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Neuromodulation of cognitive-behavioral control

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Chapter Ten

The effect of cerebellar tDCS on sequential response selection processes

Jongkees, B. J., Immink, M.A., Boer, O. D., Yavari, F. B., Nitsche, M. A., & Colzato, L. S. (Submitted). The effect of cerebellar tDCS on sequential motor control and learning.

Abstract

In recent years, transcranial direct current stimulation (tDCS) has received considerable attention as a means to transiently alter cortical excitability and synaptic plasticity. So far, only few studies have investigated the cognitive-behavioral effects of applying tDCS to the cerebellum. Given the role of the cerebellum in fine motor control and motor coordination, we investigated whether cerebellar tDCS modulates response selection processes. Seventy-two participants received either anodal (excitatory), cathodal (inhibitory) or sham (placebo) tDCS while performing a serial reaction time task (SRTT). To compare acute and long-term effects of tDCS on response selection, participants came back for follow-up 24 hours after stimulation. Results indicate that the three groups did not differ in performance prior to tDCS. Although tDCS did not affect implicit motor learning, anodal as compared to cathodal and sham stimulation did modulate response selection processes as evidenced by overall increased response latencies both during stimulation and at 24 hours follow-up. These results are consistent with the notion that the cerebellum exerts an inhibitory effect on primary motor cortex (M1), which results in delayed movement when this inhibition is strengthened by tDCS.

Introduction

Recent years have seen a substantially growing interest in non-invasive methods of brain stimulation. In particular, transcranial direct current stimulation (tDCS) has received considerable attention as a means to transiently alter cortical excitability and synaptic plasticity (Nitsche & Paulus, 2000, 2001; Nitsche, Nitsche, et al., 2003; Plewnia et al., 2015). Although many studies have examined the cognitive-behavioral effects of stimulating cortical areas such as dorsolateral prefrontal cortex and primary motor area (M1), very recent studies have begun to investigate the cerebellum as a potential site of stimulation (van Dun, Bodranghien, Mariën, & Manto, 2016). The cerebellum plays a critical role in sensorimotor control, such as planning, initiation and organization of movement (Manto et al., 2012). This raises the question whether cerebellar tDCS can modulate response selection processes. Investigating this issue has the potential to further our knowledge of the cerebellum's involvement in sensorimotor control and offer rehabilitation strategies for patients with cerebellar dysfunction. Therefore, in the present study we set out to clarify the effects of cerebellar tDCS by assessing response selection and motor sequence acquisition in the serial reaction time task (SRTT) both during stimulation and at 24 h follow-up.

tDCS is typically applied by mounting two electrodes on the scalp, with a current of 1-2 mA running between the electrodes. This is thought to alter the resting membrane potential of neurons in a polarity-dependent manner: neurons beneath the anode are slightly depolarized and thus have an increased likelihood of firing, whereas neurons beneath the cathode are slightly hyperpolarized and thus have a reduced likelihood of firing (Nitsche & Paulus, 2000). At longer stimulation periods, tDCS can also affect neural plasticity for minutes or hours following stimulation (Nitsche & Paulus, 2001; Nitsche et al., 2008; Nitsche, Nitsche, et al., 2003) by producing changes in levels of glutamate and GABA (Bachtiar et al., 2015; Soyoung Kim et al., 2014; Nitsche, Fricke, et al., 2003; Stagg et al., 2009).

Of relevance to the present study's objective, previous research has demonstrated that cerebellar tDCS modulates a phenomenon referred to as

cerebello-brain inhibition (CBI) in a polarity-dependent manner. That is, Purkinje cells in the cerebellum exert an inhibitory tone over M1 via the dentate-thalamo-cortical pathway (Kelly & Strick, 2003; Middleton & Strick, 2000), and this inhibition is strengthened by anodal tDCS and weakened by cathodal tDCS relative to sham stimulation (Galea, Jayaram, Ajagbe, & Celnik, 2009). Combined with the fact that motor sequence acquisition is typically associated with an increase in excitability of M1 (Lin et al., 2011), this suggests that anodal tDCS could hinder the initiation of movement and impair acquisition of motor sequences, whereas cathodal tDCS could facilitate these processes.

Studies on cerebellar tDCS have not yet unequivocally confirmed or falsified these hypotheses. In support of these expectations, anodal tDCS has previously produced a delay in the initiation of muscle activity (Dutta, Paulus, & Nitsche, 2014) and impaired handwriting legibility with the non-dominant hand (Foerster et al., 2013). However, these findings contrast with two reports that anodal tDCS enhanced implicit motor sequence learning (Ehsani, Bakhtiary, Jaberzadeh, Talimkhani, & Hajihasani, 2016; Ferrucci et al., 2013). Unfortunately, these studies report only that the stimulation produced a larger difference in reaction time (RT) between random and sequenced response blocks, but do not clarify whether this difference is driven by an increase in RT for random responses, a decrease in RT for sequenced responses, or both. Furthermore, one of these studies used a symbolic rather than spatial stimulus-response mapping (Ehsani et al., 2016), which further complicates the interpretation of the results, whereas the other study observed no sequence learning in the group receiving sham stimulation (Ferrucci et al., 2013). Considering also the fact that these studies have primarily focused on anodal rather than cathodal tDCS, there is still much uncertainty about the effects of cerebellar tDCS on sensorimotor control.

In the present study we set out to clarify this issue by examining the effects of anodal, cathodal and sham tDCS of the cerebellum on response selection and motor sequence acquisition in a SRTT with a spatial stimulus-response mapping. The SRTT is a 4-choice RT task (Nissen & Bullemer, 1987)

that involves response selection, inhibition of non-target responses and implicit formation of response sequence structures, each of which may be sensitive to a modulation of cerebellar excitability (and indirectly, M1 excitability) via tDCS. Typically, a second-order conditional (SOC) response sequence is embedded in the SRTT unbeknownst to the participants. Implicit acquisition of this sequence structure results in increasingly shorter RT and less response errors as the task progresses (Abrahamse & Noordzij, 2011; Nissen & Bullemer, 1987; Schwarb & Schumacher, 2012). However, there is potential difficulty in disentangling the nature of these improvements (Jongkees, Immink, et al., 2017) as performance improvements might not necessarily be due to implicit learning processes but rather reflect general practice effects (Abrahamse & Noordzij, 2011). For this reason, a transfer approach is commonly used to judge the extent to which performance improvements rely on the practiced sequence (Abrahamse & Noordzij, 2011; Robertson, 2007; Willingham, 1999). This was implemented in the present experiment by presenting 10 out of 13 SRTT blocks that exclusively contained the same repeating SOC response sequence. The remaining three blocks (1, 7 and 13) were probe blocks that consisted predominantly of the trained SOC sequence, but also an untrained SOC sequence in order to disentangle sequence-specific learning from general practice effects. In light of the effects of cerebellar tDCS on CBI, we expected anodal relative to sham tDCS to impair overall RT and sequence acquisition, whereas cathodal relative to sham tDCS was expected to produce the opposite behavioral results. Furthermore, to investigate the effect of tDCS on consolidation processes following training, we assessed SRTT performance not only during stimulation but also at 24 h follow-up.

