Dynamic modulation of intrinsic functional connectivity by transcranial direct current stimulation

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Abstract. Transcranial direct current stimulation (tDCS) is a non-invasive brain stimulation technique capable of modulating cortical excitability and thereby influencing behavior and learning. Recent evidence suggests that bilateral tDCS over both primary sensorimotor cortices (SM1) yields more prominent effects on motor performance in both healthy subjects and chronic stroke patients than unilateral tDCS over SM1. To better characterize the underlying neural mechanisms of this effect, we aimed to explore changes in resting-state functional connectivity during both stimulation types. In a randomized single-blinded cross-over design, 12 healthy subjects underwent functional magnetic resonance imaging at rest before, during and after 20 minutes of uni-, bilateral and sham tDCS stimulation over SM1. Eigenvector centrality mapping (ECM) was used to investigate tDCS-induced changes in functional connectivity patterns across the whole brain. Uni- and bilateral tDCS over SM1 resulted in functional connectivity changes in widespread brain areas as compared to sham both during and after stimulation. Whereas bilateral tDCS predominantly modulated changes in primary and secondary motor as well as prefrontal regions, unilateral tDCS affected prefrontal, parietal and cerebellar areas. No direct effect was seen under the stimulating electrode in the unilateral condition. The time course of changes in functional connectivity in the respective brain areas was non-linear and temporally dispersed. These findings provide evidence towards a network-based understanding regarding the underpinnings of specific tDCS interventions.

Key words: centrality, graph-based analysis, non-invasive brain stimulation, primary sensorimotor cortex (SM1), resting-state fMRI
Introduction

Transcranial direct current stimulation (tDCS) is a non-invasive brain stimulation technique known to modulate cortical excitability in a polarity-specific manner (Nitsche et al. 2008). For example, anodal tDCS applied over the primary sensorimotor cortex (SM1) increases corticospinal excitability even beyond the stimulation period whereas cathodal tDCS decreases it (Nitsche and Paulus 2000). Studies using excitability measurements of the living human brain with transcranial magnetic stimulation (TMS) as well as pharmacological interventions suggested that an increase of excitability induced by anodal and a decrease of excitability induced by cathodal stimulation depends on changes in the neuronal membrane potential (Nitsche et al. 2003a; Nitsche et al. 2005). More specifically, anodal tDCS has been shown to result in a depolarization, while cathodal stimulation leads to a hyperpolarization of the resting membrane potential. Furthermore, at least for anodal stimulation a study using magnetic resonance spectroscopy provided evidence that anodal tDCS leads to locally reduced GABA while cathodal stimulation causes reduced glutamatergic neuronal activity with a highly correlated reduction in GABA (Stagg et al. 2009).

Based on these findings, the application of tDCS has re-emerged in the last decade as a tool to effectively modulate brain function. Until now, behavioural effects of tDCS have been extensively studied in motor control and motor learning (for review, please see (Reis et al. 2008)). For example, anodal tDCS delivered over SM1 has been consistently shown to transiently improve performance and/or learning of various motor tasks in both healthy subjects (Nitsche et al. 2003c; Stagg et al. 2011) and chronic stroke patients (Hummel et al. 2005; Lindenberg et al. 2010). Furthermore, when applied in multiple sessions on 5 consecutive days,
long-term improvements in a sequential pinch force task for up to 3 months were observed (Reis et al. 2009). These results, together with findings in animals studies showing that tDCS acts upon brain-derived neurotrophic factor (BDNF)-dependent synaptic plasticity, further strengthen its potential as an adjuvant tool in neurorehabilitation (Fritsch et al. 2010).

One important yet open question relates to the optimal arrangement of the tDCS electrodes in order to achieve maximum stimulation effects. In the motor domain, a commonly used tDCS setup consists of a unilateral anodal tDCS electrode over SM1 contralateral to the moving/learning extremity (unilateral tDCS), while the other electrode is applied to the contralateral supraorbital region. More recently, a new tDCS electrode arrangement, that uses simultaneous anodal tDCS of one SM1 and cathodal tDCS of the homologous SM1 (bilateral tDCS), yielded more prominent behavioural effects in healthy subjects during a finger sequence task (Vines et al. 2008) and lead to an improvement of the motor deficit in chronic stroke patients (Lindenberg et al. 2010). The more powerful effects of bilateral tDCS over SM1 have been assumed to be related to a more pronounced interference with interhemispheric information processing as compared to unilateral tDCS over SM1 (Vines et al. 2008). However, the exact underlying neural mechanisms still remain elusive and certainly require further investigation.

