More focal is not always better: effects of conventional versus high-definition transcranial direct-current stimulation on implicit motor sequence learning

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Abstract

Conventional transcranial direct-current stimulation (tDCS) delivered to the primary motor cortex (M1) has been shown to enhance implicit motor sequence learning (IMSL). Conventional tDCS targets M1 but also the motor association cortices (MAC), making the precise contribution of M1 to IMSL presently unclear. We aimed to address the roles of these areas by comparing conventional tDCS of M1 and MAC to High-Definition (HD) tDCS, which more focally targets M1. In this sham-controlled, crossover study in 89 healthy adults, we used mixed-effects models to analyze sequence-specific and general learning effects in the acquisition, short- and long-term consolidation phases of IMSL, as measured by the serial reaction time task. Conventional tDCS did not influence general learning, improved sequence-specific learning during acquisition (anodal: M=42.64 ms, sham: M=32.87 ms, p=.041) and deteriorated it at long-term consolidation (anodal: M=75.37 ms, sham: M=86.63 ms, p=.019). HD tDCS did not influence general learning, slowed performance specifically in sequential blocks across all learning phases (all p's<.050), and consequently deteriorated sequence-specific learning during acquisition (anodal: M=24.13 ms, sham: M=35.67 ms, p=.014) and long-term consolidation (anodal: M=60.03 ms, sham: M=75.01 ms, p=.002). Our findings indicate that generalized stimulation of M1 and MAC enhanced acquisition, but hindered consolidation of IMSL. In contrast, focal M1 stimulation by HD tDCS worsened overall performance, likely due to cathodal inhibition of MAC as induced by the return electrodes. Consequently, this disruption of performance supports the notion that these areas fundamentally contribute to IMSL as an integral part in the cortico-basal ganglia-thalamo-cortical network.

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Neural mechanisms of sequence learning

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ABSTRACT

Conventional transcranial direct-current stimulation (tDCS) delivered to the primary motor cortex (M1) has been shown to enhance implicit motor sequence learning (IMSL). Conventional tDCS targets M1 but also the motor association cortices (MAC), making the precise contribution of M1 to IMSL presently unclear.

We aimed to address the roles of these areas by comparing conventional tDCS of M1 and MAC to High-Definition (HD) tDCS, which more focally targets M1. In this sham-controlled, crossover study in 89 healthy adults, we used mixed-effects models to analyze sequence-specific and general learning effects in the acquisition, short- and long-term consolidation phases of IMSL, as measured by the serial reaction time task.

Conventional tDCS did not influence general learning, improved sequence-specific learning during acquisition (anodal: M =42.64 ms, sham: M =32.87 ms, p= .041) and deteriorated it at long-term consolidation (anodal: M =75.37 ms, sham: M =86.63 ms, p= .019). HD tDCS did not influence general learning, slowed performance specifically in sequential blocks across all learning phases (all p 's<.050), and consequently deteriorated sequence-specific learning during acquisition (anodal: M =24.13 ms, sham: M =35.67 ms, p= .014) and long-term consolidation (anodal: M =60.03 ms, sham: M =75.01 ms, p= .002).

Our findings indicate that generalized stimulation of M1 and MAC enhanced acquisition, but hindered consolidation of IMSL. In contrast, focal M1 stimulation by HD tDCS worsened overall performance, likely due to cathodal inhibition of MAC as induced by the return electrodes. Consequently, this disruption of performance supports the notion that these areas fundamentally contribute to IMSL as an integral part in the cortico-basal ganglia-thalamo-cortical network.

Keywords : tDCS, primary motor cortex, premotor cortex, supplementary motor area, motor association cortex, implicit motor sequence learning

INTRODUCTION

Implicit motor sequence learning (IMSL) is defined as the ability to incidentally learn sequential actions , as expressed in many of our daily activities (e.g., reaching, typing, driving) . We determined whether IMSL can be improved by transcranial direct-current stimulation (tDCS), which entails the administration of a weak electrical current at the scalp, traditionally between two electrodes (one anode, one cathode). Although the exact mechanisms underlying tDCS are not entirely understood, it is commonly assumed that tDCS exerts its immediate effects by shifting the resting membrane potential of neurons .