Materials and methods

Participants

Seventy-two right-handed, healthy undergraduate students from Leiden University were offered partial course credit for participation in a study on brain stimulation. Participants were randomly assigned to receive either anodal ($N = 24$), cathodal ($N = 24$), or sham ($N = 24$) stimulation. Group demographics

are presented in Table 1. The groups were comparable with respect to age, $F(2,69) = .675$, $p = .512$, gender distribution, $X^2(2, N = 72) = .572$, $p = .751$, and hours of sleep, $F(2, 69) = .118$, $p = .888$. Participants were screened individually using the Mini International Neuropsychiatric Interview (MINI), a short, structured interview of approximately 15 min that screens for several psychiatric disorders and drug use (Sheehan et al., 1998), and has been used previously in research on tDCS (Jongkees, Sellaro, et al., 2017) and the SRTT (Jongkees, Immink, et al., 2017). Participants were included if they met the following criteria: (i) between 18 and 30 years; (ii) no history of neurological or psychiatric disorders; (iii) no history of substance abuse or dependence; (iv) no chronic or acute medication; and (v) no implants or cardiac disorders for safety reasons concerning the tDCS. Before the start of the study, participants were informed of the procedure and potential side-effects of the tDCS (i.e., itching, stinging or burning sensation from the electrodes, reddening of the skin and head ache). None of the participants reported major side-effects. The study conformed to the ethical standards of the declaration of Helsinki with written informed consent from all subjects and the protocol was approved by the local ethical committee (Leiden University, Institute for Psychological Research).

Table 1. Group demographics

	Stimulation		
	Anodal	Cathodal	Sham
Male-to-female ratio	7:17	7:17	5:19
Age in years	19.8 (1.6)	19.5 (1.5)	19.3 (1.8)
Sleep session #1 in h	7.3 (1.8)	7.3 (1.1)	7.6 (1.0)
Sleep session #2 in h	7.4 (1.5)	7.1 (1.2)	7.2 (1.2)

Standard deviation in parentheses

Cerebellar transcranial direction current stimulation

Cerebellar tDCS was applied using three electrodes of 35 cm^2 (5 cm x 7 cm), with the target electrode centered over the inion and the two reference electrodes placed over bilateral mastoid to limit the effects of the reference electrodes on cortical activity. Whereas previous studies typically placed the target electrode lateral to the inion to investigate effects on unimanual performance (Ehsani et al., 2016; Ferrucci et al., 2013), others have centered the target electrode over the inion for bilateral stimulation of the cerebellum

(Ho et al., 2014; Martin et al., 2015; Panouillères, Miall, & Jenkinson, 2015). As the SRTT in the present study required bimanual performance, we also opted to center the target electrode over the inion. Stimulation consisted of a current of 1 mA delivered by a DC Brain Stimulator Plus (NeuroConn, Ilmenau, Germany), a device complying with the Medical Device Directive of the European Union (CE-certified). The current was built up during a fade-in of 10 s, after which stimulation lasted for precisely 20 min and then ended with a 10 s fade-out. All participants finished the SRTT task within the 20 min of stimulation. Impedance was below 15 kΩ throughout the stimulation.

SimNIBS, a freely available software software (www.simnibs.org), was used to develop the head model for finite element modeling (Thielscher, Antunes, & Saturnino, 2015; Windhoff, Opitz, & Thielscher, 2013). SimNIBS uses FreeSurfer and FSL BET to segment the head. SimNIBS pipeline was applied on a realistic head model which has been provided by SimNIBS as the example dataset (http://simnibs.de/version2/documentation). Five tissue segments are considered in the model: scalp, skull, cerebrospinal fluid (CSF), gray matter (GM), and white matter (WM). Electrodes, modeled as saline-soaked 5×7 cm^2 rectangular sponges, were positioned over the inion and mastoids. Current intensity was set to 1mA for the electrode over inion and 0.5 mA for each of the electrodes over mastoids. Finite Element Method (FEM) in SimNIBS pipeline was employed to calculat electric field (EF) distribution. Spatial distribution of the normalized EF values calculated by the computational analysis of the head model are shown in Figure 1. Electric field strength exhibits high values in the surface and deep layers of the cerebellum.

The experience of side-effects due to tDCS was assessed through self-report ratings on a five-point scale for the following symptoms: head ache, neck pain, nausea, muscle contractions in the face or neck, stinging sensation under the electrodes, burning sensation under the electrodes, and a nonspecific, uncomfortable feeling. Consistent with previous studies the most prominent side-effects were stinging and burning sensations under the electrodes (Bikson et al., 2009), although none of the participants voiced major complaints.

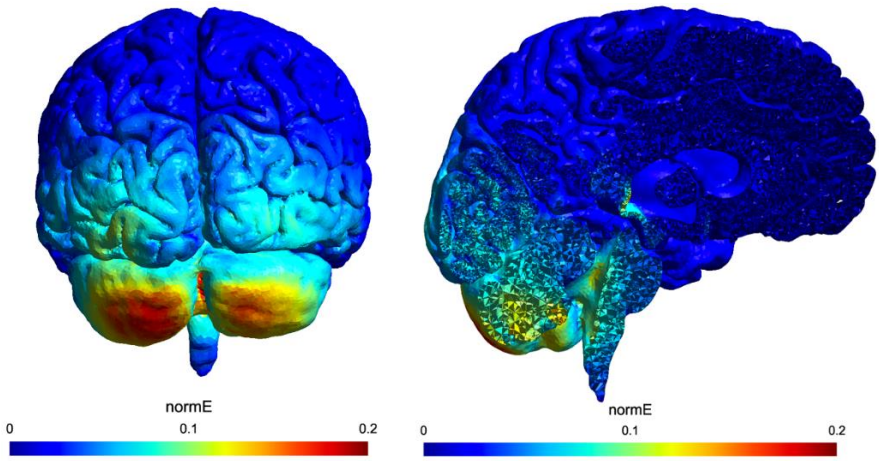

Figure 1. Spatial distribution of the normalized electric field calculated using SimNIBS pipeline; Anode: 5cmx7cm, centred over the Inion, 1mA current, two cathodes over mastoids, 5cmx7cm, 0.5mA current each.

