM group in that study displayed a roughly comparable (-2% versus -6.5%) total

learning value to the current SHAM group. In another study, we applied c-tDCS during only one day of practice of the same overhand throwing task, although a retention test on the next day was used to quantify motor learning. The main findings were that the c-tDCS group enhanced total learning (-13.3% vs +5.4% endpoint error) almost exclusively through online effects (-22% vs -6.4%) compared to the SHAM group. Taken together, the results of that c-tDCS group contrast to those of the tACS group in the current study, although the results of the SHAM groups were similar between the two studies despite the use of one versus three days of stimulation and practice. However, a third study performed in our laboratory provided conflicting results to both of these prior studies. This study incorporated elements of both studies in that it involved the same 3-day experimental design and overhand throwing task but applied c-tDCS. Contrary to our expectations, the endpoint error significantly declined over the three days of practice, but the magnitude of reduction was not significant between the c-tDCS and SHAM groups. Thus, although the difference in endpoint error between the initial pre-test blocks and final post-test blocks were significant and indicated greater overall total learning effects, the between-group total learning effects were not significant indicating that c-tDCS application was not effective. Finally, the total learning effects that emerged independent of group were due to online effects, as the offline effects partly negated the online effects. Thus, the general results of that study show some similarities with the current study in that the stimulation groups did not outperform the SHAM groups. Collectively, our prior studies and the current results suggest that M1-tACS may not be as effective as c-tDCS, and especially M1-tDCS in improving motor learning in this difficult overhand throwing task. However, the challenges presented by the utilization of a complex motor task in a work-intensive experimental design comprising three-days of experiments in different groups of participants suggests caution in the interpretation of the

combined results. Ultimately, multi-day studies incorporating a complex motor task and a within-subjects design with an appropriate washout period may be needed to determine the relative effectiveness of c-tDCS, M1-tDCS, and M1-tACS.

The absence in the literature of any other multi-day M1-tACS studies involving either a simple or a complex motor task precludes direct comparisons with the current results. However, some useful information may be able to be drawn from mixed nature of the current findings relative to the four studies aforementioned multi-day studies by Reis and colleagues [17-19, 76] that involved the SVIPT. Briefly, their initial seminal study found that M1-tDCS applied over five days during practice significantly improved total learning of the SVIPT, almost exclusively due to offline effects [19]. These results were replicated in a subsequent study involving three groups of participants that received M1-tDCS over three days of SVIPT practice [18]. In contrast, three days of c-tDCS applied during practice significantly improved SVIPT total learning, but in this case, it was primarily due to offline effects. Finally, another study reported that SVIPT total learning was significantly enhanced in a M1-SO tDCS group, a bihemispheric M1-tDCS group, and a M1-SO transcranial random noise stimulation (tRNS) group relative to a SHAM group. In all cases, the total learning effects were due to offline effects. Collectively, the lack of M1-tACS effects in the current study are not congruent with the set of findings in all of those four studies. Once again, the most likely explanation for the disparate findings is the relative difficulty of the overhand throwing task. Although the SVIPT is arguably much more challenging than the motor tasks used in the preponderance of tDCS and tACS studies, it cannot be viewed to be as complex as the overhand throwing task (see section *2.5*). Accordingly, further tACS studies comprising both simple and complex motor tasks practiced over several days will

be needed to determine the viability of tACS as an intervention to improve motor skill and learning.

tDCS applied to M1 usually increases M1 excitability as indicated by enhancements in MEP amplitudes evoked by TMS for up to 90 minutes after stimulation [50, 83], although this is not consistent finding as a significant minority of studies have not reported significant effects [84]. However, a much fewer number of studies have assessed the influence of M1-tACS on these outcomes. The few available have also shown that M1-tACS administered with a variety of sets of parameters can also increase M1 excitability [22, 34, 35], although perhaps to a lesser extent compared with tDCS. Accordingly, a unique paradigm that proved successful in two prior studies was used to assess the effects of M1-tACS on MEP amplitude. It initially hypothesized that the M1-tACS group would exhibit enhanced MEPs after stimulation, whereas MEPs would be unchanged in the SHAM group. This expectation was based not only on the aforementioned tDCS and tACS results reported above by numerous research groups, but also the results of our prior 3-day M1 overhand throwing study. In that study, M1-tDCS significantly increased MEP amplitudes on all three days using the identical paradigm, while MEP amplitudes in the SHAM group were unchanged. In contrast, the MEP results of the current study are exceedingly difficult to interpret and were contradictory relative to our original hypothesis. Specifically, MEPs amplitudes increased rather considerably following tACS application, however, the SHAM group also displayed large MEP increases that were not too much lower relative to the tACS group. This resulted in a significant main effect of *test* as MEP amplitudes increased similarly for both groups between the pre- and post-tests, but the increase was not significantly different between the two groups.

