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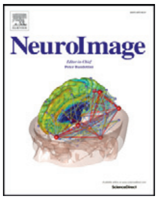

Full Length Article

Effects of transcranial direct current stimulation on the functional coupling of the sensorimotor cortical network

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ABSTRACT

Transcranial direct current stimulation (tDCS) is well established—among the non-invasive brain stimulation techniques—as a method to modulate brain excitability. Polarity-dependent modulations of membrane potentials are detected after the application of anodal and cathodal stimulation, leading to changes in the electrical activity of the neurons. The main aim of the present study was to test the hypothesis that tDCS can affect—in a polarity-specific manner—the functional coupling of the sensorimotor areas during the eyes-open resting condition as revealed by total EEG coherence (i.e., coherence across the average of all combinations of the electrode pairs placed around the stimulation electrode). The changes in the total EEG coherence were evaluated pre-, during, and post-anodal and cathodal tDCS. While no differences were observed in the connectivity characteristics of the two pre-stimulation periods, a connectivity increase was observed in the alpha 2 band in the post-anodal tDCS with respect to pre-anodal and post-cathodal tDCS. The present study suggests that a specific approach based on the analyses of the functional coupling of EEG rhythms might enhance understanding of tDCS-induced effects on cortical connectivity. Moreover, this result suggests that anodal tDCS could possibly modify cortical connectivity more effectively with respect to cathodal tDCS.

Introduction

Transcranial direct current stimulation (tDCS) is well established—among the non-invasive brain stimulation techniques—as a method to modulate neural activity. Polarity-dependent modulations of membrane potentials are detected after the application of anodal/cathodal stimulation, as reflected by a transient increase/decrease of cortical excitability (Bindman et al., 1964; Nitsche and Paulus, 2000; Paulus, 2011).

Altering brain functions with tDCS while simultaneously assessing those functions with neuroimaging is essential to determining whether and how tDCS affects brain functions. Modulation of sensorimotor function associated with tDCS has been previously investigated through several neuroimaging procedures (e.g., functional magnetic resonance imaging—fMRI, electroencephalographic—EEG spectral analysis, coregistration of transcranial magnetic stimulation, and EEG—TMS-EEG), to track the involvement of complex excitatory and inhibitory processes (Hunter et al., 2013). Moreover, changes induced by tDCS over several neurophysiological outcome measures have also been investigated (Stagg and Nitsche, 2011; Nitsche et al., 2008).

Methods directly probing the cortical activity changes underlying the polarity-induced effects of tDCS aim to investigate the overall correlations between specific neural network recruitment and behavioural changes (Shafi et al., 2012; Luft et al., 2014; Bestmann et al., 2014; Bortoletto et al., 2015a, 2015b). In order to achieve this goal, the study of cortical activity and connectivity—pre-, during, and post-tDCS—by means of EEG, operates as an important tool for correlating time-varying dynamic changes in brain connectivity/excitability with transient behavioral modifications (Keeser et al., 2011; Zaehle et al., 2011; Notturno et al., 2014). Recent studies have highlighted that different EEG measures (e.g., evoked potentials, event-related desynchronization/synchronization, and functional connectivity) can be used to probe the state of the cortical area stimulated by tDCS by adopting a multimodal approach that combines tDCS with EEG, both off- and online (e.g., Matsumoto et al., 2010; Polania et al., 2010; Pellicciari et al., 2013; for a review, see Miniussi et al., 2012). Specifically, tDCS has enabled the modulation, in a polarity-dependent manner, of local neural activity, altering ongoing brain activity in the frequency domain—with topographic dependency

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as a function of the stimulated sites (Antal et al., 2004; Spitoni et al., 2013; Mangia et al., 2014; Song et al., 2014).

