Using high-definition transcranial direct current stimulation to elucidate the role of the cerebello-thalamo-prefrontal network in explicit sequence learning

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Abstract

Though we have a general understanding of the brain areas involved in motor sequence learning, there is more to discover about the neural mechanisms behind which a new skill is learned. Initial skill acquisition may be subserved, in part, by interactions between the cerebellum (CBLM) and prefrontal cortex (PFC) through a cerebello-thalamo-prefrontal network. We investigated the consequence of stimulating the PFC using high-definition transcranial direct current stimulation (HD-tDCS) before administering an explicit motor sequence learning paradigm. Using a mixed within- and between-subjects design, we employed anodal (n = 24) and cathodal (n = 25) tDCS (relative to sham) to temporarily alter brain function and examine the effects on skill acquisition. In order to gain a comprehensive perspective on the role of this network in sequence learning, we compared the current findings to our recent research investigating the CBLM using the same experimental design. The PFC results indicate that anodal stimulation negatively impacts explicit motor sequence learning, relative to sham, while the effects of cathodal stimulation are minimal, though indicative of either a stabilizing or facilitatory influence. This is consistent with past CBLM results. Our findings, therefore, suggest polarity-specific effects of tDCS on the acquisition of a sequential pattern of finger movements, both when applied to the PFC and CBLM. Collectively, this supports the involvement of a cerebello-thalamo-prefrontal network in initial skill acquisition when cognitive processes, such as working memory, are utilized. Exploring methods that may improve motor learning is important in developing therapeutic strategies for motor-related diseases and rehabilitation.

Keywords: cognition; PFC; HD-tDCS; motor skill; sequence learning

Introduction

Movement coordination and motor skill acquisition are important for routine functioning and day-to-day tasks (i.e., riding a bike, playing a musical instrument, and playing sports). The ability to adequately learn and perform a skill, therefore, is crucial for the execution of conventional actions that impact everyday tasks. Moreover, the capacity to relearn motor skills after injury or infarct is also of interest considering the impact of motor-related issues on daily functioning. As such, investigating the neural circuitry and underlying mechanisms of motor sequence learning is important in advancing our current understanding of this behavior and informing protocols and therapeutic strategies for improved motor function and rehabilitation.

Skill acquisition typically develops over distinct learning phases beginning with an early, or fast, phase during the initial stages of learning and transitioning into a late, or slow, phase as automaticity develops (Karni et al., 1998). The early learning phase is characterized by more cognitively-focused processes and requires active thinking and working memory (WM) while the skill is initially being acquired (Anguera et al., 2012). The late learning phase is more motor-focused as the skill becomes automatic through repetition and practice. Different brain areas are involved in each learning phase, assisting via their unique functions (Doyon et al., 1997). The striatum is particularly active during the late learning phase when a motor skill becomes implicit, whereas the prefrontal cortex (PFC) and cerebellum (CBLM) have been implicated in the WM and cognitive aspects of early learning (Aizenstein et al., 2004; Anguera, Reuter-Lorenz, Willingham, & Seidler, 2010; Bernard & Seidler, 2013; Doyon, Gabitov, Vahdat, Lungu, & Boutin, 2018; Ungerleider, Doyon, & Karni, 2002). In fact, research suggests that the CBLM impacts cognitive processes through a closed-loop cerebello-thalamo-prefrontal circuit (Bernard et al., 2012; Kelly & Strick, 2003; Krienen & Buckner, 2009; O'Reilly, Beckmann, Tomassini, Ramnani, & Johansen-Berg, 2010; Salmi et al., 2010; Strick, Dum, & Fiez, 2009), which may be particularly important during initial skill acquisition. Notably, we have recently suggested that the PFC is the nexus of this circuit, governing the operations of early sequence learning, while the CBLM may serve more of a supporting role (Ballard, Goen, Maldonado, & Bernard, 2019). The PFC coordinates the cooperation of

other brain areas involved in sequence learning (Albouy et al., 2012) and is considerably engaged in the early stages of skill acquisition when cognitive and WM resources are primarily at play (Anguera et al., 2012, 2010; Bo & Seidler, 2009; Schendan, Searl, Melrose, & Stern, 2003).

Though we have a general understanding of the brain areas involved in sequence learning (Doyon et al., 2018), there is still more to discover about the neural mechanisms underlying the learning of a new skill. Skill acquisition may be aided by interactions between the CBLM and PFC through the proposed cerebello-thalamo-prefrontal cognitive network (Eliassen, Souza, & Sanes, 2001); however, this idea remains generally speculative. Our goal in the current study is to investigate the notion that this network, and especially the PFC, is involved in initial skill acquisition, while also shedding light on the cognitive aspects of explicit sequence learning. Thus, our current work focuses on the role of the PFC in the cognitive aspects of sequence learning in comparison to prior work investigating the role of the CBLM (Ballard et al., 2019). Understanding the unique and relative contributions of these two areas in a key circuit will provide novel new insight into the processes involved in sequence learning. In our previous research, we found polarity-specific effects of CBLM stimulation on the acquisition of a new motor skill. Anodal tDCS negatively impacted sequence learning and cathodal tDCS had little impact on performance (Ballard et al., 2019). Ultimately, we aim to better understand the neural underpinnings of sequence learning, particularly with respect to the involvement of cognitive systems and their associated networks.

To this end, we can investigate the role of certain brain areas and probe the underlying circuitry of sequence learning with non-invasive brain stimulation. One way to do so is with transcranial direct current stimulation (tDCS). tDCS involves applying small amounts of electrical current to the scalp with an array of electrodes targeting a specific brain area and altering its function (Nitsche et al., 2008). tDCS impacts the firing rates of neurons and can either increase or decrease neuronal activity in the targeted area, depending on the type of stimulation administered (cathodal or anodal) (Nitsche & Paulus, 2000; Takano et al., 2011). This technique of non-invasive brain stimulation has been successful in improving cognition and motor learning in multiple experimental settings (Buch et al., 2017; Israely & Leisman,

2019; Ke et al., 2019). Research employing stimulation of the dorsolateral PFC (DLPFC) has demonstrated an influence of tDCS on the cognitive aspects related to motor learning (Foerster et al., 2013; Fregni et al., 2005; Leite, Carvalho, Fregni, & Gonçalves, 2011; Pope, Brenton, & Miall, 2015; Wu et al., 2014; Zhu et al., 2015), likely due to the impact on WM processes that provide essential contributions to skill acquisition. To enhance our knowledge regarding the neural mechanisms underlying sequence learning, we implemented high-definition tDCS (HD-tDCS) that achieves increased focality and improved targeting compared to the traditional 2-pad stimulation technique. Though some recent studies have taken advantage of this advanced method (Denis, Zory, & Radel, 2019; Gbadeyan, McMahon, Steinhauser, & Meinzer, 2016; Nikolin, Lauf, Loo, & Martin, 2018), more work is needed to fully comprehend the capacity with which HD-tDCS impacts activity in the PFC with respect to explicit motor sequence learning and initial skill acquisition.

Here we targeted the left DLPFC to get at the WM processes and cognitive aspects of explicit motor sequence learning, using both anodal and cathodal HD-tDCS. We chose this particular location for its role in the control of cognition and WM, specifically in the context of sequence learning, as several investigations using functional magnetic resonance imaging (fMRI) and positron emission tomography (PET) have indicated activation in the DLPFC during the early stages of skill acquisition (Honda et al., 1998; Jenkins, Brooks, Nixon, Frackowiak, & Passingham, 1994; Sakai et al., 1998; Schendan et al., 2003). Further, to better understand the relative contributions of the CBLM and PFC to initial skill acquisition, we compared the results of our recent work targeting the CBLM (Ballard et al., 2019) with stimulation to the DLPFC. Notably, all analyses of the data set included here are unique to this investigation and do not duplicate our prior work. In line with results from the previously collected data on the CBLM, we predicted differing impacts of stimulation type (cathodal vs. anodal) on performance wherein cathodal tDCS would provide stabilizing, or facilitatory, effects and anodal tDCS would impede sequence learning.

Materials and Methods

Participants

This study was carried out on two distinct groups of individuals, resulting in two completely independent samples. Each individual was screened for exclusion criteria associated with tDCS (Nitsche et al., 2008) in order to ensure the safety of those that participated. Both groups underwent the same experimental procedures, but differed in the type of stimulation received (cathodal vs. anodal). The first group consisted of twenty-five individuals that underwent cathodal and sham stimulation during two separate HD-tDCS sessions, counterbalanced in order. Though data was originally collected for thirty-five subjects, one individual was discontinued due to discomfort from stimulation and four individuals did not return for the second session of the study. Additionally, we applied an *a priori* exclusion criterion based on behavioral performance in order to avoid the influence of outliers, such that those with performance 3 standard deviations below the group mean were excluded. In the cathodal sample, this resulted in the exclusion of five subjects, and accuracy was at 34% or less. Thus, the final sample for the first group consisted of twenty-five healthy young adults (15 female, mean age 19.2 years $\pm$ 0.9 S.D., range 18-21 years).