Serial reaction time task

To assess response selection and sequence learning, participants performed a SRTT (Vaquero et al., 2006) presented using E-Prime 2.0 software (Psychology Software Tools, Inc., Pittsburgh, PA, USA). In this task four horizontally-aligned empty squares are presented in the centre of the screen. On each trial one of the squares turns red and the participant must press a corresponding button on the QWERTY keyboard (from left to right: V, B, N, M) using the index and middle fingers of the left (V, B) and right (N, M) hand. An error sound is presented if the wrong button is pressed, along with the Dutch words “Verkeerde toets!” (“Wrong button!”). RT is measured in ms as the latency in the key press to the stimulus and if RT exceeds 3,000 ms, the Dutch words “Te langzaam!” (“Too slow!”) are presented. Following the response, the four empty squares appear for a 50 ms response-stimulus interval before the next stimulus is presented. Participants were instructed that accuracy and response speed were equally important in the task.

All participants completed one task familiarization block of 120 randomly sequenced trials prior to stimulation to check for pre-existing group differences in response selection efficiency. Subsequently, participants

performed 13 training blocks that each consisted of 10 cycles of 12 trials while stimulation was applied. Blocks 2-6 and 8-12 consisted of 10 cycles of the same repeating 12-item SOC response sequence (VBVNMBNVMNBM) (Reed & Johnson, 1994). In order to disentangle sequence-specific performance from general practice effects, blocks 1, 7 and 13 were probe blocks. These blocks always started and ended with two cycles of the same SOC sequence in training blocks. Randomly inserted in the remaining six cycles were two consecutive cycles of an untrained transfer SOC sequence. This transfer sequence limits anticipation of responses and thus RT and response errors are expected to be higher for transfer sequences, but performance is expected to recover on the trained SOC trials. After completion of each block, performance feedback indicated the number of errors and mean RT followed by a 30 s rest interval.

At 24 h follow-up, participants completed the test phase of the SRTT consisting of 3 blocks, the first and third being probe blocks while the second exclusively contained the trained SOC, to investigate whether cerebellar tDCS affected overnight consolidation processes.

Procedure

Upon entering the lab, informed consent was obtained and participants completed the familiarization block of the SRTT. Subsequently, tDCS was applied for 20 min, during which participants completed 13 blocks of the SRTT. Stimulation was applied throughout the entire task, which took no more than 20 min to complete. After the task participants were asked to rate, on a five-point scale, to what extent they experienced adverse effects due to the stimulation. None of the participants reported major side-effects. All participants came back to the lab 24 h after the first session to complete 2 probe blocks and one block with the trained SOC without stimulation. The two sessions together took an approximate total of 60 min to complete.

Analysis

To compare SRTT performance between groups, percent accuracy (PAC) was calculated for each participant in familiarization, training and test phases of the SRTT. PAC for each phase was separately submitted to one-way analysis of variance (ANOVA) using the aov function.

For analysis of RT performance in SRTT phases, all incorrect trials were removed. RT data in familiarization, training and test SRTT phases were analysed using linear mixed-effects modelling (LMM) with the lme4 package in R (Bates, Mächler, Bolker, & Walker, 2015). The LMM approach does not require data averaging like traditional ANOVA analysis approaches and so LMM provides a more selective approach to investigating experimental effects and interactions (Lo & Andrews, 2015). This is because LMM allows for control of variance associated with random factors (Baayen, Davidson, & Bates, 2008). In the present LMM analyses, we treated participants and response stimuli as random factors. For fitted LMM models, we used the car package in R (Fox & Weisberg, 2011) to conduct type III Wald *F* tests with Satterthwaite degrees of freedom approximation (Luke, 2017).

LMM for RT in familiarization included Group (Sham, Anodal, Cathodal stimulation) as a fixed factor. Training RT data was first analysed with LMM on the 10 training blocks that involved only the target SOC sequence (blocks 2-6 and 8-12) to evaluate overall performance improvements with the training sequence. For this, we included Group and Block as fixed factors. We then conducted separate LMM on training RT data from the three probe blocks (blocks 1, 7 and 13) to evaluate sequence-specific learning by comparing performance on the target SOC sequence and the transfer SOC sequence. Here, we included Group, Block and Sequence Type (trained and transfer SOC) as fixed factors. To evaluate sequence-specific learning outcomes at test (24 h follow-up) relative to the end of training, we conducted LMM on RT data for the three Groups across the third and final probe block of training (training block 13) and the two probe blocks at test (test blocks 1 and 3) with Group, Block and Sequence Type as fixed factors. Finally, to evaluate test RT performance when only the SOC trained sequence was

present, we conducted LMM on the second test block with Group as a fixed factor. Significant effects from LMM were graphed using the effects (Fox, 2013) and ggplot2 (Wickham, 2009) R packages.

Results

We observed no significant group differences for PAC in familiarization (M = 97.20%, SD = 2.50, p = .71), training (M = 96.94%, SD = 1.65, p = .58) and test (M = 97.31%, SD = 1.75, p = .34).