Contemporaneous theoretical models distinguish three stages in IMSL: an*acquisition* phase, a *consolidation* phase, and a*retention* phase . Multiple studies and meta-analyses suggest a positive impact of anodal primary motor cortex (M1) tDCS, especially on the *consolidation* of sequential knowledge . This is consistent with the notion that M1 plays a key role in the early consolidation and retention of motor learning .

Recent evidence, however, disputes the notion that M1 is involved in any of these stages of learning, by revealing widespread learning-related activity changes in parietal and premotor areas . This is in line with recent studies documenting the role of the motor association cortex (MAC), comprising the premotor cortex and supplementary motor areas, in IMSL . Consequently, the precise contribution of M1 to IMSL remains presently unclear.

tDCS-IMSL studies almost exclusively investigated the effects of conventional tDCS (i.e., with one anode

and one cathode) delivered over M1, based on the premise that M1 is the most essential cortical area for this skill . Typically, rather large electrodes (generally 16 to 35 cm^2) were used . Therefore, MAC, adjacent to M1, was likely additionally included in the electric field, see Figure 1 (top). This raises the question of whether existing effects of M1 tDCS on IMSL can be attributed to a generalized stimulation of M1 *and* MAC, rather than a specific stimulation of M1 alone. More recent High-Definition (HD) tDCS devices allow for stimulation of the targeted cortical region (e.g., M1) with higher specificity, see Figure 1 (bottom).

In the present study, we compared conventional tDCS, targeting M1 and MAC, with HD tDCS, focally targeting M1, to investigate the precise contribution of M1 to IMSL. We assessed (IMSL) using the Serial Reaction Time (SRT) task which taps learning of a sequence of key presses in response to a sequence of visual targets . The effects of stimulation on SRT task performance were analyzed at three time points: (1) during acquisition, (2) five minutes after stimulation (short-term consolidation), and (3) one week after stimulation (long-term consolidation). Overall response times (RTs) decrease over SRT training (general learning effect, secondary outcome), but it is the observation that RTs increase when sequenced events are interrupted that specifically denotes the sequence-specific learning effect (primary outcome).

We hypothesized that conventional tDCS would improve both sequence-specific learning and general learning (but see for null findings), particularly in the long-term consolidation phase . Superior HD tDCS effects would support the notion that M1 contributes indispensably to IMSL, independently of MAC. Alternatively, superior conventional tDCS effects would suggest that positive effects are to be attributed to a generalized stimulation of M1 and adjacent MAC, rather than M1 alone.

<<< FIGURE 1 HERE >>>

MATERIALS AND METHODS

Study design

In this single-blind, sham-controlled, counterbalanced study, half of the participants received conventional M1 tDCS and the other half received HD M1 tDCS. All participants received both anodal (active) tDCS and sham (placebo) tDCS in a random order, with the order being concealed for participants. Ethical approval was obtained from the Medical Ethics Committee of the University Hospital Brussels (identifier: B1432020000011).

Participant recruitment and inclusion criteria

Ninety volunteers (18 to 35 years) were recruited through social media and flyers. Exclusion criteria were a history of self-reported neurological and/or recent musculoskeletal diseases and known contra-indications for tDCS (deep brain stimulator; pacemaker; head wound; skin condition of the scalp; a history of epilepsy).

Conventional transcranial direct-current stimulation

For the application of conventional tDCS, a 1x1 Low-Intensity Direct-Current Stimulator (Soterix Medical Inc, New York, USA) was used. Direct current was delivered through square rubber electrodes placed in rectangular saline-soaked sponges (size 35 cm^2).To stimulate M1, electrodes were placed over C3 or C4 according to the international 10-20 electroencephalogram (EEG) system, matching with M1 contralateral to the performing, dominant hand . An electrode angle orientation of 45° (i.e., with 45° deviation from the midsagittal plane) was used as this positioning induces superior neuroplastic effects of M1 due to a better alignment with the motor cortex . The reference electrode was positioned on the supraorbital area, ipsilateral to the dominant hand .

