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RESEARCH REPORT

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Complex sequential learning is not facilitated by transcranial direct current stimulation over DLPFC or M1

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Abstract

Transcranial direct current stimulation (tDCS) is a non-invasive brain stimulation technique which was found to have a positive modulatory effect on online sequence acquisition or offline motor consolidation, depending on the relative role of the associated brain region. Primary motor regions (M1) and dorsolateral prefrontal cortices (DLPFC) have both been related to sequential learning. However, research so far did not systematically disentangle their differential roles in online and offline learning especially in more complex sequential paradigms. In this study, the influence of anodal M1 leg area-tDCS and anodal DLPFC-tDCS applied during complex sequential learning (online and offline) was investigated using a complex whole body serial reaction time task (CWB-SRTT) in 42 healthy volunteers. TDCS groups did not differ from sham tDCS group regarding their response and reaction time (online) and also not in terms of overnight consolidation (offline). Sequence specific learning and the number of recalled items also did not differ between groups. Results may be related to unspecific parameters such as timing of the stimulation or current intensity but can also be attributed to the relative role of M1 and DLPFC during early complex learning. Taken together, the current study provides preliminary evidence that M1 leg area or DLPFC modulation by means of tDCS does not improve complex sequential skill learning.

Significance statement: Understanding motor learning is helpful to deepen our knowledge about the human ability to acquire new skills. Complex

Abbreviations: a-tDCS, anodal tDCS; BI, blinding index; CWB, complex whole body; DLPFC, dorsolateral prefrontal cortex; GL, general learning; M1, primary motor cortex; NoI, number of recalled items; RB, random blocks; ReT, reaction time; RM-ANOVA, repeated-measures ANOVA; RT, response time; SB, sequence blocks; SRTT, serial reaction time task; SSL, sequence specific learning; s-tDCS, sham tDCS; TD, training day; tDCS, transcranial direct current stimulation; uv-ANOVA, univariate ANOVA; VAS, visual analogue scale.

sequential learning tasks have only been studied, sparsely, but are particularly mimicking challenges of daily living. The present study studied early motor learning in a complex serial reaction time task while transcranial direct current stimulation (tDCS) was either applied to leg primary motor cortex or bilateral dorsolateral prefrontal cortex. TDCS did not affect sequential learning, neither directly during performance nor in terms of sequence consolidation. Results provide preliminary information that M1 or bilateral DLPFC modulation does not improve early complex motor learning.

KEYWORDS
complex serial reaction time task, dorsolateral prefrontal cortex, primary motor cortex, sequential learning, transcranial direct current stimulation

1 | INTRODUCTION

Many activities in everyday living ranging from simple tasks such as getting ready for work to complex sports-related activities require performing several consecutive motor tasks in a sequential order. Most familiar motor sequences are performed in a highly automatised manner, but all have to be learned at the beginning. Additionally, injury, disease or even healthy ageing can make active relearning necessary (Malone & Bastian, 2016; Pandian et al., 2012; Sturma et al., 2019; Voelcker-Rehage, 2008). To support successful learning, understanding mechanisms of sequential task acquisition/consolidation and associated brain networks is crucial, especially because some motor sequences are established incidentally or implicitly (Cleeremans et al., 1998). Sequential learning has been operationalised most frequently by using a serial reaction time task (SRTT, Nissen & Bullemer, 1987; Robertson, 2007). SRTT learning is conceptualised by comparing responses to a repeating sequence of stimuli, which can be predicted and learned, with responses to randomly ordered stimuli, where only visuomotor associations but no temporal predictions are learned (Savic & Meier, 2016). Learning the SRTT involves motor, perceptual and declarative components (Robertson, 2007); thus, a variety of different brain regions were found to be associated with it (Dayan & Cohen, 2011; Keele et al., 2003), including frontal (Mizuguchi et al., 2019; Robertson et al., 2001), sensorimotor and cerebellar areas (Baldassarre et al., 2021; Doyon et al., 2003; Orban et al., 2010) with their relative contribution depending on task complexity (Carey et al., 2005; Gonzalez & Burke, 2018) and learning phase (Doyon & Benali, 2005). Complex sequential learning tasks have only been sparsely studied, so far especially with regard to neuronal contributions. Given that everyday situations often require multiple multi-joint motor movements with precise spatial and temporal timing, we however believe that studying more complex daily life relevant motor scenarios should become one major research focus.

Non-invasive brain stimulation techniques such as transcranial direct current stimulation (tDCS) have been widely used to investigate the causal relationship between stimulated brain regions and behaviour (Hashemirad et al., 2016; Savic & Meier, 2016). In brief, tDCS modulates cortical excitability in a polarity-specific manner (Nitsche & Paulus, 2000) and, when applied over a longer period of time, also modulates cortical plasticity and behaviour (Fritsch et al., 2010; Stagg et al., 2011). TDCS can facilitate online sequence acquisition and offline motor consolidation, depending on the relative role of the associated brain region. During SRTT learning, anodal tDCS (a-tDCS) over the primary motor cortex (M1) was mainly found to improve offline motor consolidation (Ehsani et al., 2016; Hashemirad et al., 2016; Krause et al., 2016; Reis et al., 2009), a process that is noticeable as performance maintenance or improvement between sessions (Robertson et al., 2004). A positive effect of M1 stimulation on SRTT acquisition was only found in some studies (Kantak et al., 2012; Nitsche et al., 2003; Savic & Meier, 2016), whereas others did not find any a-tDCS effect during acquisition (Ehsani et al., 2016; Reis et al., 2009) or even reported worsening of performance (Keitel et al., 2018). Besides, there is growing evidence for a substantial role of the prefrontal cortex (PFC) during sequential learning, at least in more complex learning paradigms (Galea et al., 2010; Meier et al., 2013; Mizuguchi et al., 2019). A-tDCS over the dorsolateral PFC (DLPFC) was found to improve implicit motor learning (Gladwin et al., 2012; Nakashima et al., 2021; Yamamoto et al., 2022). However, to our knowledge, DLPFC-tDCS in SRTT learning

has rarely been studied (Nakashima et al., 2021; Nitsche et al., 2003) and has not been studied at all in complex settings. Taken together, tDCS can facilitate SRTT sequence acquisition and consolidation, depending on the relative role of the associated brain region. M1 and DLPFC both substantially contribute to SRTT learning; however, research so far did not systematically disentangle their differential roles in online and offline SRTT learning especially in more complex paradigms.

