Neuromodulation: Technology at the Neural Interface

Received: February 21, 2024 Revised: May 14, 2024 Accepted: May 15, 2024

https://doi.org/10.1016/j.neurom.2024.05.002

Investigating the Working Mechanism of Transcranial Direct Current Stimulation

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ABSTRACT

Background: Transcranial direct current stimulation (tDCS) is used to modulate neuronal activity, but the exact mechanism of action (MOA) is unclear. This study investigates tDCS-induced modulation of the corticospinal excitability and the underlying MOA. By anesthetizing the scalp before applying tDCS and by stimulating the cheeks, we investigated whether stimulation of peripheral and/or cranial nerves contributes to the effects of tDCS on corticospinal excitability.

Materials and Methods: In a randomized cross-over study, four experimental conditions with anodal direct current stimulation were compared in 19 healthy volunteers: 1) tDCS over the motor cortex (tDCS-MI), 2) tDCS over the motor cortex with a locally applied topical anesthetic (TA) on the scalp (tDCS-MI + TA), 3) DCS over the cheek region (DCS-C), and 4) sham tDCS over the motor cortex(sham). tDCS was applied for 20 minutes at 1 mA. Motor evoked potentials (MEPs) were measured before tDCS and immediately, 15, 30, 45, and 60 minutes after tDCS. A questionnaire was used to assess the tolerability of tDCS.

Results: A significant MEP amplitude increase compared with baseline was found 30 minutes after tDCS-MI, an effect still observed 60 minutes later; no time*condition interaction effect was detected. In the other three conditions (tDCS-MI + TA, DCS-C, sham), no significant MEP modulation was found. The questionnaire indicated that side effects are significantly lower when the local anesthetic was applied before stimulation than in the other three conditions.

Conclusions: The significant MEP amplitude increase observed from 30 minutes on after tDCS-MI supports the modulatory effect of tDCS on corticospinal neurotransmission. This effect lasted one hour after stimulation. The absence of a significant modulation when a local anesthetic was applied suggests that effects of tDCS are not solely established through direct cortical stimulation but that stimulation of peripheral and/or cranial nerves also might contribute to tDCS-induced modulation.

Keywords: Mechanism of action, motor evoked potentials, neuromodulation, transcranial direct current stimulation, transcranial magnetic stimulation

INTRODUCTION

Transcranial direct current stimulation (tDCS) is a form of transcranial electrical stimulation (tES) in which a unidirectional constant electrical current is noninvasively delivered through a minimum of two electrodes placed on the scalp with the aim of modulating brain activity.[1] Anodal tDCS has been reported to improve cognitive abilities and other symptoms of various neuropsychiatric disorders such as drug addiction, schizophrenia, and major depressive disorder.[2–5] However, the reported outcomes of tDCS studies are highly variable, and full clinical applicability is hampered by the lack of knowledge on the mechanism of action (MOA).

tDCS-induced modulation of corticospinal excitability has been investigated through the recording of motor evoked potentials (MEPs) elicited by transcranial magnetic stimulation (TMS) before

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Source(s) of financial support: Evelien Carrette is supported by a research grant from the Geneeskundige Stichting Koningin Elisabeth. Robrecht Raedt is supported by grants of the FWO (Fonds Wetenschappelijk Onderzoek) Flanders and Bijzonder Onderzoeks Fonds (BOF)–UGent special research fund. Kristl Vonck has been funded by the BOF-UGent special research fund. Paul Boon is supported by grants of the FWO Flanders, BOF-UGent, Ghent University Hospital, and E-Epilepsy (European Union). The study was part of a collaboration project entitled "PerStim," which is cofunded by the Dutch PPP Allowance made available by Health Holland, Top Sector Life Sciences & Health to EpilepsieNL to stimulate public-private partnerships.

and after tDCS. In the "transcranial mechanism" hypothesis, the weak electrical field delivered through tDCS is believed to lead to a shift in the neuronal resting membrane potential, leading to alterations in the action potential dynamics.[6,7] The direction of this shift depends, among others, on the electrode montage. Although somewhat oversimplified, the conventional perspective poses that anodal tDCS induces depolarization of the membrane potential, leading to an increased corticospinal excitability and higher MEP amplitude. Conversely, cathodal stimulation is believed to produce contrasting effects.[8,9] Opposite effects have been shown, and anodal tDCS also can lead to hyperpolarization depending on the applied stimulation intensity and duration.[10]

It remains heavily debated whether the electrical current intensity that can be safely applied to the human scalp and that is well tolerated is sufficient to induce biologically relevant membrane potential changes in the brain cortex, considering the attenuation due to the presence of the skull and shunting through soft tissues.[11] A human cadaver brain study showed that a mere 25% of scalp-applied currents actually reach the brain, an amount that was unable to achieve significant changes in transmembrane potential and spiking rate.[11]

Several alternative, more indirect working mechanisms have been proposed.[1,7,12] The "transcutaneous mechanism" hypothesis proposes tDCS-induced effects on cutaneous peripheral or cranial nerve fibers that run in and through the scalp and skull in the vicinity of the applied current.[7] Depending on the location of the tDCS electrodes, several cranial and peripheral nerves can be affected, such as the occipital nerve, branches of the trigeminal nerve, and the cervical plexus.[13] Afferent fibers from these nerves travel to the brainstem, where they project to the nucleus of the solitary tract (NTS) and trigeminal nuclei.[14] Neurotransmission is integrated in the brainstem reticular formation, which is a brainstem neuronal network comprising clusters of nuclei including the locus coeruleus (LC). Modulation of the LC leads to an increase in noradrenaline release.[14,15] Noradrenaline can increase cortical excitability through excitatory effects of β-adrenoreceptors.[16] Noradrenaline also drives synaptic plasticity by induction of long-term potentiation leading to activation of voltage-gated ion channels. TMS depolarizes the axonal terminals of pyramidal cells and inhibitory interneurons in the cortex and induces a discharge of synchronous action potentials that travel through the corticospinal tract, causing activation of lower motor neurons innervating specific muscles, generating an MEP.[17] The MEP amplitude reflects neuronal membrane excitability and depends primarily on ion channel activity. MEP amplitude is affected by voltage-gated sodium channel blockers.[18] Therefore, indirect activation of the LC could lead to an increase in MEP amplitude.

