APPLYING ANODAL TRANSCRANIAL DIRECT CURRENT STIMULATION AT BA6 DURING REPETITIVE PRACTICE ENHANCES MOTOR LEARNING BY IMPROVING ENCODING AND POST-PRACTICE CONSOLIDATION

A Dissertation

by

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ABSTRACT

Understanding how the structure of practice influences the consolidation and long-term retention of motor skills is important to maximize learning. Learning multiple motor sequences simultaneously is facilitated by using an interleaved as opposed to repetitive training schedule typically manifest as superior consolidation and long-term retention. Recent neural imaging data has highlighted the importance of earlier and more consistent recruitment of the BA6 region, in particular the supplementary motor area (SMA) and dorsal premotor area (PMd), during IP compared to repetitive practice (RP). Indeed, the emergence of greater functional connectivity of dorsal premotor region (PMd) during IP has been reported to be predictive of subsequent learning gains. The primary aim of this work was to modify the cortical activity at SMA (Experiment 1) and PMd (Experiment 2) during RP and IP using anodal or cathodal transcranial direct current stimulation (tDCS). The expectation was that increasing activity at these neural regions during RP should enhance offline gain. Conversely, down-regulating the cortical activity at these neural sites during IP should disrupt the expect learning benefit associated with this practice format. Participants were exposed to anodal tDCS at SMA (Exp1) and PMd (Exp2) of 2 mA during approximately 20-min of RP format or cathodal tDCS at these same sites while experiencing RP. Performance of three motor sequences was assessed in a RP format prior to any practice and immediately after practice, as well as 6-hr, 24-hr, and 72-hr after the completion of RP or IP. No stimulation was present during any of the test blocks. As expected, there was a robust learning benefit from IP manifest as superior early consolidation during the initial 6-hr after practice and further performance enhancement likely a result of more effective sleep-mediated consolidation. Applying anodal tDCS during RP at SMA (Experiment 1) led to increased

offline gain both from superior early time-dependent as well as enhanced sleep-mediated consolidation. Administering anodal tDCS at PMd during RP (Experiment 2) also offered a learning benefit but surfaced from improved encoding during practice rather than from a change in post-practice consolidation processes. Cathodal stimulation at either SMA or PMd during IP failed to change the behavioral outcomes associated with their sham counterparts. These data then suggest that adequate activation of both neural regions, SMA complex and PMd, is important for skill acquisition but despite being neighboring neural sites within BA6 the specific contribution of each site to the evolution of novel motor memories is quite distinct.

DEDICATION

I would like to dedicate this culmination of work to my family, without whom I would not have been able to complete this adventure. My father, Hak-Soon Kim, mother, Yeon-Ju Na, and sister, Yun-Hee Kim have always been a source of support and comfort for me and push me to be the very best that I can. I appreciate all they have done for me and my family throughout my life. Without their unwavering support, this endeavor would have been much more difficult. I am so proud of our family and the way we each support each other and build each other up to be the best that we can be. Thank you for being by my side and helping me to complete this journey. We did it together.

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CONTRIBUTORS AND FUNDING SOURCES

Contributors

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INTRODUCTION

The evolution of motor skill learning is studied in numerous research fields including kinesiology, psychology, neuroscience, and rehabilitation medicine. Competent motor behavior resulting from skill acquisition is the foundation of many human activities such as typing, driving, using tools, and playing musical instruments. The acquisition of these skills can take an enormous commitment to physical practice. It is not surprising then that a great deal of effort is exerted trying to understand how the skill acquisition process can be expedited. Exhibiting a capacity to acquire motor skills is central to both the acquisition of, as well as, the rehabilitation of many skills that we need for everyday functioning. It is not surprising then that considerable effort has been focused on the identification of best practices to facilitate skill acquisition. One possible "best practice" is based on the notion that for an individual to learn they must be placed under high cognitive "challenge or load" when training. One such practice approach has been studied under the guise of the contextual interference (CI) effect from which it has been proposed that increasing CI during training results in memory for skill that is resilient and enduring. The interference created in practice is most often characterized by the manner in which multiple novel motor skills are scheduled during each bout [1–3]. An interleaved practice (IP) format is most commonly used to induce relatively greater interference as a result of rapid switching between the motor skills being acquired across practice trials. Conversely, a repetitive practice (RP) format creates less interference because the learner executes the same motor skill repeatedly for a fixed number of trials, or until a criterion level of performance is reached for a single motor task before practice with an alternative variation. It should be noted that the latter form of training is a common instructional format used in athletic, and/or clinical settings when improvements in motor performance are sought. This is despite the fact there is now considerable evidence indicating that the more challenging IP format is superior for developing skills that are less susceptible to forgetting as well as exhibit more broad generalizability. This finding, while frequently reported in the laboratory environment [2], has also been observed outside the laboratory for a diverse set of subject populations and has been used successfully in the clinical domain [4–6] and rehabilitative settings [7,8].

Theoretical accounts for the learning advantage afforded through exposure to greater contextual interference

Two theoretical accounts have been forwarded to explain the CI effect, specifically, the learning benefits gained from experiencing IP rather than RP. The first position, presented by Lee and Magill (1985) is referred to as the forgetting-reconstruction position [9]. This proposal focuses primarily on the extensiveness of trial-to-trial processing that occurs during IP and RP. Specifically, in IP, pertinent knowledge about each task being acquired is assumed to be frequently exchanged in working memory throughout practice such that knowledge of any one skill being learned is forgotten on later trials. This being true, when faced with any single repetition, the learner is more likely to complete a full complement of processing in preparation for each trial across the entire practice bout. In other words, forgetting task-specific knowledge from working memory occurs between trials, thus requiring frequent reconstruction of an action plan from trial to trial [9].

On the other hand, RP requires executing the same response over and over without interruption from the demand of performing a different task. Thus, the learner can maintain critical task knowledge in working memory from trial to trial throughout training. Lee and Magill suggest that the lack of exchange of information in working memory reduces the need and/or opportunities to reconstruct action plans needed to perform each task during practice. The reduction in preparatory cognitive activity is argued to impede the "the process of developing and implementing an action plan" (p. 19, Lee & Magill, 1985). The focus of the reconstruction account then is on the improvement in effectively manufacturing the appropriate plan for each upcoming response as a result of being exposed to heightened interference in practice. This account is in keeping with the relatively larger attention demands that have been noted during IP compared to RP [10]. This position is also congruent with the general finding from numerous neural imaging studies that individuals faced with IP exhibit a broader activation of neural regions central to skill acquisition including supplementary motor area, dorsal premotor area, and striatum than their RP counterparts [11–13]. Learners that encounter RP rely more extensively on activation of the default network that has been reported to be involved when an individual mind-wanders [11].

Another explanation for the CI effect has been referred to as the elaboration perspective [14]. This account focuses on the differential use of two qualitatively unique categories of information processing during IP and RP [15]. Intra-task processing consists of task analyses that exclude reference to information directly related to other motor skills concurrently being acquired and/or other existing knowledge and is assumed to be the primary mode of operation during RP. *Inter-task processing* on the other hand serves to uncover the similarities and differences between the tasks that are being acquired by engaging more elaborate between-task analyses. The consequence of this latter processing mode is thought to be more richly embellished motor sequence knowledge that includes, but is not restricted to, the relationships between each practiced task and other available knowledge. A important outcome of conducting extensive inter-task processing, assumed to occur during IP, is the formation of a more permanent and intricate memory network that supports robust access to task specific knowledge at a later time. Congruent with the basic tenet of the elaboration account are recent findings using neural imagery that highlight reduced activation of key sensorimotor regions during retention following IP coupled with extensive functional connectivity [13] and heightened cortical excitability at primary motor cortex (M1) [12,16]. These outcomes have been interpreted as more efficient movement retrieval [16,17]. Interestingly, these studies have revealed that the magnitude of functional connectivity that develops for circuits involving supplementary motor area (SMA) and dorsal premotor region (PMd) mediate the size of the offline gain advantage that results for IP [16,17].

Taken together these data reveal that practice of multiple motor skills in an IP format, such that greater CI is created during training, leads to poor performance during encoding maybe resulting from the "heavy" processing demands associated with this practice schedule. Despite this, individuals exposed to IP exhibit superior retention as compared to acquiring the same skills in a RP format. It appears that the more extensive preparatory cognitive activity demanded during IP is associated with the establishment of a richer network of task specific knowledge developed during IP thus affording the learner more efficient access to task relevant information during delayed tests which affords more successful performance [2].

More extensive superior frontal regions activation is associated with IP retention advantage

In a recent review addressing the neural underpinnings of motor skill acquisition in varied CI learning environments, Wright et al.,(2016) highlighted the importance of greater activation of lateral and medial premotor regions (Brodmann Area (BA)6) during IP compared to RP for the retention difference that has been frequently reported in the literature. Specifically, Wright et al.(2016) noted that a number of studies have demonstrated that the blood-oxygenated level dependent (BOLD) signal levels exhibited at lateral and medial premotor regions were at least 50% greater later in IP than earlier, implying significant activation of these regions as IP progresses [11,18]. Early behavioral work reported that IP results in the development of more resilient parsing of motor sequences, a behavioral signature for the formation of motor chunks [19]. The SMA, the more medial premotor region, was recently described as a domain-independent sequence processor with a key responsibility of amalgamating primitive task elements into higher order representations, which is developing motor chunks in the case of sequential motor skills, to support more efficient skill execution [20,21]. The early and persistent activation of the SMA during IP may play an important part in the effective development and implementation of motor chunks which should contribute to the successful retention performance associated with IP rather than RP [20–22].

In separate work, Tanaka et al., (2010) also highlighted the importance of the SMA for establishing motor memories that can be retrieved effectively. This work demonstrated that the administration of 1 Hz repetitive transcranial magnetic stimulation (rTMS) at SMA immediately after practice impedes subsequent retention performance for individuals trained in a RP format. This was not the case when the same stimulation was applied following IP because, according to

Tanaka et al., individuals exposed to IP more rapidly migrate information to M1 during the acquisition of novel motor skills likely as a result of relatively earlier activation of SMA and the development of motor chunks [23]. More recently, Kim and Wright (submitted) further evaluated the role of the SMA during IP and RP by simultaneously applying transcranial direct current stimulation (tDCS) during practice in an effort to either down or up-regulate SMA activity during RP and IP respectively. An underlying assumption driving the work of Kim and Wright was that broader activation of the medial premotor region during IP supports the development of motor memories, likely through the formation of more resilient motor chunks [19,24], which can be consolidated post-practice [25]. On the basis of this assumption, cathodal stimulation at SMA during IP was expected to impede the retention advantage commonly reported of this practice format whereas anodal stimulation at SMA during RP was hypothesized to increase SMA activation during training thus improve retention compared to a sham control. Retention performance in this case was assessed after both 6-hr and 72-hr to evaluate time-dependent and sleep-dependent offline performance change following RP and IP.

In the case of the sham conditions, as expected, learning was superior following IP. This benefit resulted from early stabilization of the newly formed motor memories within the initial six hours of training which were further enhanced with overnight sleep. Thus, the learning advantage afforded through IP results from improvement of both time-dependent and sleep-dependent consolidation processes compare to RP. Administering anodal tDCS at SMA throughout RP did not influence the manner in which time-dependent consolidation was conducted but had a positive effect on sleep-dependent consolidation reflected in significant improvement in offline gain following sleep compared to sham. These data imply that the extensive activation of SMA during training is important for updating newly formed motor memories during a period of sleep [26,27].

Supplementary motor cortex and/or dorsal premotor cortex contribution to the retention benefits associated with high contextual interference

In the work of Kim and Wright (submitted) the principle target of stimulation was SMA. In this work it was explicitly recognized that given the nature of the electrode montage used to administer tDCS it was impossible to distinguish if stimulation occurred at only one or both of the sub-components of the SMA namely, SMA-proper or pre-SMA. It's worth noting however that *a priori* modeling of current flow using HD-Explore software (Soterix, New York, NY) for the particular montage and stimulation parameters used by Kim and Wright (submitted) revealed a sizeable increase in the field intensity at MNI coordinates consistent with SMA-proper (X:7, Y:0, Z: 51, 0.347V/m) [28]. In contrast, using the same modeling approach, the field intensity resulting from the proposed stimulation at Pre-SMA (X: -3, Y:6, Z: 53, Na/N V/m) and PMd (X:-29, Y:-1, Z: -44, 0.17V/m) regions was smaller [28]. Vollman et al. (2013) has reported that anodal stimulation at SMA-proper, the region according to the modeling most likely impacted by the tDCS montage in the present work, but not pre-SMA facilitates visuomotor skill acquisition [29]. Despite *a priori* modeling estimates of current flow from Kim and Wright there is still some concern that dorsal premotor region was impacted by the stimulation conditions adopted in Kim and Wright's work. This is particularly important for the present work because this region, in addition to SMA, has been implicated in the offline gain advantage that is observed for IP [12,13,17].

Three factors in particular increase the possibility that SMA and/or PMd were influenced. First, in Kim and Wright, the center of the active electrode was placed slightly to the right of the vertex, in the direction of PMd, at approximately 2-cm anterior of Cz. Most previous studies have located the center of this electrode over the vertex when attempting to stimulate SMA (see Figure 1). Second, the active electrode in Kim and Wright was quite large (35 cm^2) compared to that used in other studies [29], which increases the likelihood of overlap with other regions in close spatial proximity, such as PMd. Reducing the size of the stimulating electrode centered over the vertex should decrease the possibility that this electrode lays above or close to PMd. Finally, it has been suggested that the relative size of the stimulating and return electrodes is important for maximizing the focality of stimulation at and around the active electrode [30,31]. Kim and Wright used two electrodes that were the same size (35 cm^2) for the anode and cathode in all conditions.

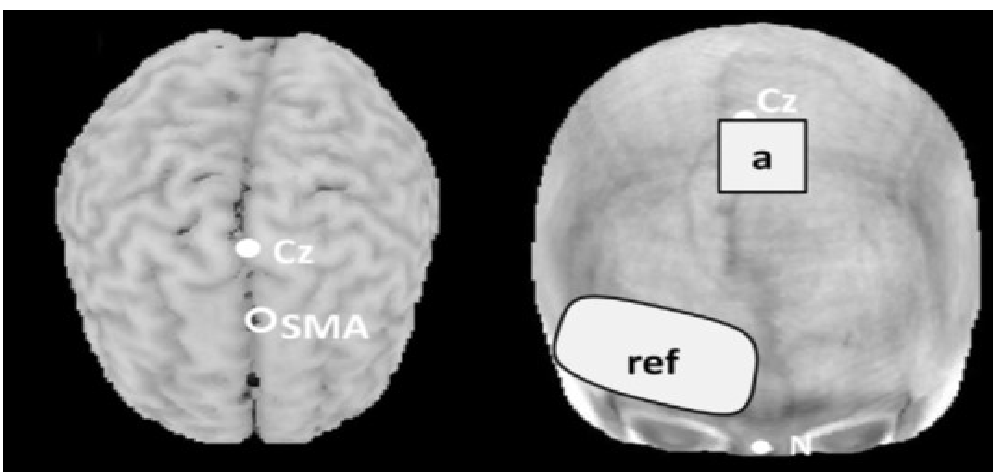

Figure. 1. Anatomical references for tDCS targeting on SMA. Identification of SMA is located 2–3 cm anterior to Cz (Cz of the international 10–20-EEG system). The figure is adapted from [32].

The present work directly focuses on two regions within the superior frontal gyrus namely the PMd and SMA that have been identified as key neural regions that differentiate the efficacy of low and high CI practice conditions for learning. Not only are these regions more extensively activated during IP compared to RP during training [11,12,17], but are part of the emergent functional connectivity that results during the later stages of practice and across the retention interval. Most importantly, the magnitude of the offline advantage observed for IP compared to RP is related to the presence of a variety of forms of functional connectivity involving specific regions within the superior frontal gyrus. The proposed experiments use tDCS in a manner similar to that adopted by Kim and Wright to examine if both SMA and PMd play a role in the learning gains promoted by IP. The initial experiment focuses on SMA by replicating the earlier study of Kim and Wright while making modifications to the electrode montage to more precisely target SMA rather than PMd stimulation. Experiment 2 applies the same approach as used in Experiment 1 in order to examine the influence of PMd during IP and RP. Experiment 2 includes conditions that consider unique contributions from left and right PMd since it has been suggested that left PMd plays a proprietary role during motor skill acquisition and much of the reported critical connectivity involving PMd resulting from IP involves the left not right hemisphere [17]. Details and expectations for each experiment follow in the next sections.

EXPERIMENT 1

When learning multiple motor sequences concurrently there is evidence that an interleaved as opposed to repetitive presentation of these skills results in superior retention and generalization. In a recent review of this literature, Wright et al. (2016) noted that an *interleaved practice (IP)* schedule was frequently associated with earlier and more consistent recruitment of the supplementary motor area (SMA) compared to its *repetitive practice (RP)* counterpart. The latter finding is consistent with SMA being implicated in supervising the organization of complex motor sequences [2,20,34]. Recently, Kim & Wright (submitted) conducted an initial attempt to use transcranial direct current stimulation (tDCS) in two novel practice contexts to evaluate the contribution of SMA to the well-documented long-term retention advantage associated with interleaving the practice of multiple new motor skills. Specifically, participants were exposed to cathodal transcranial direct current stimulation (tDCS) at SMA of 2 mA during approximately 20-min of interleaved practice or anodal tDCS while practicing within a repetitive format respectively in an attempt to modify the activity at SMA and the concomitant [1]retention outcomes commonly associated with these training formats. The basic hypothesis was that cathodal or anodal stimulation would suppress or facilitate the contribution of SMA during practice respectively. If this occurred, it was anticipated that suppressing SMA during interleaved practice should hinder performance during acquisition and retention. Conversely, facilitating SMA, via anodal stimulation, might enhance the effectiveness of repetitive practice resulting in gains in retention

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[1] The goal in the present set of experiments was to demonstrate that upregulation and downregulation of neural activity at SMA (Experiment 1) and/or PMd (Experiment 2) was central to the well-described behavioral outcomes associated with RP and IP but not to determine what combination of practice format and stimulation resulted in the most superior or inferior outcomes. For this reason, we included only anodal stimulation with RP to try to induce an improvement in outcomes and cathodal with IP to accomplish the reverse.

performance. Performance of three motor sequences in a repetitive format was administered prior to practice, and 6-hr, 24-hr, and 72-hr after the completion of practice for all participants. Results revealed the typical outcome for training and test performance for interleaved and repetitive practice conditions in sham tDCS conditions (i.e., CI effect) [1,2]. Moreover, cathodal tDCS impaired performance during training for individuals in the IP format whereas performance of the motor sequences in the RP format was enhanced via anodal stimulation. After 6-hrs, a time interval offering an opportunity to consolidate across a wake-filled interval, participants exposed to interleaved training showed performance enhancement which was absent when this training format was paired with cathodal stimulation. Individuals trained in a repetitive format exhibited forgetting after the 6-hr period irrespective of the stimulation condition. However, participants in the repetitive practice condition that received anodal tDCS during practice exhibited continued performance improvement across the subsequent three days of tests at a rate similar to individuals to IP which in turn was superior to the rate of improvement of the repetitive condition not afforded stimulation. This previous study demonstrated then that IP not RP led to superior retention of trained skills for up to 72-hr after practice was complete [5,6]. This learning benefit resulted from superior early stabilization of the memory for these novel skills within the initial six hours of training which was further enhanced across two periods of overnight sleep. These data revealed that both time-dependent and sleep-dependent consolidation processes are enhanced through exposure to greater CI present in an IP schedule. Administering anodal tDCS at SMA throughout RP had no impact on the time-dependent consolidation but had a positive effect on sleep-dependent consolidation reflected in significant offline gain with each additional sleep episode. Thus, heightened recruitment of SMA, as occurs in IP, affords a learning advantage by fostering overnight processing of new memories.

However, this work was not without limitations. Specifically, when targeting SMA for the administration of tDCS in RP and IP, slightly different montages to target SMA then commonly used in the literature were used as well as relatively large (i.e., 5x7cm^2) electrodes that reduce the focality of stimulation. As a result some overlap beyond SMA possibly including the premotor area may have occurred. Inadvertent stimulation of PMd is of particular concern because this neural site in particular has been identified as also being differentially activated during RP and IP formats and may play an important role in determining the nature of retention performance [2,17]. Nonetheless, the a priori modelling forwarded by Kim & Wright (submitted) did reveal heightened current flow (.374 V/m^2) at MNI coordinates (x: 7, y: 0, z: 51) which are congruent with the location of SMA described by Mayka et al., (2016) and left SMA proper (x:-3, y: -2, z: 57) Vollmann et al., (2013).

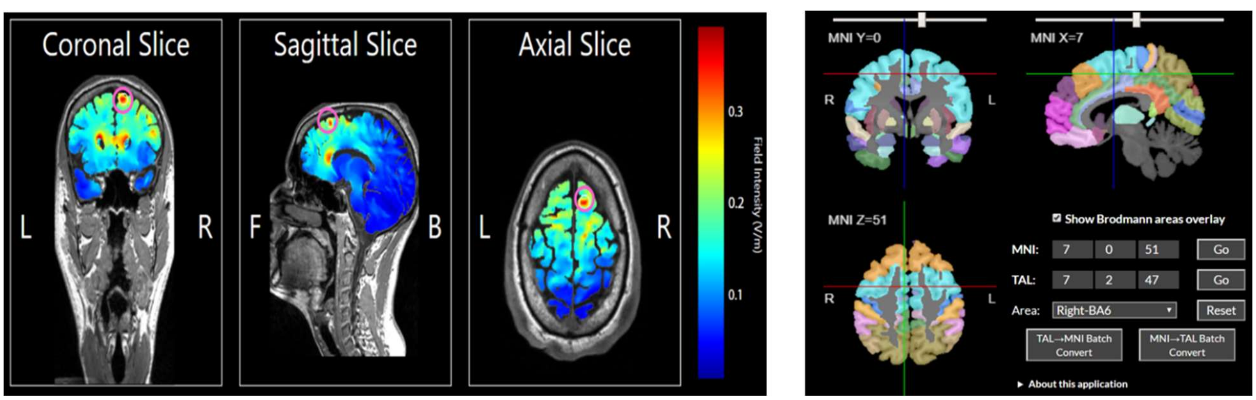

Figure 2. *Left:* Modeled field potentials in an adult male brain, revealing the distribution of the electrical field in the brain with the anodal stimulation montage designed to target the SMA complex. The pink circle represents the SMA in the head model. The modelling showed that heightened current flow (.374 V/m^2) at MNI coordinates (x: 7, y: 0, z: 51) are congruent with the location of SMA described by Mayka et al., (2016), left SMA proper (x: -3, y: -2, z: 57), Vollmann et al. (2013). The MNI coordinates are overlaid on an alternative reference source specifically the Yale BioImage Suite (*Right*). The figure is adapted from Kim & Wright (Submitted to *Neuroscience*).

Thus, clearly verifying the role of SMA in IP and RP is a critical and necessary step to more clearly explore the neural correlates underpinning IP and RP formats [28,29]. The primary purpose of Experiment1 was to verify the role of SMA during IP and RP using anodal and cathodal tDCS to delineate the learning benefits of IP associated with activation of SMA. The positioning of the electrodes, the size of the electrode, as well as the relative size of the electrodes was manipulated to increase the precision of targeting at SMA in tDCS and improve focality of the modulation at the target site [34]. Despite these changes, in general, we anticipate that the behavioral outcomes that were reported by Kim and Wright to be replicated.

Methods

Participants

Participants were right-handed undergraduate students (N=76) that received course credit for their participation. Individuals had no prior experience with the experimental tasks and was unaware of the specific purpose of the study. All participants completed an informed consent approved by an Institutional Review Board before any involvement in the experiment.

Discrete Sequence Production Task

The motor skills used in Experiment 1 were modified discrete sequence production (DSP) tasks [33]. These tasks have been used previously to study motor sequence learning [25]. Each DSP task use in Experiment 1 was executed on a standard PC keyboard and consisted of typing a predetermined set of six key-presses using only the left index finger in response to a visual signal that indicate the required key to press. The four keys used were “V”,” B”, N”, and “M.” on a standard PC keyboard. The order of key-presses for each DSP task was determined by the presentation of a black dot within one of four boxes displayed on the lower third of the computer screen in a spatially compatible manner with the keys on the keyboard. Participants were instructed to associate the appearance of the visual signal in the leftmost box with a "V" key press. In a similar vein, individuals were told that a black dot in the rightmost box required a press of the rightmost "M" key with the left index finger. The black dot remained in the same location of the display until the correct key was pressed (see Figure 3A). Three unique 6-key DSP tasks were used namely, 1-3-4-2-4-3, 4-2-1-3-1-2, and 2-4-3-1-3-4 for which 1 represents a leftmost "V" key press and 4 a press of the rightmost "M" key. There was a 300-750ms response-to-stimulus interval (RSI) after the third key press during execution of all of the DSP tasks during every practice trial to encourage participants to execute each motor sequence as two motor chunks, each containing 3 key-presses [33,34]. All other RSIs was 0-ms in duration. All features of this experiment was programmed using E-Prime® 2.0 (Psychology Software Tools, Inc., Sharpsburg, PA).

By using the DSP task in the present work, it is also possible to probe the locus of any improvement in acquisition and retention of motor sequences following previous training under different practice formats. Abrahamse et al. (2013) proposed that the execution of a DSP task involves three distinct processes. Specifically, the first process is referred to as sequence *initiation* and is reflected in the time to complete the first key-press. This process is assumed to involve selection and preparation of the sequence. A relatively slow key-press typically observed in the middle of a motor sequence is assumed to incur a cost of transitioning between motor chunks that comprise the motor sequence. This has been referred to as a *concatenation* point and is assumed to involve selection and preparation of an upcoming motor chunk. Finally, all other key-presses are usually executed considerably faster, often with an RT lower than 100 ms, than those associated with initiation and concatenation and reflect the cost of the *execution* of single elements (i.e., key-press) contained in a motor chunk. Assuming experience with prior RP contributes to improvement in a memory retrieval processes for newly learned skills it seems reasonable to assume that this benefit would most likely to be observed for the sequence production processes most intimately related to retrieval, that is, initiation and concatenation rather than execution.