The above results suggest the hypothesis that neuronal networks—as reflected by EEG activity across regional brain structures—could be modulated by tDCS (Luft et al., 2014). In this theoretical framework, the spectrum of EEG power density *per se* may not fully capture the modulation of functional neural connectivity. Nevertheless, more specific markers of functional neural connectivity can be derived by measuring the functional coupling of resting state EEG rhythms between pairs of electrodes. In fact, linear components of such coupling, functional coordination, and mutual information exchange can be evaluated by analyzing EEG spectral coherence (Gerloff et al., 1998; Gevins et al., 1998; Thatcher et al., 1986; Rappelsberger and Petsche, 1988). Spectral coherence is a normalized value that quantifies the temporal synchronization of two EEG time series between pairs of electrodes in the frequency domain of the oscillations. Its theoretical assumption is based on the observation that when the oscillatory activity of two cortical areas is functionally coordinated their EEG rhythms show a linear correlation and high spectral coherence. In general, decreased coherence either reflects reduced linear functional coupling and information transfer (i.e., functional uncoupling or unbinding) among cortical areas or reflects the reduced modulation of areas functionally bound by a third region. Conversely, an increase in EEG spectral coherence values can be interpreted as an enhancement of the linear functional connections and information transfers (i.e., functional coupling or binding), reflecting the interaction of individual cortical structures. Increased coherence in alpha or in faster EEG frequencies reflects a greater "facilitation," or functional connectivity. Meanwhile, increased coherence in the delta frequency suggests a greater "inhibition," or a functional disconnection. Pertinently, spectral coherence may reflect the integrity of cortical neural pathways (Locatelli et al., 1998). Previous EEG studies have reported a greater decrease of coherence for alpha rhythms and an increase for delta rhythms in cognitively impaired patients than in control subjects—as an effect of the brain network's disconnection (Cook and Leuchter, 1996; Jelic et al., 1997, 2000; Almkvist et al., 2001; Knott et al., 2000; Adler et al., 2003). Moreover, changes of motor cortex excitability, tested by TMS, can be predicted by evaluating the EEG fluctuations of the motor cortex connectivity patterns in the period preceding the delivery of the TMS pulse (Ferreri et al., 2014). It has also been demonstrated that EEG spectral coherence is enhanced following perceptive, cognitive, and motor processes in the cortical regions involved in task-related processing (Sauseng et al., 2005; Vecchio et al., 2007, 2010, 2012). This occurs as a function of the extension and type of the engaged neural networks (Pfurtscheller and Lopes da Silva, 1999; Von Stein and Sarnthein, 2000). In addition, recent studies introduced the concept of "total coherence," obtained by averaging the EEG spectral coherence across all combinations of electrode pairs (Tecchio et al., 2003, Babiloni et al., 2010, 2014).

The present work is the logical sequel of a previous study that addressed the hypothesis of specific cortical excitability modulations induced by the different polarity of tDCS (e.g., Pellicciari et al., 2013). It also stems from studies showing that anodal tDCS alters ongoing brain EEG activity during resting state, in the alpha band rhythm (e.g., Spitoni et al., 2013). Here, we tried to demonstrate that tDCS induces brain network modulation, particularly pertaining to the functional coupling of the alpha rhythm, moving from the evidence of a statistically significant influence of time-varying and spatially patterned synchronization of EEG rhythms in determining cortical excitability (Ferreri et al., 2014). Moreover, the activity of pyramidal cortical neurons, which contribute to the excitability levels of the related neuronal assemblies, can be inferred by EEG scalp characteristics, such as spectral frequency profiles, topographies of various rhythms, and the phase coherence of the EEG oscillations (Ferreri et al., 2011a, 2011b, 2012; Klimesch et al., 2007; Neuper and Pfurtscheller, 2001).

Given these premises, the choice to investigate the coupling of the sensorimotor areas via EEG recording during the resting state was based on the idea that this coupling might predominately involve the alpha rhythm. Therefore, the main aim of the present study was to test the hypothesis that a specific tDCS current polarity affects the functional coupling of sensorimotor areas during the eyes-open resting condition, as revealed by the total EEG coherence (i.e., coherence across the average of all combinations of electrode pairs placed around the scalp stimulation electrode; this allows for the observation of a global modulation of coupling in the considered network) recorded before (pre), during, and after (post) anodal and cathodal stimulation.

Materials and methods

Subjects

Eighteen healthy participants took part in the study. Three participants were excluded from the analysis due to excessive noise in the EEG recording during tDCS. The remaining fifteen (seven males and eight females) had a mean age of 23.2 ± 3 years. The inclusion criteria were as follows: no history of neurological, psychological, or other relevant medical diseases and no consumption of CNS-active medication at the time of the experiment. The study was approved by the Ethics Committee of IRCCS Centro San Giovanni di Dio, Fatebenefratelli, Brescia, Italy, and written informed consent was obtained from all participants before the experiment.

