

Discernible effects of tDCS over the primary motor and posterior parietal cortex on different stages of motor learning

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Abstract

Implicit motor learning and memory involve complex cortical and subcortical networks. The induction of plasticity in these network components via non-invasive brain stimulation, including transcranial direct current stimulation (tDCS), has shown to improve motor learning. However, studies showing these effects are mostly restricted to stimulation of the primary motor cortex (M1) during the early stage of learning. Because of this, we aimed to explore the efficacy of anodal tDCS applied over the posterior parietal cortex (PPC), which is involved in memory processes, on serial reaction time task (SRTT) performance. Specifically, to evaluate the involvement of both motor learning network components, we compared the effects of tDCS applied over regions corresponding to M1 and PPC during the early and late stages of learning. The results revealed a selective improvement of reaction time (RT) during anodal stimulation over the PPC in the late stage of learning. These findings support the assumption that the PPC is relevant during specific phases of learning, at least for SRTT performance. The results also indicate that not only the target area (i.e., PPC), but also timing is crucial for achieving the effects of stimulation on motor learning.

Keywords: Motor learning, Posterior parietal cortex, Primary motor cortex, Serial reaction time task, Transcranial direct current stimulation

Article Note

Supplementary Information

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Introduction

The primary motor cortex (M1) and the posterior parietal cortex (PPC) are the main components of the frontoparietal motor learning network, not only for explicit but also for implicit motor learning (Deiber et al. [1997](#); Lin et al. [2012](#); Gonzalez et al. [2016](#); Sakurada et al. [2019](#)). The involvement of this network in motor learning has been evaluated by neuroimaging (Deiber et al., [1997](#); Gonzalez et al., [2016](#); Sami, Robertson, & Miall, et al. [2014](#)) and neurophysiological records (Lin et al. [2012](#); Sakurada et al. [2019](#)). These studies have shown changes in the activity of M1 and other areas of the motor learning network, such as the premotor cortex, the supplementary motor area, and the parietal cortex, during the performance of a typical implicit motor learning task, the serial reaction time task (SRTT) (Grafton et al. [1995](#), [1998](#), [2002](#)). In particular, representations of acquired motor sequences, including late learning stages, involve activity of the parietal cortex, and this process seems to be independent from of the cortical effector (task-specific

motor responses) (Grafton et al. [1998](#)), while M1 activity is associated with the early motor sequence learning stage and is effector-specific for the task (Grafton et al. [1995](#)). Interestingly, moreover neuroimaging data obtained during rapid-eye-movement (REM) sleep, in which memory traces are supposed to be consolidated (Miller and Gehrman [2019](#)), have shown regional brain activity in the parietal and premotor cortices, among other areas (Maquet et al. [2000](#)). By SRT tasks, thus distinct learning and memory formation stages, which involve specific mechanisms of the motor network (Dudai et al. [2015](#); Gonzalez and Burke [2018](#); Klinzing et al. [2019](#)), can be evaluated by multiple session approaches.

Although M1 and PPC appear to be differentially involved in different phases of motor learning, in the sense that M1 is assumed to be more closely involved in early acquisition of motor skills, while the PPC appears to be more critically involved in later stages of learning (Honda et al. [1998](#); Catalan et al. [1998](#); Hamano et al. [2020](#)), respective evidence about the specific contribution of these network components to the temporal dynamics of motor learning is mainly based on functional imaging approaches, and thus is correlative. Methods allowing mechanistic or causal explanations are suited to strengthen respective evidence. Non-invasive brain stimulation (NIBS) techniques, such as transcranial direct current stimulation (tDCS) (de Xivry et al. [2011](#); Hashemirad et al. [2017](#)) and repetitive transcranial magnetic stimulation (rTMS) (Koch and Rothwell [2009](#); Karabanov et al. [2012](#)), have been used to alter neuronal activity and induce neural plasticity of the M1 and PPC, as well as to establish causal relationships between motor network components and behavioral outcomes (Ammann et al. [2016](#); Hashemirad et al. [2016](#)). Anodal tDCS applied over M1, that is, stimulation with the anode positioned over this region, has been shown to increase cortical excitability, while cathodal tDCS reduces it with standard intervention protocols (Nitsche and Paulus [2000a, b, 2001](#); Nitsche et al. [2003a, 2005](#); Agboada et al. [2019](#); Ghasemian-Shirvan et al. [2020](#)). With sufficiently long stimulation protocols, stimulation does not only does stimulation induce acute effects on cortical excitability, but also anodal tDCS induces long-term potentiation (LTP)-like plasticity. Since motor learning is associated with LTP (Rioult-Pedotti et al. [2000](#)), LTP-like plasticity enhancement during learning with anodal tDCS has potential to improve motor learning (Nitsche et al. [2003b](#); Reis et al. [2009](#); Reis and Fritsch [2011](#); Stagg et al. [2011](#)).

Additional cortical areas have been related to motor memory, but only a few tDCS studies have focused on areas other than the M1 (Focke et al. [2017](#); Jongkees et al. [2019](#); Katak et al. [2012](#); Nitsche et al. [2010](#); Pollok et al. [2021](#)). Stimulation over the premotor cortex during sleep-dependent consolidation, but not during initial learning, improved SRTT performance (Nitsche et al., [2010, 2003a, b](#)). This suggests a dependency of stimulation effects from its timing, which has to coincide with respective cognitive processes. Nonetheless, the effects of tDCS over the PPC or M1 on motor performance have usually been probed in early stages of learning, but not in late stages (Kumar et al. [2020](#); Pollok et al. [2020](#)). Given the discernible contribution of the M1 and PPC on different phases of motor learning, it can be assumed that different timings of stimulation over these areas result in SRTT performance modulation.

We aimed to explore if tDCS applied over the regions corresponding to M1 or PPC, which induces long-term potentiation-like plasticity relevant for learning and memory consolidation, has different effects on different phases of motor learning depending on the specific timing of stimulation, that is, depending on whether tDCS is applied during the early acquisition phase or during a late stage of learning. The SRTT, an implicit motor sequence learning task (Nissen and Bullemer [1987](#)), was used to explore performance alterations elicited by anodal tDCS over the M1, PPC, or sham stimulation during the early and late stages of motor learning. In congruence with data previously shown for the premotor cortex with respect to improvement of sleep-dependent re-consolidation of motor memories via tDCS (Nitsche et al., [2010](#)), a performance improvement associated with anodal tDCS over the PPC was expected with stimulation applied during the late stage of learning. Effects of stimulation over M1 were expected during initial learning.

