Cognitive Contributions to Motor Learning

by

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ABSTRACT

Transcranial direct current stimulation (tDCS) can facilitate motor learning. However, the tDCS literature scarcely addresses whether stimulation to prefrontal brain regions affects motor learning, whether chunking together of individual actions can be influenced by tDCS, and whether there are age differences in how stimulation affects sequence learning. Here we completed a series of studies that examined the application of tDCS to the prefrontal cortex (PFC), motor cortex (M1), or the presupplementary motor area (preSMA) and its impact on motor sequence learning to understand the neural bases of motor learning.

First, we found both left and right PFC stimulation slowed reaction time decreases and chunking. Stimulation to the preSMA lowered reaction time but came at the expense of a higher number of chunks. and tDCS over M1 helped with reaction time decreases and chunking. Further, contrasts revealed the M1 group had overall faster reaction times and fewer chunks. In order to understand the sequence learning impairment of left PFC anodal tDCS group, we added a left PFC cathodal montage. The left PFC cathodal group demonstrated impaired learning, with longer reaction time and a greater number of chunks, results similar to the left PFC anodal montage.

In experiment two, participants from the left PFC, M1, and sham tDCS groups returned for a fourth session to assess long-term effects of tDCS. Participants completed a single session of practice without tDCS on the same sequences assigned to them the year before. We found the M1 tDCS group reduced reaction time at a faster rate relative to sham and the left PFC group demonstrated less forgetting over the course of a year, but overall slower reaction times.

Finally, we determined how tDCS applied to the same four brain regions as in the first study affected sequence learning and chunking in older adults. We found no age differences regarding stimulation effects on reaction time reductions; both age groups benefited from M1 stimulation, whereas stimulation to the prefrontal cortices impaired learning. However, we did find age-group differences in chunking. Stimulation to M1 helped chunking processes for both age groups and to a greater extent for older adults.

Thus, our findings suggest that regardless of age, stimulation to prefrontal cortices impairs learning, likely interfering with the automatization of sequence, whereas stimulation to M1 facilitates learning, especially in chunk formation. In light of our findings, we suggest the Cognitive framework for Sequential Motor Behavior (C-SMB), a framework that accounts for motor sequence learning should be modified to account for our findings.

CHAPTER I: General Introduction

Overview

How the prefrontal cortex (PFC) and cognitive processes contribute to motor learning is unclear. The PFC is engaged in early motor sequence learning (Deiber et al., 1997; Jenkins, Brooks, Nixon, Frackowiak, & Passingham, 1994; Keisler & Shadmehr, 2010) as well as in working memory (Courtney, Petit, Maisog, Ungerleider, & Haxby, 1998). However, the specific role of the PFC in motor sequence learning is still unknown. Previous research has demonstrated a relationship between working memory and motor learning. For example, working memory capacity is correlated with the extent of motor learning (Bo & Seidler, 2009). Further, motor chunking, an indication of sequence learning, is defined as when two or more individual actions become grouped together. Interestingly, working memory is also correlated with chunk length (Bo, Borza, & Seidler, 2009b; Bo & Seidler, 2009). Thus, a possible link between cognitive processes and motor learning is through chunking.

Transcranial direct current stimulation (tDCS), a form of non-invasive brain stimulation, can provide insight into the relationship between brain region and function. For example, tDCS over primary motor cortex can enhance motor learning (Kincses, Antal, Nitsche, Bártfai, & Paulus, 2004; Nitsche & Paulus, 2000). Further, Vollmann and colleagues (2013) found that individuals who received stimulation to supplementary motor area (SMA) but not the pre-supplementary motor area (preSMA) demonstrated marked increases in learning magnitude. However, two issues with the current tDCS motor learning literature are: 1) a lack of targeting

prefrontal regions with tDCS (in addition to motor regions) and 2) no instances of using tDCS to understand the neural correlates of chunking. Therefore, the goal of this dissertation is to target prefrontal (in addition to motor) areas using tDCS in order to understand the cognitive contributions to motor sequence learning.

Stages of Explicit Motor Learning

Motor learning occurs in at least two, overlapping stages: a fast, early stage primarily driven by cognitive processes, and a slow, late stage that is largely automatic (Keisler & Shadmehr, 2010). Neuroimaging studies have shown that depending on the stage of motor learning, motor control is mediated by different brain regions. For instance, prefrontal brain regions, which are typically associated with cognitive processes, are more engaged early in learning. Jenkins et al. (1994) used position emission tomography (PET) to measure regional cerebral blood flow in participants performing either an unfamiliar, explicit 8-element sequence, or a previously learned sequence. Prefrontal regions (anterior middle frontal gyrus, dorsolateral prefrontal cortex, and frontopolar cortex) and premotor areas showed activity only when participants learned and encoded the novel sequence relative to baseline, but not when participants performed the previously learned sequence (retrieval). Further, Deiber et al. (1997) conducted an experiment in which participants completed a visuomotor/conditional task, where they had to move a joystick in one of four different directions with their right hand depending on the stimulus and location of the stimulus while in a PET scanner. As participants became more skilled at the task, there were decreases in regional cerebral blood flow of the right DLPFC, premotor areas, as well as in the posterior parietal cortex (Deiber et al., 1997). Deiber and colleagues (1997) proposed that the disengagement of prefrontal areas could be due to less dependence on (spatial) working memory with practice. These results compliment the previous

findings of Jenkins et al. (1994), in that, after the sequence is learned the prefrontal, premotor, and posterior parietal cortices are less engaged, consistent with a role of PFC specific primarily in the early stage of learning.

The primary motor cortex (M1) is integral to both stages of learning. Karni et al. (1998) used fMRI to demonstrate that the left primary motor cortex is involved in the first few minutes of learning during an explicit sequence learning task as well as during sequence production three and eight weeks later. Further, inhibiting M1 with repetitive transcranial magnetic stimulation (TMS) *after* participants have practiced a finger tapping task disrupts retention of improvements (Muellbacher et al., 2002). Kawai et al. (2015) demonstrated that M1 is critical for learning a complex sequence, but *not* necessary for the recall and execution of a motor skill in rodents after extensive training; this finding suggests that while M1 may be involved in both stages of learning, it's involvement does decrease for production of highly automatized sequences.

In conclusion, neuroimaging studies provide evidence that motor learning occurs in at least two stages. The early stage of motor learning often involves engagement of frontal brain regions such as DLPFC and M1. The later stage of motor learning involves a decrease in activation of prefrontal brain regions, while M1 may continue to be involved.

The Role of Cognitive Processes in Motor Learning

The PFC and cognitive processes are engaged during motor learning, but their specific role is unclear. Regions of the PFC are engaged in working memory, which in turn has been strongly linked to learning new action sequences. Single-cell recordings and BOLD activation in the DLPFC of non-human primates and humans demonstrate a sustained level of activity during the delay period in working memory tasks (Courtney et al., 1998; Funahashi, Bruce, & Goldman-

Rakic, 1989). Working memory performance is associated with sequence learning. For example, short-term working memory capacity is positively correlated with the amount of implicit sequence learning that occurs in a practice session (Frensch & Miner, 1994) and visuospatial working memory capacity is related to sequence learning performance under both implicit and explicit conditions (Bo, Peltier, Noll, & Seidler, 2011; Bo, Borza, & Seidler, 2009a). Perhaps not surprisingly, DLPFC activity has been reported for both explicit and implicit sequence learning studies (Aizenstein et al., 2004; Willingham, Salidis, & Gabrieli, 2002).

Awareness and Motor Sequence Learning

An additional role of the prefrontal cortex during motor learning may involve strategy. Gaining awareness of a sequence during practice changes the underlying neural correlates, possibly reflecting changes in strategy. In a study conducted by Grafton et al. (1995), participants underwent a PET scan while performing the serial reaction time task under implicit conditions. In this task, participants produce finger movements in response to stimuli presented in a sequential order. The left primary motor cortex and preSMA, and the right putamen showed activation increases when participants remained unaware of the sequence. Interestingly, the right DLPFC, premotor cortex, ventral putamen, bilateral parietal and occipital cortices showed increases in activation, specifically around the time when participants became explicitly aware of the sequence. The right DLPFC gradually increased in activation, building up until participants became aware, suggesting that this region is involved in other cognitive components in addition to (spatial) working memory during sequence learning (Grafton, Hazeltine, & Ivry, 1995). Prefrontal and parietal cortical areas are considered part of an attentional and cognitive network (Corbetta, Miezin, Dobmeyer, Shulman, & Petersen, 1990) and could be involved in strategic shifts in motor learning. These neural correlates of learning were later replicated in a study

conducted by Honda et al., (1998) although Honda et al. used a task where movements were not spatially cued. Honda et al. (1998) found that a frontoparietal network was associated with explicit learning, similar to Grafton's earlier findings (Grafton et al., 1995; Honda et al., 1998). Honda hypothesized that this network may store an explicit task strategy. Thus, it is unclear whether right dorsolateral prefrontal cortical contributions are specific to spatial working memory, explicit awareness, or both. It is interesting to note that the same frontoparietal network was engaged in both studies regardless of whether the task was spatially cued.

Recently, there have been experiments exploring the role of cognitive strategies in sensorimotor adaptation and the interaction between explicit and implicit processes. Mazzoni and Krakauer (2006) instructed participants to use a set strategy: to aim 45 degrees clockwise in order to counteract an applied counter-clockwise visual rotation of movement feedback. The strategy reduced initial errors, but as the number of trials increased, errors occurred in the direction of the implemented strategy, surpassing the target and continuing clockwise. The authors explained this effect by positing that an implicit process was still ongoing based on the error between the visual feedback of the cursor and the desired aiming effect, *not* the target and movement feedback (Mazzoni, 2006). To follow-up the Mazzoni and Krakauer (2006) study, Taylor and Ivry (2012) demonstrated that if given enough trials, participants eventually reduce the error to zero when given a similar strategy (Taylor & Ivry, 2012). Moreover, they developed a computational model that incorporated two potential learning processes. The model provided a good fit to the adaptation error data, suggesting that both strategy based (explicit) and adaptation based (implicit) processes are operating at the same time. Given that there are bidirectional connections between the cerebellum and prefrontal cortex (Middleton & Strick, 2002; Watson, Becker, Apps, & Jones, 2014), it is reasonable to think that these two brain regions work in

concert to produce a coordinated network that integrates across motor and cognitive processes of adaptation and learning.

One quantitative way to measure motor learning or plasticity is through motor excitability and motor cortex representation via motor evoked potentials (MEPs), which can be elicited with TMS. In a study conducted by Pascual-Leone et al. (1994), amplitudes of cortical output maps of finger muscles changed as a function of the type of knowledge (implicit vs. explicit) individuals had during sequence practice. Participants initially learned implicit sequence , however, as learning progressed, individuals became explicitly aware of the sequence. TMS was used to map the cortical motor outputs of each individual finger used in the sequence. The researchers found that the cortical maps of the muscles involved in the task not only increased in peak amplitude relative to baseline but also in the number of scalp positions that evoked a MEP (a Pascual-Leone, Grafman, & Hallett, 1994). This was not the case for task-irrelevant muscles. Interestingly, the progressive enlargement of the cortical map continued only while participants remained implicit. Once explicit knowledge was gained, motor cortical output went back to baseline. This finding suggests that the type of knowledge an individual has of a motor sequence is related limited to prefrontal cortical engagement but also motor cortical representations.

Motor Chunking

Motor sequences are organized hierarchically. Motor chunking is one feature of sequence learning that appears under some conditions; two or more individual actions become grouped together in a memory chunk. For example, a six-item sequence may be executed as two, three-item sequences with a slight pause between them. Chunking is the result of extended practice and thus reflects automaticity in motor sequence learning (Abrahamse, Ruitenberg, de Kleine, &

Verwey, 2013). Chunking facilitates the learning of sequences (Verwey, 2010) presumably by reducing memory load (Penhune, 2013).

Chunking can be observed through reaction times and error rates. For example, subjects make more errors at the beginning of a chunk (Abrahamse et al., 2013; Ashe, Lungu, Basford, & Lu, 2006; Lungu et al., 2014; Sakai, Kitaguchi, & Hikosaka, 2003). When the same sequence is plotted over many trials, an orderly subset of prolonged inter-key intervals emerges, which is assumed to reflect boundaries between chunks (see Figure 1.1). Characteristically, the first element in the sequence is slow, especially relative to the other elements, and somewhere between element two and element six (of a six item sequence) there is another relatively slow response.

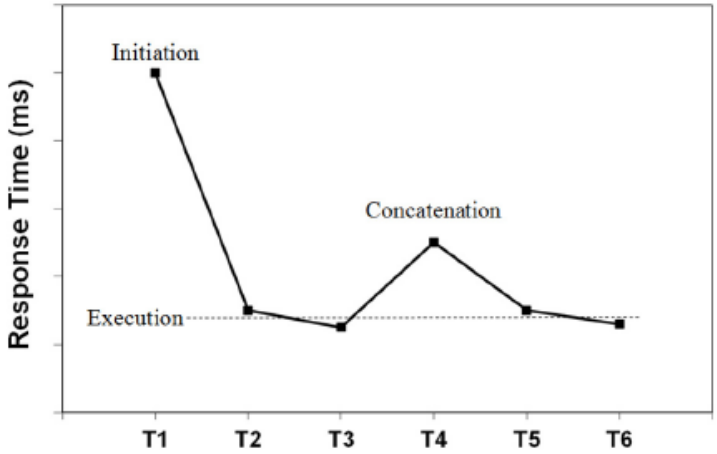

Figure 1.1 Characteristic reaction time pattern of averaged key presses of individual elements after extended practice. T4 (concatenation) is assumed to be the initiation point of a second chunk. Figure taken from Abrahamse et al. (2013).

These slow responses are considered to be chunking or concatenation points of the sequence. Overtime as participants repeatedly execute short motor responses in a small time window, the

movements eventually become yoked together into one single representation, labeled a motor chunk (Verwey, 1996).

It is common to take the average of the inter-key intervals over many trials within a sequence and subjectively compare response times of the prolonged key presses to the inter-key interval presses between pauses to determine chunk boundaries. Using this approach to determine chunk boundaries, Bo et al. (2009, 2011) observed a relationship between an individual's spatial working memory capacity and chunk length for both young and older adults (J. Bo et al., 2011; J Bo et al., 2009a). The ability to hold and manipulate spatial information in mind over a period of a few seconds was positively related to the length of elements in a sequence that can be chunked together (J Bo et al., 2009a). Thus, a possible link between cognitive processes and motor learning is through chunking.

Proposed computational models are able to detect chunks on a trial-by-trial basis instead of through multi trial averages. This new approach allows for a more informative way in to investigate the development of motor chunks. For example, a model developed by Wymbs et al. (2012) uses the consistency and correlation of the inter-key presses in order to determine chunk boundaries(Wymbs, Bassett, Mucha, Porter, & Grafton, 2012). Another model developed by Acuna et al. (2014) uses Bayesian statistics, response times, and error rates as well as their correlations across key presses to detect chunk boundaries (Acuna et al., 2014). These two recent approaches provide evidence for segregation of sequences into chunks and an increase in chunk length with extended practice (see Appendix B for a comparison of data analyzed using the Acuna model versus the t-test approach).

Neural Underpinning of Chunking

The pre-supplementary motor area (preSMA) and DLPFC exhibit a clear role in chunking. There is a negative relationship between motor sequence chunk strength and fMRI BOLD activity in the left mid-DLPFC and foci along the intraparietal sulcus (Wymbs et al., 2012). Further, there is a shift from right prefrontal regions during early learning to left DLPFC during intermediate learning, suggesting different neural correlates depending on the stage of learning (Pammi et al., 2012). Non-invasive brain stimulation studies can demonstrate a causal relationship between brain region and function. Using TMS to create a "virtual lesion" over the preSMA while participants produced an overlearned sequence resulted in increased reaction times during a chunk point (Kennerley, 2003; Ruitenberg, Verwey, Schutter, & Abrahamse, 2014). Thus, both left and right prefrontal regions are related to chunking in some capacity, but may be dependent on the stage of learning, whereas preSMA is involved in chunk loading.

Theories of Explicit Motor Sequence Learning

Models of motor sequence learning propose a multi-stage learning process. Hikosaka and colleagues (1999) posited that there is a gradual and parallel transition between two stages of motor learning. The first is an early, fast stage in which a spatial sequence is encoded in visuospatial coordinates via association cortices (prefrontal and parietal cortex and the anterior basal ganglia); the second is a later, slower stage in which the sequence is acquired predominantly as a motor representation dependent on the motor cortices and the putamen (Hikosaka et al., 1999).

Hikosaka's proposed model is also in accordance with recent neuroimaging findings that demonstrate that not only are frontal brain regions associated with early learning, but also prefrontal regions are specifically related to spatial working memory. For example, activation of

the right DLPFC and bilateral inferior parietal lobules were active in both early visuomotor adaptation and during a spatial working memory task, showing a positive relationship between performance in early adaptation and spatial working memory (Anguera, Reuter-Lorenz, Willingham, & Seidler, 2010).

In a model proposed by Doyon and Ungerleider (2002), early, fast learning involves of two loops: a cortico-striato-thalamo-cortical loop and a cortico-cerebello-thalamo-cortical loop, which operate in parallel. Functional interactions between these two systems are crucial for early, fast learning (Doyon & Benali, 2005; Doyon & Ungerleider, 2002). For example, knowledge acquired during a motor adaptation task can then be later used towards learning sequential movements (Seidler, 2004). As learning progresses the neural underpinnings sub serving motor learning become specialized. Thus, in the late, slow stages of learning the cortico-striatal system is crucial for the consolidation of motor sequence learning, whereas the cortico-cerebellar system is crucial for the consolidation of motor adaptation learning. This model posits that motor skill learning involves interactions between distinct cortical and subcortical brain regions that depend on the stage of learning.

The Dual Processor Model also posits a two-part learning process with emphasis on cognition (Abrahamse et al., 2013; Verwey, Shea, & Wright, 2014). The Dual Processor Model involves a cognitive processor and a motor processor. Verwey and colleagues suggest that the prefrontal cortex may act as the central processor, and M1 as the motor processor (Verwey, Shea, & Wright, 2015). According to the model, early in the DSP task there is an emphasis on the cognitive processor, which translates each individual stimulus into the corresponding response. The cognitive processor communicates with the motor processor, which makes appropriate motor executions. After repeated execution of a sequence, once a chunk is formed, the cognitive

processor no longer loads individual responses; instead it loads in motor chunks, still communicating with the motor processor to execute the finger movements. Thus, over many trials, the involvement of the cognitive processor is significantly reduced, while the motor processor continues to be highly involved (Hommel, 2000). The shift from the cognitive processor to the motor processor in the Dual Processor Model is consistent with the motor learning literature on stage theory (Abrahamse et al., 2013).

The Cognitive framework for Sequential Motor Behavior (C-SMB), built on the Dual Processor model, can also be useful to explain sequential motor learning processes (Abrahamse et al., 2013; Verwey et al., 2014). In the C-SMB framework, information is processed by three different processors: a perceptual processor, a central processor, and a motor processor, which communicate with each other via two overlapping storage components. The perceptual processor processes features of stimuli and transmits its output to short-term memory- the first storage component, which stores non-motor, spatial, and verbal representations of movements. The central processor, which the authors suggest is the prefrontal cortex, has access to short-term memory and is involved in preparing and initiating sequences, setting task goals, and loading the motor buffer- the second storage component. The motor buffer is limited to storing motor representations. The motor processor then executes the motor buffer content. During sequence learning, once a motor representation chunk is formed, the central processor can access and load the chunk into the motor buffer, to be executed by the motor processor. Execution of the chunk from the motor buffer does not require the involvement of the central processor. Thus, as in the Dual Processor model, the role of the central processor is reduced over the course of sequence learning.

Transcranial Direct Current Stimulation and Motor Learning

Transcranial direct current stimulation (tDCS), a form of non-invasive brain stimulation, has been shown to improve motor learning in healthy adults when applied to the motor cortex (Kincses et al., 2004; Michael A. Nitsche et al., 2003). tDCS modulates cortical excitability of populations of neurons that underlie the location of two scalp electrodes and works in a polarity specific manner (Monte-Silva et al., 2013). tDCS provides more evidence for one to interpret causal relationships between brain regions and function unlike neuroimaging studies, which rely on correlations. For example, anodal tDCS over M1 facilitates motor learning in the SRTT in young adults in a single session (Nitsche et al., 2003). Similarly, anodal stimulation over M1 results in increased retention of a newly learned visuomotor transformation within a single session of practice (Galea, Vazquez, Pasricha, Orban De Xivry, & Celnik, 2011).

tDCS modulates both cortico-cortical and cortico-sub-cortical functional connectivity. tDCS over left M1 increased connectivity degree between left M1 and the left posterior cingulate and the right DLPFC (Polanía, Paulus, Antal, & Nitsche, 2011). The same research team completed a similar study using the same approach limited to the hand/arm area within left M1. Cathodal stimulation increased local connections within M1, whereas and anodal stimulation decreased the minimum path length within M1 (Polanía, Paulus, & Nitsche, 2012b). Polania and colleagues also found cortico-striatal and thalamo-cortical functional connectivity modulations after anodal and cathodal stimulation to left M1. Further, anodal tDCS to M1 increased functional coupling between left M1 and left thalamus, whereas cathodal tDCS over M1 decreased functional coupling between left M1 and right putamen (Polanía, Paulus, & Nitsche, 2012a). In conclusion, both anodal and cathodal stimulation over left M1 modulates both global as well as local brain connectivity.

tDCS affects functional brain activity. Two separate studies have used anodal tDCS to target the left inferior frontal cortex while participants were engaged in cognitive tasks inside an MRI scanner. tDCS over left inferior frontal cortex facilitated performance in picture-naming and there was a reduction in the BOLD response in the left inferior frontal cortex (Holland et al., 2011). In the second study,the active tDCS group showed an improvement in semantic word generation (measured by reducing errors) as well as a reduction of the BOLD response in the left ventral inferior frontal gyrus (Meinzer, Lindenberg, Antonenko, Flaisch, & Flöel, 2013). Thus, tDCS paired with cognitive tasks not only improves performance but also reduces BOLD activity in task-specific brain regions.

Purpose

There is limited literature regarding the neural correlates of sequence chunking. To date, only one neuroimaging study has investigated the neural correlates while quantitatively defining chunks (Wymbs et al., 2012). There are currently no published studies, to our knowledge, which pair tDCS with sequence learning to understand the neural correlates of chunking. Further, no study has investigated the neural correlates of chunking in older adults. Older adults show reduced (Bo et al., 2009a) or no (Verwey, 2010) evidence of sequence chunking with practice; tDCS in this group could therefore greatly facilitate sequence learning. Thus, the broad, overarching goal of this thesis is to understand the neural underpinnings of and cognitive contributions to explicit motor sequence learning by using tDCS in young and older adults. We used a between-subjects design and randomly assigned participants to receive anodal tDCS at one of four regions of the brain while they learned two different sequences in the DSP task. In the first experiment, young adult participants practiced the DSP task while receiving tDCS over two days. Participants were also brought in for a third day to measure retention of the learned

sequences. This design allowed us to examine the effects of tDCS on different stages of motor learning. Four groups received real tDCS to either left prefrontal, right prefrontal, left M1, or preSMA brain regions and one group received sham tDCS using the same montage as left M1. A follow-up experiment was performed to understand the polarity specific effects of tDCS on motor learning and chunking. The follow-up study to experiment 1 used a similar study design, however, a group of participants were now assigned to receive cathodal tDCS over left PFC. We then compared learning in terms of reaction time and number of chunks of the left PFC cathodal group to the left PFC anodal and sham tDCS groups. In experiment 2, we brought back participants from experiment 1 over a year later to understand the long term effects of tDCS on motor learning. The left PFC, M1, and sham tDCS participants were invited back to complete another session of DSP practice using the same sequences they had learned a year earlier. Finally, experiment 3 comprised the same design as experiment one, however participants were older adults allowing us to determine age differences in the neural bases of motor sequence learning.

CHAPTER II: The Effect of tDCS on Motor Learning and Chunking

Conceptual Frameworks of Sequence Learning

The Dual Processor model and the Cognitive framework for Sequential Motor Behavior (C-SMB) provide a conceptual framework for motor sequence learning in the discrete sequence production (DSP) task (Abrahamse et al., 2013; Verwey et al., 2015). The authors of these models posit that communication occurs between a central and a motor processor for successful sequence learning. Communication between the two processors is thought to occur via a temporary storage unit, termed the motor buffer. The central processor is thought to be versatile and involved in many roles such as stimulus identification, response selection, loading the motor buffer, setting current goals, and preparing and initiating familiar and unfamiliar sequences. The role of the motor processor is limited to sequence execution. Early in motor sequence learning, there is an emphasis on the central processor as it is loading each individual movement into the motor buffer. However, after significant practice, when individual elements have been grouped into motor chunks, the central processor reduces its contributions by then loading chunks- rather than individual movements- into the motor buffer.

The authors of the Dual Processor model and C-SMB hypothesize that the central processor is controlled by the prefrontal cortex. The prefrontal cortex, in concert with the basal ganglia, organize activity of regional networks connecting or decoupling cortical regions with each other in order to optimize learning. With practice, cortico-cortical connections develop; these are thought to ultimately be responsible for motor skill execution. Thus, according to the

Dual Processor model and C-SMB framework, the prefrontal cortex plays a prominent role throughout sequence learning and chunking.

The Dual Processor model and the C-SMB model posits that learning in the DSP task requires the use of two separate execution modes. The first is a reaction mode, occurring when participants are first exposed to the DSP task and are responding to each individual stimulus separately. The second is a chunking mode, occurring later in learning when emphasis is put on the first stimulus and subsequent stimuli are largely ignored. Verwey et al., the authors of the Dual Processor model and the C-SMB framework, posit that the prefrontal cortices and the preSMA play key roles in the chunking mode, albeit for separate purposes.

Prefrontal Cortex Involvement in Sequence Learning

Neuroimaging and non-invasive brain stimulation studies largely support a role for the prefrontal cortices in sequence learning. Neuroimaging studies demonstrate that prefrontal cortices are engaged in an explicit version of artificial grammar learning (Yang & Li, 2012), explicit versions of the serial reaction time task (Hazeltine, Grafton, & Ivry, 1997; Honda et al., 1998; Willingham et al., 2002), and probabilistic sequence learning (Aizenstein et al., 2004). Transcranial direct current stimulation (tDCS) and transcranial magnetic stimulation (TMS), both non-invasive forms of brain stimulation, also support the role of the prefrontal cortices in motor learning. A recent study used anodal tDCS either over right or left DLPFC as participants practiced a probabilistic sequence learning task that was spatially cued (Janacsek, Ambrus, Paulus, Antal, & Nemeth, 2015). Janacsek and colleagues found an advantage in learning for individuals that received right DLPFC stimulation measured two and twenty-four hours after the completion of the task, but not in the left DLPFC group. In another study, Pascual-Leone and colleagues used TMS to disrupt the DLPFC while participants completed several blocks of the

serial reaction time task. Participants who received TMS over the DLPFC, but not other brain regions, showed impaired procedural learning (A. Pascual-Leone, Wassermann, Grafman, & Hallett, 1996). Thus, a large body of research provides support for the Dual Processor model and C-SMB framework proposals that the prefrontal cortices are integral for sequence learning.

In accordance with the Dual Processor model and C-SMB framework, neuroimaging work also supports the role of the prefrontal cortices in sequence chunking, specifically chunk segmentation. One neuroimaging study used the m x n task, where m is a set of illuminated squares in a matrix and n corresponds to the number of sets needed to be completed, to investigate the neural correlates of chunking and found activation in bilateral DLPFC and parietal cortex early in learning (Pammi et al., 2012). In a separate neuroimaging study using a sequence learning task, Wymbs et al. (2012) found an association between chunk strength and left DLPFC activity (Wymbs et al., 2012). These two studies support that the prefrontal cortices are involved early in the chunking process and are likely involved in the segmentation of sequences.

In addition, a behavioral study conducted by Debarnot et al. (2012) supports the involvement of the prefrontal cortices in the proceduralization of motor sequence learning. In their study, participants first practiced a finger sequence tapping task, then half of the participants did nothing, while the other half memorized a list of words. The participants who memorized the list of words immediately following sequence practice showed a reduction in the number of accurately completed sequences when tested twelve hours later, an indication that the word list memorization process interfered with retention of the learned sequence. The group of participants who did not memorize the word-list

Immediately following practice showed an improvement in the number of sequences produced later, an indication of no interference and an effect of successful consolidation. Thus, reallocating the declarative memory system (prefrontal cortices) to an alternate task likely interferes with the proceduralization, or automaticity, of motor sequence learning.

