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Cerebellar Transcranial Direct Current Stimulation for Motor Skill Acquisition in a Throwing Task

Austuny Jackson
University of Nevada, Las Vegas

CEREBELLAR TRANSCRANIAL DIRECT CURRENT STIMULATION FOR MOTOR SKILL ACQUISITION IN A THROWING TASK

By

Austuny K. Jackson

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School of Allied Health Sciences
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Austuny K. Jackson

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Brach Poston, Ph.D.
Examination Committee Chair

Kathryn Hausbeck Korgan, Ph.D.
Graduate College Interim Dean

Richard Tandy, Ph.D.
Examination Committee Member

Mark Guadagnoli, Ph.D.
Examination Committee Member

Daniel Young, Ph.D.
Graduate College Faculty Representative

ABSTRACT

Transcranial direct current stimulation (tDCS) is a non-invasive brain stimulation technique in which a very weak electrical current is applied to the scalp to either increase (anodal stimulation) or decrease (cathodal stimulation) the excitability of a selected brain region, most commonly the motor cortex. tDCS is a promising intervention that can modulate cortical excitability, enhance motor learning, and improve motor function in healthy subjects, older adults, stroke patients, Parkinson's disease, and in other cognitive and motor disorders. Recently, cerebellar transcranial direct current stimulation (c-tDCS) has started to be examined using similar protocols as existing ones used in studies of tDCS applied to the motor cortex and has been able to improve performance in simple arm movement tasks in young and old adults. This study was is set out to evaluate the influence that c-tDCS has on accuracy and variability of a complex, multi-joint throwing task in younger adult population. A total of 24 (n = 12 per group) healthy young adult males were allocated to either a c-tDCS group or a SHAM stimulation group. Each subject participated in two experimental sessions (practice session, retention session) performed on consecutive days. In the first session (practice session), subjects performed the throwing task in a baseline testing block, followed by 6 practice trial blocks. The practice blocks were followed by a post-testing block. For the practice blocks only, subjects performed the throwing task for 25 minutes in combination with either c-tDCS or SHAM stimulation. In the second session (retention session), subjects perform a retention test (1 block of trials of the throwing task) 24 hours after the practice session to quantify the magnitude of motor learning experienced by the two groups.

The primary dependent variable was the endpoint error, whereas the endpoint variance was selected as the secondary dependent variable. For the test blocks, the dependent variables were analyzed by two-factor repeated measures ANOVAs: 2 *Group* (c-tDCS, SHAM) x 3 *Test* (BASELINE, POST, RETENTION). For the practice blocks, the dependent

variables were analyzed by two-factor repeated measures ANOVAs: 2 *Group* (c-tDCS, SHAM) x 6 *Block* (1, 2, 3, 4, 5, 6). For the endpoint error in the test blocks, there were no significant differences between the two groups of subjects for any of the testing blocks. However, independent of group, endpoint error was significantly lower for the post-test block compared with the baseline test block (P = 0.004). Furthermore, endpoint error was similar between the retention test block and the baseline test block. Finally, the difference in endpoint error between the retention test block and the post-test block barely failed statistical significance (P = 0.063). For the practice blocks, the results indicated that there were no significant differences in endpoint error between the c-tDCS and SHAM groups (P = 0.148). Furthermore, endpoint error was not different for any of the practice blocks, which indicated that endpoint error did not decrease significantly with practice. For the endpoint variance in the test blocks, there was a significant (P = 0.034) GROUP x TEST interaction. Conversely, the post hoc analysis shows that the interaction came close, but missed statistical significance (P = 0.107 and P = 0.067) for lower endpoint variance in the c-tDCS group compared with the SHAM group for the post test block and retention test block, respectively. However, the difference between the groups for the baseline test was not significant (P = 0.824). For endpoint variance in the practice blocks, the results indicated that there were no significant differences between the c-tDCS and SHAM groups (P = 0.152). Furthermore, endpoint variance was not different for any of the practice blocks, which indicated that endpoint variance did not decrease significantly with practice. The data suggest that a one time acute application of c-tDCS does not improve the motor skill acquisition or retention in a complex, multi-joint throwing task in young adults compared to practice alone (SHAM stimulation).

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CHAPTER 1

INTRODUCTION

Non-invasive brain stimulation methods have recently emerged as interventions to improve motor performance in both healthy subjects and a number of patient populations[1-13]. In particular, transcranial direct current stimulation (tDCS) appears to be the most effective and practical non-invasive brain stimulation method based on the available literature. tDCS application involves passing a constant, direct current between two electrodes placed on the scalp with the aim of either increasing (anodal stimulation) or decreasing (cathodal stimulation) excitability of a specific brain region[14,15]. The most common finding is that a single 10-20 minute application of anodal tDCS to the primary motor cortex usually results in increases in motor performance of approximately 10-15% in tasks involving the hand and arm musculature. These acute performance enhancements are thought to be at least partially due to the increases in the cortical excitability induced by the stimulation[14,15]. Furthermore, the observed cortical excitability increases mimic those seen following motor practice and are thought to represent use-dependent plasticity in the motor cortex[16]. Most importantly, tDCS may be able to improve motor performance to a greater degree than can be achieved practice alone, which would have significant implications for motor learning in healthy populations as well as in rehabilitation therapy for patient populations[11,12].

The vast majority of experimental tDCS studies have involved tDCS of the primary motor cortex while other important motor areas that are accessible to stimulation have received much less attention. For example, tDCS applied to the premotor cortex and supplementary motor area have each only been investigated in one study. In addition, several recent experimental studies have shown that tDCS applied to the cerebellum (cerebellar tDCS; c-tDCS) can also lead to improvements in motor function that are similar or even greater than tDCS applied to the primary motor cortex[17-21]. For example, two studies by

Celnik and colleagues found that c-tDCS improved motor performance in young adults on 2-dimensional arm movement tasks performed in the horizontal plane[17,20]. Furthermore, c-tDCS improved arm movement performance in older adults to such an extent that performance became equal to that of young adults[20]. However, these studies all involved rather simple laboratory tasks that were novel to the subject. Thus, it is unknown whether c-tDCS can improve motor skill acquisition and motor leaning to a greater extent than can be achieved by practice alone in a familiar, complex motor task in healthy young adults.

Purpose of study

Despite the promising findings of the currently available c-tDCS studies, all of these investigations have only examined simple laboratory motor tasks such as seated planar arm movements. Based on these aforementioned limitations, this study was intended to determine the influence of c-tDCS on accuracy and variability in young adults, while they performed a complex, multi-joint throwing task. This was accomplished by having two groups of subjects perform tennis ball throws to a target over a 25 minute practice session while either real c-tDCS or SHAM stimulation was applied, followed by a retention session 24 hours later involving follow-up testing of throwing performance. Thus, the practice session quantified motor skill acquisition, whereas the retention session quantified the amount of motor learning that occurred. A throwing task was chosen because cerebellum's involvement in throwing tasks has been well-characterized. Furthermore, the cerebellum has been implicated in tasks that involve the coordination of multi-joint movements, planning and compensation for the effects of joint interaction torques, and the refinement of motor commands to increase accuracy on a trial by trial basis. It was hypothesized that c-tDCS would significantly improve the rate of motor skill acquisition and the amount of motor learning compared to motor practice alone.

Research hypotheses

Hypothesis 1

H_{01}: c-tDCS will have no effect on both accuracy and variability in the throwing task.

H_{A1}: c-tDCS will improve accuracy and lower variability in the throwing task.