MethodMethods

Subjects

An interview with each subject was performed before participation in the study. We explicitly asked for the tendency to prefer the use of one hand over the other. Participants reporting right-handedness in this interview were included in the study. Forty-eight non-smoking right-handed healthy subjects, 21 men and 27 women, aged between 18 and 45 years (mean age = 24 ± 8 years), participated in this mixed, partial crossover, randomized and counterbalanced, sham-controlled, single-blind study. Twenty-four subjects (11

men and 13 women) were included in the early learning + tDCS group (stimulation during the early stage of motor learning). The remaining subjects (10 men and 14 women, mean age) were included in the late learning + tDCS group (stimulation during the late stage of motor learning). None of the participants was taking any medication, and none reported previous or present neurological or psychiatric diseases. All subjects gave informed written consent before participation. The study was approved by the Ethics Committee of the University of Huelva (05/2014) and conforms to the principles of the World Medical Association Declaration of Helsinki.

Transcranial direct current stimulation (tDCS)

tDCS was provided by a battery-driven constant-current stimulator (TCT Research tDCS Stimulator, TST Kowloon, Hong Kong) (Wexler 2015; Brennan et al. 2017), with conductive rubber electrodes placed between two saline-soaked sponges. The tDCS electrode size targeted to the parietal or motor cortex was $20 \text{ cm} \times 20 \text{ cm}^2$ ($4 \times 5 \text{ cm}$). The cathode return electrode size was in both cases $35 \text{ cm} \times 35 \text{ cm}^2$ ($7 \times 5 \text{ cm}$), and was placed over the right supraorbital ridge. To stimulate the left PPC or left M1, the respective anodal electrode was placed over the P3 or C3 position according to the international 10–20 EEG system for electrode placement (Klem et al. 1999; Herwig et al. 2003), based on individual head measures. The electrodes were fixed onto the head by elastic rubber bands.

Direct current stimulation was applied during task performance at 0.5 mA current intensity (anode: 0.025 mA/cm^2 , cathode: 0.014 mA/cm^2 at the electrode–skin interface), with gradual increase and decrease for 8 s at the beginning and the end of stimulation, respectively. tDCS was applied online (during SRTT performance), since offline-off-line applications seem to be ineffective to improve SRTT performance (Chen et al. 2020; Kuo et al. 2008). For sham tDCS, current was increased and then decreased over 8 s at the beginning and the end of stimulation, but the participants did not receive stimulation during the remaining session. Subjects were blinded to the specific tDCS condition. Similar stimulation protocols result in excitability changes stable for about 1 h after motor and parietal cortex tDCS (Nitsche & Paulus 2000a, b, 2001; Rivera-Urbina et al. 2015). During the tDCS procedure, a mild tingling sensation under the active and return electrodes was reported indistinctly in both stimulation conditions during the first minutes. Each subject received direct current stimulation over two different cortical areas, the left PPC (P3) and left M1 (C3), both anodal and sham, in randomized order and on separate days at least 1 week apart. After each intervention, participants were orally asked about any sensation related to tDCS application during stimulation. In all cases, itching, tingling, and slight warmth beneath the area of the electrode was reported at some time point during the stimulation session. No serious adverse effects during or after the study were reported. SimNIBS 3.1.2 (Simulation of Non-Invasive Brain Stimulation), a tool based on the finite element method for simulation of electric fields, was used for modeling the intensity of the electric field at the brain level induced by the respective electrode configurations (Saturnino et al. 2019). The SimNIBS coordinates for the electrode position C3 were: $x = -67$, $x = -67.16$; $y = -16$, $y = -16.60$; $z = 82.76$. For the electrode position P3, we used the coordinates: $x = -54$, $x = -54.66$; $y = -85$, $y = -85.61$; $z = 64.84$, and for the return cathodal electrode position, corresponding to the right supraorbital ridge, we used the coordinates: $x = 29.18$; $y = 86.77$; $z = 34.05$. All these coordinates correspond to the specific magnetic resonance images used in this software to create a head model for which the regions of interest can be localized via the international 10–20 EEG electrode positioning system. We used the C3 electrode position for targeting the left M1 region, and the P3 electrode position for the left PPC. Electrode sizes used for modeling were: $5 \times 4 \text{ cm} = 20 \text{ cm} \times 20 \text{ cm}^2$ and $7 \times 5 \text{ cm} = 35 \text{ cm} \times 35 \text{ cm}^2$ for the anodal and cathodal electrodes, respectively. For both models (with M1 and PPC as cortical targets), the return electrode (cathode) was positioned over the Fp2 EEG electrode position, corresponding to the right supraorbital region. According to the stimulation protocol of this study, the parameters of current intensity in both modeling approaches were set at 0.5 mA. SimNIBS default tissue isotropic conductivity values for scalar brain anisotropy are 0.126 S/m for white matter, 0.275 S/m for grey matter, 1.654 S/m for cerebrospinal fluid (CSF), 0.010 S/m for the bone (skull), 0.465 S/m for the scalp, 0.5 S/m for the vitreous bodies of the eyes, and 1.0 S/m for the saline-soaked sponges (Saturnino et al. 2015).

Serial reaction time task (SRTT)

Subjects performed the SRTT (running on SuperLab, Cedrus Corporation, CA, USA) in front of a computer screen located at about 50 cm eye distance and at eye level. To perform the task, a keyboard was used in

which four selected continuous keys (index ~~finger—Z finger—Z~~ key, middle ~~finger—X finger—X~~ key, ring ~~finger—C finger—C~~ key, pinky ~~finger—V finger—V~~ key) served as response keys. In each trial, an asterisk appeared on the computer screen as a signal at one of the four positions (each corresponding to the order of the four keys). Subjects were instructed to press the corresponding key according to the position of the signal (asterisk) on the screen as fast and correct as possible. The asterisk disappeared once a key was pushed, and 500 ms ~~afterwards—afterward~~ the next asterisk appeared. The SRTT included ~~8—eight~~ blocks of 120 trials each. Stimuli in blocks 1 and 6 had a pseudo-random order of the cues, in which asterisks were presented equally frequently in each position without a repeating sequence. These blocks were used to discern sequence-independent from sequence-specific motor performance improvement. The pseudo-random order of stimuli in these blocks, especially block 6, might also interfere with sequence learning in the following sequence blocks, although this could be considered an incidental effect. Blocks 2, 3, 4, 5, 7 and 8, contained a sequence of 12 trials, which was repeated ~~10—ten~~ times. Subjects were not informed about the sequences. Since the study included four tDCS sessions for each participant, four different versions of the SRTT with specific trial sequences were applied in counterbalanced order across all participants.

Procedure

To explore a possible differential involvement of the M1 and PPC in different stages of motor learning, specifically in the early and late stages of acquisition, as previously suggested (Grafton et al. [1995](#); Deiber et al. [1997](#); Clark and Ivry [2010](#); Hamano et al. [2020](#)), we applied tDCS separately over each area during the early stage of learning (first day of motor ~~learning—early learning—early learning group-~~), or during a late stage (the second day of motor ~~learning—late learning—late learning group-~~).