Several studies provide evidence in opposition of the Dual Processor model and C-SMB framework, though, suggesting that the prefrontal cortices are not involved in the automatization of sequences. For example, Galea and colleagues demonstrated that inhibiting the prefrontal cortices using TMS immediately after learning facilitates sequence learning. Researchers disrupted either the left or right DLPFC immediately after participants learned a sequence and found that regardless of hemisphere, disruption of the DLPFC enhanced sequence learning (Galea, Albert, Ditye, & Miall, 2010). Another study provides similar findings (Zhu et al., 2015a); participants received cathodal tDCS over left PFC or sham stimulation while attempting to sink golf putts. The cathodal tDCS group showed an *advantage* in golf putting performance as measured by successful putts relative to the sham group. In summary, contrary to the assertions of the Dual Processor model and C-SMB framework, these studies suggest that disrupting prefrontal regions via non-invasive brain stimulation immediately following or during learning enhances retention.

In sum, two lines of evidence provide disparate predictions regarding the effects of prefrontal stimulation during sequence learning. According to the Dual Processor model and C-SMB framework, the central processor, controlled by the prefrontal cortex, is robustly involved throughout sequence learning. Thus, in the context of the Dual Processor model and C-SMB framework, stimulating the prefrontal cortices should accelerate motor sequence learning and chunking throughout the learning process. However, another line of evidence suggests that

prefrontal disruption or inhibition during or immediately following learning facilitates motor learning. Here, we sought to adjudicate these two competing views regarding prefrontal cortical contributions to motor sequence learning by applying anodal tDCS to the left or right prefrontal cortex and assessing the impact on motor sequence learning.

preSMA Involvement in Sequence Learning

In addition to the prefrontal cortices, the Dual Processor model and the C-SMB framework also posit that the preSMA also plays a critical role initiating action sequences in the chunking mode (Abrahamse et al., 2013). Evidence of preSMA involvement in learning new action sequences and chunking has been well established. The preSMA is critical for learning new action sequences in non-human primates (Nakamura, Sakai, & Hikosaka, 1998, 1999) as well as in humans (Grafton et al., 1995; Willingham et al., 2002). Further, the preSMA is engaged in explicit sequence learning in humans (Honda et al., 1998)

Consistent with the Dual Processor model and the C-SMB framework, previous studies suggest the preSMA is also involved in chunking, specifically chunk loading. Two separate studies using TMS have corroborated the role of the preSMA in chunk loading (Kennerley, 2003; Ruitenberg et al., 2014). These studies involved creating a transient lesion over preSMA during sequence production, thereby disrupting the preSMA while participants produced an overlearned sequence. PreSMA disruption resulted in significantly slower reaction times at a chunk point, suggesting the preSMA has two roles in sequence learning: it is involved in initiating or aborting a new action sequence and loading in chunks.

M1 Involvement in Sequence Learning

The Dual Processor model and the C-SMB framework posit that motor processor functions (motor execution) are performed by primary motor cortical areas; neuroimaging and tDCS work supports this notion. Using fMRI, Karni et al. (1998) reported activity in the left primary motor cortex in the first few minutes of explicit motor sequence learning as well as during sequence production three and eight weeks later, suggesting a role for M1 involved in both online and offline learning (Karni et al., 1998). Nitsche (2003) found that stimulation to M1 via tDCS facilitated motor learning in the serial reaction time task within a single session (i.e. online gains). In addition to single session benefits (online effects), tDCS may also influence consolidation of motor learning (offline effects). In a study conducted by Reis and colleagues, participants received tDCS stimulation over M1 while learning an isometric pinch force sequence task over the course of five consecutive days (Reis et al., 2009). Participants that received tDCS showed greater motor skill learning (captured by a model which accounts for speed and accuracy) that was primarily driven by offline effects. That is, while there were no immediate, beneficial effects of tDCS within a single day of stimulation relative to sham, during the subsequent session, participants exhibited benefits from the prior day's stimulation. Thus, using tDCS over M1 during learning can yield both online and offline gains in motor learning tasks. These findings are compatible with the Dual Processor model and the C-SMB framework view of the motor cortex playing a role in motor sequence execution.

Current Study

We investigated the cognitive and neural bases of the development of automaticity in an explicit sequence-learning task. We examined sequence learning and the development of motor chunks over the course of three days while participants learned to perform key press sequences

in the discrete sequence production task (DSP). Participants received anodal tDCS over either left M1, left prefrontal, right prefrontal, preSMA, or sham while they practiced one simple and one complex 6-item sequence. We hypothesized that preSMA stimulation would aid sequence learning as represented in more efficient chunking of elements. Additionally, we predicted that M1 stimulation would facilitate learning as evidenced by online and offline gains in response time but, in line with the Dual Processor Model and the C-SMB model, we did not expect M1 stimulation to change chunk characteristics. Finally, we hypothesized that stimulating either the left or right DLPFC would facilitate sequence learning and chunking, based on the Dual Processor Model and the C-SMB framework. Investigating whether tDCS to these brain regions has differing effects on motor sequence learning parameters will further elucidate the neurocognitive processes of sequence learning.

Method

Participants

Sixty-five young adult participants (age range 18-30 yr, 27 male; age = 20.5 ± 2.4 (mean ± SD)) were recruited from the University of Michigan campus and greater Ann Arbor area. All participants were right handed, reported no history of mental health events, drug abuse, neurological, or psychiatric disorders. During the first session, all participants signed a consent form approved by the University of Michigan Institutional Review Board, verbally answered an alcohol and drug abuse questionnaire, completed the Beck Depression inventory (Beck, Steer, & Brown, 1996), a custom tDCS screening form, and the Montreal Cognitive Assessment (Nasreddine et al., 2005). All Participants scored >23 on the MOCA, had no self-reported history of alcohol or drug abuse, and scored <13 on the Beck Depression Inventory.

tDCS Setup

Participants were randomly assigned into one of five tDCS groups for the duration of the study. Four of the five tDCS groups received real, anodal stimulation, whereas the fifth group was a sham group. The electrode placement was determined using the 10-20 EEG system. For right and left prefrontal cortex stimulation groups, the anode was either placed over scalp location F4 or F3 and the cathode over the contralateral orbit. For the real, left M1 stimulation group, the anode was placed over the scalp location C3 and the cathode over the contralateral orbit. For the preSMA stimulation group, we took 8.7% of the measured distance between the nasion and inion and placed the anode anterior to that distance from Cz with the cathode over Fpz. The reference electrode for the preSMA montage was different from the other conditions, as previous literature has demonstrated this to be an effective montage for preSMA (Vollmann et al., 2013). The sham stimulation group received the same montage as the real, M1 tDCS group. Stimulation current was 2 mA and was administered using a conventional tDCS device (Soterix Medical Inc, New York, NY) for a maximum of twenty minutes via two rubber electrodes which were placed inside two saline-soaked sponges. For the sham group, the current ramped up to 2 mA, then immediately ramped back down over a period of thirty seconds. The anode electrode size was always 5x5 cm and the cathode was 5x5 cm except for the preSMA group, where it was 5x7 cm. tDCS setup was identical during sessions one and two, and tDCS was not administered during session three.

Task Order

During the first session, participants completed a variety of paper and pencil and computerized neuropsychological assessments. First, we administered Thurston's card rotation task (two-dimensional mental rotation), followed by a custom computerized version of a visual

search task presented using the software Presentation. Then participants completed the digit symbol substitution task (Wechsler, 1956), a modified version of the visual array change working memory assessment (J Bo et al., 2009a; Luck & Vogel, 1997), then three trials of the Purdue pegboard task (one trial consisting of right hand only, left hand only, left and right hands simultaneously, and bimanual assembly) (Tiffin & Asher, 1948), and then finally we measured the participant's grip strength. The purpose of these assessments was to better characterize the participants and to examine correlates of sequence learning and tDCS responsiveness. We offered participants a break approximately every 20-30 minutes and we had participants take a mandatory 3-5 minute break before we began tDCS set-up. After tDCS set-up, we turned on the stimulation to 1 mA for fifteen seconds (pre-stimulation tickle) to ensure satisfactory contact quality and to ensure participants could tolerate the stimulation. After this brief stimulation period, participants completed a shortened 10-item PANAS mood inventory, then the experimenter explained the instructions for the DSP task as the participant followed along on the screen. Once participants heard the instructions and had no further questions, we started the tDCS stimulation and let it ramp up to full intensity (always 2 mA) and asked whether participants were comfortable with the stimulation (including sham participants). Once participants confirmed they were comfortable and the stimulation could be tolerated, we started the DSP task. After six blocks of practice in the DSP task, which typically ended before the 20 minutes of tDCS had expired, we administered a second version of the digit symbol task, the 10-item custom made PANAS mood survey, and a custom tDCS side effects questionnaire. After the participants completed the tDCS questionnaire, we removed the electrodes, and sent the participants home with an exercise questionnaire as well as the Edinburgh handedness questionnaire (Oldfield, 1971). Session one lasted approximately two hours and thirty minutes.

During session two, participants practiced their assigned sequences for another six blocks, then were tested on their assigned sequences via a paper and pencil questionnaire (see below) and computerized test portion of the DSP task. First participants completed the card rotations task, followed by the digit symbol substitution task. After the paper and pencil tasks, tDCS was set-up and the pre-stimulation tickle was administered. Like session one, participants were asked if the stimulation was tolerable and additionally, whether it felt like session one. We then administered the 10-item mood questionnaire and summarized instructions of the DSP task emphasizing a balance between speed and accuracy. Before starting another six blocks of DSP practice (blocks 7-112), tDCS was started, then once the stimulation reached full intensity and the participant was comfortable, the DSP task was started by the experimenter. After six blocks of sequence practice, the DSP questionnaire was administered (tDCS stimulation is off at this point), followed by instructions of the test portion of the DSP task. Once participants understood the test portion of the DSP task and completed all four conditions, participants completed the digit symbol substitution coding task again, the mood survey, and the tDCS side effects questionnaire.

On the third day of testing, participants started the DSP task, completed two blocks of practice (blocks 13-15), followed by the DSP questionnaire, which was followed by the test portion of the DSP task. After the DSP test portion of the task, participants were offered a break, then completed the card rotations test, the visual search task, the digit symbol substitution coding task, and the visual array change task. Afterwards, participants completed an exit survey, which asked whether they thought they were in the sham or real tDCS group.

Discrete Sequence Production (DSP) Task

The DSP task used for this study was a slightly modified version of that used by Ruitenberg and colleagues (Ruitenberg et al., 2014). Each participant was randomly assigned two, six-item, sequences to practice for the duration of the study. One of the sequence pairs was considered simple and had an imposed structure (e.g., cvncvn, vbcvbc, ncbncb, and bnvbnv), whereas the other sequence was complex and did not have an imposed structure (e.g., nvbcbv, cbnvnb, vncbcn, bcvnvc). The purpose for having two sequences is that sequence complexity differentially taxes cognition according to the Dual Processor Model (Ruitenberg et al., 2014; Verwey et al., 2014). Furthermore, in order for the sequences to be unpredictable for the participant, two sequences are necessary. In order to investigate the role of the central processor and the role of the prefrontal cortices in sequence learning we decided to limit our analyses to the complex sequences. Participants placed their index, middle, ring, and pinky fingers of their right hand on the C, V, B, and N keys of a keyboard, respectively. Four horizontally aligned white squares with black trim were presented in the middle of the screen of a computer monitor with a white background. The blank squares were randomly presented for either 500 or 1000ms before the first stimulus was displayed. As soon as one of the squares was filled in by a light green color (for up to 2000ms), participants were told to make a response with the spatially corresponding key. Once a correct response was given, the green square returned to white for 50ms and then the next square in the sequence would turn light green. Once all six squares of the sequence were successfully pressed, the display turned to white for 1000ms to indicate completion of the sequence. If participants made an incorrect key press, the message “mistake, again” was displayed in red at the bottom of the screen for 1000ms. If a participant did not respond within the 2000ms window, the message, “no response, again” was displayed in red at the bottom of the

screen for 1000ms. Participants had the opportunity to practice each of their two sequences eight times during each block of practice. If a participant made an error either by pressing the incorrect key or not responding to the stimulus at all during the first trial, the participant would have fifteen trials remaining, eight from one sequence, seven from the other. Participants had six blocks of practice during session one, six blocks of practice during session two, and two blocks of practice during session three.

Halfway through a block (eight trials), or sub-block, participants observed a feedback screen for ten seconds. The feedback screen displayed three pieces of information from top to bottom: percent error, mean reaction time, and a numerical countdown starting from ten. At the top of the screen it read "mistakes x.xx%" along with one of two messages depending on the amount of errors made by participants during the last sub-block. It read "too many mistakes" if the percentage of errors exceeded 13%, or "< 13% Good," if the percentage of errors made within the last sub-block was <13%. Incorrect key presses and no responses were combined to determine the amount of errors made. Below the displayed error feedback it read, "Mean reaction time: xxx ms." Below the displayed mean reaction time in the middle of the screen was an ongoing numerical countdown which started at ten and counted down to zero. Once zero was reached participants immediately started the next sub-block. At the end of a block, participants observed another feedback screen for fifty seconds. The feedback screen had the same information as when it was presented during the end of the first sub-block, and additionally, text at the bottom of the screen that read, "After this, practice block x will start."

Before blocks 2, 3, 4, 5, and 6 during sessions one and two, immediately following the feedback screen after the second sub-block, participants observed another screen that read, "As you have noticed, there are 2 fixed sequences. Please learn them! We will continue with the same

task." At the bottom of the screen was a prompt that read, "Press the space bar to continue." After the participant pressed the space bar there was a final prompt before participants started practice that read, "1) please place the fingers of your right hand on C V B N keys and 2) respond quickly, but don't make too many mistakes (less than 13%)," with a prompt at the bottom of the screen that said press space to continue.

During sessions two and three immediately following the DSP questionnaire (description below), participants completed the test phase of the DSP task. The test phase consisted of four conditions, each comprised 48 trials (24 trials of each sequence) and followed the same structure as practice. For example, there was a 10 second break between the two sub-blocks and a 50 second break between each testing condition. Two of the four test conditions used the same two sequences the participants had practiced. In the *familiar* condition participants responded to the green squares in the same way they had during practice, the two sequences hadn't changed and the stimuli were presented in the same way. In the *single-stimulus* condition, participants performed their practiced sequences; however, only the first square of the sequence turned green. Immediately after the participant pressed the correct corresponding key, the squares remained white, and participants had to complete the rest of the sequence (5 key presses) without the help of the squares turning green. In the *mixed-familiar* condition, 75% of the trials had changes to the sequences such that two of the six stimuli were changed whereas in 25% of the trials the sequences were the same as practice. The two changes to the sequences were never consecutive and never included the first item. Thus, participants saw familiarities in the sequences, but often experienced deviations. In the fourth condition, *mixed-unfamiliar*, there were two sequences that the participant had never experienced before.

DSP Questionnaire

At the end of block 6 during session two and block 2 of session three of the DSP task, a white screen with black text read, “Practice is now finished for today, you will now be tested on your knowledge of the sequences.” We then administered a custom 6-item questionnaire testing the participants’ explicit knowledge of the learned sequences, their confidence, as well as strategies. The first question had two sets of six empty boxes and asked participants to write the two sequences they practiced and below to indicate how confident they were in the correctness of the sequences from 0-100%. Participants were told that the order of the sequences did not matter. For question two, we displayed the squares on the computer screen with the letters in them, and asked them to point to the squares and verbally tell us what the two sequences were along with their confidence in their correctness. We confirmed each sequence by repeating it back to the participant (see Appendix A for explicit awareness results). The third question was a multiple choice displaying eighteen possible sequences. The participant was told to choose the two that they had practiced by checking the empty column beside the sequences. The fourth question asked participants, “In what way did you recognize your sequences in the previous question?” Participants could choose from four options: 1) by remembering the order of the letters on the keys, 2) by finger-tapping the sequences on the table or in my mind, 3) by remembering the positions of the squares and the keys, or 4) in a different way, namely:” where the participant could offer an alternative answer. The fifth question asked, “Have you previously participated in an experiment with key press sequences?” Followed by, “Were those the same sequences?” We told participants this did not include the previous session of the current study. The sixth question asked, “Did you realize that there were two fixed sequences during practice.” Participants could choose from “No” or “Yes (I did, at some moment during practice).”

ROAST Current Density Modeling

Realistic volumetric-Approach to Simulate Transcranial Electric Stimulation, or ROAST, is an open source pipeline available for modeling the current produced by transcranial electric stimulation. The software uses a T1 image of the 6th gen MNI-152 head to build a model of the electric field and voltage in the brain (here we only use the electric field output). We ran the model a total of four times to account for the four different tDCS electrode montages used in this experiment (right PFC, left PFC, M1, preSMA). The input parameters of the model for the left PFC, right PFC, and left M1 were consistent between our set-up and what the model allowed as input. However, the model did not allow for different electrode sizes (e.g., 5x5 cm and 5x7 cm), which were used in the preSMA montage. Also, the input for the electrodes in ROAST was limited to the 10-10 EEG system, however, we determined the site of the anode for the preSMA montage as anterior to Cz by 8.7% of the distance between the nasion and the inion. Thus, the anode for the preSMA was *slightly* posterior of FCz. As a result, the output of the model for the preSMA should be interpreted with caution.

Data Analyses

As we are interested in learning and chunking differences between real tDCS and sham our primary outcomes for this study were reaction time, number of chunks, and number of errors for the complex sequences. Having an imposed structure in the sequence would create artificial chunk points and likely impact the results. Thus, we implemented a linear mixed model using the statistical software, Stata, for reaction time and chunk number using trials as a continuous factor. We chose a linear mixed model because every participant will have a different number of trials due to the removal of errors. In the mixed model, we used random intercepts and fixed slopes for each participant. In order to identify the number of chunks for each key press, we used a

computational model developed by Acuna et al., (2014). The model uses reaction times as well as the covariation across key presses in order to detect chunk boundaries (Acuna et al., 2014). An advantage of using the Acuna model (Acuna et al., 2014) to define sequence chunk points is that it allows us to investigate how chunking changes on a trial by trial basis, unlike the traditional t-test method, which averages over hundreds of trials. Likewise, we investigated how reaction time changes across trials to compare the two dependent variables. For the number of errors, we used a repeated measures ANOVA using sequence (simple, complex), session (session one, session two, session three), and stimulation group in the full model. We also used two one-way ANOVAs with four contrasts (right PFC vs sham, left PFC vs sham, etc.) to investigate offline learning gains as well as overall differences in reaction time and number of chunks. Offline learning gains were calculated by subtracting the mean of six key presses from the first trial of a session from the mean of six key presses from the last trial within a session (e.g., mean RT trial 192 session one – mean RT trial 193 session two). Beta and standard error values are presented relative to sham. It should be noted that we did not correct for multiple comparisons.

Results

Errors

A 2 (sequence type: simple, complex) by 3 (session: one, two, three) repeated measures ANOVA revealed a significant main effect of session ($F(2, 114)= 113.33$, $p < 0.001$) and sequence ($F(1, 57)= 4.792$, $p = 0.033$) on errors. Participants committed an average number of 8.3 errors during session one, 8.1 errors during session two, and 2.3 errors during session three. Participants committed an average number of 5.8 errors for the complex sequences and 6.8 for the simple sequences. All other main effects and interactions for errors, including those with stimulation group, did not reach significance.

Reaction Time

Regardless of sequence type and across all tDCS stimulation groups, the linear mixed model revealed that reaction time changed faster across trials in the first session than in the second ($\beta = -.84$, SE = .02, $p < 0.001$). Reaction time across trials in session three changed significantly faster relative to the trials in session two ($\beta = -.27$, SE = .066, $p < 0.001$). The model also revealed a significantly faster rate of reaction time decrease across all three sessions for the complex sequence relative to the simple sequence ($\beta = -.11$, SE = .04, $p = 0.003$). Further, reaction time for complex trials changed at a significantly faster rate relative to simple trials during sessions one ($\beta = -.10$, SE = .02, $p < 0.001$) and three ($\beta = -.23$, SE = .11, $p = 0.039$).

We found several significant differences in the slopes of reaction time across trials within session by tDCS stimulation group. In the first session, the left PFC group reduced reaction time more slowly across trials relative to the sham group ($\beta = -.09$, SE = .03, $p = 0.006$), whereas stimulation to M1 resulted in a significantly faster change in reaction time across trials in the first session relative to sham ($\beta = -.07$, SE = .03, $p = 0.048$). In the second session, stimulation to left PFC resulted in a significantly faster rate of change in reaction time across trials relative to the sham group ($\beta = -.16$, SE = .03, $p < 0.001$).

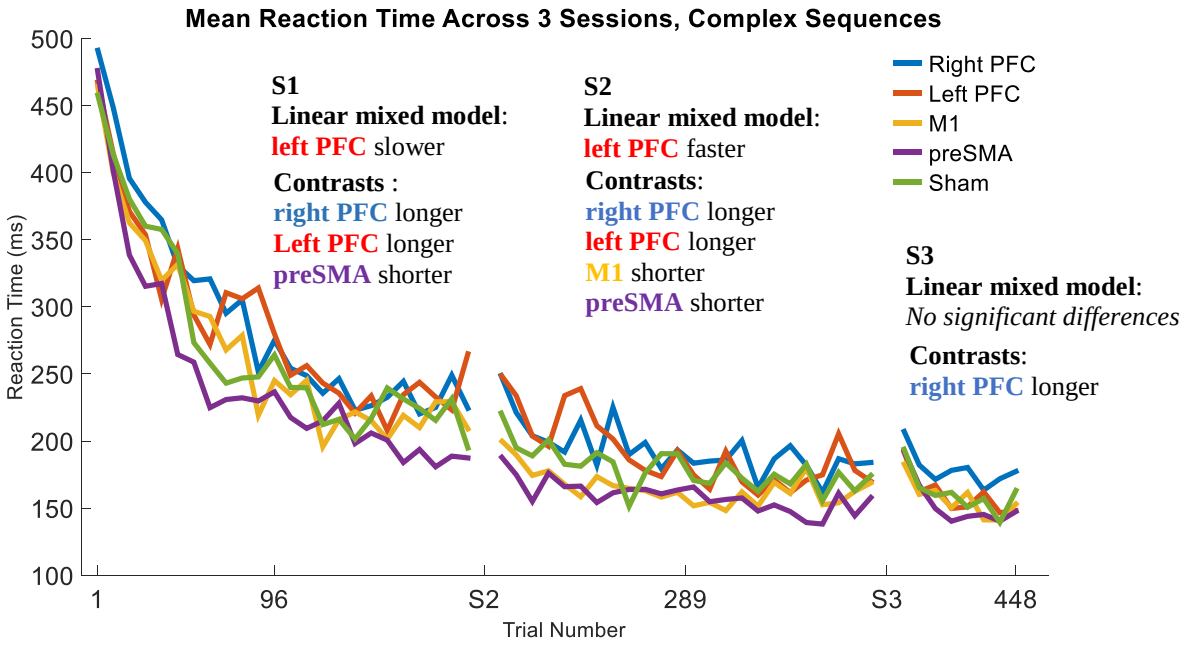

Figure 2.1. Mean reaction time (ms) as a function of trial number for complex sequences. Displayed means were binned across every 8 trials. Blue lines denote the right PFC tDCS group, red lines denote the left PFC tDCS group, yellow lines denote the M1 tDCS group, purple lines denote the preSMA tDCS group, green lines denote the sham tDCS group. S2 and S3 on the x-axis represent the start of session two and session three, respectively. The linear mixed model revealed that the left PFC group changed reaction time at a significantly slower rate relative to sham during session one, but changed at a significantly faster rate relative to sham during session two. Contrasts revealed both the left and right PFC groups had longer reaction times in sessions one and two. Stimulation to preSMA resulted in shorter reaction times during sessions one and two and stimulation to M1 resulted in shorter reaction times during session two.

During the first session, individuals in the left PFC group reduced their reaction time for complex sequences at a significantly slower rate relative to individuals in the sham group (β = .10, SE = .05, $p < 0.038$; Figure 2.1). There was a trend demonstrating that individuals who received stimulation to right PFC during the first session showed a faster rate of change while practicing the complex sequences relative to sham (β = -.08, SE = .05, $p < 0.087$; Figure 2.1). In the second session, the left PFC group decreased reaction time at a faster rate relative to the sham group (β = .18, SE = .05, $p < 0.001$; Figure 2.1).

Contrasts on Reaction Time

Hypothesis driven contrasts revealed that both the right PFC ($t(5148) = 4.102$, $p < 0.001$) and the left PFC ($t(5148) = -2.270$, $p = .023$) tDCS groups were significantly slower than the sham group in session one. In contrast, the preSMA tDCS group ($t(5148) = -3.471$, $p = 0.001$) had significantly shorter reaction times than the sham group. In session two, both the right PFC

(t(5147) = 3.558, p < .001) and left PFC (t(5147) = 2.837, p = .005) tDCS groups had significantly longer reaction times than the sham group. In contrast, the M1 (t(5147) = -3.151, p = .002) and preSMA (t(5147) = -5.272, p < .001) groups had significantly shorter reaction times than sham in session two.

In the third session, the right PFC tDCS group was significantly slower than the sham group (t(1719) = 3.185, p = .001).

In summary, linear mixed model analyses demonstrated that the left PFC tDCS group changed reaction time at a significantly slower rate during session one, but at a faster rate during session two. However, contrasts revealed the left PFC tDCS group had significantly longer reaction times in both session one and session two. The right PFC tDCS group had significantly longer reaction times across all three sessions. Thus, stimulation to either the right or left PFC slowed sequence production. The preSMA tDCS group had significantly shorter reaction times during session one and session two, whereas the M1 tDCS group had significantly shorter reaction times limited to session two.

Offline gains

Planned contrasts revealed tDCS to M1 did not significantly modify offline gains in reaction time regardless of the session.

Chunks

Learning was evident as a decrease in the number of sequence chunks across the three days of practice. The number of chunks across all trials within the second session decreased at a faster rate relative to the first session (β = -.00, SE = .00, $p < 0.001$), and the number of chunks in the third session decreased at a faster rate relative to the second session (β = -.00, SE = .00, p =

0.026). The number of chunks for complex sequences decreased faster than for simple sequences across trials (β = -.00, SE = .00, p = 0.002). The number of chunks reduced more quickly for complex than simple sequences in both the first session (β = -.00, SE = .00, p = 0.001) and the third session (β = -.00, SE = .00, p = 0.001). In contrast, the number of chunks reduced faster for simple than complex sequences in the second session (β = .00, SE = .00, p = 0.001).

Within session one, across all trials, the right PFC group (β = .00, SE = .00, p = 0.039) and the left PFC group (β = .00, SE = .00, p < 0.001) reduced the number of chunks at a significantly slower rate relative to the sham group (Figure 2.2). In contrast, stimulation to the preSMA resulted in a significantly faster rate of reduction in the number of chunks relative to sham (β = -.00, SE = .00, p = 0.013) within the first session (Figure 2.2). In the second session, the right PFC group (β = 00, SE = .00, p < 0.001), the left PFC group (β = .01, SE = .00, p < 0.001) and the M1 group (β = .01, SE = .00, p < 0.001) reduced the number of chunks across trials at a significantly slower rate relative to sham. In the third session, stimulation to preSMA resulted in a faster rate of reduction in the number of chunks relative to sham (β = .02, SE = .00, p < 0.001) and a trend for the right PFC group to reduce chunks at a faster rate (β = -.01, SE = .00, p = 0.056; Figure 2.2.

Across all the trials within the first session, stimulation to left PFC resulted in a reduction of the number of chunks at a slower rate relative to sham for the complex sequence (β = .003, SE = .000, p < 0.001; Figure 2.2). In contrast, stimulation to M1 (β = -.003, SE = .000, p = 0.001) and the preSMA (β = -.004, SE = .000, p < 0.001) resulted in a faster rate of reduction in the number of chunks across all trials within the first session (Figure 2.2). The right PFC (β = -.01, SE = .000, p = 0.001) and left PFC (β = -.00, SE = .000, p = 0.001) tDCS groups produced chunks at a faster rate relative to the sham group in the second session for the complex sequences

(Figure 2.2). The right PFC (β = -.013, SE = .004, p = 0.002) and the preSMA (β = -.017, SE = .004, p < 0.001) stimulation groups reduced the number of chunks at a significantly faster rate relative to sham across all trials within the third session while practicing the complex sequence (Figure 2.2).