A similar MOA has been revealed for other nerve stimulation techniques that show effects on cortical excitability and cognitive functions such as vagus nerve stimulation.[19] Other potential working mechanisms of tDCS are effects on blood vessels, cortical astrocytes, and placebo effects.[20,21] These potential alternative mechanisms have gradually gained more interest, but they have been poorly investigated in mechanistic studies or controlled for in experimental designs.

The goal of this study was to further elucidate the MOA of anodal tDCS and investigate the potential contribution of peripheral and/or cranial nerve stimulation. An experimental design in which four conditions (cfr infra) were tested within subjects allowed disentangling of the transcranial and transcutaneous mechanism and is therefore of great added value to the ongoing debate regarding the MOA of tDCS. As a read-out parameter, we measured the peak-to-peak amplitude and onset latency of TMS-evoked MEPs, which has been shown to be the most reliable neurophysiological parameter to investigate tDCS effects.[22,23] We hypothesized that the MOA of tDCS is not solely dependent on a transcranial direct cortical effect but that transcutaneous stimulation of peripheral/cranial nerves also might contribute to its net effects. We estimated to find a smaller effect of anodal tDCS on the MEP amplitude when eliminating the transcutaneous mechanisms using a topical anesthetic.

The findings of this study contribute to the ongoing debate on the exact MOA of tDCS and thereby provide valuable implications regarding future tDCS research. These findings show the importance of adding control conditions for potential nontranscranial mechanisms in an experimental design, which will lead to a better understanding of the driving factor in tDCS-induced effects. Moreover, the results can serve as a guide to optimize electrode montages to make maximal use of one or both mechanisms in clinical trials.

MATERIAL AND METHODS

Participants

Nineteen healthy subjects participated in this study (11 women, mean age ± SD: 25.4 ± 4.3 years). This sample size was based on previous research[12] and on an a priori power calculation assuming a moderate effect size, a power of 0.8, and significance level of 0.05. All participants gave written informed consent before the experiment and met the inclusion criteria (age 18–45 years, right-handed, no neurologic or psychiatric disorder; the Supplementary Data list all in- and exclusion criteria). A screening visit was planned during which participants completed a tDCS and TMS safety screening questionnaire and underwent a clinical neurologic exam.[24–26] All subjects were informed about the potential (side) effects of tDCS. The study protocol was approved by the medical ethics review board of Ghent University. All experimental procedures were conducted in accordance with the Declaration of Helsinki.

Experimental Design

In this single-blind, randomized, cross-over controlled trial, each subject underwent four experimental sessions. Testing was performed at the same time of the day to minimize circadian effects, and sessions were separated by ≥ one week to prevent carry-over effects. Participants were seated reclined in a comfortable chair with their hands supine on their laps and were asked to remain silent during the measurements to avoid speech-induced modulation of brain excitability. During each experimental session, anodal direct current stimulation (DCS) was delivered in one of the following conditions to the subjects in a randomized order. One condition comprised the transcranial delivery of DCS over the motor cortex (MI) region (tDCS-MI; Supplementary Data Fig. S1 presents an electrical field modeling of this montage). Another condition comprised anodal DCS over the MI region in combination with prior application of a topical anesthetic (TA) ointment comprising lidocaïne 2.5% + prilocaïne 2.5% (EMLA, Aspen, London, UK) and covering both electrode sites on the head to temporarily block peripheral/cranial nerve conduction (tDCS-MI + TA). A third condition comprised DCS over the cheek region with the aim of affecting cutaneous nerve endings of the peripheral/cranial nerves (eg, the trigeminal nerve) in the vicinity of the electrode, while avoiding the induction of transcranial effects (DCS-C; the

Supplementary Data present an electrical field modeling of this montage). A final condition comprised sham DCS, in which only in the first 15 seconds an electrical current was delivered (sham). A figurative overview of the four different conditions is presented in Figure 1.

Each session started with a preparation. First, the position of the head of the participant in addition to the TMS coil was registered within the neuronavigation system for frameless stereotaxy (Localite, Bonn, Germany). This allowed online tracking of the TMS coil relative to the head in space and ensured consistent coil positioning throughout the entire session. TMS was applied with the MagPro X100 TMS device and a statically cooled figure-of-eight coil (MagVenture, Farum, Denmark) to determine the left-sided motor hotspot for the right first dorsal interosseus (FDI) muscle. The location of the optimal stimulation site was determined by systematically delivering single TMS pulses over the left MI until the location that elicited the largest peak-to-peak MEP amplitude was detected. The coil was held tangentially to the scalp with the handle pointing backward at 45° from the midline. MEPs were recorded using Synergy EMG software (Natus, Middleton, WI) using two adhesive Ag/AgCl electrodes (Natus) overlying the left FDI muscle, and a ground electrode placed over the styloid process of the ulna. The resting motor threshold (rMT) (ie, the minimum stimulus intensity to elicit MEPs ≥50μV in exactly 5/10 consecutive trials, which was an iterative process starting from 50% maximum stimulator output and increasing with 3% until the threshold was reached) and TMS stimulation intensity (ie, the intensity required for eliciting consecutive MEPs with an average peak-to-peak amplitude of 1mV) were determined; this was performed at the beginning of each session. The obtained TMS intensity was used throughout the entire session. Depending on the condition, a carbomer gel or TA was applied, covering the area where the tDCS sponge electrodes were later to be placed (5.5 × 5.5 cm; 5 g/site). In the tDCS-MI + TA condition, the TA ointment was applied over the areas to be covered by the electrodes attached to the head, whereas a carbomer gel was applied to the areas to be covered by the electrodes over the cheeks. In all three other conditions, only carbomer gel was applied to all four electrode sites. The carbomer gel contained the same components as the TA ointment, excluding the active substances. The ointment was applied for 45 minutes, after which it was removed, and the skin was dried to proceed with the baseline recording. A maximal anesthetizing effect is obtained after 30 to 60 minutes of application. The 45 minutes application duration is sufficient to obtain an anesthetizing effect of approximately 2 hours.[27]

One baseline MEP recording containing 25 single TMS pulses was performed. This was immediately followed by the application of four saline-soaked sponge-electrodes (5 × 5 cm; Soterix Medical Inc, Woodbridge Township, NJ): one anode over the left motor hotspot, which was determined during the preparatory part using TMS, one cathode over the right supraorbital area, one anode over the left cheek, and one cathode over the right cheek (Fig. 1). All four sponge electrodes with attached wires were placed on the head to assure consistency across conditions. Only one pair of electrodes was connected to the tDCS stimulator (Soterix Medical Inc) to deliver active or sham tDCS either to the cheeks or over the MI. This depended on the condition (Fig. 1) and was invisible to the participants for blinding purposes.

tDCS was administered for 20 minutes at a stimulation intensity of 1 mA, with the stimulation intensity gradually increasing to this value during a ramp-up period (15 seconds) at the beginning of stimulation and gradually decreasing during a ramp-down period (15 seconds) at the end of stimulation. For the sham condition, the stimulation intensity was gradually increased to the maximum intensity (1 mA) with a ramp up of 15 seconds, after which it started to gradually decrease to 0 mA with a ramp down of 15 seconds. Participants experienced a similar tingling sensation at the beginning of the stimulation session in both conditions.