In the early learning + tDCS group every subject participated in four different ~~two~~2-day sessions. Each session consisted of 2 consecutive days of task performance, corresponding with the early stage (the first day) and late stage (the second day, 24 h later) of learning. For stimulation, the PPC/M1 regions were individually located according to the international 10–20 EEG system, as described above. On the first day of each session, participants received anodal or sham stimulation over the P3 or C3 regions, in randomized order across sessions, during performance of one of the four counterbalanced SRTT versions (one for each of the stimulation conditions: anodal C3, Sham C3, anodal P3, Sham P3). ~~Twenty-four hours—24 h~~ later (that is, the second day of the respective session), every subject performed again the SRTT version of the previous day to evaluate performance in the late stage of motor learning, but no tDCS was applied on this day (Fig. [1A](#)). All subjects of the late learning + tDCS group also participated in four ~~two~~2-day sessions (one for each stimulation condition). Subjects performed one of the four counterbalanced SRTT versions without stimulation during the first day of each session (day of early learning), and 24 h later (the second day of each session, i.e., the day of late learning) they received anodal or sham stimulation over P3 or C3, in randomized order, during performance of the same SRTT version (Fig. [1B](#)). After completion of the task, participants were asked about the occurrence of any motor sequence. Verum or sham tDCS was applied during task performance only in the corresponding learning phase (early or late) of each experimental condition.

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Fig. 1 Experimental design. Four ~~two~~2-day sessions were conducted in each group in randomized order. For the early learning group (**A**), participants received anodal or sham tDCS (in different sessions) on day 1 over the cortical target (posterior parietal cortex or motor cortex) during performance of the computer-based serial reaction time task (SRTT). Four different keys of the computer keyboard were used for responding. In each trial, an asterisk appeared on the screen at one of four positions as a signal or cue for ~~the~~ respective responses. The four possible positions of the stimulus (asterisk) are shown in the left column. One day later (day 2), the participants performed again the task with the identical sequence but without tDCS. For the late learning group (**B**), the procedure was identical, except for the fact that participants performed the respective SRTT without stimulation on the first day. One day later, anodal or sham tDCS was applied over the parietal or primary motor cortex during task performance. In both groups, the four different SRTT versions used in the study were counterbalanced to avoid any repetition effect of the task throughout the four experimental sessions

Data Analysisanalysis

Since tDCS was applied during the whole task performance period, a one-way ANOVA was conducted to analyze differences between experimental conditions in each group (early and late learning) with respect to

the total time required for task performance, which defined the duration of tDCS, to exclude inter-group differences of stimulation duration. Reaction time (RT) was recorded for each trial. RTs above 3 standard deviations of the individual subject's mean RT and incorrect responses were discarded. For each experimental condition, an error rate (ER) was calculated for each participant representing the number of errors conducted in each block of the task. The standard deviation of RT for each subject in every block was calculated as an index of individual variability of RT. Statistical analyses were performed for variability of RT and the RT and ER values via repeated measures ANOVAs. A $2 \times 2 \times 2 \times 2 \times 8$ mixed model ANOVA (with tDCS timing, i.e., early learning + tDCS vs. late learning + tDCS, as between-subject factor) was conducted for RT values standardized to block 1 of the first intra-session day (absolute value of each block/absolute value of block 1) as dependent variable, thus allowing to control for data variability and generate scales of comparable values with respect to the first block of the task (random stimuli block without a sequential order). The following within-subject factors were included: stimulation area (C3 and P3), stimulation condition (anodal and sham), intra-session time (early learning - day - day 1, and late learning - day - day 2), and block (1–8). In case of significant results of this overall ANOVA (primary outcome parameter), additional exploratory repeated measures ANOVAs were conducted to analyze the standardized RT values of each stimulation condition (anodal vs. sham) in each tDCS group (early learning + tDCS vs. late learning + tDCS), with respect to stimulation over C3 and P3 for day 1 and day 2, resulting in eight exploratory 2-way repeated measures ANOVAs with the within-subject factors stimulation (anodal vs. sham) and block. In case of significant results, Fisher's LSD post hoc tests were conducted to analyze differences between anodal and sham stimulation in each experimental condition and block. Additionally, 2 separate exploratory post hoc repeated measures ANOVAs for day 1 and day 2, with the between group factors stimulation (anodal vs. sham), group (early vs. late learning), and area (C3 vs. P3), were conducted to analyze the standardized RT values throughout the task blocks (within-subject factor). In case of significant results, Fisher's LSD post hoc tests were conducted to analyze between block differences in each experimental condition.

The ER values and variability were analyzed by the same overall mixed model ANOVA as the RT values, and separate ANOVAs and Fisher's LSD post hoc tests (anodal vs. sham and C3 vs. P3) were also conducted for each block in each experimental condition in case of significant results. Absolute RT values obtained in block 1 were analyzed by an ANOVA with the factors group, stimulation condition, stimulation area and day, to evaluate possible initial differences between the experimental conditions. In addition, a $2 \times 2 \times 2 \times 2 \times 8$ mixed model ANOVA was conducted to analyze absolute values across all blocks. The results of these analyses and respective post hoc tests for absolute values are available in the Supplemental material (Table 1S-3S). Mauchly's test of sphericity was conducted in all ANOVAs. The critical level of significance in all tests was set to $p < 0.05$. All analyses were carried out using SPSS software.

Results

Figure 2 shows the current flow calculated by the finite element method according to the electrode configurations used in this study. Participants were not aware of distinguishable motor sequences between learning and random blocks when they were asked in each case. There were no differences between experimental conditions with respect to the duration required for performance of the whole task, and thus there were no differences with respect to stimulation duration, for the early ($F_{3,92} = 0.326, p = 0.807$) and late ($F_{3,91} = 0.285, p = 0.836$) learning groups (day 1 and 2, respectively) (anodal C3, day 1: $\overline{\text{RT}} = 14.21$ min, SD = 2.66; anodal P3, day 1: $\overline{\text{RT}} = 14.29$ min, SD = 3.0; anodal C3, day 2: $\overline{\text{RT}} = 14.42$ min, SD = 2.22; anodal P3, day 2: $\overline{\text{RT}} = 13.92$ min, SD = 2.62; sham C3, day 1: $\overline{\text{RT}} = 13.57$ min, SD = 2.91; sham P3, day 1: $\overline{\text{RT}} = 14.23$ min, SD = 3.02; sham C3, day 2: $\overline{\text{RT}} = 14.27$ min, SD = 2.22; sham P3, day 2: $\overline{\text{RT}} = 14.50$ min, SD = 2.37). The analyses of the absolute and standardized RT values conducted by the Lilliefors-corrected Kolmogorov–Smirnov test showed a normal distribution of the data for the overall measures of the early ($p > 0.05$) and late ($p > 0.05$) learning groups. Shapiro–Wilk tests confirmed the normal distribution of the absolute and standardized RT data ($p > 0.05$ for each data set and group). The analyses of the ER conducted by the Lilliefors-corrected Kolmogorov–Smirnov test also showed a normal distribution of the data for the overall measures of the early ($p > 0.05$) and late ($p > 0.05$) learning groups, and Shapiro–Wilk tests confirmed normal distribution of the data ($p > 0.05$ for each group).