CHAPTER 2

REVIEW OF RELATED LITERATURE

Non-invasive brain stimulation overview

Transcranial direct current stimulation is a non-invasive brain stimulation technique in which a very weak electrical current is applied to the human scalp to either increase (anodal stimulation) or decrease (cathodal stimulation) the excitability of a selected brain region[22,8-10,23,13]. The vast majority of motor system studies have targeted the primary motor cortex with tDCS, although the pre-motor cortex, dorsolateral prefrontal cortex, supplementary motor area, posterior parietal cortex, and the cerebellum have also been stimulated in a few studies. Regardless of the stimulation site, tDCS is painless and depending on the stimulation parameters, any acute effect induced by tDCS from a single application wears off within 5 to 90 minutes after stimulation ceases[14,15]. Recently, tDCS has emerged as a promising intervention that can influence cortical excitability, enhance motor learning, and improve motor function in healthy subjects, older adults, stroke patients, Parkinson's disease, and in other cognitive and motor disorders[8-10,23,13,1-5,24,6,7]. Accordingly, there are over 100 clinical trials involving tDCS listed on www.clinicaltrials.gov that have been recently completed or are currently active. Furthermore, the number of research studies that have utilized tDCS has increased dramatically in the past few years from a little over 100 in 2008 to well over a thousand at the present time.

The history of tDCS

The use of electrical currents to impact brain function surprisingly goes back for at least approximately 2000 years[10]. The Roman doctor, Scibonius Largus, and the Greek physician Claudius Galen, both reportedly placed live torpedo fish on the scalps of human patients to relieve headaches. Since these initial applications, the technique of electrical brain stimulation has been forgotten and periodically revived several times over the past 2000

years. For example, around 1790, the Italian scientists, Galani and Volta, made numerous important electrophysiological discoveries including the use of torpedo fish to treat depression[10]. In the late 1800's and early 1900's, scientists in the United States and England both successfully used electric and magnetic fields to stimulation the visual and taste areas of the brain. Most importantly, animal studies in the 1950's and 1960's demonstrated several important aspects of electrical brain stimulation such as the ability of direct currents to increase (anodal stimulation) or decrease (cathodal stimulation) the spontaneous firing rates of neurons in the exposed cortex of rodents. However, this research was not pursued in humans at the time as complimentary techniques did not exist to non-invasively study the effects of tDCS in humans.

In the last 10-12 years, tDCS research has been revived again by researchers such as Doctors Priori of Italy and Nitsche of Germany. Modern techniques such as functional magnetic resonance imaging (fMRI) and transcranial magnetic stimulation (TMS) have now allowed the effects of tDCS to be easily, painlessly, and non-invasively studied in humans[24,8,9,23,13]. The vast majority of these original and the currently available studies have targeted the primary motor cortex with tDCS due to its importance in movement control and its easy accessibility for study with TMS. Thus, modern tDCS studies have been able to provide a large amount of physiological, behavioral, and clinical data regarding the effects of tDCS on humans when coupled with contemporary, complimentary neurophysiology techniques.

tDCS application involves passing a constant, direct current through a pair of rubber electrodes encased in saline soaked sponges that are placed on the scalp. The electrodes most commonly vary in size from 25 cm^2 and 35 cm^2 with the most common arrangement involving placing the anode over the motor cortex and the cathode over the contralateral eyebrow. In this case, this electrode montage is referred to as anodal stimulation as the

current flows from cathode (negatively charged) to the anode (positively charged) electrode. The net result of this montage is an increase in the excitability of the cortical neurons under the anode. These results are similar to the aforementioned animal studies where the electrode was applied directly on the surface of the exposed cortex. Accordingly, human studies have demonstrated similar effects by application of tDCS by the method described above when applied non-invasively to the scalp.

The intensity of tDCS (current strength) is another important parameter of stimulation that has received considerable scrutiny and can now be applied within certain guidelines[25]. To be effective, the current must be large enough to change neuronal activity and behavior. Studies involving monkeys have shown that approximately 50% of the current applied transcranially enter the brain through the skull and these results seem to hold in humans[8]. Thus, relatively weak currents between 0.5 mA and 2 mA seem to be adequate to change cortical excitability and influence motor performance in humans. Accordingly, most studies have used and found a current strength of 1 mA to be effective, although a current strength of 2 mA is being increasingly used, especially in cognitive studies. Under these conditions, tDCS does not directly induce neuronal action potentials and excitability modifications in the way that repetitive transcranial magnetic stimulation does, but instead modifies the spontaneous firing rates of neurons. Thus, this enhances the net excitability of the population of neurons impacted by the electrical field. These increases in excitability are important because they resemble the increases in excitability of motor cortex neurons following normal practice of a motor task[16]. Thus, it seems that the external electrical field may augment this process and that this may be one physiological mechanism underlying the effects of tDCS on motor performance, at least during acute, one-time applications (see below).

The timing effects of tDCS application have also been relatively well-defined in recent studies. In a classic study by Nitsche and colleagues, tDCS was given for various

periods of time, and cortical excitability was increased following tDCS scaled with the amount of time that tDCS was applied. In the longest time period studied, tDCS applied for 13 minutes at 1 mA led to excitability increases for at least 90 minutes following the end of stimulation[14]. Further studies involving both performance and excitability measures seemed to generally support this basic finding[3,15]. Therefore, tDCS is most commonly applied for 20 minutes in current studies with a current strength of 1-2 mA. Another important timing issue is whether tDCS should be applied before, during, or after a motor task to induce increases in motor performance. Accordingly, a numerous studies that have applied anodal tDCS to the hand area of the motor cortex either before or during motor training have enhanced motor performance in a variety of populations[22,8,9,23,13]. However, application of tDCS after motor training does not seem to improve performance compared to practice alone without tDCS.

Another important area of tDCS research is the ability to provide SHAM stimulation, which is especially important in clinical trials as novel interventions are well-known to elicit significant placebo effects. Fortunately, it has been shown that it is much easier to successfully perform SHAM-control tDCS studies compared to other brain stimulation methods such as TMS[8,23]. This is because in tDCS studies the current can be ramped up and down over a 30-60 second period, a protocol that elicits no measurable physiological or performance effects but leads to nearly identical skin sensations as real stimulation that lasts for 20 minutes. In both cases, the subject normally feels a light itching, burning, or warm sensation for 1-2 minutes. Thus, SHAM and real stimulation are not able to be discriminated between for the vast majority of subjects[17].

tDCS to improve skill acquisition and learning in healthy adults and motor disorders

Anodal tDCS applied over motor cortex in a single application of sufficient magnitude and duration can increase cortical excitability and improve performance in a variety of laboratory tasks involving hand and arm movements in healthy subjects[11,12], older

adults[6], stroke[4,5,7], PD[1-3], and other populations. In these studies, the performance improvements usually reach 10-15%, whereas the excitability increases are on the order of 20-40%. As mentioned previously, the ability to safely and reliably alter cortical excitability is important because increased cortical excitability following practice of a task has been interpreted as an indicator of use-dependent plasticity in the motor cortex[16]. Accordingly, the short-term increases in cortical excitability have been associated with improvements in motor function. For example, tDCS improved United Parkinson's Disease Rating Scale (UPDRS) scores, increased cortical excitability[3], and the increased cortical excitability was associated with the improved UPDRS scores[3]. However, this study and the other aforementioned studies were all acute studies that measured performance when cortical excitability was transiently increased during and after tDCS. Nonetheless, chronic tDCS applied for 5 straight days increased the total amount of motor learning in healthy adults by a magnitude of almost 40%[12] greater than practice alone and the effects persisted for 2 weeks after stimulation[12]. A similar study by the same researchers found similar effects after 3 consecutive days of tDCS of the motor cortex in healthy young subjects[11]. Collectively, these studies highlight the potential of tDCS as an adjunct therapeutic intervention to improve motor function in a variety of populations, especially the elderly or those with motor disorders who seem to experience even greater positive effects than young adults as they have more room to improve[6]. Finally, tDCS has advantages compared to other brain stimulation techniques that are used to improve motor function. For example, tDCS offers several important clinical and scientific advantages over repetitive TMS such as portability, safety, ease of administration, ability to be delivered during motor activities or task practice, a superior ability to blind subjects with sham stimulation, and low cost (as low as $400 versus $20,000-100,000 for rTMS)[8,23].

