

Assessing cortical synchronization during transcranial direct current stimulation: A graph-theoretical analysis



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ABSTRACT

Transcranial direct current stimulation (tDCS) is a neuromodulation technique that can alter cortical excitability and modulate behaviour in a polarity-dependent way. Despite the widespread use of this method in the neuroscience field, its effects on ongoing local or global (network level) neuronal activity are still not foreseeable. A way to shed light on the neuronal mechanisms underlying the cortical connectivity changes induced by tDCS is provided by the combination of tDCS with electroencephalography (EEG). In this study, twelve healthy subjects underwent online tDCS-EEG recording (i.e., simultaneous), during resting-state, using 19 EEG channels. The protocol involved anodal, cathodal and sham stimulation conditions, with the active and the reference electrodes in the left frontocentral area (FC3) and on the forehead over the right eyebrow, respectively. The data were processed using a network model, based on graph theory and the synchronization likelihood. The resulting graphs were analysed for four frequency bands (theta, alpha, beta and gamma) to evaluate the presence of tDCS-induced differences in synchronization patterns and graph theory measures. The resting state network connectivity resulted altered during tDCS, in a polarity-specific manner for theta and alpha bands. Anodal tDCS weakened synchronization with respect to the baseline over the fronto-central areas in the left hemisphere, for theta band ($p < 0.05$). In contrast, during cathodal tDCS a significant increase in inter-hemispheric synchronization connectivity was observed over the centro-parietal, centro-occipital and parieto-occipital areas for the alpha band ($p < 0.05$). Local graph measures showed a tDCS-induced polarity-specific differences that regarded modifications of network activities rather than specific region properties. Our results show that applying tDCS during the resting state modulates local synchronization as well as network properties in slow frequency bands, in a polarity-specific manner.

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Introduction

Transcranial direct current stimulation (tDCS) is a neuromodulation technique that alters cortical excitability by means of weak direct currents. Experiments in animals (Bindman et al., 1964; Purpura and McMurtry, 1965; Brunoni et al., 2011) and humans (Nitsche and Paulus, 2000) have shown that the use of such currents induces polarity-specific effects that are not strictly restricted to the stimulated site (Stagg et al., 2009; Stagg and Nitsche, 2011; Polania et al., 2011a, 2011b; Keeser et al., 2011; Pellicciari et al., 2013). These findings suggest

that neuronal mechanisms underlying the described effects should involve synaptic strength changes and possibly the modulation of synaptic connectivity, leading to a viable way to induce changes in excitability (Stagg and Nitsche, 2011) and, therefore, the functionality of specific networks. Several review studies have been published demonstrating some possible applications of this neuromodulator technique (e.g., Bestmann et al., 2014; Driver et al., 2009; Miniussi et al., 2013; Zaghi et al., 2009). However, it is still not clear how tDCS induces neural activity alterations, especially at the network level. Because electrical stimulation affects large areas of the brain cortex, tDCS-induced cortical modification should result in a complex combination of both local and global excitation/inhibition phenomena (Borchers et al., 2012). To try to explain these alterations and to gain new insight into these mechanisms, tDCS and electroencephalographic (EEG) recordings have been used in sequential offline (Miniussi et al., 2012), and online tDCS-EEG approaches (e.g., Accornero et al., 2014; Baxter et al., 2014; Mangia et al., 2014), or in combination with other techniques (Hunter et al.,

Abbreviations: tDCS, transcranial direct current stimulation; EEG, electroencephalography; SL, synchronization likelihood; ICA, independent component analysis.

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2013), such as transcranial magnetic stimulation (TMS) (Pellicciari et al., 2013; Romero et al., 2014), magnetoencephalography (e.g., Garcia-Cossio et al., 2016) and electromyography (EMG) (Dutta et al., 2014). Until now, studies using EEG have shown how the tDCS induces polarity-specific on brain activity oscillations in different frequency bands (Kirov et al., 2009; Miller et al., 2015; Notturmo et al., 2014; Song et al., 2014; Spitoni et al., 2013; Ulam et al., 2014). However, a network approach could be used to obtain a better understanding of the ongoing effects induced by neuromodulation (Bortoletto et al., 2015; Luft et al., 2014) and to capture brain functions in a multi-dimensional manner (i.e., from a network perspective). Considering that perceptual, motor and cognitive processes are built from complex patterns of activity sustain by brain circuitry, analysing the tDCS induced effects at the network level is more promising than doing so only at the level of specific brain regions.

Using a network based approach could allow us to track the ongoing functional connectivity changes induced by tDCS during both spontaneous neural activity and task activity. If we consider the brain as a graph, in case of functional brain networks the nodes represent regions, while edges represent the presence and the strength of functional connections between different nodes (i.e., areas). As a result, graph theory can be applied to analyse the relation between nodes via edges using EEG data, by means of specific connectivity measures (for a review see, Deco et al., 2015). In this perspective, measures derived from graph theory can be useful for characterizing the tDCS-induced modulation on brain systems topology.