A postintervention MEP recording was performed immediately after removing the tDCS electrodes (time point 0) and 15, 30, 45, and 60 minutes after stimulation. At the end of each session, participants were asked to fill in an international standardized questionnaire concerning the tolerability of tDCS and potential side effects.[26]

Figure 2 presents an overview of the study protocol of one experimental session.

At the end of the experiment, once the last session was completed, the participants were asked to indicate per session whether they received real or placebo tDCS, or whether they had no idea.

Analysis and Statistics

For each participant, the peak-to-peak amplitudes and onset latencies of 25 single-pulse MEPs were calculated and averaged for each time point (baseline, 0, 15, 30, 45, and 60 minutes post-tDCS). We compared changes in MEP amplitude and latency recorded from the FDI muscle across the various conditions (tDCS-MI, tDCS-MI + TA, DCS-C, sham) using a two-way repeated measures analysis of variance (rmANOVA) with time (six levels) and condition (four levels) as within-subject factors. The Mauchly test was used to

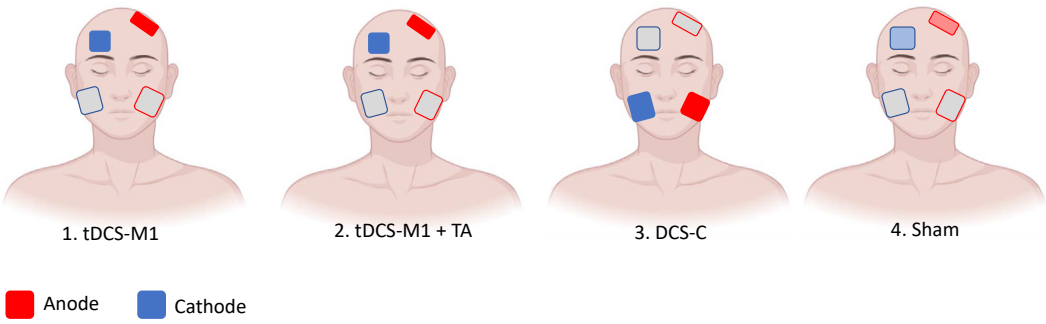

Figure 1. A schematic overview of the four different conditions. The fully colored electrodes depict electrodes used for active tDCS (red = anode; blue = cathode). The gray electrodes depict inactive electrodes, which were not connected to the tDCS stimulator.

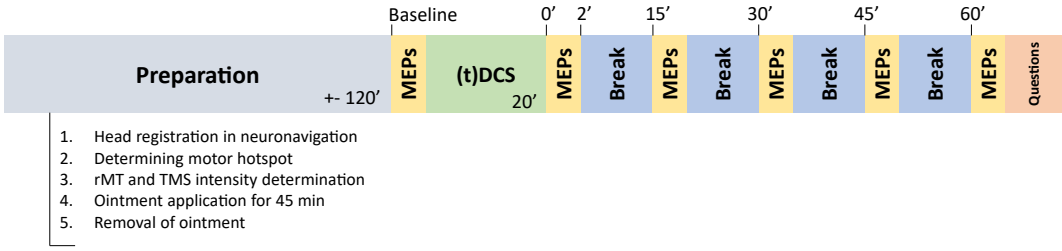

Figure 2. Schematic overview of one experimental session.

assess the validity of the sphericity assumption for rmANOVA, requiring equal variances for each set of difference scores. Greenhouse-Geisser–corrected significance values were used when sphericity was violated. In case of significant main effects, post hoc comparisons were performed using the least significant difference (LSD) adjustment for multiple comparisons.

To evaluate the response rate in all active tDCS conditions (tDCS-MI, tDCS-MI + TA, and DCS-C), three categories were classified: increase, no change, and decrease, in which the SD of the post sham measurements averaged across individuals ($1\sigma = 0.37$) served as the Sham Variability-Based Threshold. Per participant, sessions with a mean MEP ratio (averaged over postmeasurements) >1.37 were classified as "increase;" sessions with values between 0.63 and less than a ratio <1.37 were considered as the "no change" group, and sessions with values <0.63 were classified as "decrease."[28]

The results of the questionnaire for the tolerability of tDCS were categorized regarding intensity and duration. The reported intensity (mild, moderate, severe) and duration (1/3, 2/3, all the time) of each local side effect (itching, tingling, burning, pain, and warmth) were summed per participant to calculate the total intensity and duration score. A summary score was calculated reflecting the total impact of the side effects, considering both intensity and duration. Differences among conditions were analyzed using a nonparametric Friedman test. In case of significant results, a post hoc Wilcoxon rank test was performed. The correlation between the reported side effects and the MEP changes were analyzed using a Spearman and Pearson correlation test.

We considered the results of all statistical analyses significant at $p < 0.05$. Statistical analyses were performed using IBM SPSS (Statistics version 20.0, IBM, Armonk, NY).

RESULTS

Effects of Condition and Time on Corticospinal Excitability

A significant main effect of time on the peak-to-peak MEP amplitude ($F = 3.749$; $p = 0.004$) was found, but no significant difference among conditions was detected ($F = 2.307$; $p = 0.087$) (Fig. 3). No interaction between time and condition ($F = 0.724$; $p = 0.760$) was found. Post hoc tests were analyzed in an exploratory manner. The within-subject contrasts revealed a significant effect for time ($F = 6.993$; $p = 0.016$) and for condition ($F = 4.582$; $p = 0.046$), but no interaction effect ($F = 1.206$; $p = 0.287$). The pairwise comparisons (LSD corrected) showed that in the tDCS-MI condition, compared with baseline, the peak-to-peak MEP amplitude was significantly increased at 30, 45, and 60 minutes after tDCS (30 minutes: $p = 0.028$, 45 minutes: $p = 0.013$, 60 minutes: $p = 0.047$). At 30 minutes after stimulation, a significant difference was found between tDCS-MI and sham ($p = 0.020$) and between tDCS-MI and DCS-C ($p = 0.029$) (Fig. 3).