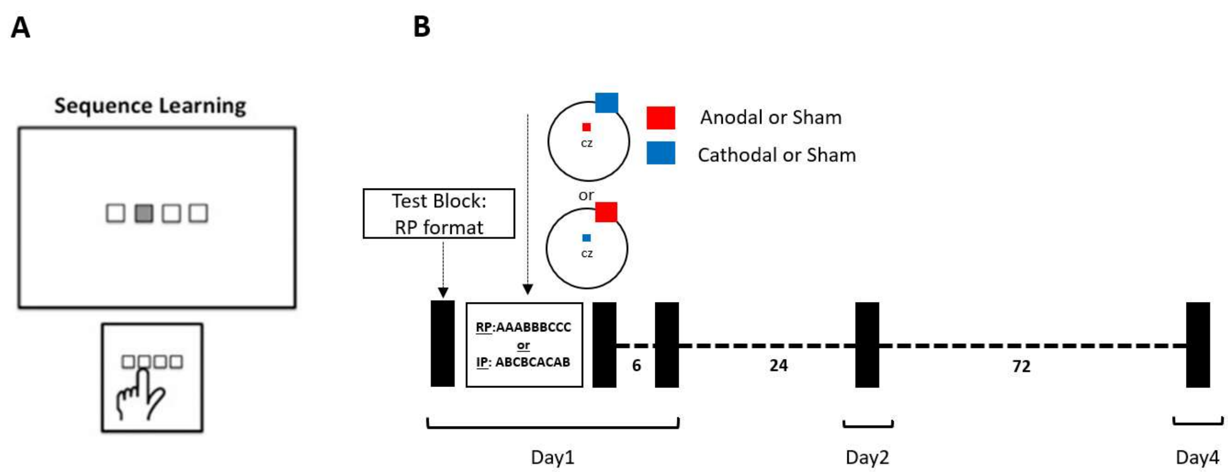

Figure 3. (A) Individuals learn three distinct 6-element Discrete Sequence Production (DSP) Tasks in either an IP or RP format. Each of these tasks involved executing a string of key presses using the left index finger only in response to a visual signal that direct the learner to the location of the next response. (B) Timeline and description of the electrode montages used to anodal and cathodal stimulation at the SMA complex for Experiment 1. Participants took four days to complete all sessions in the experiment. The filled black box represents a test block that was administered in a repetitive format and consisted of 21 trial that included seven trials for each of three target DSP tasks. After initial test block, all individuals were exposed to real or sham stimulation during nine blocks of RP or IP practice. RP or IP took approximately 20-min to complete which was the duration of anodal (RP-AtDCS), cathodal (IP-CtDCS), or sham (RP-Sham, IP-Sham) stimulation. The same RP format 21-trial test block was administered at several time points after the completion of either RP or IP, namely post training, as well as 6-hr, 24-hr, and 72-hr following the conclusion of practice.

Transcranial Direct Current Stimulation (tDCS)

Participants were assigned to one of two stimulation conditions (RP-AtDCS, IP-CtDCS) which involved 20-min of either anodal or cathodal stimulation at SMA while two other conditions involved only sham stimulation (RP-Sham, IP-Sham). All real stimulation conditions consisted of a 2mA current being administered through a 5x5 cm electrode for the stimulation and a 5x7 cm electrode as the reference [30,35] covered by saline-soaked sponges resulting in a maximum current density of 0.04 mA/cm^2 that was administered using a battery-driven stimulator (tDCS Stimulator; TCT Research Limited, Hong Kong). *Anodal* stimulation used for the RP-AtDCS condition involved placement of the anode 2.5cm anterior of Cz (determined based on the International 10-20 system) [36,37]. The cathode for this condition was placed above the forehead over the contralateral supraorbital area (see Figure.3B). Figure 4 shows the current flow associated with this electrode montage arrangement modeled using HD-Explore™ (Soterix Medical Inc., New York, NY). Cathodal tDCS targeting SMA for the IP-CtDCS condition involved reversing the locations of the anode and cathode described for the IP-AtDCS condition. That is, the anode was located on the forehead over the contralateral supraorbital area whereas the cathode was located on the skull in a manner described earlier. The sham stimulation condition involved the anodal stimulation electrode montage described previously but stimulation only delivered for 30-sec at the beginning and end of the 20-min training period.

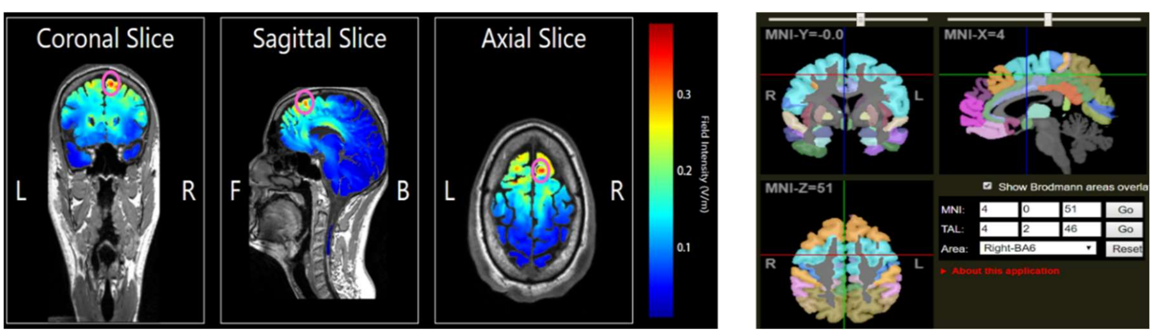

Figure 4. *Left*: Modeled field potentials in an adult male brain for the distribution of the electrical field in the brain with the selected montage used for anodal stimulation used in RP. The pink circle represents the location of the SMA in the head model. Modelling revealed heightened current flow (.331 V/m^2) at MNI coordinates (x: 4, y: 0, z; 51) congruent with the location of SMA as described by Mayka et al., (2016) and left SMA proper (x:-3,y: -2,z: 57) reported by Vollmann et al., (2013). These MNI coordinates are overlaid on an alternative reference from the using Yale BioImage Suite *(Right).*

Procedure

Participants first read and signed an informed consent. Individuals were randomly assigned to one of four stimulation conditions namely repetitive practice with sham stimulation (RP-Sham), interleaved practice with sham stimulation (IP-Sham), repetitive practice with anodal stimulation (RP-AtDCS), or interleaved practice with cathodal stimulation (IP-CtDCS) (see Figure 3B). Before RP or IP, a 21 trials baseline test block was administered including seven trials for each of three target DSP tasks, presented in a repetitive format. This same 21-trial test block was administered again at several intervals after the completion of either RP or IP, namely, immediately post training (Post-Imm), 6-hr (Post-6), 24-hr (Post-24), and 72-hr (Post-72) following the conclusion of practice. Training consisted of nine blocks of practice with each block consisting of 21 trials resulting in a total of 189 trials that include 63 trials for each of the target DSP tasks. For RP, all trials within a trial block (i.e., 21 trials) involved only one DSP task. In the case of IP, each 21-trial block consisted of seven trials of each of the target DSP tasks (see Figure 3A). There was 2-sec interval between trials and a 30-sec interval between blocks during RP and IP.

Throughout training but not the test sessions, individuals were exposed to real (i.e., IP-CtDCS, RP-AtDCS) or sham (IP-Sham, RP-Sham) stimulation. Training was lasted approximately 20-min which determined the duration of anodal (RP-AtDCS), cathodal (IP-CtDCS), or sham (RP-Sham, IP-Sham) stimulation. Prior to the beginning of the training period, all participants were prepared with the electrode montage that was specific to their assigned condition. Immediately on completion of training, the two electrodes were removed from the participant's head. At this point each subject was administered the immediate posttest trials (see Figure. 3B). No stimulation was presented during any of the test trials. For all tests trials, the 300 to 750-ms RSI was removed between response 3 and stimulus 4.

Measurement and Analyses

For all trials during training and test blocks, the primary dependent was *sequence total time* (TT) which was the interval from the presentation of the first stimulus to the execution of the final key-press for the DSP task being performed. Since mean TT it typically is not normally distributed, for all the analyses that were conducted, the median TT was used as an estimate of performance for each training and test block for each individual. Moreover, when using a DSP task, the latency associated with the first key press captures the costs of sequence initiation referred to herein as *initiation time* (IT). The response time to the key press following the random non-0 RSI (key press 4) reflects the transition between two motor chunks and is referred to as *concatenation time* (CT). The remaining key presses (2, 3, 5 and 6 for DSP tasks in this experiment) each depended solely on execution demands and contributed only to execution time (ET) [25,33]. Analysis of IT, CT, and ET offered the opportunity to further probe the influence of practice schedule (i.e., RP and IP) and stimulation (i.e., anodal and cathodal) on how motor sequences are implemented with practice.

The initial analyses focused on the traditional CI effect by assessing online (i.e., during blocks of RP and IP) and offline (i.e., during test blocks) performance of the sham conditions only. To evaluate online performance, the TT data for the baseline and PostI test blocks for individuals assigned to the sham conditions were submitted to a 2 (Schedule: RP-Sham, BP-Sham) × 2 (Block: baseline, PostI) ANOVA with repeated measures on the last factor. A second analysis focused on the change in TT that occurred from the end of RP or IP (i.e., PostI) to a set of delayed test trials to evaluate the retention benefit of the RP and IP schedules. This initial analysis of delayed test performance only incorporated data from the 24-hr test block because most previous studies assessing the impact of CI for offline administered retention tests the day after practice concluded. Thus, TT data for each individual in the RP-sham and IP sham stimulation conditions for the PostI and Post24 test blocks were submitted to 2 (Schedule: RP-Sham, IP-Sham) × 2 (Block: postI, post24) ANOVA with repeated measures on the last factor.

To access the impact of tDCS at SMA during RP and IP for online performance, TT for the Baseline and PostI test blocks for individuals that experienced RP and IP was separately submitted to a 2 (Stimulation: RP-Sham, RP-AtDCS) × 2 (Block: Baseline, PostI) and 2 (Stimulation: IP-Sham, IP-CtDCS) × 2 (Block: Baseline, PostI) ANOVA with repeated measures on the last factor.[2] The impact of stimulation for online performance during IP and RP was further probed by submitting. TT data during training for each individual to separate 2 (Stimulation: RP-Sham, RP-AtDCS) × 9 (Block: 1-9) and 2 (Stimulation: IP-Sham, IP-CtDCS) × 9 (Block: 1-9) ANOVA with repeated measures on the last factor.

Evaluating the impact of tDCS at SMA during RP and IP on early time-dependent consolidation was accomplished by submitting the TT data from PostI and Post6 test blocks for individuals trained with RP and IP to either a 2 (Stimulation: RP-Sham, RP-AtDCS) × 2 (Block: PostI, Post6) or 2 (Stimulation: IP-Sham, IP-CtDCS) × 2 (Block: PostI, Post6) ANOVA with repeated measures on the last factor. The impact of tDCS at SMA during RP and IP on long-term retention was evaluated using two separate approaches. First, TT data for the Post6, Post24, and Post72 test blocks for individuals that experienced RP and IP were submitted to either a 2 (Stimulation: RP-Sham, RP-AtDCS) × 3 (Block: Post6, Post24,

[2] It is important to note that the goal of the present work was not to demonstrate that performance for individuals assigned to RP-AtDCS could match IP-Sham or that behavioral outcomes for IP-CtDCS were equivalent to those observed for RP-Sham. Rather, we addressed a more straightforward comparison involving the stimulated condition (RP-AtDCS, IP-CtDCS) with their associated control condition (RP-Sham, IP-Sham) to probe the relative impact on performance of changing SMA recruitment for each practice format. For this reason, most of the analyses conducted and reported in the result section focused on a practice schedule (IP or RP) separately rather than comparing performance of all stimulation conditions in each analysis.

Post72) or to a 2 (Stimulation: IP-Sham, IP-CtDCS) × 3 (Block: Post6, Post24, Post72) ANOVA with repeated measures on the last factor. To further examine the impact of training context and stimulation on long-term retention of a set of novel motor skill an approach previously adopted by Reis et al. (2009) was used [38]. Specifically, a linear function was fit to the TT data for Post6, Post24, and Post72 for each individual and the slope was used to represent the rate of gain or loss following the completion of early consolidation. The slope for each individual was subjected to separate one-way t-tests for the RP (RP-AtDCS, RP-Sham) and IP (IP-CtDCS, IP-Sham) conditions. Each of the aforementioned were repeated using IT, CT, and ET as dependent variables to further probe how each independent planning process, namely initiation, concatenation, and execution time were impacted by practice schedule and stimulation.

Results

Baseline DSP task performance

Total time data from the baseline test trials performed prior to any practice (see Figure 7 and 8) for all individuals was subjected to a 4 (Stimulation: RP-Sham, IP-Sham, RP-AtDCS, IP-CtDCS) between-subject analysis of variance (ANOVA) which failed to reveal a significant main effect of Stimulation, $F(3, 72) = 0.38$, $p = 0.77$, $\eta_p^2 = 0.02$. Thus, as expected, TT did not differ as a function of stimulation condition prior to any exposure to training and/or stimulation (RP-Sham, *M* = 2885 ms, *SEM* = 71 ms; IP-Sham, *M* = 2794 ms, *SEM* = 80 ms; RP-AtDCS, *M* = 2782 ms, *SEM* = 69 ms; IP-CtDCS, *M* = 2839 ms, *SEM* = 84 ms).

Contextual interference revisited: Online and offline performance of individuals that experienced RP and IP with sham stimulation

Online Performance during RP and IP with sham stimulation. To assess if the online performance observed in the present study replicated previous work addressing the influence of different levels of CI during training [1,2], TT data for the baseline and Post-I test blocks for individuals assigned to the sham conditions were submitted to a 2 (Schedule: RP-Sham, IP-Sham) × 2 (Block: baseline, PostI) ANOVA with repeated measures on the last factor. The main effect of Schedule, F(1,36) = 0.22, p = 0.64, η_p^2= .001. and the Schedule x Block interaction, F(1,36) = 3.85, p = 1.69, η_p^2= 0.1 were not significant. However, the main effect of Block, F(1,36) = 285.51, p < 0.01, η_p^2= 0.89 was significant. Subsequent post-hoc analysis of the Block main effect revealed that TT for the baseline test block (M =2840ms, SEM = 75ms) was significantly greater than TT displayed during the Post-I test block (M = 1876ms, SEM = 67ms).

Offline Gains following RP and IP with *sham stimulation***.** The initial assessment of offline gains following RP and IP involved submitting TT data for each individual in RP-sham and IP sham conditions for the PostI and Post24 test blocks to a 2 (Schedule: RP-Sham, IP-Sham) × 2 (Block: postI, post24) ANOVA with repeated measures on the last factor. The main effect of Schedule, $F(1,36) = 1.85$, $p = 0.18$, $\eta_p^2 = 0.05$ and Block, $F(1,36) = 0.09$, $p = 0.76$, $\eta_p^2 = .001$ were not significant. However, the Schedule x Block interaction, $F(1,36) = 22.26$, $p < .001$, $\eta_p^2 = 0.38$ was significant. Simple main effect analyses of this interaction revealed that TT for the PostI test block did not differ significantly for individuals in the RP-Sham (*M* = 1859 ms, *SEM* = 71 ms) and IP-Sham (*M* = 1893 ms, *SEM* = 63 ms) Schedule conditions. However, TT at the Post24 test block for individuals in the IP-Sham condition (*M* = 1736 ms, *SEM* = 65 ms) was significantly lower than the TT displayed by the individuals in the RP-Sham condition (*M* = 1997 ms, *SEM* = 61 ms), *F*(1,36) =

34.62, $p < .001$, $\eta_p^2 = 0.49$. Furthermore, the reduction in TT from the Post I to Post24 test block for the IP-Sham condition was significant, $F(1,36) = 12.61$, $p=0.01$, $\eta_p^2 = 0.26$(post-I: $M = 1894$ ms, $SEM = 63$ ms; post24, $M = 1736$ ms, $SEM = 55$ ms). In contrast, TT from the PostI to Post24 test blocks for the individuals in the RP-Sham schedule condition was not significantly different, $F(1,36) = 9.74$, $p = 0.01$, $\eta_p^2 = 0.21$. (postI: $M = 1859$ ms, $SEM = 71$ ms; post24, $M = 1997$ ms, $SEM = 61$ ms).

The offline performance profile then was in general agreement with the prevailing literature [2]. Individuals assigned to IP exhibited superior performance during delayed test compared to their RP counterparts manifest as enhanced performance across the initial 24-hr after practice following IP whereas RP was associated with some forgetting albeit non-significant in the present case [3,11].

Online gain: Performance during IP and RP in the presence of stimulation

Given the present study replicated the CI effect, the next question is if the described behavioral outcomes traditionally associated with RP and IP formats are modified by administering anodal and cathodal tDCS at SMA respectively during practice. Figure 7 and 8 depict the TT for each test blocks administered prior to (Baseline) and after the completion of training (PostI, Post6, Post24, Post72) for the individuals that were assigned to RP (Figure 7: RP-Sham, RP-AtDCS) and IP (Figure 8: IP-Sham, IP-CtDCS). Recall that stimulation (i.e., real or sham) was not present during any of the test blocks.

Influence of anodal tDCS at SMA for online performance during RP. TT data for the Baseline and PostI test blocks for individuals that experienced RP were submitted to a 2

(Stimulation: RP-Sham, RP-AtDCS) × 2 (Block: Baseline, PostI) ANOVA with repeated measures on the last factor. This analysis revealed a significant main effect of Block, $F(1,36) = 341.69$, $p < .001$, $\eta_p^2 = 0.9$. This main effect was a result of TT for the Baseline test block (*M* = 2834 ms, *SEM* = 70 ms) being significantly greater than the TT noted during the PostI test block (*M* = 1777 ms, *SEM* = 64 ms). The main effect of Stimulation, $F(1,36) = 3.14$, $p = 0.08$, $\eta_p^2 = 0.08$ and the Stimulation x Block interaction, $F(1,36) = 0.29$, $p = 0.6$, $\eta_p^2 = 0.01$ failed to reach significance. These data reveal that experiencing RP resulted in online gains across training and the observed improvement in TT was not modified by supplementing RP with anodal tDCS.

A second assessment of online performance during RP that was supplemented with anodal stimulation focused on TT data from the training blocks while stimulation was present as opposed to the test blocks that surrounded RP training (see Figure 5). TT data for each of the nine training blocks for each individual in the RP-AtDCS and RP-Sham conditions were submitted to a 2 (Stimulation: RP-Sham, RP-AtDCS) × 9 (Block: 1-9) ANOVA with repeated measures on the last factor. The main effects of Stimulation, $F(1,36) = 6.72$, $p = 0.01$, $\eta_p^2 = 0.16$, and Block, $F(8,288) = 54.11$, $p < .001$, $\eta_p^2 = 0.6$ were significant. However, the Stimulation x Block interaction, $F(8,288) = 0.39$, $p = 0.92$, $\eta_p^2 = 0.01$ was not significant. Subsequent post-hoc analyses of the significant main effects indicated that TT was lower in the presence of anodal stimulation during the training period (RP-sham, *M* = 1777 ms, *SEM* = 73 ms; RP-AtDCS, *M* = 1590 ms, *SEM* = 42 ms) and the Block main effect emerged as a function of a gradual reduction in TT across practice.

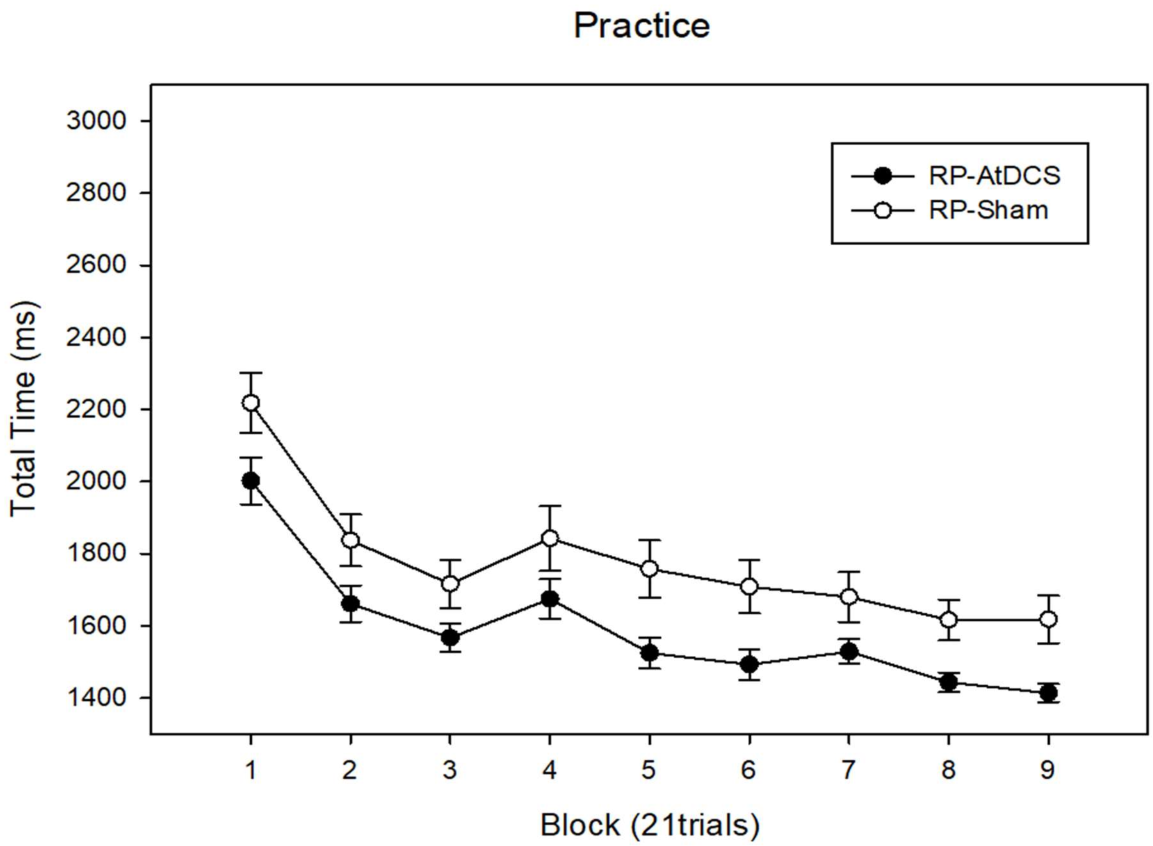

Figure 5. Total Time(ms) for the nine training blocks performed between the baseline and immediate test blocks during which real or sham stimulation was applied for the RP condition. Individuals in the RP-AtDCS condition exhibited significantly lower TT for all blocks compared to their sham counterparts

Influence of cathodal tDCS at SMA for online performance during IP. TT data for the Baseline and PostI test blocks for individuals that experienced IP were submitted to a 2 (Stimulation: IP-Sham, IP-CtDCS) × 2 (Block: Baseline, PostI) ANOVA with repeated measures on the last factor. This analysis revealed significant main effects of Block, $F(1,36) = 284.33$, $p < .001$, $\eta_p^2 = 0.89$ but failed to reveal a significant Stimulation, $F(1,36) = 0.92$, $p = 0.35$, $\eta_p^2 = 0.02$ and Stimulation x Block interaction, $F(1,36) = 0.06$, $p = 0.8$, $\eta_p^2 = 0.01$. The Block main effect was a function of TT for the baseline test block (*M* = 2831 ms, *SEM* = 87 ms) being significantly greater than the TT reported during the PostI test block (*M* = 1944 ms, *SEM* = 58 ms). Online performance during IP was also evaluated using TT data from training while stimulation was present.

Specifically, the TT data for each of the nine training blocks for each individual in the IP-CtDCS and IP-Sham conditions were submitted to a 2(Stimulation: IP-Sham, IP-CtDCS) × 9(Block: 1-9) ANOVA with repeated measures on the last factor. The main effects of Stimulation, $F(1,36) = 0.03$, $p = 0.87$, $\eta_p^2 = 0.01$, was not significant. However, the main effect Block, $F(8, 288) = 35.39$, $p < .001$, $\eta_p^2 = 0.5$ and the interaction of Stimulation x Block, $F(8,288) = 2.04$, $p < 0.05$, $\eta_p^2 = 0.05$ were significant. Subsequent simple effect analyses of the significant interaction indicated that TT for individuals in the IP-Sham (*M* = 2250 ms, *SEM* = 97 ms) and IP-CtDCS (*M* = 2233 ms, *SEM* = 58 ms) was similar across practice except during Block8, during which TT for the IP-CtDCS condition (*M* = 2024 ms, *SEM* = 42 ms) was significantly lower than that displayed by the IP-Sham condition (*M* = 2139 ms, *SEM* = 84 ms), $F(1,36) = 4.17$, $p < 0.05$, $\eta_p^2 = 0.1$ (See Figure 6).

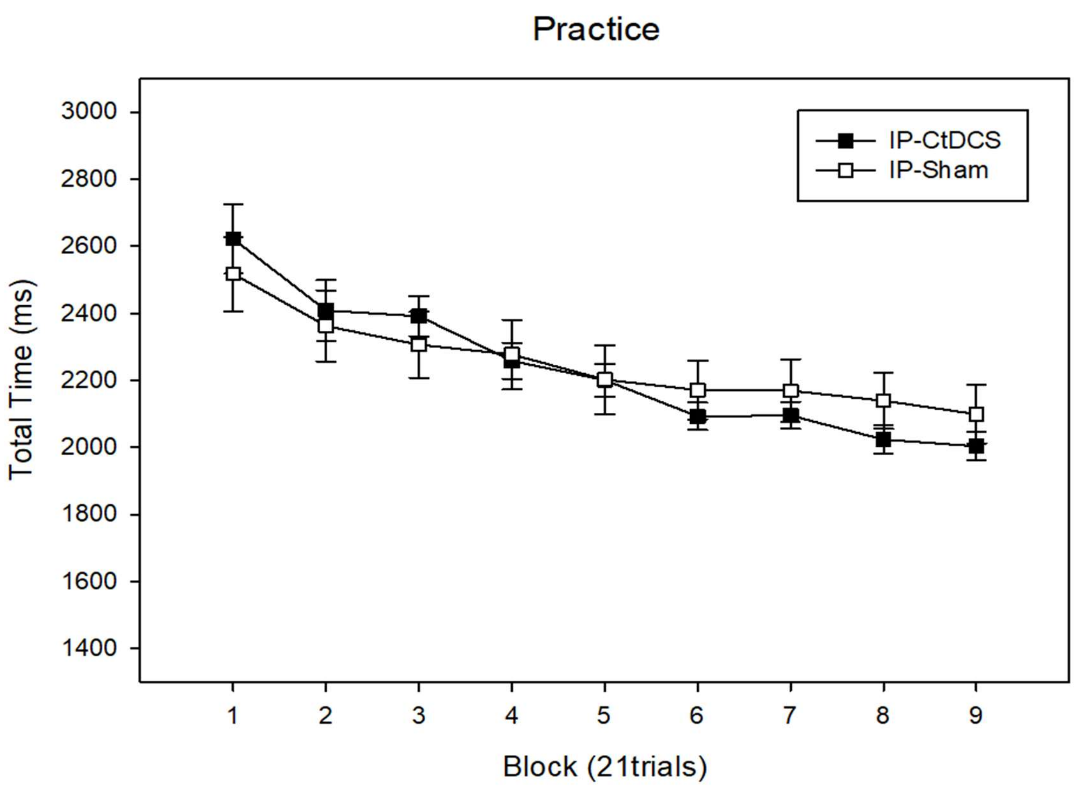

Figure 6. Total Time(ms) for the nine training blocks performed between the baseline and immediate test blocks during which real or sham stimulation was applied. Performance was very similar for all training blocks except Block 8 during which the IP-CtDCS condition led to lower TT compared to the IP-sham condition.

Offline gains resulting from early time-dependent consolidation

There is considerable literature addressing the importance of early time-dependent consolidation, that independent on sleep [39], for the development of motor memories [40]. The influence of the practice format experienced coupled with stimulation on early consolidation was evaluated using TT data from the PostI and Post6 test blocks. These data are depicted in Figure 7 and 8 for RP and IP respectively. As was the case with earlier assessments, separate analyses were conducted to evaluate the impact of the application of anodal and cathodal tDCS at SMA during RP and IP.