The first attempt that was made to characterize offline tDCS in terms of connectivity patterns was the work by Polania and co-workers (Polania et al., 2011a, 2011b); however, these attempts could be further expanded using the entire mathematical framework provided by graph theory (Bullmore and Sporns, 2009), that is, using graph theoretical measures as neurophysiological markers to characterize functional connection. In several studies (Pijneburg et al., 2004; Stam et al., 2005), synchronization likelihood (SL) has been proposed as a way to capture the spatiotemporal interactions between brain signals, and, therefore, to describe the strength of the mutual information between recorded signals. Therefore, such measures have been used in the context of network analysis (Cao et al., 2014; Kim et al., 2013). Neuroimaging has greatly benefitted from the integration of graph theory to describe the properties of complex networks; using the same approach in this context will help to better characterize tDCS-induced effects at the level of the functional network and allow a more accurate cortical targeting of neuromodulator protocol (for a review, see Sale et al., 2015). Moreover, investigating the dynamic connectivity changes during tDCS could allow assessing online the neural correlates of behavioural outcomes (for a review, see Luft et al., 2014).

Starting from this scenario, the aim of this study was to investigate the induced effects of anodal and cathodal tDCS by means of network measures, using SL as an interdependency measure. To achieve this goal, co-registered tDCS-EEG (simultaneous) was acquired from healthy subjects during the resting state, and it was compared to baseline EEG. Such signals were pre-processed, filtered in the canonical frequency bands, and used to build connectivity matrices, to use graph theoretical processing. In order to explore online polarity-dependent changes in the functional connectivity between different brain regions, a network approach was used to evaluate how the tDCS affects the functional coupling at specific frequencies. We hypothesized that tDCS could modulate the synchronization pattern of specific brain rhythms within the active brain networks of the resting human brain.

Materials and methods

Subjects

Twelve healthy volunteer subjects (nine females, three males, mean age: 29.9 ± 3.8 years) took part in the study. All subjects were right-

handed according to the Edinburgh handedness inventory test (Oldfield, 1971). None of the participants had a history of neurological or other relevant medical disease, or were on CNS-active medication at the time of the experiment. All subjects were informed of the experimental procedures and provided written informed consent before the experiment. The experimental protocol was performed in accordance with the safety procedures for non-invasive brain stimulation (Poreisz et al., 2007; Fertonani et al., 2015), and it was approved by the Ethics Committee of IRCCS Centro San Giovanni di Dio, Fatebenefratelli, Brescia, Italy.

Experimental design

The principal aim of the experiment was to determine tDCS-induced polarity-dependent effects on ongoing cortical activity and connectivity, in terms of electrophysiological changes. The experimental protocol involved three EEG recording sessions of 7 min, separated by 15 min from one another (see Fig. 1). Anodal, cathodal and sham stimulations were administered with the order of the stimulation conditions balanced between subjects.

During each session, the EEG signals were registered for 3 min before applying tDCS and co-registering EEG signals for 4 min; both recordings were performed during an eyes-open resting condition. Fig. 1 shows the experimental protocol. For a qualitative evaluation of the stimulation residual effects (offline), an EEG recording session of 3 min was acquired from six subjects right after the end of each stimulation session. During the experiment, the participants were seated in a comfortable chair in a Faraday cage, soundproofed room. To thoroughly define the features of the artefacts in terms of their frequency content and surface distributions, the same protocol was applied in a phantom experiment, using a phantom head made with a cantaloupe melon (Politte et al., 2010; Veniero et al., 2009).

EEG recordings

EEG equipment (BrainAmp 32MRplus system, Brain Products GmbH, Munich, Germany) was used to record continuous EEG activity before and during tDCS. EEG signals were recorded from 19 cortical sites (Afz, F7, F3, F4, F8, FC5, FC1, FC2, FC6, T7, C3, C4, T8, P7, P3, P4, P8, O1, O2), positioned according to the 10–20 International System, using sinterized Ag/AgCl-coated electrodes mounted on an elastic cap (EasyCap GmbH, Herrsching, Germany). Additional electrodes were used as the ground and the reference. The ground electrode was placed in the mid-occipital position, and the common reference was placed over the right mastoid. Recordings obtained from the left mastoid electrode were used off line to re-reference the scalp recordings to the average of the left and the right mastoid. Horizontal and vertical eye movements were detected by recording electrooculogram (EOG) signals, to monitor subjects' behaviour on-line and to reject off-line trials with ocular artefacts, such as blinks. The EEG and EOG signals were acquired with a band-pass filter at 0.1–1000 Hz. All signals were digitized at a sampling rate of 5 kHz using a 16-bit A/D-converter. The skin-electrode impedance was kept below 5 k Ω .

tDCS

During the data acquisition, tDCS stimulations (anodal, cathodal and sham) were administered with a battery-driven DC stimulator (BrainStim, EMS, Bologna, Italy) at current intensity of 0.6 mA, using an active electrode with an area of 9 cm² placed over FC3, according to the 10–20 International System, with a resulting current density of 0.067 mA/cm². The return electrode, with an area of 30 cm², was placed on the forehead over the right eyebrow. In the anodal and cathodal sessions, the current was delivered for 240 s, including two ramping periods of 8 s at the beginning and at the end of the stimulation. During the sham session, the electrodes were located in the same positions as

