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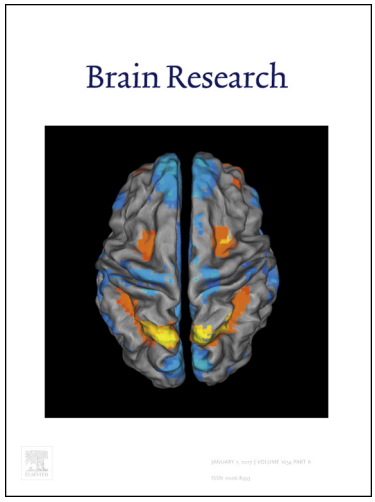

Research report

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Research Report

Concurrent anodal transcranial direct-current stimulation and motor task to influence sensorimotor cortex activation

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Running title: Cortical oxygenation and transcranial stimulation

Highlights

- Functional near infrared spectroscopy probed the sensorimotor cortex activity when using online, offline and sham high definition anodal tDCS.
- Online and offline high definition anodal tDCS induced greater cortical activation 30 min after stimulation.
- Concurrent application of high definition anodal tDCS during a motor task induced more pronounced changes in sensorimotor cortex activation

Abstract

Functional targeting with anodal high-definition transcranial direct current stimulation (HD-atDCS) of involved brain areas during performance of a motor task (online) may facilitate sensorimotor cortex neuroplasticity compared to performing the motor task after HD-atDCS (offline). The aim of this study was to employ functional near-infrared spectroscopy to compare the time course of motor task-related changes in sensorimotor cortex activation between online and offline HD-atDCS. We hypothesized that online HD-atDCS would have a greater effect on task-related sensorimotor cortex activation than offline HD-atDCS. In a within-subject sham controlled and randomized study design, 9 healthy participants underwent 3 HD-atDCS sessions (online, offline and sham) targeting the left sensorimotor cortex separated by 1 week. Functional near-infrared spectroscopy hemodynamic changes were measured from the left sensorimotor cortex during a simple finger opposition motor task before (Pre), immediately (T1) and 30 min after (T2) each session. The movement rates were not different between (online, offline, sham) or within (Pre, T1, T2) sessions. At T2, online HD-atDCS was associated with a significant increase (large effect size) in sensorimotor cortex activation (Hedges $g = 1.01$, $p<0.001$) when compared to sham; there was a nonsignificant trend to increase activation between offline and sham (Hedges $g = 0.52$, $p=0.05$) and between online and offline (Hedges $g = 0.53$, $p=0.06$). Concurrent application of HD-atDCS during a motor task may produce larger sensorimotor cortex activation than sequential application.

Keywords: online, neurovascular coupling, neuromodulation, functional neuroimaging, high-definition montage, plasticity.

1. Introduction[1]

Transcranial direct current stimulation (tDCS) is a non invasive brain technique used to deliver weak electrical currents through electrodes to modulate cortical excitability in different brain regions (Nitsche and Paulus, 2000; Stagg and Nitsche, 2011). Anodal tDCS (atDCS) is able to increase cortical excitability as reflected by an increase in amplitude of motor evoked potentials evoked at rest after tDCS (Jacobson et al., 2011). However, recent reports have suggested that around half of healthy subjects do not show the expected excitatory effect following atDCS (Chew et al., 2015; Li et al., 2015; López-Alonso et al., 2014; Strube et al., 2015; Vallence et al., 2015; Wiethoff et al., 2014). In addition, there is a relative paucity of knowledge regarding the effects of task-concurrent atDCS on cortical activity in target brain regions.

The use of functional neuroimaging methods based on neurovascular coupling mechanisms allows indirect simultaneous measures to be made of brain activity during (online) and following (offline) tDCS (Siebner et al., 2009). Some early functional magnetic resonance spectroscopy (fMRI) studies investigating offline (after 20 min at 1 mA) and online (during periods of stimulation from 20 s to 2 min at 1 mA) atDCS protocols including motor tasks have reported contrasting findings in brain activation patterns (Antal et al., 2011; Jang et al., 2009; Kwon and Jang, 2011). Offline atDCS (20 min, 1 mA)-hand movements increased activation in the targeted sensorimotor cortex (SMC) compared to sham (Jang et al., 2009). But online atDCS (8x 20 s, 1mA)-finger movements decreased activation of the supplementary motor area without notable changes over the targeted SMC (Antal et al.,

Abbreviations. fNIRS, functional near infrared spectroscopy; SFO, simple finger opposition; SMC, sensorimotor cortex; T1, time 1; T2, time 2; tDCS, transcranial direct current stimulation.

