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RESEARCH ARTICLE

Application of anodal tDCS at primary motor cortex immediately after practice of a motor sequence does not improve offline gain

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Abstract

Tecchio et al. (J Neurophysiology 104: 1134–1140, 2010) reported that the application of anodal tDCS at primary motor cortex (M1) immediately after practice of a procedural motor skill enhanced consolidation, which in turn improved offline gain. Tecchio et al. noted, however, that this study did not account for known after-effects associated with this form of non-invasive stimulation. The present study was designed to explicitly reevaluate Tecchio et al.'s claim. As in the original study, individuals experienced either anodal or sham stimulation at M1 after practice of a serial reaction time task (SRTT) followed by test trials 15-min later. Two additional novel conditions experienced the test trials after 120-min rather than 15-min thus allowing potential stimulation after-effects to dissipate. The expectation was that if anodal stimulation influences post-practice consolidation leading to offline gain, this effect would be present not only at 15-min but also after 120-min. In agreement with the working hypothesis, findings revealed offline gain at both 15-min and the longer 2-h time period. Unexpectedly, we found no interaction between real and sham conditions. The lack of difference between Real and Sham effects weakens confidence in the potential of post-practice tDCS for consolidation enhancement, while it is more consistent with other claims that decoupling practice and anodal tDCS stimulation in time can reduce the effectiveness of exogenous stimulation for procedural skill gain.

Keywords Procedural learning · Consolidation · tDCS · Motor cortex

Introduction

When a learner practices a new motor sequence and performance improves, referred to as *online performance gain*, a labile memory representation is formed which is susceptible to interference from immediate practice with other motor skills (Brashers-Krug et al. 1996). However, following the completion of practice and in the absence of interference these new motor memories are quickly stabilized and possibly enhanced as a result of consolidation (Walker et al. 2003). This process is commonly referred to as *offline performance gain*. *Consolidation* has been described as a process of strengthening memories and is assumed to be responsible for offline improvement that establishes stabile and/or enhanced motor memories (Diekelmann and Born 2007).

Considerable evidence has emerged from both neuroimaging and behavioral studies for a prominent role of the primary motor area (M1) in the development of motor memories (Doyon et al. 2009; Karni et al. 1995; Penhune Steele 2012). Muellbacher et al. (2002) noted that M1 plays a crucial role in consolidation of a motor skill in the early stages of learning. In a series of studies, Muellbacher et al. reported that the performance improvement that followed repeated bouts of practice of a relatively simple pinch-force task could be attenuated by applying 1-Hz repetitive transcranial

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magnetic stimulation (rTMS) at M1 for 15-min immediately after practice. In essence, rTMS at M1 interrupted stabilization (i.e., offline gain) of the behavioral improvement achieved during practice (i.e., online gain). Interestingly, this stimulation had no effect on the learner's subsequent ability to recover these gains via additional practice (i.e., later online gain). The importance of M1 for consolidation was verified in subsequent experiments by Muellbacher et al. that revealed no degradation in performance between practice bouts when rTMS was administered at the occipital or dorsolateral prefrontal regions. Furthermore, application of rTMS at M1 6-h after practice failed to disrupt performance suggesting that a time window of approximately 6-h after practice was important for consolidation to occur.

More recently, the contribution of M1 to offline performance gains during motor sequence learning has been explored using an alternative form of non-invasive brain stimulation called transcranial direct current stimulation (tDCS). This involves the passage of weak direct current between two electrodes placed on regions of interest within the brain (e.g., M1). The applied current flows between a positively charged anode and a negatively charged cathode. Since tDCS induces an intracerebral current flow, neuronal excitability of the targeted brain area can be modified in a polarity-specific manner. Generally, anodal stimulation (with reference to the target area) increases cortical excitability while cathodal stimulation has been reported to decrease excitability at M1 (Nitsche and Paulus 2000; Reis and Fritsch 2011, although see Batsikadze et al. 2013).

Administering anodal tDCS at M1 during physical practice has been reported to facilitate motor learning in healthy adults (Buch et al. 2017) as well as individuals with stroke (Boggio et al. 2007; Fregni et al. 2005; Hummel and Cohen 2005). Online improvement of novel motor sequences has been observed frequently when anodal tDCS at M1 occurs *concurrently with practice* of a novel motor sequence across a single day (Cuypers et al. 2013; Karok and Witney 2013; Zimerman et al. 2013; Kantak et al. 2012; Stagg et al. 2011; Nitsche et al. 2003) as well as across multiple days (Ciechanski and Kirton 2017; Waters-Metenier et al. 2014; Prichard et al. 2014; Saucedo Marquez et al. 2013; Reis et al. 2009). A few studies have failed to reveal any enhancement in motor sequence acquisition when anodal stimulation at M1 occurs during training (Ambrus et al. 2016; Amadi et al. 2015; Reis et al. 2015). In contrast, application of similar stimulation *prior to practice* has been shown to hinder subsequent performance (Amadi et al. 2015; Stagg et al. 2011).