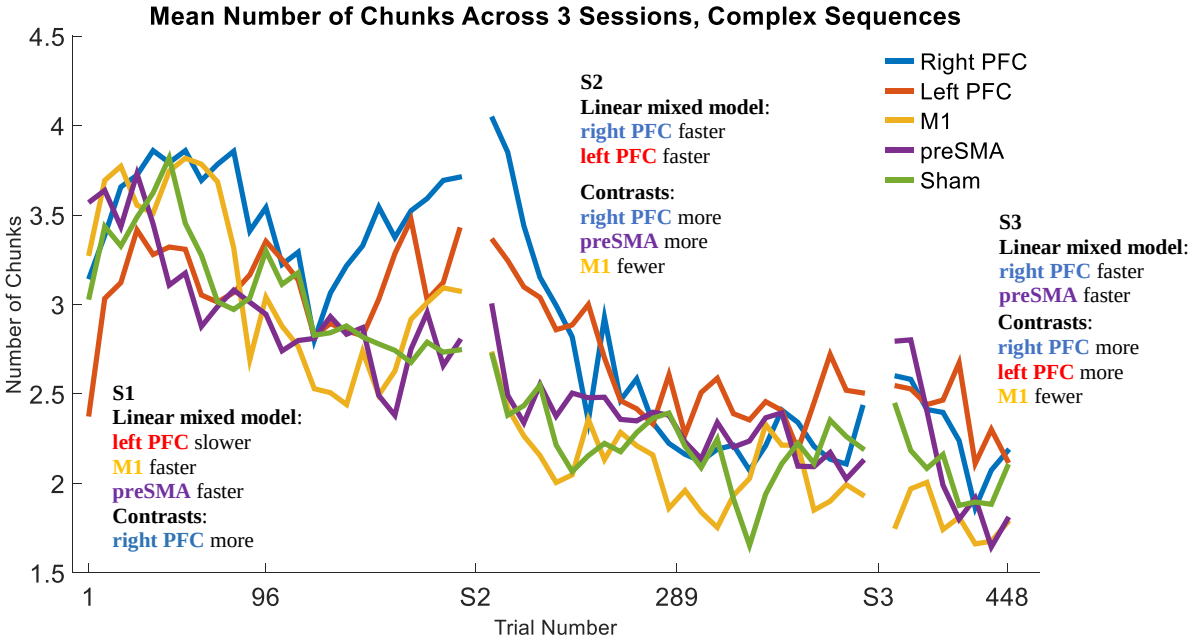

Figure 2.2. Mean number of chunks as a function of trial number for complex sequences. Displayed means were binned across every 8 trials. Blue lines denote the right PFC tDCS group, red lines denote the left PFC tDCS group, yellow lines denote the M1 tDCS group, purple lines denote the preSMA tDCS group, and green lines denote sham. S2 and S3 on thr x-axis represent the start of session two and session three, respectively. Linear mixed models revealed that stimulation to left PFC resulted in a slower rate of change in the number of chunks during session one, but a faster rate of change in session two. M1 stimulation reduced the number of chunks at a faster rate in session one. Stimulation to right PFC reduced the number of chunks at a faster rate in session two and three. Stimulation to preSMA resulted in a faster rate of change during session three. Contrasts revealed the right PFC group had more chunks across all three sessions. Stimulation to left PFC and preSMA resulted in more chunks in session two. Stimulation to M1 resulted in fewer chunks in session two and session three.

Contrasts on Chunks

Planned contrasts in session one revealed that the right PFC had significantly more chunks in the complex sequences (t(4660) = -8.146, p < 0.001) relative to sham in session one.

The right PFC (t(4739) = -7.482, p < 0.001), left PFC (t(4739) = -9.156, p < 0.001), and preSMA (t(4739) = -2.702, p = 0.007) had significantly more chunks relative to sham in the

second session. In contrast, M1 had significantly fewer chunks relative to the sham group ($t(4739) = -2.018$, $p = 0.044$) in the second session.

The right PFC ($t(1597) = -2.626$, $p = .009$) and left PFC ($t(1597) = -3.786$, $p < 0.001$) groups had significantly more chunks relative to sham in the third session. In contrast, the M1 tDCS group had significantly fewer chunks relative to sham ($t(1597) = -3.766$, $p < 0.001$) in the third session.

In summary, stimulation to left PFC resulted in a reduction in the number of chunks at a slower rate during session one, but a faster rate during session two. The right PFC group reduced chunks at a faster rate in sessions two and three. However, contrasts demonstrated that the right PFC group had a higher number of chunks across all three sessions, whereas the left PFC group had a higher number of chunks for sessions two and three. Stimulation to M1 resulted in a faster reduction of the number of chunks in session one and fewer chunks for sessions two and three, whereas stimulation to preSMA resulted in a faster reduction in the number of chunks but a higher number of chunks in session two.

Testing Conditions

A mixed 2 (session: two, three) by 2 (sequence type: simple, complex) by 4 (testing condition: single stimulus, familiar, mixed familiar, mixed unfamiliar) repeated measures ANOVA was performed on the mean reaction time for each testing condition. There was a main effect of session ($F(1,57) = 46.45$, $p < 0.001$), a main effect of sequence type ($F(1,57) = 4.15$, $p = 0.046$), and a main effect of testing condition ($F(3,171) = 1401.69$, $p < 0.001$). Reaction time in session two (M = 296.95) was higher than session three (M = 277.57). Simple sequences (M = 287.80) were produced faster than complex sequences (M = 289.72). The reaction times in the

single stimulus condition (M = 180.65) were slower than in the familiar condition (M = 166.68) (t(61) = 4.01, p = 0.001), and reaction times in the mixed familiar condition (M = 393.03) were faster than the reaction times in the mixed unfamiliar (M = 408.68) (t(61) = -6.23, p = 0.001; Figure 2.5). There were no significant interactions.

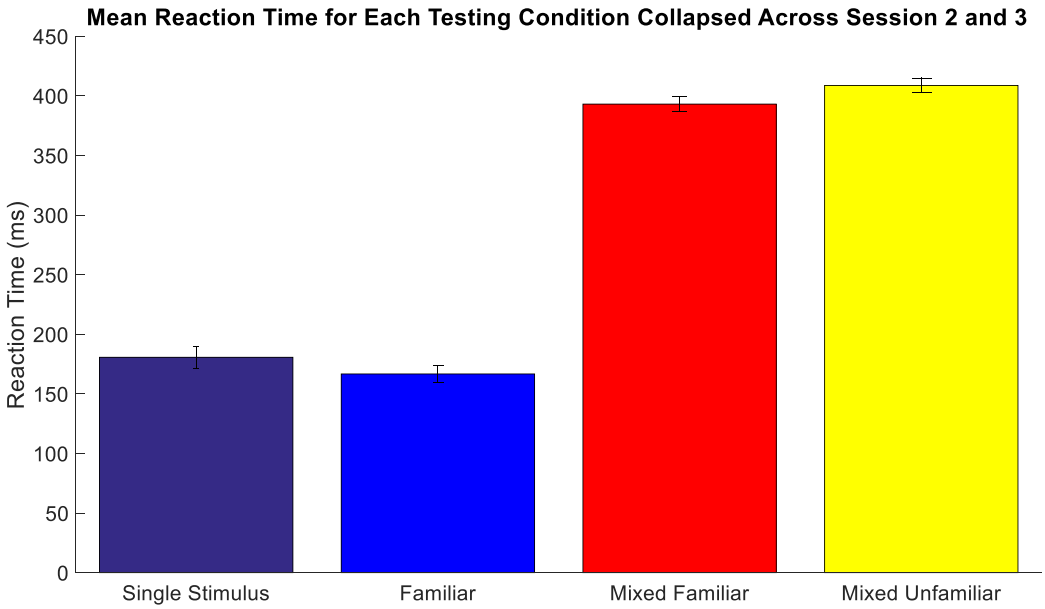

Figure 2.3. Mean reaction time (ms) for each testing condition collapsed across sessions two and three. Error bars are standard deviations.

ROAST Results

We used an open source computational model, ROAST, to simulate electrical fields generated in the brain (Huang et al., 2017). We customized the parameters of the model, changing the height of the electrode to 1 mm and sponge height to 2 mm and the radius of the electrodes to 3.5355 as we used two 5x5cm electrodes and not a high-definition tDCS system, which typically uses circular electrodes. Currently, the model does not allow input for two different sized electrodes, which we used for the preSMA montage (we used a 5x5 cm electrode for the anode and a 5x7 cm electrode for the cathode). Thus, output for the preSMA is not an entirely accurate representation. We used the following commands for each electrode montage.

The command, roast('example/subject1.nii',{'F3',2,'Fp2',-2}) was used for the left PFC anode right orbitofrontal set-up (Figure 2.4). The command roast('example/subject1.nii',{'F4',2,'Fp1',-2}) was used for the right PFC anode left orbitofrontal set-up (Figure 2.5). The command roast('example/subject1.nii',{'C3',2,'Fp2',-2}) was used for the left M1 anode, right contralateral orbit cathode set-up (Figure 2.6). We used the command roast('example/subject1.nii',{'FcZ',2,'FpZ',-2}) for the preSMA anode and the forehead cathode montage (Figure 2.7).

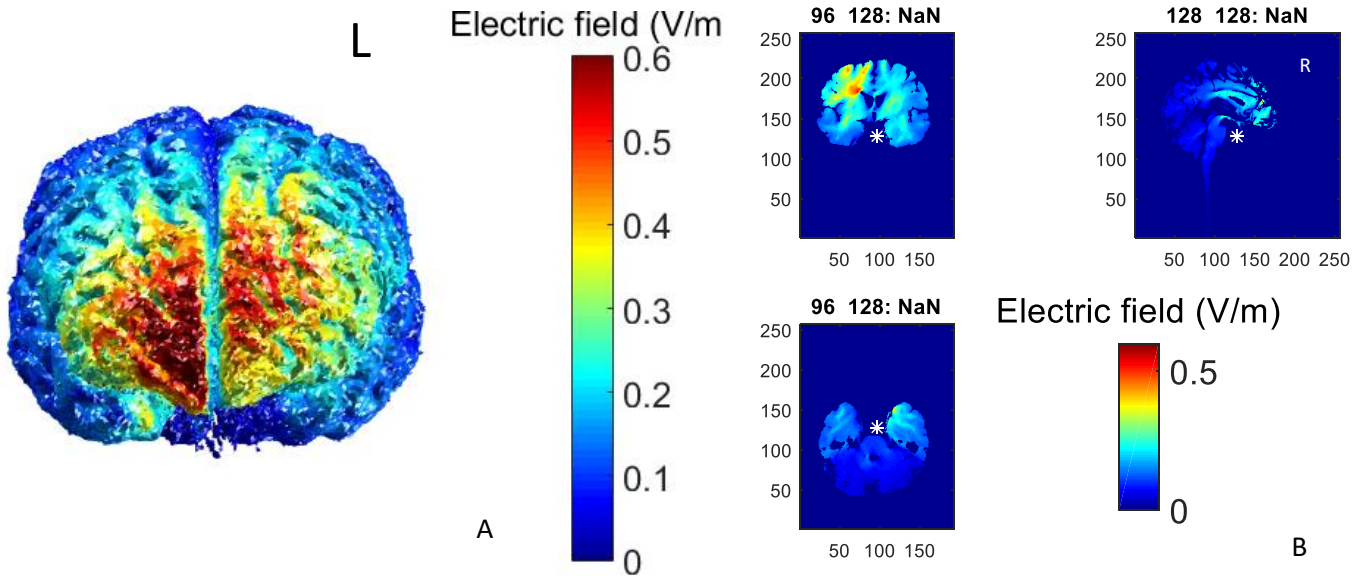

Figure 2.4. A) Electric field magnitude distribution in the whole brain for the left PFC montage. The left hemisphere (L) is depicted on the right side. B) Electric field magnitude distribution in coronal, sagittal, and horizontal planes of the brain for the left PFC montage.

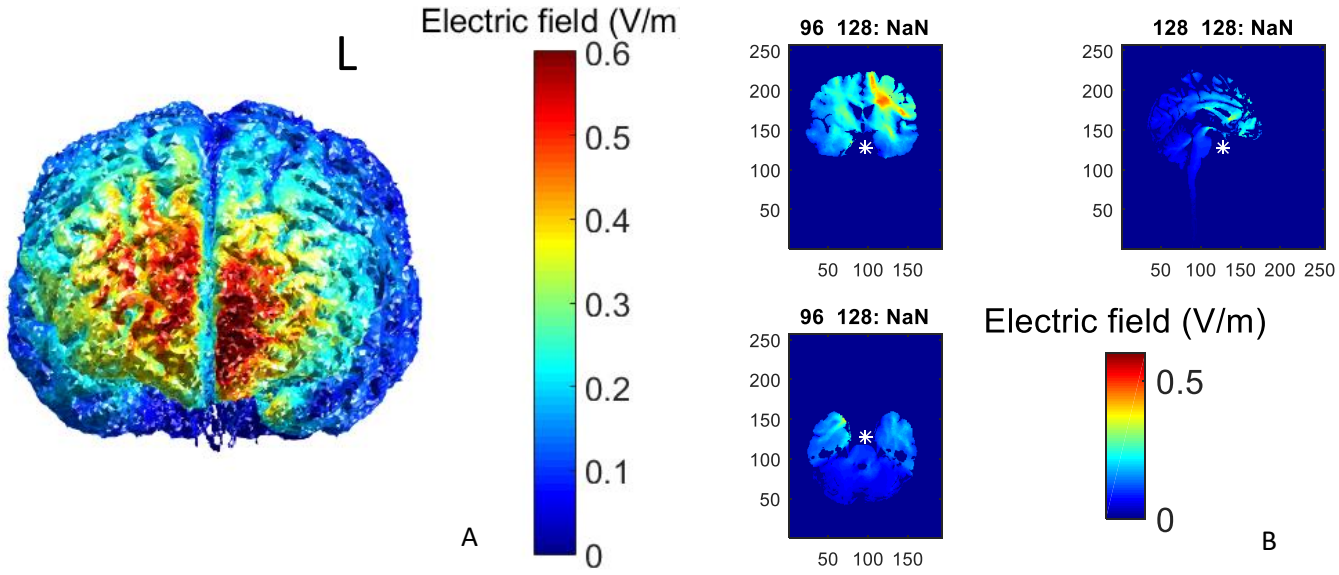

Figure 2.5. A) Electric field magnitude distribution in the whole brain for right PFC montage. The left hemisphere (L) is depicted on the right side. Electric field magnitude distribution in coronal, sagittal, and horizontal of the brain for right PFC montage.

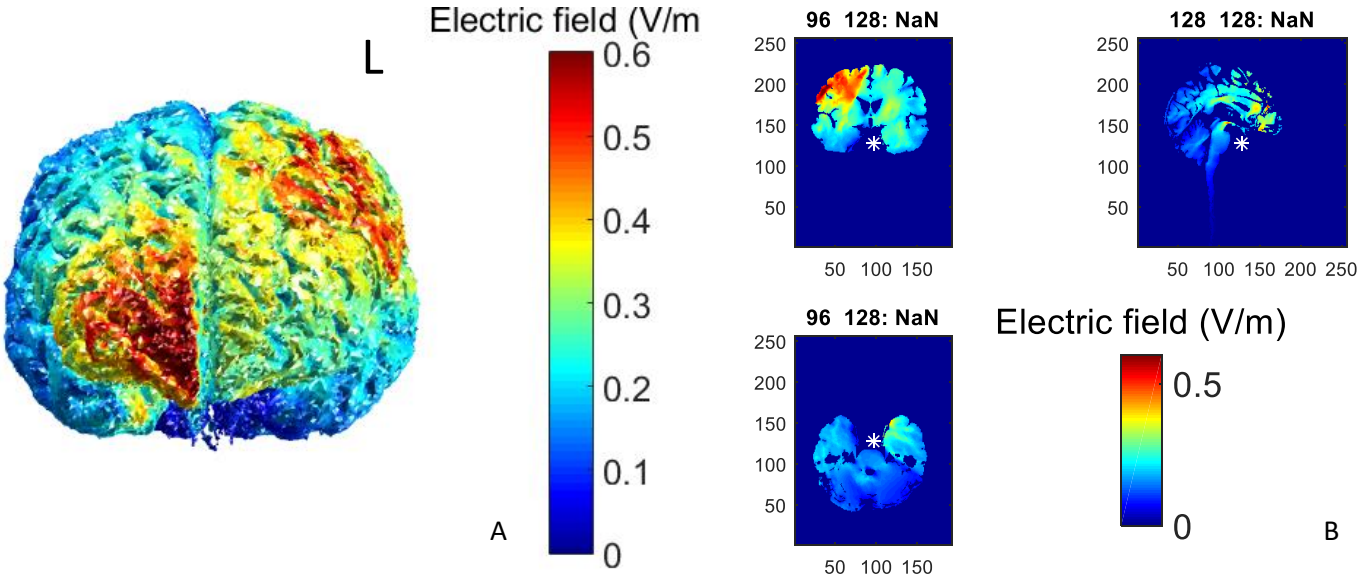

Figure 2.6. A) Electric field magnitude distribution in the whole brain for M1 montage. The left hemisphere (L) is depicted on the right side. B) Electric field magnitude distribution in coronal, sagittal, and horizontal planes of the brain for M1 montage.

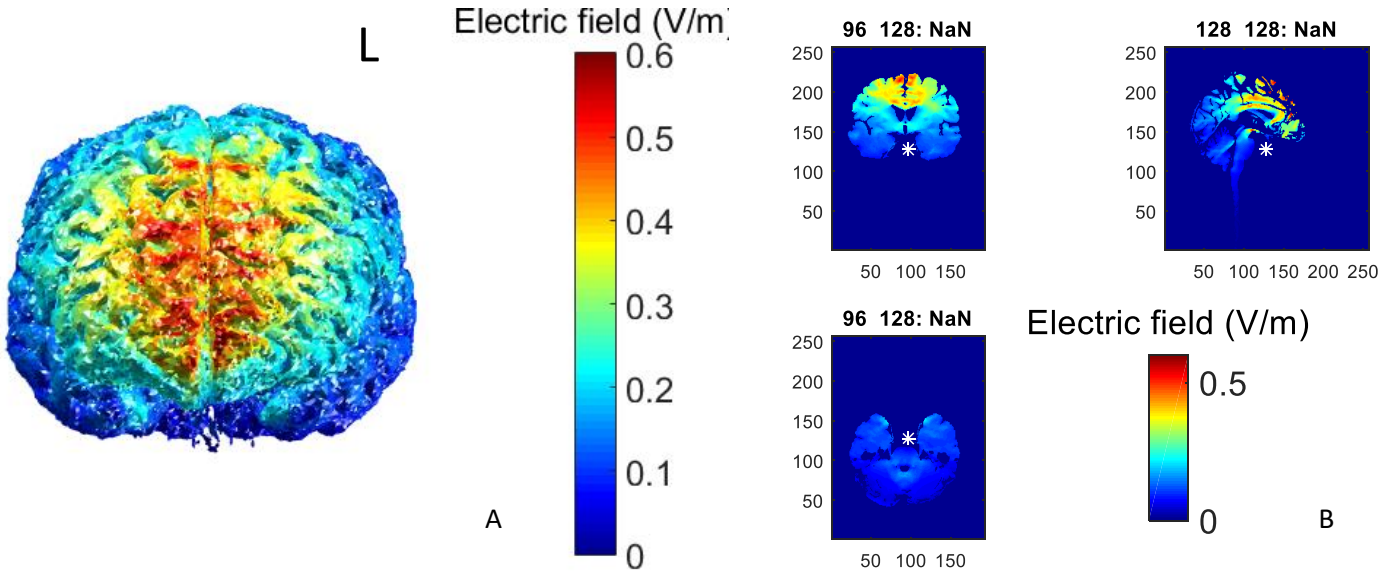

Figure 2.7. A) Electric field magnitude distribution in the whole brain for preSMA montage. Left hemisphere (L) is depicted on the right side. Electric field magnitude distribution in coronal, sagittal, and horizontal planes of the brain for preSMA montage.

Discussion

In the current study, we investigated the neural bases of motor sequence learning and chunking within the perspective of the Dual Processor model and the C-SMB framework. Participants received tDCS during practice on the discrete sequence production task over three sessions (tDCS was applied during the first two). In contrast with our hypothesis and the predictions of the Dual Processor model and the C-SMB framework, we found that stimulation to prefrontal regions impaired sequence learning and chunking. We also found that stimulation to preSMA showed a tradeoff between reaction time and number of chunks such that there was a higher number of chunks, but shorter reaction times in session two. Our results suggest that the preSMA plays a robust role in both sequence learning and chunk formation. This novel finding expands the role of the preSMA beyond the findings of previous literature demonstrating preSMA involvement in chunk loading (Kennerley, 2003; Ruitenberg et al., 2014).

Stimulation to either left or right PFC did not facilitate sequence learning or chunk formation. The Dual-Processor model and the C-SMB framework propose that the prefrontal cortices play a multi-faceted, robust role throughout the DSP task. Thus, we anticipated that stimulation to prefrontal regions would facilitate learning. Although the linear mixed model analysis indicated that the left and right PFC groups "catch up" in session two in terms of reaction time, they remained slower than the sham group, overall. Additionally, in session 2, the left and right PFC tDCS groups had more chunks and longer reaction times.

The Dual Processor model and the C-SMB framework hypothesize that the prefrontal cortex prepares and initiates movement, especially once chunks have been formed. Given the specific and time-dependent role of the prefrontal cortex proposed by the Dual Processor model and the C-SMB framework, it may be that tDCS is too temporally crude of a technique for testing such a hypothesis. That is, in the present study, stimulation before, during, and after sequence initiation may be the cause of the observed learning and chunking impairments; such constant stimulation may cause the central processor to stay online when- according to the Dual Processor model and C-SMB framework- it is not needed and may instead impair performance. To investigate this possibility, future studies could pair a burst of stimulation either using TMS or direct alternating current over the prefrontal cortices before and/or during the first stimulus of a sequence to determine whether this more temporally refined approach would interfere with learning as well or facilitate it in accordance with the models.

Another potential explanation for why stimulation in our study interfered with sequence learning may be the decoupling of prefrontal areas from subcortical regions critical for sequence learning. According to the Dual Processor model and the C-SMB framework, the prefrontal cortices may act as a central processor, with connections to subcortical structures including the

thalamus and the basal ganglia. tDCS to either left or right prefrontal regions may thus indirectly affect subcortical structures such as the basal ganglia and thalamus, which have previously been implicated in chunking and/or motor sequence learning. Chunking is impaired in stroke patients who had a stroke in or near the basal ganglia (Boyd et al., 2009), and individuals with subcortical lesions in the thalamus show deficits in measures of long-term explicit memory performance, and implicit visual motor sequence learning (Exner, Weniger, & Irle, 2001). Further, previous literature supports the idea that non-invasive brain stimulation to prefrontal regions may affect the basal ganglia and thalamus. Symptoms of Parkinson's disease, a neurodegenerative disease of the basal ganglia, can be transiently improved through non-invasive brain stimulation; anodal tDCS over left DLPFC for patients with Parkinson's disease results in improved balance and gait, and reduced Timed Up and Go times (Lattari et al., 2017), as well as improved working memory performance (Boggio et al., 2006). Further, bilateral prefrontal tDCS with the anode over right PFC decreases resting blood perfusion not only in the orbitofrontal cortex, but also in the right caudate in healthy young adults (Weber, Messing, Rao, Detre, & Thompson-Schill, 2014). Another study showed that tDCS with the anode over left PFC decouples the left PFC from the thalami (Stagg et al., 2013). Thus, it is possible that in our study, stimulating the prefrontal cortices decoupled the prefrontal cortex from subcortical structures, such as the basal ganglia and thalami, that are critical for successful sequence learning and chunking.

However, in contrast with the predictions of the Dual Processor model and the C-SMB framework, an alternative explanation of our findings may be that prefrontal cortex engagement directly negatively impacts performance- regardless of coupling with subcortical structures. This explanation is consistent with previous literature demonstrating that engagement of the prefrontal cortices can interfere with motor sequence learning and retention. Disruption of either the left or

right DLPFC with TMS immediately following sequence learning results in greater retention assessed 12 hours later (Galea et al. 2010). Cathodal stimulation over the left PFC facilitates performance and retention in a golf putting task (Zhu et al., 2015b). Another example of prefrontal activity correlating with poorer performance comes from a study by Lee and Grafton (2015) who demonstrated that a high monetary incentive during a bimanual motor task leads to 'choking', as indicated by reduced accuracy. Immediately prior to movement onset for these high-incentive trials, there was an increased BOLD response in the DLPFC and an increase in functional connectivity between the bilateral motor cortex and prefrontal brain regions. Similarly, a previous study found that inhibiting the prefrontal cortices enhanced automaticity in sequence learning (Galea et al., 2010). Consistent with these previous findings, in the present study, tDCS stimulation to increase spontaneous excitability of prefrontal cortices during learning impaired motor sequence learning. Thus, over-involvement of frontal brain regions may negatively impact motor performance.

As currently described, the Dual Processor model and the C-SMB framework do not account for situations in which engagement of the prefrontal cortex would impair learning and execution. These models should be revised to account for the present findings along with previous neuroimaging, tDCS, and TMS research indicating that while the prefrontal cortex may play an important role in motor sequence learning and execution, its involvement is not unconditionally beneficial. As a result, the Dual Processor model and the C-SMB framework should be revised to consider existing prefrontal tDCS and TMS literature. Our results are consistent with these previous findings, in that stimulation to the prefrontal cortices, interferes with sequence automatization.

It is noteworthy to point out that stimulation to the right and left PFC did not produce identical behavioral outcomes. Although the right PFC tDCS group had an overall higher number of chunks in session one relative to sham, the left PFC group did not. Further, the rate of change in reaction time and chunking was negatively impacted by left PFC stimulation during session one; however, there were no differences between sham and the right PFC group. These differences may be explained by the electric field distribution figures produced by the ROAST computational model. For the left PFC group, there is a higher electric field in the right hemisphere and the distribution of that electric field is greater than the distribution in the left hemisphere; in contrast, for the right PFC group, there is a smaller distribution of the electric field in the left hemisphere. Thus, left v. right PFC tDCS differentially affects the hemispheres. It is possible that the electric field distribution asymmetry between the two prefrontal tDCS groups is responsible for the behavioral differences observed. Future studies should consider scanning a subset of participants from each tDCS group and using current density modeling software to enable further comparison and interpretation of electric field distribution in study participants. In additional, the Dual Processor model and C-SMB framework should be further defined to consider the differential contributions of the left and right hemispheres.

Unexpectedly, stimulation to M1 accelerated chunk formation. Contrasts demonstrated overall faster reaction times and fewer chunks for the M1 group in session two and selective benefits to reaction time and chunking in sessions one and three, relative to sham. Moreover, statistical contrasts did not reveal a single instance in which the M1 group was at a disadvantage relative to sham. Stimulation to M1 has previously been shown to facilitate motor learning in a wide variety of explicit sequence learning tasks (Saucedo Marquez, Zhang, Swinnen, Meesen, & Wenderoth, 2013; Stagg & Nitsche, 2011; Waters-metenier, Husain, & Wiestler, 2014). Further,

the Dual Processor model and the C-SMB framework put emphasis on the motor processor during execution; therefore, it is not surprising that stimulation over M1 facilitated online learning in our task. In addition, Penhune & Steele (2010) proposed that the striatum, responsible for motor chunking, and M1, responsible for the representation of learned sequences, work in concert to learn explicit, spatial motor sequences (Steele & Penhune, 2010). Indeed, Polania and colleagues (2012) have demonstrated that tDCS over left M1 modulates cortico-striatal functional connectivity (Polanía et al., 2012a). Thus, it is possible that stimulation to M1 in our study indirectly affected the striatum, thought to largely be responsible for chunking.