2011). In the latter study, the inability to measure alterations of activation in the targeted SMC during online atDCS might have been due to the low intensity (1 mA) and the short duration (20 s) of the stimulation protocol. Conversely, it was observed that online atDCS (2 min, 1 mA)-hand movements induced more SMC activation than sham (Kwon and Jang, 2011). These contradictory findings using short duration and lower intensity atDCS protocols stem from the technological limitation of combined atDCS-fMRI techniques that cause possible distortions in fMRI signals by the tDCS electrical/magnetic fields, as well as subject safety due to heating of tDCS electrodes by the fMRI magnetic field. These limitations have therefore encouraged the search for alternative functional neuroimaging methods to determine the effect of task-concurrent atDCS on SMC activation.

In contrast to fMRI, motor-task related changes in the concentration of oxygenated (O_2Hb) and deoxygenated (HHb) hemoglobin in the SMC measured by functional near-infrared spectroscopy (fNIRS), reflect with good sensitivity the hemodynamic response to neuronal activity (Leff et al., 2011) without interference from the tDCS environment. The combined use of atDCS with fNIRS as a relatively simple and safe method offers the possibility to investigate continuously the online and offline effects of atDCS on resting-state (Muthalib et al., 2017) and task-related SMC hemodynamic response (Choe et al., 2016; Gözenman and Berryhill, 2016). atDCS using a high-definition (HD-atDCS) electrode montage (4x1) has been shown to increase the focality and intensity of stimulation at the primary motor cortex target (Datta et al., 2009). Our preliminary fNIRS study (Muthalib et al., 2016) using HD-atDCS (2 mA, 20 min) during a sequential finger opposition (SFO) task found a decrease in task-related activation in the targeted left SMC compared to pre-stimulation. However, since the after effects of HD-atDCS show peak changes in cortical excitability after a delay of ~30 minutes from the cessation of the stimulation (Kuo et al., 2013), it is not known whether task-related SMC activation would also show greater

neuromodulatory effects up to 30 min. Moreover, the relative effectiveness of online and offline HD-atDCS protocols to modulate motor task-related SMC activation needs to be clarified in order to develop the most optimal protocol.

Therefore, the aim of the present study was to compare the time course of SFO motor task-related modulation of SMC activation between online and offline HD-atDCS protocols in a within-subjects sham-controlled and randomized design. It was hypothesized that online HD-atDCS would impact SFO motor task-related activation in the targeted SMC to a greater extent than both sham and offline HD-tDCS.

2. Results

2.1. Subjective sensation and anxiety when using tDCS

As indicated in Table 1, no difference ($F(2,16)=0.633$, $p=0.544$) was observed among the sessions for the resting state cutaneous sensation over the scalp during HD-atDCS, indicating that the participants were unable to differentiate real HD-atDCS from sham sessions. There was no significant difference ($F(2,16)=0.440$, $p=0.652$) in STAI values between the sessions.

2.2. Movement rate

As indicated in Table 2, there were no significant differences in the SFO movement rate over time ($F(2,16)=1.377$, $p=0.692$), between the experimental sessions ($F(2,16)=1.167$, $p=0.337$), and between the right and left hands ($F(2,16)=1.651$, $p=0.235$).

2.3. Functional near-infrared spectroscopy

Figure 1 shows the normalized (only for illustrative purposes) changes in HHb and Hb_{diff} for the online, offline, and sham sessions over time. For HHb (Fig. 1A), there was no effect of Session ($F(2,16)=0.098$, $p=0.907$), but there was a Session x Time interaction effect ($F(4,32)=3.228$, $p=0.025$, $\eta^2p=0.288$) and an effect of Time ($F(2,16)=9.616$, $p=0.002$, $\eta^2p=0.546$). *Post hoc* analysis revealed significantly lower HHb (i.e., increased SMC activation) from Pre to T2 for both the online ($p<0.0006$) and offline ($p<0.02$) sessions, while there was no significant change for sham. At T2, HHb for the online session was significantly ($p<0.01$, $g=1.08$) lower than the sham session, but there was no significant difference in HHb between online and offline ($g=0.54$) or between offline and sham ($g=0.38$). At T1, although HHb was significantly ($p<0.02$, $g=-0.63$) higher (i.e., decreased SMC activation) for the online than offline session, these changes in HHb were not significantly different to sham ($g=-0.48$ vs. online, $g=0.25$ vs. offline).

