THE EFFECT OF TIMING OF ANODAL TRANSCRANIAL DIRECT CURRENT STIMULATION (TDCS) ON ONLINE AND OFFLINE MOTOR SEQUENCE LEARNING

A Thesis

by

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ABSTRACT

Transcranial direct current stimulation (tDCS) has emerged as a noninvasive brain stimulation technique that may facilitate the acquisition, consolidation, and long-term retention of motor skills. The present study aims to examine the effect of timing of anodal tDCS at primary motor cortex (M1) for online and offline changes in motor sequence learning. At this time, the extant literature provides some support for the efficacy of tDCS applied during motor training for increased online and offline gain in skill, while administration of exogenous stimulation before or after training provides no benefit beyond a sham stimulation control. Unfortunately, this conclusion is made on the basis of multiple cross-experiment comparisons within which tDCS was applied in very different ways (e.g., electrode montage, duration, current, etc.). The main hypothesis of the present study is that only application of anodal tDCS at M1, while also practicing a serial reaction time (SRT) task, will result in greater online and/or offline gains manifest as faster response times. Ninety right-handed undergraduate students participated in this study. They were randomly assigned to one of four tDCS condition groups: (1) tDCS before practice (BEF), (2) tDCS during practice (DUR), (3) tDCS after practice (AFT), and (4) no tDCS (NO) condition group. The non-dominant hand (left hand) was used to perform 15-min of practice with an SRT. Participants in the BEF condition group received 2-mA of bi-hemispheric tDCS over contralateral M1 before practice. tDCS was administered during the 15-min practice period for the DUR group. The AFT condition group received tDCS immediately after they finished practice. Participant assigned to the NO condition group did not receive tDCS and only performed the SRT task. The retention test was conducted 1 hr and 24 hr after the practice session was concluded. Online, offline, and total changes in response time were analyzed, and the results indicated that the online, offline, and total change in response time for the SRT between the four tDCS timing conditions was not different. These data question the effectiveness of a single dose of tDCS, regardless of its temporal placement relative to physical training, for inducing improvement in skill.

DEDICATION

This thesis is dedicated to my parents in the Republic of Korea.

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CONTRIBUTORS AND FUNDING SOURCES

Contributors

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NOMENCLATURE

AFT	tDCS After Practice
ANOVA	Analysis of Variance
AT	Adaptation Task
BEF	tDCS Before Practice
DSP	Discrete Sequence Production
DUR	tDCS During Practice
M1	The Primary Motor Cortex
MST	Motor Sequence Task
NIBS	Noninvasive Brain Stimulation
NO	No tDCS
RT	Response Time
SEM	Standard Error of the Mean
SRT	Serial Reaction Time
tDCS	Transcranial Direct Current Stimulation
TMS	Transcranial Magnetic Stimulation

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CHAPTER I

INTRODUCTION AND LITERATURE REVIEW

The acquisition, consolidation, and long-term retention of motor skills is central to human functioning. Motor skills such as typing, those needed for playing musical instruments as well the range of motor behaviors exhibited in the sporting arena are typically enhanced through experience with extensive practice or training (Dayan & Cohen, 2011; Doyon et al., 2018). In the laboratory, the learning process is commonly probed by manipulating some feature (e.g., presence of reward, Abe et al., 2011) assumed to be important for learning during a period of training. The impact of this feature for performance (e.g., improved speed-accuracy relationship, Reis et al., 2009) for (a) acquisition, (b) the period between the end of acquisition and test (i.e., consolidation, Abe et al., 2011), and (c) multiple tests administered over an extended time period without further training (i.e., long-term retention, Abe et al., 2011; Reis et al., 2009) is often assessed. Changes that are observed during the acquisition phase are referred to as *online change* and are considered distinct from adaptation (i.e., positive or negative) that occur outside the actual practice environment, the latter of which are called *offline change* (Reis et al., 2009). Offline changes are associated with consolidation and long-term retention.

Much of the work that has focused on identifying features of training that result in a positive impact for acquisition, consolidation, and/or long-term retention of motor skills has almost exclusively relied on the use of either (a) motor sequence tasks (MST) (Doyon et al.,2018; Wright et al., 2016), or (b) adaptation tasks (AT) (Galea et al., 2011). MSTs are sequential motor behaviors that at first are executed as a set of independent movement elements (e.g., key-presses) that are eventually performed as a set of unitary groups of elements (i.e., motor chunks, Abrahamse et al., 2013) with sufficient practice. Examples of MSTs include, but are not limited to, the finger-to-thumb opposition task (e.g., Karni et al., 1995), the discrete sequence production (DSP) task (Abrahamse et al., 2013; Verwey, 2001), serial reaction time (SRT) task (Brown & Robertson, 2007; Walker et al., 2002, 2003), sequences of movements of the whole arm (Kovacs, Buchanan, & Shea, 2009), or sequential movements of the oculomotor system (Albouy et al., 2006). On the other hand, ATs requires the performer take an existing, typically well-learned, movement capability (i.e., reaching) and perform it in a novel context that demands some form of compensation to account for environmental change. The most common ATs require participants to adapt to either visual (Imamizu et al., 2000, 2003; Tseng et al., 2007) or motor perturbations (e.g., Brashers-Krug, Shadmehr, & Bizzi, 1996). The present work focuses exclusively on learning of an MST which in this case involves a modified SRT task.

Recently the use of noninvasive brain stimulation (NIBS) techniques, such as transcranial magnetic stimulation (TMS) and transcranial direct current stimulation (tDCS), have been touted as potential adjuncts to physical practice to enhance motor learning (Buch et al., 2017). tDCS, the NIBS that is the focus of the present work, involves the passage of a weak direct current between two electrodes that are used to target a region of interest within the brain (e.g., the primary motor cortex (M1)). The applied current flows between a positively charged anode and a negatively charged cathode. Since tDCS induces an intracerebral current flow, it is assumed that neuronal excitability of the targeted brain area can be modified in a polarity-specific manner. Generally, anodal stimulation (with reference to the target area) increases cortical excitability, while cathodal stimulation has been reported to decrease excitability at M1 (Nitsche & Paulus, 2000; Reis & Fritsch, 2011).

With respect to online change, it has been reported that anodal tDCS over M1 can facilitate motor performance during initial acquisition. For example, Nitsche et al., (2003) had individuals perform a 12-element SRT task while also experiencing (a) anodal tDCS, (b) cathodal tDCS, or (c) sham stimulation at contralateral M1, premotor area, or prefrontal cortex. Only anodal tDCS at M1 resulted in enhanced sequence learning toward the end of the training period reflected in a larger reaction time difference between a random and trained sequence. Since many of the participants were unable to verbalize the nature of motor sequence for which they had shown online gain, the learning was assumed to be implicit. The finding that M1 was an important neural region with respect to observing improvements in motor performance was consistent with other related findings using TMS (e.g., Muellbacher et al., 2002).