The current stimulation was gradually ramped up from 0 mA to 2 mA in 60 seconds. In the anodal tDCS condition, this intensity was maintained for the duration of the Serial Reaction Time (SRT) task (median duration +- 17 minutes; extracted from E-Prime), resulting in a current density of 0.057 mA/cm^2. After completion of the SRT task, the current was gradually ramped down again to 0 mA. Although electrode placement was identical for the sham tDCS condition, unbeknown to the participant, stimulation was gra-

dually decreased to 0 mA immediately after the one-minute ramp-up. This gradual ramping-up and -down was repeated after the completion of the task to optimize participant blinding.

High-definition transcranial direct-current stimulation

The Soterix 4x1 Multichannel Stimulation Adapter (Soterix Medical Inc, New York, USA) was used to deliver HD tDCS over M1. HD tDCS was delivered through five Ag-sintered ring electrodes placed in plastic encasings embedded in an EEG cap: one central anode and four return-electrodes (Figure 2, following). Everything else was identical to the conventional tDCS set-up. Computational modelling of the electric field strengths demonstrates that HD tDCS had a much higher focality of stimulation and higher peak electric stimulation in M1, particularly in the more superior M1 where the upper extremity is represented in the Penfield motor homunculus , see Figure 1.

<<< FIGURE 2 HERE >>>

Serial Reaction Time Task

The SRT task, identical to , was performed on laptops using E-Prime® software (Psychology Software Tools, Inc., Pittsburgh, PA, USA). The full-length SRT task consisted of eight blocks of 72 trials (sequential blocks 1-6 and 8, random block 7). A shortened version consisted of three blocks (sequential blocks 1 and 3, random block 2). There was an optional thirty-second break between consecutive blocks.

<<< FIGURE 3 HERE >>>

Experimental procedure

Following collection of baseline demographic characteristics (sex, age, dominant hand) during screening, all participants underwent six experimental sessions, see Figure 4. In Sessions 1 and 4, the full eight-block SRT task was carried out during the application of either anodal or sham tDCS (*acquisition phase*). In Sessions 2 and 5, five minutes post-tDCS, participants carried out the short three-block SRT task without the application of tDCS (*short-term consolidation phase*). Sessions 3 and 6 were planned one week after Session 1 and 4, respectively, and consisted of the same full eight-block SRT task from one week earlier – this time without the application of tDCS (*long-term consolidation phase*). A washout period of at least three weeks was included to control for carry-over effects between the two tDCS conditions (anodal/sham) 11Additionally, to control for possible carry-over of sequential knowledge, the SRT-task followed a different sequence in each stimulation condition (e.g. 132342134142 for anodal versus 243413241213 for sham).. After cross-over, the experimental procedure was repeated with the opposite stimulation condition. Immediately after Session 6, participants completed a post-SRT-task questionnaire to determine their awareness of the sequential nature of the task .

<<< FIGURE 4 HERE >>>

Outcome measures and statistical analyses

All statistical analyses were carried out using IBM SPSS Statistics (version 28) and R (version 4.2.1). The *lme4* package (version 1.1-30) for R was used to fit generalized linear mixed-effect models (GLMM) and the *car* package was used to conduct type III Wald tests for the fitted GLMM models . Post-hoc tests for significant interaction effects were carried out using the *emmeans* package . The *ggplot2* package was used for the graphical representation of significant interaction effects . Level of significance was set at $\alpha = 0.05$. Appropriate corrections for multiple comparisons were made.

Baseline demographical variables

Pearson correlation analyses, Bonferroni-corrected for multiple comparisons, were performed to investigate if IMSL correlated with demographical variables: sex, age, education level, and dominant hand. If assumptions for parametrical testing were violated, the non-parametric alternative Spearman's Rho was calculated.

Response times

Analyses of the SRT task performance were based on response times (RTs). Practice trials (16.72% of all data), RTs shorter than 100 ms (0.71%), RTs longer than 2000 ms (0.05%), and erroneous responses (3.00%) were excluded from the RT analyses. A series of GLMM were fitted to the RTs of each trial , as this approach does not require data averaging or discarding of data in case of subject attrition .