Impact of Anodal tDCS at SMA during RP on early time-dependent consolidation. TT data from PostI and Post6 test blocks for individuals trained with RP were submitted to a 2 (Stimulation: RP-Sham, RP-AtDCS) × 2 (Block: PostI, Post6) ANOVA with repeated measures on the last factor. This analysis revealed a significant main effect of Block, $F(1,36) = 7.26$, $p < 0.01$, $\eta_p^2 = 0.17$ and Stimulation, $F(1,36) = 8.09$, $p < 0.01$, $\eta_p^2 = 0.18$. In addition, the interaction of Stimulation x Block, $F(1,36) = 4.18$, $p < 0.05$, $\eta_p^2 = 0.1$ was also significant. Simple main effects analyses of the interaction revealed that TT for the individuals assigned to RP-Sham (postI: M = 1859 ms, SEM = 71 ms, 6-hr: M = 2043 ms, SEM = 84 ms) was significantly greater than the TT for the participants in the RP-AtDCS condition (postI: M = 1695 ms, SEM = 56 ms, 6-hr: M = 1714 ms, SEM = 52 ms) in postI and 6-hr test blocks. Interestingly, RP-Sham showed a significant increase in TT from the PostI to Post 6 test block, $F(1,36) = 11.95$, $p < 0.01$, $\eta_p^2 = 0.25$. In contrast, individuals that experienced anodal stimulation with RP exhibited no significant change in TT during the initial 6-hr after practice, $F(1,36) = 0.13$, $p = 0.73$, $\eta_p^2 = 0.02$ (See Figure 7).

*Impact of Cathodal tDCS at SMA during IP for early time-dependent consolidation***.** TT data for the postI and post6 test blocks for individuals that experienced IP were submitted to a 2 (Stimulation: IP-Sham, IP-CtDCS) × 2 (Block: postI, post6) ANOVA with repeated measures on the last factor. These analyses indicated that the main effect of Stimulation, $F(1,36) = 0.68$, $p = 0.41$, $\eta_p^2 = 0.02$, Block, $F(1,36) = 2.26$, $p = 0.14$, $\eta_p^2 = 0.06$, and the Stimulation x Block interaction, $F(1,36) = 1.85$, $p = 0.18$, $\eta_p^2 = 0.15$ all failed significance. These analyses revealed that the TT for the IP-CtDCS (*M* = 1953ms, *SEM* = 44ms) participants performed similar to the TT observed for their IP-Sham (*M* = 1892ms, *SEM* = 65ms) during post-I and post6 test blocks.

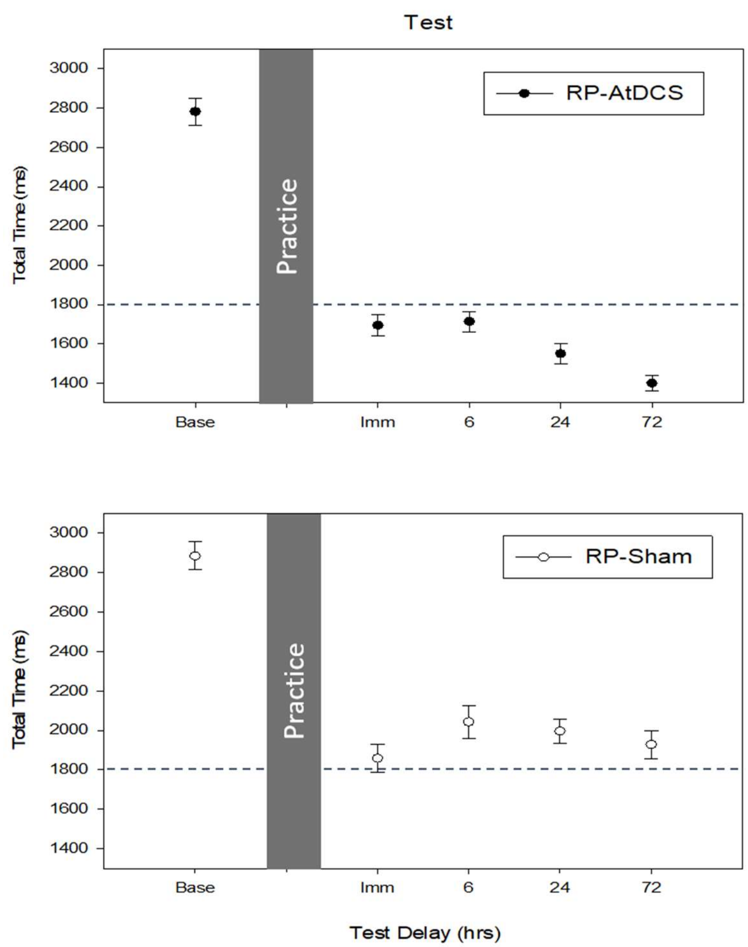

Figure 7. Total Time(ms) at Baseline (Base), immediately after practice (Imm), 6-hr post practice (6), 24-hr post practice (24), and 72-hr post practice (72) test blocks for individuals assigned to the **RP-AtDCS (top)** and **RP-sham (bottom)** conditions. Error bars are standard errors. Letters indicate scores differed significantly, p < .05.

Taken together these data reveal that the administration of anodal tDCS at SMA during RP had significant impact on the unfolding of early time-dependent consolidation that occurs independent of sleep, with the presence of stimulation fostering stabilization in performance in the first 6-hr after practice (see Figure 7). In contrast, administration of cathodal stimulation during IP had no impact on TT across the 60hr retention interval reflected in both the IP-CtDCS and IP-Sham conditions displaying no change in TT across the first 6-hr after practice (see Figure 8).

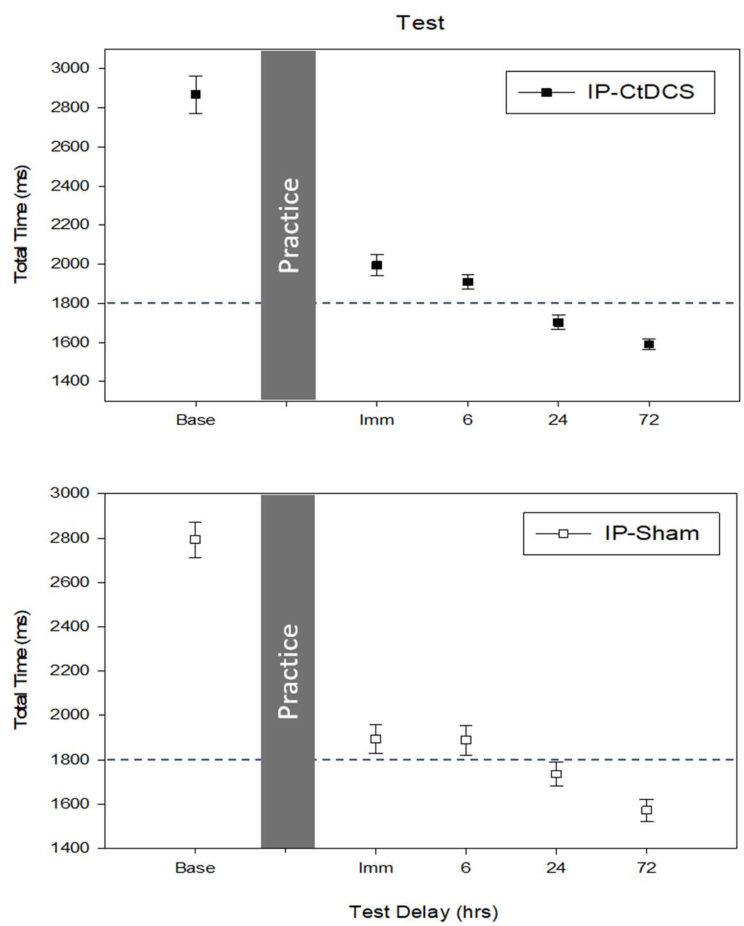

Figure 8. Total Time(ms) at Baseline (Base), immediately after practice (Imm), 6-hr post practice (6), 24-hr post practice (24), and 72-hr post practice (72) test blocks for individuals assigned to the **IP-CtDCS (top)** and **IP-sham (bottom)** conditions. Error bars are standard errors. Letters indicate scores differed significantly, $p < .05$.

Long-term retention following RP and IP in the presence of stimulation

Evaluation of the impact of practice schedule and stimulation on long-term retention involved examination of performance during the Post6, Post24, and Post72 test blocks. As noted earlier, these data are displayed in Figure 7 and 8 for RP and IP respectively. Congruent with the assessments reported in earlier sections, separate analyses were conducted to evaluate the impact of anodal and cathodal tDCS at SMA during RP and IP respectively.

Impact of Anodal tDCS at SMA during RP on long-term retention. The TT data for the Post6, Post24, and Post72 test blocks for individuals that experienced RP were submitted to a 2 (Stimulation: RP-Sham, RP-AtDCS) × 3 (Block: Post6, Post24, Post72) ANOVA with repeated measures on the last factor. This analysis revealed significant main effects of Stimulation, $F(1,36) = 34.82$, $p < .001$, $\eta_p^2 = 0.49$ and Block, $F(2,72) = 12.25$, $p < .001$, $\eta_p^2 = 0.25$. However, the Stimulation x Block interaction failed to reach significant level, $F(2,72) = 2.65$, $p = 0.08$, $\eta_p^2 = 0.07$.

Subsequent post-hoc analysis of the main effect of Stimulation revealed that TT for individuals in the RP-AtDCS condition was significantly lower during the Post6, Post24, and Post72 test blocks compared to the TT displayed by the individuals in the RP-Sham condition. Interpretation of the significant Block main effects revealed that TT did not differ significantly across test blocks for the individuals in the RP-Sham condition, $F(2,72) = 1.79$, $p = 0.17$, $\eta_p^2 = 0.05$ (Post6, M = 2043 ms, SEM = 84 ms; Post24, M = 1997 ms, SEM = 61 ms; Post72, M = 1928 ms, SEM = 70 ms). In contrast, TT across the test blocks for the participants assigned to the RP-AtDCS condition differed significantly, $F(2,72) = 13.11$, $p < .001$, $\eta_p^2 = 0.27$. Subsequent post-hoc assessment revealed that these individuals exhibited a significant reduction in TT from the previous test block such that TT at Post6 (M = 1714 ms, SEM = 52 ms) was greater that the TT observed at the Post24 test block (*M* = 1550 ms, *SEM* = 52 ms) which in turn was greater than the TT reported for the Post72 test block (*M* = 1340 ms, *SEM* = 39 ms).

Impact of Cathodal tDCS at SMA during IP on long-term retention. The TT data for the Post6, Post24, and Post72 test blocks for individuals that experienced IP were submitted to a 2 (Stimulation: IP-Sham, IP-CtDCS) × 3 (Block: Post6, Post24, Post72) ANOVA with repeated measures on the last factor. This analysis revealed a significant main effect of Block, $F(2,72)$ = 68.95, $p < .001$, $\eta_p^2 = 0.66$. Subsequent post-hoc assessment of the Block main effect revealed that TT at the Post6 test block (*M* = 1900 ms, *SEM* = 51 ms) was significantly greater than the TT displayed during the Post24hr test block (*M* = 1720 ms, *SEM* = 46 ms) which in turn was significantly greater than the TT observed for the Post72 test block (*M* = 1582 ms, *SEM* = 38 ms). The main effect of Stimulation, $F(1,36) = 0.00$, $p = 0.97$, $\eta_p^2 = 0$, and the Stimulation x Block interaction, $F(2,72) = 0.63$, $p = 0.53$, $\eta_p^2 = 0.02$, failed to reach significance.

To further examine the impact of practice schedule and stimulation on long-term retention an approach previously adopted by Reis et al. (2009) was used. Specifically, a linear function was fit to the TT data from the Post6, Post24, and Post72 test blocks for each individual and the slope was used as an index of rate of gain or loss over time following the completion of early consolidation (see Figure.9). The slope for each individual was subjected to separate one-way t-tests for the RP conditions (i.e., RP-AtDCS, RP-Sham) and IP conditions (i.e., IP-CtDCS, IP-Sham). The analysis for RP revealed that the greater rate of improvement in TT across test blocks for individuals in the RP-AtDCS condition (M = 4.42 ms/hrs, SEM = 0.63 ms/hr) compared to their RP-sham stimulation counterparts (M = 1.68 ms/hrs, SEM = 1.34 ms/hr) approached conventional level of significance, $t(36) = -1.85$ $p = 0.07$. A similar assessment for the IP revealed that the rate of improvement in TT across test blocks for the individuals in the IP-CtDCS condition (M = 4.32 ms/hrs, SEM =0.59) did not differ from that observed for the IP-Sham condition (M = 4.51 ms/hrs, SEM = 0.68), $t(36) = -0.22$, $p = 0.83$.

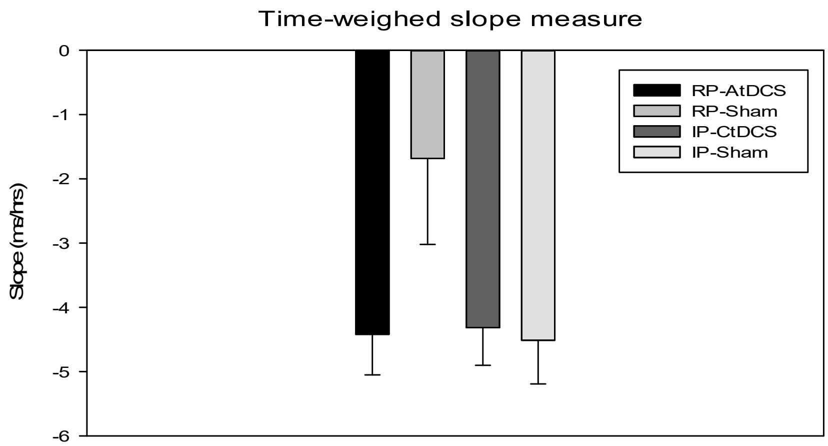

Figure 9. The slope of linear function in TT data from Post6 to Post72 test blocks. The slope reflects the rate of improvement in TT across time such that a more negative slope represents greater gain in DSP task performance. Error bars are standard errors.

As a whole these data indicated that modifying neural activity at SMA using anodal-tDCS during RP influenced long-term retention for novel motor memories. RP result in greater benefit from time dependent consolidation as reflected in non-significant change in TT from the Post-I to Post6hrs. Moreover, administration of anodal tDCS during RP facilitates overnight consolidation by inducing significant improvements in TT with each additional sleep episode. In contrast, the individuals assigned to the RP-Sham condition did not show any improvement from sleep-dependent consolidation while also revealing significant forgetting of the trained DSP tasks during the early consolidation phase. For IP, cathodal stimulation failed to influence the behavioral outcomes associated with IP observed for participants of the IP-sham condition. Specifically, IP resulted in a stable memory during the initial early time-dependent consolidation period and fostered enhancement of this memory with exposure to multiple sleep episodes. Supplementing IP with cathodal stimulation was not sufficient to disrupt these outcomes.

Impact of tDCS at SMA for initiation, concatenation, and execution processes used during DSP task production during RP and IP

Additional analyses were conducted to evaluate if the influence of stimulation of SMA during RP and IP was restricted to specific planning processes that have been described as central to the production of DSP tasks such as those used in the present work (Abrahamse et al., 2013). Recall that implementation of a discrete motor sequences involves a number of independent planning processes, namely initiation, concatenation, and execution that can be isolated by using pre-structured DSP tasks such as those used in the present work [33,34]. As noted earlier, when using a DSP task, the latency associated with the first key press captures the costs of sequence initiation referred to herein as initiation time (IT). The response time to the key press following the random non-0 RSI (key press 4) reflects the cost of transitioning between two motor chunks and is termed concatenation time (CT). The remaining key presses (2, 3, 5 and 6 for DSP tasks in Experiment1) each depend solely on execution demands and contributed only to execution time (ET) [33].

Baseline DSP task performance

Initiation (IT), concatenation (CT), and execution time (ET) data from the baseline test trials prior to any practice for all individuals was subjected to a 4 (Stimulation: RP-Sham, IP-Sham, RP-AtDCS, IP-CtDCS) between-subject (ANOVA) which failed to reveal a significant main effect of

Stimulation for IT, $F(3, 72) = 0.04$, $p = 0.99$, $\eta_p^2 = .001$, CT , $F(3, 72) = 1.89$, $p = 0.14$, $\eta_p^2 = 0.07$ and ET , $F(3, 72) = 2.23$, $p = 0.09$, $\eta_p^2 = 0.08$. Thus, as expected, IT, CT and ET did not differ as a function of stimulation condition prior to any exposure to training and stimulation.

Contextual interference revisited: Online and offline changes in initiation, concatenation, and execution for individuals that experienced RP and IP with sham stimulation

Online changes in initiation, concatenation, and execution during RP and IP with sham stimulation. The IT, CT, and ET for each individual assigned to the RP and IP sham conditions were submitted to separate 2 (Schedule: RP-Sham, IP-Sham) × 2 (Block: baseline, PostI) ANOVA with repeated measures on the last factor.

For IT, the main effect of Schedule $F(1,36) = 2.25$, $p = 0.14$, $\eta_p^2 = 0.06$ was not significant. However, the main effect of Block, $F(1,36) = 89.43$, $p < .001$, $\eta_p^2 = 0.71$ and the Schedule x Block interaction, $F(1,36) = 5.11$, $p < .05$, $\eta_p^2 = 0.12$ were significant. Simple main effect analyses of the Schedule x Block interaction indicated that IT for the baseline test block did not differ significantly for individuals in the RP-Sham (M = 544 ms, SEM = 24 ms) and IP-Sham (M = 536 ms, SEM = 23 ms) schedule conditions. However, IT at PostI for individuals in the RP-Sham condition (M = 336 ms, SEM = 14 ms) was significantly lower than the IT observed for the individuals in the IP-Sham condition (M = 408 ms, SEM = 15 ms), $F(1,36) = 8.26$, $p < .01$, $\eta_p^2 = 0.19$.

For CT, the main effects of Schedule, $F(1,36) = 5.17$, $p < .05$, $\eta_p^2 = 0.13$, and Block, $F(1,36) = 70.46$ $p < .001$, $\eta_p^2 = 0.66$ were significant. However, the Schedule x Block interaction, $F(1,36) = 1.08$, $p = 0.31$, $\eta_p^2 = 0.03$ was not significant. CT for participants in the RP-sham condition (M = 414 ms, SEM = 19) was significantly greater than observed for their IP (M = 368 ms, SEM = 15) counterparts. The Block main effect was a function of CT for the baseline test block (*M* = 449 ms, *SEM* = 18) being significantly greater than the CT reported during the PostI test block (*M* = 333 ms, *SEM* = 16).

For ET, the main effects of Block, $F(1,36) = 301.2$, $p < .001$, $\eta_p^2 = 0.89$ was significant. This main effect was a result of ET for the Baseline test block (*M* = 467 ms, *SEM* = 15) being significantly greater than the ET reported during the PostI test block (*M* = 286 ms, *SEM* = 12). The main effect of Schedule, $F(1,36) = 1.46$ $p = 0.23$, $\eta_p^2 = 0.04$ was not significant. The Stimulation x Block interaction, $F(1,36) = 3.96$, $p = 0.06$, $\eta_p^2 = 0.1$ approached conventional level of significance. Simple main effect analyses of the Schedule x Block interaction indicated that ET at baseline test block for individuals in the IP-Sham condition (*M* = 446 ms, *SEM* = 16 ms) was significantly lower than the ET observed for the individuals in the RP-Sham condition (*M* = 487 ms, *SEM* = 15 ms), $F(1,36) = 7.56$, $p < .01$, $\eta_p^2 = 0.19$. However, ET at PostI test block did not differ significantly for individuals in the RP-Sham (*M* = 285 ms, *SEM* = 11 ms) and IP-Sham (*M* = 286 ms, *SEM* = 12 ms) schedule conditions.

Offline gain in initiation, concatenation, and execution during RP and IP with sham stimulation. The next analysis focused on the change in IT, CT and ET from the end of the training (i.e., PostI) to the time of test following RP and IP. As was the case when evaluating TT, this initial analysis of delayed test performance used data from the 24-hr test block as most previous studies assessing the impact of CI for offline gain addressed this issue by conducting retention tests the day after practice concluded. Thus, IT, CT and ET data for each individual in the RP-sham and IP sham stimulation conditions for the PostI and Post24 test blocks were submitted to separate 2

(Schedule: RP-Sham, IP-Sham) × 2 (Block: postI, post24) ANOVA with repeated measures on the last factor.

For IT, the main effect of Schedule approached conventional significance levels, $F(1,36) = 3.94$, $p = 0.06$, $\eta_p^2 = 0.1$ but the Block main effect was not significant, $F(1,36) = 0.95$, $p = 0.34$, $\eta_p^2 = 0.02$. However, the Schedule x Block interaction, $F(1,36) = 13.18$, $p < .001$, $\eta_p^2 = 0.27$ was significant. Simple main effect analyses of this interaction revealed that IT at PostI for individuals in the RP-Sham condition (*M* = 336 ms, *SEM* = 14 ms) was significantly lower than the IT for the individuals in the IP-Sham condition (*M* = 408 ms, *SEM* = 15 ms), $F(1,36) = 35.54$, $p < .001$, $\eta_p^2 = 0.5$. In contrast, IT for the Post24 test block did not differ significantly for individuals in the RP-Sham (*M* = 359 ms, *SEM* = 18 ms) and IP-Sham (*M* = 369 ms, *SEM* = 16 ms) stimulation conditions. The reduction in IT from the PostI to Post24 test block for the IP-Sham condition was approached significant, $F(1,36) = 10.60$, $p=0.01$, $\eta_p^2 = 0.23$. IT from the PostI to Post24 test approached significance for the individuals in the RP-Sham stimulation condition, $F(1,36) = 3.53$, $p = 0.07$, $\eta_p^2 = 0.09$, with a greater IT at the post24 test block indicating some forgetting.

For CT, the main effect of Schedule, $F(1,36) = 7.10$, $p = 0.01$, $\eta_p^2 = 0.16$ and Block, $F(1,36) = 5.32$, $p < .05$, $\eta_p^2 = 0.13$ were significant. However, the Schedule x Block interaction, $F(1,36) = 2.54$, $p = 0.12$, $\eta_p^2 = 0.07$ was not significant. Post-hoc analysis of the Schedule main effect indicated that CT was lower for individuals in the IP-Sham condition (*M* = 297 ms, *SEM* = 11) than for those assigned to RP-Sham (*M* = 345 ms, *SEM* = 17). The Block main effect was a result of CT for the PostI test block (*M* = 333 ms, *SEM* = 16) being significantly greater than the CT reported during the Post24 test block (*M* = 309 ms, *SEM* = 12).

For ET, the main effect of Schedule, $F(1,36) = 3.05$, $p = 0.08$, $\eta_p^2 = 0.1$ and Block, $F(1,36) = 0.74$, $p = 0.4$, $\eta_p^2 < 0.01$ were not significant. However, the Schedule x Block interaction, $F(1,36) = 17.24$, $p < .001$, $\eta_p^2 = 0.32$ was significant. Simple main effect analyses of this interaction revealed that ET for the PostI test block did not differ significantly for individuals in the RP-Sham (*M* = 285 ms, *SEM* = 11 ms) and IP-Sham (*M* = 286 ms, *SEM* = 12 ms) stimulation conditions. However, ET at Post24 for individuals in the IP-Sham condition (*M* = 266 ms, *SEM* = 9 ms) was significantly lower than the ET for the individuals in the RP-Sham condition (*M* = 316 ms, *SEM* = 10 ms), $F(1,36) = 33.21$, $p < .001$, $\eta_p^2 = 0.48$. The reduction in ET from the PostI to Post24 test block for the IP-Sham condition was significant, $F(1,36) = 5.41$, $p < .05$, $\eta_p^2 = 0.13$. Whereas, ET from the PostI to Post24 test blocks for the individuals in the RP-Sham stimulation condition showed that ET significantly increased, $F(1,36) = 12.58$, $p = 0.01$, $\eta_p^2 = 0.26$.

Analyses of IT, CT, and ET were conducted to investigate if the well-documented impact of IP and RP for online and offline performance is restricted to a specific process that have been delineated as central to the implementation of discrete motor sequences. Taken together these analyses revealed that the benefit from IP that develop across a 24-hr retention interval are quite broad emerging for initiation, concatenation, and execution processes. It is worth noting however that the benefit of IP for concatenation emerged during encoding and remained thereafter. In contrast, improvements in initiation and execution only surfaced following a period of consolidation. That is, initiation and execution processes were similar at the conclusion of practice for the RP-sham and IP-sham conditions but only the individuals exposed to IP showed improvement in the implementation of these processes after 24-hr. In contrast, following RP, significant degradation in the initiation and execution was observed.

Online change in initiation, concatenation, and execution processes during RP and IP in the presence of stimulation

Figure 10 and 11 display IT, CT, and ET for all test blocks administered prior to (Baseline) and after the completion of training (PostI, Post6, Post24, Post72) for the individuals that were assigned to RP (Figure 10: RP-Sham, RP-AtDCS) and IP (Figure 11: IP-Sham, IP-CtDCS) conditions.

Influence of anodal tDCS at SMA on initiation, concatenation, and execution processes during RP. IT, CT and ET data for the Baseline and PostI test blocks for individuals that experienced RP were separately submitted to a 2 (Stimulation: RP-Sham, RP-AtDCS) × 2 (Block: Baseline, PostI) ANOVA with repeated measures on the last factor. For IT, this analysis revealed a significant main effect of Block, $F(1,36) = 170.1$, $p < .001$, $\eta_p^2 = 0.83$. This main effect was a result of IT for the Baseline test block (M = 539 ms, SEM = 24 ms) being significantly greater than the IT noted during the PostI test block (M = 320 ms, SEM = 13 ms). The main effect of Stimulation, $F(1,36) = 0.89$, $p = 0.36$, $\eta_p^2 = 0.02$ and the Stimulation x Block interaction, $F(1,36) = 0.41$, $p = 0.53$, $\eta_p^2 = 0.01$ failed significance. Experiencing RP resulted in online gains in IT across training and this improvement in IT was not modified by supplementing RP with anodal tDCS. For CT, this analysis revealed a significant main effect of Block, $F(1,36) = 98.56$, $p < .001$, $\eta_p^2 = 0.73$. This main effect was a result of IT for the Baseline test block (M = 460 ms, SEM = 19 ms) being significantly greater than the CT noted during the PostI test block (M = 331 ms, SEM = 15 ms). The main effect of Stimulation, $F(1,36) = 3.05$, $p = 0.09$, $\eta_p^2 = 0.08$ and the Stimulation x Block interaction, $F(1,36) = 0.00$, $p = 0.99$, $\eta_p^2 = 0$ failed significance. As was the case for IT, experiencing RP resulted in a significant reduction in CT across practice but this improved was not influenced by the administration of anodal tDCS.