The efficacy of anodal tDCS for inducing gains across training does not appear to be restricted to an MST that is acquired implicitly. Stagg et al. (2011) informed participants that a 10-element motor sequence would be practiced and required subjects to memorize the order of the elements within the sequence prior to practice. As was the case in Nitsche et al. (2003), the sequential motor skill was executed faster following anodal stimulation at M1 when compared to sham or cathodal stimulation. Such online benefit from anodal stimulation at M1 for MST learning appears to be quite robust as the enhancement has been reported when stimulation is applied only once (Cuypers et al., 2013; Kantak, Mummidisetty, & Stinear, 2012; Karok & Whitney, 2013; Nitsche et al., 2003; Reis et al., 2009; Stagg et al., 2011, Vines, Cerruti, & Schlaug, 2008; Zimerman et al., 2013) or across multiple days (Reis et al., 2009; Water-Metenier et al., 2014). In general, then, there is some support for the claim that anodal stimulation at M1 administered in conjunction with training leads to improved performance during the acquisition of an MST. It should be noted however that there are studies that have failed to elicit this online benefit from tDCS during this period (see Buch et al., 2017, Table 1).

In contrast to the concurrent application of anodal tDCS during practice of an MST, administering this form of neuromodulation prior to practice has been reported to be ineffective at inducing the same positive effect previously noted when practice of the MST and stimulation occur simultaneously. For example, Stagg et al. (2011), in an experiment discussed earlier, had individuals practice an MST following anodal stimulation at M1. In this case, online change for the MST was significantly less than that observed for the individuals that were exposed to sham stimulation. Moreover, pre-training application of tDCS resulted in significantly poorer motor performance compared to the same stimulation presented concurrently with training. The failure to improve motor skill acquisition or enhance online change has been noted in several other studies (Amadi et al., 2015; Kuo et al., 2008). Taken together, the extant data suggest that if online change is observed, it is most likely to occur when there is temporal coupling of the application of tDCS and practice.

Offline gain, improvement that emerges outside the boundaries of the practice period, have also been observed quite extensively following application of anodal tDCS at M1 during practice. Kang & Paik (2011) examined the impact of both unilateral and bi-lateral stimulation of M1 for implicit MST learning. Unilateral tDCS consisted of the anode located at contralateral M1 with the cathode placed at the ipsilateral supraorbital site. In contrast, for bi-lateral stimulation, the anode remained at contralateral M1 while the cathode was placed at ipsilateral M1. While the electrode montage (uni vs. bi-lateral stimulation) failed to impact the efficacy of tDCS, velum stimulation resulted in superior performance when compared to a sham condition in terms of offline change. Specifically, tDCS stimulation was associated with stable performance across the 24-hr retention interval whereas sham stimulation led to significant forgetting across this period. These data suggest that post-practice processes critical for determining the eventual fate of a newly acquired motor memory were enhanced by the administration of anodal tDCS during practice. This outcome has been verified in a number of subsequent studies (Cuypers et al., 2013; Kantak et al., 2012; Karok & Whitney, 2013; Zimerman et al., 2013).

As was the case for stimulation applied during training, applying tDCS for more than a single day also leads to an enhancement in performance across the test interval (Reis et al., 2009, 2015; Schmabra et al., 2011; Waters-Metenier, 2014). For example, Reis et al. (2009) had individuals practice a sequential force-pinch skill over the course of five days while paired with either anodal or sham tDCS at M1. Greater offline gains in skill were associated when practice occurred in the presence of anodal tDCS compared to sham stimulation. Thus, while the reported gain appeared to be associated with enhanced post-practice consolidation, no further benefit surfaced from exposure to the tDCS for long-term retention. That is, the rate of loss in motor performance for the velum and sham conditions did not differ for additional tests that were administered across the next three months.

Tecchio et al. (2010) considered the possibility that memory consolidation could be influenced directly by exposing the learner to anodal stimulation during the post-practice period. Thus, as opposed to application of tDCS prior to (e.g., Stagg et al., 2011) or during practice (Nitsche et al., 2003; Reis et al., 2009), Tecchio et al. (2010) examined the impact of administering stimulation at M1 at the conclusion of a period of training. Essentially, individuals practiced a 9-element finger sequencing task for approximately 10 min. At the completion of practice, individuals were exposed to anodal stimulation at M1 or sham stimulation for 15 min. Contralateral M1 was the selected site for the anode paired with cathode placed over contralateral shoulder. Immediately after stimulation (i.e., tDCS or sham), the subject performed an additional set of trials with the practiced finger sequencing task. Individuals that were privy to tDCS at M1 exhibited superior performance at the time of test compared to those receiving sham. Tecchio et al. (2010) claimed that exogenous stimulation immediately following practice expedited consolidation thus improving performance.

Despite reporting offline gain from the application of anodal tDCS at M1, Tecchio et al. (2010) alluded to the possibility that this outcome may have been a consequence of the well-documented after-effects of tDCS (Nitsche & Paulus, 2000) as opposed to a direct effect on post-practice consolidation. This possibility was confirmed by data from a control condition included in the work of Reis et al. (2015) that applied anodal tDCS for 15 min following practice of a sequential visual isometric pinch force skill and failed to reveal offline gain at a subsequent 24-hr delayed test. These data then question whether post-practice stimulation can influence consolidation and as a result impact fate of a newly acquired motor memory.

With respect to the application of tDCS at M1, the most frequently utilized neural site for this form of NIBS (see Dayan & Cohen, 2011; Doyon et al., 2009), the most robust evidence for enhanced online and offline gains emerges only when the stimulation occurs concurrently with practice. However, a significant shortcoming when assessing the impact of tDCS timing for motor skill acquisition is that any conclusion drawn must be made on the basis of multiple cross-experiment comparisons. What makes this particularly problematic in this context is the vast array of features that vary across these experiments with respect to the manner in which tDCS is applied (e.g., electrode montage and size, intensity (mA), current density, current duration, etc., see Buch et al., 2017). In order to directly address this shortcoming, the present experiment was designed to assess, within a single study, whether the time frame during which anodal stimulation at M1 is applied, relative to a bout of training with an MST, plays a role in determining the emergence of online and offline gains in motor performance. On the basis of the extant literature it is expected that, at a minimum, online and offline gains will be observed when velum stimulation is present during physical practice.

CHAPTER II

METHODS

Participants

Ninety right-handed undergraduate students were recruited from and received course credit for an undergraduate kinesiology class for participation in the study. Table 1 provides demographic information for these participants. Prior to any involvement in this study, all participants gave written informed consent that was approved by the Texas A&M University Institutional Review Board.

Table 1. Sample size, gender, and age of participants assigned to tDCS before practice, tDCS during practice, tDCS after practice, and no tDCS experimental conditions.

Group	*n*	Male	Female	Age (*SD*)
tDCS Before Practice	23	9	14	20.78 (2.41)
tDCS During Practice	22	6	16	20.73 (1.83)
tDCS After Practice	23	6	17	20.83 (1.47)
No tDCS	22	10	12	20.64 (1.50)
Total	90	31	59	20.74 (1.81)

Prior to the experiment, a handedness inventory was administered, and participants were excluded if they were identified as left-handed. In addition, individuals did not participate if they indicated a previous adverse response to tDCS, had a history of epilepsy, experienced a traumatic brain injury, had metal in their brain or skull, had cochlear implants, implanted neurostimulator or pacemaker, used an infusion pump, underwent a surgical procedure to the spine, were pregnant, or consumed recreational drugs or alcohol within the previous 24 hr.