Alternatively, M1 tDCS could have affected chunking through the premotor cortex. In animal models, the premotor cortex is densely connected to M1 (Fang, Stepniewska, & Kaas, 2005; Godschalk, Lemon, Kuypers, & Ronday, 1984; Godschalk, Lemon, Kuypers, & Van Der Steen, 1985). The motor learning literature suggests that premotor cortex is also engaged during chunking (Abe et al., 2007; Bor, Duncan, Wiseman, & Owen, 2003; Pammi et al., 2012). Thus, it is possible that in the present study, stimulation over M1 positively impacted M1-premotor connectivity, resulting in online gains. The Dual Processor model or the C-SMB framework predict a role of M1 in execution and do not have a role of M1 in chunking. Given the present and previous findings, the Dual Processor model and the C-SMB framework could be further modified to incorporate the role of the primary motor cortex, or motor processor, beyond simple sequence execution.

Contrary to our hypothesis, we found that M1 stimulation did not affect offline learning gains. This is inconsistent with the findings of Reis et al. who found an acceleration of motor learning through the selective enhancement of offline gains during an isometric pinch force sequence task (Reis et al., 2009). The enhancement of offline gains in the isometric pinch force

sequence task but not in the present study's DSP task might be due to task-specific effects of tDCS. For example, Marquez et al (2013) found a double dissociation of the enhancement of motor skills using two different tasks. Stimulation to M1 enhanced online gains, but not offline gains for a finger sequence learning task, whereas offline gains, but not online gains were enhanced for an isometric pinch force task (Saucedo Marquez et al., 2013). The DSP task used in our study is more comparable to the finger sequence-learning task employed by Marquez and colleagues; thus, our findings are consistent with theirs.

The present study is not without limitations. In the current study, we used a single-blind design with the experimenter aware of tDCS assignment. However, the participants were relatively poor at guessing whether or not they received stimulation. Another potential issue is that the statistical contrasts performed during the analysis were not corrected for multiple comparisons. While this is an important consideration especially for exploratory studies, we had a specific set of hypotheses behind the study and selectively ran these contrasts. In fact, some discourage the use of adjustments entirely (Gelman, Hill, & Yajima, 2012; O'Keefe, 2003). Finally, it is possible that other regions of the brain that were not directly targeted were affected by the tDCS current due to the size of the electrodes and the non-focal electric field in the brain produced by tDCS, as well as by network propagation effects. Previous studies pairing tDCS and fMRI have found widespread BOLD activity in both cortical and subcortical regions of the brain that are far from the anode electrode (Park et al., 2013; Peña-Gómez et al., 2012; Charlotte J Stagg et al., 2013). Thus, our results cannot be attributed to any one brain region with certainty. Future studies should consider pairing tDCS with other neuroimaging methods in order to better understand how tDCS influences the brain and behavior.

The results from the testing conditions are consistent with a previous study applying TMS to preSMA during the same task (Ruitenberg et al. 2014). We found sequences were performed fastest in the familiar and single stimulus conditions. The single stimulus condition (181 ms) produced slower reaction times compared to the familiar testing condition (167 ms). These reaction time numbers are nearly identical to those of Ruitenberg et al. (2014), who found that the single stimulus condition (192 ms) produced slower reaction times relative to the familiar condition (160 ms). Slower response times in the single stimulus condition are an indication that sequences were not completely automatized.

In conclusion, tDCS to four different cortical regions yielded differential effects dependent on the site of stimulation during an explicit sequence learning task. Stimulation to preSMA showed a chunking and reaction time trade-off. tDCS either over right or left PFC impaired learning as evidenced by longer reaction times and an increased number of chunks. M1 stimulation did not yield offline gains, but did yield online gains as indicated by a reduced number of chunks.

Cathodal Follow-up

Introduction

Inhibiting the prefrontal cortices facilitates retention and consolidation in motor sequence learning. For example, disrupting either the right or left DLPFC via TMS immediately after participants learned an explicitly cued twelve-item sequence, significantly improved retention (Galea et al. 2010). Likewise, attending to the execution of a well proceduralized, or fully automated skill results in poorer performance (Beilock & Carr, 2001; Beilock, Carr, MacMahon, & Starkes, 2002; Gray, 2004). The underlying mechanism of the facilitation of retention observed in the study conducted by Galea et al. (2010) is likely the competitive interaction

between the declarative and procedural memory systems. These studies in combination with the findings from our first study suggest that engaging the declarative memory system interferes with the proceduralization of sequences. Therefore, disrupting the prefrontal cortices with tDCS might be expected to facilitate learning. An example of this comes from a study by Zhu et al (2015) who used cathodal stimulation to inhibit the left PFC and found better performance in a golf putting task (Zhu et al., 2015b). Given the findings from our first experiment, which demonstrated that anodal tDCS to prefrontal regions impaired learning, we anticipated that cathodal stimulation to prefrontal regions should result in enhanced learning in the DSP task as well. To test this prediction and to better understand the behavioral effects produced by the anodal left PFC tDCS montage in the first experiment, here we tested a left PFC cathodal tDCS group performing the same tasks. If engaging prefrontal regions results in task interference, we would expect that cathodal stimulation to left PFC would produce the opposite behavioral effects to those observed for the left PFC anodal group in the first experiment. That is, we hypothesized that left PFC cathodal stimulation would facilitate reaction time decreases and enhance chunking relative to the sham group.

Method

We used a near identical experimental design as the first experiment with a few exceptions. The polarity of the tDCS montage was reversed, with the cathode placed over F3 and the anode placed over the contralateral orbit. Thirteen participants were recruited for the cathodal tDCS group. They did not complete the MOCA, Purdue pegboard, or visual search tasks; they only completed the digit span task. We used the same 24 participants from the first experiment for the anode left PFC and sham tDCS groups.

Results

Errors

A mixed, three (session) by two (sequence type: simple or complex) repeated measures ANOVA was run on the amount of errors produced during the DSP task. tDCS group was the between subjects factor. There was a main effect of session (F (2,114) = 18.440, $p < 0.001$) and a session by sequence type interaction (F (2,114) = 63.856, $p < 0.001$). Two paired sample t-tests were run in order to understand the main effect of session. The t-tests revealed a significant difference between the amount of errors committed in session two (M = 4.18, SD = .32) and session three (M = 5.12, SD = .50; t(60) = 12.01, p < 0.001). In order to understand the session by sequence type interaction, two one-way repeated measure ANOVAs were run, one with the simple sequences and the other with the complex sequences. Using only the simple sequences over the three sessions, a significant main effect of session was revealed (F (2,114) = 41.74, $p <$ 0.001). Paired sample t-tests revealed a significant difference between session one (M = 8.0, SD = 5.24) and session two (M = 6.4, SD = 4.33; t(59) = 2.92, p = 0.005) and between session two and session three (M = 1.52, SD = 1.50; t(59) = 9.15, p < 0.001. There were no main effects or interactions of stimulation group on errors.

Reaction time

Hypothesis driven pairwise comparisons in the linear mixed model demonstrated that the rate of change in reaction time in session two was significantly slower relative to the rate of change in session one (β = .77, SE = .02, p < 0.001). The rate of change in the reaction time in session three was significantly faster than the rate of change in session two (β = -.24, SE = .07, p = 0.001). Complex trials were produced at a significantly faster rate relative to simple trials (β = -.11, SE = .05, p = 0.038).

Hypothesis driven pairwise comparisons for the first session revealed that anodal stimulation to left PFC produced a significantly slower rate of change in reaction time for the complex sequences (β = .16, SE = .05, p = 0.001; Figure 2.8) relative to sham (results previously reported). Anodal stimulation to left PFC during session two affected the rate of change in reaction time such that it was significantly faster for the complex sequences (β = -.21, .05, p < 0.001) relative to sham. Similarly, cathodal stimulation to left PFC produced significantly faster changes in reaction time for the complex sequences (β = -.1, .05, p = 0.045; Figure 2.8) relative to sham in session two. Two follow-up contrasts were performed between the left PFC cathodal group and left PFC anodal group to determine whether the stimulation groups differed from each other during sessions two and three for the complex sequences. The contrast between the left PFC anode and the left cathode for the complex sequences in session two was significantly different, such that the left PFC anodal group changed the rate of reaction time at a significantly faster rate relative to the left PFC cathodal group (β = -.11, .05, p = 0.028). There were no significant findings for the third session.

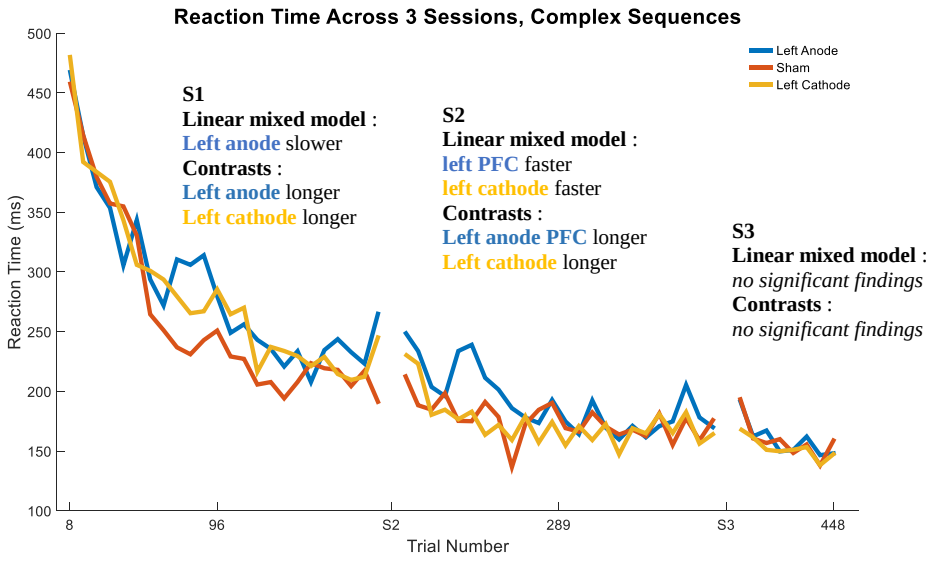

Figure 2.8. Mean of reaction time (ms) as a function of trial for complex sequences. Displayed means were binned every 8 trials. Blue lines denote left PFC anodal group, orange lines denote the sham group, and the yellow lines denote the left cathodal tDCS group. S2 and S3 denote the start of session two and session three. The linear mixed model revealed that left anodal PFC stimulation reduced reaction times at a slower rate relative to sham in session one, but at a faster rate relative to sham during session two. The left cathode group reduced reaction times at a faster rate relative to sham during session two. One-way ANOVA contrasts revealed that both the left anode and cathode groups had significantly longer reaction times relative to sham during session one and session two.

Contrasts for Reaction Time

The left PFC anode ($t(2975) = 3.483$, $p = .001$) and the left PFC cathode ($t(2975) = -3.324$, $p = .001$) tDCS groups had significantly longer reaction times compared to the sham group for the complex sequences in the first session.

The left PFC anodal ($t(2869) = 3.350$, $p = .001$) as the left PFC cathodal ($t(2869) = 3.196$, $p = .001$) tDCS groups were significantly slower compared to the sham group for the complex sequences in the second session.

No statistical differences were found between the real left PFC tDCS groups and sham for the complex sequences in session three. And the left PFC anode and the left PFC cathode tDCS group never differed from each other across all sessions and sequence types.

Chunks

Hypothesis driven pairwise comparisons revealed that the number of chunks over all trials in session two reduced at a significantly faster rate than in session one ($\beta = -.00$, SE = .00, $p < 0.001$). Within the second session, participants reduced the number of chunks for complex sequences faster than for simple sequences ($\beta = .00$, SE = .00, $p = 0.003$).

Cathodal stimulation to left PFC resulted in a significantly faster reduction in the number of chunks relative to anodal stimulation ($\beta = .-00$, SE = .00, $p = 0.039$) but not sham for the

simple sequences. Cathodal left PFC stimulation resulted in a significantly slower rate of change for the complex sequences relative to sham (β = .00, SE = .00, p = 0.033).

Within session one, anodal stimulation to left PFC significantly slowed the rate of chunking for the complex sequences (β = .01, SE = .00, p < 0.001) relative to sham. Similarly, cathodal stimulation to left PFC also significantly slowed the rate of change in the number of chunks for the complex sequences (β = .01, SE = .00, p < 0.001). Pairwise comparisons between the two real stimulation groups revealed no significant differences for the complex sequences (β = -.00, SE = .00, p = 0.58). In session two, anodal stimulation to left PFC significantly reduced the number of chunks over trials at a faster rate relative to sham for the complex sequences (β = -.00 SE = .00, p < 0.001; Figure 2.9). Both anodal and cathodal stimulation to left PFC lead to a reduced rate of chunking during session one, but a faster rate of chunking in session two.

Contrasts for Chunks

The left PFC cathode group had significantly more chunks compared to the sham group ($t(2610) = -3.082$, $p = .002$) for the complex sequences in session one.

The left PFC anodal group had significantly more chunks compared to sham ($t(2674) = 10.103$, $p < 0.001$) as well as the left PFC cathodal ($t(2674) = 8.284$, $p < 0.001$) tDCS groups for the complex sequences in the second session.

Both the left PFC anodal ($t(930) = 5.273$, $p < 0.001$) and left PFC cathodal ($t(930) = -2.135$, $p = .033$) tDCS groups had significantly more chunks compared to the sham group for the complex sequences in session three. The left PFC anodal had significantly higher number of chunks compared to the left PFC cathodal tDCS group ($t(960) = 2.918$, $p = 0.004$) for the complex sequences in session three.

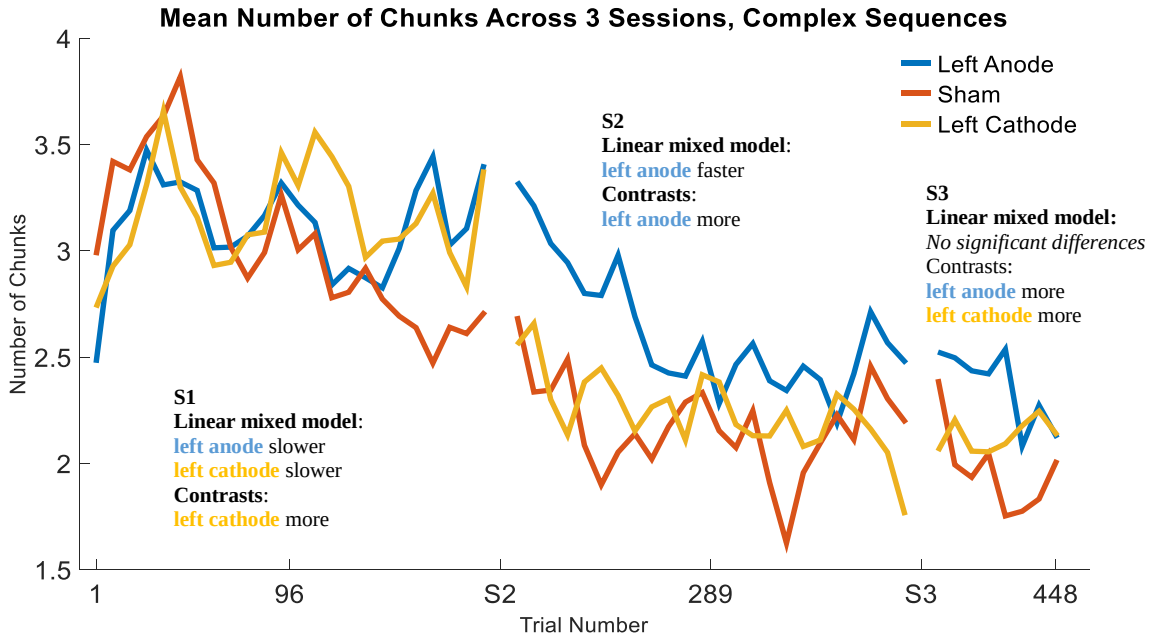

Figure 2.9. Mean number of chunks as a function of trial for complex sequences. Blue lines denote the left PFC anodal group, orange lines denote the sham group, and the yellow lines denote the left cathodal tDCS group. S2 and S3 denote the start of session two and session three. The linear mixed model revealed both the left anode and left cathode tDCS groups reduced the number of chunks at a slower rate relative to sham in session one. The left anode group reduced the number of chunks at a faster rate during session two. One-way ANOVA contrasts revealed the left anode has significantly more chunks throughout the sessions, whereas the left cathode tDCS group had more chunks during session two and three.

Offline Gains

Planned contrasts revealed no significant differences in offline gains between any real tDCS stimulation group and sham for both the reaction time or for the number of chunks between session one and two and between session two and three.

Testing Conditions

A mixed 2 (session: two, three) by 2 (sequence type: simple, complex) by 4 (testing condition: single stimulus, familiar, mixed familiar, mixed unfamiliar) repeated measures ANOVA was performed on the mean reaction time for each testing condition. The repeated measures ANOVA revealed a main effect of session ($F(1,32) = 30.09$, $p < 0.001$), a main effect of sequence type ($F(1,32) = 4.30$, $p = 0.046$), and condition ($F(3,96) = 933.72$, $p < 0.001$). Session two (M = 290.29) was slower than session three (M = 271.17), the simple sequences (M = 277.67) were significantly faster than the complex sequences (M = 283.80), and the familiar testing condition (M = 160.62) was significantly faster than the single stimulation testing

condition (M = 171.48), the mixed familiar condition (M = 390.14) and the mixed unfamiliar condition (M = 400.68). There was also a significant session by testing condition interaction ($F(3,96) = 4.54$, $p = 0.005$; Figure 2.10), a significant session by sequence type by testing condition ($F(3,96) = 3.31$, $p = 0.023$).

In order to better understand the three-way interaction, we ran two additional 2 (sequence type: simple, complex) by 4 (testing condition: single stimulus, familiar, mixed familiar, and mixed unfamiliar) repeated measure ANOVAs, one excluding the data from session two and one excluding the data from session three. Excluding the data from session three, we found a significant sequence type by testing condition interaction ($F(3,96) = 2.80$, $p = 0.044$). Excluding the data from session two revealed no significant interaction between sequence type by testing condition ($F(3,96) = .20$, $p = .90$). We ran two additional repeated measure one-way ANOVAs to further break up the two-way interaction between sequence type and testing condition. We found a significant main effect of testing condition for both the simple sequences $F(3,96) = 1059.135$, $p < 0.001$ and the complex sequences $F(3,96) = 422.01$, $p < 0.001$. There were no main effects or interactions involving the stimulation groups.

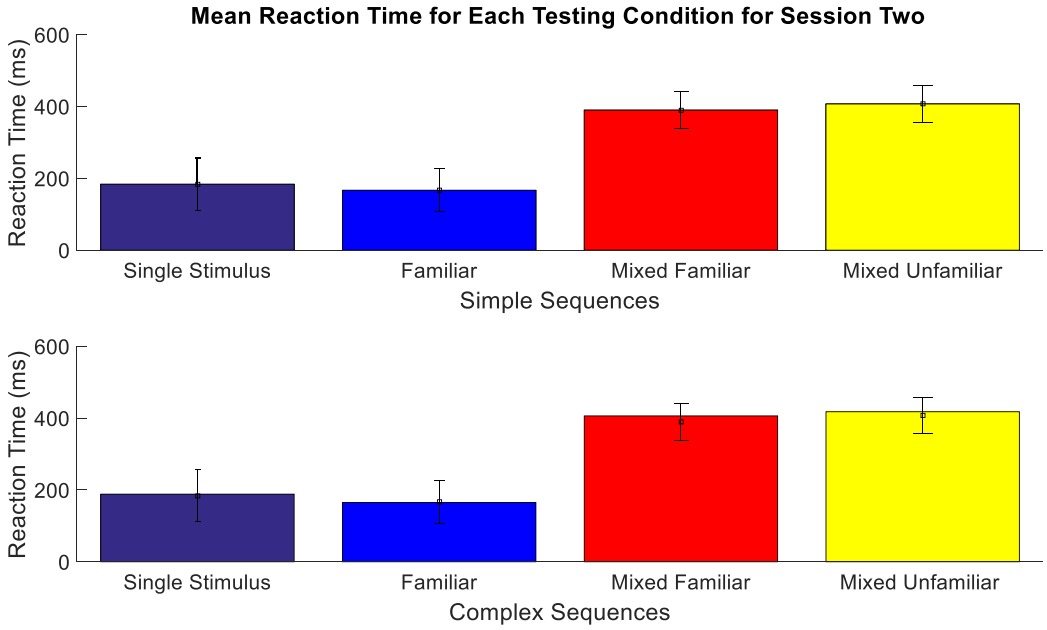

Figure 2.10. Mean reaction times for each testing condition within session two separated by sequence type. Top panel is simple sequences, bottom panel is complex sequences.

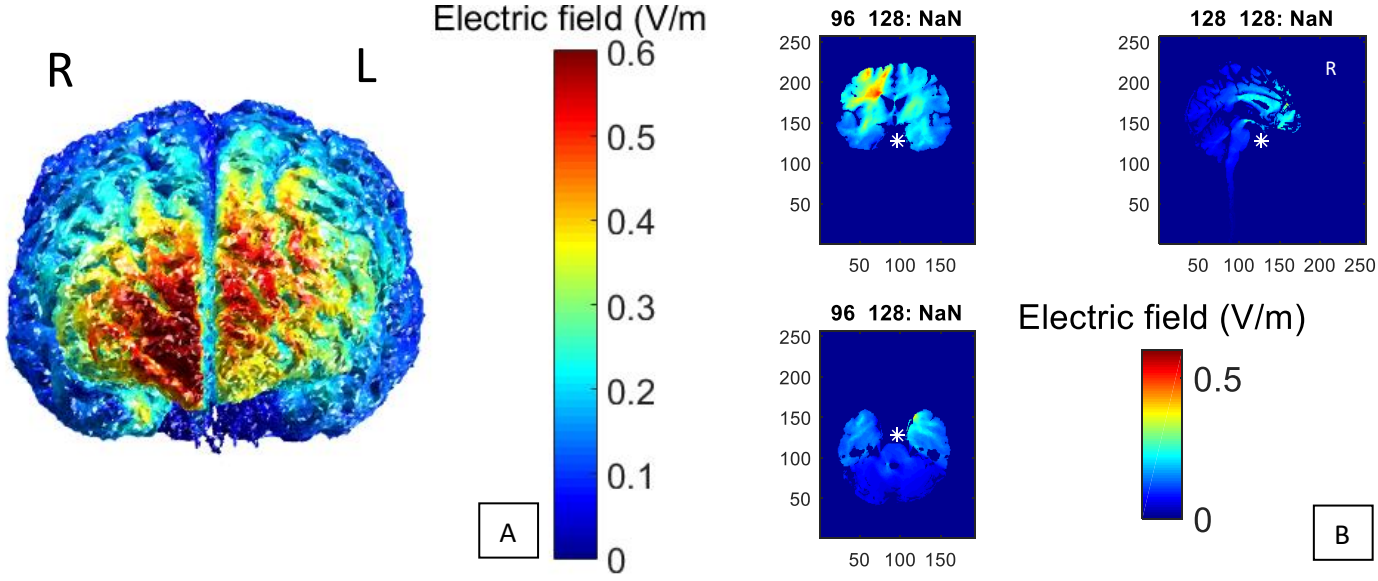

Figure 2.11. A) Electric field magnitude distribution in the whole brain for left PFC cathodal montage. B) Electric field magnitude distribution in coronal, sagittal, and horizontal planes for left PFC cathodal montage.

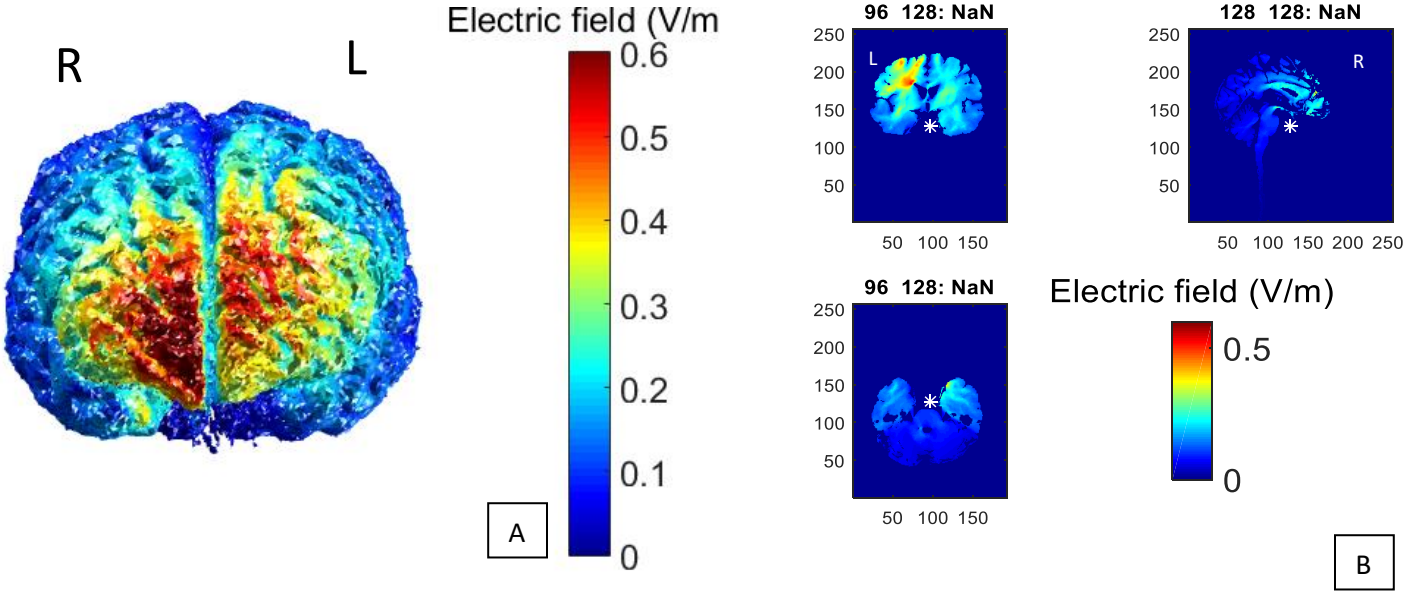

Figure 2.12. A) Electric field magnitude distribution in the whole brain for left PFC anodal montage. B) Electric field magnitude distribution in coronal, sagittal, and horizontal planes for left PFC anodal montage.

Discussion

We hypothesized that left PFC cathodal stimulation would enhance sequence learning resulting in faster reaction times and fewer chunks; however, the results do not support this prediction. We found that both anodal and cathodal tDCS over the left PFC yielded similar behavioral outcomes, producing slower reaction times and a higher number of chunks relative to sham. Similar to experiment one, we observed an impairment of learning as evidenced by a slower rate of change in reaction time and the number of chunks during session one. In session two, linear mixed model analysis suggested that stimulation groups may have had enhanced learning, based on a faster rate of reaction time decrease relative to sham. However, the one-way ANOVA contrasts revealed the real stimulation groups on average had longer reaction times and more chunks relative to sham, indicating that overall, left or right PFC stimulation does not positively impact learning.

Our results are inconsistent with previous findings which demonstrate that inhibiting the prefrontal cortices enhances learning (Joseph M. Galea et al., 2010; Zhu et al., 2015b). The disagreement between our findings and previous findings may be due to methodological differences. In the Galea et al. (2010) study, TMS was used to disrupt the prefrontal cortices *after* sequences had been learned. Similarly, Debarnot et al. (2012) had participants memorize a word-list *after* learning occurred. Here, participants received stimulation *while* they simultaneously learned the sequences. Thus, either engagement or disruption of the prefrontal cortices and the declarative memory system via tDCS during learning resulted in slower reaction times and shorter chunks. Another explanation for why the different electrode polarities did not induce opposite behavioral results might be due to the similar electric fields. The ROAST computational model output suggests that the electric magnetic field distribution across the brain

was nearly identical across both the left anode montage and the left cathodal montage. Based on the output of the model, it is possible that the electric field induced in the brain is similar, which could indicate that cathodal stimulation did not simply suppress PFC as predicted.

Another likely explanation for our findings is that cathodal stimulation may result in cortical excitability under higher intensities. Batsikadze et al. (2013) stimulated left M1 with cathodal tDCS for twenty minutes either at 1 mA or 2 mA and then monitored cortical excitability via MEPs (Batsikadze, Moliadze, Paulus, Kuo, & Nitsche, 2013). The surface area of the cathode was $35cm^2$ and the reference electrode was $100cm^2$. Batsikasdze and colleagues found that 1 mA of cathodal stimulation resulted in cortical inhibition, leading to significant lower MEPs, whereas 2 mA of cathodal stimulation resulted in increased cortical excitability, leading to significantly greater MEPs. Given that we used 2 mA of stimulation coupled with smaller electrodes (yielding a higher current density), it is reasonable to think that we induced cortical excitability underneath the cathode instead of suppressing it.