Offline enhancement in sequence learning also occurs following the application of anodal tDCS at M1 during training especially when the stimulation is paired with practice over multiple days of training (Naros et al. 2016; Reis et al. 2015; Waters-Metenier et al. 2014; Saucedo Marquez et al. 2013; Reis et al. 2009; although see Prichard et al. 2014). For example, Reis et al. (2009) required individuals practice a sequential force-pinch skill over the course of 5 days in the presence of either anodal or sham tDCS at M1. Greater performance gains were associated when practice occurred in the presence of anodal tDCS compared to sham stimulation. Of particular interest, for the present work, was the finding that a significant proportion of the behavioral improvement was manifested offline. That is, performance enhancement from the application of anodal tDCS at M1 emerged during the 24-h intervals between practice sessions. These data were further elaborated by Reis et al. (2015) noting that similar offline gain can be manifested with exposure to just sufficient time, between 15-min and 3-h following stimulation, as opposed to being dependent on overnight sleep. The latter of which has been documented to be influential in supporting offline improvement during procedural skill learning (Diekelmann and Born 2007). These data then suggest that the exogenous application of tDCS at M1 over a number of days of practice can modify post-practice consolidation such that a more stable or even enhanced motor memory is developed across the entire course of training which remains relatively stable over longer periods of retention (see Reis et al. 2009).

Tecchio et al. (2010) adopted a more direct approach to assess if post-practice consolidation can be influenced via exogenous neuromodulation. Specifically, they considered the possibility that application of anodal tDCS at M1 immediately following rather than during practice but prior to administering a retention test is sufficient to improve post-practice consolidation thus leading to increased offline gain. To evaluate this proposal Tecchio et al. had individuals practice a nine-element serial reaction time task (SRTT) interspersed with execution of a random set of key presses. Test performance (i.e., offline gain) was evaluated approximately 30-min after the initial training period. For the individuals that received 15-min of anodal tDCS at M1 following practice, test performance was superior to those that experienced sham stimulation suggesting that the administration of tDCS had indeed enhanced post-practice consolidation. Despite this observation, Tecchio et al. noted that it is possible that this outcome could have been a consequence of the well-documented after-effects of tDCS (Nitsche and Paulus 2000) as opposed to a direct effect on post-practice memory processes. This possibility was confirmed by data from a control condition included in the work of Reis et al. (2015) that applied anodal tDCS for 15-min following practice of a sequential visual isometric pinch force skill and failed to reveal offline gain at a subsequent test given following a sleep-filled interval.

The data from Reis et al. (2015) are not in agreement with Tecchio et al.'s (2010) findings and more broadly question the efficacy of exogenous stimulation during the consolidation period as a means of garnering offline gain.

Rather, these data argue that simultaneous application of tDCS with training is required for offline skill gains to emerge (see also Fritsch et al. 2010). Unfortunately the test interval used in the control condition in the work of Reis et al. (2015) included a period of sleep which may have influenced the reported outcomes given sleep's well-documented impact on consolidation. For this reason, it seems appropriate to revisit Tecchio et al.'s study and related findings. Specifically, it is important to directly evaluate the impact of anodal tDCS at M1 for test trials at a time point at which increase in M1 excitability attributable to the administration of the stimulation is likely extinguished while also eliminating the potential contribution of sleep-related consolidation. The primary goal for the present study was to directly address this issue. Individuals were exposed to one of four experimental conditions each of which involved an initial bout of motor training with a target motor sequence task similar to that used by Tecchio et al. (2010). This training was then followed by the administration of either 15-min of anodal or sham tDCS at contralateral M1. Either 15-min (see Tecchio et al. 2010) or 120-min after the receipt of tDCS or sham stimulation, test trials of the target motor sequence were conducted. This design resulted in four separate experimental conditions that involved the administration of test trials 15-min after AtDCS (AtDCS_15), 120-min after AtDCS (AtDCS_120), 15-min after sham stimulation (Sham_15), or 120-min after sham stimulation (Sham_120). If Tecchio et al. are correct in their assertion that anodal tDCS applied on contralateral M1 soon after training can improve early consolidation of motor sequence learning, it was anticipated that an offline gain would be observed when testing was conducted after a 120-min interval (AtDCS_120 condition). Alternatively, if the claim of Reis et al. (2015), that exogenous stimulation after practice does not impact ongoing consolidation, there should be no offline gain for the delayed test administered across a wake-filled interval despite the possibility that stimulation after-effects might still contribute to improved performance during the 15-min test delay.