Tasks

Serial Reaction Time Task

Participants placed the four fingers (pinky, ring, middle, and index fingers) of their non-dominant (left) hand on the “V”, “B”, “N”, and “M” keys that correspond to the visual signals “1”, “2”, “3”, and “4” respectively. Each participant was required to execute a string of key presses that constituted a motor sequence. Each motor sequence that was executed consisted of eight key presses on a PC keyboard (e.g., 4-1-3-2-3-1-2-4). The number string that represented the keys to be pressed was displayed in the center of a computer monitor. One motor sequence was designed as the training sequence (i.e., 4-3-1-2-4-2-1-3). An additional set of five motor sequences were also used and presented randomly throughout training (see Figure 1). Each of these motor sequences required eight key presses that were distinct from the training sequences, and the other motor sequences that were presented randomly throughout practice. Participants were expected to perform the string of key presses as quickly and accurately as possible for 30-s. During each 30-s trial, the participant was provided real-time feedback in the form of remaining trial time, the total number of key presses, the number of incorrect key presses, and the number of correct sequences.

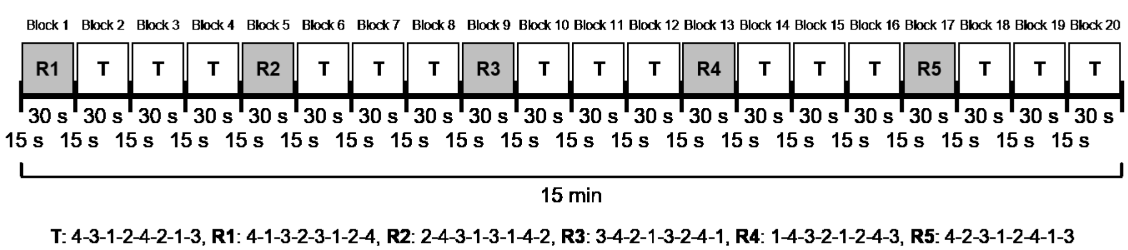

Figure 1. Each practice and test period involved trial blocks with the repeating target (T) sequence and random (R) sequences. In both practice and test periods one training sequence (T: 4-3-1-2-4-2-1-3) and five random sequences (R1: 4-1-3-2-3-1-2-4, R2: 2-4-3-1-3-1-4-2, R3: 3-4-2-1-3-2-4-1, R4: 1-4-3-2-1-2-4-3, and R5: 4-2-3-1-2-4-1-3) were included. Participants performed the required sequence for 30 s and then experienced a 15-s rest interval between blocks. An entire practice or test period took 15 min.

Transcranial Direct Current Stimulation

Two silicon conductive electrodes (electrode size 40 X 40 mm; sponge sleeve size 50 X 50 mm) that were soaked in saline solution were placed on the subjects' scalp regardless of group and task session. The anode was placed over right M1 (C4 in the 10-20 system), and the cathode (reference) electrode was placed over the left M1 (C3 in the 10-20 system) (see Figure 2). The electrodes were connected to a tDCS device (TCT, Hong Kong). Skin resistance was continuously monitored throughout the period of stimulation and was maintained under 5.99 kΩ. The target current was 2 mA (Current Density = 0.08 mA/cm^2), but the actual mean current across all participants was 1.95 mA. The duration of stimulation for all participants was 14-15 min.

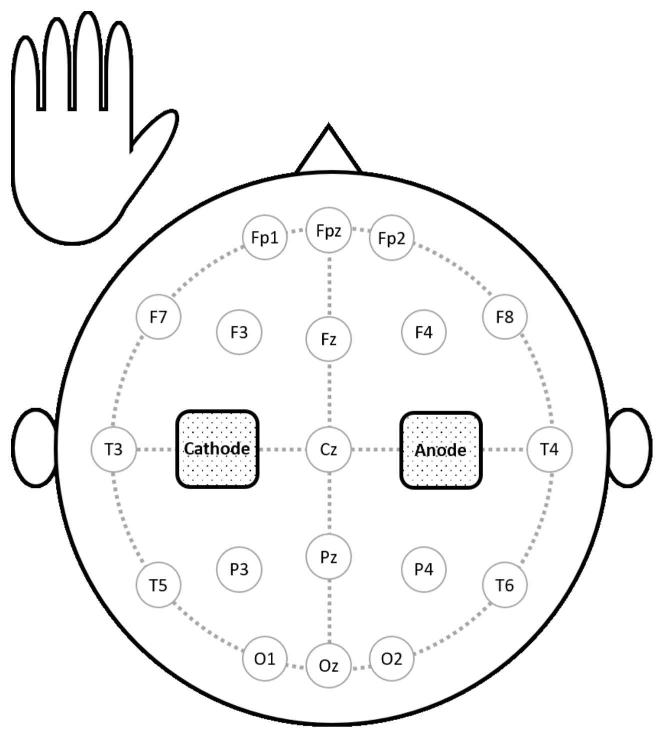

Figure 2. The bi-hemispheric stimulation montage. The anode was placed contralateral to the responding limb (left hand), and the cathode was placed ipsilateral to the responding limb (Anode: over C4, Cathode: over C3).

Procedure

The experiment was conducted across a two-day period and is depicted in Figure 3. All participants completed the consent form and pre-experiment questionnaires prior to participation. Each participant was randomly assigned to one of four stimulation condition groups: (1) tDCS before practice (BEF), (2) tDCS during practice (DUR), (3) tDCS after practice (AFT), or (4) no tDCS (NO). Individuals assigned to the BEF condition received tDCS for 15 min prior to any practice with the SRT task. Immediately following stimulation these individuals practiced the SRT task for 15 min and then remained seated for an additional 15 min before being released from the experiment. For the participants assigned to the DUR condition, 15-min of tDCS was administered during the sequence task which was followed by remaining in the laboratory for 15 min prior to being dismissed. Subjects from the AFT group received stimulation right after they finished the 15-min practice and left the laboratory after the application of tDCS. Participants in the NO group did not receive stimulation at all and only completed the sequence task. They also remained in the laboratory for 15 min after they completed practice.

For all individuals, the left limb was used to perform the motor sequence. A trial with a motor sequence consisted of executing an eight-element sequence as accurately and quickly as possible for 30 s. Some trials were performed with the target motor sequence, while others with alternative novel sequence that were used on “random” trials interspersed through training. Fifteen minutes of total practice involved fifteen 30-s trials of the repeated target sequence and 30-s trials with a random sequence (see Figure 1). Performance change (in ms) of the repeated sequence across training, 1 hr after training, and 24 hr after training were assessed for all individuals.