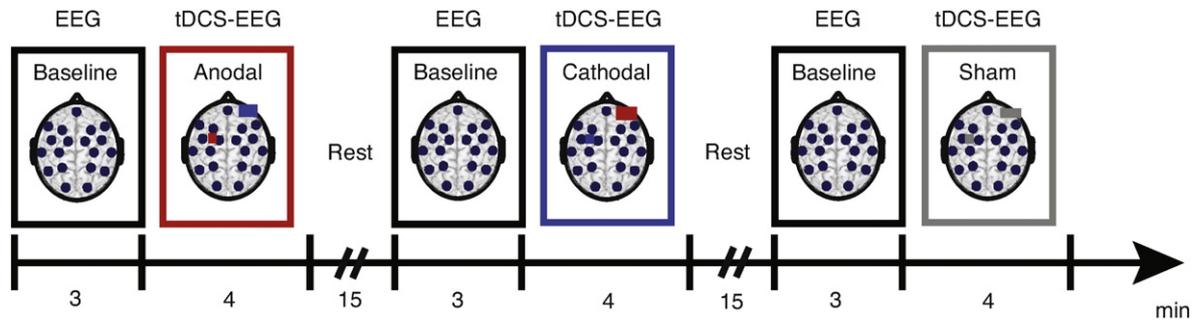


Fig. 1. A schematic representation of the experimental procedure. Three electroencephalography (EEG) recording sessions were made for each subject, separated by resting phases of 15 min. In each session, baseline EEG was recorded for 3 min before acquiring co-registered transcranial direct current stimulation (tDCS)-EEG for 4 min. The order of the stimulation conditions (anodal, cathodal, sham) were balanced between subjects and were randomly administered to each subject.

in the active stimulations, but the current was supplied for the first 8 s ramp up, 10 s of stimulation, 8 s ramp down and 230 s of pause. This procedure ensured that the subjects felt the tingling sensation at the beginning of the stimulation. At the end of the experimental protocol, the participants completed a questionnaire about sensations experienced during each stimulation session to evaluate whether the tDCS caused any discomfort (Fertonani et al., 2015).

Data pre-processing

The acquired EEG signals were pre-processed using MATLAB and the EGLAB toolbox (Delorme and Makeig, 2004). First, data were re-referenced to the mastoid channels, then they were low-pass filtered using a cut-off frequency of 250 Hz, resampled from 5000 Hz to 250 Hz and band-pass filtered between 2 and 48 Hz to exclude very low-frequency artefacts and line noise. For tDCS-EEG co-registered signals, the first and the last segments were rejected because of high amplitude noise and saturation phenomena due to the current ramping up and down. Independent Component Analysis (ICA) was applied to remove ocular artefacts. After the removal, eye channels were discarded, reducing the dataset for each subject to the 19 cephalic channels, modelled by 19 independent components (ICs). The phantom data were pre-processed in the same way. To define the spectral characteristics and the spatial distribution of tDCS-related non-physiological artefacts, the anodal and cathodal stimulation data retrieved from the phantom were compared with the sham ones using ICA (for a detailed explanation see supplementary material). In this way, artefact-related ICs were identified in terms of affected electrodes and frequency content. Such ICs were therefore identified in subjects' data and removed. EEG was then segmented into 2 s baseline-corrected epochs, where the baseline was estimated using the first 50 samples of each epoch. Artefact epochs (with excessive drift or muscle artefacts) were identified by visual inspection and then removed. Finally, artefact-free data were band-pass filtered to obtain signals related to four different frequency bands (theta: 4–8 Hz, alpha: 8–12 Hz, beta: 12–30 Hz, gamma: 30–40 Hz). Before data analysis, the power spectrum density for the whole dataset was calculated in order to compare every stimulation condition (anodal, cathodal and sham) with each other. In this way, we could assess residual noise differences between conditions (details are available in the Supplementary material).

Synchronization likelihood

Among the measures of functional connectivity, we chose to use SL which is capable to estimate both linear and non-linear dependencies between the EEG time courses of different brain areas. Specifically, SL was computed for each frequency band using the first 10 artefact-free epochs for each subject and between all pairs of channels during each

tDCS session. SL was chosen due to its capacity to detect both linear and non-linear coupling between signals (Stam and van Dijk, 2002; Montez et al., 2006; Rosales et al., 2014). The methods for the calculation of SL as well as the choice of its parameters are reported in the supplementary material. A 19x19 network matrix was obtained for each frequency band, for each subject and for each session. In this way, the EEG synchronization patterns were represented as graphs, formed by nodes (19 electrodes) and edges (SL values between channels).