Therefore, in the present study, we were aiming at investigating the relative contribution of DLPFC and M1 on complex sequential learning using a complex whole-body SRTT (CWB-SRTT). The CWB-SRTT was previously developed by our group (Mizuguchi et al., 2019) and is a modified version of a SRTT performed with the lower extremities. Therefore, it places higher demands on whole-body postural control and requires a specific motor output that relies on spatial orientation and coordination abilities. Single-session tDCS over DLPFC or M1 was applied during CWB-SRTT acquisition, and both online and offline gains were investigated using a 2-day interventional paradigm. With regard to previous studies, we hypothesised, that (i) bilateral DLPFC-tDCS enhances online CWB-SRTT learning, indicated by faster completion time reduction in sequence blocks compared to sham tDCS (Gladwin et al., 2012). Furthermore, we expected (ii) bilateral DLPFC-tDCS to enhance CWB-SRTT sequence specific learning (SSL), indicated by a time difference between sequence and random blocks (Mizuguchi et al., 2019). For M1-tDCS, we mainly hypothesised (iii) a beneficial effect on motor consolidation, indicated by a between-session improvement or maintenance of completion time values.

2 | MATERIAL AND METHODS

2.1 | Ethical approval

This study was approved by the local ethics committee of Leipzig University (ref. nr. 191/19-ek). All participants provided written informed consent and all procedures were conducted in accordance with the Declaration of Helsinki.

2.2 | Participants

We enrolled a total of 42 volunteers in our experiment (14 per group, mean age: 24,29 ± 2,76 years, range: 20–31 years, 18 female). None of the participants had a history of neurological illness, and during the time of the experiment, no participant was taking any central-acting drugs. All participants were right handed. Before and after the experimental procedure, all participants rated their levels of attention, fatigue and discomfort on a visual analogue scale (VAS) to rule out unspecific effects of these factors on behavioural performance.

2.3 | Sensorimotor task—whole-body serial reaction time task (CWB-SRTT)

A detailed description of the four-directional CWB-SRTT can be found in previous publications of our group (Maudrich et al., 2021; Mizuguchi et al., 2019). In brief, the participants stood on two centre plates, which were surrounded by a four-directional plate array. The participants were instructed to look at a monitor in front of them, which displayed the target cue in one of four squares, corresponding to the four surrounding plates and respond to the cue by stepping onto the corresponding plate as quickly as possible after its appearance. Left foot usage was instructed for left-side plates and right foot usage for right-side plates. After each response, the participants were asked to move back to their initial position, where 500 ms later the next cue appeared. Target cues remained visible until the correct response was made, and incorrect responses were indicated by a red flashing on the monitor. In total, 12 cues per block were presented. Sequence blocks (SB) included the following cue order: 2-3-2-4-1-3-1-4-3-4-2-1 (1: front left, 2: front right, 3: back left, 4: back right), thus providing a balanced number of movements to each of the four directions. All participants were naïve regarding the learning sequence and were explicitly not instructed about the presence of a sequence to maximise the possibility of performing an implicit motor learning task. To evaluate explicit sequence awareness, sequence knowledge was tested after the second training day by asking the participant (a) whether they recognised a sequential pattern in the visual cue order and (b) to recall as many elements of the sequence as possible without external help. Random blocks (RB) included a pseudo random cue order with equal probabilities regarding each number and a limit of maximally three consecutive repetitions per item. Each block lasted for a maximum of 25 s, and a 25-s interblock interval was included. After each block, the participants received feedback about the total time they took to complete the sequence. A custom-made script operated the CWB-SRTT (C#, Microsoft Visual Studio 2017). Please see Figure 1 for CWB-SRTT setup details.