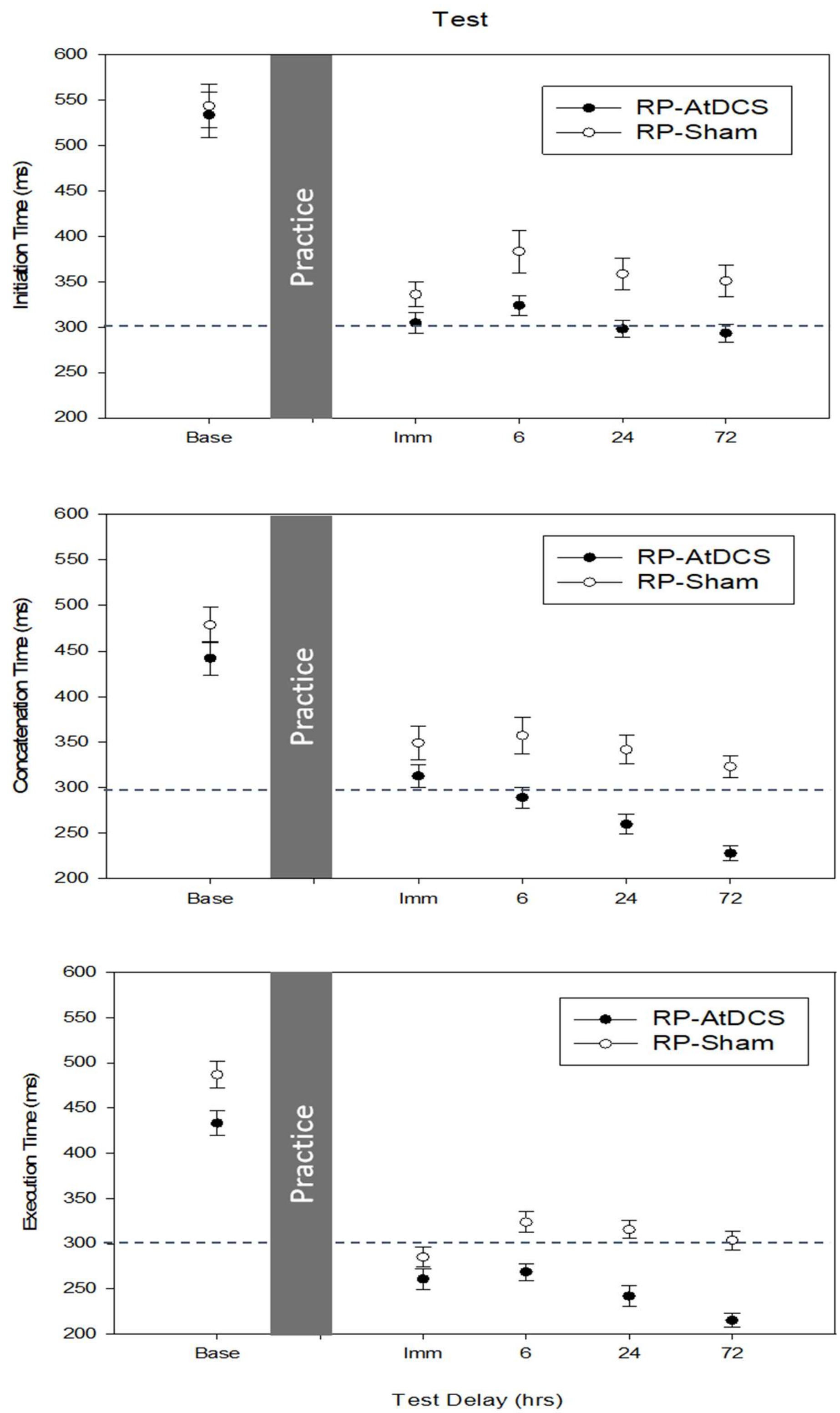

Figure 10. IT (top), CT (middle), and ET (bottom) at Baseline (Base), immediately after practice (Imm), 6-hr post practice (6), 24-hr post practice (24), and 72-hr post practice (72) test blocks for individuals assigned to the RP- AtDCS and RP-sham conditions. Error bars are standard errors. Letters indicate scores differed significantly, $p < .05$.

For ET, this analysis revealed a significant main effect of Block, $F(1,36) = 268.3$, $p < .001$, $\eta_p^2 = 0.88$ and Stimulation, $F(1,36) = 7.85$, $p = 0.01$, $\eta_p^2 = 0.03$. This main effect of Block was a result of ET for the Baseline test block (*M* = 460 ms, *SEM* = 14 ms) being significantly greater than the ET observed during the PostI test block (*M* = 273 ms, *SEM* = 11 ms). The main effect of Stimulation revealed that ET can be lowered by practicing in the presence of anodal tDCS during RP compared to just being exposed to RP (RP-AtDCS: *M* = 347 ms, *SEM* = 12 ms; RP-Sham: *M* = 386 ms, *SEM* = 13 ms). The Stimulation x Block interaction, $F(1,36) = 1.65$, $p = 0.21$, $\eta_p^2 = 0.04$ failed significance.

Influence of cathodal tDCS at SMA on initiation, concatenation, and execution processes during IP. IT, CT and ET data for the Baseline and PostI test blocks for individuals that experienced RP were separately submitted to a 2 (Stimulation: IP-Sham, IP-CtDCS) × 2 (Block: Baseline, PostI) ANOVA with repeated measures on the last factor.

For IT, this analysis revealed a significant main effect of Block, $F(1,36) = 69.28$, $p < .001$, $\eta_p^2 = 0.66$. This main effect was a result of IT for the Baseline test block (*M* = 535 ms, *SEM* = 23 ms) being significantly greater than the IT noted during the PostI test block (*M* = 369 ms, *SEM* = 16 ms). The main effect of Stimulation, $F(1,36) = 0.35$, $p = 0.56$, $\eta_p^2 = 0.01$ and the Stimulation x Block interaction, $F(1,36) = 1.24$, $p = 0.27$, $\eta_p^2 = 0.03$ failed significance. Thus, while IT improved with IP, this improvement was not influenced by the supplementation of cathodal tDCS.

For CT, this analysis revealed a significant main effect of Block, $F(1,36) = 62.18$, $p < .001$, $\eta_p^2 = 0.63$ and Stimulation, $F(1,36) = 4.61$, $p < 0.05$, $\eta_p^2 = 0.11$. The main effect of Block was a result of CT for the Baseline test block (*M* = 438 ms, *SEM* = 18 ms) being significantly greater than the CT noted during the PostI test block (*M* = 345 ms, *SEM* = 17 ms). The main effect of Stimulation was a result of CT being increased when cathodal stimulation was paired with IP (IP-CtDCS: *M* = 415 ms, *SEM* = 20 ms; IP-Sham: *M* = 386 ms, *SEM* = 15 ms). The Stimulation x Block interaction, $F(1,36) = 0.59$, $p = 0.45$, $\eta_p^2 = 0.02$ failed significance.

For ET, this analysis revealed a significant main effect of Block, $F(1,36) = 210.5$, $p < .001$, $\eta_p^2 = 0.85$. This main effect was a result of ET for the Baseline test block (*M* = 456 ms, *SEM* = 17 ms) being significantly greater than the ET noted during the PostI test block (*M* = 287 ms, *SEM* = 11 ms). The main effect of Stimulation, $F(1,36) = 0.33$, $p = 0.57$, $\eta_p^2 = 0.01$ and the Stimulation x Block interaction, $F(1,36) = 0.36$, $p = 0.55$, $\eta_p^2 = 0.01$ failed significance. ET was reduced with IP and was not modified by supplementation of cathodal tDCS.

In general, for RP, practice led to a reduction in the time to initiate, concatenate, and execute elements of the practice motor sequence. The inclusion of anodal stimulation during RP appeared to result in a reduction in time to execute elements in the sequence. It should be noted however that the lack of an interaction for ET indicates that the improvement in ET was present at both tests suggesting that individuals assigned to the real stimulation condition were generally more effective at implementing the execution process. Similar outcomes emerged for IP. That is, there was a general improvement for all processes as a result of practice. Improvements from real stimulation in the IP condition for CT and ET once again appeared to reflect that individuals assigned to this condition were initially faster during the baseline test block.

Offline gains resulting from early consolidation in initiation, concatenation, and execution processes

There is considerable literature addressing the importance of early time-dependent consolidation, independent on sleep [39], for the development of motor memories [40]. The influence of the practice format experienced coupled with stimulation on early consolidation was evaluated using IT,CT, and ET data from the PostI and Post6 test blocks. These data are depicted in Figure 9 and 10 for RP and IP respectively. As was the case with earlier assessments, separate analyses were conducted to evaluate the impact of the application of anodal and cathodal tDCS at SMA during RP and IP.

Impact of Anodal tDCS at SMA during RP on early time-dependent consolidation. IT, CT, and ET data from PostI and Post6 test blocks for individuals trained with RP were submitted to separate 2 (Stimulation: RP-Sham, RP-AtDCS) × 2 (Block: PostI, Post6) ANOVA with repeated measures on the last factor.

For IT, this analysis revealed a significant main effect of Block, $F(1,36) = 13.10$ $p < .001$, $\eta_p^2 = 0.27$ and Stimulation, $F(1,36) = 5.01$, $p < 0.05$, $\eta_p^2 = 0.12$. This main effect of Block revealed that IT was slowed across the first six-hrs following practice (Post6 test block, *M* = 354 ms, *SEM* = 17 ms; PostI test block *M* = 320 ms, *SEM* = 18 ms). The main effect of Stimulation showed that individuals that had RP supplemented with anodal tDCS continued to exhibit a reduced IT across the 6-hrs after practice concluded (RP-AtDCS: *M* = 314 ms, *SEM* = 11 ms) compared to the individuals that merely experienced RP (RP-Sham: *M* = 360 ms, *SEM* = 19 ms). The Stimulation x Block interaction, $F(1,36) = 2.36$, $p = 0.13$, $\eta_p^2 = 0.06$ failed significance.

For CT, the main effect of Block, $F(1,36) = 0.96$, $p = 0.33$, $\eta_p^2 = 0.03$ was not significant. However, the main effect of Stimulation, $F(1,36) = 5.96$, $p < .05$, $\eta_p^2 = 0.14$ and Stimulation x Block interaction, $F(1,36) = 4.05$, $p < .05$, $\eta_p^2 = 0.1$ were significant. Simple main effect analyses of this interaction revealed that individuals in the RP-AtDCS showed significantly lower CT at PostI and Post6 compared to RP-Sham. In addition, the reduction in CT from the PostI (M = 312 ms, SEM = 12 ms) to Post6 (M = 289 ms, SEM = 11 ms) test block for the RP-AtDCS condition was significant, $F(1,36) = 4.47$, $p <.05$, $\eta_p^2 = 0.11$. In contrast, CT from the PostI (M = 357 ms, SEM = 20 ms) to Post6 (M = 341 ms, SEM = 16 ms) test blocks for the individuals in the RP-Sham stimulation condition were not significantly different, , $F(1,36) = 0.54$, $p =0.47$, $\eta_p^2 = 0.01$.

For ET, the main effect of Block, $F(1,36) = 18.52$, $p < .001$, $\eta_p^2 = 0.34$, and Stimulation, $F(1,36) = 7.71$, $p = 0.01$, $\eta_p^2 = 0.17$ were significant. Moreover, the Stimulation x Block interaction, $F(1,36) = 8.20$, $p = 0.01$, $\eta_p^2 = 0.19$ was significant. Simple main effect analyses of this interaction revealed that individuals in RP-AtDCS showed significantly lower ET at PostI and Post6 test block than individuals in the RP-Sham. In addition, individuals in RP-AtDCS exhibited stable performance across the PostI (M = 261 ms, SEM = 11 ms) to Post6 (M = 269 ms, SEM = 9 ms) test block, $F(1,36) = 1.05$, $p = 0.31$, $\eta_p^2 = 0.03$. However, individuals in RP-Sham condition exhibited a significant increase in ET between the PostI (M = 285 ms, SEM = 11 ms) to Post6 (M = 324 ms, SEM = 11 ms), $F(1,36) = 25.73$, $p <.001$, $\eta_p^2 = 0.42$.

Impact of Cathodal tDCS at SMA during IP on early time-dependent consolidation. IT, CT, and ET data for the postI and post6 test blocks for individuals that experienced IP were submitted to separate 2 (Stimulation: IP-Sham, IP-CtDCS) × 2 (Block: postI, post6) ANOVA with repeated measures on the last factor.

For IT, this analysis revealed a significant main effect of Block, $F(1,36) = 7.31$ $p = 0.01$, $\eta_p^2 = 0.17$. This main effect of Block was a result of IT for the PostI block (M = 422 ms, SEM = 15 ms) being significantly greater than the IT noted during the Post6 test block (M = 400 ms, SEM = 15 ms). The main effect of Stimulation, $F(1,36) = 1.35$ $p = 0.26$, $\eta_p^2 = 0.04$ and Stimulation x Block interaction, $F(1,36) = 0.35$, $p = 0.56$, $\eta_p^2 = 0.02$ failed significance. For CT, the main effect of Block, $F(1,36) = 4.91$, $p < .05$, $\eta_p^2 = 0.12$, and Stimulation, $F(1,36) = 6.25$, $p < .05$, $\eta_p^2 = 0.15$ were significant. Subsequent post-hoc analyses of the significant Block main effect resulted from CT being lower for the Post6 (M = 323 ms, SEM = 14 ms) compared to that observed at PostI (M = 345 ms, SEM = 17 ms) test block. In the case of the Stimulation main effect, CT was lower for individuals that were exposed to IP only (M = 311 ms, SEM = 12 ms) compared to those that were privy to IP supplemented with cathodal stimulation (M = 357 ms, SEM = 19 ms). For ET, the main effect of Block, $F(1,36) = 0.01$, $p = 0.92$, $\eta_p^2 = 0$, Stimulation, $F(1,36) = 0.00$, $p = 0.99$, $\eta_p^2 = 0$, and the Stimulation x Block interaction, $F(1,36) = 0.59$, $p = 0.45$, $\eta_p^2 = 0.02$ were not significant.

Thus, the administration of cathodal stimulation in conjunction with IP had no impact on the evolution of ET across the initial six-hrs following the completion of practice.

Two important findings emerged with respect to the component processes and early time dependent consolidation. First, recall that for TT, supplementing RP with anodal stimulation at SMA led to improved time-dependent consolidation which was manifest as stable performance (i.e., no change in TT) across the first six hours after practice concluded. This is in contrast to significant forgetting that is frequently observed following RP during the early consolidation phase. The present set of analyses focused on the component processes reveals that this the improvement from stimulation at SMA results from enhanced concatenation and execution. For both of these component processes, anodal stimulation eliminated forgetting associated with their RP-sham counterpart's performance. Improving concatenation is congruent with the described role of SMA for fostering the development of and transitioning between motor chunks [20]. Second, contrary to the influence of anodal stimulation in RP, the administration of a cathodal stimulation during IP had no functional consequence for early time-dependent consolidation.

Long-term changes in initiation, concatenation, and execution processes associated with administration of tDCS at SMA during repetitive and interleaved practice

As was the case when addressing TT, evaluation of the impact of practice format and stimulation on long-term retention involved examination of performance from the Post6, Post24, and Post72 test blocks. Congruent with earlier assessments, separate analyses were conducted for IT, CT, and ET to evaluate the impact of anodal and cathodal tDCS at SMA during RP and IP respectively.

Impact of Anodal tDCS at SMA during RP on long-term change in IT, CT, and ET. IT, CT and ET data for the Post6. Post24, Post72 test blocks for individuals that experienced RP were submitted to separate 2 (Stimulation: RP-Sham, RP-AtDCS) × 3 (Block: Post6, Post24, Post72) ANOVAs with repeated measures on the last factor.

For IT, this analysis revealed significant main effects of Stimulation, $F(1,36) = 8.71$, $p < .01$, $\eta_p^2 = 0.19$ and Block, $F(2,72) = 7.76$, $p < .01$, $\eta_p^2 = 0.18$. Subsequent post-hoc analysis for the main effect of Stimulation indicated that RP-AtDCS ($M = 305$ ms, $SEM = 10$ ms) showed significantly lower IT across Post6, Post24, and Post72 test blocks than individuals in the RP-Sham condition

(M = 364 ms, SEM = 20 ms). Interpretation of the significant main effects of Block revealed that IT at Post6 (M = 354 ms, SEM = 17 ms) was significantly greater than Post24 test block (M = 328 ms, SEM = 14 ms), but which was not significant compared to Post 72 test block (M = 322 ms, SEM = 14 ms) for the participants assigned to the both RP conditions. The Stimulation x Block interaction failed to reach significant level, $F(2,72) = 0.02$, p =0.98, $\eta_p^2 < 0.01$.

For CT, this analysis revealed significant main effects of Stimulation, $F(1,36) = 25.85$, $p < .001$, $\eta_p^2 = 0.42$ and Block, $F(2,72) = 13.43$, $p < .001$, $\eta_p^2 = 0.27$. Subsequent post-hoc analysis of the Stimulation main effect indicated that RP-AtDCS (M = 259 ms, SEM = 10 ms) showed significantly lower CT across Post6, Post24, and Post72 test blocks than individuals in the RP-Sham condition (M = 340 ms, SEM = 16 ms). Interpretation of the Block main effect revealed that CT was gradually reduced across post6 (M = 323 ms, SEM = 16 ms) to post72 (M = 257 ms, SEM = 10 ms) test blocks. The Stimulation x Block interaction failed to reach significant level, $F(2,72) = 1.10$, p =0.34, $\eta_p^2 = 0.03$.

For ET, this analysis revealed significant main effects of Stimulation, $F(1,36) = 35.42$, $p < .001$, $\eta_p^2 = 0.5$, Block, $F(2,72) = 15.09$, $p < .001$, $\eta_p^2 = 0.27$ and the Stimulation x Block interaction, $F(2,72) = 3.08$, $p < .05$, $\eta_p^2 = 0.08$. Simple main effect analyses of the interaction revealed that RP-AtDCS (M = 242 ms, SEM = 10 ms) showed significantly lower ET across Post6, Post24, and Post72 test blocks than individuals in the RP-Sham condition (M = 314 ms, SEM = 11 ms) as expected. Moreover, ET for the PostI, Post24, and Post72 test blocks did not differ significantly for individuals in the RP-Sham, $F(2,72) = 2.30$, $p = 0.11$, $\eta_p^2 = 0.06$. In contrast, individuals in RP-AtDCS exhibited a significant reduction in ET, $F(2,72) = 15.88$, $p < .001$, $\eta_p^2 = 0.3$ from the Post6 (M = 269 ms, SEM = 9 ms) was greater that the ET observed at the Post24 test block (M = 242 ms, SEM =

12 ms) which in turn was greater than the ET reported for the Post72 test block (*M* = 215 ms, *SEM* = 8 ms).

Impact of Cathodal tDCS at SMA during IP on long-term change in IT, CT, and ET. IT, CT, and ET data for the Post6, Post24, Post72 test blocks for individuals that experienced IP were submitted to separate 2 (Stimulation: IP-Sham, IP-CtDCS) × 3 (Block: Post6, Post24, Post72) ANOVA with repeated measures on the last factor.

For IT, this analysis revealed a significant main effects of Block, $F(2,72) = 22.56$, $p < .001$, $\eta_p^2 = 0.39$. Subsequent post-hoc assessment of Block main effect revealed that IT at the Post6 test block (*M* = 400 ms, *SEM* = 15 ms) was significantly greater than the IT at the Post24hr (*M* = 366 ms, *SEM* = 16 ms) which was significantly greater than IT Post72 (*M* = 344 ms, *SEM* = 17 ms). The main effect of Stimulation, $F(1,36) = 0.13$, $p = 0.72$, $\eta_p^2 <.01$, and Stimulation x Block interaction, $F(2,72) = 0.96$, $p = 0.39$, $\eta_p^2 = 0.02$, failed to reach significance level.

For CT, this analysis revealed significant main effects of Block, $F(2,72) = 39.29$, $p < .001$, $\eta_p^2 = 0.52$. Subsequent post-hoc assessment of Block main effect revealed that CT at the Post6 test block (*M* = 323 ms, *SEM* = 14 ms) was significantly greater than the CT at the Post24hr (*M* = 281 ms, *SEM* = 8 ms) which was significantly greater than CT Post72 (*M* = 252 ms, *SEM* = 7 ms). The main effect of Stimulation, $F(1,36) = 2.57$, $p = 0.12$, $\eta_p^2 =0.07$, and Stimulation x Block interaction, $F(2,72) = 0.96$, $p = 0.39$, $\eta_p^2 = 0.02$, failed to reach significance level.

For ET, this analysis revealed significant main effects of Block, $F(2,72) = 55.55$, $p < .001$, $\eta_p^2 = 0.6$. Subsequent post-hoc assessment of Block main effect revealed that ET at the Post6 test block (*M* = 287 ms, *SEM* = 9 ms) was significantly greater than the ET at the Post24hr (*M* = 264 ms, *SEM* = 8 ms) which was significantly greater than ET Post72 (*M* = 240 ms, *SEM* = 6 ms). The main effect of Stimulation, $F(1,36) = 0.10$, $p = 0.76$, $\eta_p^2 < .01$, and Stimulation x Block interaction, $F(2,72) = 0.26$, $p = 0.77$, $\eta_p^2 < .01$, failed to reach significance level.

As noted earlier, TT continued to be reduced across the delayed test blocks when administration of anodal tDCS at SMA accompanied RP suggesting an impact of exogenous stimulation of long-term retention. Unlike early consolidation enhancement in TT being a result of improved concatenation and execution, in the case of long-term retention, it appears this is solely a function of more effective implementation of the execution process. As was the case in earlier analyses, application of cathodal tDCS at SMA during IP appear inconsequential with respect to long-term retention of newly formed memories.

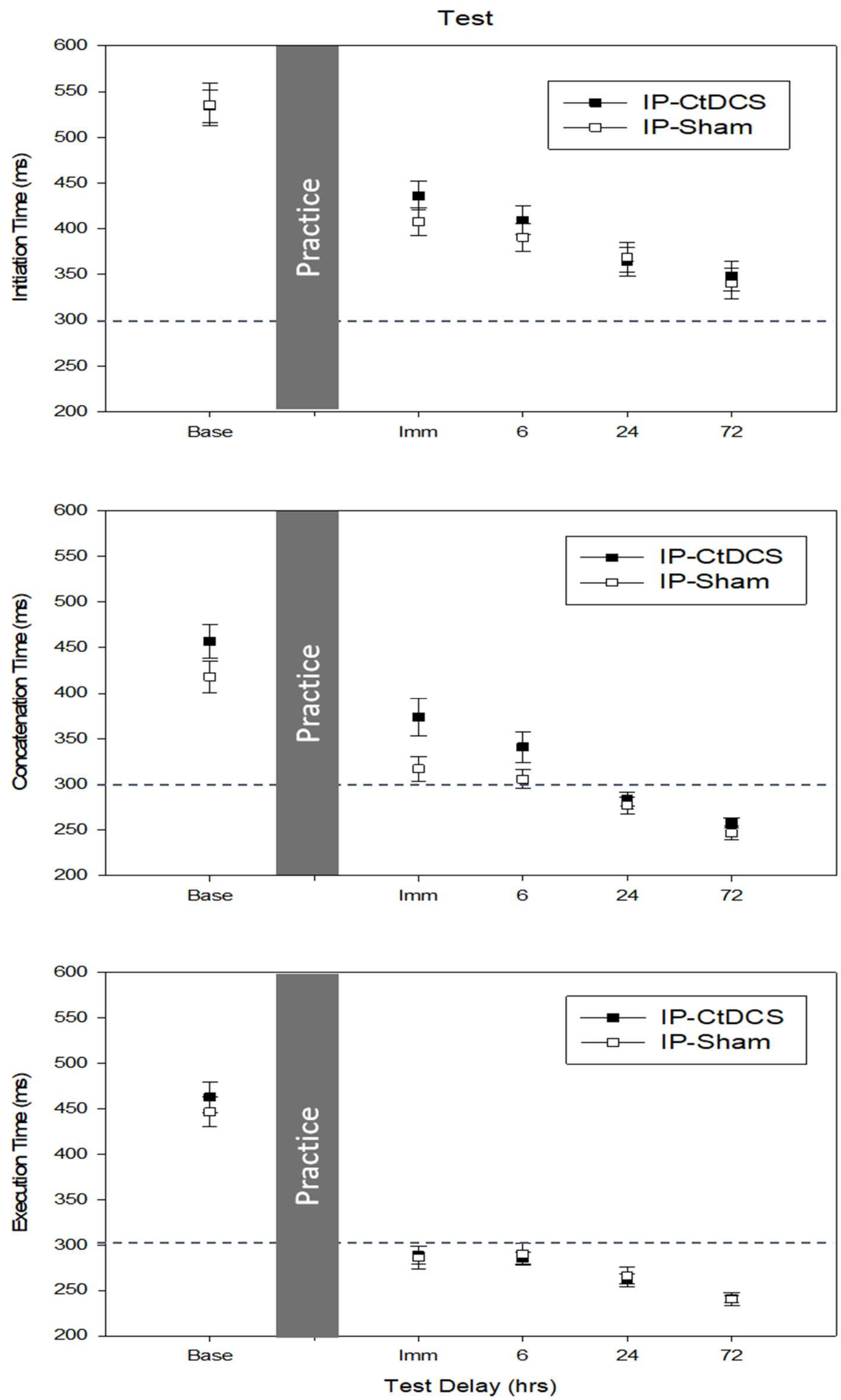

Figure 11. IT (top), CT (middle), and ET (bottom) at Baseline (Base), immediately after practice (Imm), 6-hr post practice (6), 24-hr post practice (24), and 72-hr post practice (72) test blocks for individuals assigned to the IP- CtDCS and IP-sham conditions. Error bars are standard errors. Letters indicate scores differed significantly, p < .05.

Discussion

Experiment 1 was designed to examine a potential limitation in earlier work by Kim & Wright (submitted) that the SMA region was not appropriately targeted during anodal and cathodal transcranial direct current stimulation (tDCS) during interleaved and repetitive practice formats. Specifically, there were concerns regarding both the positioning of and size of the electrodes used in the previous work to facilitate or inhibit participation of the SMA complex during practice. Kim and Wright (submitted) used 5 x 7 cm^2 electrodes for stimulation and reference. Given the size of the target electrode it's possible that substantial overlap existed with the premotor area that is closely located next to the SMA. This is particularly concerning given PMd in particular has been identified as being differentially activated during RP and IP formats and may play an important role in determining retention performance [2,17]. Kim and Wright (submitted) also used slightly different montages to target SMA than has been adopted previous in the literature by placing the anode 3 cm anterior of Cz (determined based on the International 10-20 system) and 2 cm to the right of this location.

In the present case, 5 x 5 cm^2 electrodes were used for the target area and placed 3 cm anterior of Cz [36,37]. It was anticipated that the use of smaller size of electrode at the target site would increase focality at SMA thus maximizing the current distribution at the target location compared to the previous study (Kim & Wright, submitted). A priori modeling using HD-Explore™ did indicate sizeable current distribution at MNI coordinates congruent with the SMA complex that was similar to that observed in the earlier study (see Figure 2). The a priori modelling in the previous study revealed heightened current flow (.374 V/m^2) at MNI coordinates (x: 7, y: 0, z; 51), a location congruent with pre-SMA described by Mayka et al., (2006). In the present case with the new montage heightened current flow was slightly lower (.331 V/m^2) at MNI coordinates again consistent with SMA complex (x: 4, y: 0, z; 51).

Despite the modification in application of tDCS at the SMA complex used herein, the general behavioral outcomes that emerged in Experiment 1 mirrored those previously reported by Kim and Wright (submitted). The global measure of performance, TT, revealed the frequently observed retention benefit for the individuals assigned to the IP-Sham compared to RP-Sham condition (See Figure. 7 and 8 bottom). As reported by Kim and Wright (submitted) this results from rapid stabilization across the initial 6-hr following practice, a timeframe during which time-dependent consolidation process are reported to occur [41]. A novel finding reported in Kim and Wright was the effectiveness of IP to foster additional improvements across a sizeable amount of time following the conclusion of practice (i.e., up to 72 hours). Presumably IP is more effective at fostering additional consolidation that is ongoing after the first 6-hrs which is likely related to additional time and sleep [3,17]. The findings from the sham conditions in the present study replicated these findings reported earlier and are in line with the broader literature that supports the claim that IP not RP supports long-term evolution of new motor memories.