Familiarization and training (day 1)

At the outset of the experiment, the groups did not differ in RT performance, as the Group effect was not significant for familiarization RT (p = .48). For RT in training blocks, in which only the target SOC sequence was performed, there was a significant Group x Block interaction, $F(18,83831) = 3.06$, $p < .001$. The source of this interaction was based on the anodal stimulation group demonstrating longer RT than sham and cathodal stimulation groups. The difference in RT for the anodal group compared to sham and cathodal groups was larger in the initial training blocks but decreased as training progressed, see Figure 2. Analysis of RT in probe blocks revealed a significant Block x Sequence Type interaction, $F(2, 24936.9) = 204.10$, $p < .001$, and a significant Group x Block interaction, $F(4, 24936.8) = 6.71$, $p < .001$. The significant Block x Sequence Type interaction (see Figure 3) follows a typical sequence learning pattern: in the first probe block, RT is equivalent between training and transfer sequences, but then RT decreases across probe blocks for the training sequence while RT for transfer sequence remains relatively unchanged. The significant Group x Block interaction (see Figure 4) follows a similar pattern as that observed for training blocks as depicted in Figure 2. Specifically, RT in probe blocks 1 and 2 is longer under anodal stimulation than sham and cathodal stimulation, but RT in the last probe block is equivalent between stimulation groups. The anodal group demonstrated a larger decrease in RT between probe blocks 2 and 3 than sham and cathodal groups across both training and transfer sequences. It does not appear that cerebellar tDCS influenced sequence

specific learning as in probe blocks, neither the Group x Sequence Type or Group x Block x Sequence Type interactions were significant (p = .64 & .58, respectively).

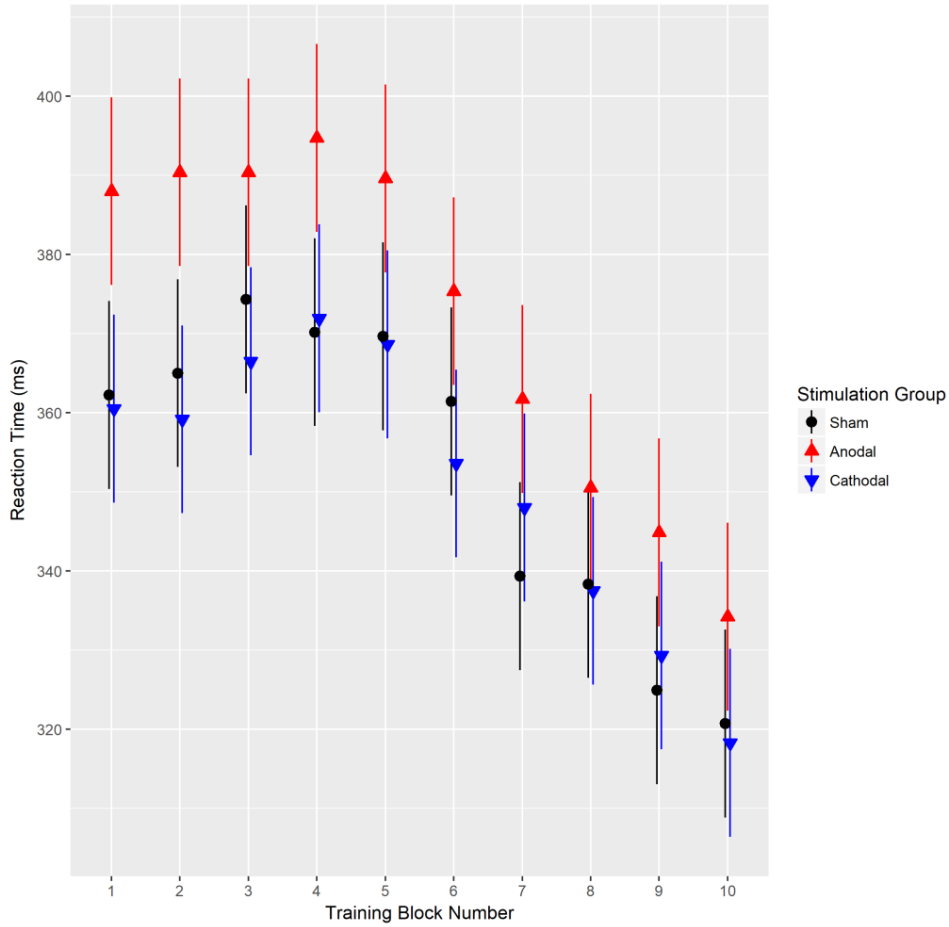

Figure 2. Mean RT in ms as a function of stimulation group and training blocks that only include the trained SOC (blocks 2-6 and 8-12). The anodal stimulation group demonstrates longer RT in early training blocks but no longer differs from the other groups at the end of training.