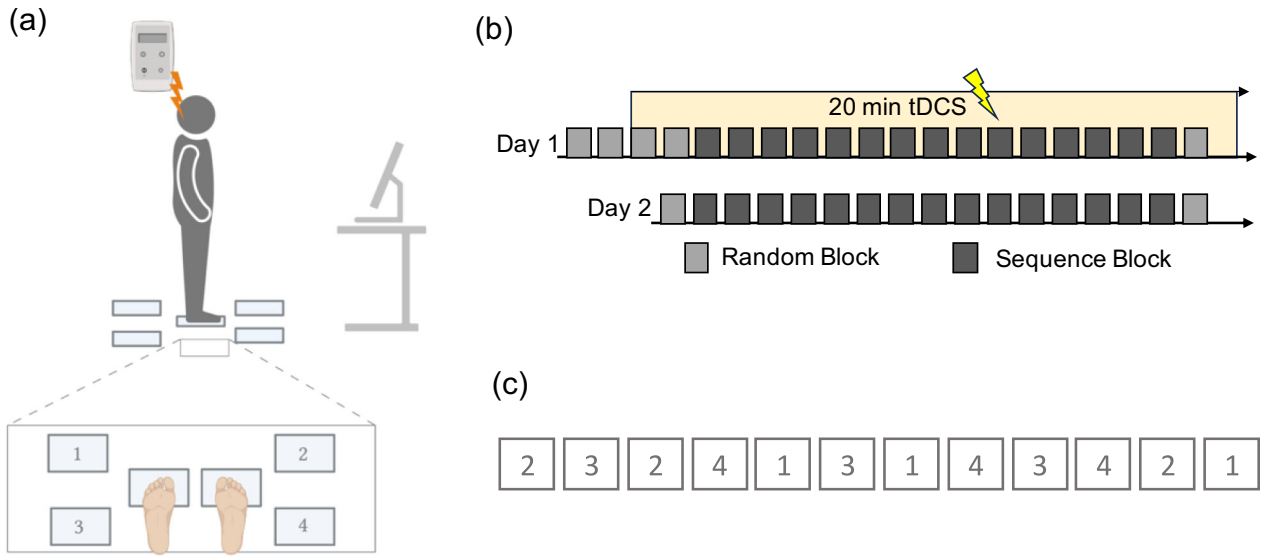

FIGURE 1 Complex whole-body serial reaction time task (CWB-SRTT). (a) Experimental setup: the participants received 20 min of tDCS while they performed the CWB-SRTT. Created with BioRender.com (2023). (b) Timeline experimental setup. (c) Sequential elements ($N = 12$). Number corresponds to plate number. tDCS, transcranial direct current stimulation.

2.4 | Transcranial direct current stimulation

The tDCS was applied via a battery-driven stimulator (neuroConn GmbH, Ilmenau, Germany) with two attached electrodes. In case of DLPFC stimulation (DLPFC-tDCS), anodal and cathodal electrodes both had a size of 5 × 7 cm and were positioned to produce a bilateral stimulation pattern (DaSilva et al., 2015). Thus, according to the 10–20 system, the cathode was positioned over F3 (MNI-Coordinate: $x = -34$, $y = 26$, $z = 44$ (Keeser et al., 2011), whereas the anode was positioned over F4 (MNI-Coordinate: $x = 34$, $y = 26$, $z = 44$). TDCS of 2 mA was applied because previous research found PFC modulation using 2 mA tDCS but not smaller (Boggio et al., 2006; Teo et al., 2011). For M1 leg area stimulation (M1-tDCS), a 5 × 7 cm anode was placed over the leg area of M1 (coordinates: $x = 0$, $y = -24$, $z = 75$, (Long et al., 2014; Taubert et al., 2016), whereas a 5 × 10 cm cathode was placed over the supraorbital area. Anatomical landmarks were identified using neuronavigation (Localite TMS-Navigator, Bonn, Germany) with our predefined MNI coordinates. After localisation with the neuronavigation system, the scalp of the participants was first rubbed with alcohol, and then both electrodes were soaked in isotone saline and fixed to the scalp with rubber bands. tDCS was applied with a current intensity of 2 mA. Current was applied for 20 min during CWB-SRTT task performance with a fade-in and fade-out period of 30 s each. During sham stimulation (s-tDCS), the current was ramped up for 30 s, held constant at 1 mA for 30 s and ramped down for 30 s. This short duration of stimulation has been shown to elicit no changes in cortical excitability while it may provide the same tingling sensation on the scalp of the participant (Nitsche et al., 2008). Generally, the impedance was monitored and kept under 10 kΩ (average: 3.87 ± 4.96 10 kΩ). The participants were unaware of their group belonging. The participants' blinding was tested by asking the participants at the end of the session, whether they believed they were actively stimulated or not. While one researcher (JK) was responsible for behavioural testing, the other researcher (EK) organised randomised allocation of participants as well as tDCS setup; thereby, we ensured successful blinding of the researcher during data acquisition (double-blinding). Please note that data analysis was performed by the researcher (EK) without blinding.

2.5 | Experimental procedure

Each participant performed two consecutive training sessions of a CWB-SRTT with its lower extremity. On the first training day (TD1), tDCS was either applied (a) bilaterally over the left and right DLPFC (DLPFC-tDCS, group 1) or (b) over M1 leg area (M1-tDCS, group 2) or (c) as a sham intervention (s-tDCS, group 3), whereas the participants performed a total number of 20 blocks with four RB at the beginning, a series of 15 sequence blocks SB in the middle and one RB at the end of the session. Two RB were performed before tDCS

was started to evaluate before-stimulation baseline performance, while two RB were performed during tDCS. Since CWB-SRTT performance took approximately 15 min, including 25-s interblock intervals, tDCS administration was continued after task completion for approximately 5 min during rest at the end of the experimental session. On the second training day (TD2), the participants performed 17 CWB-SRTT blocks with one RB at the beginning,15 SBs in between and 1 RB at the end without tDCS to evaluate potential effects on CWB-SRTT skill consolidation and skill learning development.