Cerebellar tDCS (c-tDCS)

Almost all motor system tDCS studies have applied tDCS to the motor cortex and other important motor areas involved in movement control, such as the cerebellum, have only been recently investigated in a few studies. This is to be expected as the motor cortex projections to upper limb motor neurons play the predominant role in the generation and execution of skilled movements. However, motor cortex output depends on inputs from sources such as premotor cortex, contralateral motor cortex, and basal ganglia along with crucial contributions from cerebellum, which is strongly involved in movement timing, multi-joint coordination, agonist and antagonist muscle interactions, and error detection in goal-directed movements. These facts, along with evidence that tDCS can influence interconnected brain regions not directly stimulated (cerebellum has strong connections with basal ganglia and motor cortex) form the basis for targeting the cerebellum with tDCS.

Recently, c-tDCS has been examined using similar protocols to the ones used in studies of tDCS applied to the motor cortex. To date, it has been found that c-tDCS can improve motor performance in young[17] and older adults[20] primarily in simple laboratory tasks involving two-dimensional arm movements, but it also was able to improve the gait adaptation following a perturbation in young adults[21]. Most importantly, c-tDCS even lead to greater improvements in an arm movement task compared to tDCS of motor cortex in young adults[17], although the task conditions in this study may have been more dependent on the cerebellum. Collectively, these factors and the positive effects on motor performance obtained in several studies involving c-tDCS in young and older adults provide strong rationale for the further investigation of c-tDCS as a method to improve motor performance. Specifically, studies need to be done with focus on more complex, gross body movement tasks, as most studies to date have focused on relatively simple laboratory tasks.

Most of the stimulation parameters for c-tDCS (current strength, stimulation duration, stimulation timing) are similar to those used for tDCS of the motor cortex. Accordingly, nearly all studies using cerebellar tDCS have used 1mA – 2mA current strengths, 15 min – 25 min stimulation durations, stimulation concurrent with the motor task, and used electrodes that are 5cm x 5cm – 5cm x 7cm. The electrode montage for c-tDCS, however, is obviously different and there are a couple of different electrode montages for c-tDCS. The most widely used montage is placing the anode 1 – 2cm below and 3 – 4cm lateral to the inion, with the cathode referenced on the ipsilateral buccinator muscle[17,20,21]. The preparation for cerebellar tDCS is similar to the application of tDCS to the primary motor cortex. The skin at the site of the electrodes is cleansed thoroughly with alcohol before the electrodes are placed and the impedance is reduced to a minimum.

tDCS safety

The application of tDCS in humans represents an off-label use of existing clinically-approved electro-therapy devices such as iontophoresis and neuromuscular electrical stimulation system techniques that are used for peripheral nerve and muscle stimulation. These devices have been used extensively for decades in clinical practice in several settings including sports medicine, athletic training, physical therapy, stroke rehabilitation, spinal cord injury, and pain management. According to the FDA, a significant risk device is one that has the following characteristics: (1) is intended as an implant and presents a potential for serious risk to the health, safety, or welfare of a subject; (2) is purported or represented to be for the use of supporting or sustaining human life and presents a potential for serious risk to the health, safety, or welfare of a subject; (3) is for a use of substantial importance for diagnosing, curing, mitigating, or treating disease, or otherwise preventing impairment of human health and presents a potential for serious risk to the health, safety, or welfare of a subject; or (4) otherwise presents a potential for serious risk to the health, safety, or welfare

of a subject. Conversely, a non-significant risk device is one that does not meet the above definitions for a significant risk device. Thus, tDCS represents a non-significant risk device because it does not fit any of the four criteria for a significant risk device.

tDCS has been used in various related forms on humans and animals for decades and no evidence suggests that it is harmful or has ever induced a serious side effect, when used within modern specified guidelines (current strength, electrode size, and stimulation duration, etc). Accordingly, these parameters have been investigated to establish safe and effective stimulation parameters for the application of tDCS in research involving human subjects. The only side effects that have been reported when the aforementioned guidelines are followed are temporary tingling, itching, headache, or skin redness under the electrodes in some subjects[8,25]. For example, a 2008 review of the approximately 100 human tDCS studies on several thousand healthy adults and patients found that 64 of these studies reported no side effects, 24 studies reported a temporary itching or tingling under the electrodes in some subjects, and only one study reported skin redness. Furthermore, these slight side effects were of equal occurrence in subjects that received placebo stimulation in 7 studies. In addition, only two subjects in these 100 studies reported a mild headache. Similar findings have recently been reported in research and review articles[1,25,23,13].

Physiological studies have also assessed the safety of tDCS when applied within the aforementioned stimulation guidelines. For example, neuronal damage was not present when measured as serum neuron-specific enolase[13]. Furthermore, tDCS did not negatively alter measures of neuropsychological function and EEG activity[26]. Accordingly, rat studies using tDCS models emulating tDCS applied to humans[27] showed that the current density needed to damage tissues or create lesions was about 1429 mA/cm^2, whereas the current densities used in human studies are usually between 0.04 and 0.08 mA/cm^2.

Although most of these studies involved tDCS to cortical areas and not c-tDCS, it is generally believed that the same safety principles apply[18,19]. Thus, similar current strengths and durations that have been successfully used on cortical areas have also been used in all of the c-tDCS studies in humans with no adverse events[17-21]. Finally, research has shown that it would require current densities of well over a thousand times higher than the current densities used in this and other c-tDCS studies to induce damage to neurons in the brain. Specifically, as mentioned above the current density needed to induce tissue damage or lesions was about 1429 mA/cm^2, whereas the current densities used in c-tDCS studies are no higher than 0.08 mA/cm^2. In conclusion, the c-tDCS stimulation parameters used in the currently available literature are either similar or identical to the all of those used in the tDCS of the motor cortex literature and have been proven to be exceptionally safe and well-tolerated.

In summary, the safety boundaries of the stimulation parameters for tDCS and c-tDCS have been relatively well-identified in the literature and have been proven to be exceptionally safe. Nonetheless, most researchers take very conservative precautionary measures to further minimize the any risks associated with tDCS application such as excluding subjects who have had seizures, other serious uncontrolled medical conditions, metal in the eye or skull, or hearing loss. Finally, subjects should be continually monitored throughout the stimulation periods by study personnel to further reduce the risks associated with stimulation.

CHAPTER 3

METHODOLOGY

Participant characteristics

Twenty-four males were recruited for the study (age range: 18-30). All participants were free of any neurological or psychiatric condition and were right-handed according to the Edinburgh Handedness Inventory. Potential participants who were regularly engaged in throwing sports were excluded as well as those who had played a high school or college sport that involved throwing such as baseball or a quarterback in football.

Experimental design

The study was a randomized, sham-controlled, double-blind experimental design. Subjects were allocated either to a c-tDCS or a SHAM group and each subject completed two experimental sessions performed on consecutive days. In the first session (practice session), subjects practiced a throwing task in association with either c-tDCS or SHAM stimulation. In the second session (retention session), subjects performed retention testing of the throwing task (no c-tDCS applied) to quantify the amount of motor learning that was potentially elicited by the two types of stimulation that were applied in the previous practice session.

Experimental procedures

Experimental Sessions. Each participant completed a practice session and a retention session on consecutive days. The practice session proceeded in the following order of steps: 1) a verbal explanation of the tennis ball throwing task along with the viewing of an instructional video of the task; 2) c-tDCS electrode placement; 3) baseline test block; 4) practice blocks; 5) post-test block. In the retention session on the following day, one block of trials of the throwing task was performed. The order of experimental procedures for the two days is depicted in Figure 1.