For Hb_{diff} (Fig. 1B), there was no effect of Session ($F(2,16)=1.640$, $p=0.225$), but there was a Session x Time interaction ($F(4,32)=2.868$, $p=0.039$, $\eta^2p=0.263$) and a main effect of Time ($F(2,16)=5.823$, $p=0.012$, $\eta^2p=0.421$). *Post hoc* analysis revealed significantly lower Hb_{diff} (i.e., decreased SMC activation) from Pre to T1 ($p<0.03$) and higher Hb_{diff} (i.e., increased SMC activation) from Pre to T2 for the online ($p<0.02$) session, while there was no significant change from Pre for the offline and sham session. At T2, Hb_{diff} was significantly higher (i.e., increased SMC activation) for the online ($p<0.0004$, $g=1.01$) session compared to sham, and there was a trend for Hb_{diff} in the online session to be higher than offline ($p=0.061$, $g=0.53$), as well as for offline to be higher than sham ($p=0.053$, $g=0.52$).

For O_2Hb, there was no effect for the Session x Time interaction ($F(4,32)=1.713$, $p=0.171$) or the main effect of Session ($F(2,16)=2.000$, $p=0.168$), but there was a trend for a main effect of Time ($F(2,16)=3.570$, $p=0.052$, $\eta^2p=0.309$).

3. Discussion

This study aimed to determine whether motor task-related SMC activation as measured simultaneously by the use of fNIRS would be modulated to a greater extent while performing a simple finger opposition motor task during (online) rather than after (offline) HD-atDCS (2 mA, 20 min). Our main novel finding showed that online and offline HD-atDCS sessions induced a delayed (30 min after stimulation) increase in SMC activation after performing the same SFO task, but only the online session was found to be significantly different from the sham condition.

For the SFO task used in this study, we sought a constant motor performance without any influence of learning. Our results confirm that the SFO task was performed at a similar movement rate within and between the three experimental sessions (see Table 1). During the SFO task, specific sensorimotor cortical networks (Anwar et al., 2016) are engaged, with the SMC showing the most consistent changes (Witt et al., 2008). Such a setup allowed us to investigate how HD-atDCS effects can be enhanced when the stimulated SMC region is concurrently activated by a motor task.

In the present study, we employed fNIRS as a relatively simple and safe method to reveal the online and offline effects of HD-atDCS on SFO motor task-related hemodynamic responses, which is a proxy of SMC activation. Based on the neurovascular coupling mechanism, the hemodynamic response measured by fNIRS is usually characterized with an increase in O_2Hb and a concomitant smaller reduction in HHb in the cortical microcirculation. Patterns of O_2Hb and HHb changes are well correlated with the fMRI BOLD signal (Leff et al., 2011) and can be used to identify the level of cortical activation (Leff et al., 2011). Due to the greater influence of superficial blood vessels on O_2Hb signals (Kirilina et al., 2012), HHb

changes (Muthalib et al., 2016) and an integrated measure combining O_2Hb and HHb (i.e., Hb_{diff} = O_2Hb – HHb) (Lu et al., 2015) is the most suitable metric for accurately detecting task-related changes in SMC activation. Indeed we found much larger variability in the O_2Hb integral values between subjects, which could account for the non-significant ANOVA effects. However, normalizing O_2Hb to HHb (i.e., Hb_{diff} that is driven by increases in O_2Hb with a smaller contribution from decreases in HHb) reduced this variability, which allowed Hb_{diff} to better detect task-related changes in SMC activation during tDCS sessions. Hence a greater SMC activation is reflected in an elevated Hb_{diff} and reduced HHb. Based on this relationship, we observed that when a SFO motor task was performed concurrently with HD-atDCS it produced a significant delayed increase (large effect size for HHb and Hb_{diff}) in SMC activation (see T2 in Fig. 1) when compared to sham. Looking at Fig. 1, offline HD-tDCS also led to a delayed increase in SMC at T2 but without reaching significance (medium effect size for Hb_{diff}) compared to sham. Finally, there was a non-significant trend but with a medium effect size, for Hb_{diff} with a higher SMC activation in the online HD-atDCS session. Overall, our findings reinforce the fact that HD-atDCS elicited more pronounced effects in the stimulated region of the SMC (Lang et al., 2005) that was evident after a 30 min delay. Determining how long these effects exactly lasted requires further measurements over 30 min after HD-atDCS and in a larger sample size of subjects.

The slightly higher increase of SMC activation in the online than offline HD-atDCS session after 30 min could be explained by the greater efficacy of HD-atDCS at inducing neuroplasticity when networks are already involved in the task, since active networks are preferentially sensitive to neuromodulation (Bikson et al., 2013; Reato et al., 2010). atDCS alone increases the driving force of synaptic activity due to the synergistic effects of dendritic hyperpolarization and somatic depolarization (Lafon et al., 2016). But synaptic modifications are more pronounced when the task and tDCS are concurrent (Karok & Witney, 2013).