The second group consisted of twenty-four individuals that also underwent two HD-tDCS sessions; however, in this group, anodal and sham stimulation were administered in a counterbalanced order. Forty-three subjects were initially enrolled in the study. Five individuals were excluded due to our exclusionary criteria to ensure participant safety, and two individuals discontinued stimulation due to discomfort. Further, one individual was excluded in light of technical difficulties with software updates and five did not return for the second session. After applying the same *a priori* cutoff to exclude behavioral outliers, six additional individuals were excluded from analyses due to accuracy scores of 33% or less, leaving twenty-four healthy young adults in the final sample for the second group (11 female, mean age 18.7 years $\pm$ 0.6 S.D., range 18-20 years).

None of the subjects included in the final sample for either group had any history of neurological disease (e.g., epilepsy or stroke) nor a formal diagnosis of psychiatric illness (e.g., depression and anxiety), and, relatedly, none were taking medications that could potentially interfere with central nervous system functioning (e.g., neuroleptics, narcotics, anxiolytics, analgesics, stimulants, and antidepressants).

All subjects were right handed in both the cathodal group (mean score 92.5 ± 12.2 S.D.) and anodal group (mean score 81.2 ± 26.6 S.D.), as assessed by the Edinburgh Handedness Inventory (Oldfield, 1971). Subjects received partial course credit for their participation and were recruited through the Psychology Subject Pool at Texas A&M University. Participation in the current study was limited to individuals that had not previously participated in a study employing tDCS and were, thereby, tDCS naïve. All subjects provided written informed consent before study procedures commenced, and our protocol was approved by the Institutional Review Board at Texas A&M University.

Procedure

HD-tDCS

In each group, participants underwent two HD-tDCS sessions, separated by exactly one week, and received both active (cathodal for the first group and anodal for the second) and sham stimulation in a mixed within- and between- subjects design. In order to keep all subjects blind to study procedures, participants were not informed of which stimulation condition was administered during each session until the end of the second session. The order of stimulation condition (active or sham) was counterbalanced across all subjects in both groups. Regardless of the type of stimulation, tDCS was administered with a Soterix MxN HD-tDCS system (Soterix Inc., New York, NY) and an 8-electrode montage. The electrode array and corresponding current intensities (**Table 1**) as well as the modeled current flow (**Figure 1**) for DLPFC stimulation were obtained through HD-Targets software that allows for stimulation of a targeted region of interest with increased focality (Datta et al., 2016; Huang et al., 2017), as compared to the traditional 2-electrode tDCS system. In order to effectively deliver 2.0 mA of net current with the acquired montage, different intensities were implemented for each individual electrode location. **Table 1** and **Figure 1** depict this information for cathodal DLPFC stimulation specifically; however, the same magnitude of intensities and modeled current flow are used for the anodal montage with the sign of each current intensity flipped and the opposite direction of current flow. For the cathodal group, we used a "spiral out" approach, whereas a "spiral in" approach was used for the anodal group, which simply refers

to the direction of current flow for a specific type of stimulation when using a montage that contains multiple stimulation electrodes.

During each HD-tDCS session, and for both experimental groups, 0.1 mA was initially delivered for one minute to establish a sufficient connection, which is key for effective stimulation. The connection was considered adequate once a resistance level of 50 kilo-ohms or lower was achieved at each individual electrode. Following this pre-stimulation period, the full stimulation session began with a gradual increase in current that occurred for roughly 30 seconds until the intended intensity of 2.0 mA was reached. At this point, current remained steady for 20 minutes if the subject was assigned to the active stimulation condition (cathodal or anodal). However, for the sham condition, current gradually decreased and returned to zero for the remainder of the stimulation period before ramping back up and then down again during the last 30 seconds. Regardless of the assigned condition, subjects experienced the same standard setup for HD-tDCS during both sessions and were made unaware of the condition being administered for each session.

Sequence Learning

We used an explicit motor sequence learning paradigm, modeled directly after Kwak, Müller, Bohnen, Dayalu, and Seidler (2012) and in replication of past work (Ballard et al., 2019), in order to assess the impact of stimulation to the DLPFC on the ability to acquire a motor skill and learn a new pattern of events. As research using functional magnetic resonance imaging (fMRI) has indicated more activation in brain areas associated with cognitive processing during explicit learning compared to implicit (Grafton, Hazeltine, & Ivry, 1995; Jenkins et al., 1994; Rauch et al., 1995; Schendan et al., 2003), we chose to investigate the influence of stimulation with an explicit sequence learning paradigm to explore its effects on the cognitive, rather than solely motor, aspects of skill acquisition.

This behavioral task was administered during both experimental sessions for each group following the 20-minute tDCS period and took roughly 12 minutes to complete. The task was displayed on a computer screen using Presentation Software (2019 Neurobehavioral Systems, Inc.) and participants were instructed to place their left middle, left index, right index, and right middle fingers on the numbers

1, 2, 3, and 4 of the computer keyboard, respectively. The task was designed to be bimanual, as is common in sequence learning research (Berner & Hoffmann, 2008; Bhakuni & Mutha, 2015; Bo & Seidler, 2009; Kwak, Müller, Bohnen, Dayalu, & Seidler, 2010; Kwak et al., 2012; Sun, Miller, Rao, & D'Esposito, 2007), in order to allow for potential translation to an MRI environment for future work. During the task, four white rectangles outlined in black were presented on the screen, and these rectangle locations corresponded to the instructed finger placements from left to right. A single rectangle was shaded to black during each individual trial, and participants were instructed to press the appropriate button matching the location of that rectangle as quickly and accurately as possible. Each stimulus was displayed for 200 milliseconds and participants were given 800 milliseconds to make a response before the next stimulus appeared. For each trial, a different rectangle location was shaded to black. The task consisted of 15 total blocks, with some being random and some containing a repetitive sequence of events. Random blocks were dispersed throughout the sequence blocks in order to account for baseline motor function, and, as the task was intended to be clearly explicit, each block was preceded by either an "R" or an "S", depending on the nature of the upcoming trials. Relatedly, our sequence learning paradigm did not include extensive practice or follow-up retention assessments and was relatively short in length to further maintain its explicit character. As such, each participant completed the following block design during both sessions: R-S-S-S-R-R-S-S-S-R-R-S-S-S-R, amounting to 6 random and 9 sequence blocks overall (Kwak et al., 2012). Each random block contained 18 trials while each sequence block contained 36 trials, totaling to 432 trials (Kwak et al., 2012). During the sequence blocks, a 6-element pattern was repeated throughout the task, and this pattern was distinct for each experimental session in order to avoid any interference from practice effects. The same pattern was presented for each participant during the first session (2-4-1-3-2-4) and a different pattern was used for the second session (3-2-4-1-2-4). The two sequences did not differ in complexity according to the Kolmogorov-Smirnov test ($D = 0.184$, $p = 0.346$), and neither contained any sequential numbers or trills.

Data Analysis

Accuracy

Behavioral performance was primarily assessed using average total accuracy (ACC) per block on the sequence trials, specifically. ACC scores are represented by the percent of correct button presses out of the total number of trials for each sequence block. Correct, incorrect (e.g., commission errors), and missed (e.g., omission errors) responses were included in the final number for total trials. Each triplet of sequence blocks was concatenated into one respective learning phase, creating three subsequent learning phases denoted by early, middle, and late. This was done in order to replicate the primary behavioral measure used in Kwak et al. (2012) and in the interest of considering timing-specific effects of stimulation on motor sequence learning. This approach allows us to investigate the broader changes in skill acquisition over the course of the learning task as our paradigm was relatively brief with only 9 sequence blocks total. Rather than modeling ACC over each individual sequence block, we chose to simplify and streamline our analyses in the effort of avoiding redundant comparisons that could potentially obscure the interpretation of results. This particular approach, in the context of ACC during a sequence learning paradigm, has been similarly adopted in prior studies of the same scope (Bo & Seidler, 2009; Kwak et al., 2010, 2012), including our own recent work (Ballard et al., 2019). Nonetheless, our experimental design for behavioral performance reflects an overall early learning paradigm as the task is considerably short in length, lasting only 12 minutes on average. The task was deliberately devised to evaluate initial skill acquisition as the cognitive components of sequence learning were of primary concern in the current study.