2.6 | Data analyses

Signals were recorded at a sampling rate of 1000 Hz. All cues which produced an error response were removed from the further analysis. We did not include the first and the third random block on day 1 into our final analysis because we consider them practice or habituation blocks. Thus, we included the second random block (R2), the fourth random block (R4) and the last random block (R5) as random and blocks 5 to 19 (S1–S15) as sequence blocks. CWB-SRTT learning was classified by calculating the average response time (RT) for each individual block, resulting in 18 relevant RT values on TD1 and 17 relevant RT values on TD2 per individual. RT was measured as the total time participants took from onset of the visual cue until they pressed the target plate with the respective foot. Furthermore, our CWB-SRTT setting allowed RT differentiation into reaction and movement time. Reaction time (ReT) was the main outcome parameter previous studies found to be associated with CWB-SRTT learning (Maudrich et al., 2021; Mizuguchi et al., 2019). ReT was operationalised by classifying the total time participants took from onset of the visual cue until they lifted the target foot from one of the middle plates. We averaged ReT for each participant, resulting in 18 relevant ReT on TD1 and 17 relevant ReT values on TD 2 per individual. SSL was evaluated by calculating the reaction time difference (Δ_{ReT}) between last SB and last RB on TD1 (R5–S15) and TD2 (R2–S15). General non-sequence-specific learning (GL) was evaluated by calculating Δ_{ReT} between the second and the last RB on TD1 (R2–R5) and the first and the last RB on TD2 (R1–R2). To evaluate consolidation, we compared ReT from the last SB on the first day (S15, TD1) with ReT on the first SB on the second day (S2, TD2). Explicit sequence knowledge was evaluated by (a) counting the number of 'yes' answers per group and (b) by summing the number of correctly recalled consecutive items of the learning sequence. VAS data were analysed by comparing the pre–post differences of attention, fatigue and discomfort between groups (ΔA, ΔF and ΔD).

2.7 | Statistical analyses

Statistical analyses were performed using JASP (Version 0.16, JASP Team 2021). The normality of the data was assessed by Shapiro–Wilk testing ($\alpha = .05$). VAS data were analysed using one-way analyses of variance (ANOVAs) with factor GROUP on pre–post difference values. To compare initial performance between groups, RT and ReT at R2 were evaluated using one-way ANOVAs. Skill learning was assessed within and between groups by evaluating RT and ReT on TD1 and TD2 using separate RM-ANOVAs with between-subject factor GROUP (DLPFC-tDCS, M1-tDCS, s-tDCS) and within-subject factor TIME (S1–S15). Consolidation was evaluated using a RM-ANOVA on ReT data with factor GROUP and TIME (S15 TD1, S2 TD2). Greenhouse–Geisser correction was implemented in case of sphericity violation. SSL and GL were evaluated separately for TD1 and TD2 using one-way ANOVAs. Equal properties between groups regarding sequence awareness were tested using a multinomial χ^2 test. Number of recalled items were compared between groups using a Kruskal–Wallis test. To test whether blinding of participants was successful, we calculated the James blinding index (BI, Arroyo-Fernandez et al., 2021; Bang et al., 2004) using the BI function in R 4.3.2, in which blinding is scaled from 0 to 1 with increasing values indicating increasing blinding success. The statistical threshold for all analyses

TABLE 1 Demographics.

Group	Age (years)	Gender (f/m)	H sports/week	Δ A TD1	Δ F TD1	Δ D TD1
DLPFC-tDCS	23.43 ± 2.6	7/7	7.25 ± 2.73	−.18 ± .77	−.11 ± 1.11	−.14 ± .36
M1-tDCS	24.93 ± 3.3	6/8	8.04 ± 4.78	−.53 ± 1.37	−.79 ± 1.25	.07 ± .27
S-tDCS	24.5 ± 2.1	5/9	6.71 ± 3.61	−.57 ± 1.04	−.21 ± .7	−.07 ± .27

Note: TDCS groups did not differ regarding age (F [2,39] = 1.102, $p = .34$), number of female participants (χ^2 [3] = 1.17, $p = .76$) and hours of sports per week (F [2,39] = .36, $p = .7$). Furthermore, changes in attention (A), fatigue (F) and discomfort (D) levels (Δ A, F, D TD1) on TD1 did not differ between groups (A: F [2,39] = .56, $p = .58$, F: F [2,39] = 1.72, $p = .19$, D: F [2,39] = 1.82, $p = .18$).

was set at $p < .05$. Effect sizes were expressed using partial eta squared (η_{p2}) for ANOVAs.

3 | RESULTS

3.1 | Demographics

Demographic variables did not differ between groups (see Table 1 for details). All participants tolerated the stimulation well and none reported any unexpected side effects from tDCS stimulation. Regarding our blinding, we found a James BI of .375 ± .078, [.222, .527], indicating a medium range of blinding. Our upper bound confidence interval was found to be slightly above .5; thus, we are confident to have achieved a sufficient level of blinding (Bang et al., 2004).

3.2 | TDCS current flow simulation

Electric field distributions were simulated based on a finite element model of a representative head using the example subject 'Ernie' of the open-source SimNIBS software (Thielscher et al., 2015) to approximate current flow. For (a) bilateral DLPFC-tDCS, both anode and cathode were defined according to our anatomical landmarks (anode: 34, $y = 26$, $z = 44$, cathode: $x = -34$, $y = 26$, $z = 44$), each with a size of 7 × 5 cm. For (b) M1-tDCS, anode was defined accordingly using predefined coordinates ($x = 0$, $y = -24$, $z = 75$), whereas a 5 × 10 cm cathodes centre location was established at Fpz. For both stimulation conditions (a) and (b), a current intensity of 2 mA was selected. For bilateral DLPFC-tDCS, maximal normalised electrical field strength (.403 V/m) was determined between the two electrodes, covering both