Alternatively, rather than inducing synaptic plasticity, atDCS paired with a motor task may have a modulatory role (Kronberg *et al.*, 2017). In addition, the fact that we combined both motor task and electrical stimulation with sufficient current (2 mA for 20 min) may have induced a "gating mechanism" that increased the calcium levels above a threshold to induce enhanced synaptic plasticity (Moriyoshi et al., 1991). In an fMRI study, Kwon and Jang (2011) observed a higher SMC activity when the motor task was applied during short tDCS application when compared to sham and the motor task alone. It may be hypothesized that when there is motor activity during prolonged HD-atDCS involving the same brain areas, the amount of current that enters the sensorimotor cortex triggers further changes in brain activity patterns for at least 30 min.

However, based on the theory of homeostatic plasticity (Turrigiano and Nelson, 2004), we might speculate that the increase of SMC activation after 30 min could be a consequence of the modification of excitation/inhibition balance at T1 requiring adjusting of their synaptic strengths (Pozo and Goda, 2010). Note that at T1, hemodynamic changes for online HD-atDCS (i.e. reduced consumption of oxygenated blood) were quite consistent with the neural efficiency hypothesis associated with lower brain activation for completion of the same task. But later, the increase in SMC activity to perform the same motor task 30 min after HD-atDCS could represent a reduced neural efficiency, which is counterintuitive to the known enhancements of motor learning after tDCS and motor task application (Reis and Fritsch, 2011). We would rather consider that the delayed increase in SMC activation after HD-atDCS could represent a type of motor memory consolidation process (Galea and Celnik, 2009). Previous work (Reis et al., 2009; Saucedo Marquez et al., 2013) highlighted the beneficial effect of online tDCS and motor task training on consolidation of the motor task after a delay period from stimulation. This consolidation results in part from memory stabilization and as such requires energy with subsequent increases in cerebral blood flow (Lisman et al., 2002).

A limitation of the current study is that although we measured the cortical activity of the stimulated region, anodal stimulation can increase connectivity patterns near the stimulation electrode as well as to more distant sites intra- and interhemispherically (Polania et al., 2011). Another limitation of this work is the final number of subjects retained for the analysis (9 subjects) and the presence of only male participants. Further studies could utilize more subjects to examine the reproducibility of these first findings and examine the interactions with the rest of the motor network through cortico-cortical connections both intrahemispherically and across the corpus callosum.

In conclusion, this study highlights the importance of the relative timing of HD-atDCS and motor task in modulating brain activation of the targeted SMC. The novel finding suggests that functional targeting of motor task-concurrent atDCS is likely more effective at producing changes in SMC activation that lasted at least 30 min after stimulation. The increase in activation of the functionally targeted SMC could be the result of several neuroplastic mechanisms that modify excitation/inhibition balance. Future research with combined neurophysiological and neuroimaging techniques is needed to fully understand this phenomenon at a larger scale.

4. Experimental procedure

4.1. Design

In a single blind randomized within-subjects design, subjects participated in three HD-atDCS sessions (online, offline and sham; see Fig. 2). The order of the sessions was randomized and counterbalanced using an online algorithm (http://www.randomization.com/). Sessions were

separated by at least 1-week and were performed at the same time (± 1 hour) of the day in a quiet and dimly lit room in order to prevent fNIRS channels contamination by ambient light.

4.2. Participants

Fifteen healthy males (mean age ± SD, 33.4 ± 12.2 yrs.) voluntarily participated in this study. Subjects were right handed (laterality index 82.8 ± 14.0, range from 58 to 100) as determined by the Edinburgh handedness inventory (Oldfield, 1971). All subjects had no history of neurology or physical disorders or any upper extremity muscle or joint injuries. The study was approved by the local Research Ethics Committee (IRB EuroMov, n°1701B) and was in accordance with the Declaration of Helsinki. All participants gave written informed consent after a description of the study procedures and associated risks.

4.3. Protocol

The subjects were seated comfortably at a desk on a height-adjustable chair in front of a LCD monitor. Both forearms were placed in supination position upon the surface of the table. Subjects were then familiarized to perform a self-paced SFO task (i.e., sequential tapping of the index, middle, ring and fourth finger against the thumb) with their left and right hands at a rate of 2-3 Hz. Following the familiarization and a 3 min rest period the subjects were required to perform the SFO task before the stimulation with their right and left hands in an alternative block design (30-s rest and 30-s task, repeated five times for each hand). The start hand was randomized and counterbalanced across the subjects. The start and the stop of the SFO task was displayed on a LCD monitor for each block to better control the duration of the task, alertness of the participants and task-related hemodynamic response (Colier et al., 1999).