The findings in Experiment 1, with respect to the use of exogenous tDCS during RP, were again very consistent with those reported by Kim and Wright. Specifically, administering anodal tDCS at SMA during RP resulted in improved time-dependent consolidation and long-term retention. Up-regulating SMA activity during RP eliminated the forgetting that is commonly observed immediately after this practice format. When RP was accompanied by anodal stimulation at SMA, TT remained stable across the first six-hrs which is similar to that observed for RP-sham participants. Thus, stimulation during practice in this case was important for fostering subsequent consolidation processes that occur at a later time after practice has concluded.

The further benefit of supplementing RP with anodal stimulation at SMA for long-term retention, first observed by Kim and Wright, was replicated in Experiment 1. Thus, increasing activity at SMA during RP can induce additional time-dependent and/or sleep-dependent retention benefits customarily not available from this training format. The benefit from real stimulation during RP emerged during the test that was administered after the initial night of sleep and was further enhanced with further sleep episodes. These data suggest that sufficient recruitment of SMA during the encoding of a motor skill is intimately involved in preparing newly formed motor memories for modifications that occur during subsequent periods of overnight sleep. Kim and Wright argued that the retention benefits might result from promoting the establishment of R-R associations (i.e., motor chunks), a process that has previously been ascribed to heightened SMA complex recruitment [20]. Moreover, there is behavioral evidence that motor chunks that are formed during IP are more resilient than those developed during RP. Administering anodal stimulation at SMA appears to be an alternative strategy to promote sufficient SMA activity to nurture the establishment of more resilient motor chunks even during RP.

A novel but important finding emerged from Experiment 1 when evaluating changes in the implementation of component processes central to motor sequence implementation resulting from anodal stimulation at SMA during RP [33]. Specifically, the early time-dependent consolidation benefit, noted for the TT data, afforded by supplemental stimulation during RP was restricted to optimizing concatenation and execution processes. That is, both CT and ET were reduced across the first 6-hr after practice for the RP-AtDCS condition as opposed to increasing (i.e., forgetting) as was the case for the RP-Sham participants. The reduction observed for CT during the early consolidation phase is consistent with the general claim that SMA recruitment is important for motor chunk development and use. In describing the component processes involved during motor sequence implementation, Abrahamse et al., (2013) proposed that the concatenation process is critical for allowing distinct motor chunks within a sequence to be executed in rapid succession. CT is seen as an index of the effectiveness of the transition from one motor chunk to the next allowing the movement sequence to unfold smoothly. This description is quite similar to that offered for the role of the SMA complex by Kennerly and colleagues [20].

While the concatenation process was the most likely process that would benefit from anodal stimulation during RP, the additional benefit afforded the execution process was less anticipated. Similar to CT, ET was reduced from consolidation that occurs during the early hours following the termination of practice. It is possible of course that the SMA has multiple functions, beyond those associated with motor chunking, with one of these being more intimately tied to the execution of individual elements contained within each motor chunk. However, an alternative possibility is that the SMA exerts an influence on remote neural site(s) that are more directly involved in execution. For example, if the stimulation used in Experiment 1 induced a broad up-regulation of the SMA complex that included SMA proper, there are well described connections with M1, a neural center likely heavily involved with execution-type processes, from this region [21]. It is feasible then that the SMA complex, when stimulated in a less focal manner as is most likely the case when using tDCS, can impart multiple functional benefits, beyond motor chunk development, that includes a role in the actual execution of newly acquired motor sequences.

The importance of SMA for execution processes is further highlighted by the effectiveness of anodal stimulation during RP for improving long-term retention. In this case, the continued improvement in TT for the RP-AtDCS condition was entirely accounted for by further reductions in ET and not CT. Thus, any offline benefits beyond the initial 6-hrs are not related to facilitating cognitive operations that supervise successful transition between motor chunks that make up a motor sequence. Rather eliciting greater SMA activity during a practice period has important implication for post practice consolidation that targets the ongoing updating of planning activities critical for efficient execution of the specific elements that make each chunk. These data then argue for a far broader impact of SMA on post practice consolidation that is viewed as crucial for the ongoing evolution of motor memories. Clearly, such potential additional role played by the SMA complex for skill acquisition deserves more experimental effort in future work.

Finally, it is important to note that while anodal stimulation at SMA during RP was effective at inducing changes in the neural environment encountered by the learner which fosters superior skill acquisition and retention, the application of cathodal stimulation during IP was not effective at reversing or altering the commonly observed functional benefits commonly associated with IP. In general, experiencing IP alone or in conjunction with cathodal stimulation mattered little with both conditions resulting in effective early time-dependent consolidation and additional improvements that ensued over the next 3-days. It is possible that SMA activity is adequately high during IP that any reduction induced via cathodal stimulation does not down-regulate neural activity to an extent that motor planning processes governed by SMA are hindered. Alternatively, there is considerable evidence that cathodal stimulation is a far less robust method to mediate neural activity than anodal stimulation [42,43]. As such, the actual change in cortical down-regulation in the case of the IP-CtDCS may have been negligible thus playing no role in the resultant behavioral outcomes.

EXPERIMENT 2

While the initial efforts of this work have focused on the SMA complex a recent review addressing the neural underpinnings of the contextual interference (CI) highlighted a potential role of the dorsal premotor cortex (PMd) via the development of critical functional connectivity involving this site that has been reported to predict retention benefits from IP [2,17]. For example, Lin et al. (2011) noted that greater functional coupling between PMd and M1 developed during IP compared to RP which was accompanied by increased excitability at M1 that continued up to 72hr after practice. It was proposed that greater PMd activity during IP may remotely influence the magnitude of M1 excitability which may aid long-term retrieval [2,12,13,17]. Moreover, the magnitude of offline gain from consolidation following IP has been associated with increased resting state connectivity between L-PMd and other key sites motor learning including hippocampus, putamen, and the cerebellum [17]. Stronger resting-state connectivity between the L-PMd and L-DLPFC, bilateral posterior cingulate cortices, and angular gyri/inferior parietal lobule has been reported after interleaved but not repetitive practice. These regions are connected as part of the frontal motor network, involved in motor planning and execution [11,44]. Taken together, these findings implicate PMd as an alternative motor planning region that is differentially recruited during IP which accounts for, at least some, of the retention advantages afforded by IP [2].

The primary purpose of Experiment 2 was to investigate the influence of PMd during RP and IP by administering anodal or cathodal tDCS respectively. Consistent with Experiment 1, which addressed SMA, smaller electrodes and specific montages thought to improve focality of stimulation and increase the precision of targeting were used. Moreover, this experiment included separate experimental conditions that involved anodal and cathodal stimulation of both right and left PMd when performing the motor skill with the left index finger. This was incorporated into the design of Experiment 2 because of the suggestion that the left PMd plays a proprietary role during motor skill performance [45–47]. As a result, while contralateral stimulation (i.e., right side for the present experiment), either anodal or cathodal, would be anticipated to be most critical for impacting left finger movement production, if indeed left PMd has a special role during movement production, then similar behavioral benefits or shortcomings, observed from contralateral stimulation, might also be observed from ipsilateral stimulation (i.e., left side for the present experiment).

Methods

Participants

Participants were right-handed undergraduate students (N=96) that received course credit for their participation. Individuals had no prior experience with the experimental tasks and was an unaware of the specific purpose of the study. All participants completed an informed consent approved by an Institutional Review Board before any involvement in the experiment.

Discrete Sequence Production Tasks

The motor task used in Experiment 2 were identical to those used in Experiment 1 and are described in detail in an earlier section.

Transcranial Direct Current Stimulation (tDCS)

Participants assigned to one of four PMd stimulation conditions (Left or right anodal; left and right cathodal) or one of two Sham conditions (RP-Sham, IP-Sham) experienced 20-min of real or sham stimulation while experiencing RP or IP (see Figure 12). All real stimulation conditions consisted of a 2mA current being administered through a 5x5 cm^2 electrode for the stimulation and 5x7 cm^2 electrode for the reference electrode covered by saline-soaked sponges resulting in a maximum current density of 0.04 mA/cm^2 that was administered using a battery-driven stimulator (tDCS Stimulator; TCT Research Limited, Hong Kong).

Anodal stimulation involved placement of the anode 2.5 cm anterior to the right or left primary motor cortex (C3 or C4), which is the premotor hand area, referenced as FC3 and FC4 in the international 10-20 electrode system[48–50]. Specifically, anodal stimulation of right PMd was coupled with the reference electrode on the left supraorbital site whereas anodal stimulation of left PMd was paired with a reference at right supraorbital site. For the *cathodal* stimulation conditions involved reversing the locations of the anode and cathode just described (see Figure 14). The sham stimulation condition involved the anodal stimulation electrode montage described previously but stimulation was only delivered for 30-sec at the beginning and end of the 20-min training period.

Figure 12 displays the current flow associated with the anodal stimulation electrode montage arrangement modeled using HD-Explore™ (Soterix Medical Inc., New York, NY). The modelling indicated heightened current flow (.449 V/m^2) and (.367 V/m^2) at MNI coordinates (x: -21, y: -1, z; 44) and (x: 21, y: -1, z; 44) which is congruent with the location of left-PMd and right-PMd respectively as described by Mayka et al. (2006).

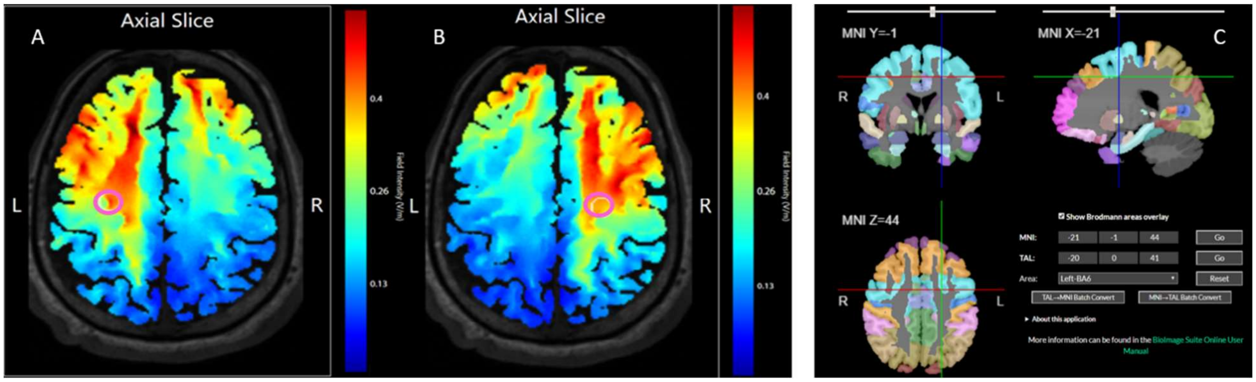

Figure 12. Modeled field potentials in an adult male brain stimulating distribution of the electrical field in the brain with the anodal stimulation electrode montage described in the text. (A) The pink circle represents the Left PMd and (B) Right PMd in the head model. (C) MNI coordinates overlaid on a reference using Yale BioImage Suite.

Procedure

The procedures followed were identical to those adopted in Experiment 1 with the only exception being the neural locale (i.e., PMd as opposed to SMA) at which real and sham stimulation was applied.

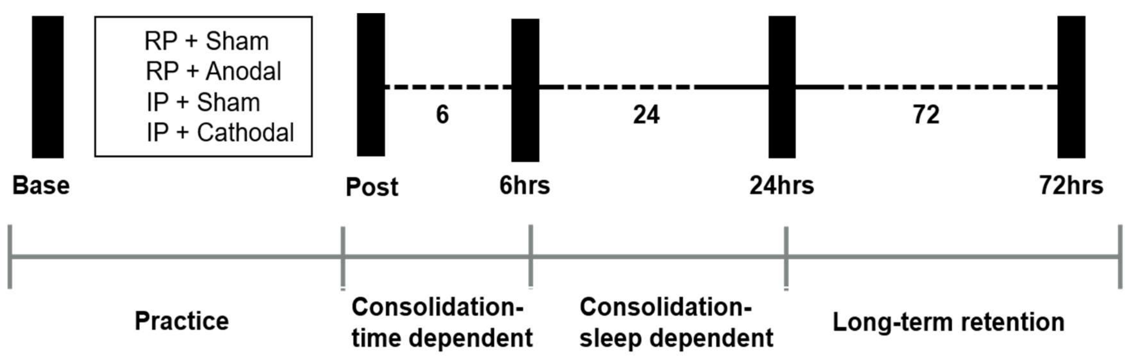

Figure 13. Timeline for Experiment 2. Participants took a total of four days to complete all phases of Experiment 2. Filled black boxes represent a test block presented in a repetitive format which is consist of 21 trial that included seven trials for each of three target DSP tasks. After the baseline test block (Base), all individuals were immediately exposed to real or sham stimulation at left or right PMd during 9 blocks of RP or IP practice. Training sessions took approximately 20-min which determined the duration of anodal (Right-RP-AtDCS, Left-RP-AtDCS), cathodal (Right-IP-CtDCS, Left-IP-CtDCS), or sham (RP-Sham, IP-Sham) stimulation. The same 21-trial test block was administered again at several intervals after the completion of either RP or IP, immediately post training, 6-hr, 24-hr, and 72-hr following the conclusion of practice.

Measurement and Analyses

The dependent variable used were identical to those used in Experiment 1. Specifically, total sequence time (TT) was the overall measure of performance for each sequence. Based on work of Abrahamse et al. (2013) initiation time, concatenation time, and execution times were calculated for each trial for each individual in order to evaluate the impact of practice schedule and stimulation on the component processes central to motor sequence production. The analyses conducted were identical to those performed during Experiment 1 with the exception that there were additional stimulation conditions included in the design for Experiment 2.

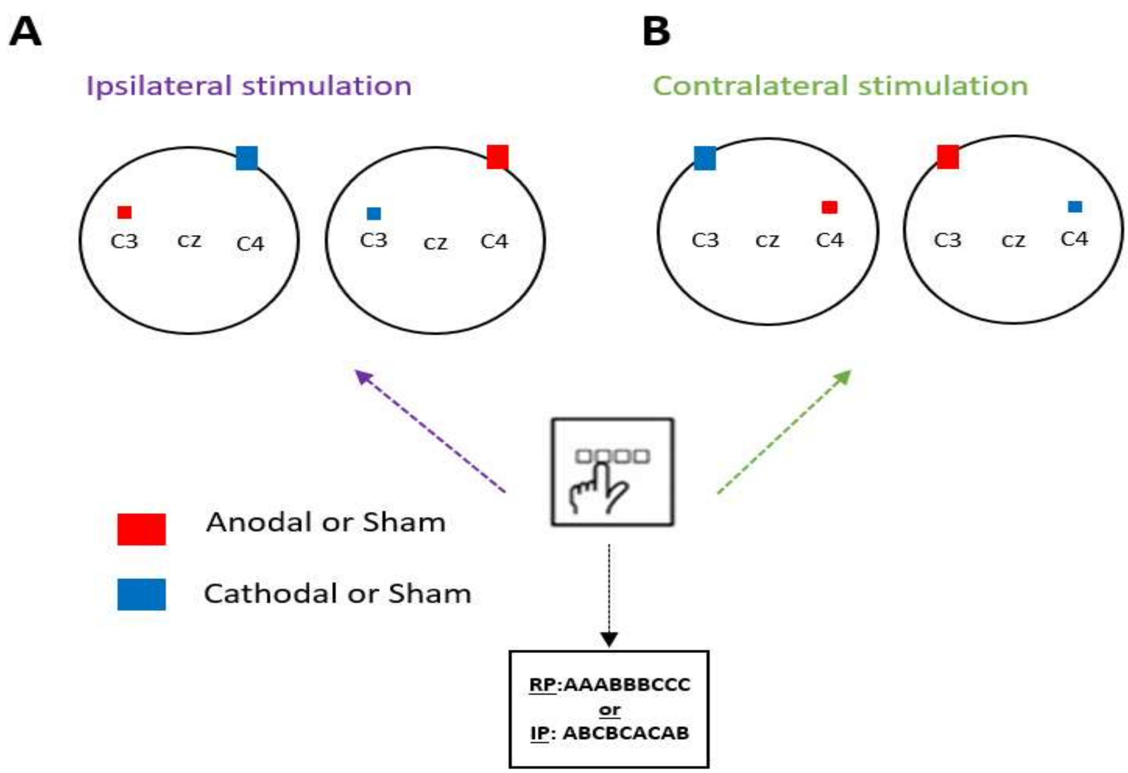

Figure 14. Schematic drawing of electrode montages organized to target either left (FC3 which is 2.5 cm anterior of C3) or right (FC4 which is 2.5 cm anterior of C4) dorsal premotor motor (PMd) area. Individuals learn three unique 6-element discrete sequence production tasks were executed with the left index finger in either an IP or RP format. (A) Anodal or Sham stimulation was administrated via two different size of electrodes with the anode placed on left PMd and the reference located at the contralateral supraorbital site. In contrast, cathodal tDCS involved the cathode placed on left PMd (cathode) and the reference electrode at contralateral supraorbital site. (B) The corresponding electrode montages were used to target right PMd. During Experiment 2 RP was only paired with anodal stimulation at either left or right PMd whereas IP was only used in conjunction with Cathodal stimulation of left or right PMd.

Results

Baseline DSP task performance

Total time data from the baseline test trials prior to any practice for all individuals was subjected to a 6 (Stimulation: RP-Sham, IP-Sham, R-RP-AtDCS, R-IP-CtDCS, L-RP-AtDCS, L-IP-CtDCS) between-subject (ANOVA) which failed to reveal a significant main effect of Stimulation, $F(5, 90) = 0.61$, $p = 0.7$, $\eta_p^2 = 0.03$. Thus, as expected, TT did not differ as a function of stimulation condition prior to any exposure to training and stimulation (RP-Sham, M = 2870, SEM = 52ms; IP-Sham, M = 2718ms, SEM = 127ms; R-RP-AtDCS, M = 2706, SEM = 52; R-IP-CtDCS, M = 2774ms, SEM = 65ms; L-RP-AtDCS, M = 2749ms, SEM = 101ms; L-IP-CtDCS, M = 2699ms, SEM = 69ms).

Contextual interference revisited: Online and offline performance of individuals that experienced RP and IP with sham stimulation

Online Performance during RP and IP with sham stimulation. To assess if the online performance observed in the present study replicated previous work addressing the influence of different levels of CI during training [1,2], the TT data for the baseline and PostI test blocks for individuals assigned to the sham conditions were submitted to a 2 (Schedule: RP-Sham, IP-Sham) × 2 (Block: baseline, PostI) ANOVA with repeated measures on the last factor. The main effect of Schedule $F(1,30) = 0.35$, $p = 0.65$, $\eta_p^2 = 0.01$. and the Schedule x Block interaction, $F(1,30) = 1.81$, $p = 0.19$, $\eta_p^2 = 0.06$ were not significant. However, the main effect of Block, $F(1,30) = 148.4$, $p < .001$, $\eta_p^2 = 0.83$ was significant. Subsequent post-hoc assessment of the Block main effect revealed that TT for the baseline test block (M =2794, SEM = 90ms) was significantly greater than the TT observed for the PostI test block (M = 1928, SEM = 68ms). The improvement in performance as a result of training within the RP and IP formats was in keeping with previous reports addressing CI and online gain [1,2].

Offline Gains following RP and IP with sham conditions. The next analysis focused on the change in TT from the end of the training (i.e., PostI) to the time of test following RP and IP. As was the case for Experiment 1, data from the 24-hr test block was used because many studies assessing the impact of CI for offline gain addressed this issue by conducting retention tests the day after practice concluded. Thus, TT data for each individual in the RP-sham and IP-sham stimulation conditions for the PostI and Post24 test blocks were submitted to 2 (Schedule: RP-Sham, IP-Sham) × 2 (Block: postI, post24) ANOVA with repeated measures on the last factor. The main effect of Schedule, $F(1,30) = 1.53$, $p = 0.23$, $\eta_p^2 = 0.05$ was not significant. However, the Block main effect, $F(1,30) = 19.39$, $p = .001$, $\eta_p^2 = 0.4$ and the Schedule x Block interaction, $F(1,30) = 15.47$, $p = .001$, $\eta_p^2 = 0.34$ were significant. Simple main effect analyses of the interaction revealed that TT for the PostI test block was similar for individuals assigned to the RP-sham (M = 1909ms, SEM = 78ms) and IP-sham (M = 1948ms, SEM = 59ms) conditions, $F(1,30) = 0.51$, $p = 0.48$, $\eta_p^2 = 0.02$. However, TT for the Post24 test block for individuals in the IP-Sham condition (M = 1624ms, SEM = 45ms) was significantly lower than the TT for the individuals in the RP-Sham condition (M = 1890ms, SEM = 91ms), $F(1,30) = 23.49$, $p = .001$, $\eta_p^2 = 0.4$. Moreover, the reduction in TT from the PostI to Post24 test block for the IP-Sham condition was significant, $F(1,30) = 34.76$, $p = .001$, $\eta_p^2 = 0.5$. In contrast, TT from the PostI to Post24 test blocks for the individuals in the RP-Sham stimulation condition did not reveal a reduction in TT, $F(1,30) = 0.11$, $p = 0.74$, $\eta_p^2 < .01$.

These data verify the frequently observed overnight improvement in performance associated with IP as well as forgetting over the same time period for individuals exposed to RP[3,11]. These data also replicate the findings from Experiment 1 for the RP-sham and IP-sham in conditions in which the electrode montage used for the same stimulation was located at SMA as opposed to PMd.

Online gain: Performance during IP and RP in the presence of stimulation

Given the present study replicated the CI effect, the next question is if behavioral outcomes traditionally associated with RP and IP formats can be modified by administering anodal and cathodal tDCS at PMd respectively during practice. Figure 17 and 18 depict TT for all test blocks administered prior to (i.e., Baseline test block) and after the completion of training (i.e., PostI, Post6, Post24, Post72) for the individuals that were assigned to RP (Figure 17: RP-Sham, RP-AtDCS) and IP (Figure 18: IP-Sham, IP-CtDCS). Recall that stimulation (i.e., real or sham) was not present during any of the test blocks. Real or sham stimulation was only present during the nine training blocks that made up either RP or IP.

Influence of anodal tDCS at right and left PMd for online performance during RP. TT data for the Baseline and PostI test blocks for individuals that experienced RP were submitted to a 3 (Stimulation: RP-Sham, R-RP-AtDCS, L-RP-AtDCS) × 2 (Block: Baseline, PostI) ANOVA with repeated measures on the last factor. This analysis revealed a significant main effect of Stimulation, $F(2,45) = 8.43$, $p < .001$, $\eta_p^2 = 0.27$, and Block, $F(1,45) = 444.9$, $p < .001$, $\eta_p^2 = 0.91$. Subsequent post-hoc analyses of the significant main effects indicated that TT for the Baseline test block (*M* = 2778, *SEM* = 68ms) was significantly greater than the TT observed for the PostI test block (*M* = 1672ms, *SEM* = 60ms).

The main effect of Stimulation revealed that TT during the PostI test block for the individuals assigned to the R-RP-AtDCS (*M* = 1551, *SEM* = 42ms) and L-RP-AtDCS (*M* = 1556, *SEM*

= 60ms) did not differ but was significantly lower than the TT revealed by the RP-sham condition (*M* = 1909ms, *SEM* = 78ms). The Stimulation x Block interaction, $F(2,45) = 1.88$, $p = 0.17$, $\eta_p^2 = 0.08$ failed significance. Experiencing RP resulted in online gains across training and the observed improvement in TT was modified by supplementing RP with anodal tDCS.

A second assessment of online performance during RP focused on TT from the training blocks while stimulation was present. TT data for each of the nine training blocks for each individual in the Left and Right RP-AtDCS and RP-Sham conditions were submitted to a 3 (Stimulation: RP-Sham, R-RP-AtDCS, L-RP-AtDCS) × 9 (Block: 1-9) ANOVA with repeated measures on the last factor. The main effects of Stimulation, $F(2,45) = 17.30$, $p = .001$, $\eta_p^2 = 0.43$, and Block, $F(8,360) = 91.27$, $p < .001$, $\eta_p^2 = 0.7$ were significant. In addition, the Stimulation x Block interaction, $F(16,360) = 1.72$, $p < .05$, $\eta_p^2 = 0.07$ was significant. Simple main effect analyses indicated that TT for individuals in the left (*M* = 1504, *SEM* = 44ms) and right (*M* = 1541, *SEM* = 37ms) RP-AtDCS conditions did not differ across nine practice blocks, but the stimulation conditions showed significantly lower TT during the training period (RP-AtDCS, *M* = 1522ms, *SEM* = 40ms) compared to RP-Sham (*M* = 1802ms, *SEM* = 59ms). In the presence of anodal stimulation in RP emerged as a function of a gradual reduction in TT across practice, but RP-sham revealed significantly slower TT in block 4, but quickly emerged as a function of a training effect across blocks (see Figure 15).

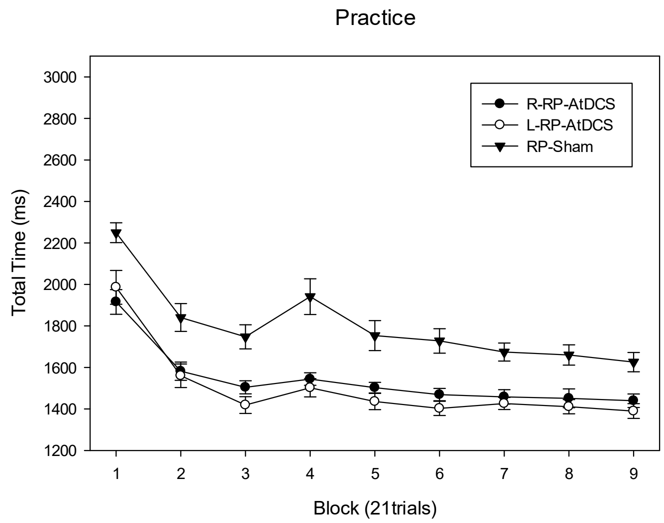

Figure 15. Total Time(ms) for the nine training blocks performed between the baseline and immediate test blocks during which real or sham stimulation was applied at PMd for the **RP** condition.

Influence of cathodal tDCS at right and left PMd for online performance during IP. TT data for the Baseline and PostI test blocks for individuals that experienced IP were submitted to a 3 (Stimulation: IP-Sham, R-IP-CtDCS, L-IP-CtDCS) × 2 (Block: Baseline, PostI) ANOVA with repeated measures on the last factor. This analysis revealed a significant main effects of Block, $F(1,45)$ = 242.28, $p < .001$, $\eta_p^2 = 0.84$ but failed to reveal a significant main effect of Stimulation, $F(2,45)$ = 0.03, $p = 0.97$, $\eta_p^2 < 0.01$ or Stimulation x Block interaction, $F(2,45) = 1.22$, $p = 0.3$, $\eta_p^2 = 0.05$. The Block main effect was a function of TT for the baseline test block (*M* = 2730ms, *SEM* = 87ms) being significantly greater than the TT reported for the PostI test block (*M* = 1942 ms, *SEM* = 59ms).