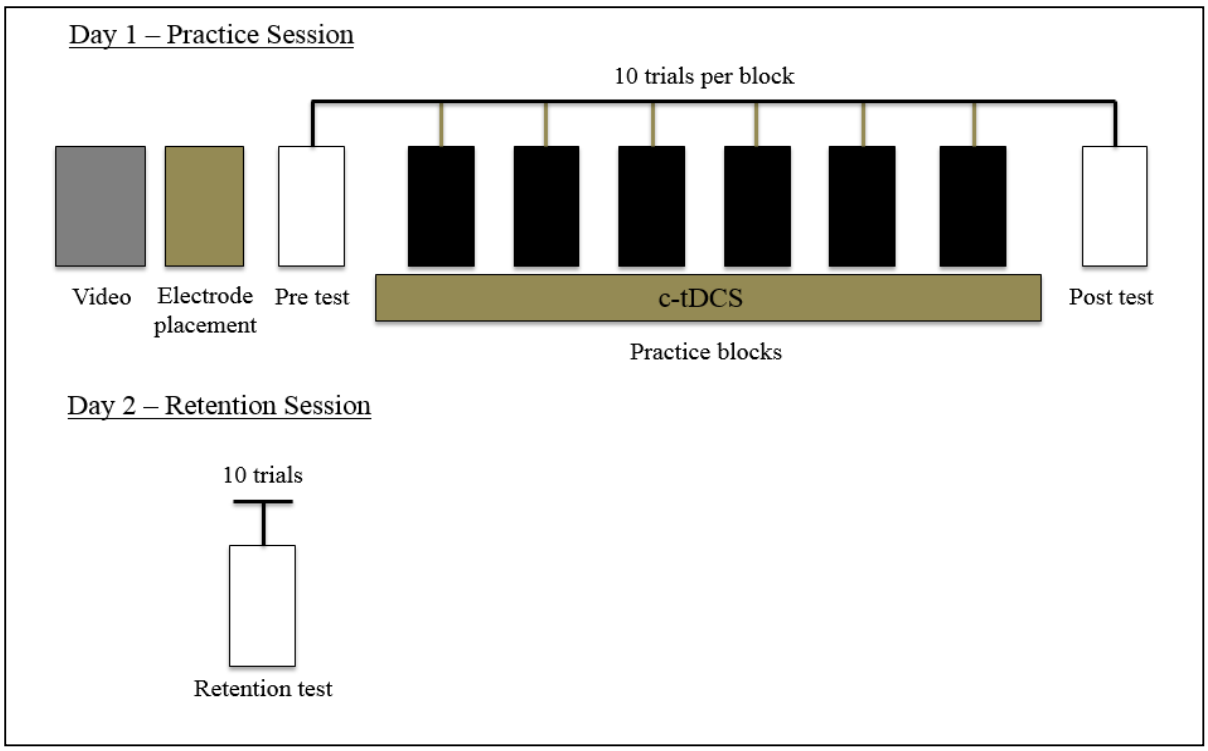

Figure 1. Experimental design

Explanation of the tennis ball throwing task and instructional video. All subjects were given the same set of verbal instructions on how to perform the tennis ball throwing task by the same investigator. Subjects were told to stand behind the line and not cross it at any point during the throw, throw the ball using an overhand motion with the right (dominant) arm, and perform each throw as accurately as possible by trying to hit the center of the target. Subjects were told to perform the throw from a stationary foot position and to not take a step or a "crow hop". In addition, subjects were instructed to throw at whatever velocity they felt would allow them to throw as accurately as possible. Finally, subjects watched an instructional video of an experienced thrower performing the throwing task. The video's purpose was to demonstrate to these relatively novice throwers on how to perform the throwing task within the context of the experimental constraints of the study.

c-tDCS application and electrode placement. A battery-driven electrical stimulator (NeuroConn DC Stimulator MR) applied tDCS through two rubber electrodes (5 x 5 cm) encased in saline soaked sponges. The anode was placed over the right cerebellum (3 cm

lateral to the inion) and the cathode was placed on the ipsilateral (right) buccinator muscle. The current strength was 2 mA and the duration of stimulation was 25 minutes. These stimulation parameters have been shown to be effective for increasing motor skill in a previous study[20]. For the SHAM group, the current was ramped up and down over 60 seconds according to standard SHAM stimulation procedures. The electrodes were held in place by rubber elastic straps and the stimulation device was placed in a small backpack worn by the subject.

Tennis Ball Throwing Task. Subjects threw tennis balls in an identical manner in the baseline, practice, and retention blocks. Subjects stood behind a line located 6 meters away from a target placed on a cement wall. The target was a large circle with a bull's eye in the center. Specifically, the target was printed on a laminated large poster hung on a cement wall (Figure 2). All participants threw a Wilson ATP tennis ball and were instructed to perform each throw as accurately as possible. Subjects did not receive verbal feedback from the instructors after each trial or trial block, but visual feedback of their performance was available. After each throw they were able to see where the ball hit the poster relative to the target located in the center of the poster. Following each trial, a mark was made at the final ball position relative to the target on the target by red colored pool chalk that was placed on the tennis ball between each trial block. This mark was then recorded with a small trial-numbered sticker after each trial of a given block of trials (Figure 2). After each block of trials endpoint coordinates of the marks of the trials was measured by one of the investigators and recorded by another investigator. During this time, the subject stood resting quietly. Each trial block lasted ~ 1.5 minutes and there was ~ 2 minutes of rest given between trial blocks (Figure 1).