The experimenter counted the number of SFO taps during each of the experimental task blocks.

Three minutes after the pre-stimulation SFO task, subjects received one of 3 HD-atDCS sessions. Each session consisted of four phases (see Fig. 2): (i) Pre: SFO task before tDCS (ii) tDCS: 20 min tDCS or sham, iii) Time 1 (T1): SFO task with Online, Offline, or Sham tDCS, and (iv) Time 2 (T2): SFO task at 30 min after tDCS. For sham tDCS, 50% underwent Online and 50% underwent Offline. The current was always ramped up or down over the first and last 30 s of stimulation. All of the subjects were instructed that they would feel senseless or a mild tingling sensation under the electrodes that fades over seconds depending on the variability of individuals, who were blinded to tDCS protocols. The current was turned off after 30 s in the two sham protocols or continued for a total of 20 min during HD-atDCS sessions (with online- or offline-motor task). Even if HD-tDCS is well tolerated (Turski et al., 2017), a questionnaire containing rating scales of 11 unpleasant sensations compared to resting state was filled out after the stimulation sequence. This questionnaire was based on the tDCS safety guidelines proposed by Poreisz et al. (2007). As variability in physiological measures can be due to psychological states (Wehrwein and Carter, 2016), the State-Trait Anxiety Inventory (STAI, Spielberger et al., 1970) for assessing trait and state anxiety was completed at the beginning of each session.

4.4. Transcranial direct current stimulation

Direct current was generated by a current stimulator (Startim®, Neuroelectrics NE, Spain) and delivered to the left SMC of the subject through a 4x1 anodal HD-tDCS montage (active anode electrode on C3 surrounded by four return electrodes on FC1, FC5, CP5 and CP1; each at a distance of ~4 cm from the active electrode (Muthalib et al., 2016). The five electrodes (3.14 cm^2 AgCl electrodes) were secured on the scalp according to the 10-10 EEG electrode

system positions using conductive paste (Ten20®, Weaver and Company, USA) and held in place using a specially designed plastic headgear to arrange the HD-tDCS electrodes and fNIRS probes on the head (see Fig. 3 for layout).

4.5. Functional near-infrared spectroscopy

Hemodynamic responses during rest and SFO task periods were recorded continuously using a continuous wave multi-channel fNIRS system (Oxymon MkIII Artinis, Medical Systems, The Netherlands) utilizing two wavelengths (~765 and 856 nm) at a sampling of 10 Hz. NIR light was delivered via fiber optic cables to a customized plastic headgear. Two receivers (avalanche photodiode) and two transmitters (pulsed laser) probes were placed, creating a 4 channel array (each channel represented by a receiver-transmitter combination separated by ~3 cm). Based on 10-20 EEG electrode system (Klem et al., 1999), the headgear was aligned with the vertex (Cz) and channels covered the stimulated SMC regions (see Fig. 3).

The fNIRS system calculates the changes in O_2Hb and HHb concentration values (expressed in μM) according to a modified Beer-Lambert Law and including an age-dependent constant differential pathlength factor (Duncan et al., 1996). During the recordings, the time course of changes in O_2Hb and HHb concentration values were displayed in real time, and the signal intensity was verified for each channel before data collection.

4.5.1. Location of fNIRS probes and HD-atDCS electrodes

A 3-dimensional digitizer (Fastrack, Polhemus, USA) was used to measure the location of each fNIRS optode probe and tDCS electrode with a stylus marker in relation to the veridical landmarks of the participant's head (nasion, Cz, the pre auricular points anterior to the left and right ears). Subsequently, these coordinates were registered over a reference MRI atlas in the Montreal Neurological Institute (MNI) coordinates system (Singh et al., 2005), and the points

on the scalp were projected over a three-dimensional reconstruction of the brain cortex (see Fig. 3) using the NIRS-SPM toolbox (Ye et al., 2009). The Brodmann areas corresponding to the region were further determined using the Anatomy 1.8 toolbox for SPM (Eickhoff et al., 2005). No difference in the location of fNIRS probes and HD-tDCS electrodes was found for the locations between sessions for each subject.

4.6. Data Analysis

4.6.1. SFO Movement rate

SFO Movement rate at each time point for each subject was calculated as the average of the number of SFO taps completed by the left and right hands divided by 300 s. Three participants out of 15 with an intra-individual coefficient of variation (CV) up to 5% for the movement rate were excluded from further analysis because they did not follow correctly the instructions of the experimental design.