Experimental design

Each participant took part in two experimental sessions, during which anodal and cathodal tDCS were delivered, respectively. The order of tDCS polarity conditions was counterbalanced among participants. The two experimental sessions were conducted on the same day (with a 4-h break between the two tDCS conditions), and the schedule was kept constant across participants to control for potential circadian effects (Sale et al., 2007). Fig. 1 shows the experimental protocol.

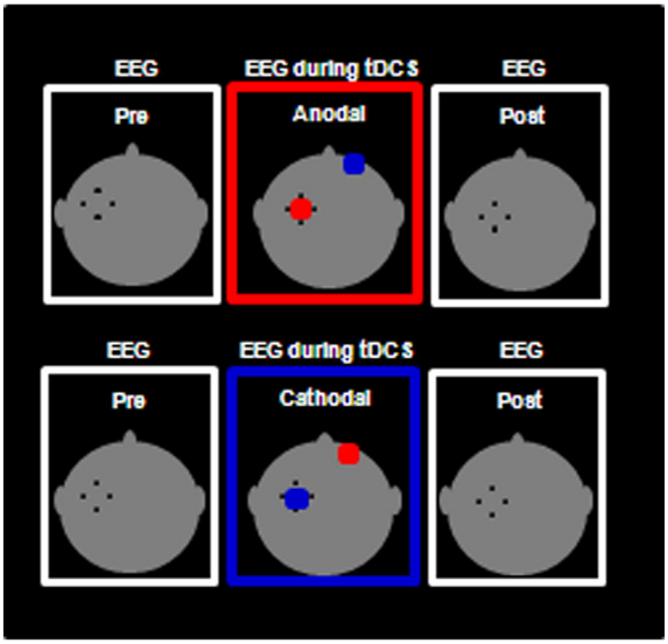

Fig. 1. Experimental procedure. Each experimental session consisted of an EEG block before (3 min), during (13 min), and after tDCS (3 min). Each block consisted of a EEG activity recording during a resting state with eyes open. Anodal and cathodal tDCS were applied to the left primary motor cortex in separate sessions, on the same day (with a 4-h break between the two tDCS conditions). Direct current stimulation (1 mA) was given through two large-sized electrodes placed (25 cm^2) over M1 and the contralateral frontopolar cortex.

The cortical state was evaluated by recording EEG activity for each experimental session before, during, and after anodal/cathodal tDCS. The EEG session before and after tDCS (pre- and post-tDCS) comprised three minutes of recording during a resting state, with eyes open. During the recording, the participants were seated on a comfortable armchair in a soundproof room and were instructed to passively sit while focusing upon a visual target directly in front of them. The EEG recording during tDCS lasted for 13 min, although the first and last minutes were excluded from the analysis because of excessive artifacts induced by the stimulation.

tDCS

The stimulation was delivered by a battery-driven electrical stimulator (NeuroConn GmbH, Ilmenau, Germany) through a couple of conductive rubber electrodes with a surface area of 25 cm^2. The electrodes, placed inside saline-soaked sponges, were fixed with a rubber band placed over the electrode, and an electro-conductive gel was applied under the saline-soaked sponges to reduce contact impedance. The rubber bands reduced electrode impedance and prevented the electrodes from moving during the experiment. The impedance was kept below 10 kΩ. The active electrode was placed over the left motor cortex: specifically, the tDCS electrode was fixed over the representational field of the right first dorsal interosseous (FDI), as previously identified by TMS. Specifically, we targeted the left motor cortex using single TMS pulses and evaluated the motor-evoked potentials (MEPs). A standard figure-of-eight-shaped coils with an outer winding diameter of 70 mm connected to a Magstim Super Rapid magnetic stimulator (Magstim Company, Whitland, UK) was used. The coil was placed tangentially to the scalp, the handle pointing backwards and laterally, about a 45° angle from the mid-sagittal axis of the participants' heads, and was oriented to elicit a posterolateral–anteromedial current flow in the brain tissue. The stimulation began at a supra-threshold intensity. The optimal stimulus site to elicit MEPs in the right FDI, termed the “motor hotspot,” was identified by positioning the coil approximately over the central sulcus and moving it on the scalp by 0.5 cm steps on the left M1. The hotspot was then marked directly on the scalp with a soft-tip pen. On this site, the RMT was assessed as the lowest stimulus intensity needed to produce a response of at least 50 μV in amplitude in the relaxed muscle for at least five out of ten consecutive stimulations, at a resolution of 1% of the maximal stimulator output (Rossini et al., 1994, 2015). The reference electrode was placed over the right frontopolar cortex and was oriented so that it was approximately parallel to the central sulcus and the eyebrow. For anodal and cathodal stimulation, the current was delivered with an intensity of 1 mA (current density 0.04 mA/cm^2) for 13 min, with a ramping period of eight seconds both at the beginning and at the end of the stimulation. The participants were blind to the tDCS conditions as highlighted by the questionnaire about sensations experienced during the two stimulations and completed at the end of each tDCS stimulation (Fertonani et al., 2010). No differences between the sensations experienced during the anodal and the cathodal tDCS were reported, as shown by the non-parametric Wilcoxon rank sum tests (all ps > 0.18).