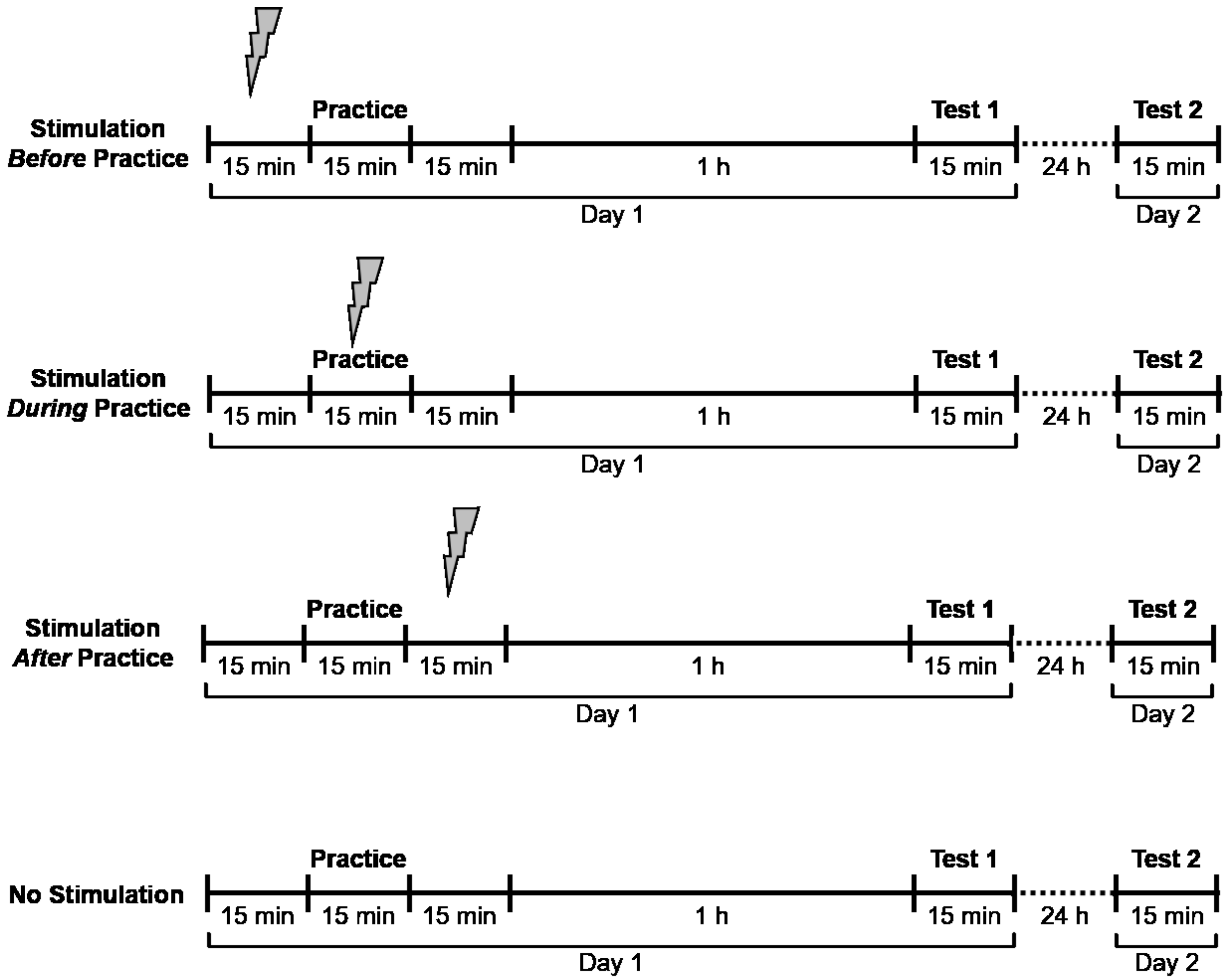

Figure 3. The timeline of events for the experiment. Participants were randomly assigned to one of four experimental conditions: tDCS before practice, tDCS during practice, tDCS after practice, or no tDCS. During the 15-min of practice individuals executed target and random motor sequences and were subsequently tested 1 hr and 24 hr later. The tests were conducted in the absence of any stimulation.

One hour after a participant was dismissed from the experiment, they returned to the laboratory to complete Test 1 which consisted of the same sequence of events described in Figure 1. Twenty-four hours later Test 2 was conducted again consisting of the same set of events described in Figure 1.

Data Analyses

Response time (RT) per correct key press (in millisecond) was the primary dependent variable that was analyzed for practice and test periods of the experiment. First, the number of correct sequences (CS) was measured in each 30-s block, and then the RT was indirectly calculated as follows: $RT = 30000/[8(CS)]$.

The primary assessment in the present work involved examining the impact of tDCS timing (BEF, DUR, AFT, NO) on total performance change, online performance change, and offline performance change. Total performance change was defined herein as the change in mean RT for the target motor sequence between the first three practice blocks and mean RT for the first three blocks in Test 2 (see Figure 4). Online change was defined as the difference in mean RT between mean RT for the first three and last three training blocks. Finally, offline change was determined as the difference in mean RT for the last three blocks in the training phase and the first three blocks in Test 2. A total, online, and offline change scores were determined for each individual in each experimental condition and subjected to subsequent analyses using SPSS (Version 25) and JMP Pro (Version 14).

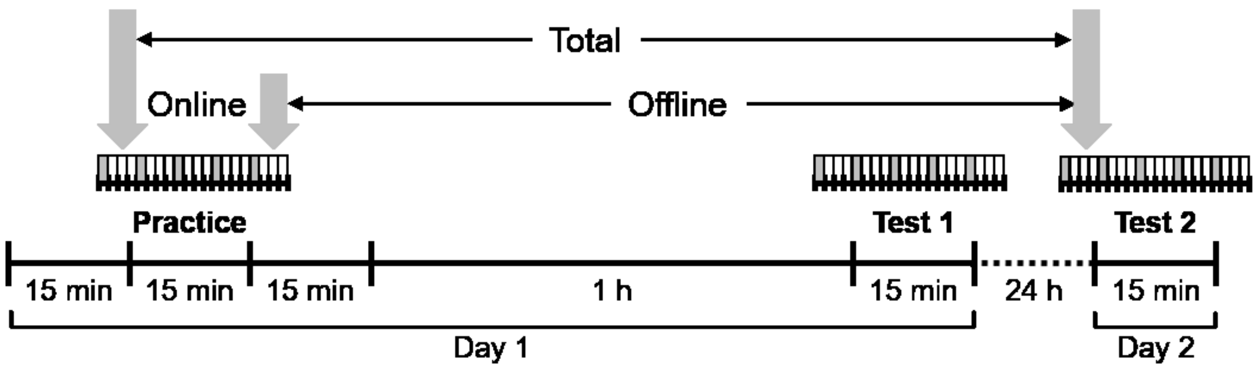

Figure 4. Training blocks used to examine online, offline, and total learning effects. The grey arrows indicate the training blocks. The first three training blocks and the last three training blocks in Practice session were employed for online learning effects. The last three training blocks in Practice session and the first training blocks in Test 2 session were employed for offline learning effects. For the total learning effects, the first three training blocks in Practice session and the first three training blocks in Test 2 session were employed.

CHAPTER III

RESULTS

Baseline Test

For this experiment, given the nature of the timing of tDCS administration, it was difficult to implement a baseline test for the target sequence. For example, if participants in BEF condition performed a baseline test and subsequently receive tDCS between the baseline test and practice session, they would in essence be considered as an AFT condition although the number of trials of the baseline test will be less than the number of trials of the practice session. Obviously comparing the first three trials for the DUR condition with other conditions' initial trials would also be problematic. Despite this shortcoming, a two-independent samples *t*-test was conducted to analyze the difference of the mean RT of the first three training blocks (Block 2, 3, and 4) between the AFT and NO conditions ($\alpha = .05$). In this analysis, the first three training blocks were considered as the baseline because participants in those two groups did not receive the stimulation before or during the first three training blocks in the practice session.