Network analysis

Functional connectivity changes were evaluated comparing SL network matrices at each frequency band. The comparisons were made between baseline and co-registered EEGs in all the stimulation conditions (anodal, cathodal and sham). As in previous network study (Kim et al., 2013), significant differences were determined using network-based statistics (NBS) (10,000 permutations, $p < 0.05$), which is more powerful than conventional mass-univariate testing (Zalesky et al., 2010). The same approach has been used to compare the baseline and post-stimulation EEGs as well as examine differences directly between anodal and cathodal stimulation conditions, each of them normalized by the respective baseline. Further analysis was conducted using the Brain Connectivity Toolbox to perform graph theoretical analysis in terms of global and local measures (Rubinov and Sporns, 2010). Using global measures, we took into account the whole network, describing the integration and segregation network capabilities (Bullmore and Sporns, 2009). Therefore, characteristic path length, mean clustering coefficient, and small-worldness were computed. Normalization was carried out on the clustering coefficient and characteristic path length using random graphs. While using local measures that related to specific nodes and characterized their network centrality, nodal degree, betweenness centrality and local efficiency were computed (for a detailed explanation see Supplementary material). Because comparing networks composed of a different number of edges could lead to spurious differences due to different network topologies (Van Wijk et al., 2010), different thresholds were used to binarize the connectivity matrices to compare graphs with a fixed network density (i.e., number of connections). The range for density was chosen to be between 0.1 and 0.9, with a range step of 0.05. To obtain scalar values for each measure, the area under the curve (AUC) was evaluated over the density range. Significant differences were defined using permutation testing (10,000 permutations, $p < 0.05$) and false discovery rate (FDR) for taking into account multiple comparisons in the case of local measures (Benjamini and Yekutieli, 2001). The statistical analysis is explained in more details in the supplementary material. Finally, in order to take into account spurious effects due to volume conduction phenomena, we reanalysed the data using a different synchronization measure, the debiased weighted phase lag

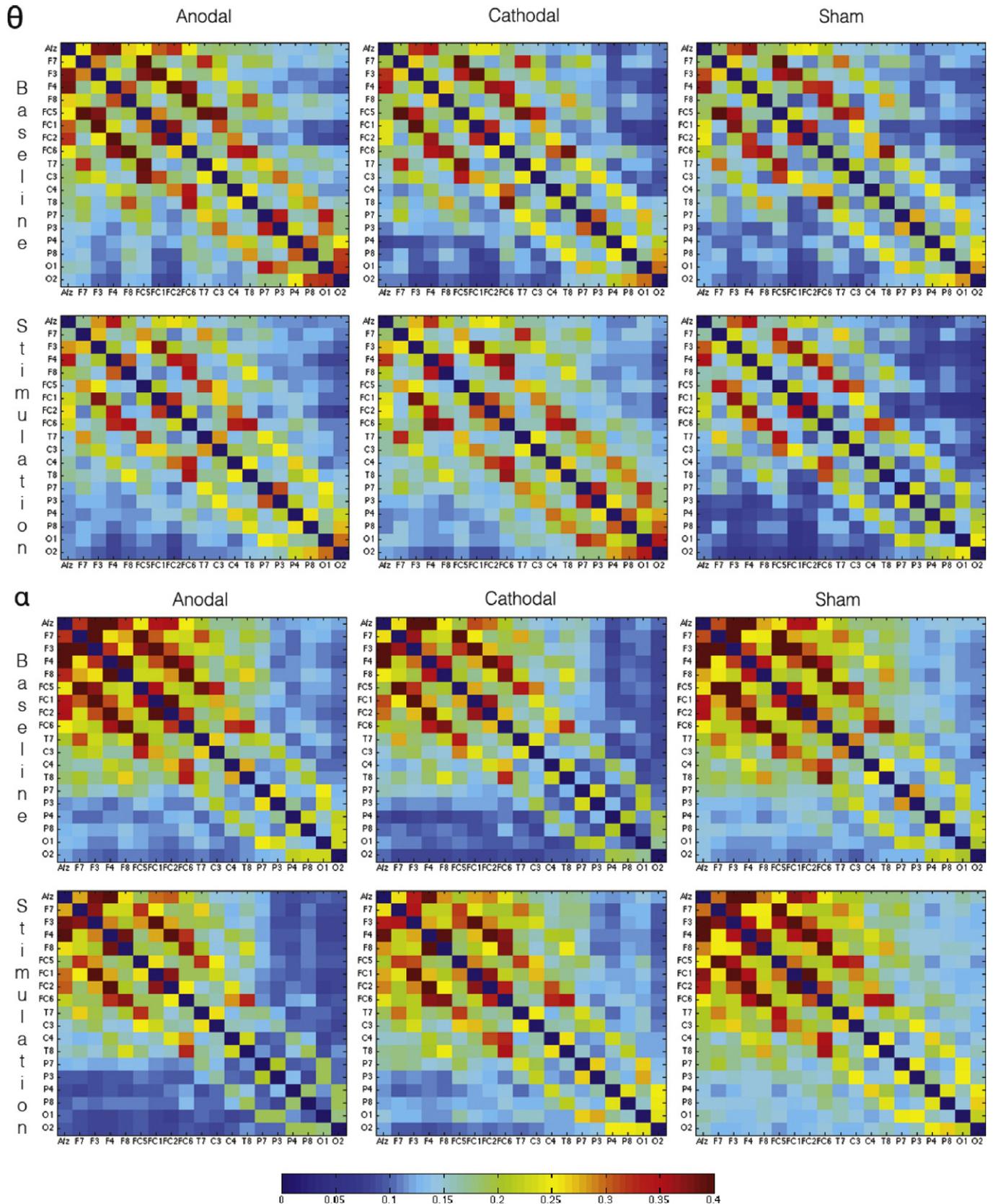


Fig. 2. Mean synchronization likelihood (SL)-based connectivity matrices for all the subjects in the theta and alpha bands during the baseline (first row: theta, third row: alpha) and the stimulation (second row: theta, fourth row: alpha) for all stimulation conditions (anodal: first column, cathodal: second column, sham: third column).

index (dWPLI) (see supplementary material), that has shown reduced sensitivity to this type of artefacts (Vinck et al., 2011).