The effect of tDCS on the MEP onset latency across conditions was assessed. A two-way rmANOVA showed no significant main effect for time ($F = 1.90$; $p = 0.102$), condition ($F = 0.606$; $p = 0.614$), or for the interaction between time and condition ($F = 0.889$; $p = 0.577$).

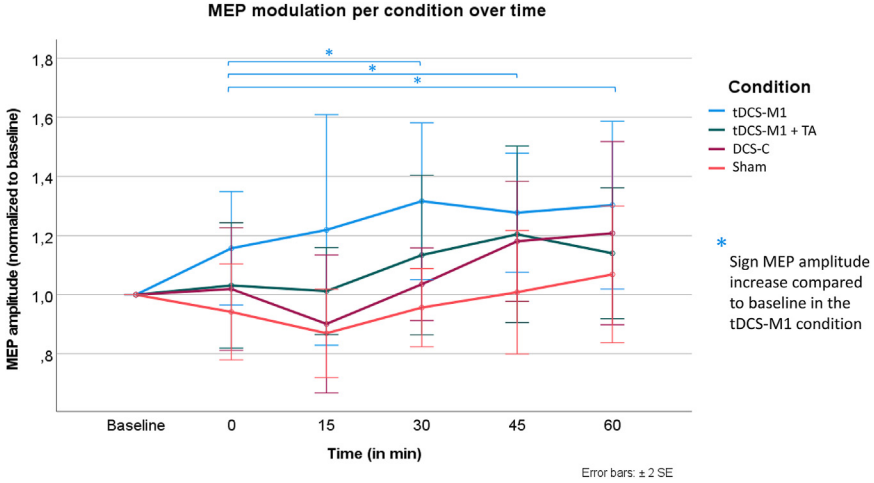

Figure 3. Changes in peak-to-peak MEP amplitude per condition over time. An increase in MEP amplitude was found in the tDCS-MI condition, with significance reached at 30, 45, and 60 minutes after stimulation. Error bars ± standard error.

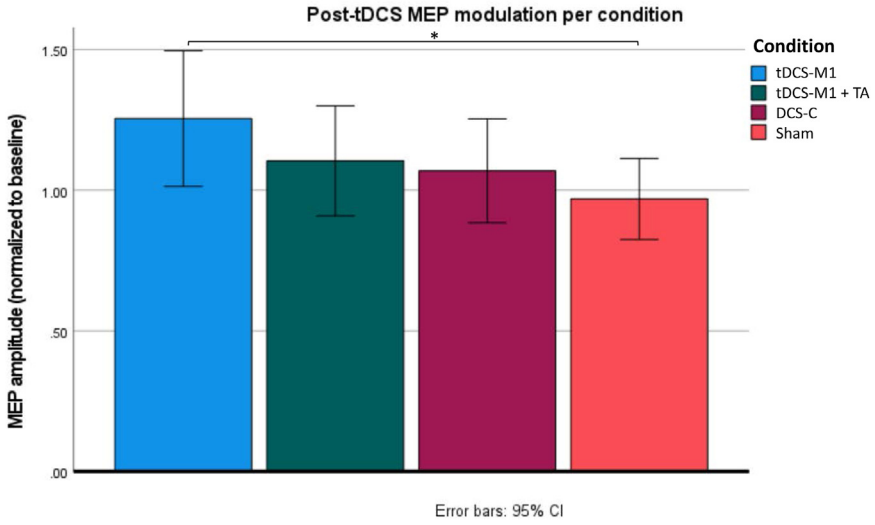

Figure 4. Normalized MEP peak-to-peak amplitude per condition (pooled MEP amplitudes: all post–time point measurements were averaged). A significant increase in MEP amplitude of 25.5% was found in the tDCS-MI condition. *Significant difference in MEP amplitude ($p < 0.05$).

When pooling the post-MEP measurements (average of MEP amplitude at time point 0, 15, 30, 45, and 60 minutes) and comparing pre and post measurements, no significant differences among the four conditions were found. There was no main effect of time (F = 2.45; $p = 0.135$), condition (F = 2.307; $p = 0.087$), or interaction (F = 2.307; $p = 0.087$). Within-subject contrasts showed a significant effect of condition (F = 4.582; $p = 0.046$) and a significant interaction effect (F = 4.582; $p = 0.046$). Pairwise comparisons showed a significant increase in peak-to-peak MEP amplitude of 25.5% in the tDCS-MI condition ($p = 0.04$), which differed significantly from the sham condition ($p = 0.039$) (Fig. 4).

Response Rate

In the tDCS-MI condition, 37% of subjects showed an increase in MEP amplitude; 5% showed a decrease, and 58% showed no change in MEP amplitude. In the tDCS-MI + TA condition, 26% showed an increase in MEP amplitude, and 74% showed no change in MEP amplitude. In the DCS-C condition, 26% showed an increase in MEP amplitude; 5% showed a decrease, and 69% showed no change in MEP amplitude. In the sham condition, 10% showed an increase in MEP amplitude; 11% showed a decrease, and 79% showed no change in MEP amplitude.

Tolerability and Blinding Assessment of the Different Conditions

Participants reported the following side effects: tingling (63%), burning (57%), itching (47%), pain (15%), warmth (15%), fatigue (15%), metallic taste (5%), and concentration issues (5%). A statistically significant difference was detected across conditions regarding the intensity ($\chi^2(3) = 14{,}87$; $p = 0.002$), duration ($\chi^2(3) = 17.65$; $p < 0.001$), and total impact score ($\chi^2(3) = 24{,}97$; $p < 0.001$) of the side effects. Post hoc analysis with Wilcoxon signed-rank tests was conducted with a Bonferroni correction applied, producing a significance level set at $p < 0.008$. Regarding the total impact score, the reported side effects in the tDCS-MI + TA condition were significantly lower than in tDCS-MI (Z = −3.522; $p < 0.001$), DCS-C (Z = −3.054; $p = 0.002$), and sham (Z = −3.221; $p = 0.001$). A significantly lower intensity score was reported in the tDCS-MI + TA than in tDCS-MI (Z = −3.128; $p = 0.002$). The duration of side effects in the tDCS-MI + TA condition was significantly shorter than of side effects in tDCS-MI (Z = −3.316; $p = 0.001$), DCS-C (Z = −3.054; $p = 0.002$), and sham (Z =−3.002; $p = 0.003$). No significant correlation was found between the intensity, duration, or total impact of the reported side effects and MEP modulation.