4.6.2. Cortical hemodynamic changes

4.6.2.1. Pre-processing

Since the presence of cardiac pulsations in fNIRS O_2Hb signals is indicative of a good contact between the optical probes and the scalp (Themelis et al., 2007), the quality of each of the four channels was checked using two pre-processing methods. First, we analyzed the power spectrum of each time series, where the detection of a peak value around 1 Hz reflects the presence of the cardiac pulsations in the fNIRS signal at rest. Then we used the continuous wavelet transform (Grinsted et al., 2004) which is a time-frequency analysis of the signal, where the presence of a strong power-band around 1 Hz reveals a good signal over time. After

these preliminary pre-processing steps, 3 participants out of 12 were removed from further analysis due to many bad channels along sessions. Then the four channels (Fig. 3) were pooled because they covered the stimulated SMC region.

4.6.2.2. Data processing

The data processing was performed for each subject using some of the Homer2 processing package functions (http://homer-fnirs.org/) based in MatLab (version 2014a, Mathworks, USA) (see Appendix A. Supporting document). The fNIRS values retained for statistical analysis were changes in the averaged O_2Hb and HHb computed over the 10 task blocks using the integral between 5 to 25 seconds out of the 30 seconds of the task. This integral analytic approach allows quantifying the concentration changes over time while being sensitive to task-related changes on O_2Hb and HHb regardless of the shape of the hemodynamic response profile (Näsi et al., 2010; Safi et al., 2012). An index of hemoglobin differential (Hb_{diff} = O_2Hb – HHb) was also used to evaluate the level of cortical activation (Lu et al., 2015). Since the SMC activation (O_2Hb, HHb and Hb_{diff}) and movement rate for the two sham sessions (sham online and sham offline conditions) were not significantly different, we pooled the data to represent one sham session.

4.7. Statistical Analysis

We used the Kolmogorov-Smirnov test to check for normal distribution. A repeated measures ANOVA ($ANOVA_{RM}$) was used to compare STAI values and sensation when using tDCS, the SMC activation (O_2Hb, HHb and Hb_{diff}) with one within-subject factor (Session: online, offline and sham) and movement rate with three within-subject factors (Hand: left, right; Time: Pre, T1, T2 and Session: online, offline and sham). In case of a significant main or interaction effect, follow-up ANOVAs with *post-hoc* LSD Fisher tests for multiple

comparisons were conducted. All statistical analyses were performed using Statistica version 7.1 (StatSoft France, 2006). In all statistical tests a significance level of 0.05 was used. The effect sizes were reported in the results section as follows: the partial-eta squared values (η^2_p) (Lakens, 2013) for the main and interaction effects of $ANOVA_{RM}$ and the magnitude of Hedges' g for the simple comparisons (post hoc) among sessions for a given time (T1 or T2). Hedges' g is a variation of Cohen's d that corrects for biases due to small sample sizes (Hedges & Olkin, 1985) and the magnitude of Hedges' g may be interpreted using Cohen's convention as small (0.2), medium (0.5) and large (0.8).

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Author Contributions

PB, MM, and JR designed the study. PB conducted the study, data collection and data analysis under the supervision of MM, GD and SP. PB and SP prepared the manuscript draft with important intellectual input from JR, GD and MM. All authors approved the final manuscript.

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Figure legends

Figure 1. Group mean (±SEM) motor-task related changes normalized to the respective baseline values (Pre) in deoxygenated (HHb, panel A) and differential (Hb_{diff}, panel B) hemoglobin concentration in the left sensorimotor cortex for the online, offline and sham HD-atDCS sessions immediately (T1) and 30 min after (T2) stimulation. * $p < 0.05$; ** $p < 0.001$; + $p = 0.053$; ++ $p = 0.061$; # T2 > Pre for Online; ł T2 > T1 for Online; $ T1 < Pre for Online.

Figure 2. Experimental timeline. All subjects underwent three HD-atDCS (2 mA, 20 min) sessions (online, offline and sham) with one week washout between each session. For each session, subjects performed a simple finger opposition (SFO) motor task before (Pre), immediately (T1) and 30 min (T2) after cessation of stimulation. SFO was performed either during the last 10 min of the stimulation period (online) or after the 20 min simulation period (offline). Sham condition was performed in either online or offline condition. See Methods for further details.