It is likely that the interplay between the prefrontal cortices and other cortical and subcortical structures is necessary for successful motor learning as posited by the Dual Processor model and C-SMB framework. Evidence of this comes from a PET study in which the anterior cingulate / mesial PFC exerted control on striatal activity during retrieval of an explicit sequence, whereas the activity between these two brain regions was uncoupled during the retrieval of sequences that had been learned implicitly (Destrebecqz, Peigneux, Laureys, & C, 2005). Therefore, tDCS may be impairing learning directly or indirectly via the striatum. In an animal model study, cathodal stimulation over the prefrontal cortex of rodents resulted in a significant increase in striatal dopamine levels (Tanaka et al., 2013). Thus, it is possible that in our study,

prefrontal stimulation disrupted its interaction with subcortical structures, negatively impacting learning.

Existing current density modeling literature of tDCS including the ROAST model we used suggests that the electric field magnitude distribution across the cortex and underlying brain regions in both anodal and cathodal stimulation over left PFC are similar (Bai, Dokos, Ho, & Loo, 2014; Datta, Truong, Minhas, Parra, & Bikson, 2012). This similarity may be due to the close proximity of the electrodes. A similar electric field distribution across anodal and cathodal left PFC stimulation might explain the similar behavioral results produced in this and other studies. Similar behavioral effects regardless of the polarity of stimulation have been demonstrated in previous tDCS literature. For example, both anodal and cathodal stimulation over the cerebellum impaired performance in a working memory task (Ferrucci et al., 2008). In another study, both anodal and cathodal tDCS to Wernicke's area improved semantic processing (Brückner & Kammer, 2017). Thus, anodal and cathodal stimulation may not consistently yield opposing effects on the brain and behavior, but rather in some instances, anodal and cathodal stimulation may have similar impacts.

While the behavioral results for the anodal and cathodal groups were similar, it is likely that they were mediated through different networks. Perfusion and functional connectivity studies using tDCS demonstrate differential network activation based on the polarity of stimulation. After twenty minutes of either anodal or cathodal tDCS over left PFC with the reference electrode over the contralateral orbit, anodal stimulation resulted in increased perfusion to primary sensory and paracingulate cortices and decreased coupling between the left PFC and thalami, brain stem, and cerebellum. Cathodal stimulation over the left PFC resulted in decreased perfusion to the thalami and decreased coupling between the left PFC and ipsilateral temporal,

parietal, and occipital cortices (Stagg et al., 2013). In the present study, we used the same electrode montage as that of Stagg and colleagues and compared the behavioral effects of anodal and cathodal stimulation; while we observed similarities in behavioral effects of anodal and cathodal stimulation, the findings of Stagg and colleagues suggest that the neural underpinnings of the observed impairments in our study likely differ depending on the stimulation type. That is, it is likely that the impairments observed in the anodal group and the impairments observed in the cathodal group were mediated by different brain networks. Contrasts between the two tDCS groups in our study also support this. The left PFC anodal group often produced sequences or formed chunks at a faster rate compared to the left PFC cathodal group, but overall the left PFC cathodal group often had fewer chunks compared to the left PFC anodal group. Likewise, in session one, cathodal stimulation resulted in an overall higher number of chunks, whereas anodal stimulation resulted in an overall higher number of chunks for session two. Had the cathodal and anodal tDCS affected the same brain networks in an identical manner we should have observed no differences between the two groups.

These findings coupled with the conflicting results in the tDCS literature suggest that the canonical assumption of 'anodal excitatory, cathodal inhibitory' is oversimplified (Bestmann, de Berker, & Bonaiuto, 2015). Confirming this notion, a meta-analysis by Jacobson and colleagues in 2012 calculated that the probability of getting the 'anodal excitatory, cathodal inhibitory' effect in the motor system was 0.67. The probability for the same tDCS effect in cognitive studies was a mere 0.16 (Jacobson, Koslowsky, & Lavidor, 2012). The lower probability in cognitive studies might be due to brain state dependent effects of tDCS (Jacobson et al., 2012); that is, different cognitive task conditions may alter the brain state in a manner that impacts whether tDCS stimulation yields excitatory or inhibitory effects (Feurra et al., 2013; Shahbabaie

et al., 2014). Future studies should adopt designs which include both anodal and cathodal tDCS groups and varied task conditions to further test these assumptions.

Another explanation for our behavioral results might be that tDCS stimulation may cause an imbalance to develop between the two frontal hemispheres. There are two lines of evidence to support this possibility. One line of evidence is based on the idea of interhemispheric inhibition, which may be influenced by tDCS. It is likely that tDCS affects brain regions distant from the anode, possibly through inhibitory interneurons. Insight gained from tDCS experiments with stroke patients help us understand interhemispheric inhibition. After a stroke, the unaffected hemisphere may be disinhibited and thus *increase* inhibition of M1 in the affected hemisphere, increasing movement difficulty (Di Pino et al., 2014). One way to normalize the interhemispheric balance is to inhibit the unaffected side and/or to excite the affected hemisphere with tDCS. The concept of bihemispheric tDCS has been demonstrated to facilitate regaining motor function in stroke patients (Lindenberg, Renga, Zhu, Nair, & Schlaug, 2010; Nowak, Grefkes, Ameli, & Fink, 2009). The idea that tDCS to left or right PFC may have interhemispheric and far reaching affects in the brain is further supported by previous evidence that the prefrontal cortex provides a balance of excitatory and inhibitory input to distant brain regions (Knight, Staines, Swick, & Chao, 1999) and a TMS study, in which inhibiting the prefrontal cortex was related to decreased inhibition of M1 in the opposing hemisphere (Duque et al., 2012). In addition to the possibility of tDCS impacting interhemispheric inhibition, a second line of evidence suggests that tDCS can alter interhemispheric connectivity. In a resting state functional connectivity study, participants that received 10 minutes of left DLPFC anodal tDCS showed increased left DLPFC connectivity to the right hemisphere and decreased connectivity to brain regions around the left DLPFC (Park et al., 2013). Thus, whether via

alterations in interhemispheric inhibition or connectivity, tDCS to prefrontal regions may cause an imbalance in activity between the two hemispheres and indirectly affect other brain regions.

Relating these ideas of tDCS induced interhemispheric imbalance to our findings, there are two possible mechanisms underlying our behavioral results. It is possible that the anodal stimulation to left PFC in our study *increased* inhibition of right PFC whereas cathodal stimulation to left PFC *decreased* inhibition of right PFC. The other possibility is that anodal left PFC tDCS increased connectivity to the right PFC and decreased connectivity within the left PFC, whereas cathodal left PFC tDCS decreased connectivity to the right PFC and increased connectivity to the brain regions around the cathode electrode. Regardless, in our study, stimulation to prefrontal regions could have resulted in an imbalance of inhibition and connectivity, ultimately impairing sequence learning and chunking.

It is also apparent from the results that reaction time changes and chunk formation do not occur in parallel or over the same timeframe. Although the rate of change in reaction time was slower in PFC stimulation groups relative to sham, the slopes of the change in reaction time were in the same direction as that of sham. However, the slopes for the chunking data of the left PFC tDCS stimulation groups are mostly flat, or even positive during session one, suggesting that stimulation to left PFC is especially harmful to chunk formation during session one. Further, comparing the figures for reaction time and number of chunks, the reaction time of the anodal as well as the cathodal left PFC group appears to remain close to the sham group throughout each session. In the chunking data, however, there are clear disadvantages of anodal left PFC stimulation in sessions two and three, as indicated by a greater number of chunks. This suggests that impairing a component of sequence learning such as reaction time does not necessarily lead to a proportional impairment in the number of chunks, or vice versa.

In conclusion, regardless of the polarity of stimulation, left PFC tDCS impaired learning as evidenced by slower reaction times and a higher number of chunks relative to sham. These results are likely due to tDCS induced perturbation of different networks important for sequence learning and chunking, cathodal stimulation causing excitation, and/or tDCS induced imbalance between the right and left prefrontal hemispheres. Future studies should include both cathodal and anodal groups paired with neuroimaging techniques to determine when, and for what tasks, cathodal v. anodal tDCS may have a positive impact.

CHAPTER III: Long Term Effects of tDCS

Introduction

The Dual Processor model and the C-SMB framework posit that the central processor (prefrontal cortices) orchestrates the transition of motor sequences from short-term memory to long-term memory. The authors of these models argue that the prefrontal cortices orchestrate this transition via their connections to the basal ganglia and hippocampus (Abrahamse et al., 2013; Verwey et al., 2014). Indeed, the prefrontal cortices interact with the medial temporal lobe during both encoding and retrieval of sequences, potentially facilitating long-term memory (Simons & Spiers, 2003). However, while these findings are consistent with a role of prefrontal cortices in consolidation and long-term memory, neuroimaging alone does not clarify the specific and causal impact of the prefrontal cortices on consolidation and long-term memory in the context of sequence learning.

Limited non-invasive brain stimulation research has been conducted to investigate the assumption that the prefrontal cortices are critical for the transition from short- to long-term memory. Evidence from one tDCS study shows that stimulating the prefrontal cortex facilitates the retention of a learned sequence. Janacsek et al. (2015) used anodal tDCS either over the left or right DLPFC during a probabilistic sequence learning task and found an advantage in sequence retention for the right DLPFC tDCS group, but not the left DLPFC tDCS group, at two and twenty-four hours post stimulation (Janacsek et al., 2015). The findings of Janacsek and colleagues suggest that stimulation of the prefrontal cortices may have long-term effects, a week or longer, but no tDCS motor learning studies have implemented a longitudinal design to

investigate the long-term efficacy of tDCS over prefrontal cortices. Thus, we aimed to address limitations in previous research by conducting a longitudinal study of the impact of tDCS to the prefrontal cortices on motor sequence retention. In accordance with the Dual Processor Model and C-SMB framework, along with the findings of Janacsek et al (2015), in the current study we anticipated that anodal stimulation to the prefrontal cortices would facilitate the long-term memory of sequences practiced a year earlier.

The long-term duration of the efficacy of tDCS is unknown. The few tDCS studies that have implemented longitudinal designs show long lasting, tDCS-linked effects. For example, Kadosh and colleagues (2010) used anodal or cathodal tDCS over the parietal lobes while participants learned artificial numerical symbols over the course of six days. Anodal tDCS enhanced numerical processing during the six days of practice, and importantly, this improvement was still evident six months after training (Kadosh et al. 2010). Another study conducted by Reis et al. (2009) demonstrated significant motor skill improvements during a five session study while participants practiced an isometric pinch force task while receiving stimulation to M1 (Reis et al., 2009). The improvement observed in the M1 group was still apparent relative to the sham group up to 3 months later. More recently, Au et al. (2016) reported significant gains in verbal working memory after participants received tDCS over either left or right DLPFC. The same research team demonstrated that 12 months later, individuals who had received tDCS as opposed to sham during the initial study showed substantial benefits to long-term retention (Katz et al., 2017). Thus, while multiple tDCS studies revealed the duration of the long-term efficacy of tDCS at least 12 months, the duration of the efficacy of prefrontal tDCS in motor learning studies is unknown.

Repetitive TMS studies with stroke patients also indicate that non-invasive brain stimulation can yield long-term effects. While most TMS studies typically assess participants 24 hours to 1 week after a TMS intervention, there are studies that have shown beneficial effects of TMS lasting at least one-week post-intervention (Takeuchi, Tada, Toshima, Matsuo, & Ikoma, 2009), two-weeks post-intervention (Fregni et al., 2006), and up to 1 year post-intervention (Khedr, Etraby, Hemeda, Nasef, & Razek, 2010) on stroke patients. These findings show that in addition to tDCS, the efficacy of TMS may also have a similar duration; however it is unknown whether the previously demonstrated long-term benefits of TMS would translate to healthy populations.

The mechanism responsible for the long-term effects of tDCS may be the result of interactions with long-term potentiation. Animal models demonstrate that weak currents introduced intracerebrally or epidurally can produce long lasting effects. These effects exhibit similar features to long-term potentiation, such as modifications of intracellular cAMP and calcium levels (Nadira Islam, Aftabuddin, Moriwaki, Hattori, & Hori, 1995; N Islam & et, 1997). Thus, consistent with these animal model findings, in humans, long lasting effects of tDCS may be due to stimulation-induced cellular changes in the brain similar to those which occur during long-term potentiation.

The Dual Processor model and the C-SMB framework do not make any predictions about the role of the motor processor (M1) in retention or long-term memory in the context of sequence learning. However, numerous tDCS studies indicate a role of M1 in retention and long-term memory for motor learning. Stimulation to M1 results in increased retention of a recently learned visuomotor adaptation (Galea et al. 2011) and individuals who receive anodal tDCS over M1 while performing thumb ballistic movements show greater retention improvements thirty

minutes and one week later relative to individuals in the sham group (Rroji, Van Kuyck, Nuttin, & Wenderoth, 2015). Another tDCS study found offline but not online gains in participants who received stimulation over M1 while practicing a sequence over the course of five days, suggesting a role of M1 in consolidation (Reis et al., 2009). Further, the benefit the M1 tDCS group received relative to sham remained significant for three months. Thus, despite consistent evidence implicating a role of M1 in long-term retention, the Dual Processor model and the C-SMB framework limit the role of M1 to execution. Further, the duration of the long-term efficacy of tDCS beyond three months have not previously been reported and is unknown. Here, we aimed to test whether the role of M1 is limited to execution in the DSP task and determine whether the long-term efficacy of tDCS over M1 is viable, one year after stimulation. Building off previous tDCS literature, we predicted that stimulation to M1 would result in greater motor sequence retention effects a year later.

In this study, we invited back participants from the left PFC, M1, and sham tDCS groups of the three-session tDCS study for a single, fourth session to test whether M1 and left PFC play a role in long-term retention and to assess the validity of the Dual Processor model and the C-SMB framework. A secondary purpose of the study was to assess the duration of the long-term efficacy of tDCS on motor sequence learning. We opted to specifically invite the left PFC tDCS group to participate in the follow-up study as the right PFC group did not demonstrate any benefit of stimulation in the initial study, while our findings for the left PFC group- although still overall suggesting a negative impact of tDCS- were somewhat more complex. The M1 group was included in this follow-up based on the benefits to reaction time and chunking observed in experiment one, along with previous tDCS literature demonstrating long-term effects of stimulation to M1. The sham group was invited back as a control. Despite the findings of

experiment one, but in accordance with the Dual Processor model and C-SMB framework and limited additional research (Abrahamse et al., 2013; Janacsek et al., 2015; Verwey et al., 2014), we predicted that the prefrontal group would demonstrate long-term retention benefits in the learned sequences. Additionally, in line with previous findings, we hypothesized that the M1 tDCS group would demonstrate long lasting retention effects relative to sham.

Method

Participants

Twenty-one young adult participants from three of the tDCS groups in experiment one came back to the lab after an average of 1.3 years from their last visit (third session). Seven participants were from the left PFC anode group, five from the M1 group, and nine participants from the sham group.

Task Order

For participants' fourth session, they completed a hybrid of sessions two and three from the first experiment. First, participants completed six blocks of practice with their originally assigned sequences in the DSP task *without* tDCS. Similar to session two, after practice, participants completed the DSP questionnaire, then advanced to the testing portion of the DSP task (single stimulus, familiar, mixed familiar, mixed unfamiliar). After the test portion, participants completed the card rotations task, visual search, visual array change task, digit symbol, then completed an exit survey questionnaire.

Data Analysis

The primary outcomes for this study were offline reaction time gains, with reaction time, number of chunks, and number of errors as the secondary outcomes. We implemented a linear mixed model using reaction time and chunk number with trials as a continuous factor. For the offline reaction time gains and number of errors, we used a repeated measures ANOVA using sequence (simple, complex) and stimulation group as the between subjects factor in the full model. Offline learning gains were calculated by subtracting the mean of six key presses from the first trial of session four from the mean of six key presses from the last trial of session three (e.g., session four (mean RT trial 1-6) – session three (mean RT trial 442-448)). It should be noted that we did not correct for multiple comparisons.

Results

Retention Interval

A one-way ANOVA was performed on the time (in minutes) between session three and session four and revealed no significant differences between the left PFC group and sham ($p = .39$) or the M1 group and sham ($p = .62$).

Errors

A two way (sequence type: simple, complex) repeated measures ANOVA was run on errors, with stimulation group (left PFC, M1, and sham) as the between subject factor. There was no main effect of sequence type $F(1,18) = 3.678$, $p = .071$, and no sequence type by stimulation interaction $F(2,18) = .37$, $p = .70$.

Reaction Time

There was no main effect of stimulation group (F (2,20) = .58, p = .56) nor a stimulation group by sequence type interaction (F (2,20) = .90, p = .64), but there was a main effect of sequence type (F (1,20) = 15.89, p < 0.001).

Hypothesis driven pairwise comparisons between the stimulation groups revealed that the M1 group was significantly faster at reducing reaction time during session four relative to sham (β = -.10 SE = .04, p = 0.021). Reaction times were reduced at a significantly faster rate for complex versus simple sequences (β = -.11 SE = .03, p = 0.001). Pairwise comparisons for the stimulation group by sequence type interactions revealed that the M1 stimulation group reduced the rate of reaction time at a significantly faster rate relative to sham for the complex sequences during session four (β = -.12 SE = .06, p = 0.041; Figure 3.2).

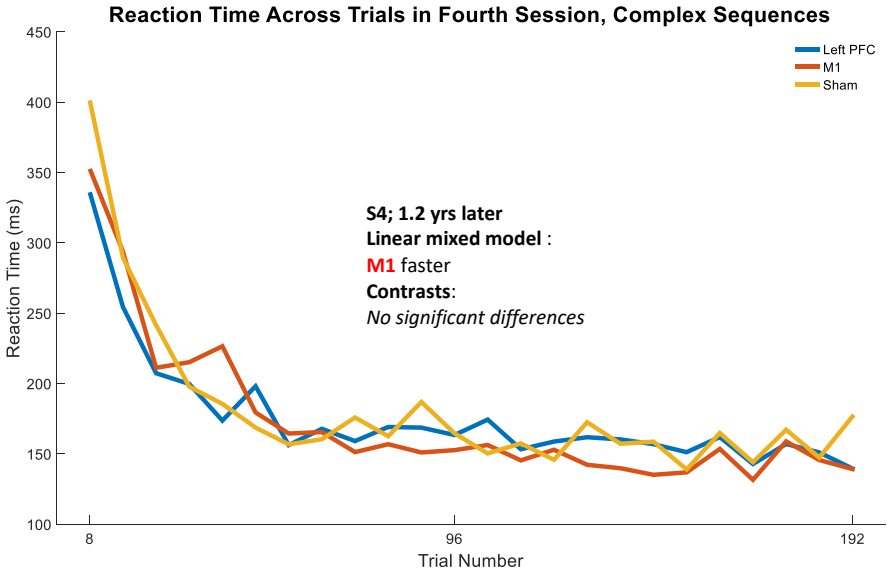

Figure 3.1. Mean reaction time as a function of trials in fourth session. Left PFC is denoted by the blue line, M1 is denoted by the red line, and sham is denoted by the yellow line. The M1 tDCS group reduced reaction times at a faster rate relative to sham.

Contrasts for Reaction Time

Contrasts between the real tDCS groups and sham revealed no significant differences.

Chunks

Chunking data is not available for this data set as the model fit failed given the limited number of trials.

Error in Testing Conditions

A two (sequence type: simple, complex) by four (testing condition: single stimulus, familiar, mixed familiar, and mixed unfamiliar) repeated measures ANOVA was run on errors, with stimulation group as a between subjects factor. There was a main effect of testing condition $F(3,54) = 7.17$, $p < 0.001$. Paired samples t-tests revealed that the single stimulus condition (M = 1.68) had significantly fewer errors relative to the mixed familiar condition (M = 2.95; t(20) = -3.58, $p < 0.001$), and the familiar condition (M = 1.62) had fewer errors relative to the mixed familiar condition (t (20) = -6.06 $p < 0.001$) as well as the mixed unfamiliar condition (M = 2.25; t(20) = 3.51, $p = .002$). All other main effects and interactions were not significant.

Reaction Time in Testing Conditions

The rate of change for reaction time for the mixed familiar testing condition was significantly slower relative to the single stimulus testing condition (β = .41 SE = .18, p = 0.020) and the familiar testing condition (β = .37 SE = .17, p = 0.032).

Across all testing conditions, the sham was slower relative to the M1 and left PFC stimulation groups. The rate of change in reaction time was significantly faster for the M1 tDCS group (β = -.57 SE = .15, p < 0.001) as well as the left tDCS group (β = -.57 SE = .14, p < 0.001)

relative to sham. It should be noted that participants did not receive stimulation at this test session; the group assignments were from one year prior.

The rate of change in reaction time for the left PFC group in the single stimulus condition was significantly faster (β = -.84 SE = .38, p = 0.028; Figure 3.2) relative to sham. The left PFC group changed reaction time over trials at a significantly faster rate in the mixed familiar condition relative to sham (β = -1.35 SE = .42, p = 0.001). The M1 tDCS group reduced the rate of reaction time in the mixed unfamiliar condition at a significantly faster rate (β = -.84 SE = .41, p = 0.040) relative to sham.

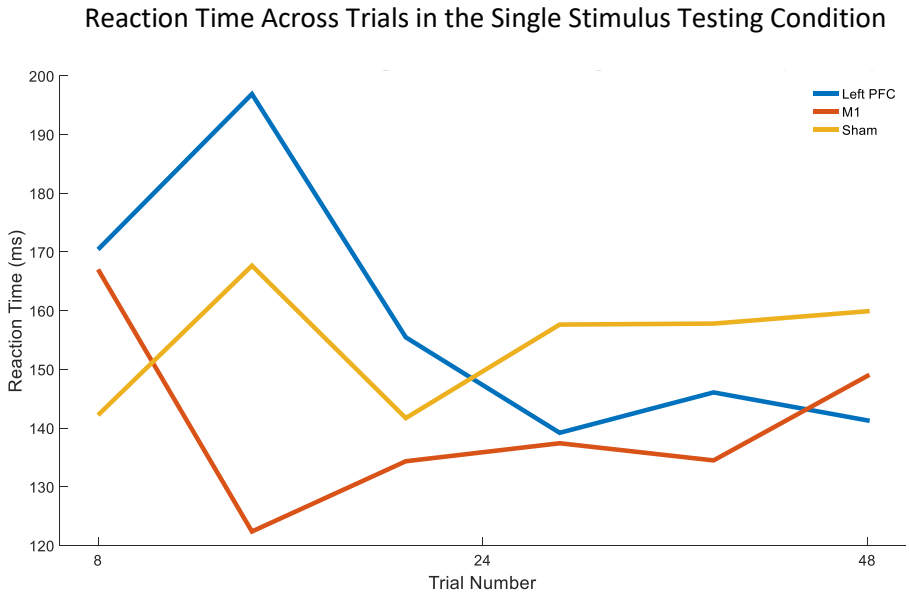

Figure 3.2. Mean reaction time as a function of trial in the single stimulus testing condition. Left PFC is denoted by the blue line, M1 is denoted by the red line, and sham is denoted by the yellow line. The left PFC tDCS group had significantly longer reaction times relative to sham.

Offline Forgetting

Offline forgetting (first trial of the fourth session – last trial of the third session) of the complex sequences showed an advantage for the left PFC group (Figure 3.3). Paired samples t-tests between left PFC and sham showed a significant difference in offline forgetting t(6) = -

2.94, p = .026 whereas there was no difference between the M1 group and sham t(4) = -.74, p = .499.

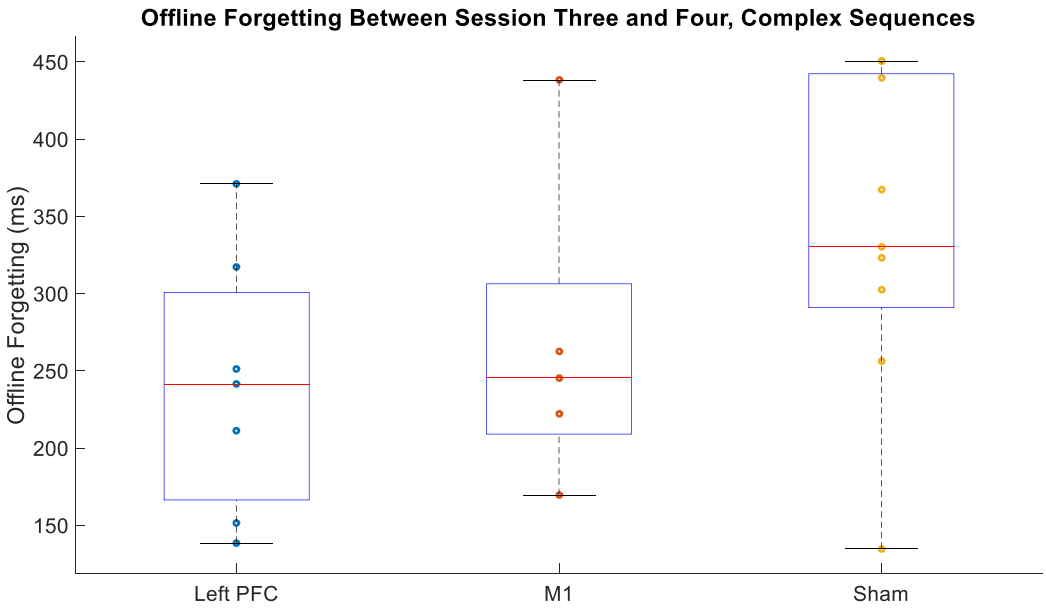

Figure 3.3. Boxplots of offline gains between sessions three and four for left PFC, M1, and sham tDCS groups. Dots within each boxplot represent each participant. The left PFC group exhibited significantly less forgetting than the sham group.

Discussion

In contrast to our hypothesis, the M1 tDCS group did not show retention effects as measured by offline forgetting. The lack of significant offline gains in the M1 group is inconsistent with previous non-invasive brain stimulation literature. The primary motor cortex has been implicated in both short- and long-term retention, likely through the process of consolidation. For example, anodal stimulation over M1 during a visuomotor adaptation task led to increased retention, or slower forgetting within a single session (Galea et al. 2011). Similarly, disruption of M1 by TMS during a visuomotor adaptation task lead to impaired consolidation 24 hours later (Hamel, Trempe, & Bernier, 2017) and anodal tDCS over M1 during a sequential force task lead to greater skill, likely by facilitating consolidation, a benefit that remained stable

up to three months later (Reis et al., 2009). The lack of a significant finding for the M1 may be due to the task specific nature of tDCS. Marquez et al. (2013) found stimulation to M1 produced offline gains for a sequential pinch force task, but not for a sequential finger tapping task (Saucedo Marquez et al., 2013). Although there wasn't significant offline forgetting for the M1 group in our study, it is noteworthy to mention that inspection of Figure 3.5 shows the median reaction time of the M1 group was similar to that of the left PFC group, which did show a significant long-term benefit in reaction time. Further, one participant in the M1 group with a reaction time near 450 ms may be driving the lack of significant results, coupled with the small sample size.

While we did not find significant long-term offline gains (less forgetting) from M1 stimulation one year prior, we did observe a long-term performance benefit for the M1 group as evidenced by the acceleration of reaction time change across trials. That is, the M1 group relearned their sequences at a faster rate than the sham group. This is in contrast with the results for the M1 group in the first session of experiment one, during which there were no significant differences in the rate of change in reaction time. Although we did not observe any significant offline gains, it is possible that the enhanced performance benefit observed in the M1 group was mediated through consolidation. Evidence supporting this idea comes from a one week, motor adaptation training study (Landi, Baguear, & Della-Maggiore, 2011). Using their right hand, participants trained for seven days in a visuomotor adaptation task, undergoing a structural brain scan pre- and post-training. Training led to faster relearning a year later and an increase in gray matter concentration and fractional anisotropy of white matter fibers in the left M1. Further, greater gray matter concentration changes were positively correlated to savings observed in the same task one year later, suggesting the left M1 is the likely location of the stored motor

representations specific to the task. This study supports our findings, where we found stimulating left M1 paired with practice resulted in faster relearning a year later.