For the analysis of sequence-specific learning effects (primary outcome), a series of GLMMs with an inverse Gaussian family and identity link function were fitted to the RTs of each trial . Only random blocks and their adjacent sequential blocks were included to circumvent convergence issues. *Group* (conventional, HD), *Stimulationcondition* (anodal, sham), *Session* (during tDCS, five minutes post tDCS, one week post tDCS) and *Block type*(sequential, random) were included as fixed factors. Interactions between these factors were included as predictors, random intercepts for*Participants* were added to account for between-subject variance in RTs and a maximal random effects structure was included regarding the slopes of the predictive variables .

To evaluate general learning effects (secondary outcome), a series of GLMMs were fitted to the RTs of each trial in the seven sequential blocks (i.e., Blocks 1 through 6 and Block 8) of the full-length SRT tasks (not applicable to the shortened SRT task as it consisted of three blocks only). *Group* (conventional, HD), *Stimulationcondition* (anodal, sham), *Session* (during tDCS, one-week post tDCS) and the second degree (i.e., quadratic and linear) polynomials of *Block* (1, 2, 3, 4, 5, 6, 8) were included as fixed factors. Interactions between these factors and random intercepts for*Participants* were again added to the models.

Accuracy

Response accuracy is much less sensitive to IMSL, as accuracy on the SRT task is generally very high and errors are likely to reflect motor rather than predictive errors . However, for the sake of comprehensiveness, a series of generalized linear mixed-effects models (GLMM) were fitted to the accuracy of each trial. As this dependent variable is a dichotomous one (false/correct), binomial logistic regressions were employed. In all other respects, these analyses were conducted analogously to the RT analyses.

RESULTS

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4. **Participants**

Table 1 *Baseline demographical variables and sequential reproduction score*

Variable	Group Conventional (n = 45)	Group HD (n = 44)	*p*
Sex (male:female)	19:26	22:22	.462
Age (years)	23.02 (± 1.631)	23.36 (± 3.005)	.506
Level of education* (1:2:3:4)	14:2:27:2	16:3:17:8	.102
Dominant hand (L:R)	7:38	8:36	.741
Subjective sequential awareness (Y/N/NA)	31:9:5	19:9:16	.375
Reproduction score	4.82 (± 2.926)	4.33 (± 1.033)	.704

Abbreviations. HD High-Definition; *L* Left; *R* Right; *Y* Yes; *N* No; *NA* Not Answered. * Level of education: based on an ordinal four-point scale: (1) lower secondary education; (2) professional (college) bachelor's degree; (3) academic (university) bachelor's degree; (4) academic (university) master's degree.	*Abbreviations. HD* High-Definition; *L* Left; *R* Right; *Y* Yes; *N* No; *NA* Not Answered. * Level of education: based on an ordinal four-point scale: (1) lower secondary education; (2) professional (college) bachelor's degree; (3) academic (university) bachelor's degree; (4) academic (university) master's degree.	*Abbreviations. HD* High-Definition; *L* Left; *R* Right; *Y* Yes; *N* No; *NA* Not Answered. * Level of education: based on an ordinal four-point scale: (1) lower secondary education; (2) professional (college) bachelor's degree; (3) academic (university) bachelor's degree; (4) academic (university) master's degree.	*Abbreviations. HD* High-Definition; *L* Left; *R* Right; *Y* Yes; *N* No; *NA* Not Answered. * Level of education: based on an ordinal four-point scale: (1) lower secondary education; (2) professional (college) bachelor's degree; (3) academic (university) bachelor's degree; (4) academic (university) master's degree.

Of the initial 90 participants, sixty-six (74.16%) completed all sessions. Except for one subject (who erroneously performed the SRT task with four fingers instead of the index finger), no data was discarded due to subject attrition as this is not required when using LMMs 11Three participants (3.37%) completed only half of the experimental procedure, four (4.49%) completed four out of six sessions and 16 participants (17.98%) missed only one session.. Baseline demographical characteristics and sequential awareness/reproduction scores for the remaining 89 participants are summarized in Table 1.