Fig. 2 Intensities of the electric fields induced by tDCS over the EEG electrode position corresponding to the primary motor cortex (M1) (**A**) or posterior parietal cortex (PPC) (**B**), calculated by the finite element method. Panel A shows dorsal, frontal and lateral (left hemisphere) views of the current flow induced by tDCS applied over the left M1 (C3 EEG electrode position). The electric field (normative strength: normE) intensity (V/m) is represented by the color bar (online version). The brighter colors (larger numbers depicted in the color bar) indicate higher electric field intensity, with the highest intensity corresponding with the left M1 region (0.128 V/m). Panel B shows dorsal, frontal and lateral views of the current flow induced by tDCS applied over the left PPC (P3 EEG electrode position). The highest electric field intensity under this electrode configuration was 0.11 V/m, corresponding with the left PPC region. SimNIBS 3.1.2 (Simulation of Non-Invasive Brain Stimulation) was used for modeling of the electric field. Red and blue electrodes of the SimNIBS output brain images represent the anodal and cathodal electrode positions, respectively

As expected, no significant main effects of the factors group ($F_{1,23} = 0.428, p = 0.519, \eta_p^2 = 0.018$), stimulation condition ($F_{1,23} = 0.450, p = 0.509, \eta_p^2 = 0.019$), and stimulation area ($F_{1,23} = 0.625, p = 0.437, \eta_p^2 = 0.026$) were found in the ANOVA conducted to analyze absolute RT values obtained in block 1. The main effect of the factor day was, however, significant ($F_{1,23} = 75.532, p < 0.001, \eta_p^2 = 0.767$). No interaction was significant in this ANOVA conducted over the data obtained in block 1 ($p > 0.05$ in all cases). The results of the overall mixed model ANOVA conducted for standardized RTs with the main factors group (early learning/late learning), area (C3/P3), stimulation (anodal/sham), time (day1/day2), and block (1–8) are shown in Table 1. The results of this ANOVA show significant main effects of the factor time ($F_{1,362} = 41.510, p < 0.001, \eta_p^2 = 0.643$) and block ($F_{7,362} = 39.395, p < 0.001, \eta_p^2 = 0.631$). The interaction between group, area, stimulation and time was also significant ($F_{1,362} = 4.374, p = 0.048, \eta_p^2 = 0.160$), as well as the interaction between stimulation and block ($F_{7,362} = 3.114, p = 0.004, \eta_p^2 = 0.119$), and between time and block ($F_{7,362} = 10.631, p < 0.001, \eta_p^2 = 0.316$). The results of the separate repeated measures ANOVAs conducted to analyze RT differences between anodal and sham conditions for each tDCS group, area and time (day) for the respective blocks are shown in Table 2. The results of these analyses show significant effects of the factor block in all experimental conditions ($p < 0.001$), due to RT reductions over blocks in the course of the experiment, and longer RTs in the random blocks, as compared to the sequence blocks. Additionally, for the late learning group-P3 anodal vs. sham stimulation (day 2) condition, the respective ANOVA showed significant main effects of factor stimulation ($F_{1,46} = 4.909, p = 0.037, \eta_p^2 = 0.176$), and the interaction between stimulation and block was also significant ($F_{7,161} = 3.097, p = 0.027, \eta_p^2 = 0.560$). When RT differences between real and sham stimulation in this experimental condition were compared by Fisher's LSD post hoc tests for each block separately (Table 4S4S), significantly reduced RTs in the anodal stimulation condition were found for blocks 3 ($p = 0.031$) and 8 ($p = 0.046$).

Table 1 Results of the mixed model ANOVA conducted to analyze standardized reaction time measures in each experimental condition and block

Reaction Time time (serial reaction time task)	d.f/df	F-value	F-value	Partial Eta Squared/eta squared
Group (early + tDCS vs. late + tDCS)	1	0.187	0.670	0.008
Area (motor cortex/posterior parietal cortex)	1	0.003	0.956	0.000
Stimulation (anodal/sham)	1	3.227	0.086	0.123
Time (first day—earlyday—early/24 h later—late—late—late)	1	41.510	* < 0.001	0.643
Block (1–8)	7	39.395	* < 0.001	0.631
Group x Area x area	1	2.398	0.135	0.094
Group x Stimulation x stimulation	1	0.850	0.366	0.036
Area x Stimulation x stimulation	1	0.101	0.753	0.004
Group x Area x Stimulation x area x stimulation	1	0.476	0.497	0.020
Group x Time x time	1	1.149	0.295	0.048

Area \times Time \times time	1	0.033	0.857	0.001
Group \times Area \times Time \times area \times time	1	0.370	0.549	0.016
Stimulation \times Time \times time	1	0.245	0.625	0.011
Group \times Stimulation \times Time \times stimulation \times time	1	2.478	0.129	0.097
Area \times Stimulation \times Time \times stimulation \times time	1	0.000	0.986	0.000
Group \times Area \times Stimulation \times Time \times area \times stimulation \times time	1	4.374	*0.048	0.160
Group \times Block \times block	7	0.441	0.875	0.019
Area \times Block \times block	7	0.222	0.980	0.010
Group \times area \times Area \times Block \times block	7	1.611	0.136	0.065
Stimulation \times Block \times block	7	3.114	*0.004	0.119
Group \times Stimulation \times Block \times stimulation \times block	7	0.600	0.756	0.025
Area \times Stimulation \times Block \times stimulation \times block	7	0.310	0.949	0.013
Group \times Area \times Stimulation \times Block \times area \times stimulation \times block	7	0.355	0.927	0.015
Time \times Block \times block	7	10.631	* < 0.001	0.316
Group \times Time \times Block \times time \times block	7	0.936	0.480	0.039
Area \times Time \times Block \times time \times block	7	0.356	0.926	0.039
Group \times Area \times Time \times Block \times area \times time \times block	7	0.678	0.691	0.029
Stimulation \times Time \times Block \times time \times block	7	0.925	0.489	0.039
Group \times Stimulation \times Time \times Block \times stimulation \times time \times block	7	1.723	0.107	0.070
Area \times Stimulation \times Time \times Block \times stimulation \times time \times block	7	0.537	0.805	0.023
Group \times Area \times Stimulation \times Time \times Block \times area \times stimulation \times time \times block	7	1.927	0.069	0.077

Table 2 Results of the separate repeated measures ANOVAs conducted to analyze standardized reaction time measures for real and sham stimulation conditions in each experimental condition