Our findings regarding the faster sequence relearning in the M1 group can be a useful addition to the Dual Processor model and the C-SMB framework. The Dual Processor model and the C-SMB framework limit the role of M1 (the motor processor) to the execution of sequences and make no predictions about its involvement in short- or long-term retention processes, relearning previously learned sequences, or in chunking. It is possible that the relearning benefit observed in the M1 group in the 1-year follow-up is due to our previous findings showing that stimulation to M1 facilitates chunking. Further, the limited role of M1 in the Dual Processor model and the C-SMB framework as involved in execution is in direct contrast with at least one animal model study that found M1 is not necessary for the execution of a complex skill once the skill has been learned (Kawai et al., 2015). In light of this animal model finding, along with our current and previous findings and previous tDCS literature, the Dual Processor model and the C-SMB framework should be revised to consider the role of M1 in the long-term storage of hierarchical memory structures.

Consistent with our hypothesis and the Dual Processor model and the C-SMB framework, we observed offline gains (less forgetting). Although the left PFC group showed overall longer reaction times in the single stimulus condition, this was unchanged from our initial reaction time findings, and we found reduced offline forgetting for the left PFC group. These findings suggest that the representation of the sequences learned approximately a year earlier decayed at a slower rate for the left PFC group. Thus, the original findings remained stable a year later, consistent with the idea that overall, enhancing excitability of left PFC enhances long-term memory. The idea that the left DLPFC has a role in long-term memory is not new.

Evidence suggests that the DLPFC may be involved in reordering pieces of information in working memory and subsequently enhancing memory for associations among items in long-term memory (Blumenfeld & Ranganath, 2007). Further, the prefrontal cortices are thought to work in tandem with subcortical structures such as the hippocampus and basal ganglia to promote long-term memory (Simons & Spiers, 2003), an idea incorporated into the Dual processor model and the C-SMB framework. A potential mechanism for the slower decay in the left PFC group observed in our study might be through the enhancement of the explicit or declarative memory system, thereby making motor representations resistant to decay. Indeed, explicit memory performance is related to both the fast and slow processes of motor adaptation and poorer retention is associated with individuals who have poorer explicit memory (Trewartha, Garcia, Wolpert, & Flanagan, 2014). Thus, stimulation to prefrontal cortices is a viable solution to facilitate retention or slow the decay of sequences likely through the enhancement of the declarative memory system.

It is noteworthy that there are no previous accounts of stimulation to prefrontal cortical regions during motor learning that demonstrate long-term retention or consolidation effects over a period greater than a week. A possible mechanism underlying long-term retention in the prefrontal cortices could involve plasticity-related protein synthesis. Plasticity-related protein synthesis is required in M1 for successful motor learning in a multi-day reaching task in non-human primates (Luft, 2004). Likewise, improvements in performance in a spatial working memory task in mice required the synthesis of proteins in the medial PFC, the same brain regions active during task performance (Touzani, Puthanveettil, & Kandel, 2007). These studies suggest that M1 and the medial PFC are involved in the consolidation and long-term retention of motor skills and spatial working memory strategies, respectively, potentially via an influence on protein

synthesis. Based on this previous literature, it is possible that tDCS promotes protein synthesis in the left prefrontal cortices of humans, promoting long-term retention of motor sequences. The Dual Processor model and the C-SMB framework do not address how non-invasive brain stimulation affects motor learning or the neurobiological mechanisms of motor learning. Considering our results and the overlap between the mechanisms of tDCS and long-term potentiation, revising the models to incorporate tDCS and the neurobiological mechanisms of motor learning, would add considerable strength to the models.

Another potential explanation of the the left PFC group may be due to an imbalance of excitation / inhibition of the prefrontal hemispheres. Our electrode montage (anode on the left PFC and the cathode on the right forehead) coupled with the neurophysiological mechanism of interhemispheric inhibition, where one hemisphere inhibits the other, there was likely inhibition of the right hemisphere. Also, previous research suggests that prefrontal regions of the brain play a role in interhemispheric inhibition. For example, the prefrontal cortex provides a balance of excitatory and inhibitory input to distant brain regions (Knight et al., 1999). Further, a non-invasive brain stimulation study using TMS, showed inhibiting the prefrontal cortex was related to decreased inhibition of M1 in the opposing hemisphere (Duque et al., 2012). Thus, less forgetting in the left PFC group may be due to the increased inhibition to the right PFC, increased excitation of the left PFC, or both. Future studies, should consider using both anodal and cathodal tDCS montages and long-term follow-up study designs to help answer this question.

In contrast to the strengths of the study, a potential limitation might be selection bias. For example, it could be that participants in the left PFC group were not representative of the participants who were in the first three sessions, thus driving the offline gains. However, we

believe this is inaccurate. For example, the median of the left PFC group and the median of the M1 group (Figure 3.3) are identical with the main difference between the two tDCS groups is one participant near 450 ms in the M1 group. Thus, the main difference between the left PFC and M1 tDCS groups is one participant. Second, the best performers (individuals that demonstrated the least forgetting) in session four of the left PFC group are in the same range of best performers in the M1 and sham tDCS groups. Thus, it is not that the left PFC group had one or multiple participants that were abnormally good performers. Third, participants in the left PFC group showed a slower rate of change in reaction time for the single stimulus testing condition and the M1 group showed a faster relearning during practice in the fourth session. These findings are consistent with the initial group of participants in the first three sessions. Thus, participants that came back for the fourth session are likely representative of the entire group.

In summary, we found offline gains, or less forgetting, for the left PFC group. These findings are consistent with previous models which support the notion of the PFC involved in long-term memory. However, stimulation to left PFC resulted in overall longer reaction times when participants were tested on their sequences without visual cues, inconsistent with sequence learning models. We also found faster relearning of sequences in the M1 group after receiving tDCS one year previously, a finding that is currently not accounted for in motor sequence learning models, which limit the role of M1 to sequence execution.

CHAPTER IV: Age Differences in Motor Sequence Learning

The Dual Processor Model and C-SMB framework

The Dual Processor Model and the Cognitive framework for Sequential Motor Behavior (C-SMB) are helpful frameworks for explaining sequence learning, presumably both in older and younger adults (Abrahamse et al., 2013; Verwey, Shea, & Wright, 2014). In the Dual Processor model and the C-SMB framework, information is processed mainly by two processors: a central processor and a motor processor, which communicate with each other via temporary storage components. The central processor, which Verwey et al. suggest is the prefrontal cortex, has access to short-term memory, and is involved in preparing and initiating sequences, setting task goals, and loading the motor buffer- a storage component. The motor buffer is limited to storing motor representations to be executed. The motor processor, suggested by Verwey et al. to be the primary motor cortex, executes the content of the motor buffer. According to the Dual Processor model and C-SMB framework, early in the DSP task the cognitive processor communicates with the motor processor, which makes appropriate motor executions. After repeated execution of a sequence, and once a chunk is formed, the cognitive processor loads in motor chunks, still communicating with the motor processor to execute the finger movements. Thus, over many trials, the involvement of the cognitive processor is significantly reduced, while the motor processor continues to be highly involved (Hommel, 2000). It should be noted that the authors of the Dual Processor model and the C-SMB frameworks make no mention as to whether the frameworks are age independent. Thus, the main purpose of the current study is to understand

whether the Dual Processor model and the C-SMB framework can be applied to older adults in addition to young adults.

Aging is Associated with Reduced Motor Performance

Advanced age is typically accompanied by impairments in sensorimotor, cognitive, and perceptual functioning (Raz, 2000; Rodrigue, Kennedy, & Raz, 2005). More specifically, older adults experience significant declines in movement ability such as reduced walking speed, poorer hand-eye coordination, and compromised motor skill learning (Studenski et al., 2011; Yan, Abernethy, & Li, 2010). For example, Shea, Park, and Braden (2006) found that when moving a lever to either a random or repeated sequence of targets, older adults showed no difference in performance during acquisition and retention relative to young adults for randomly presented target locations; however, the researchers found age-related performance differences for the repeated sequences, with poorer performance in older adults as indicated by slower reaction time; these differences increased over practice. Shea and colleagues interpreted this finding as reflecting an inability of older adults to use sequence information to decrease reaction time. Further, Liu, Cao, and Yan (2013) found that although older adults exhibited evidence of learning a new motor skill, their learning gains were smaller than those observed in young adults. These findings are consistent with the idea that motor learning is compromised in older adults.

Although older adults experience declines in motor learning, additional research suggests that this may not impact performance universally, or under every circumstance. Howard & Howard (1989) demonstrated that older adults exhibited comparable learning to that of young adults when motor learning was measured as the difference between reaction time in a sequence block and the reaction time in a random block in the serial reaction time task. However, when learning was measured in terms of accuracy during a generation block, older adults showed

markedly reduced learning compared to the young adults (Howard & Howard, 1989). Another motor learning study used the serial reaction time task and replicated Howard & Howards' findings that older adults show motor sequence learning, as indicated by reaction time differences (Brown, Robertson, & Press, 2009). However, when participants were re-tested 24 hours later, only the young adults exhibited a beneficial change in skill, indicating that between-session (offline) gains were reduced relative to those normally observed in young adults; these findings are consistent with the idea that neural plasticity and consolidation may be reduced with advancing age (Wilhelm, Prehn-Kristensen, and Born 2012; Wilhelm, Diekelmann, and Born 2008). In another study, Seidler (2006) had older and young adults complete a visuomotor sequence-learning task in which they made a sequence of movements with a joystick. Although older adults had slower reaction times overall, they showed no deficit in sequence learning, in line with the idea that age-related learning deficits may be task specific (Seidler, 2006). Further, Bo and Seidler (2009) had both young and older adult participants perform the alternating serial reaction time task at various difficulties and only found age differences with the more challenging task conditions. A review by Voelcker-Rehage also came to the same conclusion (Voelcker-Rehage, 2008). Thus, previous research shows that despite age related decline, older adults can learn new motor tasks, albeit not as well as young adults. The effect of age on motor learning is likely dependent on task difficulty or complexity and may be related to impaired consolidation processes.

Chunking and Older Adults

Older adults exhibit declined or limited chunk use that may be due to diminished working memory. For example, after practice in the DSP task, young adults exhibited evidence of chunking, whereas many older adult participants did not (Verwey, 2010). In another study

investigating the mechanisms behind chunking, working memory capacity was positively correlated with chunk length in both young and older adults. However, older adults showed significantly reduced working memory capacities and chunk lengths relative to young adults (Bo et al., 2009a). Moreover, only 7% of young adults showed no evidence of chunking, whereas 22% percent of older adults showed no evidence of chunking. The link between the prefrontal cortices and working memory is well established (Kane & Engle, 2002) and it is known that older adults show significant reductions in prefrontal volume and gray matter (Esiri, 2007; Scahill et al., 2003). Thus, it is possible that chunking limitations in older adults may be linked to prefrontal function.

Neuroimaging in Older Adults

fMRI studies consistently demonstrate that relative to young adults, older adults show overactivation of some regions of the brain, under-recruitment of others, and overall more bilateral activation. For example, one fMRI study had young and older adults perform an isometric hand grip task using either their dominant or non-dominant hand (Ward & Frackowiak, 2003). The investigators found that during this task, there was a positive relationship between a participant's age^2 and the number of voxels in the left postcentral sulcus, the left inferior central sulcus, and left precentral gyrus. When participants used their non-dominant hand, there were positive relationships between age^2 and the number of voxels in the left ipsilateral inferior postcentral gyrus, the left inferior central sulcus, and the left superior central sulcus. Further, regardless of the hand used, older adults showed greater activation in the left hemisphere relative to young adults, suggesting increased bilateral recruitment in older adults. More support for overactivation in older adults comes from an fMRI study, in which older and young adults performed a serial reaction time task with their dominant right hand (Mattay et al., 2002). Older

adults showed greater activation in the left primary motor cortex, primary and secondary somatosensory areas, the premotor and supplementary motor areas, and subcortical areas of the brain relative to young adults. Evidence for bilateral activation in older adults is supported by a spatial working memory study conducted by Reuter-Lorenz et al. (2000). In their study, young and older adults kept the location of targets in working memory during a delay period. During the task, young adults exhibited strong right lateralization of prefrontal cortex activity, whereas for older adults there was greater left prefrontal activation with overall a more bilateral pattern of activation (Reuter-Lorenz et al., 2000). These studies show that older adults recruit brain regions bilaterally for tasks in which young adults only recruit one hemisphere.

Bilateral Activity in Older Adults is Likely Due to Compensatory Mechanisms

Studies incorporating non-invasive brain stimulation have shown that the bilateral activation commonly observed in older adults is likely compensatory. Rossi et al. (2004) used TMS to create a transient lesion either over the right or left DLPFC as young and old participants encoded pictures, then again when participants retrieved them (Rossi et al., 2004). During the retrieval phase, young adults that had received a transient lesion over left DLPFC were more impaired, relative to the right DLPFC stimulation condition, whereas older adults were equally impaired regardless of the hemisphere. Similar results were found in a cathodal tDCS, a form of brain stimulation where an area of the brain underneath the electrode is inhibited, study in which researchers targeted the right primary motor cortex as young and older adult participants learned a motor sequence using their right hand (Zimerman, Heise, Gerloff, Cohen, & Hummel, 2014). Inhibiting the right primary motor cortex resulted in a marked decrease in the number of correct sequences produced in older adults, whereas it left young adults unaffected. Further, the researchers found a relationship between age and impact of cathodal tDCS, such that the older

the participant the larger the impairment. The findings from these two studies suggests that both hemispheres are functionally utilized in older adults; thus, targeting either hemisphere with anodal tDCS in our current study, rather than cathodal as in these previous studies, may be a viable strategy for enhancing performance in older adults.

tDCS with Older Adult Populations

The use of tDCS in older adult populations is relatively scarce, but tDCS does show promise for ameliorating age-related cognitive and motor declines both during stimulation (online) and later, without stimulation (offline). An example of online effects comes from Meinzer et al. (2013), who paired tDCS with a semantic word generation task in older and young adults. tDCS to left inferior frontal gyrus not only improved older adult performance in the task to the level of young adults, but also changed their functional brain activity and connectivity to patterns that more closely resembled those of young adults (Meinzer, Lindenberg, Antonenko, Flaisch, & Flöel, 2013). Another illustration of tDCS mitigating cognitive deficits in older adults comes from a within-subjects, multi-session study using an object-location learning task (Flöel et al., 2012). Older adults received tDCS to the right temporoparietal cortex for 20 minutes while learning the location of buildings on a street map. Results showed no differences in the learning rate between the sham and tDCS groups nor any immediate recall (online) effects. However, performance a week after stimulation (offline) showed a large, significant and beneficial difference in the performance of the tDCS group relative to the sham group, suggesting an effect of tDCS on consolidation (Flöel et al., 2012). This finding is consistent with another study that revealed that older adults have a delayed plastic response to tDCS relative to young adults (Fujiyama et al., 2014b).

Motor learning paradigms have also been coupled with tDCS to make older adults more youth-like in their performance. For example, in a five session study during which older adults practiced a serial reaction time task while receiving anodal tDCS to left M1, or sham, researchers showed that only participants that received real stimulation over M1 exhibited sequence specific learning effects, or online gains (Dumel et al., 2016). In another motor learning study conducted with older and young adults, participants received stimulation over M1 while learning a five element sequence (Zimerman et al., 2013). Without stimulation, older adults demonstrated a marked difference in motor performance relative to young adults. However, the older adults who received stimulation during practice showed a significant motor performance improvement. Importantly, the boost gained from tDCS by older adults made it so older adults no longer had a motor performance deficit relative to young adults. Further, both young and older adults exhibited enhanced retention effects 90 minutes and 24 hours later (offline). Thus, tDCS can have both online (Dumel et al., 2016; Meinzer, Lindenberg, Antonenko, Flaisch, & Floel, 2013; Zimerman et al., 2013) and offline effects (Flöel et al., 2012; Zimerman et al., 2013) in older adults. The contradictory findings between Zimerman et al. (both offline and online effects) and Floel et al. (only offline effects) might be due to task-specific effects of tDCS. Marquez and colleagues found that in young adults, keeping the electrode montage constant (over M1) but changing the task between an isometric pinch force task or a finger sequence tapping task, resulted in online but not offline effects for the finger sequence task, and offline but not online gains for the pinch force task (Marquez et al., 2013); the impact of tDCS may be similarly task-dependent in older adults. Overall, these studies suggest that while the specific impact of tDCS may be task dependent, tDCS in older adult populations has potential for reducing cognitive and motor declines.

The Dual Processor Model and the C-SMB Framework in the Current Study

Despite clear neural and motor learning differences between young and older adults, the Dual Processor model and the C-SMB framework do not account for age differences. The Dual Processor model and C-SMB framework emphasize central processor (prefrontal) involvement during sequence learning. Specifically, as the models currently stand, the central processor plays an influential role early in sequence learning but its role tapers off as learning continues. Consistent with these models, previous research implicates the prefrontal cortex in chunking in young adults (Pammi et al., 2012; Wymbs et al., 2012). However, in older adults, the central processor may play a more dominant role throughout sequence learning, continuing to stay online even as learning continues. Consistent with the assertion of greater central processor involvement in older adults, previous research has shown increased bilateral frontal activation in older adults during tasks that typically engage one hemisphere in young adults. In addition, previous studies show promise for tDCS as a means of reducing age-related cognitive and motor declines. Building off these previous findings, in the present study we anticipated that stimulation to prefrontal cortices would be especially helpful to older adults in both reaction times and chunk formation due to the strong possibility that their engagement is compensatory. Thus, differential impact of tDCS to prefrontal cortices for younger v. older adults in our study would provide additional evidence for a need to revise the Dual Processor model and C-SMB framework to account for age-related differences.

Hypotheses

In this study, we implemented the same design and stimulation sites as in the first study in a group of older adults. Based on previous tDCS findings showing enhanced motor sequence learning in older adults after left M1 stimulation (Dumel et al., 2016; Zimerman et al., 2013) as

well as neuroimaging evidence showing the M1 involvement during sequence learning in older adults (Mattay et al., 2002), we hypothesized that stimulation to M1 would facilitate learning across the three days of practice relative to the sham group. We predicted that this would be evidenced by faster reaction times and a steeper, negative slope for number of chunks over time. Based on greater bilateral- and over- recruitment of prefrontal regions in older adults, as well as the positive relationship between working memory capacity and chunk length, we also hypothesized that anodal stimulation to either right or left PFC would facilitate learning in older adults. Further, we hypothesized that stimulation to preSMA would result in overall faster reaction times, consistent with our first experiment as well as previous literature.

Method

Participants

We used the same sixty-five young adult participants (age range 18-30 yr, 27 male; age = 20.5 ± 2.4 (mean ± SD)) as experiment one. Additionally, we recruited sixty-one older adult participants (age range 64-84 yr, 29 male; age = 70.7 ± 5.76 (mean ± SD)) from the University of Michigan campus and greater Ann Arbor area. All participants were right handed, reported no history of mental health events, drug abuse, neurological, or psychiatric disorders. During the first session, all participants signed a consent form approved by the University of Michigan Institutional Review Board, verbally answered an alcohol and drug abuse questionnaire, completed the Beck Depression inventory (Beck 1988), a custom tDCS screening form, and the Montreal Cognitive Assessment (Nasreddine 2005). All Participants scored at least >23 on the MOCA, had no self-reported history of alcohol or drug abuse, and scored <13 on the Beck Depression Inventory. Additionally, all participants were not taking any medications that could interact with the central nervous system.

Results

Cognitive Asessment, Purdue Pegboard, Spatial Working Memory

Age differences were observed across all secondary tasks. Scores on the MOCA, Purdue pegboard, and spatial working memory capacity were all significantly lower compared to young adults (Table 4.1)

Table 4.1. Mean values (with standard deviation) for each task performed for young and older adults. Young adults had significantly slower MOCA scores ($p < 0.001$), placed significantly more pegs with their right hand ($p < 0.001$) and left hand ($p < 0.001$) in the Purdue Pegboard task and had higher spatial working memory capacities in session one ($p < 0.001$) and session two ($p < 0.001$) than older adults.

	MOCA	**Purdue Right**	**Purdue Left**	**VAC S1**	**VAC S3**
Young	**28.42 (1.5)**	**16.11 (1.7)**	**14.84 (1.6)**	**4.7 (1.0)**	**4.8 (1.3)**
Old	**27.16 (1.9)**	**13.00 (1.7)**	**12.37 (1.7)**	**3.0 (1.1)**	**3.3 (.95)**

Reaction time

We ran a linear mixed model which included session, stimulation group, age group, sequence type, and trials. Results of the linear mixed model revealed several significant main effects. There was a main effect of session $F(2, 248) = 613.22$, $p < 0.001$, a main effect of sequence $F(1, 124) = 14.23$, $p < 0.001$, and a main effect of age group $F(1, 124) = 245.51$, $p < 0.001$.

We performed follow-up pairwise contrasts for each significant main effect. These revealed a significantly slower change in the rate of reaction time for the second session compared to the first session ($\beta = .74$, SE = .01, $p < 0.001$). Session three was significantly faster than session two ($\beta = -.60$, SE = .05, $p = 0.002$). The rate of change in reaction time was

significantly faster for the complex sequences than it was for the simple sequences (β = -.10, SE = .03, p = 0.003). We also found older adults changed their reaction times at a significantly faster rate relative to the young adults (β = -.48, SE = .03, p < 0.001).

The model also revealed several significant interactions. There was an interaction between stimulation group and session (F(8, 992) = 8.64, p < 0.001), session and sequence type (F(2, 248) = 3.11, p = 0.04), session and age group (F(2, 248) = 107.7, p < 0.001), and stimulation group by session by age group (F(2, 248) = 107.7, p < 0.001). We followed up the stimulation group, session, and age group interaction by collapsing across sequence type and running the model again for each session including one real stimulation group and the sham group with both age groups. Regardless of the session and combination of real stimulation group and sham (e.g., right PFC and sham tDCS groups in session one), we found no significant stimulation by age group interactions. Thus, we ran a series of pairwise comparisons pooling the data across age groups in order to understand the stimulation by session interaction. Pairwise compairsons revealed the left PFC (β = .20, SE = .03, p < 0.001), right PFC (β = .14, SE = .03, p < 0.001), and the preSMA (β = .25, SE = .03, p < 0.001) tDCS groups reduced reaction times at a significantly slower rate relative to sham during session one (Figure 4.1). In contrast, the M1 tDCS group reduced reaction time across trials at a significantly faster rate relative to sham (β = -.12, SE = .03, p < 0.001) during session one (Figure 4.1). In session two, both the left PFC (β = -.08, SE = .03, p = 0.010) and right PFC (β = -.10, SE = .03, p = 0.001) reduced reaction times at a significantly faster pace relative to sham. Stimulation to preSMA reduced reaction times at a slower pace relative to sham in session two (β = .09, SE = .03, p = 0.002). In session three, the left PFC group reduced reaction time at a significantly faster rate relative to sham (β = -.31, SE =

.15, $p = 0.044$). All other stimulation groups were not significantly different from sham in session three.

Thus, stimulation to either left PFC or right PFC hinders learning in session one, but facilitates learning in session two. Additionally, stimulation to left PFC in session three accelerates learning relative to sham. Stimulation to M1 facilitates learning only in session one, while stimulation to preSMA hinders learning across the first two sessions.

Contrasts on Reaction Time (Collapsed Across Young and Older Adults)

Collapsing across the age groups as there was no stimulation group by age group interaction, The M1 group was significantly faster relative to the sham group in session one ($t(10133) = 1.979$, $p = .048$; Figure 4.2).

The right PFC ($t(10133) = 6.499$, $p < 0.001$) and the left PFC ($t(10133) = 2.362$, $p = 0.018$Figure 4.2) tDCS groups were all significantly slower than the sham group in the first session.

Stimulation to the right PFC ($t(10109) = 5.933$, $p < 0.001$), the left PFC ($t(10109) = 4.332$, $p < 0.001$), and the preSMA ($t(10109) = -1.460$, $p < 0.001$) all resulted in significantly longer reaction times relative to sham in the second session (Figure 4.2).

The right PFC ($t(3389) = 3.280$, $p = 0.001$)and the preSMA ($t(3389) = -3.154$, $p = 0.002$) tDCS groups were all significantly slower than the sham group in the third session..

In summary, the left and right PFC tDCS groups had significantly longer reaction times in session one and two. Additionally, the right PFC group had longer reaction times during session three. The m1 group displayed shorter reaction times limited to session one, whereas the preSMA tDCS group displayed longer reaction times in session two and session three.

C**hunks**

The linear mixed model revealed several significant main effects for the number of chunks. There was a significant main effect of stimulation group $F(4,496) = 10.75$, $p < 0.001$, session $F(2,248) = 220.29$, $p < 0.001$, sequence type $F(1, 124) = 6.87$, $p < 0.001$, and of age $F(1, 124) = 82.15$, $p < 0.001$.

We performed follow-up pairwise contrasts between each real stimulation group and sham and found the M1 ($\beta = .00$, SE = .00, $p = 0.002$) and the left PFC ($\beta = .00$, SE = .00, $p < 0.001$) tDCS groups were significantly slower reducing the number of chunks over trials relative to sham. Follow-up contrasts comparing each session revealed a significantly faster reduction in the change of number of chunks in session two relative to session one ($\beta = -.00$, SE = .00, $p < 0.001$). The change in the number of chunks within session three was significantly faster than the rate of change in the number of chunks in session two ($\beta = -.01$, SE = .00, $p < 0.001$). A contrast between sequence types revealed that the change in the number of chunks for the complex sequences was significantly faster relative to the simple sequences ($\beta = -.00$, SE = .00, $p = 0.005$).

The model also revealed several significant interactions. There was a stimulation group by session interaction ($F(8, 992) = 29.42$, $p < 0.001$), a session by sequence type interaction ($F(2, 248) = 8.34$, $p < 0.001$), a stimulation group by age group interaction, ($F(4, 496) = 3.64$, $p = 0.006$), a session by age group interaction ($F(1, 248) = 143.71$, $p < 0.001$), a stimulation group by session by age group interaction ($F(8, 992) = 8.05$, $p < 0.001$), a stimulation group by sequence type by age group interaction ($F(4, 496) = 3.72$, $p = 0.03$), a stimulation group by session by age group interaction ($F(8, 992) = 2.19$, $p < 0.001$) and a stimulation group by session by sequence type by age group interaction ($F(8, 992) = 7.77$, $p < 0.001$).

In order to understand the stimulation group by session by sequence type by age group interaction we ran a series of linear mixed models. The model included all stimulation groups, both age groups, and all trials, but omitted sequence type and session. Thus, two of the six models ran only included data from session one, two models included data from session two, and two models included data from session three. Further we ran each model pair for each sequence separately (e.g., session one, complex sequence; session one, simple sequence; etc). We found no significant stimulation group by age group interaction for the complex sequence ($F(4, 496) = 1.57$, $p = 0.18$) or the simple sequence ($F(4, 496) = .18$, $p = 0.95$) in session one. In session two there was a significant stimulation group by age group interaction for the complex sequence ($F(4, 496) = 6.14$, $p < 0.001$) as well as for the simple sequence ($F(4, 496) = 4.29$, $p = 0.002$). Contrasts for session three revealed a significant interaction between the stimulation groups and age for the complex sequences ($F(4, 496) = 10.01$, $p < 0.001$) as well as the simple sequences ($F(4, 496) = 5.53$, $p < 0.001$).