Shapiro-Wilk test revealed that these data are normally distributed ($p = .284$). According to the result of Levene's test for equality of variances, the group variances are unequal ($p = .015$). The results of Welch's *t*-test indicated that there was no significant difference between the two groups, $t(35.321) = 0.78$, $p = .442$. Thus, participants in AFT condition ($M = 626$ ms, $SEM = 17$ ms) and NO condition ($M = 601$ ms, $SEM = 27$ ms) revealed similar performance for the first three training blocks in the practice session.

Online Change in Performance of the Target Motor Sequence

Figure 5 depicts the total (leftmost panel), online (middle panel), and offline (rightmost panel) response time changes as a function of Timing condition (BEF, DUR, AFT, NO). To examine the online performance change, the difference between the mean RT of the first three training blocks (Block 2, 3, and 4) and the mean RT of the last three training blocks (Block 18, 19, and 20) for the practice period for each individual was calculated (see Figure 4).

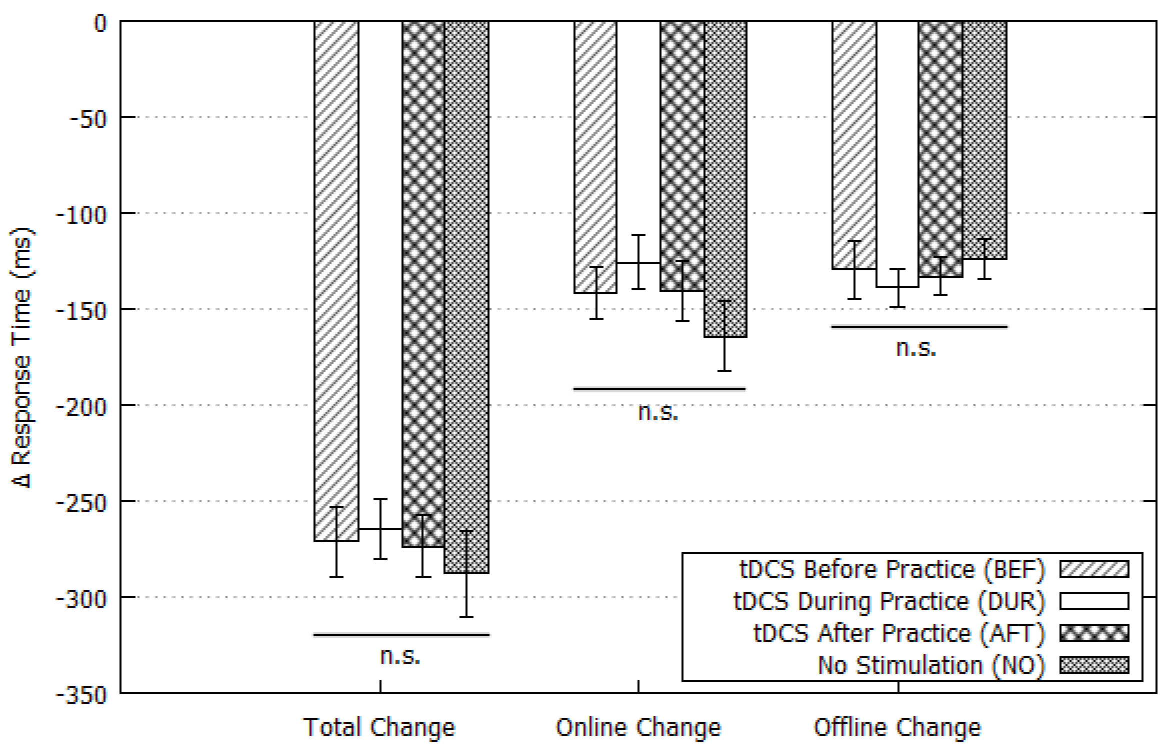

Figure 5. Total, online, and offline changes as a function of BEF, DUR, AFT, and NO timing conditions. Δ Response Time (ms) indexes the average of the difference between post- and pre-blocks (Total Change: Online Change plus Offline Change or the average of Block 2, 3, and 4 in Test 2 session minus the average of Block 2, 3, and 4 in Practice session; Online Change: the average of Block 18, 19, and 20 in Practice session minus the average of Block 2, 3, and 4 in Practice session; Offline Change: the average of Block 2, 3, and 4 in Test 2 session minus the average of Block 18, 19, and 20 in Practice session). The error bars represent the standard error of the mean (*SEM*).

These data were analyzed with the Shapiro-Wilk test, Levene's test, and one-way Timing (BEF, DUR, AFT, NO) analysis of variance (ANOVA). These analyses indicated that the data are normally distributed (p = .195), and the group variances are equal (p = .490). The one-way Timing ANOVA failed to detect differences between the four conditions, $F(3, 86) = 1.02$, $p = .387$, $\eta^2 = .034$ (BEF: M = -142 ms, SEM = 14 ms, DUR: M = -126 ms, SEM = 14 ms, AFT: M = -140 ms, SEM = 15 ms, and NO: M = -164 ms, SEM = 18 ms) (see Figure 5, middle panel).

The biggest difference in the response time changes was observed between DUR and NO conditions (see Figure 5, middle panel). Hence, two-independent samples t-test was additionally conducted to compare the response time changes in the two groups using an alpha level of .05. However, this analysis failed to reveal a significant difference between these two groups ($t(42) = 1.66$, $p = .104$). Figure 6 displays the individual data for online change for each individual in each of the four timing conditions (BEF, DUR, AFT, NO).

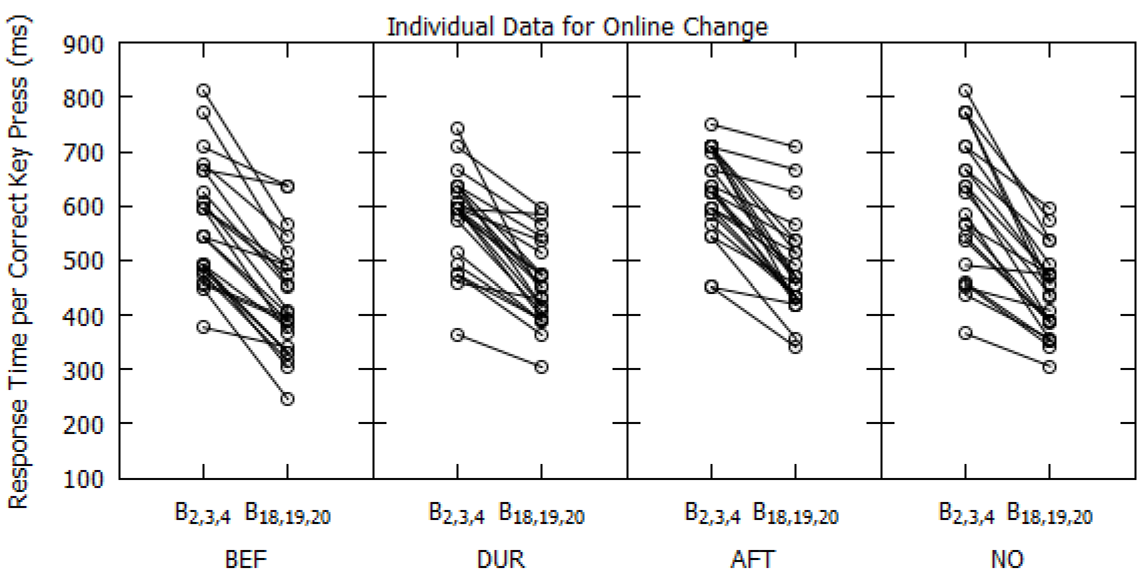

Figure 6. The individual data for online change. The response time from the first three training blocks (Block 2, 3, and 4) and the last three training blocks (Block 18, 19, and 20) in Practice session are displayed by each group.