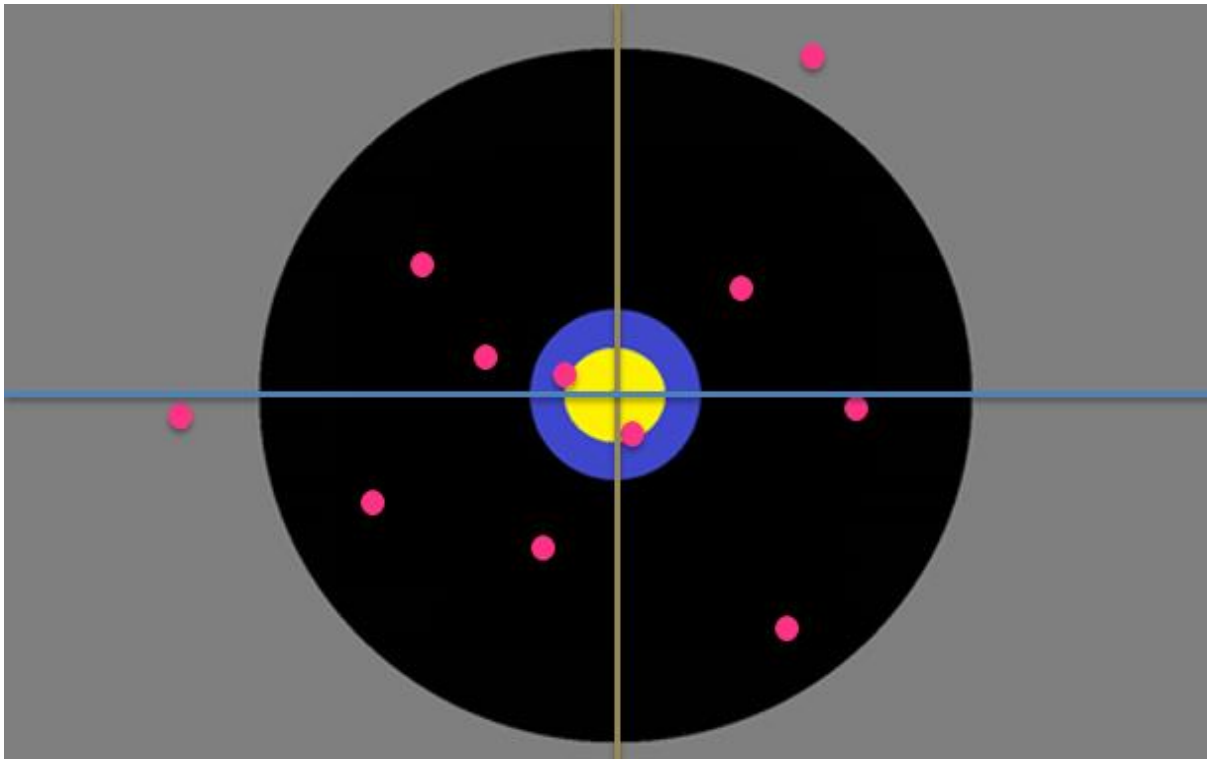

Figure 2. Target schematic

Baseline test block. The baseline test block consisted of one block of 10 trials to confirm that the randomization process resulted in both groups demonstrating a similar initial performance level in the task and to serve as a baseline for comparing potential performance improvements in the subsequent post-test and retention test blocks. Ten trials were chosen because this number of trials was viewed as sufficient to obtain accurate baseline data without significantly influencing subsequent performance curves for the practice blocks. Finally, a block of 10 trials was also consistent with the blocks of 10 trials used during the practice blocks, the post-test block, and the retention test block.

Practice blocks and c-tDCS. The practice blocks were performed during application of c-tDCS for a total practice and stimulation period of 25 minutes (Figure 1). A total of 6 practice blocks of 10 trials were performed. Each block took ~1.5 minutes to complete and a rest period of ~2 minutes was given between each practice trial block. Thus, each subject performed a total of 60 trials of the throwing task in the practice blocks.

Post-testing blocks. After the last practice block, subjects rested for ~5 minutes before performing one block of ten trials.

Retention testing blocks. On the next day, subjects were required to come back to the laboratory to perform a retention test block. The retention test block was conducted in the same manner as the baseline test block and post-test block (1 block of 10 trials) and subjects were reminded to perform the task in an identical manner as they did on the previous day. In this session, c-tDCS was not applied and the instructional video was not replayed.

Data analysis

Endpoint error and endpoint variance. The endpoint error was the primary dependent measure of interest. Endpoint error was quantified as the shortest distance between the *x* and *y* coordinates of the middle of the target and the final endpoint of ball contact for each trial the Pythagorean Theorem was used. Thus, endpoint error corresponded to the absolute distance of the final endpoint of ball contact from the target and gave a measure of endpoint accuracy. Endpoint variance was determined as the sum of the variances of the absolute values of the *x* and *y* endpoints for a given block of trials. In contrast to endpoint error, endpoint variance provides a measure of within-subject performance variability. Since it is possible that a subject can have relatively low performance variability yet be relatively far from the target on average (low accuracy), endpoint error and endpoint variance can have low correlations and potentially provide different performance information. On the other hand, it is also possible that the two measures are relatively highly correlated if there if subjects do not display a consistent pattern of endpoint bias relative to the target.

Statistical analysis

Test blocks: Endpoint error and endpoint variance were analyzed by two-factor repeated measures ANOVAs: 2 *Group* (c-tDCS, SHAM) x 3 *Test* (BASELINE, POST-TEST, RETENTION).

Practice blocks: Endpoint error and endpoint variance were analyzed by two-factor repeated measures ANOVAs: 2 *Group* (c-tDCS, SHAM) x 6 *Block* (1, 2, 3, 4, 5, 6).

CHAPTER 4

RESULTS

Endpoint error for the test blocks

The main effect for GROUP was not significant (P = 0.290) as the endpoint error was similar for the c-tDCS group and SHAM stimulation group when averaged over the three test blocks. However, there was a significant main effect for TEST (P = 0.003) and post hoc analysis showed that the endpoint error was significantly lower for the post-test block when they were compared to the baseline test block (P = 0.004). Differences between the post-test block and the retention test block missed statistical significance (P = 0.063). Furthermore, endpoint error was similar between the retention test block and the baseline test block. Finally, the GROUP x TEST interaction was not significant (P = 0.217).

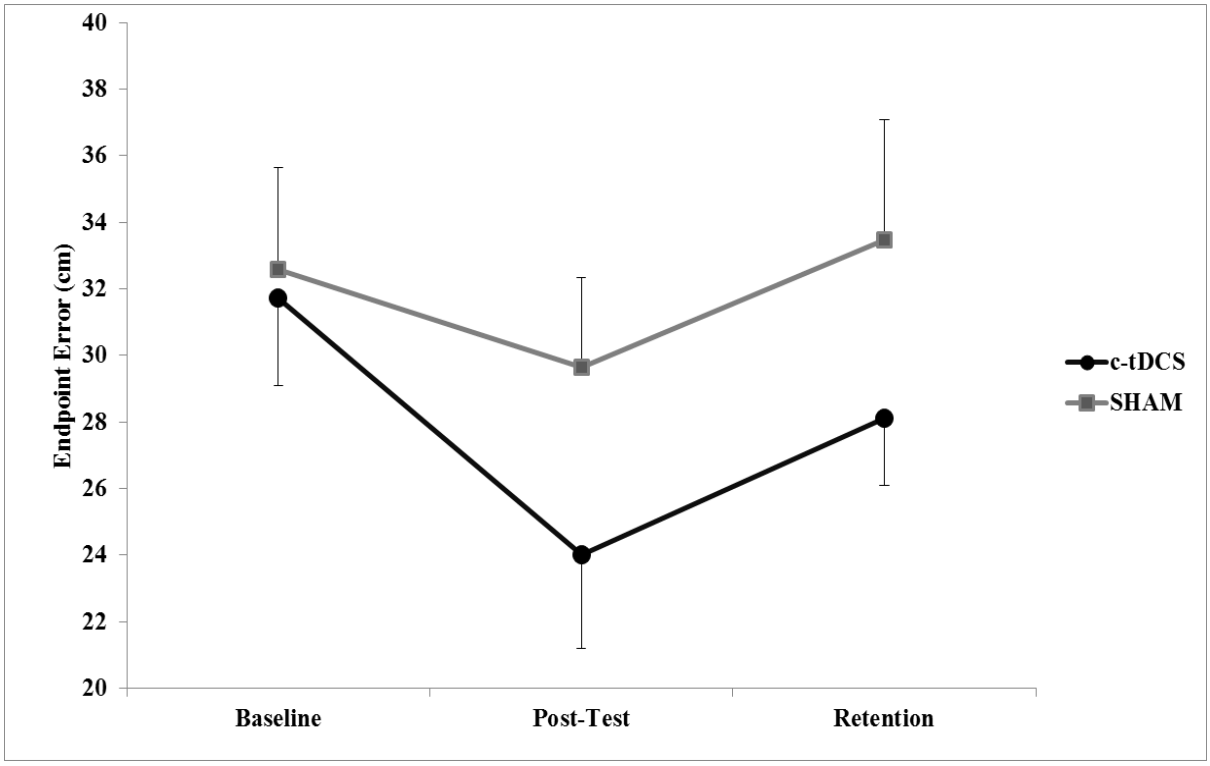

Figure 3. Endpoint error for the test blocks

Endpoint error for the practice blocks

The main effect for GROUP was not significant (P = 0.148) as the endpoint error was similar for the c-tDCS group and SHAM stimulation group when averaged over the six

practice blocks. Furthermore, the main effect for BLOCK was not significant (P = 0.534), which indicated that endpoint error did not decrease significantly with practice. Finally, the GROUP x BLOCK interaction was not significant (P = 0.275).