Methods

Participants

One-hundred right-handed, as assessed using the Edinburgh handedness inventory, young adults (mean age = 22 years, SD = 2 years, 68 females) served as participants in the experiment. Participation in this study fulfilled a research requirement for university-level class and informed consent was obtained prior to any involvement in the study.

Motor sequencing task

All participants practiced a 8-key motor sequencing task that required the repetition of the same string of key presses for 30 s (e.g., 4-2-1-3-1-4-2-3) on a standard PC keyboard using the C, V, B, N keys where "1" was associated with the leftmost key (C key) and "4" with the rightmost key (N key) (see Walker et al. 2003). The participant was informed the goal was to produce as many accurate reproductions of the sequence of key presses as possible during the 30-s trial. Four white squares were displayed on a black background on the computer monitor that represented the four keys (C, V, B, and N). As a key was pressed a black circle appeared in the box as feedback that a correct key press had been made. As the participant executed the set of required key presses the black circle moved back and forth across the four boxes. If an erroneous key press was made, the black circle did not move until the correct key was pressed. All individuals performed all 30 s trials of the motor sequencing task with their non-dominant (left) hand throughout the practice and test phases. The order in which these keys should be depressed during a trial was illustrated with the string of numbers presented at the top of the computer monitor for the entire duration of a trial. All participants were instructed to execute the key presses accurately and as fast as possible. Some trials involved a random string of 8-key presses that was also produced for 30 s. The goal again was to press the keys in the correct order and as fast as possible. Each of these trials involved a different string of eight key presses.

Transcranial direct current stimulation

Following the initial bout of practice, anodal transcranial direct current stimulation (tDCS) involved delivery of the current for 15-min by a battery-driven stimulator (tDCS Stimulator; TCT Research Limited, Hong Kong) using two saline soaked 5×5 cm^2 sponge electrodes. Stimulation intensity was 1 mA resulting in a current density of 0.04 mA/cm^2. The anode was placed over right M1 (i.e., this electrode was centered over C4 in accordance with the International 10/20 System) with the cathode placed on the right shoulder (see Fig. 1) (also see Tecchio et al. 2010). In the case of sham stimulation, the electrode montage was the same but stimulation was delivered for only 30-s at the beginning and end of the 15-min stimulation period.

Procedures

On arrival at the laboratory each participant completed an informed consent prior to engagement in any phase of the experiment and pre-experiment screening to exclude individuals with contra-indications for tDCS. Individuals were assigned to one of four experimental conditions (a) anodal