Results

No adverse effects or discomforts were reported by the participants during the tDCS sessions. Moreover, none of the subjects were able to determine whether they had received real (anodal or cathodal) or sham tDCS.

As a result of the first processing step, SL-based connectivity matrices were obtained. Fig. 2 shows the mean matrices for all of the subjects in the theta and alpha frequency bands, comparing the baseline and the stimulation SL values in all three of the stimulation conditions (anodal, cathodal and sham).

The altered functional networks in terms of synchronization are represented in Fig. 3 in all three of the stimulation conditions respectively for the theta, alpha and gamma frequency bands. No functional connections were significantly altered in the other bands.

During anodal stimulation, the resting state network connectivity was significantly modulated in the theta band. A general decrease in the left-hemispheric synchronization with respect to the baseline was highlighted. Specifically, weakened functional connections were located over the frontal and central areas (FC5-F7, FC5-AFz, FC5-F3 and FC5-C3; $p = 0.0435$). No synchronization change was highlighted in the other frequency bands.

In the case of cathodal stimulation, a significant increase in inter-hemispheric synchronization connectivity was observed over the centro-parietal, centro-occipital and parieto-occipital areas for the alpha band (C3-P8, C3-O1, O1-P4 and O1-O2; $p = 0.0410$).

Finally, in the sham stimulation, theta and alpha bands did not show any significant modulation; whereas the gamma band showed a desynchronized intra and inter-hemispheric network over the left centro-parietal areas (FC1-P7, FC2-P7, C4-P7, P7-P4, P7-P8, FC2-C4, FC2-T8, C4-P4, C4-P3, C3-C4 and FC1-C4; $p = 0.0239$).

The comparison between baseline and post-stimulation data using NBS showed no significant alteration in all the stimulation conditions and frequency bands. On the other hand, the direct comparison between cathodal and anodal conditions revealed a significant synchronized network in the alpha band and mainly included fronto-occipital and parieto-occipital connections (FC2-FC6, F3-T7, FC5-P7, T7-P7, Afz-

O1, F7-O1, F4-O1, F8-O1, FC1-O1, FC2-O1, FC6-O1, T7-O1, C3-O1, C4-O1, P7-O1, P3-O1, P4-O1, P8-O1, C3-O2, P7-O2, P3-O2, O1-O2, $p = 0.0024$).

Using connectivity matrices as a network representation, global and local measures were calculated. Global measures in terms of AUC are reported in the supplementary material. No significant differences were observed in this case or in the other two stimulation conditions.

Regarding the local measures, as shown in Fig. 4, a significant increase in the nodal degree (i.e., number of connections) was observed during the cathodal stimulation condition with respect to the baseline, located in right hemisphere over the central area within the theta band ($p = 0.0011$, FDR-corrected). Although other nodes showed different degree mean values as well, these differences did not reach significance because of their higher variances. No significant differences were observed for the sham condition, or for the other frequency bands. The other local measures, betweenness centrality and local efficiency, were not altered by the stimulations.

Notably, the dWPLI-based analysis did not show significant differences when comparing baseline and stimulation sessions across all the stimulation conditions.

Discussion

This study aimed to evaluate the ongoing (i.e., online) effects of short periods of anodal and cathodal tDCS on the functional connectivity of a resting-state brain network. Specifically, our aim was to explore tDCS polarity-dependent changes in the network dynamics and underlying cortical activity during rest. To achieve this goal, a network model based on graph theory was used on EEG data. The tDCS-induced effects by means of altered cortical connections and graph theoretical measures were quantified. The results show that both anodal and cathodal stimulation affected mainly the low (theta and alpha) frequency EEG bands, modulating specific cortical connections in intra- and inter-hemispheric networks. Considering that the resting state patterns of brain connectivity are the result of robust and specific intrinsic neural activity (Cabral et al., 2014; Deligianni et al., 2014), our results highlight that tDCS is able to affect selectively the spontaneous neuronal fluctuations, modulating the dynamics of the brain at rest. Although tDCS is able to change network properties, it doesn't seem to affect the topological organization of the brain activity at a global level.