Offline Change in Performance of the Target Motor Sequence

The difference between the mean RT of the last three training blocks (Block 18, 19, and 20) in Practice session and the mean RT of the first three training blocks (Block 2, 3, and 4) in Test 2 session was used to evaluate the offline change that occurred for each of the timing conditions (see Figure 4). To examine offline learning change, the first three blocks in Test 2 session were adopted instead of the first three blocks in Test 1 because about 1-hr interval between Practice (initial acquisition) and Test 1 session is not sufficient for motor memory consolidation.

According to the Shapiro-Wilk test, the data were not normally distributed (p = .015). Therefore, the data were analyzed with the Kruskal-Wallis test, a non-parametric method. The analysis failed to reveal a significant main effect of Timing condition, $\chi^2(3) = 1.983$, p = .576 (see Figure 5). Thus, the offline response time change was similar for the BEF (M = -130 ms, SEM = 15 ms), DUR (M = -139 ms, SEM = 10 ms), AFT (M = -133 ms, SEM = 10 ms), and NO (M = -124 ms, SEM = 11 ms) conditions. Figure 7 displays the individual data for offline change for each individual in each of the four timing conditions (BEF, DUR, AFT, NO).

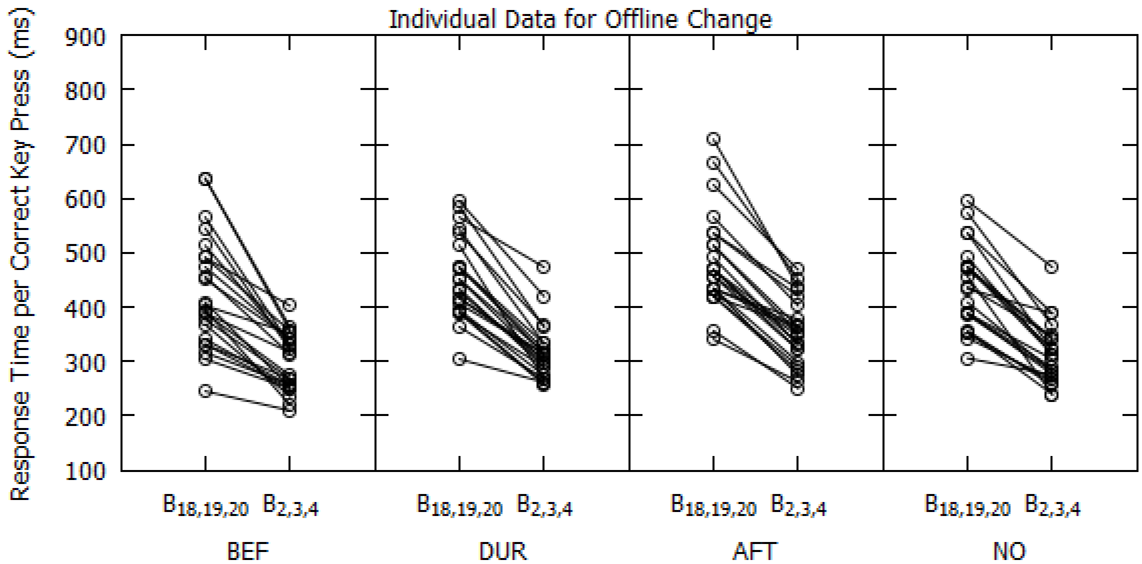

Figure 7. The individual data for offline change. The response time from the last three training blocks (Block 18, 19, and 20) in Practice session and the first three training blocks (Block 2, 3, and 4) in Test 2 session are displayed by each group.

Total Change in Performance of the Target Motor Sequence

To examine the total learning effects, the difference between the mean RT of the first three training blocks (Block 2, 3, and 4) in Practice session and the mean RT of the first three training blocks (Block 2, 3, and 4) in Test 2 session was used (see Figure 4). The Shapiro-Wilk test revealed that the data are normally distributed (p = .227). Levene's test indicated that the assumption of homogeneity of variances is not violated (p = .239). The one-way Timing ANOVA failed to reveal a main effect of Timing, $F(3, 86) = 0.283$, $p = .837$, $\eta^2 = .010$ (see Figure 5). Thus, the total response time change was similar for the BEF (M = -271 ms, SEM = 18 ms), DUR (M = -265 ms, SEM = 16 ms), AFT (M = -274 ms, SEM = 16 ms), and NO (M = -288 ms, SEM = 22 ms) conditions. Figure 8 displays the individual data for total change in performance for each individual in each of the four timing conditions (BEF, DUR, AFT, NO). Figure 9 displays performance across all training and test blocks for the four timing conditions and it was from these data that the total, online, and offline changes were extracted.

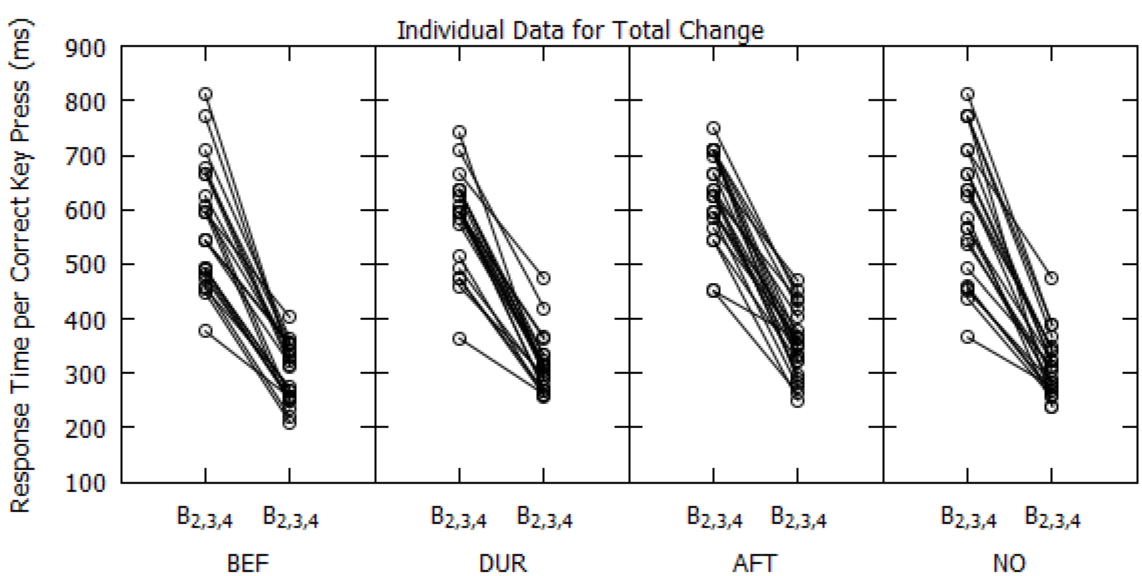

Figure 8. The individual data for total change. The response time from the first three training blocks (Block 2, 3, and 4) in Practice session and the first three training blocks (Block 2, 3, and 4) in Test 2 session are displayed by each group.