Reaction Time

We used mean reaction time (RT) for correct responses as an additional measure of behavioral performance, considering both random and sequence trials for this variable. With RT, trials in which the participant did not respond in time, or missed, were excluded and only those trials in which a correct response had been made during a random or sequence block were used to calculate averages.

Statistical Models

In order to quantify any potential differences in performance between stimulation types (cathodal vs. anodal), mixed model ANOVAs and independent samples *t*-tests were performed across the two

experimental groups to directly compare performance after sham stimulation and active stimulation. Following the direct stimulation condition comparisons, performance differences between stimulation types relative to baseline (sham), were carried out using ACC difference scores (i.e., active ACC – sham ACC) that were calculated for each group to better understand the impact of tDCS on motor sequence learning when taking performance in the absence of stimulation into account. Additionally, repeated-measures ANOVAs and paired samples *t*-tests were conducted within each individual group to investigate performance differences on the sequence learning paradigm between active (cathodal or anodal) and sham stimulation conditions. And finally, mixed model ANOVAs and independent samples *t*-tests were performed on RT difference scores (i.e., active RT – sham RT) and ACC difference scores, collapsing across both stimulation locations and types, to compare the current PFC data to our prior work with the CBLM. For more information on the sample demographics for the CBLM data, please see Ballard and colleagues (2019). Critically, the experimental design exactly paralleled what was described here, and there was no overlap in participants across groups or studies. All analyses were *a priori* and performed using the ezANOVA and t.test packages in R Programming Software.

Results

Prefrontal Stimulation Effects on Sequence Learning

Comparing Cathodal and Anodal

In order to ensure that the sham condition does indeed serve as a reliable measure of baseline performance, we first confirmed that there were no differences in performance between the two groups in the absence of stimulation. A 2 x 3 mixed model ANOVA (stimulation group by learning phase), considering the sham groups only, revealed a significant main effect of learning phase on ACC for sequence blocks, F (2, 94) = 3.63, p = 0.030, η^2 = 0.020, and, critically, no effect of stimulation group (cathodal vs. anodal) on ACC, F (2, 47) = 0.07, p = 0.789, η^2 = 0.001. Further, the interaction between learning phase and stimulation group was not statistically significant, F (2, 94) = 1.98, p = 0.143, η^2 = 0.011. This indicates that, though ACC does change over the course of the task, performance does not significantly differ between the two stimulation groups after the sham condition. Thus, because ACC does

not differ between groups in the absence of stimulation, this substantiates the use of the sham condition as a baseline measure that can then be contrasted with active stimulation to allow for comparisons in performance after cathodal or anodal tDCS.

As such, we directly compared performance on the sequence learning paradigm between active stimulation groups (cathodal vs. anodal). A 2 x 3 mixed model ANOVA (stimulation group by learning phase), on active groups only during sequence blocks, revealed a significant main effect of learning phase on ACC, F (2, 94) = 16.90, $p < .001$, $\eta^2 = 0.087$, and an interaction between learning phase and stimulation type (cathodal vs. anodal), F (2, 94) = 4.35, $p = 0.016$, $\eta^2 = 0.024$, however the main effect of stimulation type was not statistically significant, F (1, 47) = 1.17, $p = 0.285$, $\eta^2 = 0.018$. These results suggest that performance, in terms of ACC, changes considerably over the course of the task after both cathodal and anodal stimulation, however the nature of this change may depend, in part, upon the type of active stimulation received. We conducted follow-up independent samples *t*-tests on each individual learning phase in order to further investigate any potential differences in performance between stimulation types. Though a significant difference in performance between cathodal and anodal groups was not observed in either the early, t (37.63) = 0.55, $p = 0.587$, or middle, t (46.79) = -0.73, $p = 0.466$, learning phases, a *trending* difference in ACC between cathodal and anodal groups in the late learning phase, specifically, was of note, t (45.76) = -1.90, $p = 0.064$. As observed in **Figure 2**, ACC is higher after cathodal stimulation (M = 90.96%) compared to anodal (M = 84.88%) towards the later sequence learning blocks when directly comparing active groups. These results suggest that anodal stimulation may negatively influence ACC as compared to cathodal, though, again, this effect was only trending towards statistical significance.

Finally, we evaluated differences in performance between the two active stimulation types after normalizing ACC scores relative to baseline (sham) performance. However, a 2 x 3 mixed model ANOVA (stimulation group by learning phase) that was carried out on the difference scores (active ACC – sham ACC) revealed no significant effects on ACC (all *ps* > 0.611). Thus, when comparing cathodal

and anodal PFC stimulation groups, specifically relative to performance in the absence of stimulation, no significant differences emerge in regards to ACC.

Cathodal

Following the between-group analyses, we looked within each individual group to examine any potential differences in behavioral performance when comparing active and sham stimulation. Focusing first on RT for the cathodal group, a 2 x 2 repeated measures ANOVA (block type by stimulation condition), collapsing across all 15 random and sequence blocks, revealed a significant main effect of block type (random vs. sequence) on RT, F (1, 24) = 108.72, $p < .001$, $\eta^2 = 0.321$, demonstrating that subjects perform the task more quickly, as indicated by lower RTs, on sequence blocks compared to random (**Figure 3**). However, we did not find a significant main effect of stimulation condition (cathodal vs. sham) on RT, F (1, 24) = 2.95, $p = 0.099$, $\eta^2 = 0.008$, nor an interaction between block type and stimulation condition, F (1, 24) = 2.02, $p = 0.168$, $\eta^2 = 0.004$.

In line with the initial between-group analyses, we looked at the effect of stimulation on ACC using a learning phase perspective, mirroring the mixed model ANOVAs performed when comparing across groups and stimulation types. Through this similar within-subjects model, a 3 x 2 repeated measures ANOVA (learning phase by stimulation condition), on sequence trials only, revealed a *trending* effect of learning phase, F (2, 48) = 3.08, $p = 0.055$, $\eta^2 = 0.010$, but failed to reveal a significant effect of stimulation condition (cathodal vs. sham), F (1, 24) = 0.39, $p = 0.537$, $\eta^2 = 0.001$, on ACC, or an interaction between learning phase and stimulation condition, F (2, 48) = 1.01, $p = 0.372$, $\eta^2 = 0.004$ (**Figure 4**). We suspect this result could, in part, be due to high inter-individual variability in ACC as illustrated in **Figure 4** by noticeably large standard error bars across both groups.

Anodal

The same within-group analyses performed on the cathodal group were repeated with the anodal group. A 2 x 2 repeated measures ANOVA (block type by stimulation condition), across all blocks, revealed a significant main effect of block type (random vs. sequence) on RT, F (1, 23) = 95.38, $p < .001$,

η^2 = 0.409, and an interaction between block type and stimulation condition (anodal vs. sham), F (1, 23) = 6.09, p = 0.021, η^2 = 0.013, though there was not a significant main effect of stimulation condition on RT, F (1, 23) = 1.76, p = 0.197, η^2 = 0.006. As in the cathodal group, the main effect of block type with the anodal group indicates that subjects maintain lower RTs and, thereby, demonstrate improved performance on sequence blocks compared to random (**Figure 5**). In order to further investigate the interaction between block type and stimulation condition, follow-up paired samples *t*-tests were conducted on each block type. When comparing RT after anodal vs. sham stimulation in the sequence blocks, we did not find a significant difference in performance between stimulation conditions, t (23) = -0.45, p = 0.658. However, when comparing RT on the random blocks between stimulation conditions, we found a significant difference in performance such that individuals have higher RTs, indicative of worse performance, after active stimulation compared to sham (or baseline), t (23) = 2.99, p = 0.006. This effect of stimulation on RT during random blocks, specifically, could be due to the possibility that anodal tDCS to the DLPFC is impacting different processes, in addition to the cognitive and learning-based behaviors, that may be involved in more motor-focused or stimulus-response functions.

As with the cathodal group, we also evaluated performance differences in the anodal group using ACC. A 2 x 3 repeated measures ANOVA (stimulation condition by learning phase), on sequence trials only, revealed a significant main effect of learning phase on ACC, F (2, 46) = 13.41, p < .001, η^2 = 0.128, and an interaction between learning phase and stimulation condition (anodal vs. sham), F (2, 46) = 3.82, p = 0.029, η^2 = 0.019, however the main effect of stimulation condition was merely *trending* towards significance in this analysis, F (1, 23) = 3.86, p = 0.062, η^2 = 0.023 (**Figure 6**). In order to parse out the interaction between learning phase and stimulation condition, we conducted follow-up paired samples *t*-tests on each individual learning phase. Though a significant difference in ACC between stimulation conditions did not emerge in the early, t (23) = 0.30, p = 0.768, or middle, t (23) = -1.21, p = 0.240, learning phases, a significant difference in performance was observed between anodal and sham stimulation in the late learning phase, t (23) = -2.51, p = 0.020. Anodal stimulation negatively impacts

sequence learning performance compared to sham, as the task progresses (*M* anodal = 84.87%, *M* sham = 90.19%; **Figure 6**). Paralleling the trending difference found between cathodal and anodal stimulation when directly comparing active groups in the late learning phase, the results from this analysis are consistent with the idea that anodal stimulation may have a negative impact on ACC and, thereby, impede sequence learning.