Online performance during IP was also evaluated using TT data from the training blocks. TT data for each of the nine training blocks for each individual in the R-IP-CtDCS, L-IP-CtDCS and IP-Sham conditions were submitted to a 3(Stimulation: IP-Sham, R-IP-CtDCS, L-IP-CtDCS) × 9(Block: 1-9) ANOVA with repeated measures on the last factor. The main effect of Block, $F(8,360)$

= 76.46, p = .001, η_p^2 = 0.63, was significant. Post-hoc analyses of the Block main effect indicated that there was a gradual reduction in TT across practice (see Figure 16). The lack of a significant main effect of Stimulation, $F(2, 45)$ = 0.98, p = 0.38, η_p^2 = 0.04 and Stimulation x Block interaction, $F(8,360)$ = 0.93, p = 0.53, η_p^2 = 0.02 revealed that cathodal stimulation at left or right PMd failed to influence improvements in TT that occur across practice (See Figure 16).

Offline gains resulting from early time-dependent consolidation

There is considerable literature addressing the importance of early time-dependent consolidation, that is independent on sleep [39], for the development of motor memories [40]. The influence of the practice format coupled with stimulation on early consolidation was evaluated using TT data from the PostI and Post6 test blocks. These data are depicted in Figure 17 and 18 for RP and IP respectively. As was the case with earlier assessments, separate analyses were conducted to evaluate the impact of the application of anodal and cathodal tDCS at PMd during RP and IP respectively.

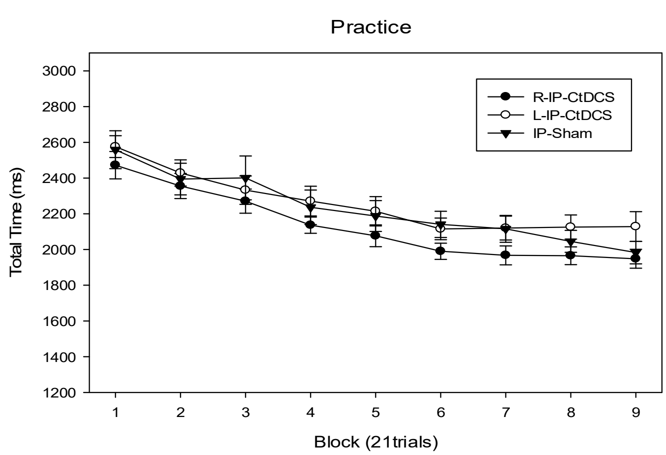

Figure 16. Total Time(ms) for the nine training blocks performed between the baseline and immediate test blocks during which real or sham stimulation was applied at PMd for the **IP** condition.

Impact of Anodal tDCS at left and right PMd during RP on early time-dependent consolidation. TT data from the PostI and Post6 test blocks for individuals trained with RP were submitted to a 3 (Stimulation: RP-Sham, R-RP-AtDCS, L- RP-AtDCS) × 2 (Block: PostI, Post6) ANOVA with repeated measures on the last factor. This analysis revealed a significant main effect of Block, $F(1,45) = 6.62$, $p = 0.01$, $\eta_p^2 = 0.13$ and Stimulation, $F(2,45) = 15.53$, $p < .001$, $\eta_p^2 = 0.41$. Post-hoc assessment of the Stimulation main effect revealed that TT for the individuals in the R-RP-AtDCS (M = 1564, SEM = 41ms) and L-RP-AtDCS (M = 1592ms, SEM = 61ms) did not differ significantly but was significantly lower than the TT for individuals in the RP-Sham condition (M = 1966, SEM = 77ms). The Block main effect resulted from the TT that was observed for PostI test block (M = 1672, SEM = 60ms) being significantly greater than the TT observed for the post6 test block (M = 1742, SEM = 59ms). The interaction of Stimulation x Block, $F(2,45) = 0.87$, $p = 0.43$, $\eta_p^2 = 0.04$ was not significant.

Impact of Cathodal tDCS at left and right PMd during IP on early time-dependent consolidation. TT data for the postI and post6 test blocks for individuals that experienced IP were submitted to a 3 (Stimulation: IP-Sham, R-IP-CtDCS, R-IP-CtDCS) × 2 (Block: postI, post6) ANOVA with repeated measures on the last factor. These analyses failed to reveal a significant main effect of Stimulation, $F(2,45) = 0.58$, $p = 0.56$, $\eta_p^2 = 0.02$, or a Stimulation x Block interaction, $F(2,45) = 2.51$, $p = 0.09$, $\eta_p^2 = 0.1$. However, a significant main effect of Block, $F(1,45) = 5.77$, $p < .05$, $\eta_p^2 = 0.11$ did emerge. Post-hoc analysis of this main effect indicated that TT for the PostI test block (M = 1942ms, SEM = 62ms) was significantly greater than TT observed during the post6 test block (M = 1889ms, SEM = 59ms).

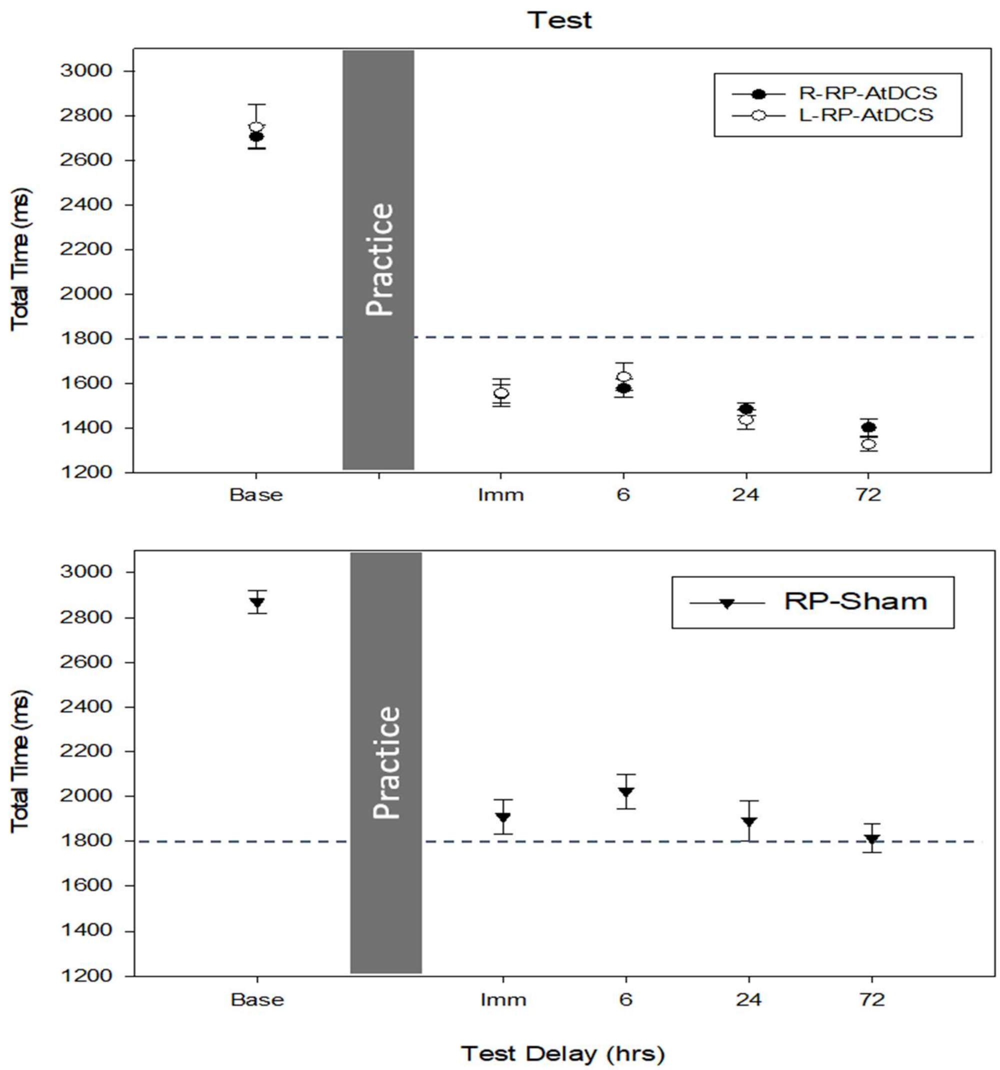

Figure 17. Total Time(ms) at Baseline (Base), immediately after practice (Imm), 6-hr post practice (6), 24-hr post practice (24), and 72-hr post practice (72) test blocks for individuals assigned to the **RP-AtDCS (top)** and **RP-sham (bottom)** conditions. Error bars are standard errors. Letters indicate scores differed significantly, $p < .05$.

Taken together, these data reveal that administration of anodal stimulation during RP and cathodal stimulation during IP failed to modify the general profile of early consolidation, or lack thereof, which characterizes IP and RP. That is, following IP, all individuals whether they were exposed to real or sham stimulation exhibited offline gain during the initial six hrs. Alternatively, for RP, supplementing practice with anodal stimulation at PMd did not eliminate the forgetting that is customarily observed following this practice format in the initial hours after practice. It is also worth noting that, for all cases, applying stimulation, either anodal or cathodal, to either hemisphere results in the same outcome.

Long-term retention following RP and IP in the presence of stimulation

Evaluation of the impact of training context and stimulation on long-term retention involved examination of performance from the Post6, Post24, and Post72 test blocks. As noted earlier, these data are displayed in Figure 17 and 18. Congruent with earlier assessments, separate analyses were conducted to evaluate the impact of anodal and cathodal tDCS at left and right PMd during RP and IP.

Impact of Anodal tDCS at left and right PMd during RP on long-term retention. TT data for the Post6. Post24, and Post72 test blocks for individuals that experienced RP were submitted to a 3 (Stimulation: RP-Sham, R-RP-AtDCS, L-RP-AtDCS) × 3 (Block: Post6, Post24, Post72) ANOVA with repeated measures on the last factor. These analyses revealed significant main effects of Stimulation, $F(2,45) = 24.62$, $p < .001$, $\eta_p^2 = 0.52$ and Block, $F(2,90) = 41.49$, $p < .001$, $\eta_p^2 = 0.48$. Subsequent, post-hoc analysis for the main effects of Stimulation indicated that TT for anodal stimulation at left (*M* = 1463, *SEM* = 45ms) and right (*M* = 1487, *SEM* = 36ms) PMd did not differ but exhibited TT that was significantly lower than the TT for individuals in the RP-Sham condition (*M* = 1909, *SEM* = 77ms). The significant main effect of Block indicated that TT for the Post6 test block (*M* = 1742ms, *SEM* = 59ms) was significantly greater that the TT observed at the Post24 test block (*M* = 1603ms, *SEM* = 55ms) which in turn was greater than the TT reported for the Post72 test block (*M* = 1513ms, *SEM* = 44ms). The Stimulation x Block interaction failed to reach significant level, $F(4,90) = 1.22$, $p =0.31$, $\eta_p^2 = 0.05$.

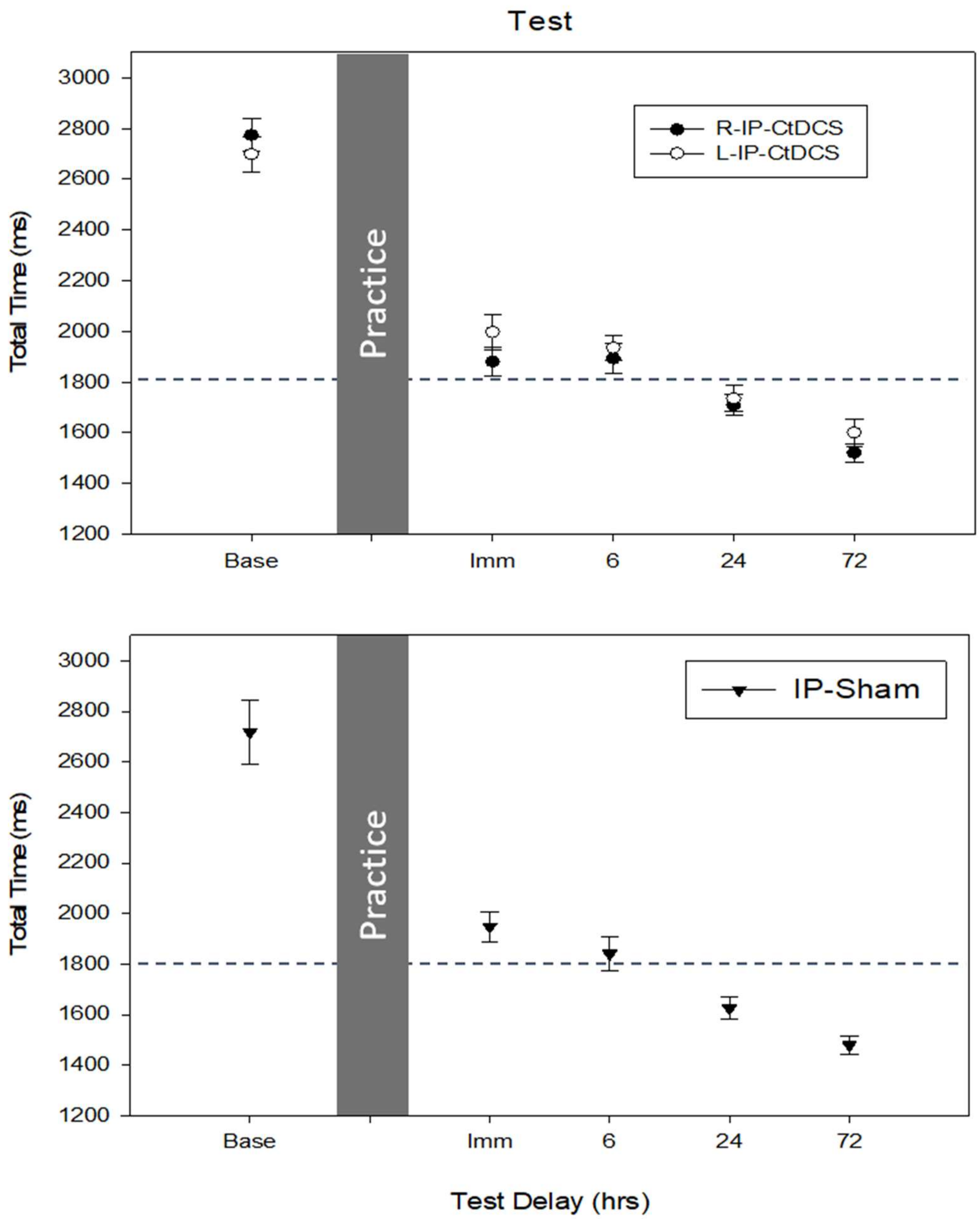

Figure 18. Total Time(ms) at Baseline (Base), immediately after practice (Imm), 6-hr post practice (6), 24-hr post practice (24), and 72-hr post practice (72) test blocks for individuals assigned to the **IP-CtDCS (top)** and **IP-sham (bottom)** conditions. Error bars are standard errors. Letters indicate scores differed significantly, $p < .05$.

Impact of Cathodal tDCS at left and right PMd during IP on long-term retention. TT data for the Post6, Post24, and Post72 test blocks for individuals that experienced IP were submitted to a 3 (Stimulation: IP-Sham, R-IP-CtDCS, L-IP-CtDCS) × 3 (Block: Post6, Post24, Post72) ANOVA with repeated measures on the last factor. This analysis revealed significant main effects of Block, $F(2,90) = 162$, $p < .001$, $\eta_p^2 = 0.78$. Subsequent post-hoc assessment of the Block main effect revealed that TT at the Post6 test block (*M* = 1889ms, *SEM* = 62ms) was significantly greater than the TT at the Post24hr (*M* = 1690ms, *SEM* = 46ms) which was significantly greater than TT Post72 (*M* = 1533ms, *SEM* = 41ms). The main effect of Stimulation, $F(2,45) = 1.45$, $p = 0.25$, $\eta_p^2 = 0.06$ and the Stimulation x Block interaction, $F(4,90) = 0.4$, $p = 0.81$, $\eta_p^2 = 0.02$, failed to reach significance level.

To further examine the impact of training context and stimulation on long-term retention an approach previously adopted by Reis et al. (2009) was used. Specifically, a linear function was fit to the TT data for Post6, Post24, and Post72 for each individual and the slope was used to represent the rate of gain or loss following the completion of early consolidation. The slope for each individual was subjected to either a 3 (Stimulation: R-RP-AtDCS, L-RP-AtDCS RP-Sham) between-subject ANOVA for the RP condition or a 3 (Stimulation: R-IP-CtDCS, L-IP-CtDCS IP-Sham) between-subject ANOVA for the IP condition. In the case of RP, this analysis failed to reveal a main effect of Stimulation, $F(2,45) = 1.50$, $p = 0.23$, $\eta_p^2 = 0.06$. The rate of improvement in TT for individuals in the right-4.61 RP-AtDCS condition (M = -2.47ms/hrs, SEM = 0.3ms/hrs), left- RP-AtDCS condition (M = -4.11ms/hrs, SEM = 0.6ms/hrs) did not differ from that of individuals assigned to the RP-sham stimulation (M = -2.85ms/hrs, SEM = 0.99ms/hrs). For the IP condition, the analysis again failed to reveal a main effect of Stimulation, $F(2,45) = 0.39$, $p = 0.68$, $\eta_p^2 = 0.06$. The rate of improvement in TT for the individuals in the left IP-CtDCS condition (M = -4.61ms/hrs, SEM =0.55), right IP-CtDCS condition (M = -5.29ms/hrs, SEM =0.51) did not differ significantly from that observed for the IP-Sham condition (M = 4.97ms/hrs, SEM = 0.57).

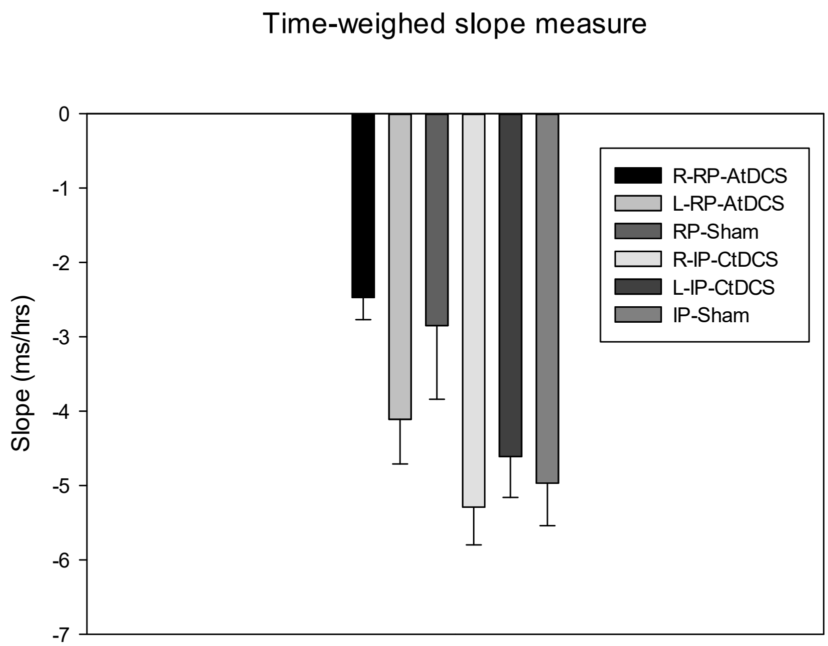

Figure 19. The slope of the linear function for TT data from Post6, Post24, and Post72 test trials. The slope index the rate of improvement that was exhibited by individuals in each experimental condition during the 72 hrs after practice was complete. The more negative the slope represents greater gains in motor sequence performance. Error bars are standard errors.

Together, these data revealed that changing activity at PMd using anodal tDCS during RP can influence remote effects related to long-term retention. Administration of anodal tDCS during RP facilitates overnight offline gains by inducing significant improvements in TT with each sleep episode. However, cathode stimulation in IP did not impact to modify PMd activation during practice, as a result IP-CtDCS condition showed the offline gain from the form of consolidation extended to long-term retention. Thus, nature of IP structure developed stronger memory trace rather than hindered with exposure to cathodal stimulation during IP.

Impact of tDCS at left and right PMd for initiation, concatenation, and execution processes used during DSP task production during RP and IP

Baseline DSP task performance. Initiation (IT), concatenation (CT), and execution time (ET) data from the baseline test trials prior to RP or IP for all individuals was subjected to a 6 (Stimulation: RP-Sham, IP-Sham, R-RP-AtDCS, L-RP-AtDCS, R-IP-CtDCS, L-IP-CtDCS) between-subject ANOVA which failed to reveal a significant main effect of Stimulation for IT, $F(5, 90) = 0.82$, $p = 0.54$, $\eta_p^2 = 0.03$, CT , $F(5, 90) = 0.37$, $p = 0.87$, $\eta_p^2 = 0.02$ or ET , $F(5, 90) = 0.58$, $p = 0.71$, $\eta_p^2 = 0.03$. Thus, IT, CT and ET did not differ as a function of stimulation condition prior to any exposure to training and stimulation (see Fig. 20 and 21).

Contextual interference revisited: Online and offline changes in initiation, concatenation, and execution for individuals that experienced RP and IP with sham stimulation

Online changes in initiation, concatenation, and execution during RP and IP in the absence of stimulation. IT, CT, and ET for each individual assigned to the sham conditions were submitted to a 2 (Schedule: RP-Sham, IP-Sham) × 2 (Block: baseline, PostI) ANOVA with repeated measures on the last factor. For IT, the main effect of Stimulation $F(1,30) = 5.87$, $p < .05$, $\eta_p^2 = 0.16$, and of Block, $F(1,30) = 59.31$, $p < .001$, $\eta_p^2 = 0.6$ were significant. Post-hoc analysis of the Block main effect indicated that IT for the baseline test block (*M* = 520ms, *SEM* = 21ms) was significantly greater than the IT for the PostI test block (*M* = 395ms, *SEM* = 20ms). The Schedule main effect resulted from individuals in RP-Sham condition exhibited a lower IT (*M* = 357, *SEM* = 20ms) compared to their IP-Sham counterparts (*M* = 433, *SEM* = 20ms). The Stimulation x Block interaction, $F(1,30) = 0.91$, $p = 0.35$, $\eta_p^2 = 0.03$ was not significant.

For CT, the main effects of Block, $F(1,30) = 17.04$ $p < .001$, $\eta_p^2 = 0.36$ was significant. However, the main effect of Stimulation, $F(1,30) = 0.16$ $p = 0.69$, $\eta_p^2 = 0.01$ and Stimulation x Block interaction, $F(1,30) = 0.10$, $p = 0.76$, $\eta_p^2 = 0.01$ were not significant. The Block main effect was a function of CT for the baseline test block (*M* = 420ms, *SEM* = 18ms) being significantly greater than the CT reported for the Post-I test block (*M* = 357ms, *SEM* = 17).

For ET, the main effect of Block, $F(1,30) = 204.8$ $p < .001$, $\eta_p^2 = 0.87$ was significant. However, the main effect of Stimulation, $F(1,30) = 0.66$ $p = 0.42$, $\eta_p^2 = 0.02$ and Stimulation x Block interaction, $F(1,30) = 0.86$, $p = 0.36$, $\eta_p^2 = 0.03$ were not significant. The Block main effect was a function of ET for the baseline test block (*M* = 436ms, *SEM* = 16ms) being significantly greater than the ET observed for the Post-I test block (*M* = 283ms, *SEM* = 11).

Offline gain in initiation, concatenation, and execution during RP and IP in the absence of stimulation. The next analysis focused on the change in IT, CT and ET from the end of the training (i.e., Post-I) to the time of test following RP and IP. For the initial analysis delayed test performance from the 24-hr test block was used because many studies assessing the impact of CI for offline gain addressed this issue by conducting retention tests the day after practice concluded. Thus, IT, CT and ET data for each individual in RP-sham and IP sham stimulation conditions for the PostI and Post24 test blocks were submitted to separate 2 (Schedule: RP-Sham, IP-Sham) × 2 (Block: postI, post24) ANOVA with repeated measures on the last factor.

For IT, the main effect of Stimulation, $F(1,30) = 0.55$, $p = 0.47$, $\eta_p^2 = 0.02$ was not significant. However, the main effect of Block, $F(1,30) = 8.01$, $p = 0.01$, $\eta_p^2 = 0.21$ and the Stimulation x Block interaction, $F(1,30) = 26.23$, $p < .001$, $\eta_p^2 = 0.47$ were significant. Simple main effect analyses of this interaction revealed that individuals in IP-Sham (*M* = 433ms, *SEM* = 79ms) performed significantly longer IT than those in RP-Sham (*M* = 357ms, *SEM* = 79ms) at the Post-I test block. However, the for the Post24 test block, IT for individuals in the IP-Sham condition (*M* = 344ms, *SEM* = 60ms) was significantly lower than the IT observed for the RP-Sham condition (*M* = 383ms, *SEM* = 92ms). The reduction in IT from the PostI to Post24 test block for the IP-Sham condition was significant, $F(1,30) = 31.61$, $p < .001$, $\eta_p^2 = 0.51$, whereas IT for the individuals in the RP-Sham stimulation condition did not show significant improvement from the postI to post24 test blocks, $F(1,36) = 2.63$, $p = 0.12$, $\eta_p^2 = 0.08$.

For CT, the main effect of Stimulation, $F(1,30) = 2.33$, $p = 0.47$, $\eta_p^2 = 0.07$ was not significant. However, the main effect of Block, $F(1,30) = 35.96$, $p < .001$, $\eta_p^2 = 0.55$ and the Stimulation x Block interaction, $F(1,30) = 7.01$, $p = 0.01$, $\eta_p^2 = 0.19$ were significant. Simple main effect analyses of this interaction revealed that CT for individuals assigned to the IP-Sham (*M* = 356, *SEM* = 15ms) and RP-Sham (*M* = 344, *SEM* = 17ms) conditions did not differ significantly during the Post-I test block $F(1,30) = 0.06$, $p = 0.81$, $\eta_p^2 = 0.01$. However, at the Post24 test block, CT for individuals in the IP-Sham (*M* = 269ms, *SEM* = 8ms) was significantly lower than the CT observed for the RP-Sham condition (*M* = 326ms, *SEM* = 18ms), $F(1,30) = 15.94$, $p < .001$, $\eta_p^2 = 0.35$. The reduction in IT from the PostI to Post24 test block for the IP-Sham and RP-Sham conditions showed significantly improvement, $F(1,30) = 21.48$, $p < .001$, $\eta_p^2 = 0.42$.

For ET, the main effect of Stimulation, $F(1,30) = 3.43$, $p = 0.07$, $\eta_p^2 = 0.1$ was not significant. However, the main effect of Block, $F(1,30) = 4.26$, $p < .05$, $\eta_p^2 = 0.12$ and the Stimulation x Block interaction, $F(1,30) = 10.16$, $p = 0.01$, $\eta_p^2 = 0.25$ were significant. Simple main effect analyses of the interaction revealed that ET for individuals in the IP-Sham and RP-Sham conditions did not differ significantly at the Post-I test block $F(1,30) = 0.13$, $p = 0.72$, $\eta_p^2 = 0.01$. However, for the

Post24 test block, ET for individuals in the IP-Sham (*M* = 244, *SEM* = 7ms) was significantly lower than RP-Sham (*M* = 292ms, *SEM* = 14ms) stimulation conditions, $F(1,30) = 23.69$, $p < .001$, $\eta_p^2 = 0.44$. The reduction in ET from the PostI (*M* = 281, *SEM* = 11ms) to Post24 (*M* = 244, *SEM* = 7ms) test block for the IP-Sham condition was significant, $F(1,30) = 13.78$, $p < .001$, $\eta_p^2 = 0.31$. In contrast, ET from the PostI (*M* = 285, *SEM* = 11ms) to Post24 (*M* = 292, *SEM* = 14ms) test block for the individuals in the RP-Sham stimulation condition did not show significant improvement, $F(1,30) = 0.63$, $p = 0.43$, $\eta_p^2 = 0.02$.