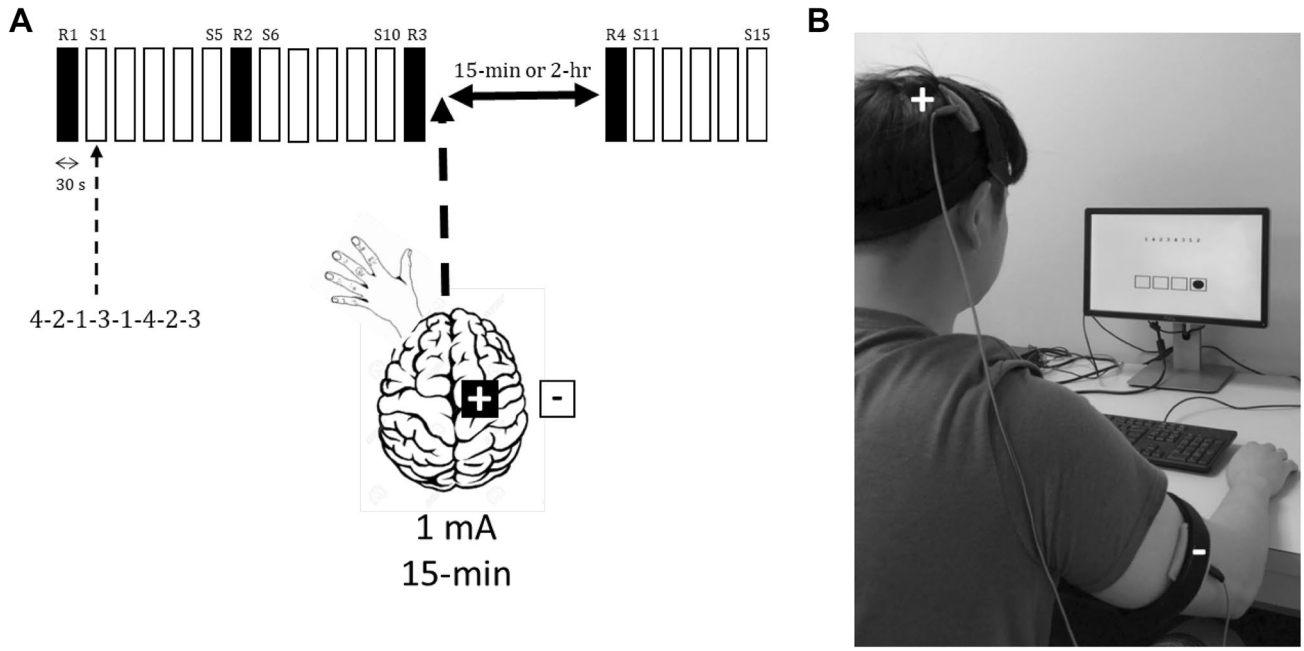

Fig. 1 Individuals experienced trials (S_n) of a motor sequencing task for 30 s intervals across a period of practice and test. These trials were interspersed with trials with random key press sequences (R_n). At the conclusion of practice some individuals (AtDCS_15, AtDCS_120) were exposed to 15-min of transcranial direct current stimulation (tDCS) whereas others were administered sham stimulation (Sham_15, Sham_120). The anodal tDCS or sham was administered either 15-min (AtDCS_15, Sham_15) or 2-h (AtDCS_120, Sham_120) after the conclusion of training (see text for further details) (**a**). Real and Sham tDCS was administered via two electrodes placed on right M1 (anode) and the right shoulder region (cathode) (**b**)

tDCS after motor training followed by test trials 15-min later (AtDCS_15), anodal tDCS after motor training followed by test trials after 120-min (AtDCS_120), sham stimulation after motor training followed by test trials after 15-min (Sham_15), or sham stimulation after motor training followed by test trials administered after 120-min (Sham_120). Prior to any practice with the target motor sequence task all individuals were first prepared for the subsequent stimulation protocol (either real or sham conditions) which involved placement of electrode at right M1 and the right shoulder area.

Following electrode placement, participants began motor training with the target motor sequence task. In a manner similar to Tecchio et al. (2010), each individual was first exposed to a trial with a random set of keys (R_1) followed by five trials of practice with the target motor sequence task ($S_{1–5}$). As noted earlier, each trial duration was 30 s with a 10 s rest interval between each trial. A trial was initiated with the presentation of the string of numbers that represented the motor sequence task to be performed for that trial. Following the initial five trials with the target motor sequence task a second trial with a novel 8-key set of key presses (R_2) was performed. On completion of R_2, an additional five trials with the target motor sequence task ($S_{6–10}$) were completed. The initial training phase was completed with one final trial with new random set of eight key presses (R_3) (see Fig. 1).

On completion of motor training, individuals were then exposed to either anodal or sham tDCS stimulation. Individuals assigned to anodal (AtDCS_15, AtDCS_120) conditions were exposed to 15-min of stimulation at right M1 (i.e., contralateral to the responding left limb). The sham (Sham_15, Sham_120) conditions on the other hand were merely exposed to stimulation for the initial 30 s of the 15-min interval. On completion of the delivery of anodal or sham stimulation, participants assigned to the most immediate test condition (i.e., AtDCS_15, Sham_15) performed a set of test trials. Specifically, the test phase began with one 30 s trial with a novel set of eight key presses (R_4). This was followed by five trials with the target motor sequence task ($S_{11–15}$). All individuals assigned to the more delayed test (AtDCS_120, Sham_120) were administered the same set

of trials but presentation occurred 2-h after the administration of anodal or sham stimulation. All trials during the test were again 30 s in duration with a 10 s rest interval. For all trials in the training and test phases, the primary dependent variable was response time (RT) for each keystroke.

Data analyses

Median RT for each training and test trial was determined for each individual for random and target motor sequences.[1] Online learning for the target and random motor sequences was separately evaluated by submitting RT data from either the S_1 and S_{10} (target motor sequence, see Fig. 1) or R_1 and R_3 (random motor sequence, see Fig. 1) trials to a repeated measures analysis of variance (ANOVA) with Group (AtDCS_15, AtDCS_120, Sham_15, Sham_120) as the between-subject factor and Training (Early, Late) as a within-subject factor. In a similar fashion, offline learning of the target and random sequence tasks was evaluated by submitting median RTs from the end of training (S10 or R3, see Fig. 1) and the initial test trial (S11 or R4, see Fig. 1) to a repeated-measures ANOVA with Stimulation Type (AtDCS, Sham) and Test Delay (15-min, 120-min) as the between-subject factors and Trial (Training, Test) as a within-subject factor.

In addition to the aforementioned analyses we also included some supplementary analyses that were similar to those included in Tecchio et al. (2010). Specifically, for online gain, RT data for S_1 and S_{10} (i.e., early and late) were normalized by the RT for initial trial with a random sequence (i.e., R1) (see Fig. 1 here and Fig. 3 in Tecchio et al. 2010). The normalized data were submitted to a repeated measures analysis of variance (ANOVA) with Group (AtDCS_15, AtDCS_120, Sham_15, Sham_120) as the between-subject factor and Training (Early, Late) as a within-subject factor. A second analyses addressed offline gain which again involved normalizing RTs from the end of training (i.e., S10) and for the initial test trial (i.e., S11) by their partner random trials, R3 and R4, respectively (see Fig. 1). The normalized RT data were submitted to a repeated measures ANOVA with Stimulation Type (AtDCS, Sham) and Test Delay (15-min, 120-min) as the between-subject factors and Trial (Training, Test) as a within-subject factor. Post-hoc tests were used to explore significant effects that emerged from these analyses.