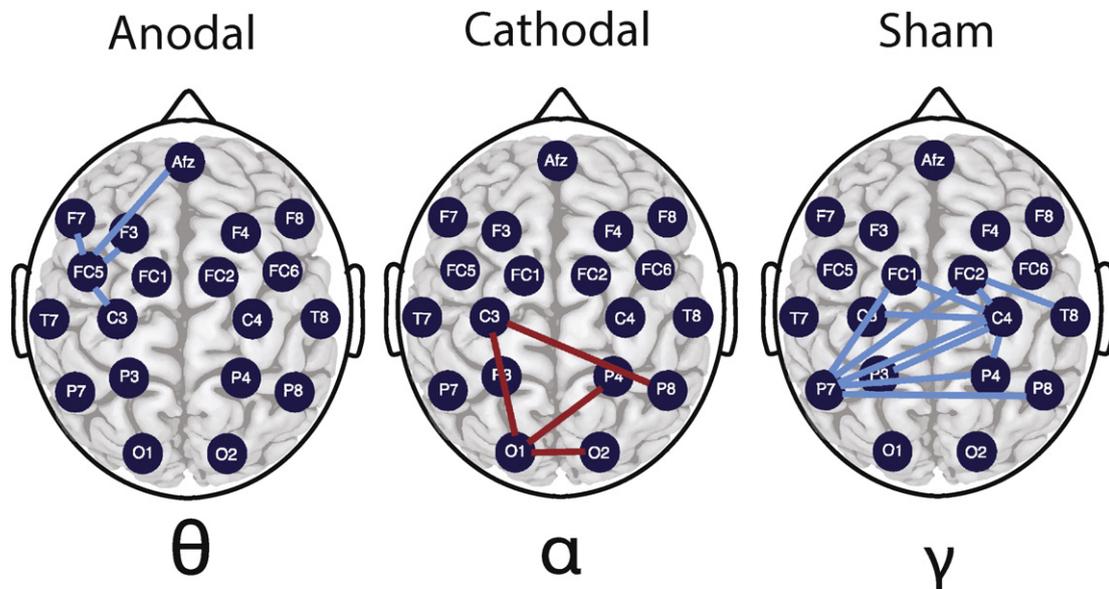


Fig. 3. EEG networks that showed significant changes in synchronization during the tDCS (red: synchronization; blue: desynchronization) for each stimulation condition (anodal, cathodal and sham) respectively at theta, alpha and gamma frequency band. No significant changes were observed in other bands.

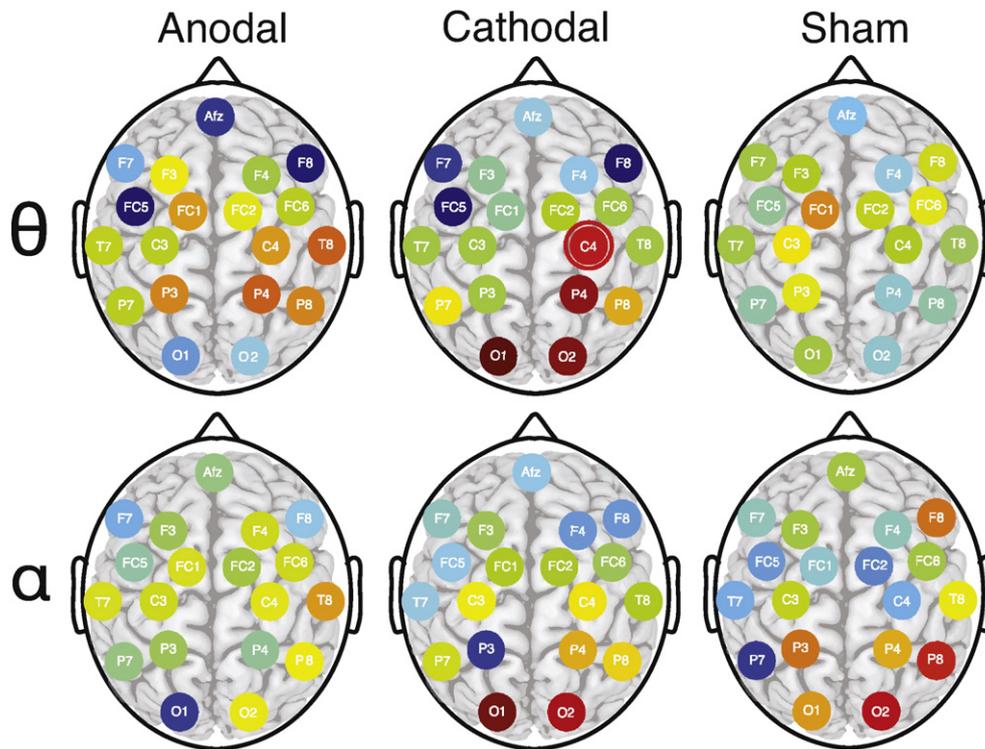


Fig. 4. Distribution of the mean AUC degree percent variation across EEG channels in the theta (first row) and alpha (second row) bands for the three stimulation conditions (anodal: left, cathodal: centre, sham: right). Circled channels showed a significant variation between the degree distribution comparing baseline and stimulation ($p < 0.05$, FRD-corrected). Although other nodes showed different degree mean values as well, these differences did not reach significance because of their higher variance.