Taken together, these data were replicated data described in Experiment 1 that exposure to IP leads to offline gain whereas RP results in significant forgetting during the 24-hr after practice. The benefit afforded through IP appear broad-based with respect to the improvement in the component processes central to motor sequence execution. This is reflected in IT, CT, and ET all being enhanced across the retention interval.

Online change in initiation, concatenation, and execution processes during RP and IP in the presence of stimulation

Figure 20 and 21 depict IT, CT, and ET for all test blocks administered prior to (Baseline) and after the completion of training (PostI, Post6, Post24, and Post72) for the individuals that were assigned to RP (Figure 20; RP-Sham, R-RP-AtDCS, L-RP-AtDCS) and IP (Figure 21; IP-Sham, R-IP-CtDCS, L-IP-CtDCS).

Influence of anodal tDCS at left and right PMd on initiation, concatenation, and execution processes during RP. IT, CT and ET data for the Baseline and PostI test blocks for individuals that experienced RP were submitted to a 3 (Stimulation: RP-Sham, R-RP-AtDCS, L-RP-AtDCS) × 2 (Block: Baseline, PostI) ANOVA with repeated measures on the last factor.

For IT, this analysis revealed that the main effect of Stimulation, $F(2,45) = 1.59$, $p = 0.22$, $\eta_p^2 = 0.07$ was not significant. However, the main effect of Block, $F(1,45) = 210.06$, $p < .001$, $\eta_p^2 = 0.82$, and the Stimulation x Block interaction, $F(2,45) = 3.28$, $p < .05$, $\eta_p^2 = 0.13$, were significant. Simple main effect of this interaction indicated that IT for all stimulation conditions did not differ for the Baseline test block, $F(2,45) = 0.3$, $p = 0.74$, $\eta_p^2 = 0.02$. While IT for the individuals in R-RP-AtDCS (*M* = 287, *SEM* = 11ms) and L-RP-AtDCS (*M* = 310, *SEM* = 12ms) did not differ significantly during the PostI test block, but it was significantly lower than that the IT observed for the RP-Sham (*M* = 357, *SEM* = 20ms) condition for the Post-I test block. All stimulation conditions exhibited a reduction in IT from the baseline to the postI test blocks.

For CT, this analysis revealed significant main effects of Stimulation, $F(2,45) = 3.65$, $p < .05$, $\eta_p^2 = 0.14$, and Block, $F(1,45) = 106.63$, $p < .001$, $\eta_p^2 = 0.7$, as well as a Stimulation x Block interaction, $F(2,45) = 4.34$, $p < .05$, $\eta_p^2 = 0.16$. Simple main effect of the interaction revealed that the CT for all stimulation conditions did not differ at the Baseline test block, $F(2,45) = 0.91$, $p = 0.41$, $\eta_p^2 = 0.03$. Moreover, CT for individuals in R-RP-AtDCS (*M* = 299, *SEM* = 15ms) and L-RP-AtDCS (*M* = 285, *SEM* = 15ms) did not differ at Post-I test blocks but exhibited CT that was significantly lower than that observed for the RP-Sham (*M* = 359, *SEM* = 18ms) condition at PostI test block. All stimulation conditions exhibited a reduction in CT from the baseline to the postI test blocks.

For ET, this analysis revealed significant main effects of Stimulation, $F(2,45) = 4.60$, $p < .05$, $\eta_p^2 = 0.17$ and Block, $F(1,45) = 495.8$, $p < .001$, $\eta_p^2 = 0.92$. The Stimulation x Block interaction,

$F(2,45) = 2.72$, $p = 0.07$, $\eta_p^2 = 0.11$ failed significance. Subsequent post-hoc analyses of these main effects indicated that ET for individuals in R-RP-AtDCS (*M* = 333, *SEM* = 9ms) and L-RP-AtDCS (*M* = 339, *SEM* = 12ms) did not differ significantly at the Baseline and Post-I test blocks. However, RP-AtDCS showed significantly lower ET than RP-Sham (*M* = 366, *SEM* = 11ms). The reduction in ET for the participants assigned to all RP conditions (Baseline: *M* = 440, *SEM* = 12ms) showed significantly improvement after nine blocks of training (PostI: *M* = 251, *SEM* = 10ms)

Influence of cathodal tDCS at left and right PMd on initiation, concatenation, and execution processes during IP. IT, CT and ET data for the Baseline and PostI test blocks for individuals that experienced IP were separately submitted to a 3 (Stimulation: IP-Sham, R-IP-CtDCS, L-IP-CtDCS) × 2 (Block: Baseline, PostI) ANOVA with repeated measures on the last factor. For IT, this analysis revealed a significant main effect of Block, $F(1,45) = 31.87$, $p < .001$, $\eta_p^2 = 0.41$. This main effect was a result of IT for the Baseline test block (*M* = 522ms, *SEM* = 26ms) being significantly greater than the IT noted during the Post-I test block (*M* = 423ms, *SEM* = 22ms). The main effect of Stimulation, $F(2,45) = 0.31$, $p = 0.73$, $\eta_p^2 = 0.01$ and the Stimulation x Block interaction, $F(2,45) = 1.90$, $p = 0.16$, $\eta_p^2 = 0.08$ failed significance.

For CT, this analysis revealed a significant main effect of Block, $F(1,45) = 20.34$, $p < .001$, $\eta_p^2 = 0.31$. This main effect was a result of CT for the Baseline test block (*M* = 425ms, *SEM* = 26ms) being significantly greater than the CT noted during the Post-I test block (*M* = 360, *SEM* = 18ms). The main effect of Stimulation, $F(2,45) = 0.49$, $p = 0.62$, $\eta_p^2 = 0.02$ and the Stimulation x Block interaction, $F(2,45) = 0.64$, $p = 0.53$, $\eta_p^2 = 0.02$ failed significance.

For ET, this analysis revealed a significant main effect of Block, $F(1,45) = 279.19$, $p < .001$, $\eta_p^2 = 0.86$. This main effect was a result of ET for the Baseline test block (*M* = 429ms, *SEM* = 15ms) being significantly greater than the ET noted during the Post-I test block (*M* = 279ms, *SEM* = 11ms). The main effect of Stimulation, $F(2,45) = 0.02$, $p = 0.98$, $\eta_p^2 < 0.01$ and the Stimulation x Block interaction, $F(2,45) = 1.16$, $p = 0.32$, $\eta_p^2 = 0.05$ failed significance.

Taken together, these data revealed that supplementing RP with anodal stimulation at either left or right PMd enhanced the performance improvements across practice. Experiencing IP resulted in online gains from training, but these improvements are not modified by supplementation of cathodal tDCS at PMd while practice occurs.

Offline gains resulting from early consolidation in initiation, concatenation, and execution processes

There is considerable literature addressing the importance of early time-dependent consolidation, that is independent on sleep [39], for the development of motor memories [40]. The influence of the practice format coupled with stimulation at left and right PMd on early consolidation was evaluated using TT data from the PostI and Post6 test blocks. These data are depicted in Figure 20 and 21 for RP and IP respectively. As was the case with earlier assessments, separate analyses were conducted to evaluate the impact of the application of anodal and cathodal tDCS at left and right PMd during RP and IP.

Impact of Anodal tDCS at PMd *during RP on early time-dependent consolidation*. IT, CT, and ET data from PostI and Post6 test blocks for individuals trained with RP were submitted to separate 3 (Stimulation: RP-Sham, L-RP-AtDCS, R-RP-AtDCS) × 2 (Block: PostI, Post6) ANOVA with repeated measures on the last factor. For IT, there was a significant main effect of Stimulation, $F(2,45) = 8.32$, $p < .001$, $\eta_p^2 = 0.27$ and Block, $F(1,45) = 20.34$, $p < .001$, $\eta_p^2 = 0.31$. Subsequent post-hoc analyses of these main effects indicated that IT for individuals in R-RP-AtDCS (*M* = 301ms, *SEM* = 11ms) and L-RP-AtDCS (*M* = 319ms, *SEM* = 12ms) did not differ significantly at the Post-I and Post-6 test blocks. However, RP-AtDCS showed significantly lower IT than RP-Sham (*M* = 385, *SEM* = 23ms). Moreover, IT at Post-6 test block (*M* = 352, *SEM* = 17ms) was significantly great than during the PostI test block (*M* = 318, *SEM* = 14ms). The Stimulation x Block interaction, $F(2,45) = 2.22$, $p = 0.12$, $\eta_p^2 = 0.09$ failed significance.

For CT, this analysis revealed a significant main effect of Stimulation, $F(2,45) = 9.06$, $p < .001$, $\eta_p^2 = 0.29$. Subsequent post-hoc analysis showed that individuals in R-RP-AtDCS (*M* = 293, *SEM* = 15ms) and L-PR-AtDCS (*M* = 281, *SEM* = 14ms) but did exhibit CT that was significantly lower than that observed for the RP-Sham condition (*M* = 358, *SEM* = 16ms). The main effect of Block, $F(1,45) = 1.16$, $p = 0.29$, $\eta_p^2 = 0.03$ and the Stimulation x Block interaction, $F(2,45) = 0.19$, $p = 0.83$, $\eta_p^2 = 0.01$ failed to reach significance.

For ET, this analysis revealed a significant main effect of Block, $F(1,45) = 10.94$, $p < .001$, $\eta_p^2 = 0.2$ and Stimulation, $F(2,45) = 11.10$, $p < .001$, $\eta_p^2 = 0.33$. Subsequent post-hoc analysis showed that individuals in R-RP-AtDCS (*M* = 240, *SEM* = 8ms) and L-PR-AtDCS (*M* = 241, *SEM* = 10ms) did not differ ET significantly at the Post-I and Post-6 test blocks. However, RP-AtDCS exhibited significantly lower ET than RP-Sham (*M* = 295, *SEM* = 12ms). The main effect of Block revealed that RP conditions exhibited significantly increased ET from the Post-I (*M* = 251, *SEM* = 10ms) to Post6 (*M* = 266, *SEM* = 11ms) test block. The Stimulation x Block interaction, $F(2,45) = 1.15$, $p = 0.33$, $\eta_p^2 = 0.05$ failed significance.

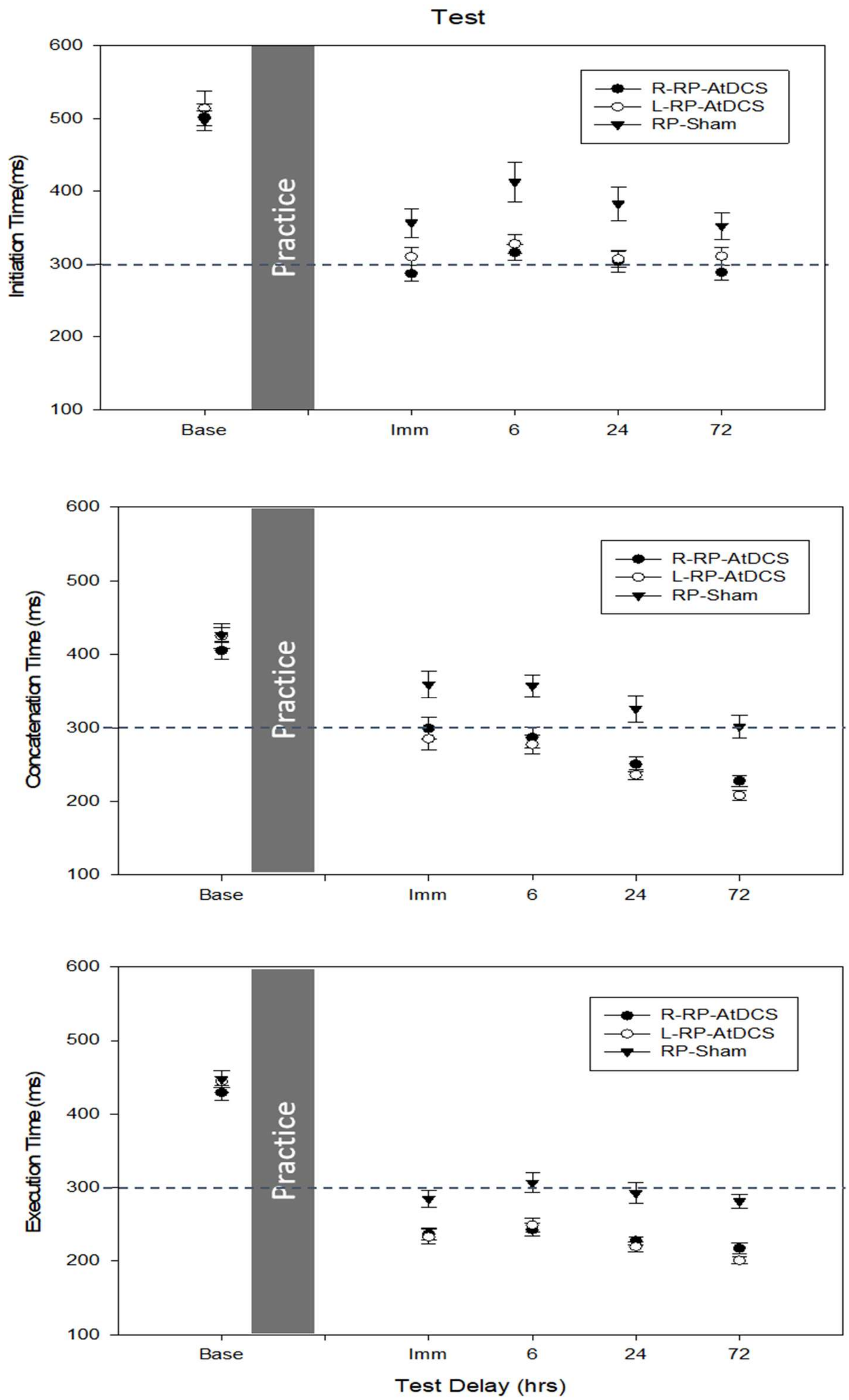

Figure 20. IT (top), CT (middle), and ET (bottom) at Baseline (Base), immediately after practice (Imm), 6-hr post practice (6), 24-hr post practice (24), and 72-hr post practice (72) test blocks for individuals assigned to the RP- AtDCS and RP-sham conditions. Error bars are standard errors. Letters indicate scores differed significantly, $p < .05$.

Impact of Cathodal tDCS at left and right PMd *during IP on early time-dependent consolidation*. IT, CT, and ET data for the postI and post6 test blocks for individuals that experienced IP were submitted to separate a 3 (Stimulation: IP-Sham, R-IP-CtDCS, L-IP-CtDCS) × 2 (Block: postI, post6) ANOVA with repeated measures on the last factor.

For IT, this analysis revealed a significant main effect of Block, $F(1,45) = 6.29$, $p < .05$, $\eta_p^2 = 0.12$. This main effect was a result of IT for the Post-I test block (*M* = 423ms, *SEM* = 22ms) being significantly greater than the IT noted during the Post-6 test block (*M* = 406ms, *SEM* = 18ms). The main effect of Stimulation, $F(2,45) = 0.44$, $p = 0.65$, $\eta_p^2 = 0.02$ and the Stimulation x Block interaction, $F(2,45) = 2.57$, $p = 0.09$, $\eta_p^2 = 0.1$ failed significance.

For CT, this analysis revealed a significant main effect of Block, $F(1,45) = 20.62$, $p < .001$, $\eta_p^2 = 0.31$. This main effect was a result of CT for the Post-I test block (*M* = 360ms, *SEM* = 18ms) being significantly greater than the CT noted during the Post-6 test block (*M* = 320ms, *SEM* = 12ms). The main effect of Stimulation, $F(2,45) = 1.27$, $p = 0.29$, $\eta_p^2 = 0.05$ and the Stimulation x Block interaction, $F(2,45) = 1.07$, $p = 0.35$, $\eta_p^2 = 0.05$ failed significance.

For ET, this analysis revealed the main effect of Block, $F(1,45) = 0.02$, $p = 0.9$, $\eta_p^2 = 0.02$, Stimulation, $F(2,45) = 0.25$, $p = 0.78$, $\eta_p^2 = 0.01$ and the Stimulation x Block interaction, $F(2,45) = 0.96$, $p = 0.39$, $\eta_p^2 = 0.04$ failed significance.

Consistent with the analysis of TT data, these data show that the application of tDCS on left or right PMd has no impact of the early time dependent consolidation that is assumed to occur in the initial hours following practice. Specifically, in the real and sham stimulated conditions in RP, performance suffers across the 6-hr test interval between postI and post6. In contrast, performance shows the expected stabilization benefit from IP across the initial few hrs after practice, but this is the same regardless of real or sham stimulation. It should not go unnoticed that administration of stimulation to either right or left PMd result in the same behavioral outcomes.

Long-term changes in initiation, concatenation, and execution processes associated with administration of tDCS at left and right PMd during repetitive and interleaved practice

Evaluation of the impact of training context and stimulation on long-term retention involved examination of performance from the Post6, Post24, and Post72 test blocks. As noted earlier, these data are displayed in Figure 20 and 21. Congruent with earlier assessments, separate analyses were conducted to evaluate the impact of anodal and cathodal tDCS at SMA during RP and IP respectively.

Impact of Anodal tDCS at PMd *during RP on long-term change in IT, CT, and ET*. IT, CT and ET data for the Post6, Post24, Post72 test blocks for individuals that experienced RP were submitted to separate a 3 (Stimulation: RP-Sham, R-RP-AtDCS, L-RP-AtDCS) × 3 (Block: Post6, Post24, Post72) ANOVAs with repeated measures on the last factor.

For IT, this analysis revealed significant main effects of Stimulation, $F(2,45) = 8.53$, $p < .001$, $\eta_p^2 = 0.27$ and Block, $F(2,90) = 10.67$, $p < .001$, $\eta_p^2 = 0.19$. Subsequent post-hoc analysis for the main effects indicated that R-RP-AtDCS ($M = 303$, $SEM = 12$ms) and L-PR-AtDCS ($M = 315$, $SEM = 12$ms) did not significantly differ IT across posttest blocks. However, RP-AtDCS exhibited significantly lower IT than individuals in RP-Sham condition ($M = 383$, $SEM = 23$ms). Post-hoc analysis for the Block main effect indicated that the Post-6 ($M = 352$, $SEM = 17$ms) was significantly greater IT than Post24 ($M = 331$, $SEM = 17$ms) test block but failed to approach significance level from Post-24 test to Post-72 (M = 317, SEM = 14ms) test block. The Stimulation x Block interaction, $F(4,90) = 1.67$, $p = 0.16$, $\eta_p^2 = 0.04$ failed significance.

For CT, this analysis revealed significant main effects of Stimulation, $F(2,45) = 18.42$, $p < .001$, $\eta_p^2 = 0.45$ and Block, $F(2,90) = 57.67$, $p < .001$, $\eta_p^2 = 0.27$. Subsequent post-hoc analysis for the main effect of Stimulation indicated that R-RP-AtDCS (M = 255, SEM = 11ms) and L-PR-AtDCS (M = 240, SEM = 9ms) did not significantly differ CT across posttest blocks. However, RP-AtDCS exhibited significantly lower IT than individuals in RP-Sham condition (M = 328, SEM = 16ms). Interpretation of the significant main effect of Block revealed that CT in for the Post6 test block (M = 307, SEM = 14ms) was significantly greater than the CT observed for the Post24 test block (M = 271, SEM = 12ms) which in turn was significantly greater than the CT reported for the Post72 test block (M = 246, SEM = 10ms). The Stimulation x Block interaction failed to reach significant level, $F(4,90) = 0.28$, p =0.89, $\eta_p^2 = 0.04$.

For ET, this analysis revealed significant main effects of Stimulation, $F(2,45) = 20.91$, $p < .001$, $\eta_p^2 = 0.48$ and Block, $F(2,90) = 32.55$, $p < .001$, $\eta_p^2 = 0.42$. Subsequent post-hoc analysis for the main effect of Stimulation indicated that R-RP-AtDCS (M = 229, SEM = 7ms) and L-PR-AtDCS (M = 223, SEM = 7ms) did not significantly differ ET across posttest blocks. However, RP-AtDCS exhibited significantly lower ET than individuals in RP-Sham condition (M = 293, SEM = 12ms). Interpretation of the significant main effect of Block revealed that ET in for the Post6 test block ((M = 266, SEM = 11ms)) was significantly greater than the ET observed for the Post24 (M = 247, SEM = 9ms) test block and Post72 (M = 233, SEM = 7ms) test blocks. ET for the latter test blocks did not differ from one another. The Stimulation x Block interaction failed to reach significant level, $F(4,90) = 1.79$, p =0.14, $\eta_p^2 = 0.07$.

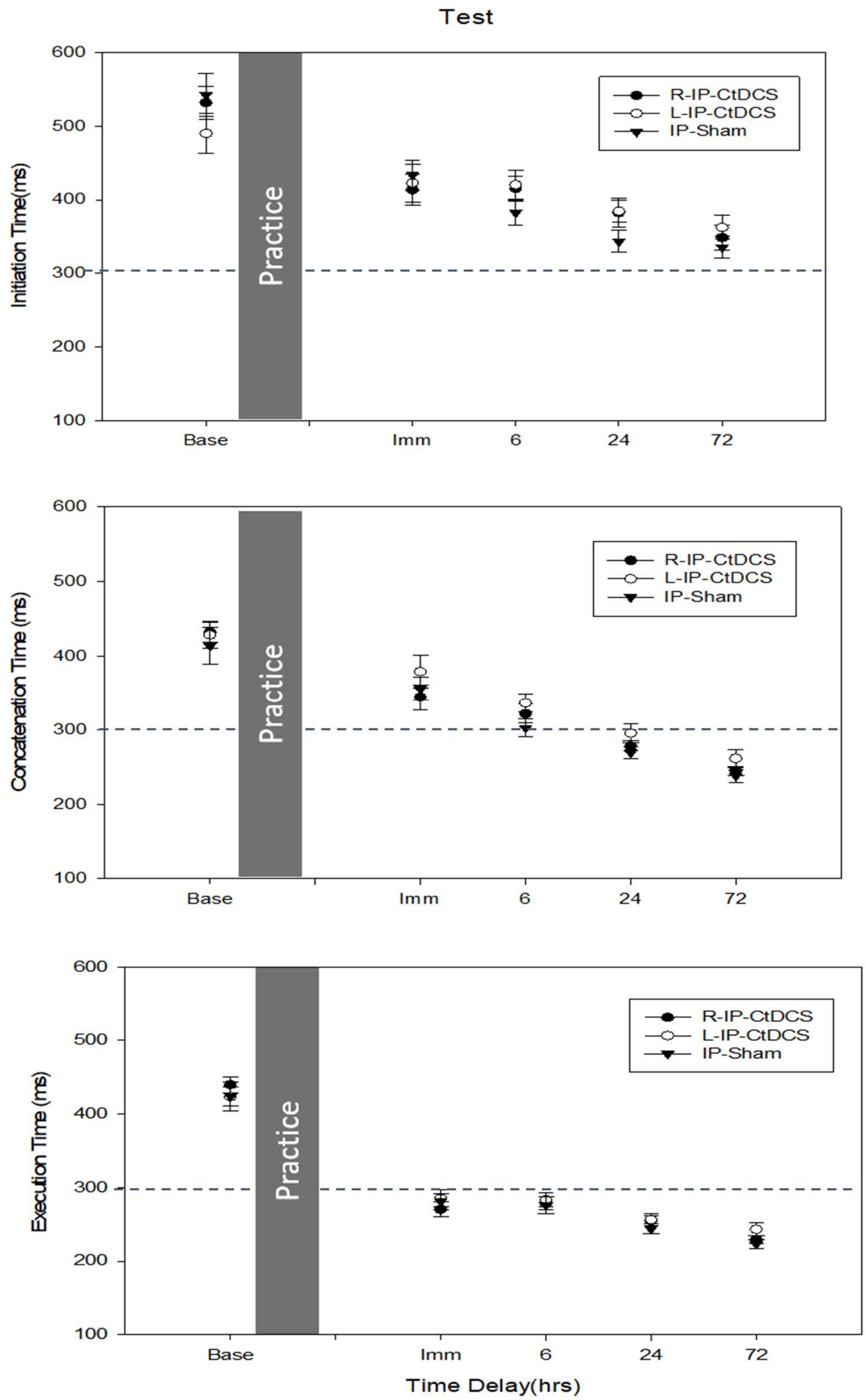

Figure 21. IT (top), CT (middle), and ET (bottom) at Baseline (Base), immediately after practice (Imm), 6-hr post practice (6), 24-hr post practice (24), and 72-hr post practice (72) test blocks for individuals assigned to the IP- CtDCS and IP-sham conditions. Error bars are standard errors. Letters indicate scores differed significantly, p < .05.

Impact of Cathodal tDCS at PMd during IP for long-term change in IT, CT, and ET. IT, CT, and ET data for the Post6, Post24, and Post72 test blocks for individuals that experienced IP were submitted to separate 3 (Stimulation: IP-Sham, R-IP-CtDCS, L-IP-CtDCS) × 3 (Block: Post6, Post24, Post72) ANOVAs with repeated measures on the last factor. For IT, this analysis revealed a significant main effect of Block, $F(2,90) = 38.54$, $p < .001$, $\eta_p^2 = 0.46$. Post-hoc assessment revealed that IT at the Post6 test block (M = 406ms, SEM = 18ms) was significantly greater than the IT at the Post24hr (M = 370ms, SEM = 17ms) which was significantly greater than IT Post72 (M = 349ms, SEM = 16ms). The main effect of Stimulation, $F(2,45) = 1.40$, $p = 0.26$, $\eta_p^2 = 0.06$, and the Stimulation x Block interaction, $F(4,90) = 0.96$, $p = 0.68$, $\eta_p^2 = 0.04$, failed to reach significance level.

For CT, this analysis revealed significant main effect of Block, $F(2,90) = 105.21$, $p < .001$, $\eta_p^2 = 0.7$. Subsequent post-hoc assessment of the Block main effect revealed that CT at the Post6 test block (M = 320ms, SEM = 12ms) was significantly greater than the IT at the Post24hr (M = 281ms, SEM = 9ms) which was significantly greater than IT Post72 (M = 248ms, SEM = 9ms). The main effect of Stimulation, $F(2,45) = 2.31$, $p = 0.26$, $\eta_p^2 = 0.09$, and the Stimulation x Block interaction, $F(4,90) = 0.34$, $p = 0.68$, $\eta_p^2 = 0.01$, failed to reach significance level. The analysis of ET also revealed a significant main effect of Block, $F(2,90) = 90.99$, $p < .001$, $\eta_p^2 = 0.67$. As was the case for IT and CT, post-hoc analysis revealed that ET at the Post6 test block (M = 279ms, SEM = 10ms) was significantly greater than the ET at the Post24hr (M = 252ms, SEM = 7ms) which was significantly greater than ET Post72 (M = 232ms, SEM = 7ms). The main effect of Stimulation, $F(2,45) = 0.77$, $p = 0.47$, $\eta_p^2 = 0.02$, and the Stimulation x Block interaction, $F(4,90) = 0.91$, $p = 0.46$, $\eta_p^2 = 0.04$, failed to reach significance level.