Comparing Prefrontal and Cerebellar Stimulation

After investigating the impact of stimulation to the DLPFC on motor sequence learning and skill acquisition, we were then interested in comparing the results of this study with those of our recent work, where the same procedures were carried out with stimulation to the CBLM (Ballard et al., 2019). The current study was intended to be a nearly identical replication of this work in order to compare the relative impacts of stimulation to the CBLM and PFC. We chose to compare the behavioral results from stimulating each location in the aim of uncovering any potential interactions or parallel outcomes that might add to our understanding of how tDCS impacts sequence learning. Specific details regarding demographic and procedural information for the CBLM study can be found in Ballard et al. (2019). Stimulation group differences are detailed in our prior work, and here the data are only used as a point of comparison for the PFC stimulation groups. Notably, all comparisons were normalized to sham as in our recent work there were also no performance differences between sham groups.

We first investigated differences in RT between the two brain locations using a 2 x 2 x 2 mixed model ANOVA (block type by stimulation type by stimulation location) on the change in RT relative to baseline (active RT – sham RT). This analysis revealed a significant main effect of stimulation location (PFC vs. CBLM) on RT after active stimulation relative to sham, F (1, 89) = 5.79, p = 0.018, η^2 = 0.037, and a *marginally* significant interaction between stimulation type (cathodal vs. anodal) and block type (random vs. sequence), F (1, 89) = 3.94, p = 0.050, η^2 = 0.018, as well as a *trending* interaction between stimulation type, block type, and stimulation location, F (1, 89) = 3.91, p = 0.051, η^2 = 0.018. When considering RT difference scores, a negative value indicates that active stimulation yields lower RTs (better performance) compared to the sham condition, and the opposite interpretation (higher RTs and

worsened performance) would result from a positive difference score. As such, for both cathodal and anodal groups, stimulation to the CBLM benefits performance in regards to RT (*M diff* = -6.12ms) whereas PFC stimulation has a negative effect on RT (*M diff* = 10.77ms), both relative to baseline (**Figure 7**). To parse out the interactions, 2 x 2 mixed model ANOVAs (block type by stimulation location) were conducted on RT difference scores for each stimulation type, separately. For the cathodal group, this analysis revealed a significant main effect of stimulation location on the change in RT after active stimulation relative to sham, F (1, 44) = 4.69, p = 0.036, η^2 = 0.055, (*M CBLM* = -8.58ms, *M PFC* = 11.79ms), though there was no significant effect of block type, F (1, 44) = 0.62, p = 0.436, η^2 = 0.006, nor an interaction between stimulation location and block type, F (1, 44) = 1.53, p = 0.222, η^2 = 0.015. For the anodal group, we found a significant main effect of block type on RT difference scores, F (1, 45) = 4.18, p = 0.047, η^2 = 0.034 (*M random* = 11.29ms, *M sequence* = -5.17ms), but no significant effect of stimulation location, F (1, 45) = 1.69, p =0.200, η^2 = 0.023, nor an interaction between block type and stimulation location, F (1, 45) = 2.46, p = 0.124, η^2 = 0.020.

Finally, we investigated performance differences between stimulation locations using ACC difference scores. A 2 x 2 x 3 mixed model ANOVA (stimulation type by stimulation location by learning phase) on the change in ACC after active stimulation relative to baseline (active ACC – sham ACC) revealed a significant main effect of stimulation type (cathodal vs. anodal), F (1, 89) = 8.75, p = .004, η^2 = 0.051, and learning phase, F (2, 178) = 4.28, p = .015, η^2 = 0.022, on ACC difference scores, as well as an interaction between stimulation type and learning phase, F (2, 178) = 3.49, p = .033, η^2 = 0.018. All remaining effects and interactions were non-significant (all *ps* > 0.144); notably, there was no effect of stimulation location on ACC difference scores, F (1, 89) = 0.001, p = 0.977, η^2 < 0.001. To investigate this result further, we broke down the ACC difference scores by each individual learning phase and compared the change in performance between stimulation types (cathodal vs. anodal) at each time point. Though a significant difference in performance was not observed between cathodal and anodal groups in the early learning phase, t (82.38) = -0.71, p = 0.482, a clear effect of stimulation type on the change in

ACC after active stimulation relative to sham is evident in the middle learning phase, t (81.71) = -2.57, p = 0.012, as well as the late learning phase, t (79.82) = -2.87, p = 0.005. These results suggest that, though there may not be differences in ACC relative to baseline between stimulation locations, a significant difference between stimulation types emerges in the middle learning phase (*M cathodal* = 1.09%, *M anodal* = -2.84%) and progresses into the late learning phase (*M cathodal* = 0.38%, *M anodal* = -4.94%). Thus, ACC after active stimulation compared to sham noticeably changes over the course of the task when considering differences between cathodal and anodal groups; however, effects are similar when stimulation is applied to the DLPFC and CBLM (**Figure 8**). Nonetheless, an apparent decline in performance throughout the task is observed in both anodal groups, and stimulation differences are present in both the middle and late learning phases.

Discussion

Summary

Applying HD-tDCS to the DLPFC significantly influenced performance on an explicit motor sequence learning paradigm. Stimulation impacted sequence learning over the course of the task as performance differences emerged in the middle to late learning phases. Additionally, the nature of the observed effects was dependent upon the type of stimulation administered (cathodal or anodal). Research concerning cathodal tDCS is generally inconclusive and largely suggests that this type of stimulation does not uniformly influence cognitive performance (Imburgio & Orr, 2018; Jacobson, Koslowsky, & Lavidor, 2012; Nozari, Woodard, & Thompson-Schill, 2014; Shilo & Lavidor, 2019; Talsma, Broekhuizen, Huisman, & Slagter, 2018). Our results from the current study broadly support this notion with respect to the PFC. Conversely, anodal tDCS is more commonly known to benefit behavioral performance as a result of increasing firing rates in the targeted brain area (Fregni et al., 2005; Ke et al., 2019; Nitsche & Paulus, 2000; Takano et al., 2011); however, here, we observed the opposite effect. We also observed differences in RT when comparing the current data to our prior work with the CBLM, indicating that the location of stimulation may be critical in certain aspects of initial skill acquisition when manipulating cerebello-thalamo-prefrontal circuits. Finally, our results indicate polarity-specific effects of tDCS on

sequence learning ACC in both the CBLM and PFC, wherein cathodal stimulation may have a weak facilitatory impact on sequence learning, or possibly rather a stabilizing effect, while anodal stimulation worsens performance, relative to sham.

Prefrontal Stimulation and Explicit Motor Sequence Learning

When directly comparing the cathodal and anodal DLPFC stimulation groups, we found that ACC changed over the course of the task, depending upon the type of stimulation administered. Anodal stimulation evoked a decline in sequence learning performance as ACC scores were over six percent lower, on average, compared to the cathodal group. Evaluating cathodal and sham stimulation in a within-subject manner, we found null effects on ACC across all learning phases, though a *trending* relationship between learning phase and ACC was present. Together with the between-subjects ACC results, this supports the idea that, in general, cathodal stimulation may not elicit reliable effects on behavioral performance (Imburgio & Orr, 2018; Jacobson et al., 2012; Nozari et al., 2014; Shilo & Lavidor, 2019; Talsma et al., 2018). However, when examining the same construct within the anodal group, we found that stimulation condition and learning phase interact to impact sequence learning performance. Performance worsened over time after anodal stimulation relative to sham, similar to our observations with the between-subject comparisons. Though unexpected in the greater context of tDCS research (Fregni et al., 2005; Ke et al., 2019; Nitsche & Paulus, 2000; Takano et al., 2011), this could be due to our distinct stimulation approach as well as improved targeting and increased focality with the HD-tDCS system employed here, as the majority of motor skill acquisition work relies upon the more traditional 2-pad stimulation technique (reviewed in Buch et al., 2017).