We followed-up the significant stimulation group by age group interactions by running another series of linear mixed models. For this analysis, we paired the stimulation groups while also keeping the session and sequence type separated for each model. For example, we maintained age group, stimulation group, and trial in the model, however, stimulation was now limited to only two groups each time the model was run: one real stimulation group and sham. The first model which was run on session two, using only the complex sequences, and including only the right prefrontal and sham tDCS groups revealed a significant stimulation by age group interaction ($F(1, 49) = 10.85$, $p = 0.001$). While the right PFC group for young adults begins session two with the highest number of chunks, the right PFC group rapidly decreases the number of chunks and by the middle of session two (trial 289), the right PFC and sham groups

are similar (Figure 4.2, top panel). However, the older adult sham tDCS group maintains an advantage over the right PFC throughout session two (Figure 4.2, bottom panel). A significant stimulation group by age interaction was also found for the left PFC and sham group $F(1, 48) = 18.26$, $p < 0.001$ and the M1 and sham group $F(1, 48) = 6.41$, $p = 0.01$. Looking at Figure 4.4, top panel, the left PFC and sham groups begin session two apart, then gradually come together as the session progresses for young adults. The older adult data show the left PFC and sham group begin the session together then as the session progresses, they separate, showing an advantage for the sham group (Figure 4.2, bottom panel). The M1 tDCS young adult group is similar to sham throughout session two (Figure 4.4, top panel), however, the older adult M1 tDCS group shows a clear advantage during most of session two (Figure 4.2, bottom panel). We found no significant stimulation group by age group interaction with the preSMA and sham data, however. Using the data from the second session and the simple sequences we found no significant interaction for stimulation group and age for right PFC and sham, left PFC and sham, M1 and sham, as well as preSMA and sham.

Several significant stimulation group by age group interactions were found using data in the third session with both complex and simple sequences. The model revealed a significant interaction between stimulation group and age group for the right PFC and sham $F(1, 49) = 21.70$, $p < 0.001$, left PFC and sham $F(1, 49) = 18.42$, $p < 0.001$, M1 and sham $F(1, 48) = 8.01$, $p = 0.005$, and preSMA and sham $F(1, 49) = 25.18$, $p < 0.001$ when including only complex sequences. On average, the young adults had fewer chunks than older adults in the second and thierd sessions across all tDCS groups. For older adults individualds in the M1 group reduced the number of chunks at a significantly faster rate relative to sham ($\beta = -.00$, SE = .00, $p = 0.022$), whereas the left PFC group reduced the number of chunks at a significantly slower rate relative

to sham (β = .00, SE = .00, $p < 0.001$). This is in contrast to the young adults for whom stimulation to M1 did not result in any significant differences in the rate of change in the number of chunks relative to sham and stimulation to left PFC resulted in a significantly faster rate of change relative to sham during session two (results previously reported in Chapter 2).

In the third session both the right (β = .01, SE = .00, $p = 0.004$) and the left (β = .01, SE = .00, $p = 0.006$) tDCS, older adult groups reduced the number of chunks at a significantly slower rate relative to sham. The M1 tDCS group reduced the number of chunks at a significantly faster rate relative to sham (β = -.01, SE = .00, $p = 0.003$). This is in contrast to the young adult groups from whom stimulation to M1 or left PFC did not result in any significiant differences in the rate of change in the number of chunks. Further, the right PFC stimulation, young adults group had a faster rate of change in the number of chunks relative to sham (results previously reported in Chapter 2).

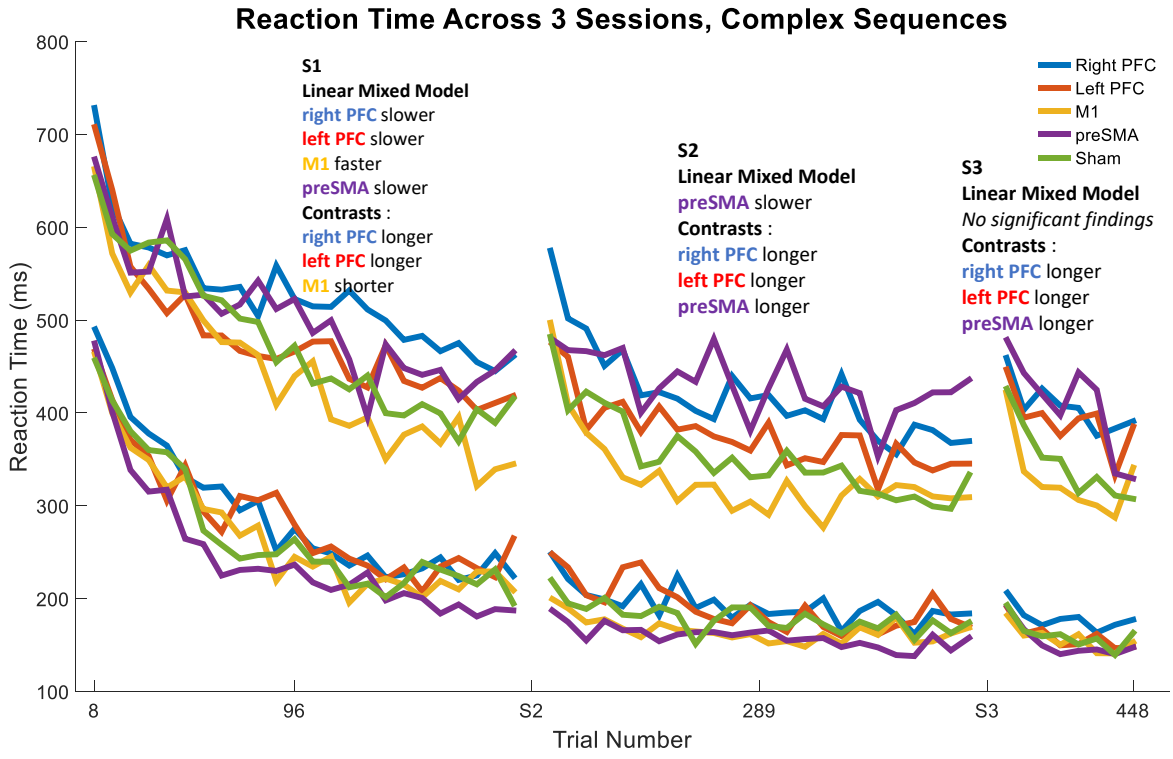

Figure 4.1. Mean reaction time as a function of trial for the complex sequences trials for young and older adults. Blue lines denote right PFC, orange lines denote left PFC, yellow lines denote M1, purple lines denote preSMA, and green lines denote sham tDCS groups. S2 and S3 labels on the x-axis represent the start of sessions two and three, respectively.

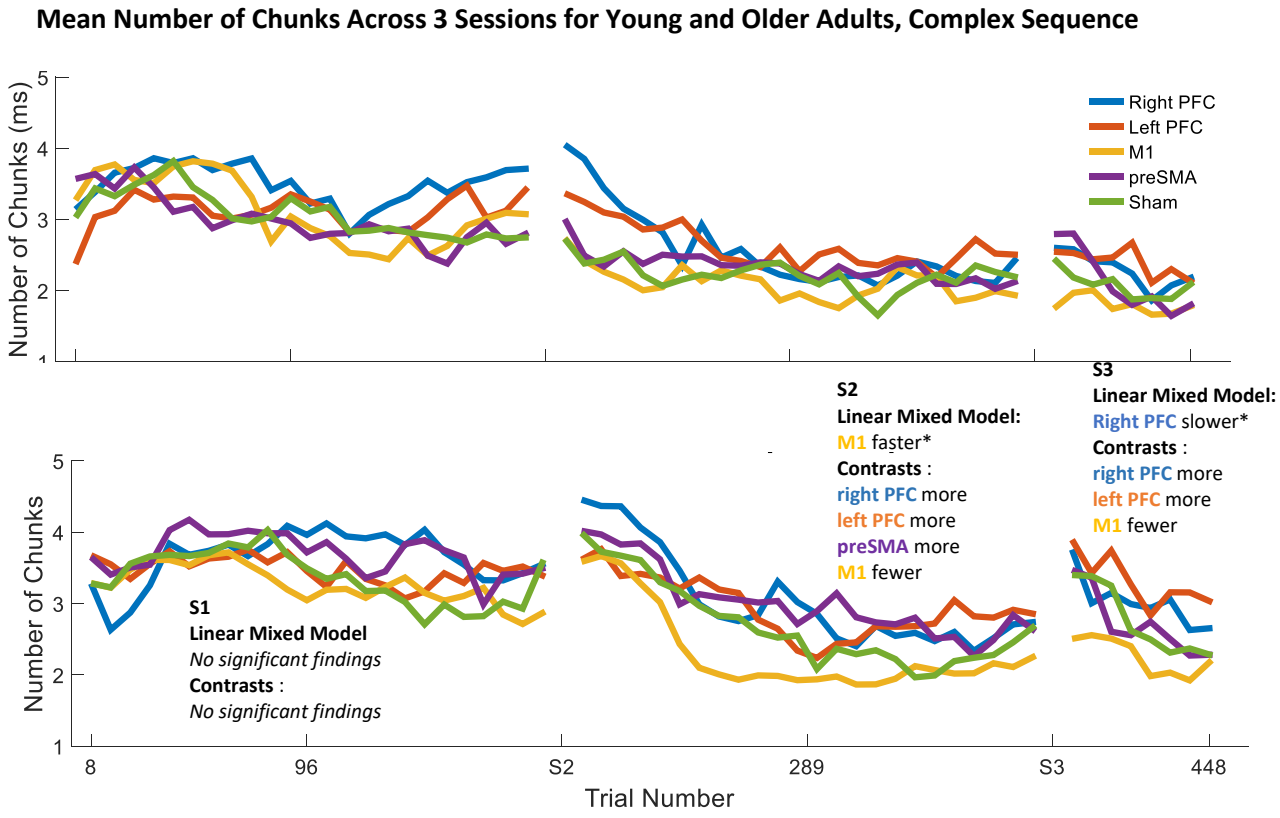

Figure 4.2. Mean number of chunks as a function of trial for the simple sequences trials for young (top panel) and older adults (bottom panel). Blue lines denote right PFC, orange lines denote left PFC, yellow lines denote M1, purple lines denote preSMA, and green lines denote sham tDCS groups. S2 and S3 labels on x-axis represent the start of session two and session three, respectively.

Contrasts on Chunks for Older Adults

Planned contrasts between each real stimulation group and sham for older adults revealed stimulation to right PFC ($t(4400) = -5.319$, $p < 0.001$), left PFC ($t(4400) = -2.836$, $p = .005$), and preSMA ($t(4400) = -2.007$, $p = .045$) resulted in significantly more chunks relative to sham, whereas Stimulation to M1 resulted in significantly fewer chunks relative to sham in the complex sequences in session one ($t(4400) = -2.836$, $p = .005$).

In the second session, the right PFC ($t(4585) = -5.980$, $p < 0.001$), left PFC ($t(4585) = -4.409$, $p < 0.001$), and preSMA ($t(4585) = -5.576$, $p < 0.001$) tDCS groups all had significantly more chunks relative to the sham group, whereas the M1 tDCS group had significantly fewer chunks relative to the sham group ($t(4585) = -6.926$, $p < 0.001$).

In the third session, contrasts revealed the right PFC ($t(1501) = -2.090$, $p = .037$) and the left PFC ($t(1501) = -4.309$, $p < 0.001$) tDCS groups had significantly more chunks compared to the sham group in the third session for the complex sequences. The M1 group had significantly fewer chunks relative to the sham group in the third session for the complex sequences ($t(1501) = -5.384$, $p < 0.001$).

In summary, the right PFC, the left PFC, and the preSMA tDCS groups all had significantly more chunks, whereas the M1 tDCS group had fewer chunks relative to sham during session one and session two. For session three, the right PFC and left PFC had more chunks, whereas the M1 tDCS group had fewer chunks relative to sham.

Discussion

Contrary to our hypothesis, the Dual Processor model and the C-SMB framework, stimulation to prefrontal cortices impaired sequence learning both in reaction time and in number of chunks. We found no age group by stimulation group interaction for the reaction time data, suggesting that stimulation to either the left or right PFC impaired sequence learning to the same extent regardless of age. In contrast, there was an age by stimulation group interaction for the chunking data; however follow-up contrasts revealed similar impairments to chunking regardless of age for the left and right prefrontal tDCS groups. The lack of a chunking benefit in the left and right PFC tDCS groups in older adults is surprising considering the neuroimaging literature, which demonstrates more bilateral activation in the prefrontal cortices in older adults relative to

young adults across many cognitive and motor tasks (Cabeza, 2002). Anodal stimulation to either the left or right PFC should facilitate learning, as bilateral hemispheric activation typically observed in older adults is thought to reflect compensation. To test this hypothesis, Zimerman and colleagues used cathodal stimulation over the ipsilateral motor cortex in both young and older adults as they trained in a complex motor skill. The logic behind targeting the ipsilateral motor cortex with cathodal tDCS is if the ipsilateral motor cortex is engaged in learning a complex task and its engagement is compensatory, then inhibiting that brain region should negatively impact learning. Indeed, cathodal stimulation harmed performance for the older adults, but did not affect performance for the young adults, suggesting that the ipsilateral primary motor cortex is engaged in a compensatory fashion for older adults (Zimerman et al., 2013). Another line of evidence suggesting that anodal tDCS to prefrontal cortices should facilitate chunking in older adults comes from a behavioral study conducted by Bo et al. (2009), which demonstrated older adults have smaller working memory capacity and diminished chunk length relative to young adults (Bo et al., 2009a). In the same study, Bo and colleagues found a positive relationship between working memory capacity and chunk length in older adults, suggesting that stimulating the prefrontal cortices may facilitate chunking through working memory. Thus, we anticipated that stimulation to either the left or right PFC would be helpful to older adults more so than young adults given that older adults typically engage both hemispheres and demonstrate compromised chunk lengths and working memory capacities.

The lack of a beneficial effect for the prefrontal tDCS groups in older adults may be due to the delayed plasticity effects observed in tDCS studies. For example, Fujiyama and colleagues measured corticospinal excitability in ten minute increments after young and older adults received tDCS over M1 for thirty minutes. While the increases in corticospinal excitability were

no different between the age groups, the older adults showed a delayed response, with the largest increase in corticospinal excitability occurring 30 minutes after stimulation. This finding is in contrast to the young adults who showed the largest increase in corticospinal excitability immediately following stimulation (Fujiyama et al., 2014). This delay in plasticity in older adults has also been supported by a recent tDCS motor sequence study in older adults. In this study, older adult participants received tDCS to M1 either immediately, an hour, or two hours following training on a motor sequence task. Only the older adult participants that received stimulation immediately following the task showed enhanced consolidation during a retest 24 hours later (Rumpf et al., 2017). The findings of Rumpf and colleagues are counterintuitive as Stagg et al. (2011) have demonstrated that the ideal tDCS protocol to enhance learning in young adults is to pair stimulation during the task (Stagg et al., 2011). Thus, the lack of prefrontal tDCS facilitating learning in older adults may be due to two possibilities. First, it may be that older adults would have exhibited a motor sequence learning benefit had we measured performance thirty minutes after the initial stimulation. Second, it may be that stimulating the prefrontal cortices during sequence learning was not an ideal protocol for older adults and instead should have been paired immediately after learning. Future studies should consider administering stimulation *after* sequence learning.

Regardless of age, stimulating the left or right PFC interfered with learning and chunking. Similar to the findings in experiment one, in which we used the same study design but with young adults, we found stimulation to prefrontal cortices in older adults impaired learning. These results are in line with previous non-invasive brain stimulation studies that demonstrate inhibiting the prefrontal cortices facilitates sequence learning (Galea et al., 2010; Zhu et al., 2015a). The findings from these studies suggest inhibiting the declarative memory system

promotes automatization of sequence learning. The concept of inhibiting the prefrontal cortices is somewhat in line with the Dual Processor model and the C-SMB framework, which posit after enough practice trials to elicit a chunk formation, the cognitive processor is less active but now its role is to load in motor chunks to be executed by the motor processor. Thus, over many trials, the involvement of the cognitive processor is significantly reduced (Hommel, 2000). As discussed in the first study, it may be that tDCS does not have the temporal specificity needed to enhance the central processor. Thus, the use of tDCS before, during, and after sequence initiation may be the cause impairment; such that the constant stimulation of the anodal electrode over the prefrontal cortex may cause the central processor to stay online when- according to the Dual Processor model and C-SMB framework- it is not needed and may instead impair performance. Future studies should consider using more temporally precise non-invasive brain stimulation techniques such as TMS or alternating current to specifically enhance the central processor (prefrontal cortex) during sequence initation.Consistent with our hypothesis, we found differential age group effects in the second and third sessions limited to the chunking data. Specifically, we found stimulation to M1 resulted in a fewer number of chunks in sessions two and three for older adults. Our findings are similar to the results of experiment one where young adults who had received stimulation to M1 had a reduced number of chunks. The Dual Processor model and the C-SMB framework limit the role of the motor processor (M1) to execution and overall, do not differentiate between age groups (young v. old). However, the findings of the current study suggest the Dual Processor model and the C-SMB framework could be revised to consider a role of the motor processor in chunking as well as examining age differences. The role of M1 in chunking is not well understood. There is evidence in one animal model study showing a potential role of the rodent secondary motor cortex in action sequence chunking (Ostlund et al.,

2009). However, the few neuroimaging studies that have investigated chunking processes in young healthy adults have not found a role of the primary motor cortex (Pammi et al., 2012; Wymbs 2012). Thus, our finding which implicate the role of M1in chunking in both young and older adults is novel.

Consistent with our hypothesis and similar to experiment one, we found stimulation to M1 resulted in a faster rate in the reduction of reaction time and overall shorter reaction times in session one. This finding is somewhat consistent with the Dual Processor model and the C-SMB framework. The framework posits that M1 is involved in sequence execution, but it is surprising that stimulation to M1 did not result in a faster rate of reduction of reaction time or overall shorter reaction times across all sessions. The results of the current study are consistent with previous literature showing that M1 plays a major role in learning complex sequences. For example, rodents are unable to learn complex sequences after M1 lesions (Kawai et al., 2015). Further evidence showing a causal relationship between M1 and sequence learning in older adults comes from two tDCS studies. Stimulating M1 while older adults practiced a motor sequence over the course of five days resulted in greater sequence specific learning effects relative to sham (Dumel et al., 2016). In another tDCS study, stimulation over M1 resulted in significant learning benefits that remained stable 24 hours later (Zimerman et al., 2013). Thus, M1 plays a central role in sequence learning in both young and older adults. However, based on our findings, the Dual Processor model and the C-SMB framework could be revised. The motor processor (M1) might have a similar time course to that of the central processor (prefrontal cortices), potentially switching roles as learning occurs, especially for older adults. For example, our data suggests M1 is needed throughout learning, initially involved in only execution, then as learning progresses, M1 is involved in the hierarchical organization of sequence as well as their

execution. This is compatible with our findings as stimulation to M1 facilitated reaction time limited to session one and lowered the number of chunks during session two and three. In contrast to our hypothesis, the Dual Processor model, and the C-SMB framework, we did not observe a single instance where stimulation to the preSMA facilitated learning. When we collapsed across age groups for the reaction time data, we observed slower reaction times and a higher number of chunks for the preSMA group relative to sham. The lack of faster reaction times and fewer number of chunks observed in the preSMA tDCS older adult group are surprising given the previous literature showing a link between chunking and the preSMA as well as literature demonstrating limited chunking abilities in older adults. In two separate studies, Kennerley et al (2004) and Ruitenberg et al (2014) demonstrated a causal relationship between preSMA and chunk loading, observing inflated reaction times at chunk points when a virtual lesion was created over preSMA in young adults (Kennerley, 2003; Ruitenberg et al., 2014). Multiple studies have also demonstrated that older adults also show limited chunking abilities as well as shortened chunk lengths (Bo et al., 2009a; Verwey, 2010), suggesting that older adults should benefit more from stimulation to preSMA. The Dual Processor model and the C-SMB framework should be revised to consider the role of the preSMA in chunking, especially for older adults, as our data do not provide support of preSMA in chunk loading. Given our results, the Dual Processor model and the C-SMB framework should put less emphasis on the preSMA and more emphasis on the motor processor (M1) for chunking processes in older adults.

In conclusion, age related differences were limited to the number of chunks and not reaction time. Unexpectedly, we found stimulation to M1 resulted in a faster rate of change in the number of chunks, especially for the older adults. Consistent with our findings in experiment one, these results implicate a role of M1 in chunking. In contrast to our hypothesis and the Dual

Processor model and the C-SMB framework, stimulation to the prefrontal cortices impaired learning for both young and older adults.

CHAPTER V: Conclusion

The first study examined how non-invasive brain stimulation affects both learning and chunk formation in the discrete sequence production task, an explicit serial reaction time task, in young adults across multiple days. Our results provide support for, yet require changes to, the Dual Processor model and the C-SMB framework, theoretical frameworks of sequence learning. We found tDCS over left and right PFC impeded learning and chunking throughout the sessions. Thus, our results revealed that regardless of the hemisphere of prefrontal stimulation, tDCS resulted in longer reaction times and a higher number of chunks. These results are not in line with what we anticipated and do not support the Dual Processor model or the C-SMB framework. Instead our results support the notion that engagement of the prefrontal cortices may interfere with learning and in the context of the DSP task, the prefrontal cortices should not be stimulated via anodal tDCS. Thus, we propose that the Dual Processor model and the C-SMB framework could be revised to consider previous non-invasive brain stimulation studies as well as our own, and that in some circumstances, prefrontal engagement can impair motor sequence learning.

In contrast with our hypothesis and the predictions of the Dual Processor model and the C-SMB framework, we found that stimulation to preSMA showed a tradeoff as evidenced by shorter reaction times, but a greater number of chunks limited to session two. Our results suggest that the preSMA plays a robust role in both sequence learning and chunk formation. This finding expands the role of the preSMA beyond the findings of previous literature demonstrating

preSMA involvement in chunk loading. Our findings that show preSMA stimulation can negatively affect the number of chunks is novel and conflict with previous research as well as the C-SMB framework.

Unexpectedly, our third major finding was that stimulation to M1 facilitated both learning and chunking, shortening reaction times and reducing the number of chunks, but did not affect consolidation. Both the Dual Processor model and the C-SMB framework limit the role of M1 to execution, however, our findings suggest the frameworks could be revised to consider the role of M1 in chunking in addition.

These results from study one elucidate the involvement of different brain regions during the motor learning and chunking process. They provide further evidence that cognitive processes may interfere with sequence learning and provide support for the role of M1 in the chunking process. The results from experiment one can help guide future tDCS studies. For example, our results can help answer questions such as when in learning should tDCS be applied and to what brain regions to maximize learning gains.

Based on our findings in experiment one, we performed a small follow-up experiment, which investigated the polarity specific effect tDCS has on learning, comparing anodal left PFC stimulation to cathodal left PFC stimulation during motor learning. We anticipated that cathodal stimulation would show the opposite pattern of results of anodal stimulation and facilitate sequence learning. However, we found mostly overlapping results between left anodal and left cathodal stimulation, with some differences between the two groups. The left anodal stimulation group learned at a faster rate and formed chunks faster relative to the cathodal group, but follow-up contrasts revealed overall slower reaction times and more chunks for the anodal group. Although the behavioral results were similar, it is likely that the anodal and cathodal stimulation

groups affected different brain regions. Further, these results challenge the canonical belief that "anodal excites, cathodal inhibits" and demonstrates this canon is an oversimplification. Future tDCS studies should include both polarities in the study design as well as neuroimaging techniques in order to understand the neural underpinnings behind stimulation polarities.

The second experiment brought back participants from experiment one over a year later to understand the long-term effects of tDCS on motor learning. Participants who had received stimulation over M1 for two sessions, demonstrated enhanced relearning of the same sequences when assessed a year later. Individuals who had initially received left PFC stimulation forgot less when compared to sham, although overall their reaction time was slower. Further, the left PFC group produced faster sequences without the help of a visual stimulus. The Dual Processor model and the C-SMB framework posit the prefrontal cortices are involved in long-term memory and our data support this. Our results show the feasibility of long lasting tDCS-linked effects on motor learning and future studies should adopt longitudinal designs.

Finally, our third experiment compared the effects of tDCS on motor learning and chunk formation in young and older adults. We found differential effects between the two age groups, limited to the rate of chunking, but not in reaction time, within session two and session three. Stimulation to M1 lowered reaction times and reduced chunks for both age groups, but to a larger extent for older adults. This is a novel finding and was unexpected given the predictions of the Dual Processor model and the C-SMB framework, which limit the role of M1 to execution. Our results paired with previous neuroimaging findings, show clear age-related brain differences between young and older adults. The Dual Processor model and the C-SMB framework should be revised to consider age-related differences in motor learning in general as well as the role M1

plays in chunking. Future tDCS studies should consider these age-related differences when using tDCS protocols to enhance learning.

Appendix A: Explicit Knowledge

In order to determine whether explicit knowledge of the sequences differed between stimulation groups for young adults we ran a nonparametric test on the first question of the DSP questionnaire in session two. The first question asked participants to produce the two sequences they had practiced over the last twelve blocks. Responses were coded as 1, correct or 0, incorrect for each sequence. Responses were then averaged between the two sequences, thus each participant could have a score between 0 and 1. The Kruskal-Wallis nonparametric t-test indicated that stimulation had no effect on explicit knowledge ($p = .54$).

Appendix B: T-Test Chunk Analysis

We ran an additional analysis using the traditional t-test method on the reaction times to determine whether the number of chunks within a sequence is markedly different from using a computational model. The alpha for the t-test method was set at .2, similar to previous studies (J Bo & Seidler, 2009). Reaction times were first averaged across key presses then across blocks for each participant for each session. We then ran the same linear mixed model with session, stimulation group, sequence type, and block in the full model.

The change in the number of chunks were significantly slower for the complex sequences relative to the simple sequences ($\beta = .01$, SE = .00, $p = 0.001$). Specifically, the complex sequences reduced the number of chunks at a slower rate relative to the simple sequences ($\beta = .02$, SE = .00, $p < 0.001$) in session two.

There were several significant session by stimulation group by sequence type interactions in the second session. Stimulation to right PFC ($\beta = .03$, SE = .01, $p = 0.001$) and left PFC ($\beta = .02$, SE = .01, $p = 0.03$) lead to a significantly slower rate of change in the number of chunks relative to sham for the simple sequences (Figure B.1). In contrast, stimulation to right PFC ($\beta = .03$, SE = .01, $p = 0.01$) and preSMA ($\beta = .02$, SE = .01, $p = 0.02$) lead a faster rate in change in the number of chunks relative to sham in the second session for the complex sequences (Figure B.2).

We found a significant session by stimulation group by sequence type in the third session. Stimulation to M1 lead to a significantly faster rate of change in the number of chunks

relative to the sham group (β = -.03, SE = .012, p = 0.008) for the complex sequences (Figure B.3).

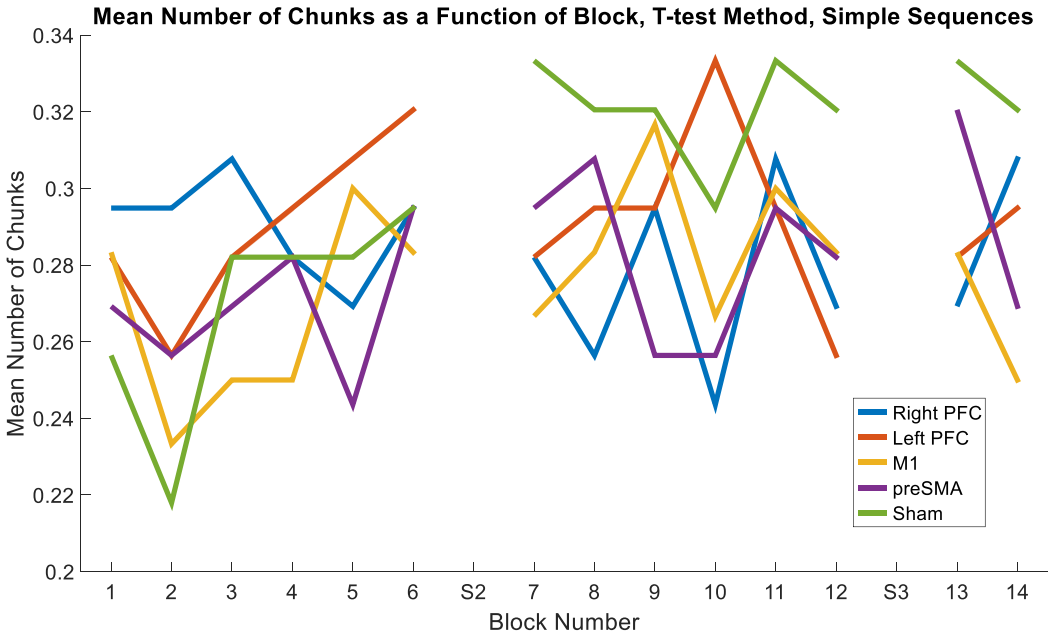

Figure B.1. Mean number of chunks as a function of block number for simple sequences across three sessions. Blue lines denotes right PFC, orange lines denotes left PFC, yellow lines denotes M1, purple lines denotes preSMA, and green lines denotes sham tDCS groups.