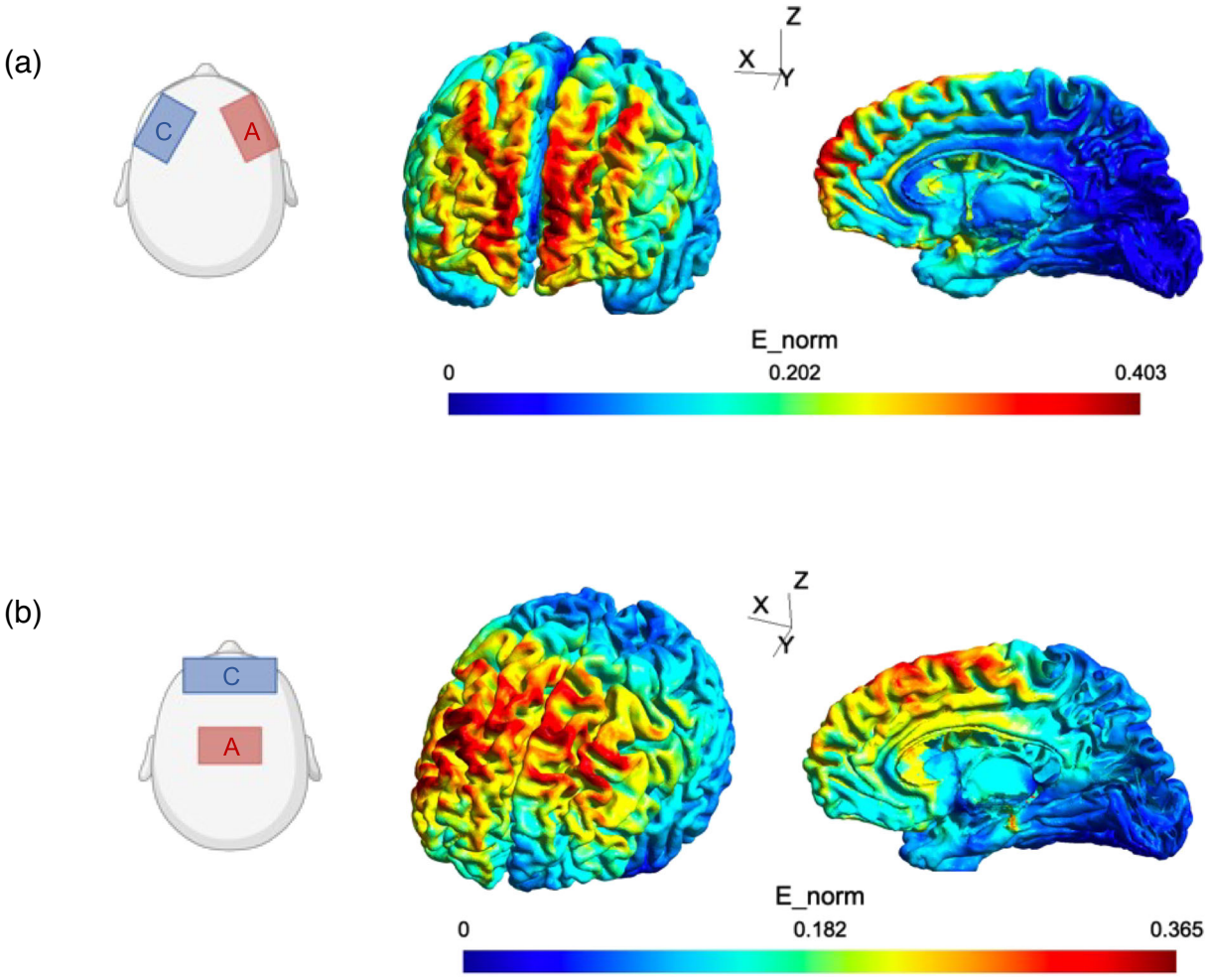

FIGURE 2 Transcranial direct current stimulation (tDCS) current flow simulation. Normalised electrical field strength (V/m) is depicted for (a) bilateral DLPFC-tDCS and (b) M1-tDCS covering the leg motor cortex. Electrode in blue colour represents the cathode (labeled with 'C'), whereas the electrode in red colour represents the anode (labeled with 'A'). Normalised electrical field strength (V/m) is indicated through colourmaps with blue representing lowest and red representing highest field strengths, respectively. Current flow image was created using the SIMNIBS software version 3.2. (Thielscher et al., 2015).

dorsolateral and medial prefrontal cortices. For M1-tDCS, maximum normalised electrical field strength (.365 V/m) was found anterior to the anode both on the cortical surface and in deeper cortical regions, corresponding to bilateral leg motor cortices and also premotor cortices (see Figure 2 for details).

3.3 | Response time (RT)

Baseline performance on R2 before tDCS onset showed no significant difference between groups (F [2,39] = .32, p = .725, η_p^2 = .02), indicating no group difference prior to the stimulation. We found a significant main