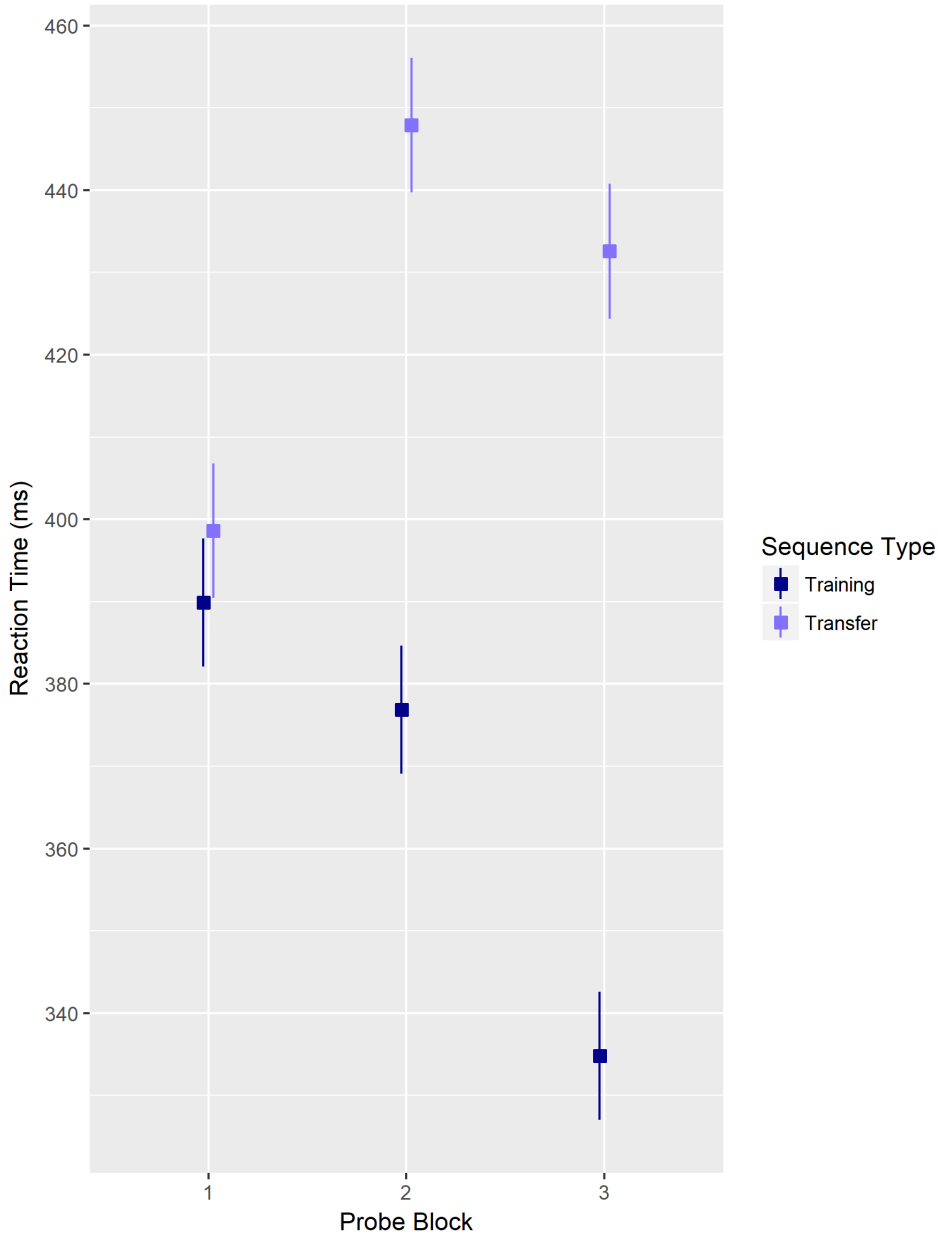

Figure 3. Mean RT in ms as a function of sequence type in the three probe blocks during training (blocks 1, 7 and 13). Performance on both sequences is comparable in the first block but diverges in the second and third probe block, demonstrating a typical sequence learning pattern.

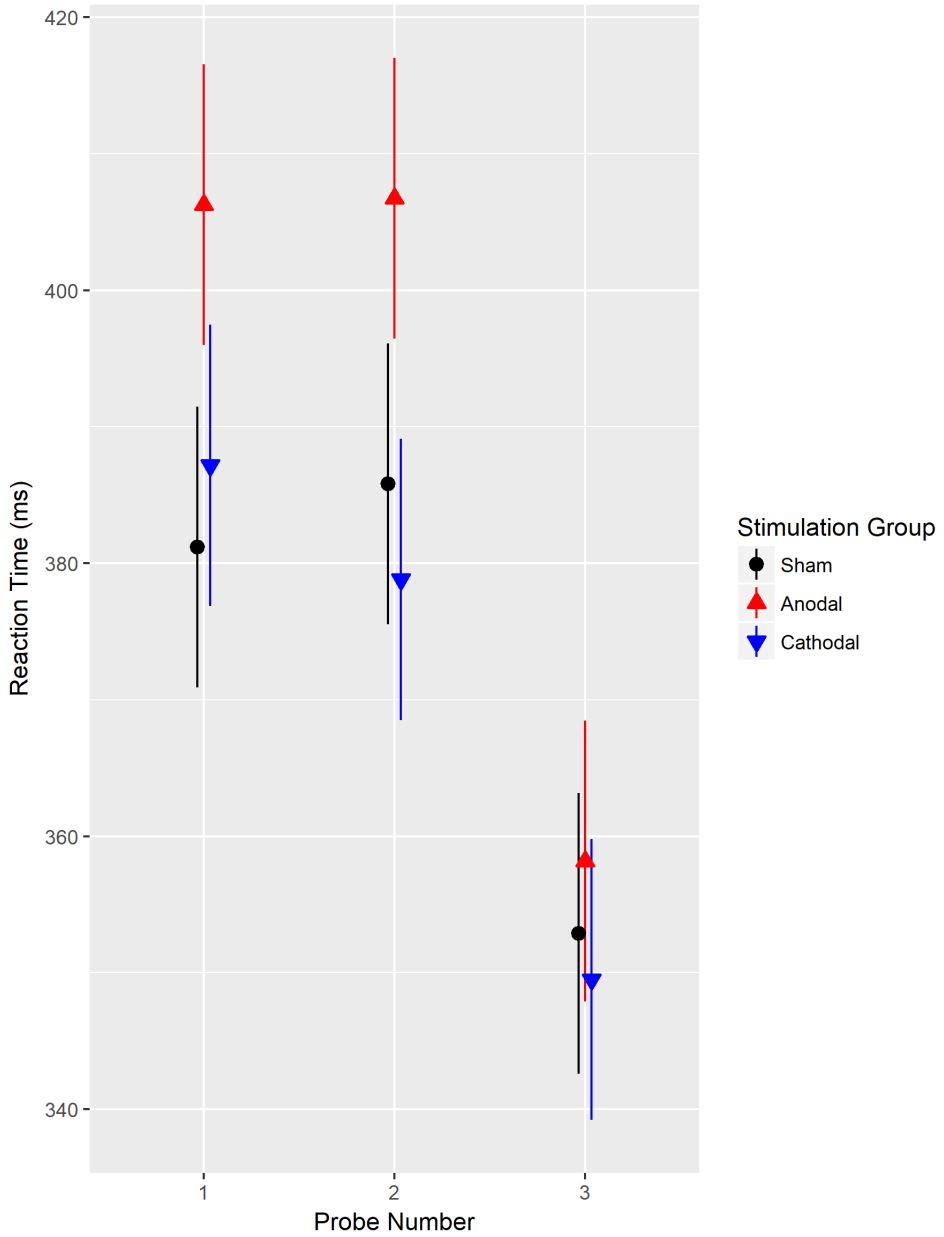

Figure 4. Mean RT in ms as a function of stimulation group and probe blocks during training (blocks 1, 7 and 13). As in the training blocks containing only the trained SOC (see Figure 1), in probe blocks 1 and 2 but not 3 the anodal stimulation group demonstrates longer RT.