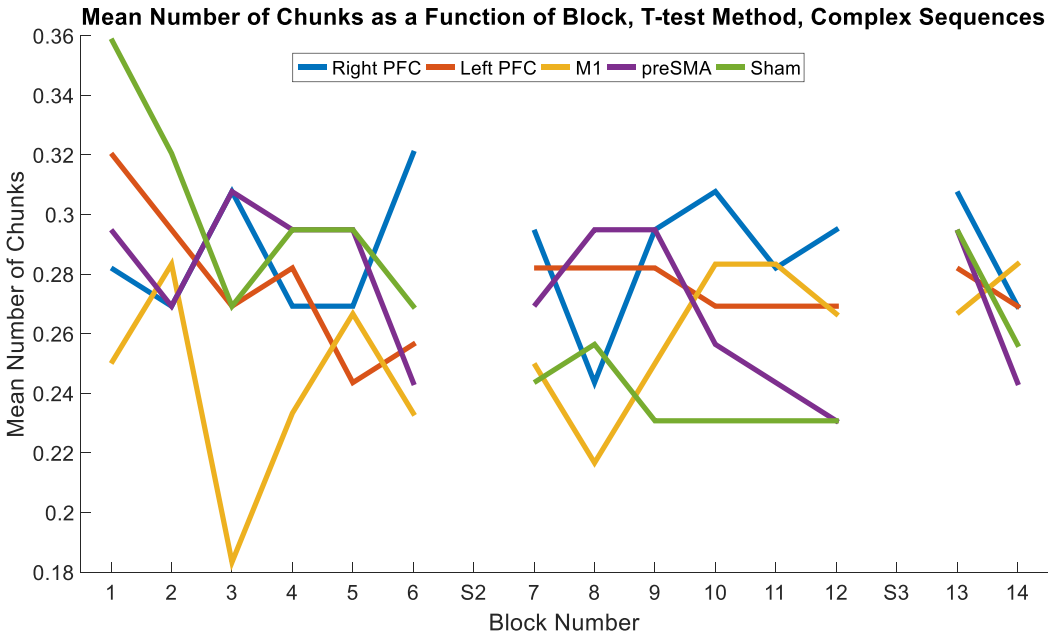

Figure B.2. Mean number of chunks as a function of block number for simple sequences across three sessions. Blue lines denotes right PFC, orange lines denotes left PFC, yellow lines denotes M1, purple lines denotes preSMA, and green lines denotes sham tDCS groups

Discussion

Traditional t-test analysis on the number of chunks revealed stimulation to right PFC and left PFC hindered the rate of change in the number of chunks in session one for the simple sequences, but stimulation to either the right PFC or preSMA group facilitated chunking in session one for the complex sequences. This is somewhat consistent with the findings of the Acuna results. When using the computational model, we found that stimulation to the right PFC harmed chunk formation during session one for the simple sequences and stimulation to preSMA helped chunk formation during session one for the complex sequences. Therefore, across both methods of analysis the only consistent finding was limited to the right PFC and preSMA group in the first session. There are many more differences between the two methods, however. When using the computational method we found the preSMA and right PFC group chunked at a faster rate during session three for the complex sequences. We found no such benefit in the t-test method. Additionally, we found that either stimulation to the right PFC or M1 benefited chunking in session two for the simple sequences and the right and left PFC group received a benefit in session two for the complex sequences. In the t-test method we found no significantly findings in session two regardless of the sequence. Thus, although we found some slight overlap between the two methods, there were more differences.

Appendix C: Simple Sequence Analysis

Reaction Time, Simple Sequences

The session by tDCS stimulation group by sequence type across all trials linear mixed model revealed that individuals who received stimulation to M1 during the first session demonstrated a significantly faster reduction in reaction time relative to the sham group (β = -.19, SE = .05, $p < 0.012$; Figure B.1). There was a trend demonstrating a slower rate for reaction time decrease for individuals in the left PFC group within the first session relative to sham (β = .09, SE = .05, $p < 0.066$). In the second session, individuals in the left PFC group decreased reaction time faster than those in the sham group (β = .14 SE = .048, $p < 0.003$; Figure B.1). The remaining contrasts for reaction time during simple sequence practice were not statistically significant.

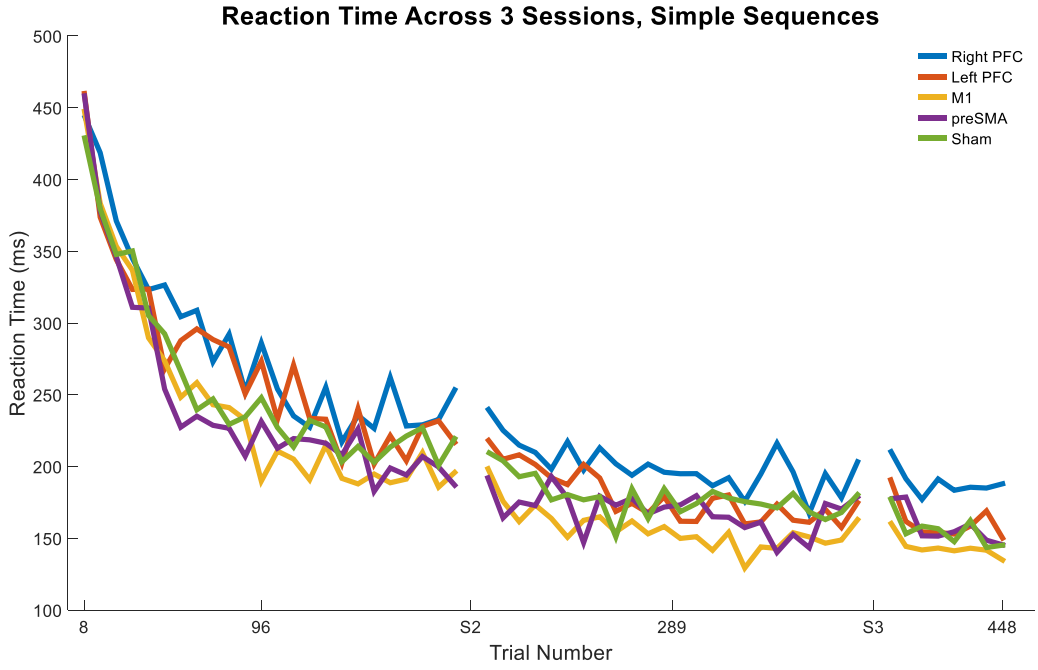

Figure C.1. Mean reaction time (ms) as a function of trial for simple sequences. Displayed means were binned across every 8 trials. Blue line denotes right PFC tDCS group, red line denotes left PFC tDCS group, yellow line denotes M1 tDCS group, purple line denotes preSMA tDCS group, green line denotes sham tDCS group. S2 and S3 on x-axis represent the start of session two and three, respectively.

Contrasts on Reaction Time, Simple Sequences

Hypotheses driven contrasts revealed the right PFC had significantly longer reaction times ($t(5157) = 4.919$, $p < 0.001$), whereas the M1 group had significantly shorter reaction times than sham ($t(5157) = -2.112$, $p = .035$) in the first session. The right PFC group had significantly longer reaction times $t(5054) = 5.817$, $p < 0.001$), however the M1 ($t(5054) = -5626$, $p < .001$) as well as the preSMA ($t(5054) = -2.728$, $p = .006$) groups had significantly shorter reaction times than sham in the second session. For the third session, the right PFC tDCS group had significantly longer reaction times ($t(1689) = 6.425$, $p <.001$) whereas the M1 tDCS group had significantly shorter reaction times relative to the sham group ($t(1689) = -2.590$, $p = .010$) for the simple sequences

Chunks, Simple Sequences

Across trials within the first session, stimulation to right PFC ($\beta = .00$, SE = .00, $p < 0.001$), left PFC ($\beta = .00$, SE = .00, $p < 0.001$), and M1 ($\beta = .00$, SE = .00, $p < 0.001$) resulted in a slower reduction of the number of chunks relative to sham (Figure C.2). There was a trend for individuals in the preSMA group to reduce the number of chunks at a faster rate within the first session relative to sham ($\beta = -.00$, SE = .00, $p = 0.065$). In session two, stimulation to right PFC ($\beta = -.003$, SE = .00, $p < 0.001$) and M1 ($\beta = -.005$, SE = .00, $p < 0.001$) resulted in a significantly faster rate in the reduction of the number of chunks relative to sham (Figure C.2). During the third session of practice, participants that had received stimulation to preSMA

reduced the number of chunks at a faster rate relative to sham (β = -.014, SE = .004, p = 0.001) (Figure C.2).

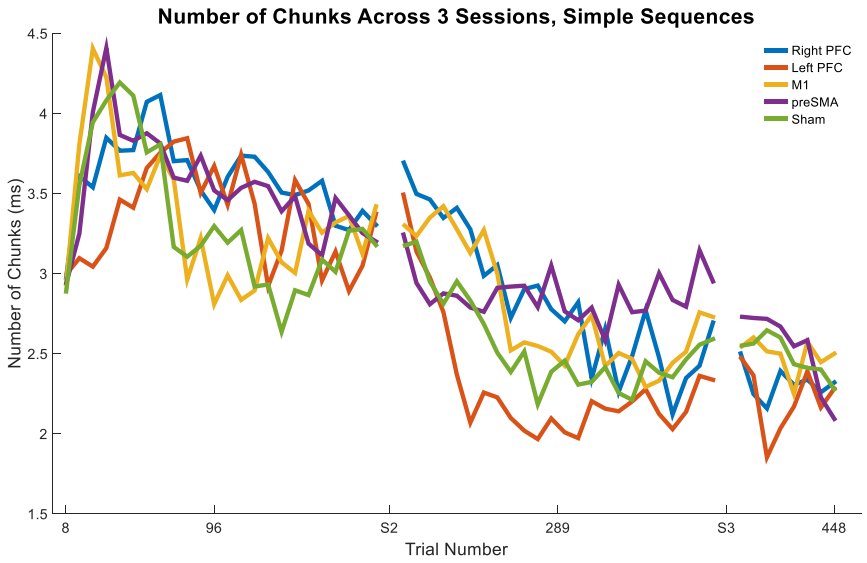

Figure C.2. Mean number of chunks as a function of trial for simple sequences. Displayed means were binned across every 8 trials. Blue line denotes right PFC tDCS group, red line denotes left PFC tDCS group, yellow line denotes M1 tDCS group, purple line denotes preSMA tDCS group, green line denotes sham tDCS group. S2 and S3 on x-axis represent the start of session two and three, respectively.

Contrasts on Chunks, Simple Sequences

The right PFC had significantly more chunks relative to sham (t(4656) = -5.095, p < 0.001). whereas the preSMA group had significantly fewer chunks (t(4656) = -3.731, p < 0.001) relative to sham in session one. Right PFC (t(4616) = -5.933, p < 0.001), M1 (t(4616) = 4.489, p < 0.001), and preSMA (t(4616) = -6.431, p < 0.001) tDCS groups had significantly more chunks relative to the sham in session two, whereas the left PFC group had significantly fewer chunks relative to sham (t(4616) = 5.937, p < 0.001) in session two. Both the right PFC (t(1588) = 2.092, p = .037) and left PFC (t(1588) = 3.185, p = .001) tDCS groups had significantly fewer chunks relative to sham in session three.

In summary, stimulation to M1 resulted in a faster reduction in reaction time limited to

session one and overall shorter reaction times in all three sessions. However, the M1 group exhibited a slower rate of change in the number of chunks in session one and overall more chunks in session two. Stimulation to left PFC resulted in a faster rate in the reduction of reaction times limited to session two. For the chunking data, stimulation to left resulted in a slower rate of change in session one, but overall fewer chunks in session two and three. Stimulation to right PFC resulted in longer reaction times in all three sessions, a slower rate of change in the number of chunks and overall a greater number of chunks in session one, and a faster rate of change in the number of chunks in session two and overall fewer number of chunks in session two and three. preSMA stimulation lead to shorter reaction times limited to session two and a faster rate of change in the number of chunks in session three, with overall fewer number of chunks in session one.

Reaction time, Cathodal Follow-up

Hypothesis driven pairwise comparisons for the first session revealed that stimulation to anodal left PFC produced a significantly slower rate of change in reaction time practicing the simple sequences ($\beta = .14$, SE = .05, $p = 0.004$; Figure C.3) Cathodal stimulation to the left PFC produced significantly slower rates in the change of reaction time ($\beta = .12$, SE = .05, $p = 0.012$; Figure C.3) relative to sham. Anodal stimulation to left PFC during session two affected the rate of change in reaction time such that it was significantly faster relative to sham ($\beta = -.17$, .05, $p < 0.001$). Similarly, cathodal stimulation to left PFC produced significantly faster changes in reaction time relative to sham ($\beta = -.12$, .05, $p = 0.015$; Figure C.3).

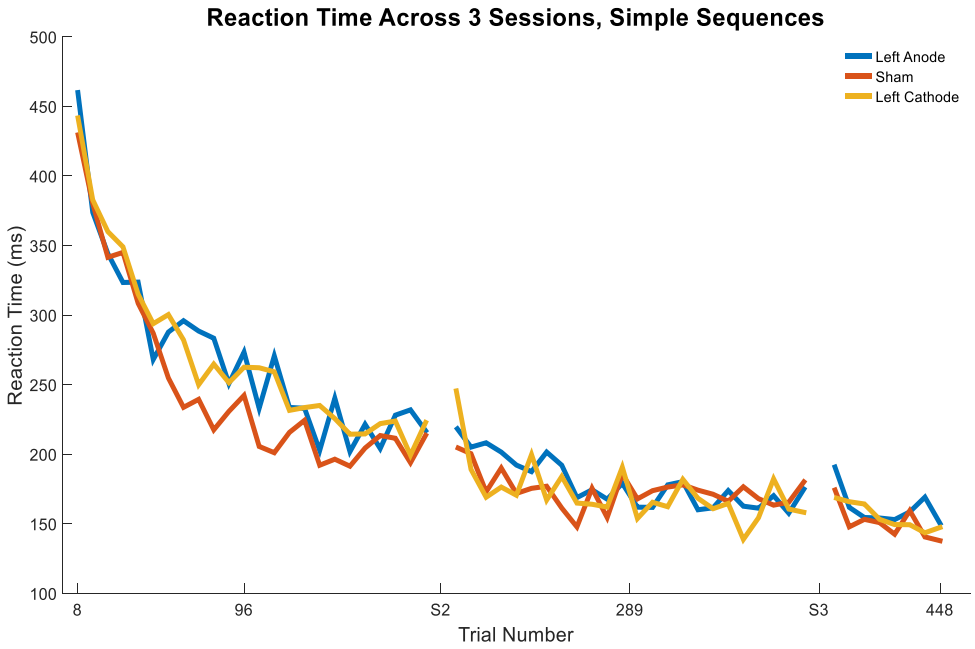

Figure C.3. Mean of reaction time (ms) as a function of trial for simple sequences trials. Displayed means were binned every 8 trials. Blue lines denote means for the left PFC anodal group, orange lines denote means for the sham group, and the yellow lines denote means for the left cathodal tDCS group. S2 and S3 denotes the start of session two and three.

Contrasts for Reaction Time, Cathodal Follow-Up

Both the left PFC anode ($t(3034) = 3.382$, $p = .001$) and the left PFC cathode ($t(3034) = -3.956$, $p < 0.001$) tDCS groups were significantly slower compared to the sham group for the simple sequences in session one. No statistical differences were found between the real left PFC tDCS groups and sham in session two or three.

Chunks, Cathodal Follow-up

Within session one, anodal stimulation to left PFC significantly slowed the rate of chunking relative to sham ($\beta = .00$, SE = .00, $p < 0.001$; Figure C.4). Similarly, cathodal stimulation to left PFC also significantly slowed the rate of change in the number of chunks ($\beta = .01$, SE = .00, $p < 0.001$). Pairwise comparisons between the two real stimulation groups revealed that the left PFC cathodal group significantly reduced the number of chunks at a slower

rate relative to left PFC anodal group (β = .00, SE = .00, p < 0.001). The left PFC cathodal group reduced the number of chunks in the simple sequences at a significantly faster rate relative to sham (β = -.00, SE = .00, p = 0.044), but slowed the rate of change in chunking (β = .00, SE = .00, p = 0.08) relative to the left PFC anode group for the simple sequences in session two (Figure C.4). In the third session, cathodal stimulation to left PFC resulted in a significantly faster rate of change in the number of chunks for the simple sequences relative to sham (β = -.01, SE = .00, p = 0.016; Figure C.4). Additionally, cathodal stimulation to left PFC resulted in a significantly faster rate of change in the number of chunks relative to the anodal left PFC group for simple sequences (β = -.01, SE = .00, p = 0.010) during session three (Figure C.4).

Contrasts for Chunks, Cathodal Follow-Up

In the second session, the left PFC anodal group had significantly fewer chunks compared to the sham ($t(2662) = -3.350$, $p < 0.001$) and the left PFC cathodal ($t(2662) = -6.534$, $p < 0.001$) tDCS groups. The left PFC cathodal group had significantly fewer chunks compared to sham ($t(911) = 8.953$, $p < 0.004$) as well as the left PFC anodal tDCS group ($t(911) = 5.982$, $p < 0.001$) in the third session.

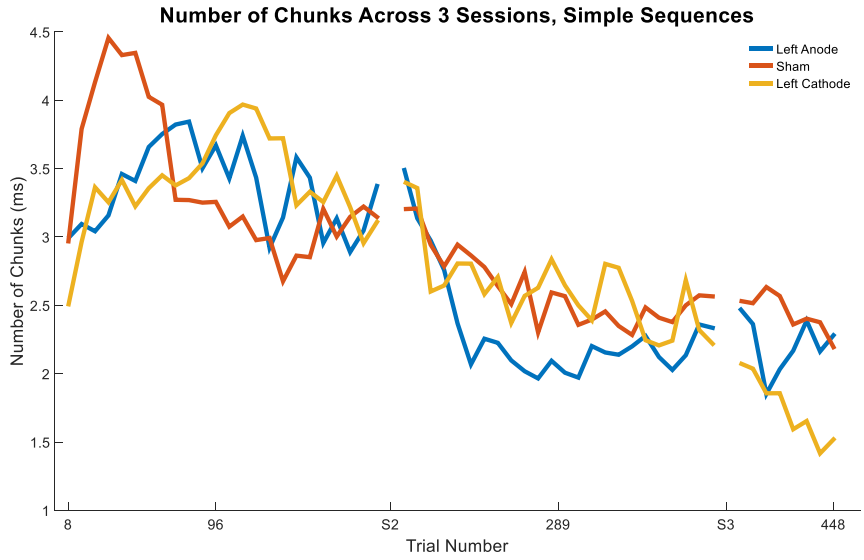

Figure C.4. Mean number of chunks across as a function of trial for simple sequences trials. Blue lines represent means for the left PFC anodal group, orange lines represent means for the sham group, the yellow lines represent mean for the left cathodal tDCS group. S2 and S3 denotes the start of session two and session three.

In summary, regardless of the polarity of stimulation, tDCS over left PFC resulted in a slower rate of change in the reaction time and the number of chunks during as well as significantly longer reaction times in session one. Cathodal or anodal stimulation to left PFC resulted in a significantly faster rate of change in reaction time in session two. Further, the anodal tDCS group had overall fewer number of chunks limited to session two, whereas the cathodal tDCS group had overall a faster rate of change in the number of chunks in session two. In session three the cathodal tDCS group displayed a fewer number of chunks.

Reaction Time, Long-Term Follow-Up

There were no significant differences between the real stimulation groups and sham.

Contrasts for Reaction Time, Long-term Follow-Up

Planned contrasts between the real stimulation (left PFC and M1) and sham revealed the left PFC group had significantly longer reaction times ($t(1779) = 3.627$, $p < 0.001$) compared to the sham group for the simple sequences

Chunking, Long-Term Follow-Up

Chunking data is not available for this data set as the model fit failed given the limited number of trials.

Reaction Time in Testing Conditions, Long-Term Follow-Up

The rate of change in reaction time for the left PFC group in the single stimulus condition was significantly faster for the simple sequences (β = -.84 SE = .37, p = 0.023; Figure C.5). The M1 tDCS group reduced the rate of reaction time in the mixed unfamiliar condition at a significantly faster rate relative to sham for the simple sequences (β = -1.62 SE = .42, p < 0.001).

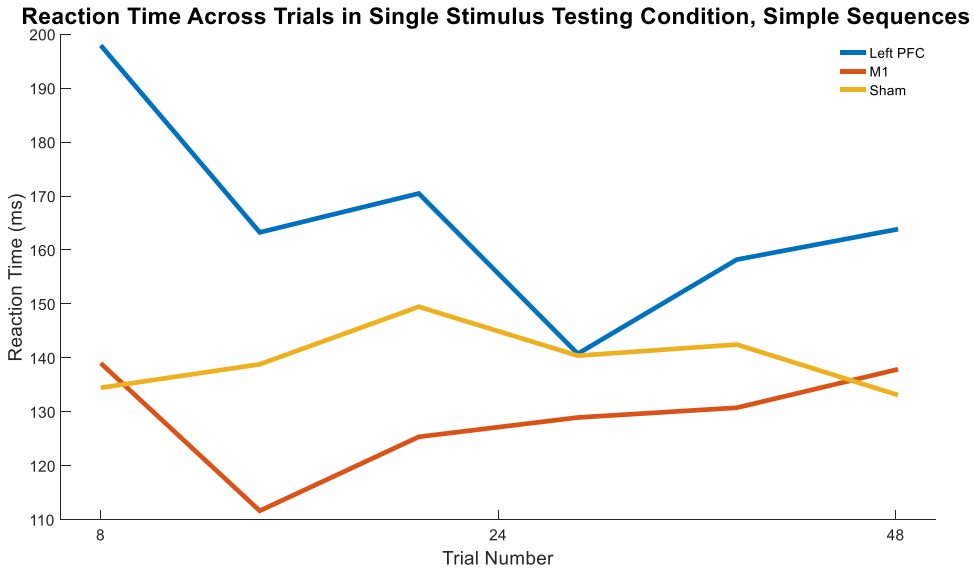

Figure C.5. Mean reaction time as a function of trial in single stimulus testing condition for simple sequences. Left PFC is denoted by the blue line, M1 is denoted by the red line, and sham is denoted by the yellow line.

In summary, stimulation to the left PFC group resulted in significantly slower rate of change when relearning the simple sequences a year later, whereas the left PFC group displayed a significantly faster rate of change in the single stimulus condition.

Reaction Time for Older Adults

There was no significant stimulation group by sequence type interaction.

Contrasts on Reaction Time for Older Adults

As there was no stimulation group by age group interaction, contrasts were pooled across age groups. The right PFC ($t(5024) = 7.371$, $p < 0.001$) and preSMA groups ($t(5024) = 6.411$, $p < 0.001$) had significantly longer reaction times than the sham group in the first session. Whereas the M1 group had significantly shorter reaction times relative to the sham group in session one ($t(5024) = 5.575$, $p < 0.001$). The right PFC ($t(4952) = 6.909$, $p < 0.001$), left PFC ($t(4952) = 2.925$, $p = 0.003$), and preSMA ($t(4952) = 11.111$, $p < 0.001$; Figure C.6) tDCS groups were all had significantly longer reaction time relative to the sham group in the second session. In contrast, the M1 had significantly shorter reaction times than the sham group in the second session ($t(4952) = -5.324$, $p < 0.001$). The right PFC ($t(1670) = 3.212$, $p = 0.001$) and the preSMA ($t(1670) = 4.760$, $p < 0.001$) tDCS groups had significantly longer, whereas the M1 group had significantly shorter reaction times relative to the sham group in session three ($t(1670) = -2.595$, $p = .010$; Figure C.6).

Figure C.6. Mean reaction time as a function of trial for young and older adults. Blue lines denote right PFC, orange lines denote left PFC, yellow lines denote M1, purple lines denote preSMA, and green lines denote sham tDCS groups. S2 and S3 labels on x-axis represent the start of session two and three, respectively.

In summary, there was no stimulation group age group interaction. Contrasts pooling across the two age groups revealed stimulation to either the right PFC or preSMA resulted in longer reaction times across all sessions, whereas stimulation to left PFC resulted in longer reaction times limited to session two. Stimulation to M1 resulted in overall shorter reaction times across all three sessions.

Contrasts on Chunks for Older Adults

Planned contrasts between each real stimulation group and sham for older adults revealed several significant results for simple sequences. Stimulation to right PFC ($t(4293) = 2.485$, $p = .013$) and M1 ($t(4293) = -4.763$, $p < 0.001$) resulted in significantly fewer number of chunks relative to the sham group in session one. While stimulation to preSMA resulted in significantly more chunks relative to sham ($t(4293) = -2.423$, $p = .015$; Figure C.7). In the second session, the right PFC ($t(4739) = -7.482$, $p <0 .001$), left PFC ($t(4739) = -9.156$, $p <0 .001$), and preSMA($t(4739) = -2.704$, $p = .007$) had all significantly more chunks relative to the sham group. The M1 group had significantly fewer chunks relative to the sham group ($t(4739) = -2.018$, $p = .044$). Contrasts revealed the right PFC ($t(1468) = -2.896$, $p = .004$) and the left PFC ($t(1468) = -2.920$, $p = .004$) tDCS groups had significantly more chunks compared to the sham group in the third session for the simple sequences. The preSMA group had significantly fewer chunks compared to sham in the third session for the simple sequences ($t(1468) = 3.675$, $p < 0.001$; C.7).

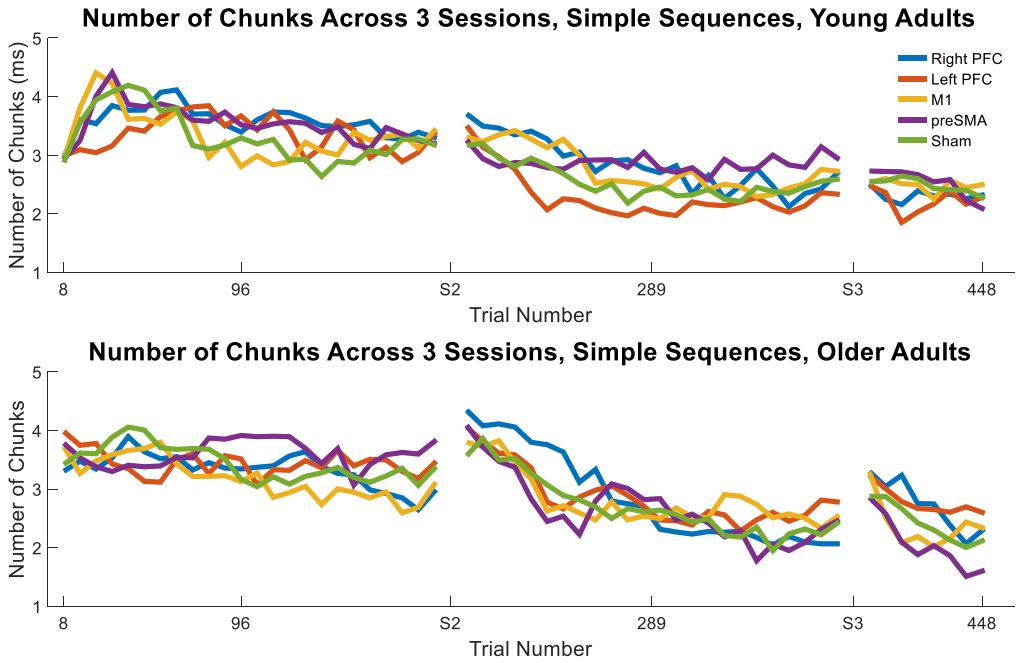

Figure C.7. Mean number of chunks as a function of trial for the simple sequences trials for young (top panel) and older adults (bottom panel). Blue lines denote right PFC, orange lines denote left PFC, yellow lines denote M1, purple lines denote preSMA, and green lines denote sham tDCS groups. S2 and S3 labels on x-axis represent the start of session two and session three, respectively.

In summary, stimulation to right PFC lead to significantly fewer chunks during session one but more chunks during session two and three. Stimulation over left PFC lead to more chunks during session two and three. preSMA stimulation lead to more chunks during session one and two, but fewer chunks on session three. And stimulation to M1 resulted in fewer chunks during session two.

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