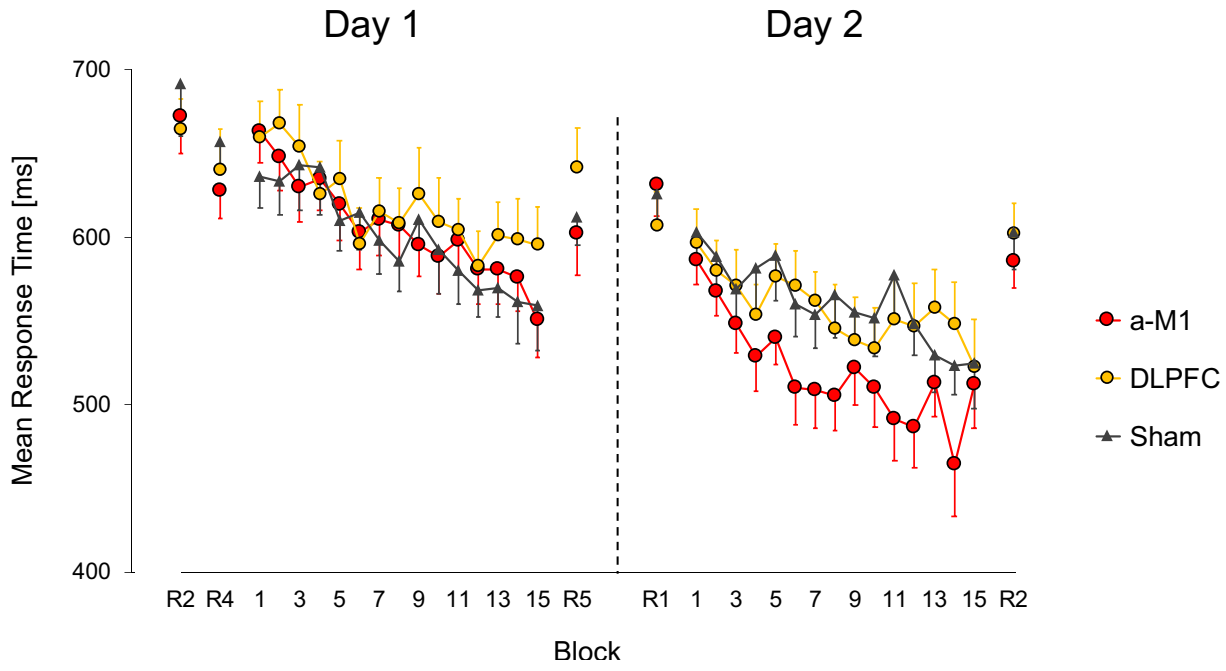

FIGURE 3 Mean response time (RT). Line graphs show average values ± standard error. Red line corresponds to a-M1-tDCS, yellow line to DLPFC-tDCS, grey line represents sham-tDCS group. Dotted line represents separation of training days. DLPFC: bilateral dorsolateral prefrontal cortex stimulation, a-M1: anodal primary motor cortex stimulation.

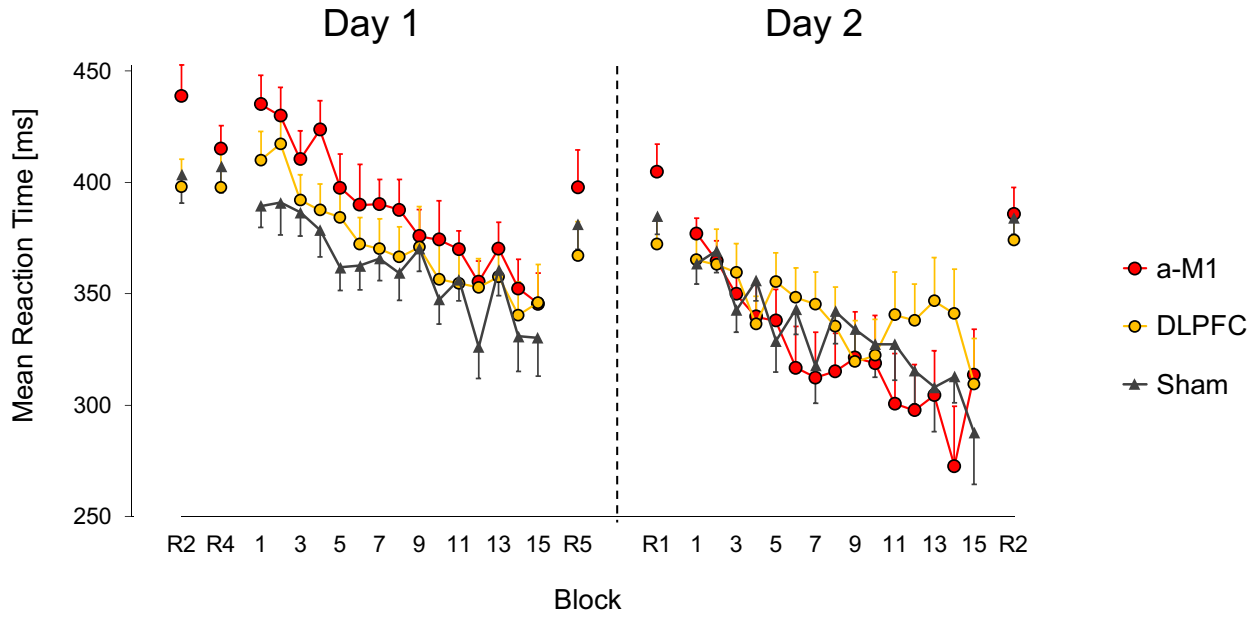

FIGURE 4 Mean reaction time (ReT). Line graphs show average values ± standard error. A-M1-tDCS is represented by the red line, DLPFC-tDCS by the yellow line, and the sham-tDCS group is shown by the grey line. Training days are separated by a dotted line. DLPFC: bilateral dorsolateral prefrontal cortex stimulation, a-M1: anodal primary motor cortex stimulation.

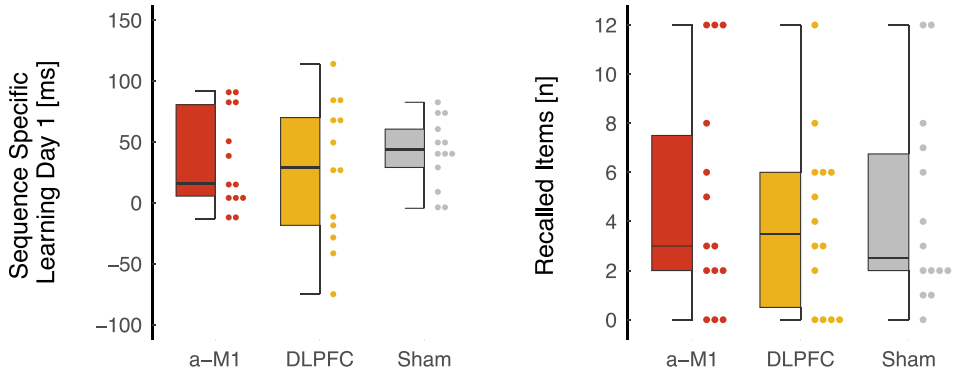

FIGURE 5 Sequence specific learning (SSL) TD 1 and number of recalled items (NoI). Boxplots show medians with 25th and 75th percentiles, dot plots showing individual values (Binwidth SSL = 7 ms). Outliers were excluded. Red box corresponds to M1-tDCS, yellow boxes represent DLPFC-tDCS, and grey boxes represent sham tDCS. DLPFC: dorsolateral prefrontal cortex stimulation; a-M1: anodal primary motor cortex stimulation.