[1] The distributions for mean RT from the training and test data were not normally distributed. For this reason the median RT for each training and test block was considered a more suitable performance measure for all the reported analyses.

Results

Online gain across initial training

Target motor sequence task

RT for the target motor sequence from early (S1) and late (S10) trials in training (see Fig. 1) as a function of Group (AtDCS_15, AtDCS_120, Sham_15, and Sham_120) is displayed in Fig. 2. Performance for the target motor sequence improved with practice but the improvement in RT was similar for individuals assigned to the AtDCS_15, AtDCS_120, Sham_15, and Sham_120 groups. This was supported by the Group (AtDCS_15, AtDCS_120, Sham_15, and Sham_120) × Training (Early, Late) ANOVA revealing a significant main effect of Trial, $F(1,86) = 106.24$, $p < 0.01$, $\eta_p^2 = 0.55$. Post-hoc assessment indicated that RT at the beginning of training (S1: $M = 516$ ms, SEM = 13 ms) was greater than that observed at the conclusion of training (S10: $M = 409$ ms, SEM = 13 ms).

Random motor sequence task

Individuals assigned to the AtDCS_15, AtDCS_120, Sham_15, and Sham_120 groups exhibited a similar change in RT for the random motor sequence at the start (R1) and end (R3) of training (see Fig. 1). The Group (AtDCS_15, AtDCS_120, Sham_15, and Sham_120) × Training (Early, Late) ANOVA was congruent with this observation

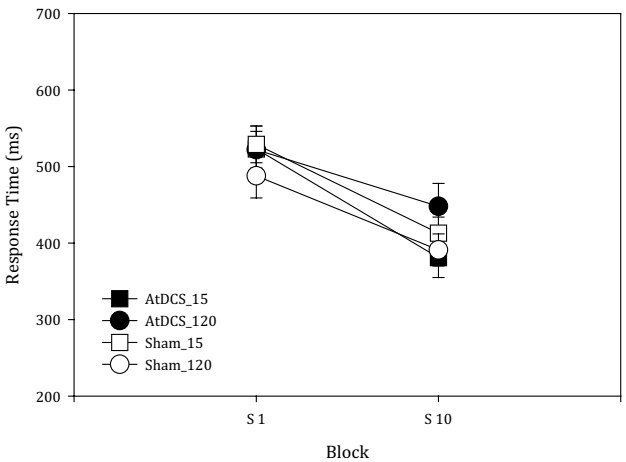

Fig. 2 Response time (ms) for the early (S_1) and late (S_{10}) blocks with the target sequence during the training phase for the AtDCS_15 (closed circle), Sham_15 (open circle), AtDCS_120 (closed square), and Sham_120 (open square) conditions

revealing only a significant main effect of Training, $F(1,86) = 12.45$, $p < 0.05$, $\eta_p^2 = 0.13$. Post-hoc assessment verified that RT for the random motor sequence task performed after the target motor sequence ($M = 539$ ms, SEM = 17 ms) was greater than the RT reported at the beginning of training phase ($M = 496$ ms, SEM = 17 ms).

Offline gain for the repeated motor sequencing task

RT for the target motor sequence task at the end of training and for the initial test trial for individuals that were administered test trials after 15-min (AtDCS_15, Sham_15; Fig. 3a) or after 120-min (AtDCS_120, Sham_ 120; Fig. 3b) are displayed in Fig. 3. These data were subjected to a repeated-measures ANOVA with Stimulation Type (AtDCS, Sham) and Test Delay (15-min, 120-min) as between-subject factors and Trial (Training, Test) as a within-subject factor which revealed only a significant main effect of Trial, $F(1,86) = 46.6$, $p < 0.01$, $\eta_p^2 = 0.35$. Post-hoc analysis indicated that the RT at test was lower ($M = 356$ ms, SEM = 15 ms) than the RT observed at the end of training ($M = 409$ ms, SEM = 18 ms).