Response times

Sequence-specific learning effects

For the analysis of sequence-specific learning effects (primary outcome), the best-fitting model included interactions between the predictors *Group* (conventional, HD), *Stimulation condition*(anodal, sham)*, Session* (during tDCS, five minutes post tDCS, one week post tDCS)*,* and *Block type (sequential, random)* as fixed factors, and an intercept and slopes for *Stimulation condition, Session,* and their interaction within*Participants* as the maximally supported random effects structure.

The three-way interaction between *Group* , *Stimulation condition* , and *Block type* was significant (X^2 (1, N = 89) = 10.10, p = .001) and revealed significantly smaller sequence-specific learning effects following anodal tDCS compared to sham in the HD group, $p<$ .0001, but not in the conventional group, p = .285 – regardless of *Session* . We also found a significant three-way interaction between *Stimulation condition* , *Session* and*Block type* (X^2 (2, N = 89) = 11.09, p = .004), showing no significant difference in sequence-specific learning between anodal and sham during acquisition,p = .793, but significantly smaller effects at short-term and long-term consolidation – regardless of *Group* . However, the significant four-way interaction between *Group* , *Stimulation condition* , *Session* and *Block type* was also significant (X^2 (2, N = 89) = 6.53, p = .038). Contrasts revealed that conventional tDCS resulted in significantly larger sequence-specific learning effects during acquisition (anodal: M = 42.64 ms, sham: M = 32.87 ms,p = .041), but smaller effects at long-term consolidation (anodal: M = 75.37 ms, sham: M = 86.63 ms, p =.019). Conversely, HD tDCS negatively impacted sequence-specific learning *both* during acquisition (anodal: M = 24.13 ms, sham: M = 35.67 ms, p = .014) *and* during long-term consolidation (anodal: M = 60.03 ms, sham: M = 75.01 ms,p = .002). Interestingly, HD tDCS resulted in significantly slower RTs in sequential blocks across all learning phases, all$p$' s < .050, and in the random block at short-term consolidation, p = .026. See Figure 5 for a visualization of sequence-specific learning effects.

To summarize, conventional tDCS significantly improved sequence-specific learning during acquisition and deteriorated it during long-term consolidation. HD tDCS significantly deteriorated sequence-specific learning during acquisition and long-term consolidation, and slowed RTs in sequential blocks across all learning phases.

11To determine the effects of explicit awareness, we followed the same procedure as in . The analyses showed that our findings were not modulated by explicit awareness.

<<< FIGURE 5 HERE >>>

General learning effects

For the analysis of general learning effects (secondary outcome), the best-fitting model included interactions between the predictors*Group* (conventional, HD)*, Stimulation Condition* (anodal, sham)*, Session* (during tDCS, one week post tDCS) and quadratic polynomials of *Block* (1,2,3,4,5,6,8) as fixed factors, an intercept and slopes for *Stimulation condition, Session,* and their interaction within *Participants* as the maximally supported random effects structure.

The interaction effect of *Group* and *Stimulation condition*bordered significance (X^2 (2, N = 89) = 3.83, p= .050), indicating a significant negative impact on RTs following HD tDCS (anodal: M = 390.40 ms, SE = 3.62; sham: M = 376.20 ms, SE = 3.24; p = .001), but no influence of conventional tDCS (anodal: M = 402.06 ms, SE = 3.30; sham: M = 401.65 ms, SE = 3.12; p = .999). The four-way interaction between *Group* ,*Stimulation condition* , *Session,* and polynomials of*Block* was significant (X^2 (2, N = 89) = 6.87, p = .032). Follow-up contrasts revealed that, for each group and in each condition, smaller general learning effects occurred at long-term consolidation compared to acquisition, all$p$'s < .050. However, these smaller effects can be attributed to overall faster RTs during consolidation for each group, as demonstrated by the significant interaction between *Group* and*Session* (X^2 (2, N = 89) = 8.61, p = .003). For each group, no significant differences occurred between stimulation conditions in either of the learning phases, all p 's > .100. See Figure 6 for a visualization of general learning effects.

In sum, whereas both conventional and HD tDCS left general learning unaffected compared to sham, HD tDCS significantly slowed overall performance.