Figure 3. Locations of the functional near-infrared spectroscopy (fNIRS) transmitter (T, in yellow) and receiver (R, in green) probes and anodal high-definition tDCS (HD-atDCS) anode (A, in red) and cathode (C, in blue) electrodes on the left hemisphere (Left panel). Each fNIRS channel was located midway between the T and R probes. MNI coordinates (x,y,z) and Brodmann areas (BA) of the 4 fNIRS channels and 5 HD-atDCS electrodes are reported on the right panel. BA1,2,3,4: sensorimotor cortex; BA6: supplementary motor area/premotor cortex; BA7: superior parietal lobule; BA40: inferior parietal lobule.

Table 1. Mean (SD) simple finger opposition (SFO) movement rate (Hz) for the online, offline and sham HD-atDCS sessions before (Pre), immediately (T1) and 30 min (T2) after stimulation.

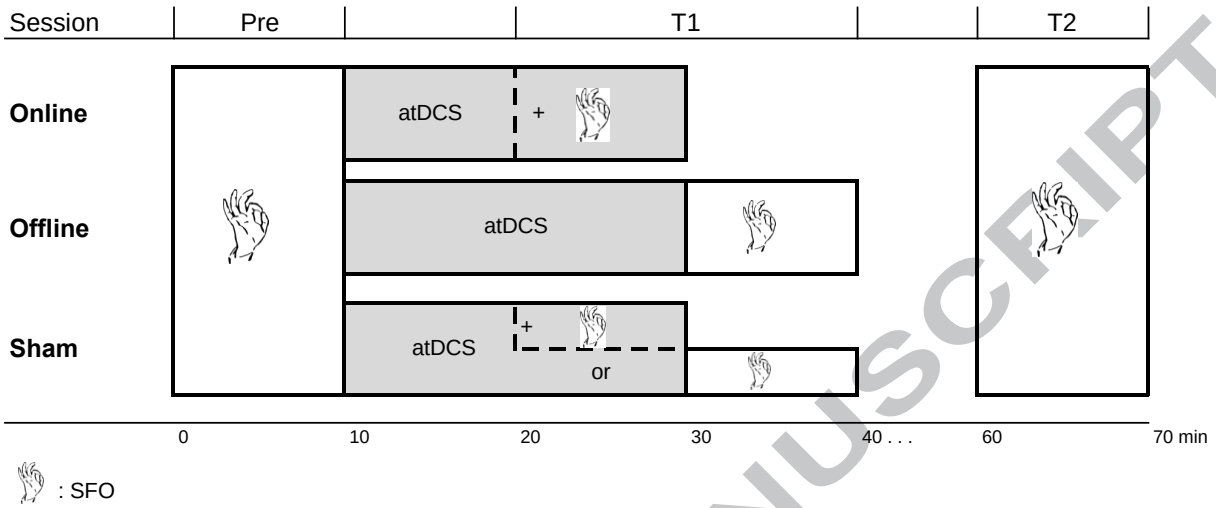
	Right hand			**Left hand**		
Session	Pre	T1	T2	Pre	T1	T2
Online	2.4 (0.2)	2.4 (0.2)	2.4 (0.2)	2.4 (0.2)	2.4 (0.2)	2.4 (0.2)
Offline	2.3 (0.2)	2.4 (0.3)	2.4 (0.2)	2.3 (0.2)	2.4 (0.2)	2.4 (0.2)
Sham	2.5 (0.2)	2.4 (0.2)	2.4 (0.2)	2.4 (0.2)	2.4 (0.2)	2.4 (0.2)

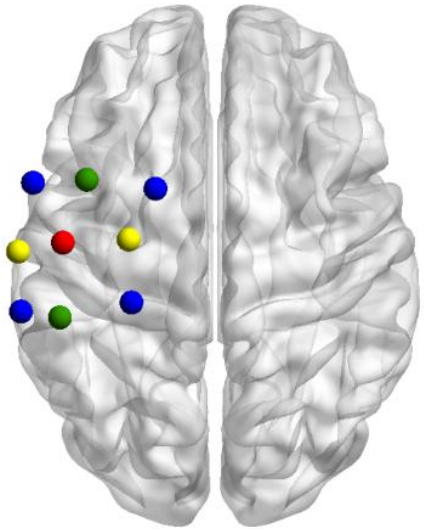
Table 2. Mean (SD) values for sensation and the state-trait anxiety inventory (STAI) completed after and at the beginning of online, offline and sham HD-atDCS sessions, respectively.