During anodal tDCS, the observed theta band alterations affected mainly the synchronization patterns in the left hemisphere, by a decrease in the fronto-central region. It is well established that tDCS alters the cortical neuronal firing rate, in a polarity-specific manner, specifically in terms of increased activity after anodal stimulation (Bindman et al., 1964; Purpura and McMurtry, 1965). Such an increase was actually coupled with decreased connectivity in the slow rhythms (i.e., theta), which could be related to local and global cortical excitability alterations (Mangia et al., 2014; Ulam et al., 2014). Although it is well established that theta rhythms increase during active movement and that theta-enhanced transmission across brain regions may be important for several different functions, such as facilitating the transfer of information from one brain region to another during different types of information processing (for a review, see Colgin, 2013), the role of theta frequency in the resting state condition is not clear. In this regard, we hypothesize that theta synchronization decrease observed during anodal tDCS tracks the dynamics underlying the functional connectivity patterns, not only related to the stimulated area but also to the areas involved in resting-state brain networks (Luft et al., 2014). In a specific manner, anodal tDCS could have reduced these resting state connections, changing the interplay between excitatory and inhibitory synaptic mechanisms (Sehm et al., 2012, 2013). Future studies should investigate the behavioural correlates of these functional connectivity modulations for understanding their functional roles (Luft et al., 2014).

Changes in connectivity were highlighted also during cathodal stimulation, although with a different modulation pattern. tDCS induced an increased synchronization over the centro-parietal, centro-occipital and parieto-occipital areas in the alpha band, in both hemispheres. These results showed that different tDCS polarities elicit different effects on functional connectivity in terms of frequency and spatial characteristics, involving more widespread changes during cathodal and more confined effects during anodal stimulation. Indeed, the topography of the

involved functional connectivity in the alpha frequency suggested that tDCS caused changes in the cortical functional networks in a polarity-specific manner, probably through different - but still unclear - neuronal mechanisms (Stagg and Nitsche, 2011).

In general, these findings support the fact that tDCS is able to modulate, in a polarity-specific manner, the functional brain resting state network connectivity (Alon et al., 2011; Sehm et al., 2012, 2013), and it emphasizes the role of neuronal synchronization/desynchronization, in the slow frequencies, that are related to the ongoing activity.

In the sham session, a decrease in synchronization was observed in the gamma band over centro-parietal regions in both hemispheres. This result is consistent with other studies where similar effects have been described for the gamma band as results of sham stimulation (Polania et al., 2011a, 2011b; Hoy et al., 2015).

Looking at the direct comparison between the anodal and cathodal stimulation conditions, we can see a synchronization effect on a distributed network that includes several cortical regions. This effect highlights the difference in terms of modulation between the two effective stimulation conditions specifically in the alpha band. However, from this comparison we can't tell if such network is driven by the cathodal, or anodal stimulation, or by both.

As shown by graph theoretical global measures, such as clustering coefficient and characteristic path length, no significant changes were observed for the different stimulation conditions or frequency bands. This means that tDCS does not alter the global topological characteristics, not affecting the synchronization of the whole brain network, but instead acts on specific regions congruently with the subject state, as it results from the local measures. We observed a significant increase in the nodal degree for the right central region in the theta band range during cathodal stimulation. Because the degree of a node is the number of its connections in respect to the rest of the network (for a review see,

Bullmore and Sporns, 2009), in this case it represents a change in the number of areas that are synchronized with the contralateral site, in relation to the stimulated area (i.e., the right central node). In this regard, we hypothesize that the increased connectivity induced by cathodal tDCS over the contralateral involved inter-hemispheric inhibitory mechanisms. Considering that nodal centrality increases are a symptom of an altered local topology (Bullmore and Sporns, 2009), these results could be taken as proof of the link between tDCS and local alteration of specialized processing (i.e., functional segregation). In this case, the central increase is the result of several synchronization phenomena. Considering the link between cortical function and neural synchronization, we can speculate that cathodal stimulation causes a site-specific decrease in the functional segregation of the brain. Moreover, even if we did not observe other significant changes, the degree variation of distribution shows a different pattern when comparing the three stimulation conditions in the alpha and theta bands. This seems to underline the polarity-dependent modulation of the synchronization mechanisms. However, from the absence of other significant changes we can also speculate that tDCS acts on the synchronization patterns more than on the region properties. In this perspective, the best way to explore the effects of the stimulation seems to be the connection-wise comparison of the brain networks using NBS.

Taken as a whole, these ongoing tDCS-induced connectivity effects are in line with previous results on inter-hemispheric changes (Pellicciari et al., 2013) and resting-state brain connectivity modulations after tDCS (Luft et al., 2014; Bortoletto et al., 2015), highlighting that the polarity-specific effects of tDCS on cortical connectivity at rest include not only the stimulated area but also the functionally connected areas.

Other local measures such as betweenness centrality and local efficiency do not show significant differences induced by tDCS protocols. This suggests that both anodal and cathodal stimulation conditions do not affect the brain region synchronization in terms of synchronization paths, at least when the subject is at rest. Because these two local measures are related to the shortest paths related to the examined node, possibly there are no reasons because such measures should change when the network is not required to increase its performance to solve a task. These results have some points in common with previous online tDCS-EEG studies (Mangia et al., 2014; Roy et al., 2014), as well as with offline tDCS-EEG studies (Polania et al., 2011a, 2011b; Pellicciari et al., 2013).