The question whether participants received real or placebo stimulation showed that 63.1% and 63.2% of the participants indicated "active" for the tDCS-MI and DCS-C conditions, respectively; 57.3% of participants believed they received placebo stimulation in the tDCS-MI + TA condition, whereas active stimulation was given. A more detailed overview of these results can be found in Supplementary Data Table S1.

DISCUSSION AND CONCLUSION

The goal of this study was to evaluate the effects of tDCS on corticospinal excitability and to investigate the MOA, in particular the contribution of peripheral and/or cranial nerve involvement. We found a significant increase in MEP amplitude 30, 45, and 60 minutes after anodal tDCS was delivered over the MI. This increase in MEP amplitude is partially in line with earlier research. The seminal study of Nitsche and Paulus reported a MEP increase of >30% after 1 mA tDCS-MI in healthy participants.[29] These findings were confirmed in other studies, showing MEP increases up to 50% after 1 mA tDCS.[30,31] However, the overall MEP increase reported in previous studies was larger than the increase found in our study (25.5%). These earlier and larger effects on MEP modulation could be due to the larger electrode size (35 cm^2 instead of 25 cm^2) in the previously mentioned studies, as was shown in Ho et al.[32]

We recorded MEPs over a time course of one hour, so our study allowed us to identify longer lasting effects of tDCS, which is of particular interest for the development of therapeutic interventions. Long-term tDCS-induced MEP modulation also has been reported in earlier studies.[8,33,34] A significant MEP size increase of 20% to 40%, after 20 minutes of 1 mA anodal tDCS, was observed up to 120 minutes after stimulation in healthy volunteers.[33,34] In these studies, other stimulation durations (15, 20, 30 minutes) and

intensities (2 mA, 3 mA) were tested, showing even longer-lasting tDCS effects until the next day when combining a high intensity with longer stimulation durations (3 mA, 20 minutes; 3 mA, 30 minutes).[33,34] The proposed underlying mechanism of this long-term offline effect is the modification of synaptic strength producing long-term potentiation (LTP). tDCS effects depend on glutamatergic mechanisms and Ca^{2+} levels.[35–37] The tDCS-induced membrane depolarization leads to an increase in glutamate release, leading to activation of postsynaptic N-methyl-D-aspartate (NMDA) receptors and thereby increased Ca^{2+} influx. Calcium is crucial for LTP induction, leading to expression of more NMDA and α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid postsynaptic receptors and thereby strengthening synaptic transmission.[36] We recorded effects over one hour, but for further development of tDCS toward therapeutic potential and identification of stimulation paradigms, more studies should include longer recordings and repeated tDCS sessions.

In contrast to our results, some previous studies have indicated a lack of effectiveness of tDCS on corticospinal excitability or even an opposite effect of tDCS.[10,38] Nuzum et al could not find any significant MEP modulation after 20 minutes of 2 mA tDCS.[38] A study by Hassanzahraee et al even found a decrease in corticospinal excitability after 26 minutes of tDCS.[10] It is known that a specific range of Ca^{2+} levels exists for optimal LTP induction. Exceeding this range, for example, owing to a longer stimulation duration or higher intensities, might activate counteracting homeostatic mechanisms, such as the activation of potassium channels and/or saturation of NMDA receptors, which may explain these diverging findings.[33]

Overall, effects of tDCS on the modulation of the MEP amplitude are associated with interindividual and intraindividual variability.[8,30,31,38–40] The lack of reproducibility of tDCS outcomes presents a barrier to clinical use. Although heterogeneity of tDCS protocols used is a likely contributor, interindividual variability in response to tDCS, partially due to differences in brain and skull anatomy, may play a large role.[40] The interindividual variability also was detected in our study cohort, with 37% of responders in the tDCS-MI condition, 5% showing a decrease in MEP amplitude, and 58% showing no change. This accords with studies reporting that only 36% to 50% of subjects responded to tDCS in the "classical" polarity-specific manner.[40–42] Personalized tDCS stimulation protocols should be further investigated to counteract this.

The MOA of tDCS and the contribution of cutaneous nerve activations to its effect remain unclear. We investigated this further by anesthetizing the scalp before tDCS administration. The MEP modulation present in the tDCS-MI condition was not detected when a local anesthetic was first applied to the scalp. Although absence of evidence does not mean evidence of absence, the fact that tDCS does not establish a statistically significant increase in MEP amplitude in this condition may suggest that the effects of tDCS are not solely established through direct effects in the cortex, but that innervation of the peripheral and/or cranial cutaneous nerves contribute to tDCS-induced effects on corticospinal excitability. Earlier studies have reported on the potential contribution of peripheral/cranial nerve stimulation to the effects of tES.[7,43,44] It is proposed that these indirect effects can be mediated through activation of brainstem nuclei, involving the LC-noradrenaline (LC-NA) system. When stimulating the second cervical nerve dermatome using tDCS, changes are induced in three different proxy measures of LC-NA activity (ie, pupil diameter, salivary α-amylase, and event-related potentials).[44] Moreover, enhanced extracellular concentrations of noradrenaline have been shown to increase motor cortical excitability by enhancing intracortical facilitation and reducing inhibition, and to drive synaptic plasticity leading to the activation of voltage-gated ion channels.[16,45] Given the MEP amplitude reflects corticospinal excitability and depends on activation of voltage-gated ion channels, this indirect activation of the LC also can explain the increase in MEP amplitude. Peripheral nerve stimulation in humans with other tES methods such as transcutaneous alternating current stimulation (tACS) was found to be responsible for the effects of tACS on enhanced physiological tremor.[12] In addition, neurostimulation devices targeting cranial nerves (trigeminal, occipital, and vagal nerve) have been successful in the symptomatic improvement of several neuropsychiatric disorders.[46–48]

Regarding the tolerability of tDCS, we found that the intensity, duration, and total impact of the reported side effects were significantly lower when the TA was first applied to the scalp, reflecting its effectiveness in suppressing peripheral sensations. This is in line with earlier findings showing that the use of the TA is a safe and effective way to suppress the peripheral/cranial nerve fiber activation while maintaining direct cortical mechanisms.[49] Thereby, it could be implemented either to decrease the side effects of tDCS or as a control condition in future tDCS studies to eliminate the transcutaneous mechanism.