Test (24 h follow-up)

Analysis of RT in the final training probe block (i.e., end of training) and the two test probe blocks (i.e., at 24 h follow-up) revealed a significant Block x Sequence Type interaction, $F(2,24944.4) = 41.88$, $p < .001$. RT for both sequence types decreased from training probe 3 to the test probe 1, suggesting a general practice effect. From test probe 1 to 2, RT further decreased for the trained sequence but increased for the transfer sequence, see Figure 5. In addition, there were significant interactions between Group and Block, $F(4,24944.4) = 5.37$, $p < .001$, and Sequence Type, $F(4,24944.4) = 6.40$, $p < .001$. The Group x Block interaction, illustrated in Figure 6, is based on all three groups demonstrating decreased RT between training probe block 3 and test probe block 1 while only the cathodal group demonstrated significantly shorter RT in test probe block 2 than test probe block 1 ($p < .01$). Underlying the Group x Sequence Type interaction was the anodal group demonstrating longer RT for trained and transfer sequences than the sham and cathodal groups, with this difference being larger for trained sequences than transfer sequences, see Figure 7. No significant group differences were observed for RT in test block 2, which involved only the trained sequence ($p = .14$).

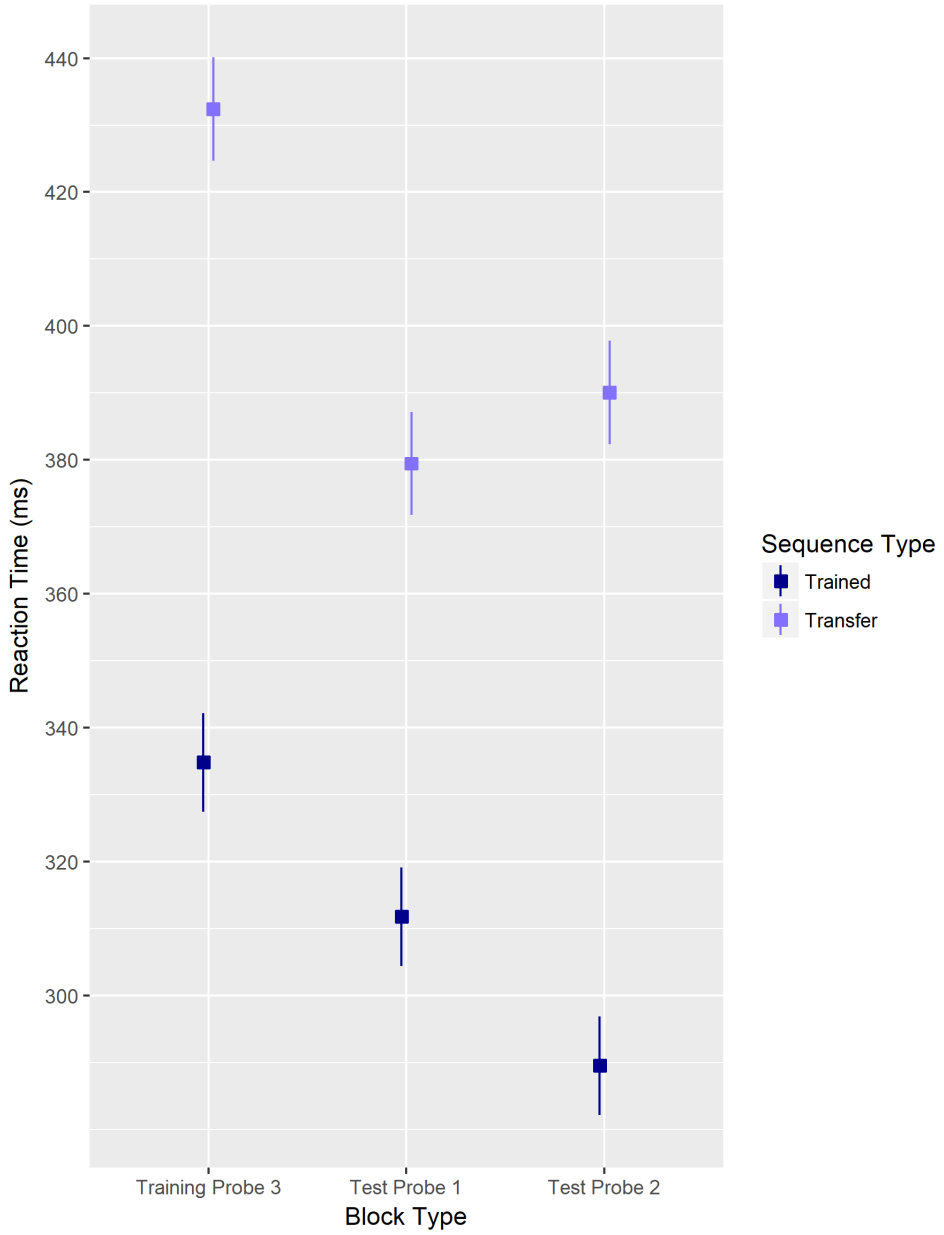

Figure 5. Mean RT in ms as a function of sequence type in the third and final probe block during training (block 13) and the probe blocks during test (at 24 h follow-up). Performance on both sequences benefits from overnight sleep, but further expose to the trained SOC facilitates performance on this sequence whereas it interferes with performance on the transfer sequence.