EEG recordings

EEG equipment (BrainAmp 32MRplus, BrainProducts GmbH, Munich, Germany) was used to record the EEG, by means of eight Ag/AgCl sintered ring electrodes. Unipolar EEG derivations were recorded from the scalp electrodes. Four electrodes were functionally positioned around the cortical motor hotspot in each orthogonal direction at a 3-cm distance (anteriorly, posteriorly, laterally to the left, and laterally to the right). Four additional electrodes were positioned over the right hemisphere, mirroring the contralateral setup. The ground electrode was placed in the mid-occipital (Oz) position. The right mastoid served as a reference for all electrodes. Recordings obtained from the left mastoid electrode were used offline to re-reference the scalp recordings to the average of the left and the right mastoids, i.e., including the implicit reference (right mastoid) into the calculation of the new reference. Horizontal and vertical eye movements were detected by recording the electrooculogram (EOG) to monitor participant behaviour online and to reject trials with ocular artifacts offline. The bipolar horizontal EOG was recorded from electrodes placed about 1 cm from the medial and lateral canthi of the dominant eye, and the bipolar vertical EOG was recorded from electrodes located about 3 cm above and below the right pupil. The EEG and the EOG signals were acquired with a band-pass filter at 0.1–1000 Hz and digitized at a sampling rate of 5 kHz using a 16 bit A/D converter. Skin/electrode impedance was kept below 5 kΩ. Data were analyzed offline with dedicated software (Brain Vision Analyzer, Brain Products GmbH, Munich, Germany). The EEG data, which included the data analyzed during tDCS, were collected in a previous experiment; more details of experimental method can be found in Pellicciari et al., 2013.

Preprocessing of the EEG data

Data were analyzed with Matlab R2014b software (Math Works, Natick, MA), using scripts based on the EEGLAB 13.4.4b toolbox (Swartz Center for Computational Neurosciences, La Jolla, CA; http://www.sccn.ucsd.edu/eeglab).

The EEG recordings were band-pass filtered from 0.1 to 47 Hz using a finite impulse response (FIR) filter and were down-sampled at 512 Hz. Imported data were fragmented in two s duration epochs, identifying and extracting visible artifacts in the EEG recordings (i.e., eye movements, cardiac activity, and scalp muscle contraction) using an independent component analysis (ICA) procedure. ICA is a blind source decomposition algorithm that enables the separation of statistically independent sources from multichannel data. It effectively separates ocular movements and blink artifacts from EEG data (Hoffmann and Falkenstein, 2008; Iriarte et al., 2003; Jung et al., 2000). ICA was performed using the Infomax ICA algorithm (Bell and Sejnowski, 1995), as implemented in the EEGLAB. Noise artifacts resulting from both resting EEG and simultaneous tDCS–EEG were dealt with via ICA. Due to the low number of electrodes and the length of recordings, we eliminated up to two components for each subject and not more than the 20% of the data trials. In fact, as a general rule, it is important to give ICA as much data as possible for successful training. Finding *N* stable components (from N-channel data) typically requires more than *kN*^2 data sample points (at each channel), where *N*^2 is the number of weights in the unmixing matrix that ICA is trying to learn (as reported in the Matlab site http://sccn.ucsd.edu/wiki/Chapter_09:_Decomposing_Data_Using_ICA).