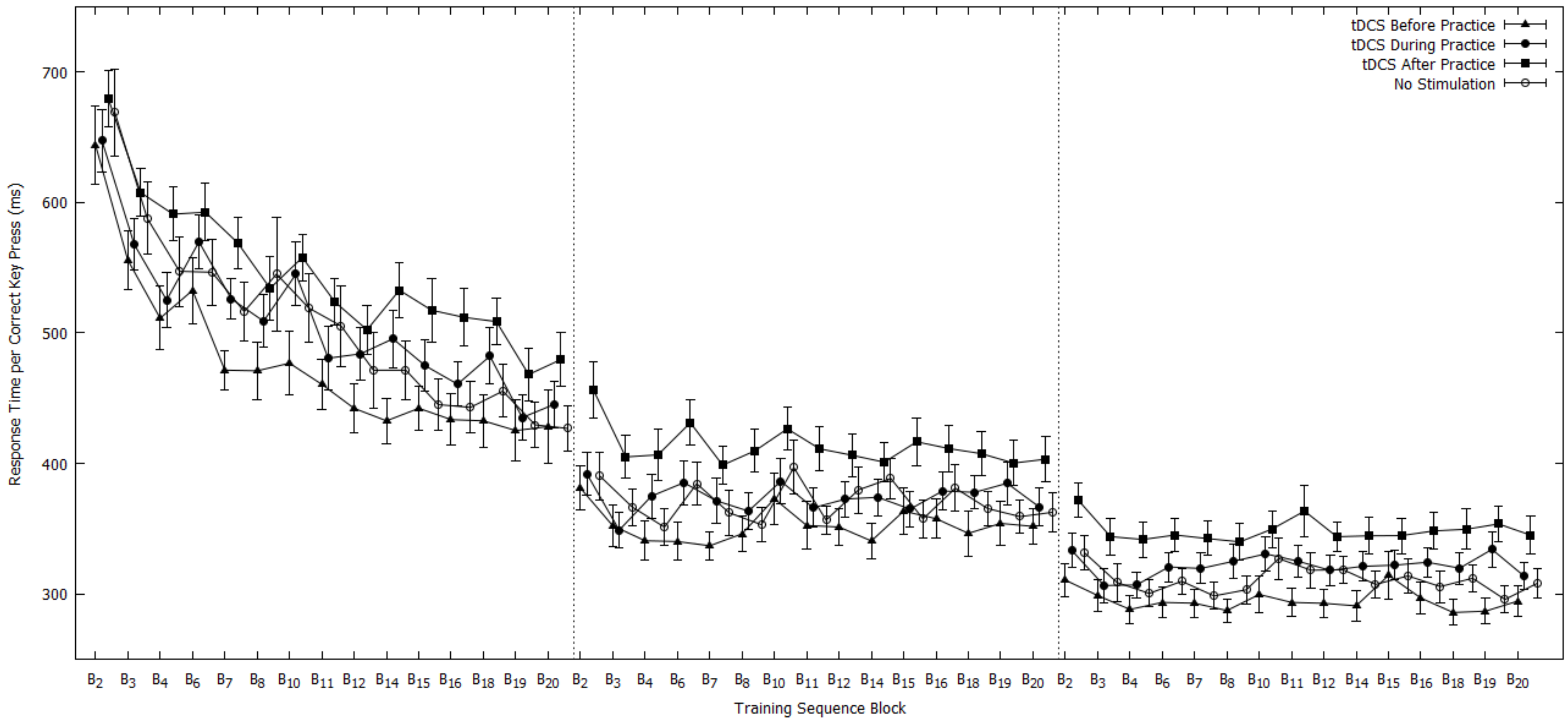

Figure 9. The data for whole training sequence blocks (Block 2, 3, 4, 6, 7, 8, 10, 11, 12, 14, 15, 16, 18, 19, and 20). The left side represents the response time for Practice session. The response time for Test 1 session is displayed in the middle section. The right part shows the result of Test 2 session. The random sequence blocks (Block 1, 5, 9, 13, and 17) were excluded here.

CHAPTER IV

CONCLUSIONS AND DISCUSSION

The purpose of the present research was to investigate the effect of timing of anodal tDCS on the motor sequence learning. To address this issue tDCS was applied at four distinct time points relative to motor training including, (a) before practice (BEF), (b) during practice (DUR), (c) after practice (AFT), or (d) a control sham condition that involved no tDCS (N). For all conditions, tDCS was applied using a bi-hemispheric electrode montage that included the anode being located above right primary motor cortex (contralateral to the moving limb which was the left hand in this experiment) and the cathode above ipsilateral M1 (see Vines et al., 2008). A 2-mA current paired with 50 x 50 electrodes was used for all tDCS conditions resulting in an anode current density of 0.08 mA/cm^2 which is consistent with other studies that have examined the impact of tDCS at M1 for motor skill acquisition.

A number of previously studied independently assessed the influence of introducing tDCS at each of the time points assessed in the present experiment. From this work, it appears that presenting tDCS while the learner is practicing the target task has the most reliable influence for both online and offline improvement in skilled performance. Indeed, there are a number of studies that have revealed enhanced online (Cuypers et al., 2013; Kantak et al., 2012; Karok & Whitney, 2013; Stagg et al., 2011; Zimerman et al., 2013) and offline (Cuypers et al., 2013; Kang & Paik, 2011; Kantak et al., 2012; Karok & Whitney, 2013; Zimerman et al., 2013) benefits during the learning of an MST. It should not go unnoticed however that there are also reports that tDCS applied during this time frame does not provide any advantage beyond sham stimulation for online change (Amadi et al., 2015; Ambrus et al., 2016; Kang & Paik, 2011) or offline improvement (Ambrus et al., 2016; Wade & Hammond, 2015).

With respect to the application of tDCS prior to and after a bout of practice with an MST, the extant literature is much less clear. Using tDCS to modify learning of an MST by exposing the learner to this form of exogenous stimulation before or after practice has, in general, failed to provide any learning benefits. There has only been one study to date that has reported improved delayed retention by administering anodal tDCS during the post-practice period. Tecchio et al. (2010) reported improved MST performance shortly after the presentation of tDCS and argued that the stimulation aided consolidation leading to better skilled behavior. This claim was refuted by Reis et al. (2015) who revealed that anodal tDCS for 15 min following practice of a sequential visual isometric pinch force skill failed to support offline gain at a subsequent 24-hr delayed test. As a result, Reis and colleagues argued that Tecchio et al. (2010)'s findings were more likely a result of well-known after-effects associated with tDCS as opposed to an influence on post-practice consolidation processes.