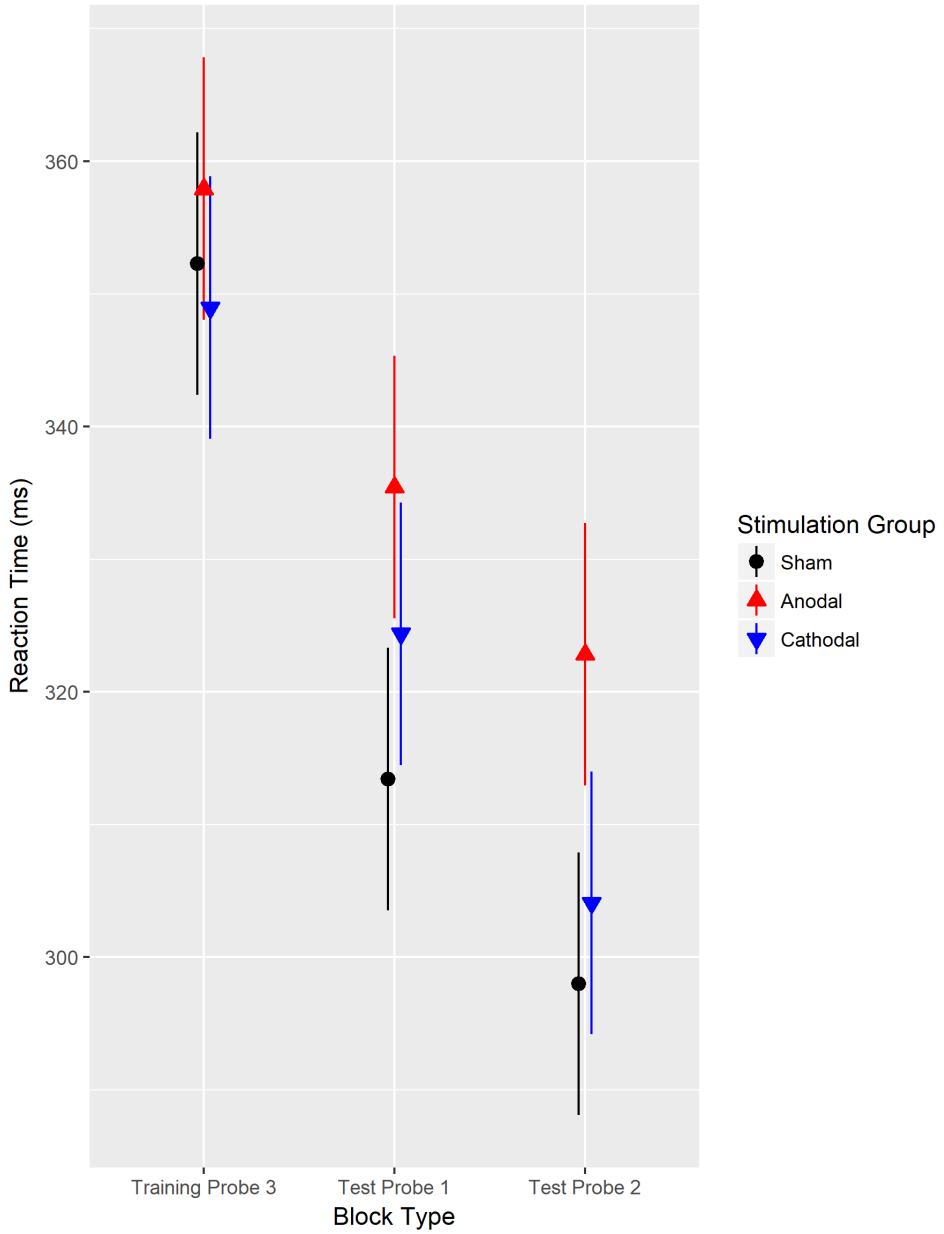

Figure 6. Mean RT in ms as a function of stimulation group in the third and final probe block during training (block 13) and the probe blocks during test (at 24 h follow-up). The groups no longer differed at the end of training on day 1, but the anodal stimulation group again demonstrated longer RT in probe blocks at 24 h follow-up.

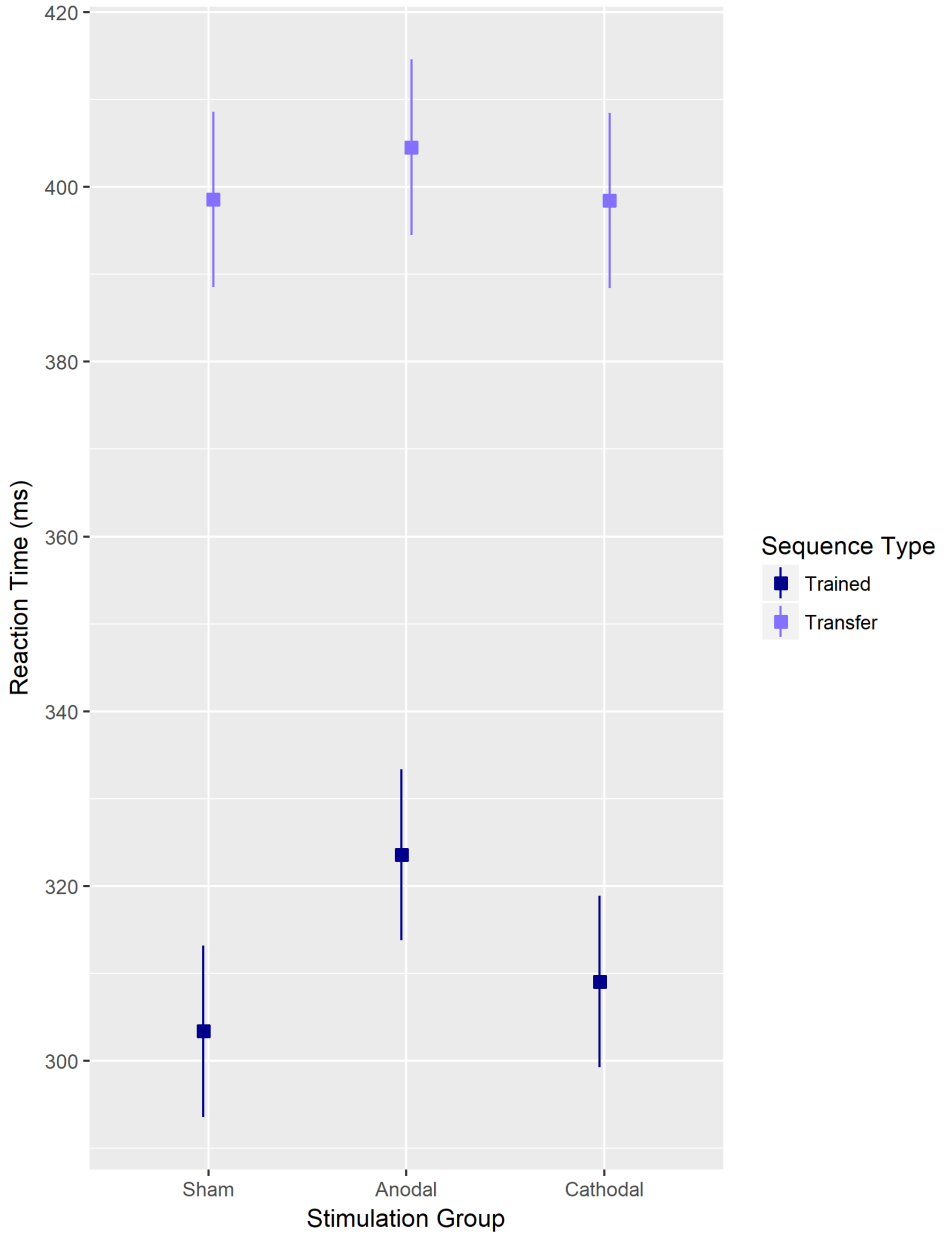

Figure 7. Mean RT in ms as a function of sequence type and stimulation group collapsed across the two probe blocks during test (at 24 h follow-up). The anodal stimulation group demonstrates longer RT than cathodal and sham groups, and this difference is greater for the trained SOC.