Coherence analysis

EEG spectral coherence is a normalized measure of the coupling between two EEG signals at any given frequency (Rappelsberger and Petsche, 1988). The coherence values were calculated at each frequency bin as

$$\mathrm{Coh}_{xy}(\lambda) = \left|R_{xy}(\lambda)\right|^2 = \frac{\left|f_{xy}(\lambda)\right|^2}{f_{xy}(\lambda) f_{yy}(\lambda)}.$$

The numerator contains the cross-spectrum of two EEG signals, *x* and *y* (fxy), for a given frequency bin (λ), while the denominator contains the respective autospectra for *x* (fxx) and *y* (fyy). The coherence value (Cohxy) is obtained by squaring the magnitude of the complex correlation coefficient R. This procedure produces a real number between 0 (no coherence) and 1 (max coherence).

As mentioned above, the global functional coupling of the EEG rhythms was indexed via spectral coherence for all combinations of electrode pairs, namely the total coherence (Babiloni et al., 2010, 2014).

The main steps for the computation of the total coherence were as follows: (i) For each subject, the spectral coherence was calculated for each pair of channels from all artifact-free EEG trials. The frequency bands of interest were delta (2–4 Hz), theta (4–8 Hz), alpha 1 (8–10 Hz), alpha 2 (10–13 Hz), beta 1 (13–20 Hz), beta 2 (20–30 Hz), and gamma (31–40 Hz). (ii) For a given frequency band of interest, the coherence value for any EEG electrode was estimated as the average of the coherence values between that electrode and each of the other electrodes ("electrode coherence"). (iii) For a given frequency band of interest, the "electrode coherence" of the four electrodes was averaged to form the total coherence.

Statistical analysis of the coherence values

Statistical analysis was aimed at evaluating the working hypothesis that the total coherence of the resting state cortical EEG rhythms, over the stimulated cortex, is modified in magnitude by different tDCS polarities. Total coherence values were considered as a dependent variable for the ANOVA design. A preliminary ANOVA was made to evaluate possible differences between the pre-stimulation periods, including the factors (levels) stimulation (anodal, cathodal) and band (delta, theta, alpha 1, alpha 2, beta 1, beta 2, gamma). The main statistical analysis included two ANOVAs. In the first one, factors (levels) were stimulation (anodal, cathodal), time (Pre, tDCS, Post), and band (delta, theta, alpha 1, alpha 2, beta 1, beta 2, gamma). In the second one, we focused only on the effect of the modulation before and after the stimulation: factors were stimulation (anodal, cathodal), time (Pre, Post), and band (delta, theta, alpha 1, alpha 2, beta 1, beta 2, gamma). Mauchly's test evaluated the sphericity assumption. The degrees of freedom were corrected through the Greenhouse–Geisser procedure. The Duncan test was used for post hoc comparisons ($p < 0.05$).

Results

The preliminary ANOVA showed no statistically significant differences between the two baselines ($F(6,84) = .09$; $p < 0.9968$), suggesting that the obtained results could be attributed to the stimulation, despite the lack of a sham stimulation condition.

In the first main ANOVA, the results showed a statistically significant effect on the main factor: time ($F(2,28) = 5.82$; $p < 0.0077$). The planned post hoc analysis indicated that the total coherence values were very similar in the pre- and post-tDCS conditions, but coherence was reduced during tDCS. This result was independent of the kind of stimulation and the frequency bands, underlining a similar trend during both anodal and cathodal tDCS.

The second main ANOVA design showed a statistically significant interaction (Fig. 2, $F(6,84) = 2.15$; $p < 0.05$) that included all factors. Duncan-planned post hoc testing showed that no differences were observed in the two pre-tDCS periods (on the left of the figure), while increased connectivity was observed in alpha 2 post-anodal tDCS with respect to both pre-cathodal ($p < 0.006$) and post-cathodal tDCS ($p < 0.00006$).

Discussion

tDCS can modify the membrane excitability of neuronal cells with a prolonged, low-intensity, electric current (1–2 mA) delivered through a pair of electrodes placed on the scalp (Paulus, 2003). The direction of changes in excitability is determined by the current direction flow and the polarity. Anodal tDCS results in a membrane depolarization (excitation), whereas cathodal tDCS induces hyperpolarization (inhibition) under the stimulating electrodes (Nitsche and Paulus, 2001, 2000; Nitsche et al., 2003).