This set of results are very counterintuitive as SHAM stimulation does not last long enough to induce real physiological effects and it is commonly also assumed that SHAM stimulation does not induce observable placebo effects. Accordingly, the majority of tDCS and tACS studies have reported non-significant changes in MEP amplitude after stimulation. However, an often overlooked and underappreciated systematic review and meta-analysis on SHAM tDCS and related techniques reported [59] that many studies have reported increases in M1 excitability following tDCS application. Although much fewer studies were available, some data were presented that suggested similar outcomes could occur after tACS. Thus, it is not uncommon for SHAM stimulation to elicit meaningful increases in MEPs relative to baseline. Accordingly, our prior three-day c-tDCS study that also used the same paradigm found the same basic pattern of results as the current study. However, the magnitude of MEP increases in the SHAM group were less pronounced than those reported here (13.9% vs 23% greater relative to baseline). In the current study, the 23% average increase in MEP amplitude in the SHAM group was not statistically different than the 35% increase in the tACS group. Thus, although MEP amplitudes increased after tACS the same result for the SHAM group greatly complicates the interpretation of these results, especially since the 35% increase in the tACS group in the range of those observed in tDCS studies [13]. Overall, we conservatively conclude that tACS likely had a real influence on M1 excitability based on prior studies, the magnitude of the effect, and because the study was a mixed-experimental design. Nevertheless, it cannot be ruled out that placebo effects or the well-known large variability and random variation common in MEP data sets were responsible for these results [13].

Finally, it was originally intended to conduct bivariate linear regression analyses to examine the relationship between changes in MEP amplitudes and changes in endpoint error, if

they were to occur, as in our prior studies with the same design [20, 42]. However, the outcomes deemed this inappropriate as explained in detail above (see section *2.7*). In short, this was due to the small number of participants who exhibited an increase in both variables on given day [20, 42]. This outcome provides further support for the tDCS studies that have shown no correlation between MEP amplitude increases and in motor skill increases [20, 42, 51, 56, 77], which is in contrast to a few small-scale early studies in patient populations [38, 39].

The potential reasons for the lack of statistically significant results are similar to many of those that are commonly given in tDCS and tACS studies that report negative findings. In addition, most of the possible explanations are not necessarily tACS specific, but rather a reflection of general limitations of tDCS related techniques. Non-significant results are often unexpected due to approximately 75% of tDCS motor skill studies having demonstrated positive findings (see tables or Buch et al. (2017) [10]). Nonetheless, it can be seen in that review that a sizable minority of studies displayed negative findings. In addition, the possible factors responsible for the lack of significant findings here are similar to those that our research group has reported in a number of prior studies using different forms of tDCS [42-44, 47, 61] and in a motor system fatigue study involving tACS [60]. Therefore, the most likely and relevant explanations based on tDCS studies and a few unique aspects of tACS are described only briefly. Finally, these same possible reasons for the lack of significant finding have extensive overlap with the limitations of the study and therefore are combined within the list below. First, it could be that the tACS current level involved was not enough to elicit significant effects or that the effects of tACS are manifested through transcutaneous stimulation of nerves on the scalp [85]. Relatedly, there could be interindividual differences [86, 87] in the amount of tACS current that reaches the brain, which would have more of an influence on between-subjects designs. Second,

it is also possible that tACS and other forms of non-invasive brain stimulation may not be as effective for improving complex or well-practiced motor tasks compared to simple or novel motor tasks. Similarly, there could have been ceiling effects due to population being healthy young adults. Third, the tACS parameters may not have been be optimal as many different sets of parameters are possible and others have produced significant improvements in motor skill [28]. Future studies will be needed to systematically isolate and address each of these individual possibilities.

CONCLUSIONS

The current study was the first to investigate the influence of M1-tACS applied over multiple days on motor learning in a complex motor task. Overall, the main findings were that the online, offline, and total learning effects in a complex motor task were not significantly different between the tACS and SHAM groups. Therefore, the results were in contrast to most of the available M1-tACS studies that have investigated simple motor tasks. Further research is needed to understand and determine the ability of M1-tACS to improve motor skill and learning in different motor tasks. There is significant room in these areas as much less work has been done on tACS in these areas compared to other non-invasive brain stimulation methods. Future research should incorporate concurrent physiological and behavioral measurements to characterize mechanisms underlying any observed the effects of tACS on motor skill and learning.