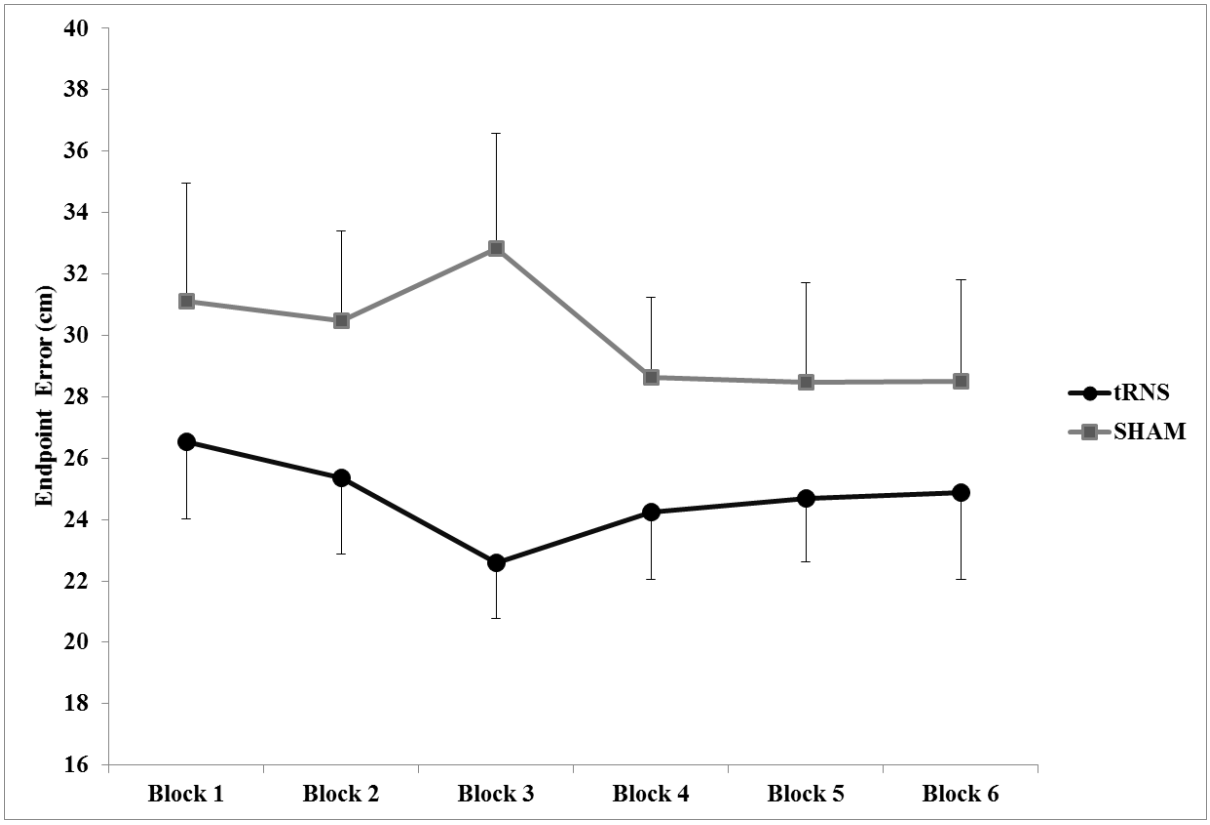

Figure 4. Endpoint error for the practice blocks

Endpoint variance for the test blocks

There was a significant (P = 0.034) GROUP x BLOCK interaction. However, post hoc analysis of the just barely failed statistical significance (P = 0.107 and P = 0.067) for lower endpoint variance in the c-tDCS group compared with the SHAM stimulation group for the post test block and retention test block, respectively. However, the difference between the groups for the baseline test was not significant (P = 0.824), which indicated that the initial level of endpoint variance was nearly identical for the two groups. The main effect for GROUP was not significant (P = 0.200), whereas the main effect for TEST just failed statistical significance (P = 0.063).

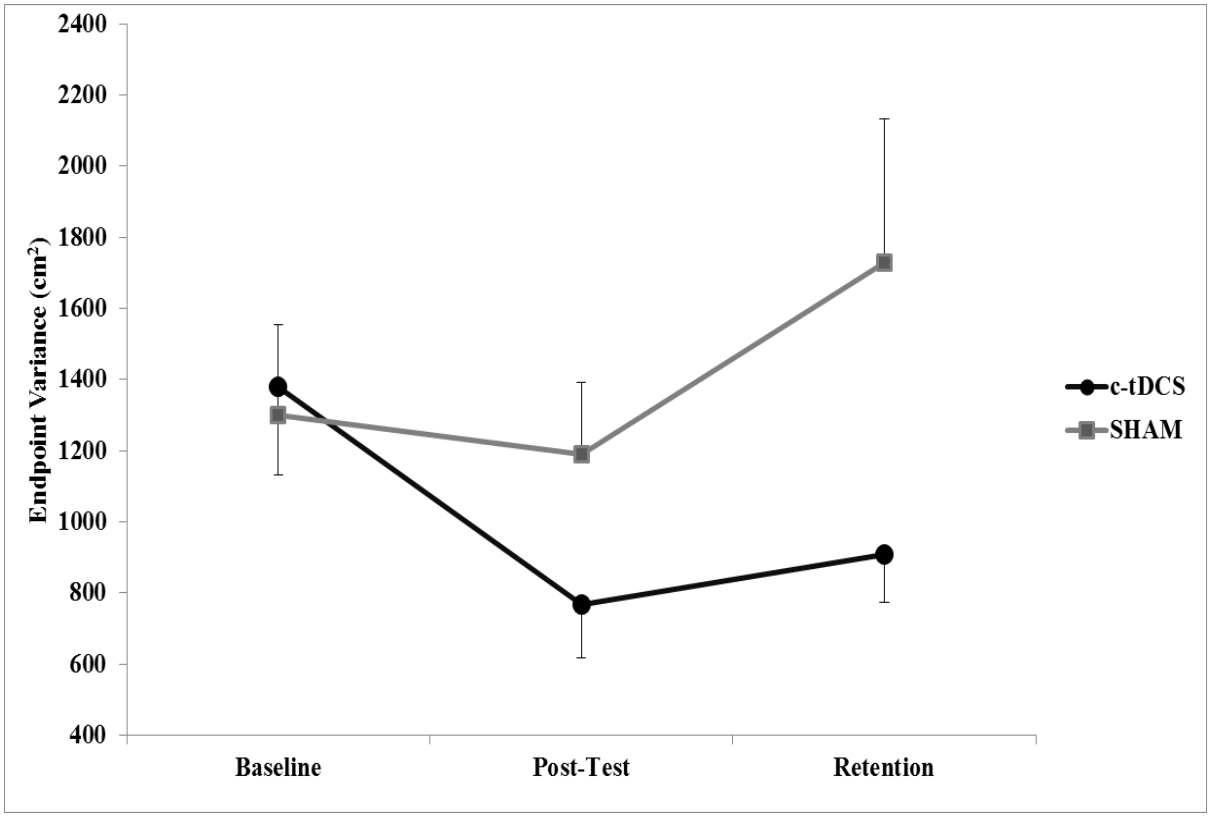

Figure 5. Endpoint variance for the test blocks

Endpoint variance for the practice blocks

The main effect for GROUP was not significant (P = 0.152) as the endpoint variance was comparable for the c-tDCS group and SHAM stimulation group after being averaged over the six practice blocks. Furthermore, the main effect for BLOCK was not significant (P = 0.326), which indicated that endpoint variance did not decrease significantly with practice. Finally, the GROUP x BLOCK interaction was not significant (P = 0.394).