Reaction Time time (serial reaction time task)	d.f	F value	F value	Partial Eta Squared
Early acquisition group-C3 stimulation-Day day 1 (Anodal anodal tDCS vs. Sham-sham tDCS)	1	0.648	0.429	0.027
Block	7	7.190	* < 0.001	0.238
Stimulation \times Block \times block	7	1.321	0.244	0.054
Early acquisition group-C3 stimulation-Day day 2 (Anodal anodal tDCS vs. Sham-sham tDCS)	1	0.049	0.826	0.002
Block	7	9.198	* < 0.001	0.286
Stimulation \times Block \times block	7	1.457	0.186	0.060
Early acquisition group-P3 stimulation-Day day 1 (Anodal anodal tDCS vs. Sham-sham tDCS)	1	1.620	0.216	0.066
Block	7	7.418	* < 0.001	0.244
Stimulation \times Block \times block	7	1.421	0.200	0.058
Early acquisition group-P3 stimulation-Day day 2 (Anodal anodal tDCS vs. Sham-sham tDCS)	1	0.730	0.402	0.031
Block	7	8.455	* < 0.001	0.269
Stimulation \times Block \times block	7	0.799	0.589	0.034
Late acquisition group-C3 stimulation-Day day 1 (Anodal anodal tDCS vs. Sham-sham tDCS)	1	0.235	0.632	0.010
Block	7	6.695	* <	0.225

			0.001	
Stimulation × Block × block	7	0.339	0.935	0.015
Late acquisition group-C3 stimulation-Day day 2 (Anodal anodal tDCS vs. Sham-sham tDCS)	1	0.402	0.532	0.017
Block	7	11.285	* < 0.001	0.329
Stimulation × Block × block	7	0.370	0.919	0.016
Late acquisition group-P3 stimulation-Day day 1 (Anodal anodal tDCS vs. Sham-sham tDCS)	1	0.444	0.512	0.019
Block	7	21.704	* < 0.001	0.486
Stimulation × Block × block	7	0.434	0.880	0.019
Late acquisition group-P3 stimulation-Day day 2 (Anodal anodal tDCS vs. Sham-sham tDCS)	1	4.909	*0.037	0.176
Block	7	17.465	* < 0.001	0.878
Stimulation × Block × block	7	3.097	*0.027	0.560

With respect to the analysis of the interaction between group, area, stimulation and time, the results of separate (day 1 and day 2) exploratory post hoc ANOVAs conducted to analyze RT differences between C3 and P3 for each stimulation condition (anodal vs. sham) and group (early vs. late learning) throughout the task blocks revealed significant main effects of the factor block on day 1 ($F_{7,17} = 16.181, p < 0.001, \eta_p^2 = 0.870$), and the factors group ($F_{1,23} = 18.502, p < 0.001, \eta_p^2 = 0.446$) and block ($F_{7,17} = 12.873, p < 0.001, \eta_p^2 = 0.841$) on day 2. The interaction between group and stimulation condition was also significant on day 2 ($F_{1,23} = 5.066, p = 0.034, \eta_p^2 = 0.180$). No other significant effects emerged.

On day 2, RT increased in block 6 (block without sequential order of stimuli) with respect to block 5 in all experimental conditions. The interference effect of the random block 6 on subsequent blocks 7 and 8 of day 2 was explored by Fisher's LSD post hoc tests to compare block 6 with the respective blocks, and block 5 was compared with blocks 7 and 8 to explore the respective effects of the intervention. The comparisons between blocks 6 and 7, 6 and 8, and 7 and 8 revealed that in the early learning group-C3 anodal, significant differences were found between blocks 6 and 8 ($p < 0.001$), and between blocks 7 and 8 ($p = 0.001$), indicating an improvement of RT after the interference block. In the early learning group-P3 anodal, significant differences were found between blocks 6 and 7 ($p = 0.039$), and between blocks 6 and 8 ($p < 0.001$), which also indicates shorter RT in the respective sequences, as compared to the random stimuli blocks. In the early learning group-C3 sham and early learning group-P3 sham, significant differences were only found between blocks 6 and 8 ($p = 0.001$ and $p = 0.006$, respectively), which suggests a somewhat slower improvement in the sham stimulation conditions. In the late learning group-C3 anodal, significant differences were found between blocks 6 and 7 ($p = 0.002$), 6 and 8 ($p < 0.001$), and 7 and 8 ($p = 0.008$), indicating significantly shortened RT in the sequence as compared to the random stimuli blocks. Likewise, in the late learning group-P3 anodal, significant differences were found between blocks 6 and 7 ($p = 0.001$), 6 and 8 ($p < 0.001$), and 7 and 8 ($p = 0.001$), also indicative for shorter RT in the respective sequence, as compared to the random stimuli blocks. These RT differences in relation to block 6 were also observed in the sham stimulation conditions. In the late learning group-C3 sham, faster RTs were found in the sequence blocks, with significant differences between blocks 6 and 7 ($p = 0.029$), and 6 and 8 ($p = 0.001$), and with faster RTs in block 8 in comparison with block 7 ($p = 0.011$). In the late learning group-P3 sham, RTs were faster in sequence blocks as well, with significant differences between blocks 6 and 7 ($p = 0.001$), and 6 and 8 ($p < 0.001$). Blocks 5 and 7, as well as blocks 5 and 8, of day 2 were also compared in order to analyze the return to pre-interference performance. In the early learning group-C3 anodal, RT was significantly increased in block 7 as compared to block 5 ($p = 0.006$), and in the early learning group-P3 anodal, RT was significantly larger in block 5, as compared to block 8 ($p = 0.008$). No differences between these blocks were found in the early learning group-C3 sham and early learning group-P3 sham. In the late learning group-P3 anodal, RT was significantly increased in block 7 with respect to block 5 ($p = 0.008$), but no differences were found in the late learning-C3 anodal group. Finally, in the late learning group-C3 sham, RT was significantly increased in block 7 compared to block 5 ($p = 0.031$), but no differences were found in the late learning

group-P3 sham. Figure 3 shows the respective standardized RTs for each experimental condition comparing anodal vs. sham stimulation, and the stimulation areas C3 vs. P3, separately for days 1 and 2. Figure 4 shows standardized RTs of each experimental condition in blocks 5, 6, 7 and 8 of day 2.