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FINAL CONCLUSIONS

The collective findings from these studies provide a foundational understanding of the effects of non-invasive brain stimulation, specifically tDCS and tACS, on M1 excitability and motor learning. Firstly, the investigation into right M1 excitability during and immediately after tDCS delivered to the left M1 revealed no significant differences in MEP amplitudes between tDCS and SHAM conditions. This suggests that tDCS does not modulate contralateral M1 excitability under the tested parameters. Similarly, the exploration of right M1 excitability during and after left M1-tACS application showed no significant differences in MEP amplitudes between tACS and SHAM conditions, indicating that tACS also does not significantly modulate contralateral M1 excitability with the current parameters.

Furthermore, the investigation into the effects of M1-tACS applied over multiple days on motor learning in a complex task found no significant differences in learning effects between tACS and SHAM groups, contrasting with findings from simpler motor task studies. These results collectively highlight that neither tDCS nor tACS, under the studied conditions, effectively modulate contralateral M1 excitability or enhance motor learning in complex tasks.

Substantial future research is needed to fully understand the influence of tDCS and tACS on both the stimulated and contralateral M1. Such studies should integrate diverse stimulation protocols with multiple physiological and behavioral measurements to elucidate the mechanisms underlying the effects of these techniques on motor skill acquisition and learning. This will be crucial for determining the viability of tDCS and tACS as interventions to improve motor skills and learning in various populations.

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University of Nevada Las Vegas, Las Vegas, Nevada *Jan 2021 - Present*

- Managed multiple concurrent projects while adhering to strict deadlines.

- Created detailed project plans outlining timelines, milestones, deliverables, resources, budgeting.

- Drafted, edited, and submitted grant proposals to large government supported/funded institutions, including the NIH.

- Designed advanced human research protocols and gained invaluable experience working with the IRB.

- Utilized advanced problem-solving skills to troubleshoot complex issues related to data collection or analysis processes.

- Provided guidance and mentorship to junior researchers (undergraduate, graduate, and medical students) on project methodology and analysis techniques.

Senior Anatomy Physiology Lab Instructor

University of Nevada Las Vegas, Las Vegas, Nevada *Jan 2020 - Present*

- Designed and implemented lab curriculum, including advanced dissection techniques, in order to bolster the retention of lecture material.
- Created and issued weekly quizzes, developed lab activities, and discussed real-world case studies in order to foster creative thinking and practical application
- Collaborated with other faculty members to develop new lab protocols in order to achieve department objectives
- Launched and methodically refined online, hybrid, and in-person Anatomy/Physiology labs during COVID-19 while strictly adhering to both state and institution guidelines.

Undergraduate Teaching Assistant (Anatomy/Physiology)

University of Nevada Las Vegas, Las Vegas, Nevada *Jan 2019 - May 2019*

- Promoted a safe, respectful, and inclusive learning environment for all students.
- Mentored pre-health students on academic success strategies, such as time management, securing internships, and study skills.
- Adapted instruction techniques based on individual student needs.

WORK EXPERIENCE

Director Of Health

Blind Center of Nevada, Las Vegas, NV *Feb 2022 - Apr 2023*

- Developed and implemented strategies to improve patient satisfaction and safety standards.
- Collaborated with other departments on initiatives such as outreach programs and community engagement initiatives.
- Oversaw recruitment process for new hires providing input on hiring decisions based on qualifications.

- Created an internal system for tracking patient outcomes which was used as a benchmarking tool for future improvement initiatives.
- Organized and managed multiple teams of health care professionals including - physicians, nurses, social workers, therapists, dieticians and pharmacists.

Outpatient Physical Therapy Manager

Kelly Hawkins, US *Oct 2012 - Aug 2017*

- Designed, implemented, executed, and recorded exercise/rehabilitative programming for patients
- Provided therapeutic modalities such as electrical stimulation, ultrasound, massage, traction, and cryotherapy
- Patient/Customer care including maintaining medical records and collaborating with healthcare providers
- Training and mentoring physical therapy technicians

SKILLS

- Student Interaction
- Educational Strategies
- Neuromodulation
- Health Science Research
- Anatomy/Physiology
- Neuroscience
- Neurophysiology
- Undergraduate/graduate/Medical student Mentoring
- Student Rapport-Building
- Student Development
- Creative Instruction Style
- Academic Lecturing
- Academic Writing
- Academic Interdisciplinary Collaboration
- Neurological Disorders
- Community Outreach

CERTIFICATIONS

- Certified Personal Trainer ISSA
- OSHA 30 Occupational Safety and Health Administration

- CPR, AED, & Basic First Aid
  American Red Cross
- Eagle Scout Award Boy Scouts of America