Though anodal tDCS typically improves performance (Fregni et al., 2005; Ke et al., 2019; Nitsche & Paulus, 2000; Takano et al., 2011), results are often specific to a distinct performance measure and may, therefore, be more limited than presumed. For instance, research conducted by Leite et al. (2011) demonstrated that anodal stimulation to the DLPFC primarily improved performance speed on a cognitive task rather than reducing number of errors or increasing efficacy. Consistent with this idea, Nikolin, Loo, Bai, Dokos, and Martin (2015) found that anodal DLPFC stimulation enhanced the rate of

verbal learning and speed of responding during a WM task, but no impacts on ACC were observed. Notably, this work was executed using the HD-tDCS method employed here, suggesting that these potentially limited effects of anodal stimulation translate to the more advanced techniques as well. Thus, the effects of anodal tDCS are most evident in performance measures related to RT in lieu of ACC. Following up on their prior work, Nikolin et al. (2018) subsequently discovered null effects of anodal HD-tDCS to the DLPFC on verbal WM performance. As such, it seems that, to an extent, inconsistent results are also observed with anodal stimulation, as with cathodal. Decreased reaction times after anodal DLPFC stimulation have been further observed in tDCS research (Wu et al., 2014); however, improvements to WM capacity were specifically observed when cognitive demand was at the highest degree. Relatedly, the anodal approach has been shown to selectively benefit cognitive performance on a difficult task (Pope et al., 2015). Thus, these distinctions indicate that anodal tDCS may exercise novel effects on less cognitively demanding tasks, such as the explicit sequence learning paradigm employed in our study. In addition, the effects of anodal stimulation may be dependent upon the specific sensory modality of the task administered as researchers have found distinct impacts of anodal HD-tDCS on visual versus auditory WM tasks (Naka et al., 2018). As such, though the effects are somewhat unexpected, our findings are not entirely inconsistent with the literature more broadly.

With respect to RT, we found that subjects performed the task more quickly on sequence blocks compared to random after both anodal and cathodal PFC stimulation. Notably, these results are consistent with our prior work on the CBLM where lower RTs were observed on sequence blocks, compared to random, after both stimulation types (Ballard et al., 2019), and with other past studies of sequence learning (Berner & Hoffmann, 2008; Julien Doyon et al., 2002; Kwak et al., 2010; Seidler, 2006). Unique to the current study, we found that individuals in the anodal group were slower after active stimulation compared to sham on the random blocks. We speculate that this negative effect of anodal stimulation to the DLPFC on RT during random blocks could be, in part, the result of an impact on motor-related processes or stimulus-response preparation functions, as the DLPFC maintains connections with the primary motor cortex (M1) (Hasan et al., 2013).

Prefrontal and Cerebellar Contributions to Explicit Motor Sequence Learning

Additionally, we compared performance after stimulation to the PFC with stimulation to the CBLM in order to investigate the relative contributions of these areas to early learning. With respect to ACC, a significant difference in the change in performance after active stimulation relative to baseline is evident between stimulation types. That is, regardless of stimulation location, polarity-specific effects of tDCS emerge when comparing cathodal and anodal stimulation. Cathodal stimulation results in small improvements, while anodal stimulation worsens performance. Consistent with our prior work (Ballard et al., 2019), and in extension of the PFC findings, this suggests that, with both locations, cathodal stimulation may have a weak facilitatory effect on sequence learning performance while anodal stimulation, again, bears a considerably negative effect.

Relevant to the cathodal findings, recent research has shown that cathodal tDCS over M1 facilitates performance on an implicit serial reaction time task (SRTT) (Shilo & Lavidor, 2019). tDCS was applied for 20 minutes during performance of the task; however, in the first 13 minutes of stimulation, the anodal condition improved performance relative to cathodal, whereas the opposite pattern was observed for the remaining 7 minutes (Shilo & Lavidor, 2019). Thus, these results indicate a shift in the polarity-specific effects of tDCS after 13 minutes of stimulation in a 20-minute protocol. Foerster et al. (2013) found improvements in motor function with anodal tDCS to the DLPFC, but stimulation was only applied for 13 minutes, which may have prevented the shift in polarity-specific effects. Further, work by Batsikadze, Moliadze, Paulus, Kuo, and Nitsche (2013) demonstrated enhanced cortical excitability in the primary motor cortex after 2 mA of cathodal tDCS was applied to the same area for 20 minutes. Thus, it may be that the polarity-specific effects of tDCS are timing-specific and dependent upon the stimulation parameters employed, thereby contributing to the inconsistency of results in tDCS research. Here, we applied 20 minutes of offline stimulation before administering a sequence learning task, thus consistent, to some degree, with a shift in polarity-specific effects over time. Cathodal DLPFC stimulation has also been shown to improve implicit motor learning during a movement control task (Zhu et al., 2015), and,

though our paradigm is explicit, this broadly supports the notion that cathodal tDCS may facilitate or stabilize performance during learning.

While the cathodal stimulation effects are small, anodal stimulation disrupts skill acquisition in both the CBLM and PFC groups. No differences in ACC were observed between stimulation locations. As both the CBLM and PFC are implicated in the cognitive aspects of initial skill acquisition (Aizenstein et al., 2004; Anguera et al., 2010; Bernard & Seidler, 2013; J Doyon et al., 2018; Ungerleider et al., 2002), it may not be entirely surprising that we detect similar effects of stimulation on explicit sequence learning after applying tDCS to each of these areas. This finding is particularly informative in further elucidating the role of the cerebello-thalamo-prefrontal network in sequence learning as it suggests that both the CBLM and PFC significantly contribute to the acquisition of a new skill. It may be the case that each area achieves an influence on skill acquisition by modulating distinct processes that provide unique inputs to learning. In work examining sensorimotor adaption, patients with CBLM degeneration and lesions to the PFC both exhibit prediction errors in a strategy-aiming task, however it is suggested that the CBLM group neglects to adapt an internal model whereas the PFC group fails to modify a strategy (Taylor & Ivry, 2014). As such, though the cerebello-thalamo-prefrontal network is involved explicit sequence learning, the relative contribution of each area in this circuit may be accomplished through the regulation of distinct processes. Importantly, this is counter to the suggestion that the CBLM may play a more supporting role in learning relative to the PFC, and broadly suggests that the nodes of this circuit play unique and complementary roles in the acquisition of new motor skills. This also has potential implications for our understanding of the CBLM's contributions to non-motor behavior, as part of the cerebello-thalamo-prefrontal circuit.

In general, the unexpected nature of our findings may partially result from methodological differences, as stimulation parameters can impact the effects of tDCS (Imburgio & Orr, 2018). For instance, whether stimulation is applied during task performance or entirely before is an important consideration as some of the excitatory effects of anodal tDCS and null effects of cathodal have specifically been observed with online protocols (Fregni et al., 2005). In the current study, we employed

offline stimulation. And again, we used a novel, more advanced tDCS approach, which may have provided a distinct impact on brain activity and behavioral performance in comparison to the traditional 2-pad technique. Importantly, individual differences in responsiveness to stimulation have also been implicated in the effect on behavioral performance (Talsma et al., 2018), and this inter-individual variability with tDCS is not yet fully understood (Buch et al., 2017). Finally, a meta-analytical review by Jacobson et al. (2012) suggests that, though excitatory effects with anodal stimulation and inhibitory effects with cathodal are persistently observed in research examining motor function, the dual-polarity theory of anodal-excitation and cathodal-inhibition with tDCS is less consistently upheld in investigations concerning cognitive performance. Given our interest in cognitive processes during learning, this may be especially pertinent here.

Limitations

While our findings offer new insights into the neural mechanisms of explicit sequence learning, a few limitations are worth noting. First, though HD-tDCS allows for improved targeting and increased focality, it is possible that stimulation reached areas other than the intended location (**Figure 1**). We suggest that our stimulation montage primarily impacted the DLPFC, however current may have spread to other regions of the PFC. In addition, as the long-term effects of tDCS are not clear, future work extending past our brief learning paradigm to include assessments of retention over time would be beneficial in defining the lasting impact of stimulation targeting the cerebello-thalamo-prefrontal network on motor sequence learning.

Conclusions

Together, our findings offer a novel perspective on the neural mechanisms underlying the cognitive aspects of explicit motor sequence learning. Results suggest that, when targeting both the PFC and CBLM, individually, anodal stimulation negatively impacts initial skill acquisition whereas the effects of cathodal stimulation are less straightforward, though universally indicative of either a facilitatory or stabilizing influence. Further, effects of applying HD-tDCS to these areas consistently emerge over the course of a brief sequence learning paradigm, implicating the cerebello-thalamo-

prefrontal network in the early stages of skill acquisition. Exploring methods that may improve the acquisition of essential motor skills is crucial in developing therapeutic strategies for motor-related diseases and recovery from injury, as well as reducing normative declines in motor functioning with advanced age.