effect of TIME across groups both on TD1 (F [7.64, 297.87] = 17.35, p < .001, η_p^2 = .31) and on TD 2 (F [7.58, 295.54] = 9.86, p < .001, η_p^2 = .21), indicating that all participants substantially reduced their RT over time. We found no significant main effect of GROUP on TD1 (F [2,39] = .26, p = .776, η_p^2 = .01) and TD2 (F [2,39] = 1.51, p = .234, η_p^2 = .07) and also no significant TIME*GROUP interaction on TD1 (F [15.28, 297.87] = .94, p = .526, η_p^2 = .05) or TD2 (F [15.16, 295.54] = 1.34, p = .176, η_p^2 = .06), see also Figure 3.

3.4 | Reaction time (ReT)

ReT performance on R2 did not differ between groups (F [2,39] = 2.86, p = .070, η_p^2 = .13). Analogue to RT, we also found a significant TIME effect on TD 1 (F [7.62, 297.01] = 19.73, p < .001, η_p^2 = .34) and TD 2 (F [6.19, 241.53] = 9.46, p < .001, η_p^2 = .20). No significant GROUP effect was found (TD1: F [2,39] = 1.79, p = .180, η_p^2 = .08, TD2: F [2,39] = .53, p = .591, η_p^2 = .03) and no significant TIME*GROUP interaction (TD1: F [15.23, 297.01] = .65, p = .840, η_p^2 = .03, TD2: F [12.39, 241.53] = 1.69, p = .066, η_p^2 = .08), see also Figure 4. For consolidation, no TIME*GROUP effect was found (F [2,39] = .22, p = .804, η_p^2 = .01) but a TIME effect (F [1,39] = 8.69, p = .005, η_p^2 = .18), with an average increase of ReT by 28 ± 21.51 ms across all groups.

3.5 | Sequence specific learning (SSL) and general learning (GL)

When comparing SSL between groups, we found no significant difference on TD 1 (F [2,39] = 1.19, p = .320, η_p^2 = .06, see also Figure 5) and no significant difference on TD 2 (F [2,39] = .65, p = .529, η_p^2 = .03). GL did not differ between groups, either (TD1: F [2,39] = .52, p = .599, η_p^2 = .03, TD2: F [2,39] = 1.03, p = .365, η_p^2 = .05).

Equal properties between groups regarding explicit sequence awareness were found (χ^2 [3] = 1.16, p = .76). Furthermore, the number of items (NoI), participants were able to recall after the end of TD2, did not differ between groups (χ^2 [2] = .09, p = .954, see also Figure 5).

4 | DISCUSSION

The main aim of the current study was to investigate the relative contribution of DLPFC and M1 on sequential learning using a complex whole-body SRTT (CWB-SRTT). Contrary to our hypotheses, DLPFC-tDCS did not enhance online CWB-SRTT learning. More precisely, we could not confirm hypothesis (i) and (ii) but found similar RT and ReT reduction in all groups and also comparable SSL ratios. These findings contradict previous studies which suggested improved sequence acquisition associated with DLPFC-tDCS (Nakashima et al., 2021; Yamamoto et al., 2022; Zhu et al., 2015). Nakashima et al. (2021) found reaction time reductions associated with DLPFC stimulation; however, effects were only found post stimulation. In the current study, post stimulation effects were not investigated. Instead, tDCS outlasted the SRTT training period in all cases; thus, one can only speculate about reaction time decreases after termination of the stimulation. Furthermore, in previous studies, DLPFC-tDCS modulated sequential learning only in motor tasks where the supervisory attention system (SAS) was involved but not in motor tasks without SAS guidance (Yamamoto et al., 2022). In the current study, no explicit

experimental manipulation of attentional parameters was performed; however, our VAS scale assessment revealed no substantial increase of attention throughout the experimental session. It seems reasonable to assume that CWB-SRTT learning does not require SAS guidance, which is why potential DLPFC-tDCS-induced enhancement of attentional parameters may not have resulted in superior CWB-SRTT motor learning performance. Moreover, Zhu et al. (2015) found implicit motor learning enhancement after cathodal tDCS was applied over the left DLPFC, which was argued by a shift in dominance from explicit to implicit memory system via reduction of explicit verbal-analytical involvement in movement control (Zhu et al., 2015). Our current flow simulation showed that bilateral DLPFC-tDCS resulted in a widespread activation increase covering large amounts of both frontal cortices. High field strength in both left and right DLPFC may have resulted in more explicit memory system usage. However, our parameter of explicit task knowledge (NoI) did not differ between groups, indicating that at least explicit CWB-SRTT learning was not specifically supported by bilateral DLPFC-tDCS. While enhancement of implicit motor learning was mainly associated with left DLPFC stimulation (Nakashima et al., 2021; Yamamoto et al., 2022; Zhu et al., 2015), little is known about right DLPFC activation and its role in motor learning subsystems. If bilateral DLPFC-tDCS activated both explicit and implicit memory systems, simultaneously, it may have resulted in a conflicting situation of both memory systems (Cohen & Robertson, 2011), thus inducing no benefit in motor learning.