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Fig. 3 Standardized reaction time (RT) for each experimental condition (panels A–DA–D) in the serial reaction time task (SRTT), comparing anodal vs. sham stimulation, and stimulation areas C3 vs. P3. **A**, early learning group, day 1; **B**, early learning group, day 2; **C**, late learning group, day 1; **D**, late learning group, day 2. The symbol (*) in panel D indicates significant differences between anodal P3 vs. sham P3 stimulation conditions, with faster RTs in the anodal tDCS condition. Error bars represent standard error of means (SEM) for each block and experimental condition

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Fig. 4 Standardized reaction time (RT) of each experimental condition in blocks 5, 6, 7 and 8 of day 2. **A**, early learning group; A_C3, anodal stimulation over C3; A_P3, anodal stimulation over P3; **B**, late learning group; B5-8, blocks 5–8; S_C3, sham stimulation over C3; S_P3, sham stimulation over P3. Lines with symbols indicate significant differences between blocks

The results of the mixed model ANOVA conducted to analyze the ER values for each experimental condition and block are shown in Table 3. The results of this ANOVA reveal significant main effects of the factor time ($F_{1,362} = 4.488, p = 0.046, \eta_p^2 = 0.176$) and block ($F_{7,362} = 20.751, p < 0.001, \eta_p^2 = 0.497$). Because also the interaction between group, stimulation, time and block was significant ($F_{7,362} = 2.427, p = 0.022, \eta_p^2 = 0.104$) (for other significant interactions, see Table 3), separate ANOVAs for each specific experimental condition were conducted to analyze anodal vs. sham differences on ER (Table 4). These ANOVAs showed significant effects of the factor block in all experimental conditions ($p \leq 0.001$), with an overall increase of the ER in block 6 of the task. The interaction between stimulation and block was significant for the early learning group-P3 stimulation condition (day 2, without tDCS) ($F_{7,161} = 2.185, p = 0.038, \eta_p^2 = 0.087$). Fisher’s LSD post hoc tests showed a reduced ER in the sham condition, as compared to anodal stimulation, specifically for block 6 ($p = 0.045$). Figure 5 shows the ER in each experimental condition comparing anodal vs. sham stimulation, and the stimulation areas C3 vs. P3. Figure 3S–Figure 3S shows the ER for each experimental condition (stimulation condition, and group), comparing anodal vs. sham stimulation, and day 1 vs. day 2.

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Fig. 5 Error rates in each experimental condition (panels A–DA–D), comparing anodal vs. sham stimulation, and stimulation areas C3 vs. P3. **A**, early learning group, day 1; **B**, early learning group, day 2; **C**, late learning group, day 1; **D**, late learning group, day 2. The symbol (*) in panel B indicates significant differences between sham P3 vs. anodal P3 stimulation conditions, with lower errors in the sham stimulation condition. Error bars represent standard error of means (SEM) for each block and experimental condition

Table 3 Results of the mixed model ANOVA conducted to analyze the error rate (ER) in each experimental condition and block

ER (serial reaction time task)				
d.f				
df		F		
value				
P				
value				
Partial				
Eta Squared				
eta squared				
Group (early + tDCS vs. late + tDCS)	1	0.037	0.850	0.002
Area (motor cortex/posterior parietal cortex)	1	0.172	0.938	0.000
Stimulation (anodal/sham)	1	1.271	0.272	0.057
Time (first day -early/24 h later -late)	1	4.488	*0.046	0.176

Block (1-8)	7	20.751	* < 0.001	0.497
Group				
x Area				
x area	1	0.188	0.669	0.009
Group				
x Stimulation				
x stimulation	1	0.170	0.684	0.008
Area				
x Stimulation				
x stimulation	1	5.261	*0.032	0.200
Group				
x Area x Stimulation				
x area x stimulation	1	1.145	2.97	0.052
Group				
x Time				
x time	1	0.160	0.693	0.008
Area				
x Time				
x time	1	5.421	*0.030	0.205
Group				
x Area x Time				
x area x time	1	0.565	0.461	0.026
Stimulation				
x Time				
x time	1	0.029	0.867	0.001
Group				
x Stimulation x Time				
x stimulation x time	1	0.930	0.346	0.042
Area				
x Stimulation x Time				
x stimulation x time	1	0.335	0.569	0.016
Group				
x Area x Stimulation x Time				
x area x stimulation x time	1	0.679	0.419	0.031
Group				
x Block				
x block	7	1.128	0.349	0.051
Area				
x Block				
x block	7	1.756	0.100	0.077
Group				
x Area x Block				
x area x block	7	1.1423	0.200	0.063
Stimulation				
x Block				
x block	7	1.597	0.141	0.071
Group				
x Stimulation x Block				
x stimulation x block	7	0.739	0.639	0.034
Area				
x Stimulation x Block				

x stimulation x block	7	0.424	0.886	0.020
Group				
x Area x Stimulation x Block				
x area x stimulation x block	7	1.674	0.120	0.074
Time				
x Block				
x block	7	6.576	* < 0.001	0.238
Group				
x Time x Block				
x time x block	7	0.939	0.478	0.043
Area				
x Time x Block				
x time x block	7	1.304	0.252	0.058
Group				
x Area x Time x Block				
x area x time x block	7	1.706	0.112	0.075
Stimulation				
x Time x Block				
x time x block	7	2.086	*0.049	0.090
Group				
x Stimulation x Time x Block				
x stimulation x time x block	7	2.427	*0.022	0.104
Area				
x Stimulation x Time x Block				
x stimulation x time x block	7	0.690	0.680	0.032
Group				
x Area x Stimulation x Time x Block				
x area x stimulation x time x block	7	0.548	0.797	0.025

Table 4 Results of the separate repeated measures ANOVAs conducted to analyze the error rate (ER) in each experimental condition

ER (serial reaction time task)	d.fdf	F-value	F-value	Partial Eta Squared
Early acquisition group-C3 stimulation-Day day 1 (Anodal anodal tDCS vs. Sham-sham tDCS)	1	2.138	0.157	0.085
Block	7	9.117	* < 0.001	0.284
Stimulation x Block x block	7	0.592	0.762	0.025
Early acquisition group-C3 stimulation-Day day 2 (Anodal anodal tDCS vs. Sham-sham tDCS)	1	0.260	0.615	0.011
Block	7	6.078	* < 0.001	0.209
Stimulation x Block x block	7	1.109	0.360	0.046
Early acquisition group-P3 stimulation-Day day 1 (Anodal anodal tDCS vs. Sham-sham tDCS)	1	0.389	0.539	0.017
Block	7	6.027	* < 0.001	0.208
Stimulation x Block x block	7	0.684	0.685	0.029
Early acquisition group-P3 stimulation-Day day 2 (Anodal anodal tDCS vs. Sham-sham tDCS)	1	1.301	0.266	0.054
Block	7	3.653	*0.001	0.137
Stimulation x Block x block	7	2.185	*0.038	0.087
Late acquisition group-C3 stimulation-Day day 1 (Anodal	1	1.533	0.229	0.065

anodal tDCS vs. Sham-sham tDCS)				
Block	7	1.864	0.079	0.078
Stimulation \times Block \times block	7	1.172	0.322	0.051
Late acquisition group-C3 stimulation-Day day 2 (Anodal anodal tDCS vs. Sham-sham tDCS)				
Block	7	6.416	* < 0.001	0.218
Stimulation \times Block \times block	7	1.492	0.174	0.061
Late acquisition group-P3 stimulation-Day day 1 (Anodal anodal tDCS vs. Sham-sham tDCS)				
Block	7	7.527	* < 0.001	0.247
Stimulation \times Block \times block	7	0.902	0.507	0.038
Late acquisition group-P3 stimulation-Day day 2 (Anodal anodal tDCS vs. Sham-sham tDCS)				
Block	7	6.862	* < 0.001	0.246
Stimulation \times Block \times block	7	1.820	0.087	0.080