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	Location	Current
1	FP1	1.0062
2	F3	-1.1757
3	F4	0.2221
4	P3	0.0761
5	C2	0.0183
6	CP2	0.1049
7	F5	-0.8243
8	TP7	0.0699
C	F9	0.5024

Table 1. Electrode array and current intensities for prefrontal stimulation. *Location*: particular brain locations for electrode placement, determined by 10-20 system; *Current*: specific intensity of electrical current applied at a given location.

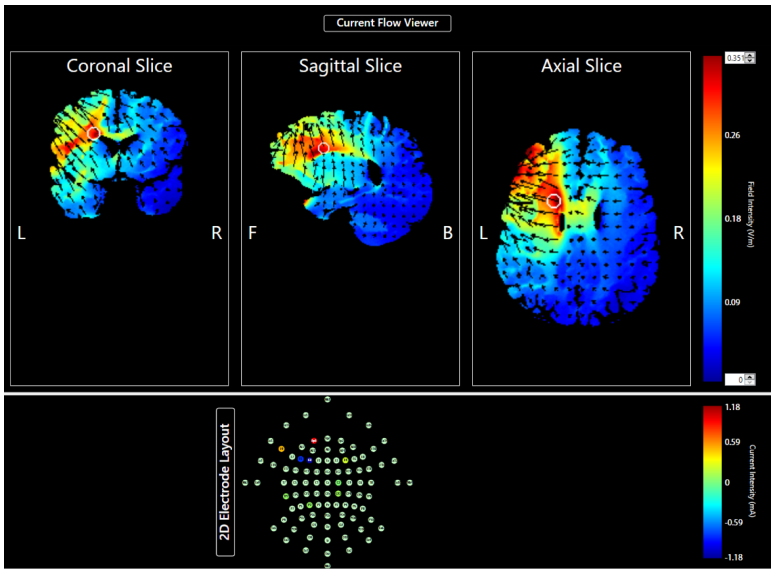

Figure 1. Modeled current flow for prefrontal stimulation (BA 46) using HD-Targets software. Color bar indicates field intensity (V/m) on a scale of 0 – 0.35 (bottom to top). *L*: Left; *R*: Right; *F*: Front; *B*: Back.

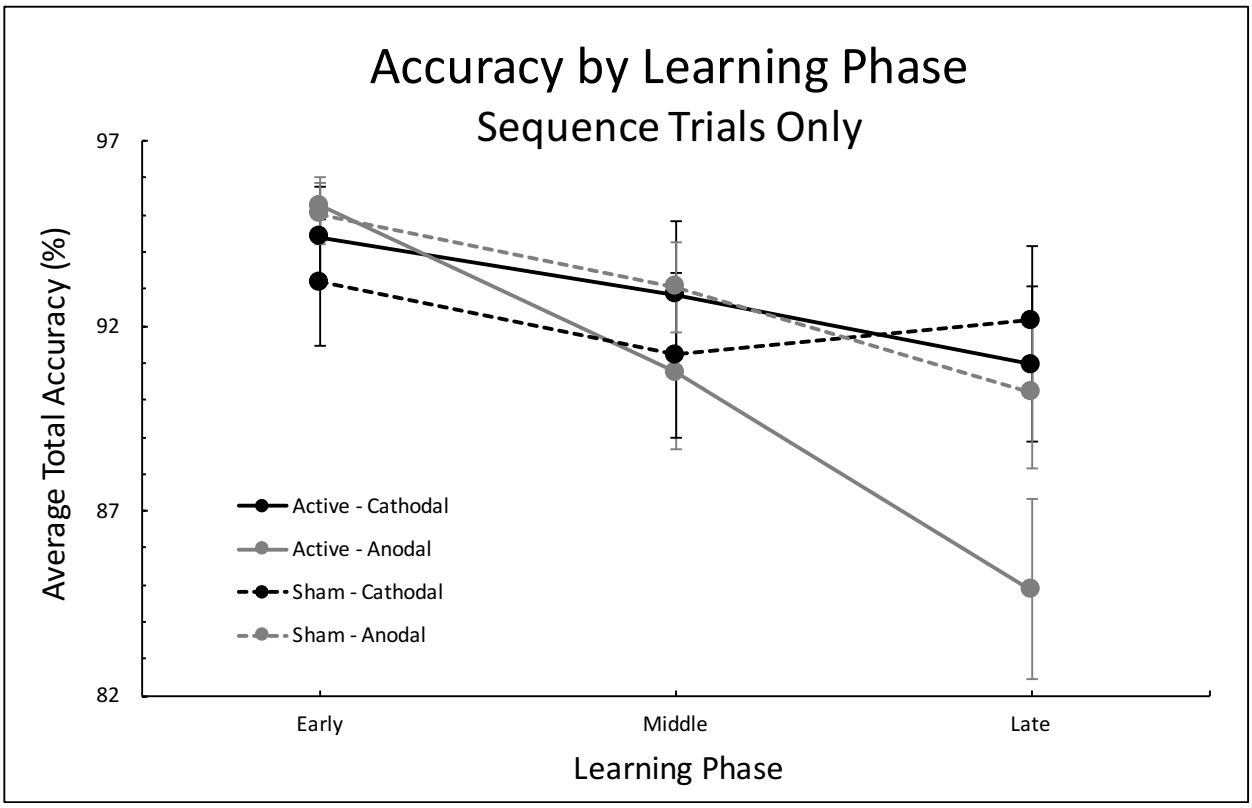

Figure 2. Group mean of total accuracy, missed trials included, across early, middle, and late sequence learning phases in cathodal, anodal, and both sham conditions for prefrontal stimulation. Error bars indicate standard error (SE).

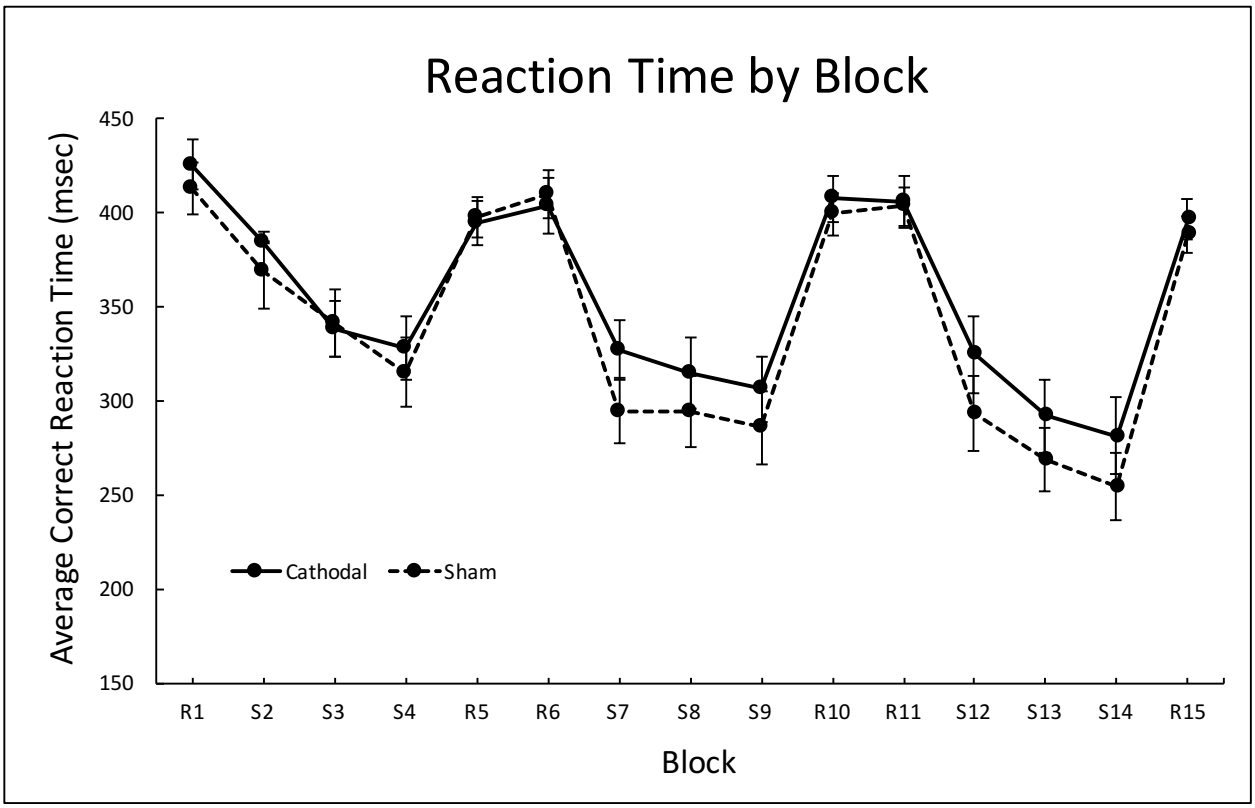

Figure 3. Group mean of correct reaction time, excluding missed trials, across all blocks in both cathodal and sham conditions for prefrontal stimulation. Error bars indicate SE.