Discussion

The present study investigated the effects of tDCS of the cerebellum on response selection and motor sequence acquisition. In brief, the results demonstrated suppressed task performance, evidenced by overall longer RT, under anodal as compared to cathodal and sham tDCS, whereas there were no differences between cathodal and sham stimulation. This pattern persisted at 24 h follow-up as indicated by longer RT under anodal tDCS, in particular for the trained as compared to a transfer SOC sequence. Crucially, this group difference was not a pre-existing one, as RT performance before stimulation did not differ between the groups. The finding that anodal tDCS of the cerebellum delayed initiation of responses is consistent with a previous study demonstrating that anodal tDCS over the cerebellum delays initiation of muscle activity (Dutta et al., 2014), and it supports the idea that excitatory stimulation of this region strengthens the inhibitory tone exerted by the cerebellum over M1 (Galea et al., 2009; Kelly & Strick, 2003; Middleton & Strick, 2000). As such, the present study provides convergent evidence that tDCS over the cerebellum can affect response selection processes.

In more detail, all groups demonstrated motor sequence acquisition during training, as evidenced by decreased RT as the task progressed and an increasing difference in RT for trained and transfer response sequences. Notably, anodal tDCS was associated with an overall increased RT during training that did not depend on the specific SOC sequence (trained or transfer) being performed. As such, anodal tDCS did not appear to selectively affect sequence acquisition but instead produced an overall delay in initiation of responses. This delay decreased across training, suggesting that participants were able to compensate for their impairment with sufficient practice. At 24 h follow-up the group that previously received anodal tDCS again demonstrated increased RT in probe blocks. This tentatively suggests that, on the long term, anodal tDCS impaired the use of the trained sequence structure when exposed to an interfering transfer sequence.

This finding that anodal tDCS effects persisted at 24 h follow-up is of particular interest, as the effect of a single 20 min bout of tDCS on cortical

excitability is supposedly of short duration (approximately 1 h) (Nitsche et al., 2008). As such, it is unlikely that this long-term impact is due to a persisting change in cerebellar or M1 excitability. Of potential relevance is the fact that the anodal group only differed in performance at follow-up when the trained and transfer sequence were presented in the same block, but not when the block contained only the trained sequence. This implies that the selective impairment of performance is related to increased interference between the trained and transfer sequences when the two are performed in close temporal succession. Hence, the detrimental effect of anodal tDCS on sequence acquisition was not immediately apparent (i.e., during training on day 1) but did render performance of the trained sequence more vulnerable to interference later on (i.e., at 24 h follow-up).

The present findings are in line with previous studies applying anodal tDCS directly over M1, which was associated with enhanced response selection as evidenced by faster responses in an SRTT (Ehsani et al., 2016; Kantak, Mummidisetty, & Stinear, 2012; Nitsche, Schauenburg, et al., 2003). Although these studies varied in their methods of analysis, they indicate that increasing excitability of M1 can facilitate overall response selection and implicit motor sequence learning. Taken together with the findings from the present study, we argue that increasing excitability of M1 by directly applying anodal tDCS to this region facilitates response selection, whereas indirectly decreasing its excitability by applying anodal tDCS to the cerebellum produces the opposite behavioral result.

However, it should be mentioned that the present findings contrast with previous reports on anodal tDCS over the cerebellum and SRTT performance (Ehsani et al., 2016; Ferrucci et al., 2013), which demonstrated a facilitation rather than impairment of response selection. Although it remains speculative what accounts for this difference in results, it should be noted that one of the studies used a symbolic stimulus-response mapping rather than a spatial one (Ehsani et al., 2016). It is therefore possible that anodal tDCS facilitated the use of such a mapping rather than response selection processes per se, which is a question that future studies should investigate systematically. Curiously,

the other study demonstrating enhanced response selection with anodal tDCS over the cerebellum based this conclusion on the comparison with a sham stimulation group that did not demonstrate sequence learning at all (Ferrucci et al., 2013). As such, this particular finding should be interpreted with caution, as SRTT performance typically does demonstrate a sequence learning pattern (Abrahamse & Noordzij, 2011; Nissen & Bullemer, 1987; Schwarb & Schumacher, 2012). In light of this heterogeneity in results, there is a strong need for systematic and independent replication of these previous and current findings.

Interestingly, the present study found behavioral effects of tDCS over the cerebellum exclusively for anodal stimulation, whereas it was previously shown that cathodal tDCS over cerebellum also affects CBI (Galea et al., 2009) and therefore could potentially produce opposite behavioral results. Notably, the previously-reported effect of cathodal tDCS on CBI was obtained with a current intensity of 2 mA, whereas in the present study we used the lower intensity of 1 mA. Hence we speculate that the stimulation intensity used in the present study was not sufficient for behavioral effects of cathodal stimulation to become apparent. As such, future studies should investigate whether the effect of cathodal tDCS over the cerebellum is dose-dependent and if at a higher current intensity it indeed produces opposite behavioral effects as those obtained with anodal tDCS.

Additionally, follow-up studies might incorporate magnetic resonance spectroscopy (MRS) to measure individual differences and changes in glutamate and GABA levels. tDCS is known to directly affect levels of glutamate and GABA in a polarity-dependent manner (Bachtiar et al., 2015; Soyoung Kim et al., 2014; Nitsche, Fricke, et al., 2003; Stagg et al., 2009), and individual differences in (the ratio between) glutamate and GABA are related to response selection efficiency (de la Vega et al., 2014; Munakata et al., 2011; Snyder et al., 2010). As such, future studies employing MRS could establish whether changes in glutamate and GABA level contribute to the behavioral effects observed in the present study, and determine whether individual

differences in baseline levels of these neurotransmitters predict behavioral responsivity to cerebellar tDCS.

To conclude, the present study adds to a very recently-established body of literature by reporting on the effects of cerebellar tDCS on response selection and motor sequence acquisition. In brief, the results are consistent with the idea that cerebellar tDCS affects CBI, thereby modulating M1 excitability and the efficiency of response selection processes.