The concurrent use of neuroimaging techniques such as functional magnetic resonance imaging (fMRI) and non-invasive brain stimulation has the potential to uncover neural mechanisms of both uni- and bilateral SM1 tDCS effects as proposed for concurrent transcranial magnetic stimulation (TMS) and fMRI (please see (Bestmann et al. 2008) for review). Likewise, a number of studies investigated tDCS-induced changes of functional activation using both fMRI and positron emission tomography during performance of a motor task (Antal et al. 2011; Baudewig
et al. 2001; Holland et al. 2011; Kwon and Jang 2011; Lang et al. 2005; Venkatakrishnan and Sandrini 2011). Unlike task-evoked fMRI changes, resting-state fMRI (rs-fMRI) measures spontaneous fluctuations of the BOLD-signal in the absence of task engagement. These fluctuations are not random but temporally coherent, thus providing a measure of the brain’s intrinsic functional architecture (Fox and Raichle 2007). Recently, a longitudinal learning study provided compelling evidence that patterns of rs-fMRI are persistently modulated by a complex motor skill training over several weeks (Taubert et al. 2011). Furthermore, using unilateral tDCS over SM1, it was demonstrated that rs-fMRI measurements (pre-post design) are capable of depicting tDCS-induced after-effects on functional connectivity (Pena-Gomez et al. 2011; Polania et al. 2011).

In the present study, we aimed to investigate changes in intrinsic functional connectivity elicited by both unilateral and bilateral tDCS over SM1 during and after stimulation without any task engagement. Only recently, a first proof of concept study validated the technical feasibility of concurrent tDCS and rs-fMRI measurements (Alon et al. 2011). Here, the authors investigated changes in functional connectivity between both SM1 using a region-of-interest approach during short blocks of anodal tDCS (7 minutes) over right SM1. Despite a highly variable response to tDCS, most likely due to the small sample size of 5 subjects, the aforementioned study revealed a decrease in functional connectivity from the right to left SM1 during tDCS.

In this study we aim at extending these findings by various important factors: First, we aimed at tracking changes in functional connectivity during the course of 20 minutes of tDCS. This stimulation duration has been most commonly used in studies of motor behaviour and learning (Reis et al. 2009; Vines et al. 2008). Second, in order to obtain information regarding potential
aftereffects of the stimulation we continued scanning for further \(~15 \) minutes. Third, we compared two different stimulation setups (bilateral and unilateral tDCS over SM1) with sham stimulation to better understand the neurophysiological underpinnings. Fourth, we aimed at investigating the effects of both stimulation approaches on large-scale brain networks by using eigenvector centrality mapping (ECM). ECM is a graph-based measure for centrality in functional brain networks that attributes a value to each voxel in the brain such that a voxel receives a large value if it is strongly correlated with many other nodes that are themselves central within the network. Thus it allows for the exploratory tracking of changes in network architecture across the whole brain (Lohmann et al. 2010; Zuo et al. 2012).

Using this experimental setup, we tested the hypothesis that bilateral and unilateral tDCS over SM1 relative to sham results in differential time-dependent engagements of intrinsic functional connectivity networks in human subjects.

**Methods**

**Subjects**

We enrolled a total number of 12 healthy, young volunteers in the study (mean age 25.8 ± 3.2 SD; 4 female). All subjects gave written informed consent to participate in the experiment according to the declaration of Helsinki and the ethic committee of the University of Leipzig approved the study. Prior to participation, all subjects underwent a comprehensive neurological examination to screen for potential exclusion criteria. They were not taking any medication. Subjects that did not meet the protocol criteria and/or had contraindications for tDCS or MRI
measurements were excluded from participation. In each subject, handedness was assessed based
on the Edinburgh Handedness Inventory (Oldfield 1971). Patients reported their hand preference
(i.e., right, left, or ambidextrous) in response to 10 questions (e.g., Which hand do you use to
light a match? Use scissors? Write?). Responses to the 10 questions were converted to a laterality
quotient (LQ) using the formula (R-L)/(R+L)* 100. LQ scores thus might range from -100
(corresponding to strong left-handedness) to +100 (corresponding to strong right-handedness).
For our study, only moderate to strong right-handed subjects, e.g. subjects with an LQ of \( \geq +60 \)
\((92.08 \pm 11.64; \text{mean} \pm \text{standard deviation})\) were included (e.g. (Isaacs et al. 2006).