Offline gain for the random motor sequence performance

RT for the random motor sequence task at the end of training and for the initial test trial for individuals that were administered test trials after 15-min (AtDCS_15, Sham_15; Fig. 4a) or after 120-min (AtDCS_120, Sham_ 120; Fig. 4b) are displayed in Fig. 4. These data were subjected to a repeated-measures ANOVA with Stimulation Type (AtDCS, Sham) and Test Delay (15-min, 120-min) as between-subject factors and Trial (Training, Test) as a within-subject factor which revealed only a significant main effect of Stimulation Timing, $F(1,86) = 88.7$, $p < 0.01$, $\eta_p^2 = 0.51$. Post-hoc analysis

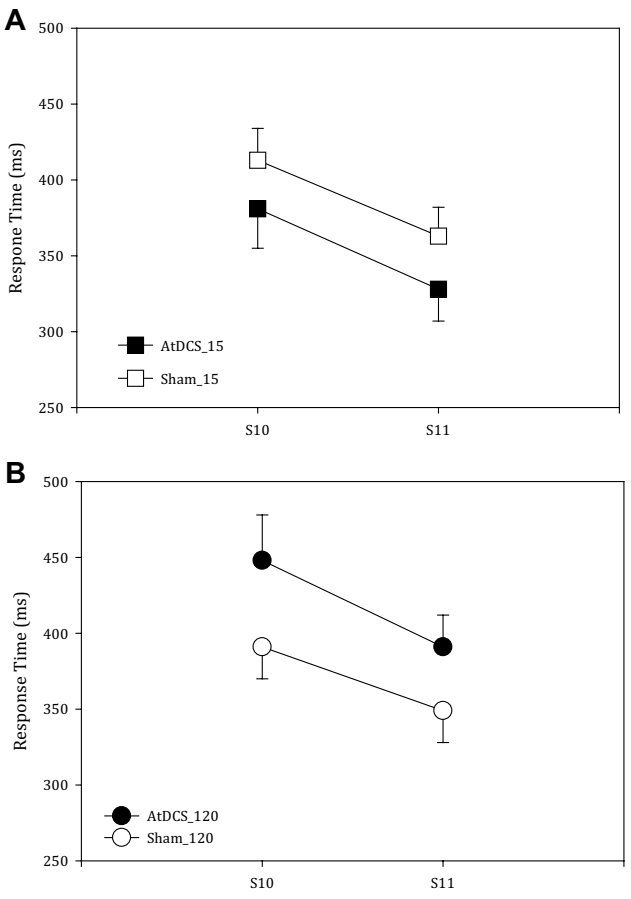

Fig. 3 Response time (ms) for the last block of training (S_{10}) and test trials (S_{11}) for the target sequence for individuals that experienced the test after 15-min (AtDCS_15, Sham_15; **a**) and 120-min (AtDCS_120, Sham_120; **b**)

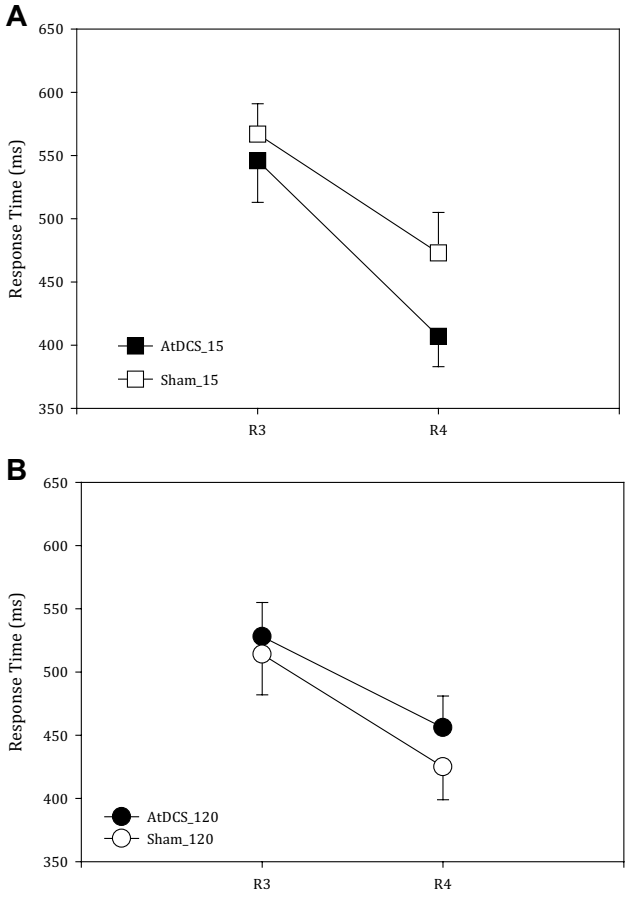

Fig. 4 Response time (ms) for the random block at the completion of training (R_3) and prior to the test trials (R_4) with the random sequence for individuals that experienced the test after 15-min (AtDCS_15, Sham_15; **a**) and 120-min (AtDCS_120, Sham_120; **b**)

indicated that the POST-stimulation RT was lower ($M = 440$ ms, SEM = 19 ms) than the RT observed PRE-Stimulation ($M = 539$ ms, SEM = 21 ms).