These data in conjunction with the corresponding TT analyses reveal that the use of stimulation at either left or right PMd has little impact on the manner in which consolidation during long-term retention unfolds. The initial impact of Stimulation per se that emerged during training remained, noted for initial post-test trials (i.e., PostI), but subsequent improvements following the 6-hr post-test block through the same test administered at 72-hr followed a similar trajectory in the absence or presence of anodal stimulation at PMd. Consistent with the previous analyses for PMd, application of anodal stimulation on the right or left side as well as cathodal stimulation did not change the general profile of the data.

Discussion

The primary purpose of experiment 2 was to manipulate the activity of Left and Right PMd during IP and RP using anodal or cathodal tDCS and examine the impact on subsequent offline memory gains and long-term retention of motor memories. Lin et al. (2018) work highlighted the importance of left PMd recruitment for this IP advantage as this neural site was part of developing functional connectivity between Left-PMd and the sensorimotor network during training and important in consolidation and retention phase. In addition, Lin et al. (2011) noted that greater functional coupling between PMd and M1 emerged during IP compared to RP which supported increasing excitability at M1 at 72-hr retention test. Therefore, greater PMd activity then appears to be a “key” neural player during IP that contributes to the formation of strengthened memory traces supporting superior long-term retention of motor memories. Thus, it was anticipated that increasing PMd activity via anodal tDCS during RP would improve offline gains by reducing the extent of forgetting often observed following RP training format (sham control group in this study). In contrast, suppressing PMd activation using cathodal stimulation should disrupt performance during IP leading to a reduction in the anticipated offline gains. Modulation of PMd activity during IP and RP would influence the development of motor memories that as consequence can be impeded and improved time-and sleep-sensitive consolidation and long-term retention processes.

As anticipated, it was replicated that superior acquisition was observed for RP-Sham, but greater offline gains emerged for IP-Sham through superior early, time-dependent consolidation as well as sleep-mediated consolidation manifest as sustained gains for 72-hr post-practice. Unexpected, all analysis failed to reveal any difference for left and right PMd in IP and RP tDCS conditions. Supplementing RP with anodal tDCS exhibited significantly lower TT rather than training under same practice condition without anodal stimulation, reflecting greater online gains. This online benefit, as increased encoding of motor memories showed increased resistant of offline forgetting over 6hr, but not enough to protect newly acquired memory reflecting offline forgetting. In other word, online benefit resulted from pairing anodal tDCS at PMd during RP was not critical for time-dependent. However, heightened PMd activity with RP-AtDCS condition showed significant offline improvement which lasted for 72hrs. Training under the RP-Sham also showed offline improvement continued for 72-hr, but significantly lower amount of offline gains occurred compared to anodal stimulation in RP. This suggest that increased PMd activity improves sleep related on long-term retention of newly acquired motor memory. The improvement in sleep-related consolidation from increased PMd activity in RP was not dependent on the location of PMd stimulation. These data are consistent with the claim that IP supports more effective time- and sleep-dependent consolidation processes the latter of which is influenced by more rigorous recruitment of PMd during this practice format. In contrast, administration of cathodal tDCS during IP led to a nonsignificant increase in mean TT reflecting failed to disrupt online gain compared to IP-Sham. This result represents cathodal stimulation during IP did not impair the motor memory development as consequently reveal offline improvement through early, time-dependent consolidation as well as sleep-mediated consolidation manifest as sustained gains for 72-hr post-practice.

In conclusion, training under interleaved format as opposed to repetitive practice is more effective to acquire motor skills and led to superior long-term retention of these skills for up to 72-hr after training was complete. This practice structure benefit impact to superior early stabilization of newly acquired memory during the initial 6hours after training period. These data intimated that early development of motor memory after IP training showed greater offline gains across two periods of overnight sleep. Administering anodal tDCS at PMd throughout RP had no impact on the time-dependent consolidation but had a positive effect on sleep-dependent consolidation reflected in significant offline gain with each additional sleep episode. Thus, heightened activity of PMd, as occurs in IP, affords a learning advantage by fostering overnight processing of new memories.

CONCLUSIONS

The overall goal of the present work was to determine the contribution of what is classically known as Brodmann area (BA) 6 to a practice scheduling phenomenon referred to as the contextual interference (CI) effect [2,51]. BA 6 is composed on a more lateral component - dorsal premotor (PMd) region, as well as a medial region which herein has been referred to as supplementary motor area (SMA) complex. While Experiment 1 focused on the SMA complex, Experiment 2 was designed to examine if PMd made a unique contribution to enhanced skill acquisition via IP. The selection of these neural regions for the application of one form of non-invasive brain stimulation (NIBS), transcranial direct current stimulation (tDCS), during IP and RP was not arbitrary. Extensive evidence is available suggesting that both SMA complex and PMd play a central role in skill acquisition, in particular when learning sequential motor behaviors such as those used in the present work [52]. More importantly, it has been recognized, from neural imaging work, that acquisition of sequential motor skills in an IP as opposed to RP schedule is associated with greater activation of these neural regions during practice [2,11]. Moreover, emergent functional connectivity between SMA and PMd with other key neural sites for motor planning and execution during and following IP is highly predictive of the offline gains that are observed during delayed retention tests [13,17,51]. On the basis of these findings it is not unreasonable then to consider both SMA and PMd as potential key “neural players” that contribute to an IP’s advantage for motor skill retention.

The basic strategy adopted in the two experiments contained herein was to attempt to modify the activity at SMA complex (Experiment 1) and PMd (Experiment 2) during RP and IP using tDCS. This form of NIBS has previously been used in conjunction with motor training to influence motor learning [38]. It should be noted however, that most previous efforts to combine tDCS and motor training have focused on modifying cortical excitability at primary motor cortex (M1). Few studies to date have targeted the more anterior motor planning sites used in the present experiments for stimulation with the intent of facilitating or hindering skill acquisition. Nor has previous work considered the use of exogenous stimulation to influence the acquisition of more than a single motor skill during training [53]. Thus, the present work entertains some interesting novel questions related to the broader issue of using NIBS to facilitate skill acquisition.

The basic expectation in both experiments was similar. Anodal tDCS was administered at SMA (Experiment 1) or PMd (Experiment 2) during RP which was expected to upregulate the activity at the targeted region within a practice schedule context (i.e., RP) which has been reported to lack involvement from this region under normal (i.e., non-stimulated) situations [11]. As a result, it was anticipated that some benefit for skill acquisition might be ascertained from the exogenous stimulation when compared to the RP-sham control. Each experiment also entertained the alternative scenario in which IP, the schedule reported to benefit from heightened activation at both SMA and PMd during training, was paired with cathodal tDCS which has been used to down-regulate cortical activity [54]. Contrary to using anodal tDCS during RP, this latter combination of IP with cathodal stimulation was expected to result in a disruption in the commonly observed skill acquisition advantage afforded through IP because of the reduced activation of important motor planning regions during training.

Given the extensive evidence that is exists in the current literature, it is not surprising that the two experiments in the present work revealed robust retention benefits for skill acquisition from IP but not RP [1–3]. In the initial section that follows some novel insight that emerged from the present work that extend the current understanding of how post-practice consolidation unfolds following IP is detailed. In the section that follows a specific role for the SMA complex during IP is described that accounts for, at least in part, the offline gains associated with IP. On the basis of findings from Experiment 2, a unique but unexpected contribution to skill acquisition from heightened activation in PMd is discussed before concluding with some general comments regarding the efficacy of tDCS as a tool for real-time modification of neural activity and its influence on resultant motor behaviors.

The contextual interference effect: A robust practice phenomenon

Increasing the extent of contextual interference (CI) during training has been argued to be an effective means of establishing motor memories that are resilient and enduring for over 30 years [1,2]. Interference is commonly modified during practice by the manner in which multiple novel motor skills are scheduled during each practice bout [1–3]. Interleaved practice (IP) is most frequently used to induce relatively greater interference as a result of rapid switching between the motor skills being acquired across practice trials. Conversely, a repetitive practice (RP) format has been argued to create less interference because the learner executes the same motor skill repeatedly for a fixed number of trials, or until a criterion level of performance is reached for a single motor task before practice with an alternative variation. There is now considerable evidence indicating that the more challenging IP format is superior for developing skills that are less susceptible to forgetting as well as exhibit more broad generalizability. This finding, while frequently reported in the laboratory environment [2], has also been observed outside the laboratory for a diverse set of subject populations and has been used successfully in the clinical domain [4–6] and rehabilitative settings [7,8].

Both Experiments 1 and 2 in the present work add to the growing literature supporting the efficacy of greater CI during practice for retention benefits. In both experiments performance at the 24-hr test block was assumed to be representative of the majority of studies that have examined this practice schedule issue in the past [1]. As expected, there was evidence that during acquisition that RP offered an advantage for sequence initiation (i.e., IT) in Experiment 1 and 2 which likely resulted from the continued presence of the information necessary to plan an upcoming motor sequence in working memory because only a single action had to be considered during any single practice block. Despite this, across both experiments, individuals exhibited a worsening of performance 24-hrs after the completion of RP. In contrast, participant's privy to IP showed a significant enhancement in performance during the 24-hr test block. Interestingly, the improvement in TT across this period resulting from IP was quite broad based with respect to the component processes that were enhanced. Facilitation in both sequence initiation and execution were observed for the IP-sham participants despite being generally poorer than their RP-sham counterparts immediately after practice terminated. To the contrary, those exposed to RP exhibited sizeable forgetting with respect to these component processes at 24-hr. Moreover, the IP schedule resulted in superior concatenation early during acquisition compared to RP and advantage continued through to the delayed test period.

The learning advantage from IP is a consequence of effective post-practice consolidation

Congruent with previous work then, the findings from Experiments 1 and 2 offered evidence of robust retention benefits from IP. Importantly, these data also implicated effective post-practice consolidation as a key contributor to this IP advantage. Specifically, in both experiments, individuals assigned to RP exhibited substantial forgetting across the 24-hr retention interval [12,25]. IP participants on the other hand revealed significant enhancement not only in overall performance (i.e., TT) but also for both initiation and execution processes that are central to the implementation of motor sequences [33].

Experiments 1 and 2 incorporated a number of additional test blocks at various temporal intervals after the conclusion of RP and IP [38,55]. These were important because they offered the opportunity to examine the importance of the each practice schedule for fostering both early time-dependent consolidation that has been reported to be critical for rapid stabilization of novel memories [56] and sleep-dependent consolidation [39] that has been associated with enhancement not just memory stabilization. Early time-dependent consolidation has been reported to occur within the first 4-6 hrs after training [57]. whereas sleep-dependent consolidation and performance enhancement has been shown to increase with the number of periods of overnight sleep [58]. In both Experiments 1 and 2, the test block administered after 6-hr was used to evaluate the extent to which early consolidation was supported. As can be seen in Figures 7 and 17, the IP-sham condition displays clear evidence that performance is stable across the initial 6-hr whereas significant degradation is associated with RP during this same time frame (see Figures 8, 18). Subsequent test blocks were incorporated at 24 and 72-hr that included overnight sleep (see Abe et al., 2013). In this case, exposure to IP resulted not in continued stable performance but performance enhancement which was incremented with each additional night of sleep. In contrast, following RP, the data generally reflected stable behavior following the initial loss in the first few after practice and little evidence of recovery of this loss through sleep-dependent enhancement.

The data from Experiment 1 and 2 then went beyond merely noting that IP facilitates post-practice consolidation [17,25]. It turns out that the post-practice benefit from IP is quite widespread influencing both time-dependent and sleep-dependent consolidation. Following IP, novel motor memories are rapidly stabilized which is then followed by further consolidation that is likely sleep-dependent. These data offer a novel account of how exposure to IP impacts critical post-practice processing that has been described as central to ongoing evolution of motor memories in the absence of practice [59].

Modifying BA6 activation during motor sequence training can influence skill acquisition

SMA plays an important role for both time-dependent and sleep-dependent consolidation

In Experiment 1, anodal tDCS was administered during RP at a location that according to our *a priori* modeling was congruent with SMA complex [28,29]. The basic tenet of this approach was that increasing SMA complex activity while the learner was engaged in practice, even in an RP format, would create a more optimal neural environment, one more like that created in IP, that would result in more effective long-term outcomes. Specifically, it was anticipated that by elevating the cortical activity at SMA during RP would provide some skill acquisition benefit beyond that observed for a RP-sham control.

While the application of anodal tDCS at SMA during did improve (i.e., reduce TT) the ongoing performance during training this was likely a performance effect due to the presence of the stimulation as no difference in the change in TT from the baseline to the immediate post-test block emerged. Despite this, there were important difference in performance of the individuals that experienced stimulation compared to those in the sham condition in the subsequent test blocks that clearly demonstrated the importance of heightened SMA activity during motor sequence skill acquisition. First, while the learners in RP-Sham exhibited the characteristic initial forgetting immediately after the conclusion of practice, being privy to stimulation was associated with very stable behavior over the first six hours. A signature for successful early time-dependent consolidation in a manner that is normally only observed for participant that are afforded IP.

Application of anodal stimulation at SMA during RP appears to be quite pervasive because not only was its presence during RP beneficial for consolidation that begins immediately after practice, it also fostered skill enhancement evidenced by the continued reduction in TT for the test blocks that occurred at 24 and 72-hrs. Again, this finding is congruent with what is typically observed for individuals exposed to IP and the fact that performance did not merely remain stable but continued to improve is characteristic of sleep-dependent gains commonplace in the literature. These data are also consistent with findings noted earlier, reported by Kim and Wright (submitted), using a different electrode montage configuration to target SMA.

Activation of SMA influences motor sequence processing that contributes to the development and use of motor chunks

The SMA has been described as a domain-independent sequence processor with a key responsibility of amalgamating primitive task elements into higher order representations. This description is in keeping with the concept of motor chunks, the development of which are considered central to optimizing sequential motor skills [20,21]. Recruitment of SMA during skill acquisition for the effective implementation of motor chunks is available [20]. Moreover, IP has been reported to support early development and more persistent use of motor chunks than RP [60]. As such, it has been speculated that retention benefits afforded through IP result from early and more prolonged activation of the SMA which acts to facilitate the development and implementation of motor chunks [20–22].

In the present experiments TT was decomposed into separate measures that have been proposed to capture unique features of motor sequence implementation. Specifically, Abrahamse et al., (2013) argued that for DSP tasks, such as those used in the present experiments, the latency associated with the first key press of the sequence captures the costs of sequence initiation. The response time to the key press following the random non-0 RSI (key press 4) reflects the costs involved in transitioning between two motor chunks. Time to perform all remaining key presses (2, 3, 5 and 6 for DSP tasks in the present experiments) each depended solely on executed individual elements that constitute any single motor chunk. Assuming these components accurately reflect the manner in which a motor sequence is implemented on a trial, if SMA activation acts to influence processes related to motor chunk use, the most likely candidate from Abrahamse's approach would be the component process targeting concatenation.

When examining how the improvements in TT unfolded across the retention tests following RP that was supplemented with anodal stimulation, two findings are particularly noteworthy. First, the most significant contribution to the time-dependent consolidation benefit (i.e., reduction in TT data from the postI to post6 test block) displayed by individuals in the RP-AtDCS condition is made by the concatenation processes. Specifically, it is only CT that shows a significant reduction, not just performance stability, across the initial 6-hr test interval, compared to IT and ET (see Figure 10). The reduction observed for CT during the early consolidation phase is consistent with the general claim that SMA recruitment is important for motor chunk development and use. In describing the component processes involved during motor sequence implementation, Abrahamse et al., (2013) proposed that the concatenation process is critical for allowing distinct motor chunks within a sequence to be executed in rapid succession. CT is seen as an index of the effectiveness of the transition from one motor chunk to the next allowing the movement sequence to unfold smoothly. These data then are consistent with the claim that heightened SMA activity during skill acquisition is critical for initial consolidation process during which improvements in motor chunk development are occurring.

While the concatenation process was the most likely process that would benefit from anodal stimulation during RP, an additional benefit was afforded from improvement in the execution process which was less anticipated. Unlike CT, ET remained stable across the early hours following the termination of practice rather than exhibit a loss as was the case for IT (see Figure 10) thus contributing to, at least in part, the observation that the individuals exposed to anodal stimulation while also experiencing RP do not reveal forgetting shortly after RP (i.e., an increase in TT during the first 6-hr after training). The potential importance of heightened SMA recruitment to improved execution processes was further highlighted by the fact it was this process alone that accounts for the long-term retention enhancement induced via stimulation. It is possible of course that the SMA has multiple functions, beyond those associated with motor chunking, with one of these being more intimately tied to the execution of individual elements contained within each motor chunk. However, an alternative possibility is that the SMA exerts an influence on remote neural site(s) that are more directly involved in execution. For example, if the stimulation used in Experiment 1 induced a broad up-regulation of the SMA complex that included SMA proper, there are well described connections with M1, a neural center likely heavily involved with execution-type processes, from this region [21]. It is feasible then that the SMA complex, when stimulated in a less focal manner as is likely the case when using tDCS, can impart multiple functional benefits, beyond motor chunk development, that includes a role in the actual execution of newly acquired motor sequences.

Increasing activation of PMd during motor training has a less robust impact on sleep dependent consolidation

In Experiment 2, while the same stimulation approach was used, namely anodal stimulation was utilized in the context of RP only, the focus was on the PMd region of BA6. Contrary, to the findings that surfaced when upregulating its neighbor, SMA, application of anodal tDCS at PMd had no impact on the unfolding of early time-dependent consolidation (see Figure 17). That is, individuals that were privy to the stimulation still exhibited rapid forgetting at the conclusion of RP in manner quite similar to that observed for the RP-Sham condition. However, in the case of later consolidation that is most likely attributed to exposure to sleep, there was some evidence that heightened activity at PMd was beneficial although it appears less robust than what was observed through SMA stimulation (see Experiment 1).

Individuals exposed to anodal stimulation did reveal a gradual, but significant, improvement in TT across the delayed test blocks administered from 6-hr to 72-hrs. However, in this case unlike in Experiment 1, there was also a small but significant improvement in TT displayed by the RP-sham participants. This combination accounted for a significant block main effect. However, the lack of stimulation x block interaction suggests that receipt of supplemental stimulation did not engender more effective sleep-dependent consolidation. This is also the case when evidence for improved sleep-dependent consolidation is examined at the component process level. Once again, only block main effects are evident and they only show improvement through the first night of sleep (i.e., the 24-hr test block) but from additional nights for initiation and execution processes. Taken together, these data offer weak support, at best, for the efficacy of upregulating PMd to induce sleep-dependent gains in skill.

Increasing activation of PMd during motor training does appear to have an important contribution to skill acquisition by improving initial encoding

While inducing greater cortical activity at PMd does little to aid post-practice consolidation and associate offline gain, there was evidence that anodal tDCS at this neural site was beneficial for skill acquisition. While upregulating activity at SMA resulted in superior performance (i.e., lower TT) during training compared to the RP-sham counterparts, this was interpreted as a temporary influence because TT for these two conditions was similar during the subsequent test block (i.e., postI). Indeed, the change in TT from the baseline to the postI test was not different across stimulation conditions.

In the case of PMd, a similar training benefit for TT during actual RP also emerged. However, this improvement in performance seemed more permanent reflected in a change in TT from the baseline test to the immediate test for the anodal tDCS supplemented conditions (~44% improvement) compared to the RP-sham condition (~30% improvement). This difference between the conditions persisted through the remainder of the tests intimating that the encoded knowledge about the learned motor sequence was more complete following stimulation at PMd compared to the learning that was accomplished in the absence of the stimulation. As a whole these data suggest that SMA is acting to support a broad set of post-practice consolidation processes that supervise latent updating of motor sequence knowledge. In contrast, activation of PMd plays a quite different role that is more directed at establishing the initial motor memory that is developed during training that given the right circumstance will further updating as a result of consolidation. Activation of both neural regions, SMA complex and PMd, appear to have important implications for skill acquisition but despite being neighboring neural sites their contribution to the evolution of novel motor memories is quite distinct.

Upregulating right or left PMd did not change the functional outcomes observed during motor training

In Experiment 2 some additional experimental conditions were included to consider recent speculation that left PMd had a proprietary role to play during motor learning. For example, Hardwick et al., (2013) have proposed that left PMd is consistently activated during acquisition of a variety of sensorimotor tasks and its recruitment was independent of movement execution and hand use. Hardwick and colleagues argue that activity of the left PMd may relate to a dominant role in movement selection via visuomotor integration and concluded that left PMd is a key structure in the network of brain areas that underlie motor learning. To consider this possibility, in Experiment 2, a condition that involved anodal stimulation during RP was applied at right PMd which is consistent with the notion of contralateral control given the motor sequence was acquired using the left hand (see Figure 14). An additional condition was included that involved anodal stimulation at left PMd. While this stimulation was ipsilateral to the responding hand, if left PMd contributed to learning in a more general manner as described by Hardwick and is colleagues, it is quite feasible that learning benefits might emerge from raising cortical activity at this site via tDCS.

Remarkably irrespective of the hemisphere to which the administration of anodal tDCS during RP was applied, the outcome was the same. This was true for the conditions involving both anodal and cathodal stimulation at PMd. These data then are consistent with the notion that left PMd is important for skill acquisition and maybe plays a proprietary role during motor learning. However, some caution is warranted in adopting such a strong position regarding the importance of left PMd for learning based on the present findings. Specifically, a stronger case for this conclusion could be made if the symmetric conditions using the right hand as the responding limb were included in this work. More importantly, if this condition were examined, one would expect that while retention benefit such as those observed in the present work should surface from anodal stimulation applied at left PMd, the corresponding benefit from the same stimulation being applied at right PMd, ipsilateral to the responding limb, would now be absent. Clearly this would be an important next step that needs to be addressed.

A word of caution about using neuromodulation to change motor behavior

Experiments 1 and 2 were designed with the primary of objective of modifying cortical activity of two specific sub-components of BA6, namely, the SMA complex and PMd, within two unique practice schedules with the intent of influencing the resultant behavioral outcomes. This approach is not unprecedented in the motor domain although most effort to date have focused almost on mediating the cortical activity at M1 and have done this in the context of learning a single motor skill. So, in this regard, focusing neuromodulation at BA6 as well as probing its effectiveness in a more complex motor learning environment is quite novel. On the basis of the discussion in the preceding section, there is considerable evidence that exogenous anodal stimulation can be used during motor training to mediate long-term motor behavior despite the novel demands of the approach used here.

It should not go unnoticed however that, in general, the use of cathodal stimulation in the present set of experiments was largely unsuccessful. In the present work cathodal stimulation at BA6 was used in an attempt to down-regulate cortical activity with the intent of interfering with behavior. In the context of the present work, the notion was that IP, a practice schedule is assumed to induce sufficient activity at BA6 to elicit positive learning outcomes, would reveal predicable learning detriments following training conducted in the presence of cathodal stimulation during practice. This was based on evidence demonstrating that the application of cathodal tDCS at M1 has been shown to down-regulate cortical activity at this site and that prolonged application such as that used in Experiment 1 and 2 has been reported to induce substantial after-effects [54]. Moreover, cathodal stimulation has been used to disrupt motor behavior, but this impact has been noted as less robust than that associated with its anodal counterpart [53]. The lack of impact from cathodal stimulation in the present work is in keeping then with the apparent inconsistency of this form of neuromodulation for mediating behavior. Nonetheless, this further highlights the need to go beyond *a priori* modeling used to "assume" the underlying changes in neural activity instigated by neuromodulation and capture the "actual" changes in neural recruitment occurring at the targeted sites. Clearly, this is a step that needs to be considered should this work continue.

Limitations and Future Directions

Recent reviews have reported that, neural imaging during IP and RP revealed that the differential cost of movement preparation reflected in the increase in attention demand is accompanied by distinct neural recruitment strategies during RP and IP formats. Therefore, increased CI during training was associated with greater recruitment of neural regions previously described as central to successful preparation and production of learned motor skills when acquiring a single novel skill. In particular, the relative contribution of the primary motor cortex (M1) as well as both lateral premotor (PMd, PMv) and medial premotor regions (pre-SMA, SMA), increased dramatically across IP but not RP.

The present work was an initial attempt to use transcranial direct current stimulation (tDCS) in two novel practice contexts to evaluate the contribution of SMA and PMd to the well-documented long-term retention advantage associated with interleaved practice of multiple novel motor skills. On the basis of the present findings it is argued that relatively greater activity at SMA and PMd during RP was associated with subsequent behavioral gains. However, it is important to recognize that a direct measure of activity at these neural regions was not obtained during training. While a priori modeling of the resultant current flow at SMA and PMd did simulate a change in activation consistent with our initial assumptions, given the retention outcome for the cathodal tDCS IP condition, it seems prudent to include a more direct assessment of activity at the target site in future endeavors.

With respect to the target location at SMA and PMd, the localization of montage and clearly understanding of current delivery needs to be considered going forward. To improve current delivery as function of tDCS one could consider the use of high definition electrode arrays that might improve focality of stimulation at target regions which could potentially lead to more effective use of tDCS protocols [61,62].

Despite the robust behavioural outcome has been reported, remarkably little is known with respect to neural mechanism underlying impact of practice structure on motor memory consolidation and long-term retention of novel or newly acquired motor skill. This knowledge gap is surprising given that impact of practice structure has been hailed as a potential intervention for motor deficits particularly pathological-related impairments. Understanding in greater detail the neural correlates of practice structure-dependent enhanced motor learning in healthy adult is a critical and necessary step prior to translation to a clinical setting. Thus, it is important to consider for future efforts to verify and advance the findings from this work.

To address these future works, comprehensive research approach will be needed to identify the evaluation of modification of emerging connectivity during distinct practice formats supplemented with functional and neurochemical brain imaging protocol coupled with non-invasive brain stimulation tools in particular TMS to investigate the impact of organization of on motor memory consolidation during motor learning. Furthermore, recently, using PPI analysis of fMRI data revealed that during IP format enhanced the development of functional connectivity with dorsal premotor (PMd) regions which are frequently considered central to motor sequence acquisition [17]. Thus, PMd seems primarily distinguished neural site to the most network activated during training and important in the consolidation that supports instrumentally giving increasing excitability at M1 leading to more consistent consolidation which is followed by during interleaved practice correlated with the benefit on retention [12,13,17]. Indeed, Experiment 2 in the present work supported that IP led to heightened PMd activity as consequently greater offline gains that lasted up to 72hrs. Together, these findings indicate that IP format has greater connectivity between the PMd and other regions in the sensorimotor network during the retention phase. Thus, the development of this network (PMd-M1) during practice phase might be modified by using tDCS. This future work might suppress and enhances the capacity of PMd interact during encoding will be reduced or increased M1 excitability, that can be examined by resting-state network (RSN). In other word, RSN might not be equal compare to both IP-sham and RP-Sham conditions. In addition, IP-Sham and RP-Anodal conditions will result in greater BOLD signals in PMd that will reflect better ongoing memory consolidation process compared to RP-sham conditions. A critical assumption made in this experiment, IP-Sham and RP-Anodal will be established robust formation of PMd functionally linked particularly sensorimotor and frontal networks that allow stronger resting-state connectivity of PMd region of networks. This will reflect in greater subsequent consolidation in terms of networks that allows memory and that promote encoded knowledge to be gained more and result on subsequent impact for the development of RSN and critical role for short and long-term retention change.

Finally, such a further investigation is essential before practice structure combination with brain stimulation tool-based interventions can be employed as a viable avenue to improve current strategies to elicit maximal skill learning in healthy and develop particularly training based neuro-rehabilitative intervention after neurologically impaired patients. The contribution of present work is expected to be accessible to understand the neural mechanisms of novel motor skill learning, recovery and to develop strategies to elicit maximal learning effects in healthy and neurologically impaired humans.

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