Although we aimed at a novel and carefully designed setup of experiments, the present study has some limitations. Because the study included 19 young and healthy participants, the results cannot be extrapolated to patients or older populations. MEPs, the main outcome measure that we used, are associated with high inter- and intraindividual variability.[50] Even though several precautions were taken to minimize this variability, such as within subject measurements, a sufficient number of pulses per measurement ($n = 25$), and neuronavigation guidance while applying TMS, variability across participants and sessions was observed, which might partly explain the absence of a significant time*condition interaction effect in our study (Supplementary Data Fig. S2 and Supplementary Data Table S2 present the results on intraindividual variability). Given MEPs only reflect corticospinal excitability, a more direct cortical outcome measure such as TMS-evoked potentials measured by electroencephalography (EEG) could be superior, although the combination of TMS-EEG and tDCS might be challenging for the practical experimental execution. Moreover, studies imply that the effects of tDCS are state dependent, meaning that the response to tDCS could be affected by the neurophysiological state of the brain.[51,52] In this study, participants were instructed to be at rest, and no experimental task was included. Asking subjects to perform an experimental task during the tDCS intervention might be beneficial to control this state across participants and sessions and thereby minimize response variability.

In conclusion, the present study showed that tDCS over the MI can modulate corticospinal excitability with effects lasting ≥ one hour after the intervention. The results suggest that the effects of tDCS are not only established through the transcranial MOA that is generally assumed, but that stimulation of peripheral and/or cranial nerves might contribute to the effects of tDCS. These findings warrant further investigation in larger cohorts and patient groups.

Acknowledgements

The Soterix Medical (Soterix Medical Inc) tDCS device used in this study was provided by Vandelanotte Consulting.

Authorship Statements

Emma Lescrauwaet and Mathieu Sprengers designed the study, in collaboration with Evelien Carrette, Debby Klooster, and Steven Beumer, and supervised by Rob Mestrom, Robrecht Raedt, Paul Boon, and Kristl Vonck. Participant recruitment and data collection were conducted by Emma Lescrauwaet. Data analysis was conducted by Emma Lescrauwaet and Mathieu Sprengers. The study was validated by Mathieu Sprengers, Debby Klooster, Steven Beumer, Rob Mestrom, Evelien Carrette, Robrecht Raedt, Paul Boon, and Kristl Vonck. Emma Lescrauwaet prepared the manuscript draft. All authors critically reviewed the manuscript draft. All authors approved the final manuscript.

Conflict of Interest

Emma Lescrauwaet, Ann Mertens, Chloé Algoet, Evelien Carrette, and Robrecht Raedt reported no conflict of interest. Debby Klooster was supported by a FWO junior postdoctoral grant (1259121N). Mathieu Sprengers, Steven Beumer, and Rob Mestrom were partially funded by the Dutch PPP Allowance made available by Health Holland, Top Sector Life Sciences & Health, to EpilepsieNL. Kristl Vonck obtained a Bijzonder Onderzoeks Fonds (BOF) grant of Ghent University for the purchase of transcranial magnetic stimulation (TMS) equipment, obtained consultancy fees from LivaNova, Synergia Medical, All Man Foundation, Precisis, and Angelini Pharma and participates in the advisory board of LivaNova, Synergia Medical, Precisis, and Angelini Pharma. Paul Boon obtained a BOF grant of Ghent University for the purchase of TMS equipment, obtained consultancy fees from LivaNova, Medtronic, and Angelini Pharma and participates in the advisory board of LivaNova, Synergia Medical, and Medtronic.

How to Cite This Article

Lescrauwaet E., Sprengers M., Carrette E., Algoet C., Mertens A., Klooster D., Beumer S., Mestrom R., Raedt R., Boon P., Vonck K. 2024. Investigating the Working Mechanism of Transcranial Direct Current Stimulation. Neuromodulation 2024; ■: 1–8.

SUPPLEMENTARY DATA

To access the supplementary material accompanying this article, visit the online version of *Neuromodulation: Technology at the Neural Interface* at www.neuromodulationjournal.org and at https://doi.org/10.1016/j.neurom.2024.05.002.