Online and offline gain for normalized motor sequence performance

Normalized RT data for the target motor sequence from early (S1) and late (S10) trials in training (see Fig. 1) for all participants were subjected to a repeated-measures ANOVA with Group (AtDCS_15, AtDCS_120, Sham_15, and Sham_120) as a between-subject factor and Training (Early, Late) a within-subject factor. Online gain for the target motor sequence was observed with training and this improvement was similar for individuals assigned to all groups. This was supported by the analysis only revealing a significant main effect of Training, $F(1,86) = 145.36$, $p < 0.01$, $\eta_p^2 = 0.63$. Post-hoc assessment indicated that the performance of the target sequence relative to a random sequence observed at the beginning of training (S1: $M = -0.04$, SEM = 0.04) increased with practice (S10: $M = -0.28$, SEM = 0.05).[2]

Normalized RT data for the target motor sequence task at the end of training and for the initial test trial for individuals that were administered test trials after 15-min (AtDCS_15, Sham_15) or after 120-min (AtDCS_120, Sham_ 120) were subjected to a repeated-measures ANOVA with Stimulation Type (AtDCS, Sham) and Test Delay (15-min, 120-min) as between-subject factor of Trial (Training, Test) as a within-subject factor. This analysis revealed significant main effects of Test Delay, $F(1,86) = 9.00$, $p < 0.01$, $\eta_p^2 = 0.11$ and Trial, $F(1,86) = 12.07$, $p < 0.01$, $\eta_p^2 = 0.12$. Post-hoc analyses indicated that RT of the target sequence relative to the random sequence was superior for the 15-min ($M = -0.29$, SEM = 0.03) compared to the 120-min delay ($M = -0.20$, SEM = 0.03) conditions and greater at the end of training (S10: $M = -0.28$, $SEM = 0.03$) than during the test (S11: $M = -0.21$, SEM = 0.02).

Discussion

The present work was designed to directly reevaluate a finding reported by Tecchio et al. (2010) that indicated greater offline gain for a procedural skill by application of anodal transcranial direct current stimulation (tDCS) rather than sham stimulation at primary motor cortex (M1) immediately after practice of the procedural task. Specifically, Tecchio et al. applied anodal tDCS at M1 for approximately 7-min shortly after a brief bout of training with a serial reaction time task (SRTT). Fifteen minutes after training was concluded and the tDCS application had ceased, Tecchio et al. reassessed performance of the SRTT. Test performance in the real stimulation condition was superior compared to a sham condition and importantly showed some improvement beyond that observed at the conclusion of acquisition. These data lead Tecchio et al. to conclude that the administration of anodal tDCS had influenced post-practice consolidation which in turn improved motor performance. However, as noted by Tecchio et al. relatively long-lasting after-effects of tDCS administration are well documented and cannot be ruled out as an alternative reason for the offline improvement reported in this study. This concern, coupled with a recent evidence revealing that tDCS stimulation cannot be temporally de-coupled from training if the intent is to influence consolidation (Fritsch et al. 2010; Reis et al. 2015), suggests that a re-examination of this issue is warranted.

To assess Tecchio et al.'s proposal explicitly, the design of the present study included the critical stimulation conditions central to Tecchio et al.'s original study that involved anodal (AtDCS_15) and sham (Sham_15) stimulation being applied right after practice followed almost immediately by a set of test trials of the practiced skill. The present work also included two novel anodal and sham experimental conditions that delayed the administration of the test trials such that the well-documented after-effects of tDCS would dissipate (i.e., AtDCS_120, Sham_120). That is, tDCS (or sham stimulation) was still applied immediately after practice finished but the test encountered by the participants occurred after 2-h not 15-min. The expectation then was that if exogenous stimulation (i.e., tDCS) impacts post-practice consolidation, the benefits reported by Tecchio et al. would still be present after the longer 2-h retention interval as well as emerging when the test trials were administered after the relatively brief 15-min interval used herein and in the original study.

As one would expect, training facilitated performance of the target motor sequence task by roughly 20%. This was revealed when assessing performance of the target sequence alone and when considering performance of this motor sequence relative to an untrained sequence. This was similar across all experimental conditions (i.e., AtDCS_15, AtDCS_120, Sham_15, Sham_120) as anticipated given that stimulation was applied after training with the target motor sequence was concluded. With respect to offline change, the findings from the AtDCS_15 and Sham_15 conditions, those conditions in the present work that mimicked those contained in the work of Tecchio et al. failed to replicate the key findings from the original work. Recall, that performance of the individuals that received anodal stimulation at M1 was expected to be superior to that displayed by their sham counterparts and that their performance would be enhanced across the short retention interval. While the analysis did reveal offline improvement, the significant trial

[2] Minus values for the normalized data used for these analyses reflects superior performance for the trained sequence compared to the random sequence.

effect intimated that this gain was present and similar for individuals in both the AtDCS_15 and Sham_15 conditions. Thus, all individuals, irrespective of the receipt of real or sham stimulation, displayed a roughly 13% additional reduction in response time beyond that accomplished online.