Finally, the analysis of variability indicated a significant main effect of the factor block ($F_{7,362} = 8.566$, $p < 0.001$, $\eta_p^2 = 0.779$), with a reduced RT variability when stimuli were displayed in pseudo-random order (block 6). There was also a significant effect of the interaction between group, area, time and block ($F_{7,362} = 2.811$, $p = 0.039$, $\eta_p^2 = 0.537$), and between group, stimulation, time and block ($F_{7,362} = 5.134$, $p = 0.003$, $\eta_p^2 = 0.679$). The results of the separate repeated measures ANOVAs showed significant effects only in the learning group-P3 stimulation condition (day 1). In this experimental condition, there was a significant main effect for the factor stimulation ($F_{1,46} = 4.608$, $p = 0.043$, $\eta_p^2 = 0.167$), with reduced RT variability for the anodal stimulation, and block ($F_{7,161} = 5.296$, $p = 0.002$, $\eta_p^2 = 0.686$), with reduced variability for block 6, but not for the interaction between these factors ($F_{7,161} = 1.378$, $p = 0.277$, $\eta_p^2 = 0.362$). No other significant effects were found.

Absolute RT values in each experimental condition comparing anodal vs. sham stimulation, and day 1 vs. day 2, are shown in Fig. 1S-1S of the Supplemental material. Figure 2S-2S shows standardized RTs for each experimental condition in the SRTT, comparing anodal vs. sham stimulation, and day 1 vs. day 2. Table 1S shows the results of the mixed model ANOVA conducted for absolute values, with the factors group, stimulation condition, stimulation area, and day as between subject factors, and block as within-subject factor. The interaction between group, stimulation, area, day, and block was significant ($p = 0.044$). Table 2S shows the results of the Fisher's LSD post-hoc tests for absolute RT values in each experimental condition, regarding blocks 5 (sequential order of stimuli) and 6 (no sequential order of stimuli). There were significant differences in performance between these blocks in all experimental conditions ($p < 0.05$), except in the early learning group-P3 anodal stimulation on day 1 ($p = 0.706$) and early learning group-P3 sham stimulation on day 1 ($p = 0.112$) and 2 ($p = 0.082$), as well as in the late learning group-C3 anodal stimulation on day 1 ($p = 0.207$) and late learning group-P3 sham stimulation on day 1 ($p = 0.168$). Performance differences between the last sequence block of day 1 and the first sequence block of day 2 were compared by contrasting absolute RT values for block 8 (day 1) and block 2 (day 2) in each experimental condition and group to identify offline-off-line learning. No significant performance differences were found between these blocks in any of the experimental conditions and group (Table 3S3S); offline-off-line learning did thus not occur. As a measure of nonspecific change of performance between sessions, absolute RT values for block 6 (day 1) and block 1 (day 2) (blocks without presentation of the sequence) were also compared in each experimental condition and group. No significant performance differences were found between these blocks in any of the experimental conditions of the early learning group ($p > 0.05$). In the late learning group, no significant performance differences were found between these blocks in any of the experimental conditions, except in the C3 anodal stimulation group, in which RT of block 1 on day 2 was significantly lower, compared to block 6 of day 1 ($p = 0.032$) (Table 3S3S).

Discussion

In the present study, we explored the contribution of M1 and PPC on the early and late stages of motor sequence learning. The findings of this study support an impact of the PPC on the late stage of motor learning. Only anodal tDCS applied over this area during the late stage of learning (second day) improved RT significantly, as compared to the sham intervention. In this experimental condition, RT was overall reduced along the blocks, and this performance improvement was significant versus sham in two of the sequence blocks (Fig. 3). This reveals an effect of anodal tDCS applied over the PPC on the late, but not early, stage of learning, and suggests that this component of the motor network is involved in selective phases of motor learning, in line with previous evidence (Honda et al. 1998; Catalan et al. 1998; Hamano et al. 2020). This stimulation effect was moreover largely restricted to RT. ER did not significantly differ between sham and anodal stimulation and between target areas in any of the experimental conditions for sequence blocks. ER was significantly lower in the early learning group-P3 sham stimulation condition, compared to anodal stimulation, in block 6 of day 2, but this effect was not found for the other sham stimulation conditions. This might be thus an incidental effect or indicate reduced interference in this group, compared to other conditions, which would be compatible with reduced sequence learning. These findings are congruent with the LTP-like plasticity effects associated with the application of anodal tDCS over cortical targets of the motor network (Nitsche and Paulus 2001; Rivera-Urbina et al. 2015; Agboada et al. 2019).

Animal (Zanatta et al. 1997) and human (Himmer et al. 2019) memory models have identified a functional brain network participating in specific stages of memory consolidation, in which the hippocampus, amygdala, entorhinal cortex and PPC are differentially involved depending on the learning modality. Different consolidation processes facilitating long-term memory, as offline-off-line improvement (Yu-Lin-Qin, Bruce L. Menaughton 1997) and memory stabilization (Kandel et al. 2014; Della-Maggiore et al. 2017), have been proposed. In humans, this brain network includes stimulus modality-dependent cortical and subcortical regions (Brodt et al. 2018). The learning process initiates specific plastic changes in these regions to generate memory engrams and consolidate memory traces after encoding. The PPC is one of the cortical regions involved in memory consolidation processes in several learning modalities (Brodt et al. 2018), including motor memory formation (Shadmehr and Holcomb 1997; Kumar et al. 2019). The contribution of this area to late learning stages, as found in the present study, is compatible with its involvement in consolidation, because late learning will make use of already existent memory content.

Neuroimaging data have pointed to M1 and PPC as relevant nodes of the motor learning cortical network (Deiber et al. 1997; Sakurada et al. 2019). These studies suggest that M1 is involved in effector-specific initial learning (Grafton et al. 1995), and PPC is relevant for largely effector-independent late learning and consolidation processes (Grafton et al. 1998). These results are supported by recent studies showing that cortical excitability-diminishing cathodal tDCS over the PPC during (Kumar et al. 2020) or after (Pollok et al. 2020) motor learning impairs inter-effector generalization and memory consolidation, respectively. Our results are in principal agreement with these studies, but additionally demonstrate that LTP-like plasticity-inducing anodal stimulation over the PPC selectively applied during the late stage of learning (day 2), but not during initial learning, improves motor performance. Considering that tDCS over the PPC modulates M1 excitability (Rivera-Urbina et al. 2015), which reveals an important neurophysiological connectivity between these nodes of the motor learning network, it might be argued that the effect of anodal tDCS over the PPC on motor performance obtained in the present study is transmitted via this cortico-cortical connectivity. Direct M1 stimulation during the late learning stage, however, did not improve performance. Moreover, the involvement of the PPC in late learning does not appear to be necessarily effector dependent (Grafton et al. 1995, 1998, 2002). The PPC has also been related to memory functions linked to spatial (Brodt et al. 2018), visual (Wang et al. 2019), and numerical (Parvizi and Wagner 2018) processing. This suggests the confluence of a multimodal functional system in the PPC supported by specific cortical networks recruited according to the modality of coded stimuli (Wagner et al. 2005; Ciaramelli et al. 2008; Hutchinson et al. 2009; Ciaramelli and Moscovitch 2020).