REFERENCES

1. Liu A, Vöröslakos M, Kronberg G, et al. Immediate neurophysiological effects of transcranial electrical stimulation. *Nat Commun*. 2018;9:5092. https://doi.org/10.1038/s41467-018-07233-7.
2. Nikolin S, Martin D, Loo CK, Boonstra TW. Effects of TDCS dosage on working memory in healthy participants. *Brain Stimul*. 2018;11:518–527. https://doi.org/10.1016/j.brs.2018.01.003.
3. Karthikeyan R, Smoot MR, Mehta RK. Anodal tDCS augments and preserves working memory beyond time-on-task deficits. *Sci Rep*. 2021;11:19134. https://doi.org/10.1038/s41598-021-98636-y.
4. Chan MMY, Han YMY. Effects of transcranial direct current stimulation (tDCS) in the normalization of brain activation in patients with neuropsychiatric disorders: a systematic review of neurophysiological and neuroimaging studies. *Neural Plast*. 2020;2020:8854412. https://doi.org/10.1155/2020/8854412.
5. Zhao H, Qiao L, Fan D, et al. Modulation of brain activity with noninvasive transcranial direct current stimulation (tDCS): clinical applications and safety concerns. *Front Psychol*. 2017;8:685. https://doi.org/10.3389/fpsyg.2017.00685.
6. Chakraborty D, Truong DQ, Bikson M, Kaphzan H. Neuromodulation of axon terminals. *Cereb Cortex*. 2018;28:2786–2794. https://doi.org/10.1093/cercor/bhx158.
7. van Boekholdt L, Kerstens S, Khatoun A, Asamoah B, Mc Laughlin M. tDCS peripheral nerve stimulation: a neglected mode of action? *Mol Psychiatry*. 2021;26:456–461. https://doi.org/10.1038/s41380-020-00962-6.
8. Dissanayaka T, Zoghi M, Farrell M, Egan GF, Jaberzadeh S. Does transcranial electrical stimulation enhance corticospinal excitability of the motor cortex in healthy individuals? A systematic review and meta-analysis. *Eur J Neurosci*. 2017;46:1968–1990. https://doi.org/10.1111/ejn.13640.
9. Nitsche MA, Cohen LG, Wassermann EM, et al. Transcranial direct current stimulation: state of the art 2008. *Brain Stimul*. 2008;1:206–223. https://doi.org/10.1016/j.brs.2008.06.004.
10. Hassanzahraee M, Nitsche MA, Zoghi M, Jaberzadeh S. Determination of anodal tDCS duration threshold for reversal of corticospinal excitability: an investigation for induction of counter-regulatory mechanisms. *Brain Stimul*. 2020;13:832–839. https://doi.org/10.1016/j.brs.2020.02.027.
11. Vöröslakos M, Takeuchi Y, Brinyiczki K, et al. Direct effects of transcranial electric stimulation on brain circuits in rats and humans. *Nat Commun*. 2018;9:483. https://doi.org/10.1038/s41467-018-02928-3.
12. Asamoah B, Khatoun A, Mc Laughlin M. tACS motor system effects can be caused by transcutaneous stimulation of peripheral nerves. *Nat Commun*. 2019;10:266. https://doi.org/10.1038/s41467-018-08183-w.
13. Majdi A, Asamoah B, Mc Laughlin M. Reinterpreting published tDCS results in terms of a cranial and cervical nerve co-stimulation mechanism. *Front Hum Neurosci*. 2023;17:1101490. https://doi.org/10.3389/fnhum.2023.1101490.
14. Tyler WJ, Boasso AM, Mortimore HM, et al. Transdermal neuromodulation of noradrenergic activity suppresses psychophysiological and biochemical stress responses in humans. *Sci Rep*. 2015;5:13865. https://doi.org/10.1038/srep13865.
15. Raedt R, Clinckers R, Mollet L, et al. Increased hippocampal noradrenaline is a biomarker for efficacy of vagus nerve stimulation in a limbic seizure model. *J Neurochem*. 2011;117:461–469. https://doi.org/10.1111/j.1471-4159.2011.07214.x.
16. Kuo HI, Qi FX, Paulus W, Kuo MF, Nitsche MA. Noradrenergic enhancement of motor learning, attention, and working memory in humans. *Int J Neuropsychopharmacol*. 2021;24:490–498. https://doi.org/10.1093/ijnp/pyab006.
17. Ortega-Robles E, Cantillo-Negrete J, Carino-Escobar RI, Arias-Carrión O. Methodological approach for assessing motor cortical excitability changes with single-pulse transcranial magnetic stimulation. *MethodsX*. 2023;11:102451. https://doi.org/10.1016/j.mex.2023.102451.
18. Ziemann U, Chen R, Cohen LG, Hallett M. Dextromethorphan decreases the excitability of the human motor cortex. *Neurology*. 1998;51:1320–1324. https://doi.org/10.1212/wnl.51.5.1320.
19. Manta S, Dong J, Debonnel G, Blier P. Enhancement of the function of rat serotonin and norepinephrine neurons by sustained vagus nerve stimulation. *J Psychiatry Neurosci*. 2009;34:272–280.
20. Monai H, Ohkura M, Tanaka M, et al. Calcium imaging reveals glial involvement in transcranial direct current stimulation-induced plasticity in mouse brain. *Nat Commun*. 2016;7:11100. https://doi.org/10.1038/ncomms11100.
21. Monai H, Hirase H. Astrocytes as a target of transcranial direct current stimulation (tDCS) to treat depression. *Neurosci Res*. 2018;126:15–21. https://doi.org/10.1016/j.neures.2017.08.012.
22. Madhavan S, Sriraman A, Freels S. Reliability and variability of tDCS induced changes in the lower limb motor cortex. *Brain Sci*. 2016;6:26. https://doi.org/10.3390/brainsci6030026.
23. Horvath JC, Forte JD, Carter O. Evidence that transcranial direct current stimulation (tDCS) generates little-to-no reliable neurophysiologic effect beyond MEP amplitude modulation in healthy human subjects: a systematic review. *Neuropsychologia*. 2015;66:213–236. https://doi.org/10.1016/j.neuropsychologia.2014.11.021.
24. Rossi S, Hallett M, Rossini PM, Pascual-Leone A, Safety of TMS Consensus Group. Safety, ethical considerations, and application guidelines for the use of transcranial magnetic stimulation in clinical practice and research. *Clin Neurophysiol*. 2009;120:2008–2039. https://doi.org/10.1016/j.clinph.2009.08.016.
25. Rossi S, Antal A, Bestmann S, et al. Safety and recommendations for TMS use in healthy subjects and patient populations, with updates on training, ethical and regulatory issues: expert Guidelines. *Clin Neurophysiol*. 2021;132:269–306. https://doi.org/10.1016/j.clinph.2020.10.003.
26. Antal A, Alekseichuk I, Bikson M, et al. Low intensity transcranial electric stimulation: safety, ethical, legal regulatory and application guidelines. *Clin Neurophysiol*. 2017;128:1774–1809. https://doi.org/10.1016/j.clinph.2017.06.001.
27. EMLA Cream 5% (5g pack) - Summary of Product Characteristics. Electronic Medicines Compendium. Accessed April 21, 2024. https://www.medicines.org.uk/emc/product/871/smpc/print.
28. Ammann C, Lindquist MA, Celnik PA. Response variability of different anodal transcranial direct current stimulation intensities across multiple sessions. *Brain Stimul*. 2017;10:757–763. https://doi.org/10.1016/j.brs.2017.04.003.