We also could not confirm hypothesis (iii), assuming a beneficial effect of M1-tDCS on motor consolidation. M1-tDCS effects on motor consolidation have been shown in a variety of different motor settings but usually using the upper limb (Hashemirad et al., 2016). However, stimulating M1 of the lower limb is more complex given the anatomical location of the leg motor cortex (Madhavan et al., 2016; Rezaee & Dutta, 2018). Therefore, transferring upper-limb findings to lower-limb findings may not meet all challenges associated with lower limb M1-tDCS, and lower limb M1-tDCS may not be as effective as upper-limb tDCS in area targeting and producing associated behavioural improvements. Nonetheless, there are also studies confirming beneficial effects of lower limb M1-tDCS even in complex motor settings (Devanathan & Madhavan, 2016; Kaminski et al., 2016). Other studies found no effect on reaction time using lower limb M1-tDCS (Seidel & Ragert, 2019). Thus, not in all cases, M1-tDCS was shown to be beneficial for learning and its relative effect may depend on the nature of the motor task. As already mentioned, CWB-SRTT can be considered a complex motor learning task with additional requirements to the postural system because the task is performed during standing. Furthermore, rear plates were invisible to the participant during task performance; thus, a relatively precise internal model about the relative plate distance and associated stride length was needed to successfully meet the plates. Internal model creation and organisation was mainly found to be acquired in the cerebellum (Imamizu et al., 2000; Imamizu et al., 2003); therefore, CWB-SRTT learning could be more related to cerebellar activation. Future studies could evaluate how cerebellar modulation interacts with CWB-SRTT learning. Additionally, even though previous studies did find a beneficial effect of M1-tDCS on SRTT learning, only effects on response times were investigated (Savic & Meier, 2016). CWB-SRTT analyses additionally allows disentangling reaction and movement time, thus making a precise interpretation of cognitive and motor mechanisms of learning possible. CWB-SRTT learning was mainly found to be associated with ReT reductions while movement times did not systematically decrease over time (Mizuguchi et al., 2019). Therefore, one can conclude that mainly cognitive processes are responsible for CWB-SRTT learning, which is one potential explanation that M1-tDCS did not result in superior CWB-SRTT performance. Furthermore, another study used 4-mA current intensity to robustly modify sequential learning using M1-tDCS (Hsu et al., 2023); thus, one could speculate that our current intensity was not sufficient to target M1 and induce a robust behavioural improvement.

Our study faces some limitations. One major issue of tDCS is its relatively low spatial focality (Buch et al., 2017), resulting in widespread co-activation of adjacent and also functionally connected brain regions. Our current flow model shows that during both DLPFC and M1 stimulation, large parts of the frontal cortex including pre-motor cortex, supplementary motor area and prefrontal regions were stimulated, even though the strongest electrical field was found anterior to the active electrode. Previous studies with similar electrode positions found tDCS-induced behavioural improvements (DaSilva et al., 2015; Kaminski et al., 2016); thus, one cannot conclude that widespread cortical activation is not efficient. However, during CWB-SRTT learning, activation of brain regions with potentially complementary functions may have resulted in a conflicting situation, not allowing relevant areas to determine the learning process. It can be stressed that isolated activation of a single hemisphere region was not measured in the current study and therefore, potentially, also behavioural effects are missing. This is also in line with recent evidence showing that placing the electrodes anterior and posterior to the M1 area produces higher field intensity and an optimised current direction compared to our conventional setup

(Evans et al., 2022). Thus, future studies should consider using a more optimised setup for M1 stimulation compared to the conventional setup. Another limitation of the current study was that no additional brain imaging was performed. Joint activation increases or decreases of brain regions induced by tDCS and movement-induced neural activation or deactivation may have resulted in a specific brain activation pattern not displayable given our current study design. We therefore strongly recommend future studies to additionally determine brain activation changes, best during task execution. The fact that the direction of stimulation (excitatory vs. inhibitory) depends on the direction of current flow in the brain, which may differ across persons and between brain areas because of anatomical differences, is another drawback of tDCS (Buch et al., 2017). Furthermore, our sample size may have been too small to detect a significant between-group difference.

Taken together, our study shows that tDCS application during complex sequential motor task acquisition is possible. However, our main aim was to disentangle roles of DLPFC and M1 during early complex sequential learning. We could not confirm a performance-enhancing effect of tDCS neither on online nor offline learning parameters. Results may be related to unspecific parameters such as timing of the stimulation or current intensity but can also be attributed to the relative role of M1 and DLPFC during early complex learning. Future studies should consider investigating neural parameters during early complex CWB-SRTT learning to gain information on changes in neural activation within sequence acquisition with a specific focus on M1 and DLPFC.

AUTHOR CONTRIBUTIONS

Elisabeth Kaminski: Conceptualisation; methodology; supervision; project administration; data curation; investigation; formal analysis; writing—original draft; writing—review and editing. **Daniel Carius**: Software; formal analysis; visualisation; writing—review and editing. **Jan Knieke**: Investigation; data curation; writing—review and editing. **Nobuaki Mizuguchi**: Conceptualisation; methodology; writing—review and editing. **Patrick Ragert**: Conceptualisation; supervision; writing—review and editing.

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CONFLICT OF INTEREST STATEMENT

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

DATA AVAILABILITY STATEMENT

Data, in an anonymous format (according to data protection policy in the ethics agreement), are available at https://doi.org/10.6084/m9.figshare.24598575.v1.

PEER REVIEW

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