On the other hand, it has been shown in previous studies that neuromodulation of M1 by tDCS improves initial motor learning (Nitsche et al., 2003b; Stagg et al., 2011). However, we could not see this effect in the present study, as stimulation over the area corresponding to M1 did not significantly improve RT when applied during the learning stage. Methodological differences regarding previous studies may explain why this expected effect was not evident. Indeed, the electrode configuration we used involved a relatively small

anodal electrode (4 × 5 cm). Although the resulting current density (0.025 mA/cm²) was similar to that of other studies (Nitsche et al. 2003b), the small size of the anode might not have been sufficiently efficient to boost M1 plasticity and specifically induce RT improvements. Here, the area covered by the stimulation electrodes might be specifically relevant and lead to different physiological and behavioral effects (Boros et al. 2008; Foerster et al. 2019). In addition, inter-individual variability of behavioral effects of tDCS may also have an impact on results of different studies. Despite this, we did not find systematic differences in variability and accuracy between experimental conditions.

Overall, our results highlight the timing-specific contribution of the PPC to motor learning. Interventions targeting this region may improve motor learning when applied in late stages of acquisition, possibly in addition or as adjunctive intervention to M1 stimulation during early stages of motor learning. This discernible function of both structures of the motor network revealed by tDCS is relevant for fine-tuning of this neuromodulation technique with respect to the temporal domain, and might be important for future experimental and applied use, particularly considering the ease of use of tDCS and its safety profile (Antal et al. 2017; Bikson et al. 2016; Brunoni et al. 2012; Giordano et al. 2017; Jackson, Bikson, Liebetanz, & Nitsche, et al. 2017a, 2017b; Nitsche & Bikson 2017; Woods et al. 2016), as well as its reported utility in motor improvement and rehabilitation (Allman et al. 2016; Esmailpour et al. 2018; Fregni et al. 2015; O'Shea et al. 2014; Reis & Fritsch 2011; Santos Ferreira et al. 2019; Stagg et al. 2012).

Some limitations of the present study should be taken into consideration. Optimization of effect size was not the objective of the present study. We used a current density comparable to that of other studies (Kuo et al. 2008; Nitsche et al. 2010). However, higher current intensities might result in stronger functional effects (Antal et al. 2017; Cuypers et al. 2013; Esmailpour et al. 2018; Evans et al. 2020; Fregni et al. 2015; Giordano et al. 2017; Nitsche & Paulus 2000a, b; Nitsche & Bikson 2017; Vignaud, Mondino, Poulet, Palm, & Brunelin, et al. 2018). Furthermore, the limited spatial specificity of tDCS as applied in the present study may be, indeed, a limitation of this method. The lack of tDCS effects on performance when applied over M1 may be related, as mentioned above, to the electrode size/current intensity configuration and its potential to induce plasticity in M1. Specific evidence is needed to precisely define the functional contribution of the M1 to general motor learning, since congruent (Nitsche et al. 2003b; Katak et al. 2012; Hamano et al. 2020) and incongruent (Berlot et al. 2020) data have been reported regarding implicit and explicit motor learning, respectively, and by different procedures. On the other hand, with lower intensities of tDCS, discrimination between real and sham stimulation conditions becomes less likely (Fonteneau et al. 2019; Neri et al. 2020). In the present study, however, we did not openly ask participants about their guesses of stimulation (real/sham), and therefore, blinding success could not be determined. Another possible limitation of this study could be that stimulation over the PPC might have had not only an effect on the PPC, but also a direct impact on M1 activity, considering that the application of anodal tDCS over the PPC by similar intervention protocols has shown to alter the M1 excitability (Rivera-Urbina et al. 2015). Since we applied tDCS over both cortical areas in separate sessions, and no significant effects were found on late learning via M1 stimulation, it is improbable that the effect of PPC stimulation on late learning was caused by current spread to M1. Finally, the non-focality of conventional tDCS electrodes is a potential limitation of this technique. The results of the electric field computational modeling based on the finite element method are congruent with this limitation, according to the calculated electric field distribution. However, simulations revealed higher electric field intensities over the cortical targets (specifically, 0.128 V/m when targeting the M1, and 0.11 V/m when targeting the PPC), as compared to other regions (which is reflected by the colors of the simulated electric field in Fig. 2). Thus, the electric field extended beyond these cortical targets, but with a considerably lower current intensity. Although SimNIBS can incorporate individual resonance magnetic images for modeling, we used a standard, non-personalized head model to get an overview of the potential electric field associated with each particular electrode configuration. Further studies using more focal approaches to optimize tDCS protocols, for example with smaller electrodes (Nitsche et al. 2007) or by 4 × 1 electrode configurations (HD-tDCS) (Alam et al. 2016; Wang et al. 2018), could provide more conclusive evidence on the precise anatomical location that mediates the effects of stimulation.

Conclusions

The PPC is primarily involved in the late stage of motor learning, but not relevantly in initial learning,

according to the results of the present study. Because selectively PPC stimulation applied during task performance on day 2, 24 h after the initial motor learning phase, improved RTs, it can be argued that tDCS over the PPC selectively improved the late stage of motor learning. These findings may help to further define the temporal dynamics of the motor network in learning and memory formation. Moreover, the application of anodal tDCS improved performance on an implicit motor learning task during reactivation of memory, and this improvement effect might have clinical and therapeutic potential. To achieve well-defined results in rehabilitation and clinical contexts, optimization of stimulation protocols would be required.

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Declarations

Conflict of interest

Michael A. Nitsche and is a member of the scientific advisory boards of Neuroelectrics and NeuroDevice. The other authors declare that they have no conflict of interest.

Ethical approval

The study was approved by the Ethics Committee of the University of Huelva (05/2014) and conforms to the principles of the World Medical Association Declaration of Helsinki.

Consent to participate

All the subjects signed informed consent to participate in the study and were informed of all the procedure.

Consent for publication

All the participants signed informed consent for the publication of research data, excluding personal identification data.

Supplementary Information

Below is the link to the electronic supplementary material. Supplementary file1 (DOCX 346 KB)

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