<<< FIGURE 6 HERE >>>

Accuracy

Participants in the conventional tDCS group responded with 96.7% accuracy in the anodal condition and 97.1% accuracy in the sham condition. Those in the HD group responded with 97.1% and 96.9% accuracy in the anodal and sham conditions, respectively. Similar analyses as mentioned above for the RT data yielded no noteworthy differences, and implied that no speed-accuracy trade-off occurred.

DISCUSSION

We aimed to unravel whether existing effects of conventional transcranial direct-current stimulation (tDCS) delivered over the primary motor cortex (M1) can be attributed to M1 or rather a generalized stimulation of M1 and its adjacent motor association cortices (MAC). To this end, we compared the effects of conventional tDCS, targeting M1 and MAC, and High-Definition (HD) tDCS, focally targeting M1, on the acquisition (during tDCS), short-term (five minutes post-tDCS), and long-term (one-week post-tDCS) consolidation of implicit motor sequence learning (IMSL).

Conventional anodal tDCS did not affect general learning (secondary outcome) but induced larger sequence-specific learning effects (primary outcome) during acquisition, compared to sham. This is in line with previous studies reporting positive effects of anodal tDCS on sequential learning in healthy adults , but see for null-findings. However, contrary to our hypothesis, smaller sequence-specific learning effects emerged in the long-term consolidation phase (i.e. one week post anodal tDCS) compared to sham. These weakened sequence-specific effects at one week post conventional tDCS are in contrast with various studies in healthy adults and one study from our own lab in individuals with Parkinson's disease , in which anodal tDCS significantly improved IMSL, particularly in the consolidation phase. While we lack the statistical power to substantiate this, RTs in the random block were lower in the consolidation phases following anodal conventional tDCS, compared to sham. Further research is warranted to explore whether this seemingly negative impact on long-term consolidation of sequential knowledge can be attributed to more flexible responses to random

stimuli following anodal tDCS. For instance, a simultaneous tDCS-EEG study could reveal whether the P1 event-related potential (ERP) component, which is known to decrease following predictable events and increase following random events, is significantly impacted by anodal tDCS .

In sum, conventional tDCS did not affect general learning, improved sequence-specific learning during acquisition, but deteriorated consolidation of sequential knowledge compared to sham.

Regarding HD tDCS, anodal stimulation did not affect general learning (secondary outcome), but negatively impacted sequence-specific learning (primary outcome) both during acquisition and at long-term consolidation. Additionally, anodal HD tDCS significantly slowed RTs across all learning phases – particularly in sequential blocks – compared to sham. In line with the only previous study investigating effects of specific M1 stimulation by means of HD tDCS on IMSL , our results showed that HD tDCS worsened SRT-performance.

The particularly negative impact of HD tDCS is counter-intuitive given the notion that excitation of the M1, a cortical region that is traditionally deemed crucial for IMSL , should improve this skill. One could argue that anodal HD tDCS resulted in hyper-excitation of M1, therefore disrupting IMSL. However, this does not seem likely as electric field simulations (Figure 1) show higher stimulation focality of HD tDCS, but comparable stimulation intensities between conventional and HD montages. A more plausible explanation for the negative effect of HD tDCS lies in inhibition of surrounding cortical areas that are also heavily involved in IMSL. The 4x1 HD tDCS configuration used in the current study required the placement of cathodes over central, frontal, parietal and temporal brain regions . The MAC, comprising the premotor cortex and supplementary motor area, are considered an integral part of the cortico-basal ganglia-thalamo-cortical network, a critical neural circuit underlying IMSL . In this network, the presupplementary motor area activates during acquisition of new sequences, but not during performance of learned sequences ; the premotor cortex is presumed to store a representation of learned sequences along with M1 , whereas the site of activation shifts to the supplementary motor area with extensive experience . In the current study, the simultaneous cathodal inhibition of MAC and anodal excitation of M1 via HD tDCS likely disrupted the neural circuits involved in IMSL.