Finally, as it is shown in the supplementary material, we did not observe any significant effect on the power spectral density in the whole dataset. Nevertheless our results could appear in partial disagreement with some previous studies in which it has been shown that tDCS increased the frequency power (Ardolino et al., 2005; Zaehle et al., 2011; Notturmo et al., 2014; Roy et al., 2014). It is important to bear in mind that slight differences in the reported frequency changes could be due to different cortical sites and protocols of stimulation, and among all on the ongoing cerebral activity during recording. Moreover, to the best of our knowledge, the present study is the first that evaluates the ongoing oscillatory activity during tDCS by means of a graph theoretical approach.

A possible limitation of this study is the low number of participants evaluated. However, the use of an experimental design in which the same participants were subjected to all the stimulation conditions, which were randomized and balanced between subjects, should have reduced any confounding effects. A further limitation could be the application of different stimulation protocols (anodal and cathodal), one after the other, with only a 15 min interval break. Though, we did not find significant changes between the baseline and the post-stimulation EEGs, and we avoid the interactions on cortical oscillatory activity using a randomized order during the protocol. Moreover, the use of short stimulation protocols at relatively low intensities might have been less effective in changing cortical activity than protocols that use higher intensities. Nevertheless, previous work has shown that

such protocols should be effective in modulating cortical activity (Nitsche and Paulus, 2000), and our results from this study confirm the effectiveness.

However, for a correct interpretation of the results we also need to take into account the results observed in the dWPLI-based analysis. Using such index, we were not able to observe differences between baseline and the stimulation conditions. Because EEG is sensitive to the volume conduction phenomena, we have taken into account the chance that the observed synchronization patterns could reflect common sources activity rather than coordinated neuronal population one. Nevertheless, although indices based on phase lag take into account volume conduction effects (Stam et al., 2007; Vinck et al., 2011) while SL does not, present results show long connections modulation effects and at this regard previous studies reported that volume conduction effects are negligible for electrodes separated by at least 2 or 4 cm (Doesburg et al., 2005; Lachaux et al., 1999; Nunez et al., 1999). We also did not observe a diffused pattern of local synchronization changes, as we could expect if we were measuring common source activity. We could speculate that such differences may be due to tDCS artefacts, but at the moment component analysis is still an effective approach to reduce such artefacts even if not totally effective (Noury et al., 2016; Roy et al., 2014). Moreover, the spatial distribution of the cathodal induced synchronization changes could hardly be attributed to a stimulation-related artefact. These considerations support the trustworthiness of present results and the potential of time domain approaches, as observed in different kind of analyses such as the EEG microstates (Khanna et al., 2015). The contrast between SL and dWPLI results could be explained keeping in mind the definition of the indices: SL implements a more general concept of synchronization and it's based on the simultaneous recurrence of patterns in time series, while dWPLI looks at the phase synchronization of the oscillations and therefore is based on the imaginary component of the cross-spectrum of the time series. Because SL allows some noncorrespondence in the waveforms of the time series, it seems able to better detect differences in synchronization patterns, as observed in previous EEG studies (Betzel et al., 2012; Kim et al., 2013). In any case, the disagreement between the two measures must be interpreted also as a caution signal towards the reliability of short connections modulation, and in this sense combining different measures is recommended. Previously, a combined phase-lag and SL study showed a large overlap between the two indices (Koenis et al., 2013), supporting the idea that SL is unlikely to be affected by volume conduction. Further studies are necessary to clarify the possibility of volume conduction effects on SL as well as to compare SL and phase lag indices.

In conclusion, our results underline that tDCS affects the topological organization of specific functional brain networks at specific frequency bands. From a neurophysiological point of view, we can only speculate that the low frequency band networks alteration could be due to a change in coupling of brain regions and might reflect changes in the signal to noise ratio of neuronal activity. According with previous evidence, we hypothesize that the local increase/decrease of spontaneous activity of a stimulated area (Bindman et al., 1964), induced by anodal and cathodal stimulation respectively, could decrease/increase the signal to noise ratio, and consequently decrease/increase the synchronization of the stimulated area with functionally connected brain regions (Polania et al., 2011a).

This study demonstrates that tDCS can change the ongoing network dynamics, and tDCS-EEG coregistration data can be analysed using graph theory to understand the induced effects of different polarities of stimulation. Moreover, connectivity patterns and graph theoretical measures show consistent results and could lead to a better characterization of brain activity patterns in terms of synchronization or desynchronization. The combination of tDCS and EEG recordings, applied not only during the resting state network activity but also in active neuronal networks, might represent an interesting and innovative approach to trace and modulate in vivo cortical excitability and effective

connectivity. Finally, the findings of this study highlight the graph theoretical approach as a method to evaluate and quantify tDCS-induced effects in terms of modulated functional networks in disease-related neuronal dysfunction.

Conflicts of interest

None.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <http://dx.doi.org/10.1016/j.neuroimage.2016.06.003>.

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