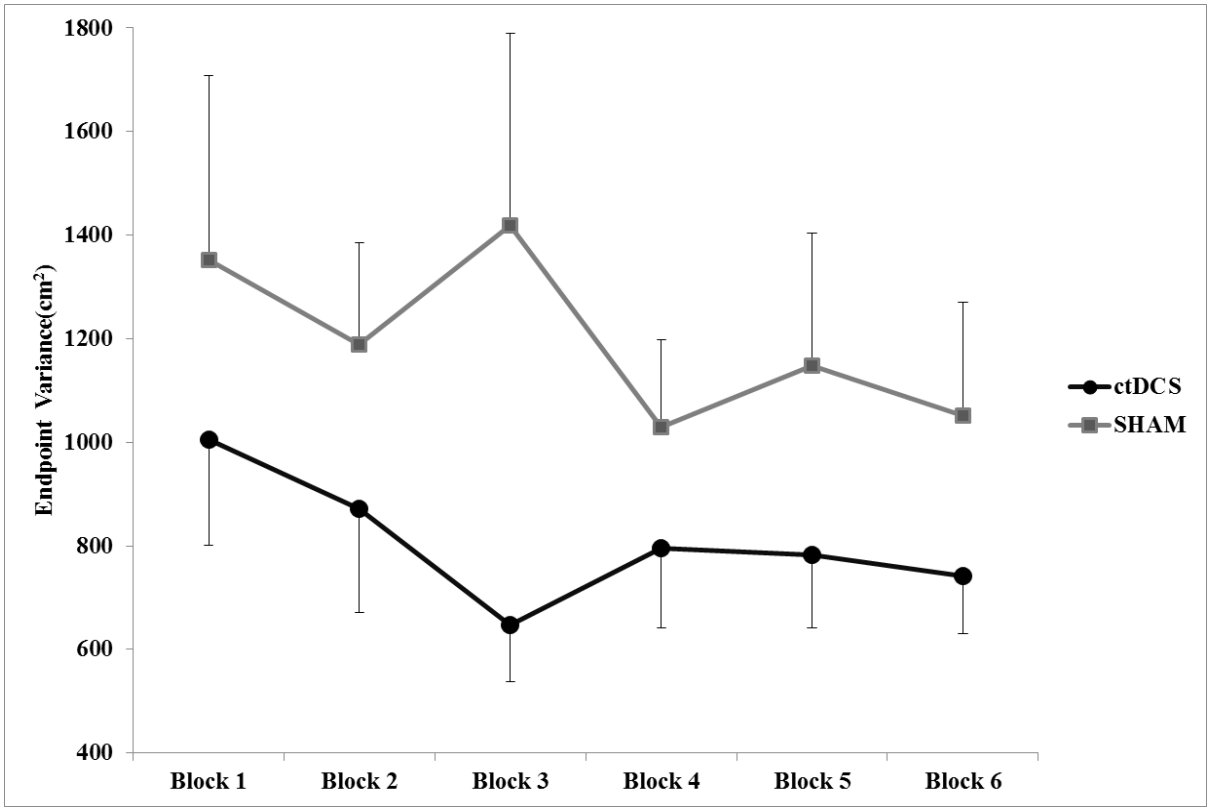

Figure 6. Endpoint variance for the practice blocks

CHAPTER 5

DISCUSSION

This study was intended to determine the influence of c-tDCS on the accuracy, and also the variability of young adults while completely a complex, multi-joint throwing task. This was accomplished by having two groups of subjects perform tennis ball throws to a target over a 25 minute practice session while either real c-tDCS or SHAM stimulation was applied. In addition, a baseline test was performed before practice along with a post-test performed 5 minutes after practice had ceased as well as a retention session that was performed 24 hours after practice. Thus, the practice session quantified motor skill acquisition, whereas the post-test measured immediate retention and the retention session quantified the amount of motor learning that occurred (longer-term retention).

There were four main findings: 1) Practice of the throwing task led to significant immediate motor learning and near significant longer-term motor learning as evidenced by the reductions in endpoint error from the baseline test block to the post-test block (5 minutes post-practice) and the retention test block (24 hours post-practice), respectively. However, these reductions in endpoint error were not different between the c-tDCS and SHAM stimulation groups, which indicated that c-tDCS did not improve motor learning as measured by throwing accuracy to a greater extent than practice alone; 2) Despite the reductions in endpoint error in the test blocks, the endpoint error did not exhibit a significant improvement over the course of the actual practice blocks for either group. Thus, c-tDCS did not improve motor skill acquisition during the practice blocks to a greater extent than practice alone; 3) Practice of the throwing task led to near significant immediate and longer-term reductions in endpoint variability as indicated by the reductions in endpoint variance from the baseline test block to the post-test block (5 minutes post-practice) and the retention test block (24 hours post-practice), respectively. Most importantly, the reductions in endpoint variance was

greater for the c-tDCS group compared to the SHAM stimulation group for the post-test and retention test blocks, although these comparisons just barely failed statistical significance; and 4) Despite the near significant reduction in endpoint variance in the test blocks, the endpoint variance did not exhibit a significant improvement over the course of the actual practice blocks for either group. Thus, c-tDCS did not significantly decrease endpoint variance during the practice blocks to a greater extent than practice alone. Collectively, the findings indicate that a single application of c-tDCS does not significantly improve motor skill acquisition or motor learning in a throwing task in young adults. The lack of ability of c-c-tDCS to elicit significant improvements in motor performance following could potentially have been due to several inter-related factors (see below).

c-tDCS and motor learning

Motor skill acquisition involves a transient alteration in motor performance during an acute practice session. In contrast, motor learning refers to a more permanent, longer-term positive alteration in motor performance that can be quantified in retention tests at various times after a practice has ended. Accordingly, the current study measured motor skill acquisition (practice) as well as immediate motor learning (post-test, 5 min after practice), and longer-term motor learning (retention test, 24 hours after practice). Based on tDCS studies in which the motor cortex was stimulated and increases in motor learning were demonstrated after several successive days of tDCS[11,12] as well as acute c-tDCS studies that increased motor skill acquisition[17-21], we hypothesized that the c-tDCS group would demonstrate a greater degree of motor learning in the immediate retention as well as the long-term retention test. In contrast to this expectation, endpoint error significantly decreased from the baseline test to the post-test, whereas the reduction in endpoint variance barely failed to reach a statistically significant difference between the baseline and post-test. Most importantly, neither endpoint error nor endpoint variance improved to a greater degree in the

c-tDCS group compared to the SHAM stimulation group. However, the reductions in endpoint variance seemed to be a stronger and more consistent than the reductions in endpoint error as the GROUP x TEST interaction reached significance for endpoint variance, but not endpoint error. Nonetheless, the post-hoc comparisons for immediate retention (post-test) and longer-term retention for endpoint variance just barely failed statistical significance. Thus, c-tDCS did not elicit a strong or consistent enough effect for clearly observable differences in movement accuracy or movement variability to be achieved compared to practice alone in the current experimental conditions.