In the present study, we used EEG recordings to measure online and offline cortical changes induced by anodal or cathodal tDCS. The aim was to evaluate the tDCS-induced effects on the functional coupling of the sensorimotor cortical networks, during the eyes-open resting state. To this end, we tested the hypothesis that the tDCS current polarity could affect total EEG coherence (i.e., coherence across the average of all combinations of the electrode pairs placed in the stimulated sensorimotor areas reflecting global modulation of coupling in the considered network). The first interesting finding was that the EEG activity during tDCS (online) was affected by the stimulation irrespective of its polarity and frequency. The second result was that after anodal tDCS (offline), there was a significant increase in the high alpha total coherence localized over the sensorimotor area.

Regarding the first result, the online effect of a global reduction of coupling in the considered network could be related to the fact that neither the anodal nor the cathodal stimulation applied during the resting state activate any specific differential network detected by the recording electrodes beneath the stimulation site. Nevertheless, it should be noted that the EEG recording during tDCS was affected by a high number of artifacts, due to the current flow (Soekadar et al., 2013). This was especially true for those electrodes close to the stimulation site. Therefore, it is possible that the change in coherence during stimulation is in part a consequence of this artifact. Specifically, the reduction of coupling during stimulation could be justified if the artifact's component was eliminated using ICA. This would mean that the content of the signal could have been impoverished.

Regarding the second offline finding, which was specific to alpha frequency, it is difficult to believe that tDCS (a constant current) is brought about by an artifact that induces frequency-specific coherency changes. Moreover, a decrease in the total coherence after cathodal stimulation was not observed, in contrast to the effects of anodal tDCS. This anodal-specific result could be related to selective excitability mechanisms underlying the tDCS-induced changes in neural activity. In this respect, it should be remembered that scalp EEG is mainly derived from the post-synaptic potentials of cortical pyramidal cells and that pioneering experiments have shown that anodal cortical hyperpolarization of pyramidal cells' apical dendrites induces their depolarization more easily than cathodal stimulation (Livingston and

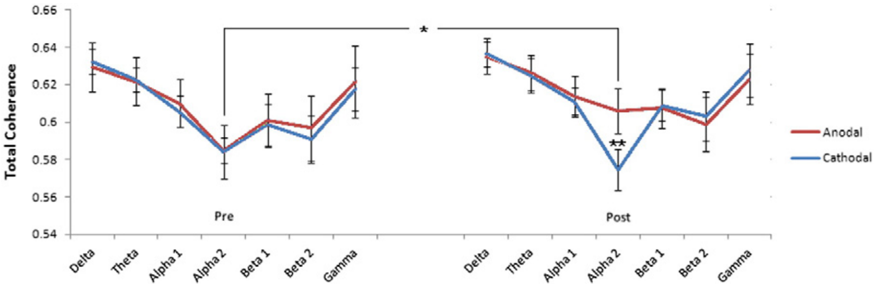

Fig. 2. Mean ± standard error relative to the ANOVA interaction ($F(6,84) = 2.15$; $p < 0.05$) between all factors stimulation (anodal, cathodal), time (Pre, Post), and band (delta, theta, alpha 1, alpha 2, beta 1, beta 2, gamma). $^{*}p < 0.006$; $^{**}p < 0.00006$.

Phillips, 1957; Hern et al., 1962; Ranck, 1975; Rossini et al., 1985). Moreover, given that continuous stimulation engages neurophysiological homeostasis mechanisms, which serve to maintain neural activity within a normal functional range (Siebner et al., 2004; Miniussi et al., 2013), it can be considered that the cortical excitability level of the sensorimotor network is not downgrading further its basal activity after cathodal stimulation. Our findings are supported not only by pioneering experimental data (Livingston and Phillips, 1957; Hern et al., 1962; Ranck, 1975) but also by recent studies in which selective effects of anodal stimulation were observed on cortical activity (Notturno et al., 2014; Spitoni et al., 2013), counteracting the dichotomy that anodal tDCS enhances cortical excitability and cathodal stimulation reduces it (Nitsche and Paulus, 2000). This result complements the finding that some cathodal montages fail to effectively modify behavioral outcomes in perceptual and cognitive tasks (Jacobson et al., 2012).