Overall, our findings are in line with the notion of MAC being a driving part of the cortico-basal ganglia-thalamo-cortical network in IMSL . Further studies are necessary to determine the specific contributions of MAC and M1 to IMSL. This issue is unlikely to be addressed through tDCS alone due to the existence of cortico-cortical connections between these areas. For instance, it was previously shown that HD stimulation of the premotor cortex also results in excitability changes in M1 . Direct comparison of HD premotor cortex versus HD M1 tDCS would therefore be tremendously challenging. In this regard, simultaneous tDCS-fMRI research could provide more clarity on the differential influences of these cortical areas on IMSL. Furthermore, our study suggests that future tDCS interventions in clinical populations should target MAC – at least in addition to M1 – to enhance IMSL, particularly in individuals with impaired IMSL, such as those with Parkinson's disease .

CONCLUSION

Our findings indicate that generalized anodal stimulation of M1 and MAC via conventional tDCS enhanced acquisition, but hindered consolidation of sequence-specific knowledge compared to sham. Our HD M1 tDCS montage had an overall negative impact on IMSL, likely due to cathodal inhibition of MAC and consequent disruption of the neural circuits involved in IMSL. These results further support the notion that MAC plays an integral part in the cortico-basal ganglia-thalamo-cortical network that underlies IMSL.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

AUTHOR CONTRIBUTIONS

M.F.: Conceptualization, Methodology, Formal analysis, Investigation, Visualization, Writing - original draft, Writing - review & editing, Funding acquisition

K.B.: Conceptualization, Methodology, Formal analysis, Writing - original draft, Writing - review & editing, Resources, Supervision, Project administration, Funding acquisition

M.S.: Investigation, Formal analysis, Writing - original draft, Writing - review & editing

C.D.: Investigation, Formal analysis, Writing - review & editing

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F.V.O.: Conceptualization, Writing - review & editing, Resources, Supervision, Project administration, Funding acquisition

E.S.: Conceptualization, Writing - review & editing, Resources, Supervision, Project administration, Funding acquisition

N.D.: Conceptualization, Methodology, Formal analysis, Writing - original draft, Writing - review & editing, Resources, Supervision, Project administration, Funding acquisition

DATA ACCESSIBILITY STATEMENT

The data that support the findings of this study will be available on a public repository.

ABBREVIATIONS

EEG: electroencephalogram

ERP: event-related potential

GLMM: generalized linear mixed-effects models

HD tDCS: high-definition transcranial direct current stimulation

IMSL: implicit motor sequence learning

M1: primary motor cortex

MAC: motor association cortices

RTs: response times

SRT: serial reaction time

1. **REFERENCES**
2. **FIGURE CAPTIONS**
3. Figure 1

Title: Simulation of normal component of field strength (V/m)

Caption: Note. Simulation of normal component of field strength (V/m) using a 1x1 conventional C3/Fp2 (top) and 4x1 HD C3/Cz/F3/T7/P3 (bottom) montage on a standard MNI 152 head. Focality entails the area of the SimNibs mesh with field norm > (peak field)/2; smaller surfaces indicate more focal stimulation. Simulation conducted with SimNIBS (Saturnino et al., 2015).

Figure 2

Title: Positioning of HD tDCS electrodes

Caption: Note. Suggested positioning for HD electrodes based on the 10-20 EEG system, for left M1 stimulation. The contralateral analogs were used for the right M1. Based on Villamar et al. (2013).

Figure 3

Title: Schematic representation of the SRT task

Caption: Note. [Left] Schematic representation of the SRT task. [Right] The response keys C, V, B, and N were the only visible keys, all other keys were covered. Taken from Firouzi et al. (2021).

Figure 4

Title: Schematic representation of the experimental design

Caption: Note. Representation of the sham-controlled, counter-balanced experimental design.

Figure 5

Title: Sequence-specific learning effects in the Conventional and HD M1 tDCS groups

Caption: Note. Interaction effects between the factors Group, Stimulation condition, Block type, and Session. Green asterisks indicate significant interactions.

Figure 6

Title: General learning effects in the Conventional and HD M1 tDCS groups

Caption: Note. Interaction effects between the factors Group, Stimulation condition, Session and Block. Although anodal HD tDCS significantly slowed overall RTs compared to sham, there was no significant influence of stimulation condition on general learning effects (secondary outcome).

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