29. Nitsche MA, Paulus W. Excitability changes induced in the human motor cortex by weak transcranial direct current stimulation. *J Physiol*. 2000;527:633–639. https://doi.org/10.1111/j.1469-7793.2000.t01-1-00633.x.
30. Bashir S, Ahmad S, Alatefi M, et al. Effects of anodal transcranial direct current stimulation on motor evoked potentials variability in humans. *Physiol Rep*. 2019;7:e14087. https://doi.org/10.14814/phy2.14087.
31. Strube W, Bunse T, Nitsche MA, et al. Bidirectional variability in motor cortex excitability modulation following 1 mA transcranial direct current stimulation in healthy participants. *Physiol Rep*. 2016;4:e12884. https://doi.org/10.14814/phy2.12884.
32. Ho KA, Taylor JL, Chew T, et al. The effect of transcranial direct current stimulation (tDCS) electrode size and current intensity on motor cortical excitability: evidence from single and repeated sessions. *Brain Stimul*. 2016;9:1–7. https://doi.org/10.1016/j.brs.2015.08.003.
33. Agboada D, Mosayebi Samani M, Jamil A, Kuo MF, Nitsche MA. Expanding the parameter space of anodal transcranial direct current stimulation of the primary motor cortex. *Sci Rep*. 2019;9:18185. https://doi.org/10.1038/s41598-019-54621-0.
34. Farnad L, Ghasemian-Shirvan E, Mosayebi-Samani M, Kuo MF, Nitsche MA. Exploring and optimizing the neuroplastic effects of anodal transcranial direct current stimulation over the primary motor cortex of older humans. *Brain Stimul*. 2021;14:622–634. https://doi.org/10.1016/j.brs.2021.03.013.
35. Nitsche MA, Jaussi W, Liebetanz D, Lang N, Tergau F, Paulus W. Consolidation of human motor cortical neuroplasticity by D-cycloserine. *Neuropsychopharmacology*. 2004;29:1573–1578. https://doi.org/10.1038/sj.npp.1300517.
36. Nitsche MA, Fricke K, Henschke U, et al. Pharmacological modulation of cortical excitability shifts induced by transcranial direct current stimulation in humans. *J Physiol*. 2003;553:293–301. https://doi.org/10.1113/jphysiol.2003.049916.
37. Liebetanz D, Nitsche MA, Tergau F, Paulus W. Pharmacological approach to the mechanisms of transcranial DC-stimulation-induced after-effects of human motor cortex excitability. *Brain*. 2002;125:2238–2247. https://doi.org/10.1093/brain/awf238.
38. Nuzum ND, Hendy AM, Russell AP, Teo WP. Measures to predict the individual variability of corticospinal responses following transcranial direct current stimulation. *Front Hum Neurosci*. 2016;10:487. https://doi.org/10.3389/fnhum.2016.00487.
39. Horvath JC, Vogrin SJ, Carter O, Cook MJ, Forte JD. Effects of a common transcranial direct current stimulation (tDCS) protocol on motor evoked potentials found to be highly variable within individuals over 9 testing sessions. *Exp Brain Res*. 2016;234:2629–2642. https://doi.org/10.1007/s00221-016-4667-8.
40. Chew T, Ho KA, Loo CK. Inter- and intra-individual variability in response to transcranial direct current stimulation (tDCS) at varying current intensities. *Brain Stimul*. 2015;8:1130–1137. https://doi.org/10.1016/j.brs.2015.07.031.
41. López-Alonso V, Cheeran B, Río-Rodríguez D, Fernández-Del-Olmo M. Inter-individual variability in response to non-invasive brain stimulation paradigms. *Brain Stimul*. 2014;7:372–380. https://doi.org/10.1016/j.brs.2014.02.004.
42. Wiethoff S, Hamada M, Rothwell JC. Variability in response to transcranial direct current stimulation of the motor cortex. *Brain Stimul*. 2014;7:468–475. https://doi.org/10.1016/j.brs.2014.02.003.
43. Vanneste S, Langguth B, De Ridder D. Do tDCS and TMS influence tinnitus transiently via a direct cortical and indirect somatosensory modulating effect? a combined TMS-tDCS and TENS study. *Brain Stimul*. 2011;4:242–252. https://doi.org/10.1016/j.brs.2010.12.001.
44. Vanneste S, Mohan A, Yoo HB, et al. The peripheral effect of direct current stimulation on brain circuits involving memory. *Sci Adv*. 2020;6:eaax9538. https://doi.org/10.1126/sciadv.aax9538.
45. Thorstensen JR, Henderson TT, Kavanagh JJ. Serotonergic and noradrenergic contributions to motor cortical and spinal motoneuronal excitability in humans. *Neuropharmacology*. 2024;242:109761. https://doi.org/10.1016/j.neuropharm.2023.109761.
46. DeGiorgio CM, Soss J, Cook IA, et al. Randomized controlled trial of trigeminal nerve stimulation for drug-resistant epilepsy. *Neurology*. 2013;80:786–791. https://doi.org/10.1212/WNL.0b013e318285c11a.
47. Cook IA, Schrader LM, DeGiorgio CM, Miller PR, Maremont ER, Leuchter AF. Trigeminal nerve stimulation in major depressive disorder: acute outcomes in an open pilot study. *Epilepsy Behav*. 2013;28:221–226. https://doi.org/10.1016/j.yebeh.2013.05.008.
48. Johnson RL, Wilson CG. A review of vagus nerve stimulation as a therapeutic intervention. *J Inflamm Res*. 2018;11:203–213. https://doi.org/10.2147/JIR.S163248.
49. Kerstens S, Orban de Xivry JJ, Mc Laughlin M. A novel tDCS control condition using optimized anesthetic gel to block peripheral nerve input. *Front Neurol*. 2022;13:1049409.
50. Spampinato DA, Ibanez J, Rocchi L, Rothwell J. Motor potentials evoked by transcranial magnetic stimulation: interpreting a simple measure of a complex system. *J Physiol*. 2023;601:2827–2851. https://doi.org/10.1113/JP281885.
51. Hsu TY, Juan CH, Tseng P. Individual differences and state-dependent responses in transcranial direct current stimulation. *Front Hum Neurosci*. 2016;10:643.
52. Dubreuil-Vall L, Chau P, Ruffini G, Widge AS, Camprodon JA. tDCS to the left DLPFC modulates cognitive and physiological correlates of executive function in a state-dependent manner. *Brain Stimul*. 2019;12:1456–1463. https://doi.org/10.1016/j.brs.2019.06.006.

COMMENT

This study holds significant interest owing to its ability to spark inquiries regarding the effectiveness of tDCS as a means to induce alterations in cortical excitability. Given the implications of such findings for the broader scientific community, I firmly believe that this study merits publication. By shedding light on the efficacy of tDCS in modulating cortical excitability, this research not only contributes to our understanding of neural mechanisms but also holds potential implications for various fields, including neuroscience, psychology, and medicine. The exploration of tDCS as a method to enact changes in cortical excitability represents a pivotal step in the advancement of neurostimulation techniques and their therapeutic applications. Furthermore, the publication of this study would facilitate scholarly discourse and encourage further investigation into the nuanced effects of tDCS on cortical excitability. It has the potential to stimulate fruitful discussions, inspire future research endeavors, and ultimately enrich the collective knowledge base of the scientific community. In light of these considerations, I advocate for the publication of this study, recognizing its relevance, significance, and potential to contribute to the ongoing dialogue within the scientific community.

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