The central issue of the present work focused on the outcome observed when the test was conducted after 120-min rather than 15-min. There was no evidence that anodal tDCS immediately after practice differentially impacted the change in performance from the end of training to test. As was the case when the test was administered after a short delay, there was also similar performance improvement across the 2-h test interval but this was again observed for both experimental conditions—AtDCS_120 and Sham_120. Thus, offline gains were still present when sufficient time had passed that would afford dissipation of any after-effects from earlier stimulation. These data further demonstrate that post-practice consolidation can positively influence behavior in the absence of interfering activity (e.g., additional practice, application of rTMS). More critically, however, the magnitude of the consolidation that was displayed is not modified by the administration of anodal tDCS in the time period during which this key memory process is ongoing. These data, in conjunction with recent data from Reis et al. (2015), are not in agreement with data from Tecchio et al. (2010) that intimated that immediate post-practice application of anodal tDCS stimulation at M1 is capable of mediating consolidation and any associated offline changes in performance.

One final issue is worth considering that addresses the nature of the information that is consolidated after practice is concluded in the present experiment. Naturally, the primary focus of many procedural skill learning studies that contemplate the acquisition and retention of motor sequences is sequence-specific learning, most commonly defined as performance improvement resulting from gaining knowledge specifically associated with the nature of the sequence that is practiced during training. Typically, this information is central to how the learner represents the newly acquired motor memory to more effectively execute the motor sequence as experience increases. Historically, the focus has been on both allocentric (e.g., visual-spatial) and egocentric (i.e., motor) sources of information that facilitate development of memory for the new skill (Hikosaka et al. 1999).

However, non sequence-specific learning can and does occur and is generally considered to involve the acquisition of a more general skill benefit that can contribute, albeit usually to a lesser extent than sequence-specific knowledge, to behavioral improvement (i.e., reduced RT). This is most likely through familiarization with features pertinent to the learning situation (Lin et al. 2010; Sidaway et al. 2016). For example, in the present experimental case, this might involve moving the hand effectively across the keyboard or understanding particular associations between stimuli and responses to form visual/spatial-motor mappings that aid the acquisition of motor sequencing tasks in general. The fact that the offline gains in the present study emerged not only in the case for the target motor sequence task but also for the random strings performed at the end of practice and the very beginning of test suggests that these improvements, at least included and possibly completely, resulted from non sequence-specific learning. This was true for individuals exposed to both anodal and sham stimulation conditions. These data then suggest that performance advantage that surfaces during the test trials was not attributable to storage of details of the trained motor sequence task but rather more likely generalized knowledge that was pertinent to the implementation of the type of skill being acquired.

In most methodological aspects such as neural site stimulated, electrode montage, and dosage, the present experiment were very similar to that of Tecchio et al. However, we would be remiss if we failed to make note of a couple of distinctions in the protocol between the present work and that of Tecchio et al's study. For example, the presence of feedback and its impact on execution correction differed across studies. Specifically, Tecchio et al. did not provide feedback about the correctness of each response while in the present work the movement of a visual signal between response locations indicated correct responses. Evidence exists demonstrating that the availability of visual feedback about the exerted pressure changes when executing of a simple weak handgrip significantly influences the profile of brain activity (Mayhew et al. 2017).[3] Moreover, the present experiment used a target motor sequence that was eight rather than nine elements in length. It is of course possible that these procedural differences might have contributed to the failure to replicate the earlier work.

In summary, the findings presented herein were not in agreement with the earlier findings of Tecchio et al. (2010) that revealed that the application of post-practice anodal tDCS at M1 can enhance offline performance gain for procedural motor skill by influencing consolidation. While consolidation of non-sequence specific knowledge was apparent, the emergence of this gain was not modified by the presence of AtDCS after training was complete. These data are consistent with those reported by Reis et al. (2015) who applied anodal tDCS for 15-min following practice of a sequential visual isometric pinch force skill and failed to reveal offline gain at a subsequent test given following a sleep-filled interval. The present data reports a similar outcome even in the absence of sleep during the retention interval. In light of the present findings and the other evidence involving stimulation over multiple days (Reis et al. 2009), it appears that decoupling practice and the application of tDCS across time

[3] We appreciate an anonymous reviewer noting the feedback protocol difference between the represent study and that of Tecchio et al. (2010) and the potential implications for the current findings.

reduces the likelihood that the stimulation will influence consolidation and as a consequence impact offline gain of procedural skill (Fritsch et al. 2010).

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