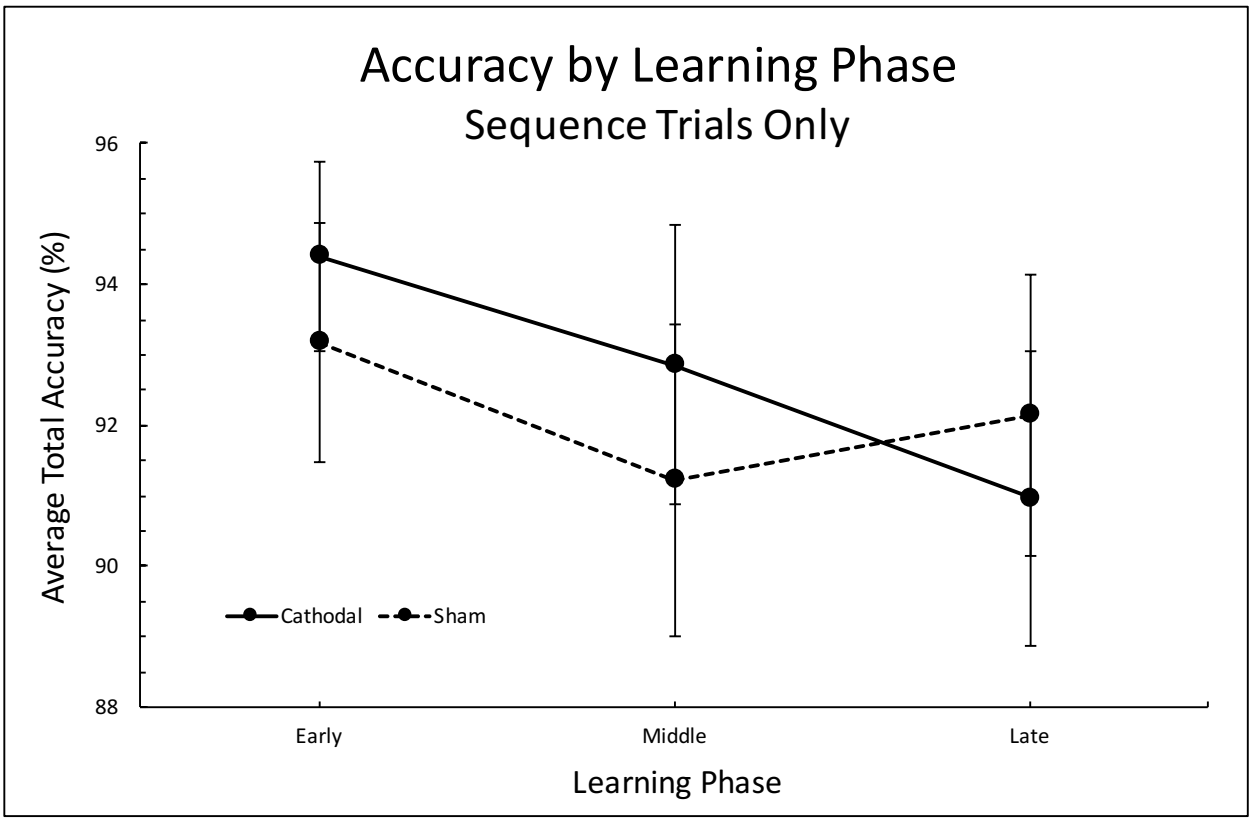

Figure 4. Group mean of total accuracy, missed trials included, across early, middle, and late sequence learning phases in cathodal and sham conditions for prefrontal stimulation. Error bars indicate SE.

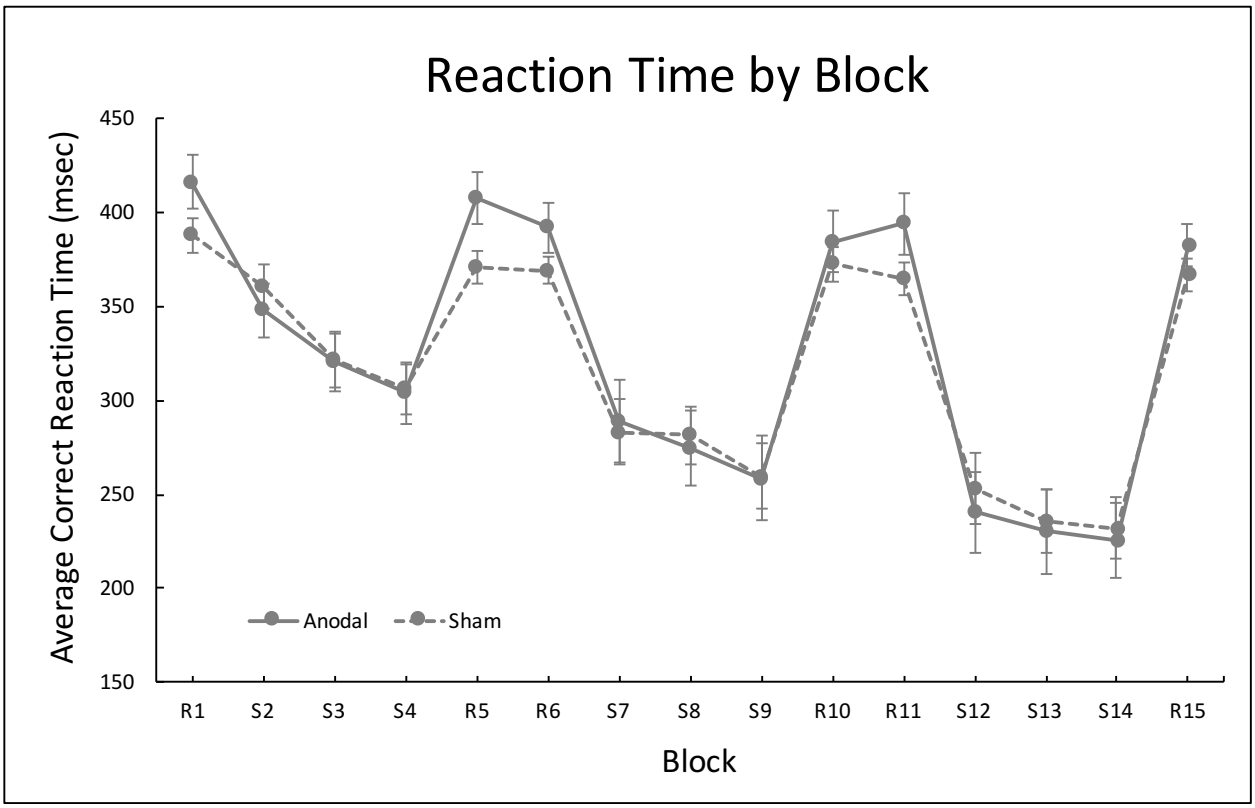

Figure 5. Group mean of correct reaction time, excluding missed trials, across all blocks in both anodal and sham conditions for prefrontal stimulation. Error bars indicate SE.