Taken together the extant data suggest that the most likely source of skill improvement from the use of tDCS should occur when it is experienced whilst practicing the to-be-learned MST. However, it is important to note that this conclusion regarding the relative effectiveness of tDCS timing (i.e., before, during, or after practice of a target skill) is made on the basis of several cross-experiment comparisons. This veracity of this conclusion is less satisfying because many of the experiments contributing to these comparisons applied tDCS in unique, but potentially important ways that may make such comparisons invalid. For example, the duration of current application ranged from 1-2 mA, the electrode montage involved different locations for placement of the return electrode, anode current density differed as a consequence of using different sizes of electrodes, and in some cases, different aged populations were evaluated.

In light of this shortcoming, the present experiment was the first attempt to evaluate the efficacy of administering anodal tDCS at M1 for support MST learning in a single experiment. Specifically, the influence of experiencing 15-min of 2 mA tDCS prior to, during, or after a period of practice with an MST was assessed. MST performance was indexed by mean response time during a set of training blocks as well as 1-hr and 24-hr delayed test blocks. The focus of the present work was on the impact of tDCS timing for online change which was defined as the change in response time from the beginning to the end of the training blocks and offline change manifest as a change in response time from the end of training to the beginning blocks 24 hr later.

As expected, individuals in all tDCS timing conditions exhibited online gain (see Figure 9). However, being privy to tDCS either before or during practice provided no benefit to performance beyond that merely evident as a result of practice as revealed by similar performance during the training blocks for the individuals in the AFT and NO timing conditions. These data, then, are congruent with previous reports suggesting anodal tDCS at M1 before practice (Stagg et al., 2011) and during practice (Amadi et al., 2015; Ambrus et al., 2016; Kang & Paik, 2011) is ineffective at inducing online performance change. The data, however, are inconsistent with a growing body of literature claiming this form of stimulation when coupled with training can be helpful (Cuypers et al., 2013; Kantak et al., 2012; Karok & Whitney, 2013; Nitsche et al., 2003; Stagg et al., 2011; Zimerman et al., 2013).

With respect to offline change, the findings from the present experiment were similar to those observed during the online phase. Once again, offline improvement did occur over the 24-

hr period suggesting some performance enhancement had occurred presumably resulting from either time-dependent (Korman et al., 2007) or sleep-mediated (Walker et al., 2002, 2003) consolidation (see Figure 9). More importantly for the present work, this outcome was not mediated in any significant manner by the introduction of tDCS at any of the time points of interest (i.e., BEF, DUR, AFT) in the present work. While we were not anticipating offline benefits to emerge from tDCS being applied before (Stagg et al., 2011) or after (Reis et al., 2015, but see Tecchio et al., 2010) practice, it was expected that pairing the tDCS with practice would result in offline improvements (Cuypers et al., 2013; Kang & Paik, 2011; Kantak et al., 2012; Karok & Whitney, 2013; Zimerman et al., 2013).

It is tempting at this point to conclude that a single exposure to tDCS is ineffective at impacting skill acquisition either during initial encoding (i.e., online) or during periods of skill consolidation (i.e., offline). This conclusion seems sound when addressing the potential of tDCS before and after practice bouts for skill acquisition but far more tenuous when contemplating the DUR condition outcome. While the presence of data in the literature supporting the efficacy of applying tDCS during practice is persuading, especially in the case of offline benefits (Cuypers et al., 2013; Kang & Paik, 2011; Kantak et al., 2012; Karok & Whitney, 2013; Zimerman et al., 2013), it is wise to remain somewhat cautious and not ignore other work, including the present study, that has failed to demonstrate the utility of tDCS at this time point (Ambrus et al., 2016; Wade & Hammond, 2015).

While the evidence for offline gain from concurrent tDCS with training is mixed when the learner is exposed to a single dose of stimulation, this is not the case when examining the findings from studies in which this same application of tDCS occurs more frequently. For example, Reis et al. (2009) had individuals practice a sequential force-pinch skill over the course of five days while paired with either anodal or sham tDCS at M1. Greater offline gains in skill were associated when practice occurred in the presence of anodal tDCS compared to sham stimulation. Indeed, of the studies that have increased the exposure to tDCS beyond a single time, most have reported an offline benefit (Reis et al., 2009, 2015; Schambra et al., 2011; Walker-Metenier et al., 2014). It is quite possible then that the frequency of the tDCS has to be sufficient to foster learning benefits. Only the case in which tDCS is paired with practice (i.e., DUR condition in the present experiment) has been examined to date.

One additional feature of the present work, in hindsight, may have been a significant contributor to the failure to demonstrate a role for tDCS at M1 for MST learning. A closer examination of studies that reported both online (Cuypers et al., 2013; Kantak et al., 2012; Karok & Whitney, 2013; Nitsche et al., 2003; Stagg et al., 2011; Zimerman et al., 2013) and offline (Cuypers et al., 2013; Kang & Paik, 2011; Kantak et al., 2012; Karok & Whitney, 2013; Zimerman et al., 2013) all applied anodal tDCS at the left M1. In the present work, the anode was located at right M1 given the participants were performing the MST with the left hand. The decision for using the left hand in the present work was made on the premise that greater improvement in performance might be observed using this hand rather than the dominant right hand. On the basis of this decision, right M1 was selected as the target region for stimulation. It is quite possible that left M1 has a proprietary role in the production of MST such as that used in the present work. Indeed, this has been alluded to in previous work addressing tDCS and motor skill acquisition (see Vines et al., 2008). Clearly, this needs to be addressed in future work to (a) examine if this accounts for our failure to observed offline benefits for the DUR condition and (b) consider if stimulating the left hemisphere also changes the effectiveness of tDCS application at other time points relative to physical training.

Finally, one other issue that is also worth considering that may have impacted the present findings and should be considered for most tDCS studies. Specifically, it appears that a relatively large sample size is required to observe positive effects of tDCS for skill acquisition (see Tecchio et al., 2010). In the present study, a total ninety subjects participated in the current study, and the average number of participants per each group was 22.5 (see Table 1). This number is not small compared to much of the existing tDCS research (see Buch et al., 2017). Nevertheless, the statistical power, calculated from G*Power (Version 3.1.9.2), was too low (Online change: .277, Total change: .106). Indeed, this is a serious issue not only for this study but also for other tDCS studies to establish the effects of tDCS.

In conclusion, the present study was designed to examine the effect of timing of anodal tDCS at M1 for online and offline changes in motor sequence learning in a single study. Specifically, the relative effectiveness of introducing this form of neuromodulation prior to, during, or after training was evaluated. As anticipated, findings from the present work confirmed the lack of utility of administering anodal tDCS at M1 prior to or after practice as a means of aiding skill acquisition. In contrast, to the extant literature, coupling tDCS with physical practice also failed to improve either online or offline performance gain. While these data caution the use of tDCS for improving motor skill learning, it is possible that more frequently pairing of the stimulation during practice targeting left M1 might prove to be more efficacious.

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