COMMUNITY SERVICE

Seminary Supervisor

Church of Jesus Christ of Latter-Day Saints, Las Vegas, NV *Jan 2019 - Present*

- Supervise seminary faculty
- Track student attendance and manage transcripts
- Counsel and provide ecclesiastical support to faculty and students

PUBLICATIONS & MANUSCRIPTS IN PREPARATION

PUBLISHED ABSTRACTS

- **Wilkins EW,** Kawana E, Lopez Mora E, Houston D, Young RJ, Boss S, Riley ZA & Poston B. The influence of transcranial direct current stimulation on contralateral primary motor cortex excitability. *Society for Neuroscience Abstracts*. Washington DC, November 2023.
- Boss R, Premyanov MI, Noorda KJ, **Wilkins EW,** Aynlender D, Davidson R, Pantovic M, Hagans T, Riley ZA & Poston B. The influence of transcranial alternating current stimulation of primary motor cortex on overhand throwing performance. *Society for Neuroscience Abstracts*. San Diego, California, November 2022.
- Pantovic M, Pudar N, Albuquerque LL, Morris D, Broeder S, **Wilkins EW**, Landers MR, Mari Z & Poston B. The influence of long-term transcranial direct current stimulation on motor learning in Parkinson's disease. *Society for Neuroscience Abstracts*. Chicago, Illinois, November 2021.
- Pantovic M, Pudar N, Albuquerque LL, Morris D, Broeder S, **Wilkins EW**, Landers MR, Mari Z & Poston B. The effects of long-term application of transcranial direct current stimulation on transfer of motor learning in Parkinson's disease. 4th International Brain Stimulation Conference. Charleston, South Carolina, December 2021.
- Morris D, Pudar N, Clingo M, Albuquerque LL, Pantovic M, **Wilkins EW**, & Poston B. The influence of inter-trial interval on transcranial magnetic

stimulation measurement of short- interval intracortical inhibition. 17/18th Annual World Congress of the Society for Brain Mapping and Therapeutics (SBMT). Los Angeles, California, July 2021.

- Albuquerque LL, Lidstone DE, Pantovic M, Munoz I, Zurowski M, **Wilkins EW**, Dufek JS, & Poston B. The influence of cerebellum transcranial direct current stimulation applied over multiple days on motor learning in a throwing task. Society for Neuroscience Abstracts. Chicago, Illinois, October 2019.

PUBLICATIONS

- Pantovic M, Lidstone DE, Albuquerque LL, **Wilkins EW,** Munoz IA, Aynlender DG, Morris D, Dufek JS, & Poston B. Cerebellar transcranial direct current stimulation applied over multiple days does not enhance motor learning of a complex overhand throwing task in young adults. *Bioengineering.* In Press, 2023.

- Pantovic M, Albuquerque LL, Mastrantonio S, Pomerantz AS**, Wilkins EW**, Riley ZA, Guadagnoli MA & Poston B. Transcranial direct current stimulation of primary motor cortex over multiple days improves motor learning of a complex overhand throwing task. *Brain Sciences.* In Press, 2023.

- Ananya S. Chauhan, Eliza Clinton, **Wilkins EW**, Richard Young & Brach Poston. The influence of Bihemispheric Transcranial Direct Current Stimulation of Primary Motor Cortex on Muscle Fatigue. *Bioengineering.* In Press, 2024

CURRENT PROJECTS (IN PREPARATION)

- **Wilkins EW**, Kawana E, Lopez Mora E, Houston D, Young RJ, Boss S, Riley ZA & **Poston B**. The influence of transcranial direct current stimulation on contralateral primary motor cortex excitability. Target Journal: *Biomedical Engineering*.

- Pantovic M, Albuquerque LL, Morris D, Broeder S, Nackaerts E, Lidstone DE, Landers MR, **Wilkins EW**, Mari ZK, & Poston B. Long-term transcranial direct current stimulation in Parkinson's disease: study protocol for a randomized, controlled clinical trial.

- The influence of transcranial alternating current stimulation of primary motor cortex on overhand
  throwing performance **(Dissertation Project)**

- The influence of transcranial alternating current stimulation of contralateral motor cortex excitability (**Dissertation Project**)

- The influence of transcranial alternating current stimulation of contralateral motor cortex excitability (**Dissertation Project**)

- The influence of bilateral dual source transcranial direct current stimulation on the progression of muscle fatigue.

- The effect of dual source premotor cortex transcranial direct current stimulation on muscle fatigue in hand muscles

- The influence of dual source cerebellar transcranial direct current stimulation on muscle fatigue

- The effect of transcranial direct current stimulation of the dorsolateral prefrontal cortex on muscle fatigue resistance

REFERENCES

Dr. Brach Poston
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