The visible improvements in endpoint accuracy and variability observed in the c-tDCS group compared to SHAM group can be seen in Figures 3 and 5, although these improvements were not statistically significant. On a closer inspection of the data, this was most likely, or at least partially, due to the wide range of inter-individual responses to the stimulation. For instance, if the baseline test block is compared to the post-test block for endpoint error, the average percentage change in endpoint error was 24% for the c-tDCS group versus 9% for the SHAM stimulation. Furthermore, 10 of the 12 subjects in the c-tDCS group showed improvements between the two tests, whereas only 8 out of 12 subjects in the SHAM stimulation group demonstrated improvements between the two tests. Most importantly, of the 10 subjects in the c-tDCS group who demonstrated an improvement (reduction) in endpoint error between the two tests, the range of percentage improvements was 51% (2-53%). In stark contrast, for the subjects in the SHAM stimulation group who improved (reduced) their endpoint error, the same calculations yield a range of percentage improvements of only 25% (0.3-25%). Similar results using the same computations between the two tests occur for the endpoint variance. In this case, all 12 subjects in the c-tDCS group showed improvements between the two tests, whereas 9 out of 12 subjects in the SHAM stimulation group demonstrated improvements between the two tests. In addition, for the 12

subjects in the c-tDCS group who demonstrated an improvement (reduction) in endpoint variance between the two tests, the range of percentage improvements was 72% (8-80%). Conversely, for the 9 subjects in the SHAM stimulation group who reduced their endpoint variance, the same calculations yield a range of percentage improvements of 42% (6-48%). Accordingly, the difference in percentage change in the measures of endpoint accuracy and endpoint variance was almost two times greater in the c-tDCS group compared to the SHAM stimulation group for those subjects that improved their performance between the tests. In summary, the wide range inter-individual responses to c-tDCS likely lead to a reduced ability to demonstrate a statistically significant difference in the retention tests between the two groups, despite relatively large differences in the group average measures of endpoint performance.

c-tDCS and motor practice

In contrast to this our original expectations based on several acute c-tDCS studies in young and old adults[17-21], the results indicated that both endpoint error and endpoint variance barely improved over the course of 6 practice blocks of 10 trials each and these improvements did not come close to approaching significance. In addition, there were no differences in the rate of reduction in endpoint error or endpoint variance between the two groups. The data and outcomes are contrary to the results of several previous studies in young and old adults, which found improved performance in arm movement and gait tasks in either young or old adults[17-21]. These conflicting results are most likely primarily due to differences in the complexity and novelty of the tasks studied in previous studies compared to the current study. Specifically, most of the aforementioned previous studies involved simple two dimensional planar arm movements in seated, immobile subjects[17,20]. In contrast, the current study involved a difficult whole body movement that involved coordination of every major joint on both sides of the body in a free standing condition. In addition, the movement was

performed in three dimensions, involved the planning and compensation for the perturbing effects of joint interaction torques in the throwing arm, the strict control of the point and timing of ball release by the digits of the hand, and likely complex computations to refine of motor commands in an attempt to increase accuracy on a trial by trial basis. Finally, in previous studies the tasks used were novel laboratory tasks that subjects had likely never done before in everyday life. Conversely, the current task involved a motor action commonly performed periodically in everyday life over the course of many years. Therefore, although the subjects were not formally trained athletes who had competed in throwing sports in the past, the task was most likely nonetheless familiar to the subjects. Thus, it would be less likely to be able to be improved to the same degree as novel laboratory tasks over the course of a single practice and stimulation session. This line of reasoning suggests that application of c-tDCS over multiple consecutive days of practice [11,12] may be needed in the type of task used in the current study, at least for young adults (see below). Accordingly, the amount of performance increase that can be observed due to c-tDCS could be highly dependent on task details, the age and initial performance level of the participants, the number of stimulation sessions, and the individual susceptibility of a given subject to non-invasive brain stimulation modalities such as tDCS.

Possible reasons for the inability of c-tDCS to significantly improve motor performance

The lack of a strong impact of c-tDCS on motor skill acquisition and motor learning was contrary to our original hypothesis and conflicts with the small number of previous studies on the influence of c-tDCS on motor function in young and old adults. Similarly, the results are also in contrast to most tDCS studies involving stimulation of the motor cortex in young adults, which have usually found an improvement in motor performance in the range of 10-15% after a one-time tDCS application[8,9]. There are a number of plausible reasons for the inability of c-tDCS to elicit enhancements in motor performance based on the available

tDCS literature (primarily studies involving tDCS of motor cortex): 1) A one-time application of c-tDCS may, at least in some cases, not be enough of a stimulus to improve motor function[11]. Accordingly, several successive days of c-tDCS may be needed, especially in young adults. For instance in a tDCS study involving motor cortex stimulation, Reis et al (2013) demonstrated that three successive days of tDCS application improved performance by about 30% versus practice alone, whereas performance was not significantly different at the end of the first day[11]; 2) The ability of c-tDCS to improve motor performance in young adults may be less than its ability to improve performance in populations such as older adults[6] and patients with motor disorders[7] as these groups have lower initial performance levels and a greater ceiling for improvement due to practice and c-tDCS. This line of reasoning, however, is based on studies involving tDCS of the motor cortex where the ability of tDCS to improve performance was correlated with the age of the subject and the level of motor dysfunction due to disease severity[6,7]; 3) The difficulty of the task used in the present study may have influenced the ability of c-tDCS to augment motor performance. While this is somewhat speculative as the ability of c-tDCS or tDCS of the motor cortex has rarely if ever been compared between simple and complex tasks. However, most successful tDCS studies have involved hand and arm muscles in simple one or two joint laboratory tasks and few if any tDCS studies have involved a whole body, multi-joint, goal-directed accuracy task such as the one employed in the current study. Therefore, it is theoretically possible that it may be much more difficult for c-tDCS to improve complex motor tasks; 4) Accumulating research from tDCS studies of the motor cortex has shown that a surprisingly large number of subjects may be non-responders to tDCS methods, especially in the short-term. Accordingly, it is possible that the same phenomenon could exist for c-tDCS and that it may be necessary to develop screening procedures for subjects most likely to respond to c-tDCS to minimize the possibility that a large number of non-responders or minimal responders could make it

difficult to find significant performance enhancements following c-tDCS application. This type of procedure has recently been applied in tDCS studies involving motor cortex; and 5) a combination of some or all of these aforementioned factors may be responsible. Finally, another explanation is that c-tDCS may simply not be as efficacious as initially believed. Future investigations will need to be undertaken to discriminate between these various possibilities.

Summary

In conclusion, c-tDCS appeared to elicit improvements in motor skill acquisition and learning compared to practice alone (SHAM stimulation), but these differences just failed to reach statistical significance likely due in large part to the wide inter-individual responses to c-tDCS. Furthermore, the reductions in endpoint variability seemed to be stronger and more consistent than the reductions in endpoint error. Taken together, the findings indicate that a single application of c-tDCS does not significantly improve motor skill acquisition or motor learning in a difficult throwing task in young adults and application of c-tDCS over multiple consecutive days may be required to improve performance in complex tasks in young adults.

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CURRICULUM VITAE

Austuny Jackson
9121 Sea Cove St.
Las Vegas, NV 89123
(702) 332-2132
jacks508@unlv.nevada.edu

Educational Experience

Masters of Science, Kinesiology **Aug 2012 – May 2016**
University of Nevada, Las Vegas Las Vegas, NV
GPA: 3.50 / 4.00

Bachelors of Science, Kinesiology **Aug 2008 – May 2012**
University of Nevada, Las Vegas Las Vegas, NV
GPA: 3.50 / 4.00

Professional Experience

Teaching Assistant **Aug 2012 – Jun 2015**
University of Nevada, Las Vegas Las Vegas, NV

- Administered and proctor exams
- Evaluated homework and exams
- Notified instructor of discrepancies with assignments and exams
- Lectures conducted:
    - Happiness in Your Profession
    - Professional Growth Through Personal Struggle
    - Memory Consolidation

Research Assistant **Jan 2014 – Aug 2014**
The Cleveland Clinic Las Vegas, NV

- Administered Jebsen-Taylor Hand Function test
- Ran statistical analysis on data from experiments
- Conducted concussion testing with C3 Protocol

Skills

- Proficient in Microsoft Office (Word, Excel, and PowerPoint)
- Excellent leadership skills
- Superior communication abilities
- Great writing competency