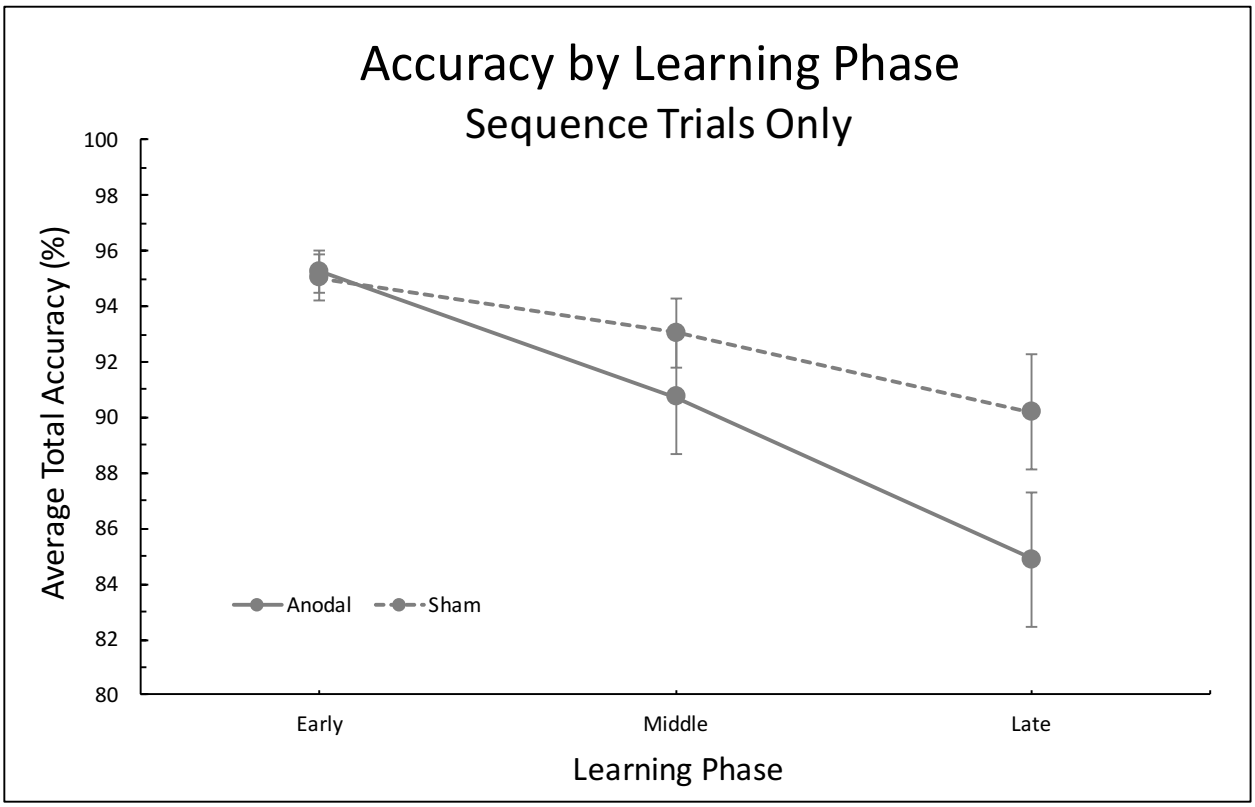

Figure 6. Group mean of total accuracy, missed trials included, across early, middle, and late sequence learning phases in anodal and sham conditions for prefrontal stimulation. Error bars indicate SE.

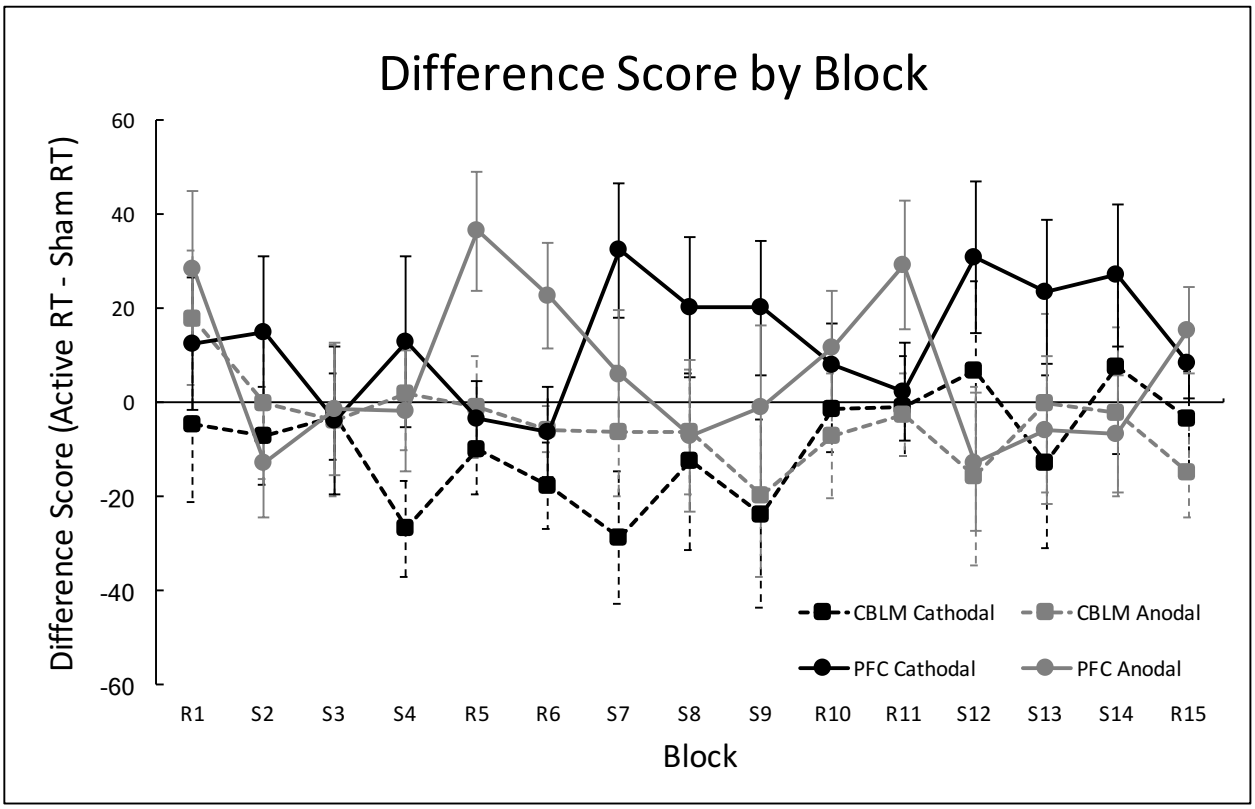

Figure 7. Difference scores (active RT – sham RT) across all blocks for cathodal and anodal conditions in both prefrontal and cerebellar stimulation groups. Error bars indicate SE.

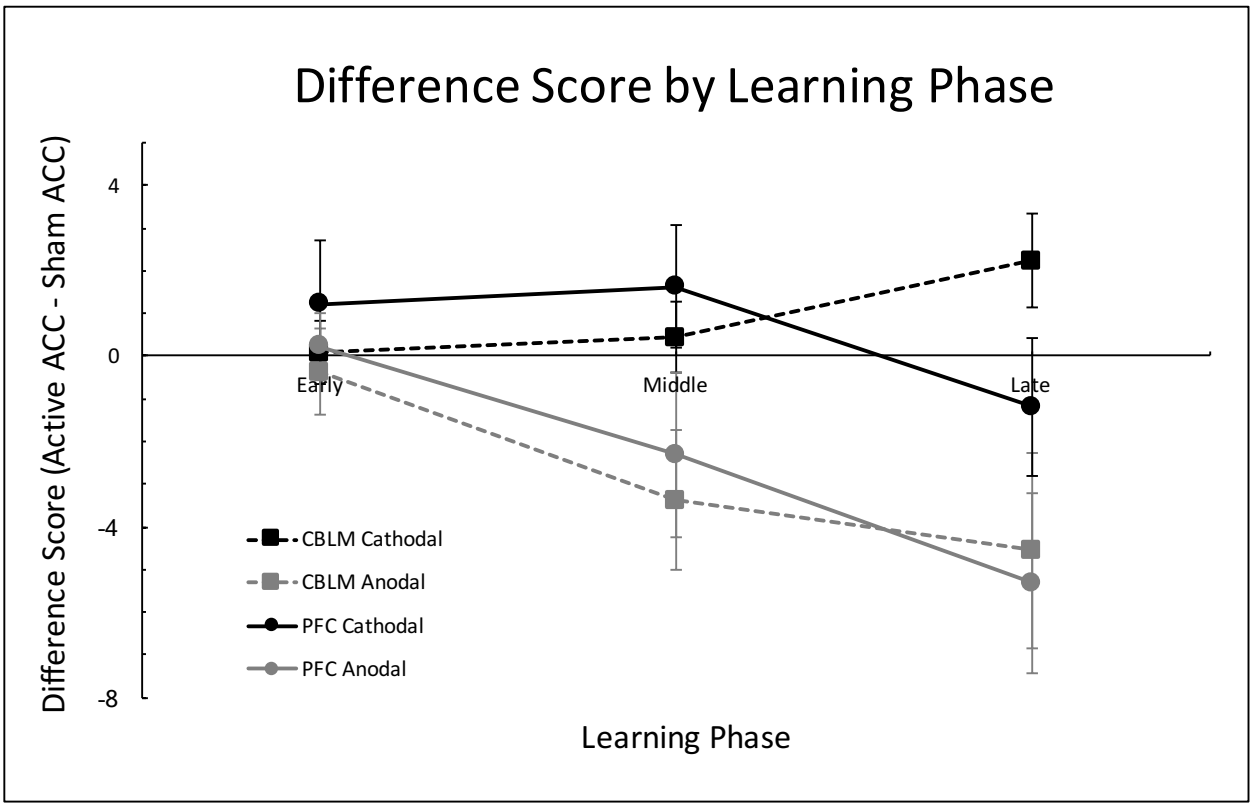

Figure 8. Difference scores (active ACC – sham ACC) across early, middle, and late sequence learning phases for cathodal and anodal conditions in both prefrontal and cerebellar stimulation groups. Error bars indicate SE.