	Online	Offline	Sham
STAI	26.1 (7.44)	25.9 (6.57)	27.0 (6.18)
sensation	1.32 (0.24)	1.26 (0.27)	1.27 (0.24)

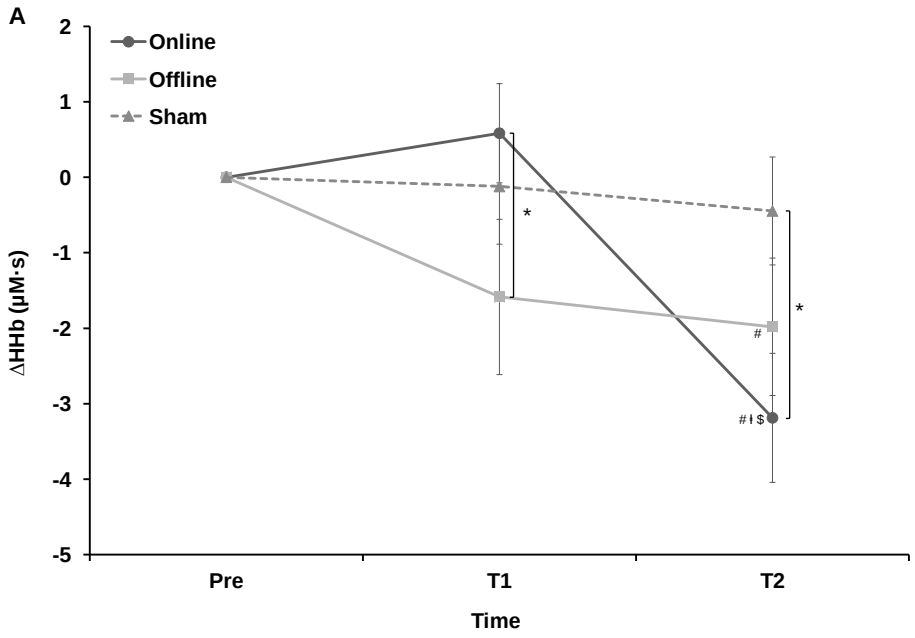
Highlights

- Functional near infrared spectroscopy probed the sensorimotor cortex activity when using online, offline and sham high definition anodal tDCS.
- Online and offline high definition anodal tDCS induced greater cortical activation 30 min after stimulation.
- Concurrent application of high definition anodal tDCS during a motor task induced more pronounced changes in sensorimotor cortex activation





		MNI coordinates			
		X	Y	Z	BA
fNIRS probes	R1	-43	11	58	3-4-6
	T1	-66	-13	42	4-6
	R2	-29	-9	71	1-2-3-40
	T2	-52	-35	59	1-3-4
HD-atDCS	A1	-51	-10	58	1-2-3-4
electrodes	C1	-20	8	68	6
	C2	-28	-30	75	7
	C3	-61	10	35	6
	C4	-65	-33	38	40


A
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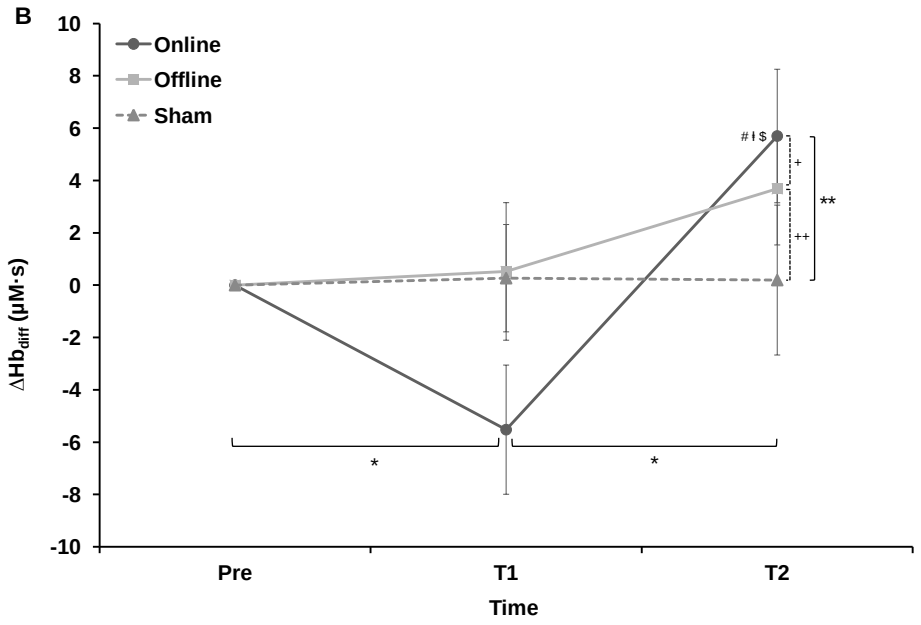
B
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