The present findings also agree with recent literature reporting an increase of alpha band after anodal tDCS with no increment after cathodal as explained in the following referenced papers. They also agree with studies reporting a higher beta coherence in the sensorimotor cortex underlying anodal tDCS (Notturno et al., 2014). Furthermore, a previous study has demonstrated that, in the resting brain, anodal tDCS alters ongoing brain activity, specifically in the alpha band rhythm (Spitoni et al., 2013). These changes may be due to the strengthening of synaptic connections in functionally linked cortical areas modulated by anodal tDCS, inducing changes in the functional architecture of the whole sensorimotor network. Such an effect should result in a local modulation of membrane polarization, as well as a long-lasting synaptic modification induced by tDCS over the sensorimotor cortex.

In fact, the setting of the stimulating electrodes (anode over dominant primary motor cortex, cathode over contralateral supraorbital region) might also suggest an electric path flowing through midline structures in the frontal lobe, including the supplementary motor area and the anterior cingulate cortex (Notturno et al., 2014). The coupling modulation of brain activity found here could also be caused in part by the direct stimulation of these brain areas, which play an important role in the planning, initiation, and execution of motor acts as well as in attention.

While low-frequency alpha rhythms (about 8–10 Hz) are supposed to reflect the regulation of global cortical arousal (Klimesch, 1999; Pfurtscheller and Lopes da Silva, 1999), there is consensus that the high-frequency alpha rhythms (10–13 Hz) reflect the activity of thalamo-cortical and cortico-cortical loops. Such activity should facilitate or inhibit the transmission and retrieval of sensorimotor information into the brain (Brunia, 1999; Klimesch, 1999; Pfurtscheller and Lopes da Silva, 1999; Steriade and Llinás, 1988) and reflect the oscillation of specific neural systems for information processing (Klimesch, 1996, 1999).

An involvement in the alpha rhythm after the application of anodal tDCS, as observed in our study, has been reported in other recent works. Alpha frequency modulations, in terms of an increase in the peak alpha frequency (Amatachaya et al., 2015) and an increase in alpha frequency power (Pellicciari et al., 2013; Spitoni et al., 2013; Mangia et al., 2014), have been reported during and post-anodal stimulation, emphasizing the role this band rhythm plays as a cortical marker of the neuromodulatory effects induced by tDCS.

The absence of a sham condition could represent a limitation of the experimental paradigm. However, if one assumes that the total coherence changes induced by tDCS were not merely a placebo effect, and that participants were blind to the current polarity stimulation condition (as reflected by no differences between the sensations experienced during the anodal and cathodal tDCS), and that EEG epochs immediately following the tDCS of different polarities were compared with those immediately preceding the stimulation, the lack of a sham condition does not seem to be of remarkable importance. In order to exclude possible difference in the baseline EEG recordings before anodal and before cathodal stimulation, we compared the baseline (pre-tDCS) period in both stimulations. The resulted analysis showed no statistical difference between the two baselines in the evaluated parameters, suggesting that the tDCS sessions performed on the same day did not affect each other. Nevertheless, the inclusion of a sham condition could help determine the effects of stimulation polarity. It should be stressed that the low number of electrodes, localized over the stimulated cortex, does not allow us to reveal fine topographic intrahemispheric effects or explore long-distance coupling (for example, by including fronto-parietal or latero-lateral connections) on the other side. Nevertheless, it should be emphasized that the aim of the present study was to test the neuromodulation effect over the stimulated hemisphere; further studies should evaluate the modulation of the intrahemispheric coherence of the non-stimulated hemisphere and interhemispheric coherence.

In conclusion, the results of our study suggest that a specific approach based on the functional coupling revelation of EEG rhythmic oscillations could be used to evaluate the effect of tDCS. This study could also be considered as a starting point to explore this coupling modification effect in tandem with a novel approach that includes graph theory (Bortoletto et al., 2015a, 2015b; Vecchio et al., 2014a, 2014b, 2014c). Finally, as suggested by previous literature (e.g., Notturno et al., 2014), the present results may support tDCS in clinical practice, particularly in cerebrovascular disease or stroke recovery, by increasing and thus strengthening synaptic connections among functionally related areas in the motor cortical network.

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None of the authors have potential conflicts of interest to be disclosed.

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