Experimental Design

Each subject participated in a total number of 3 sessions which comprised of concurrent tDCS
over SM1 and resting-state functional MRI (rs-fMRI) in a cross-over design. The only difference
between each session was the type of tDCS: either unilateral tDCS (with the anode placed over
the right SM1 and the cathode placed over the contralateral orbit), bilateral tDCS (with anodal
stimulation of right and cathodal stimulation of left SM1) or sham stimulation (here, the setup of
the unilateral or bilateral tDCS condition was randomly chosen). The order of the sessions was
randomized between and within subjects. Sessions were separated by at least one week to avoid
any carryover effects.

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tDCS was delivered by a battery-driven DC current stimulator (Neuroconn GmbH, Ilmenau, Germany) using a pair of electrodes in a 5x7cm saline-soaked sponge. The electrodes were manufactured to be compatible with the MR-scanner environment (Neuroconn GmbH, Ilmenau, Germany) and equipped with approx. 5 kΩ resistors in each wire to avoid sudden temperature increases due to induction currents from radio frequency pulses, as described previously (Antal et al. 2011). The electrode cables ran through the MR room and passed a radio frequency filter in the MR cabin wall in order to reduce potential artifacts during image acquisition. The cables were connected to a MR-compatible DC stimulator that was placed outside the scanner room. Two filter boxes (Neuroconn GmbH, Ilmenau, Germany) were placed between electrodes and stimulator.

Before MRI scanning, the electrodes were attached to the subject’s head using elastic bands. We deployed different electrode montages for each session in accordance with a previously published study (Vines et al. 2008). For unilateral right SM1 stimulation, the anode was centered over C4 according to the international 10-20 System while the cathode was attached to the forehead above the contralateral orbit. For bilateral SM1 stimulation, the anode was centered over C4 (corresponding to right SM1), while the cathode was centered over C3 (corresponding to the left SM1; please see also Figure 1A).

For all experimental conditions (uni-, bilateral tDCS over SM1 and sham), the current was increased in a ramp-like fashion over the first 30 seconds of stimulation to a maximum of 1mA eliciting a transient tingling sensation on the scalp. tDCS was delivered for 20 minutes in the uni- and bilateral tDCS conditions and for up to 30 s in the sham condition. During stimulation, a continuous monitoring of the impedance revealed no changes throughout the experiment.
current density at the stimulation electrodes at our maximum setting of 1 mA for uni- and bilateral tDCS over SM1 was 0.028 mA/cm². Total charge as expressed by current density × total stimulation duration (s) was 0.034 C/cm². Thereafter, currents were turned off slowly over a few seconds precluding sensory differences between conditions (Nitsche et al. 2003b). This strategy has been shown to be efficient in blinding of the procedure (Gandiga et al. 2006; Ragert et al. 2008).

**Scanning protocol**

FMRI data were acquired under eyes-closed condition on a Siemens Magnetom Tim Trio 3 Tesla scanner equipped with a standard 8-channel head coil. During each session, a total number of 6 blocks of echo-planar-imaging (EPI) were acquired with 200 whole-brain volumes each using the following parameters: acquisition matrix = 64 x 64, slice thickness = 3 mm (1 mm gap), voxel dimensions = 3x3x4 mm, 34 slices, TR = 2300 ms, TE = 30 ms, flip angle = 90°, bandwidth = 1825 Hz. The total time for each fMRI session was approximately 55 minutes. Before scanning, tDCS electrodes were attached to the scalp of each subject outside of the scanner room (see also tDCS procedures above). Subsequently, subjects were brought into the scanner room and one EPI sequence (duration ~7.6 min) was acquired before tDCS application (rs-fMRI at baseline). Subsequently, the respective tDCS condition (uni-, bilateral or sham tDCS over SM1) was started and applied for 20 minutes for the *verum* conditions during the next 3 blocks (total of 600 volumes, duration of ~23 min) followed by 2 additional EPI blocks (total of 400 volumes, duration of ~15.3 min) that were acquired directly after the stimulation (please also
see Figure 1B). The same procedure applied for the sham condition except that the tDCS was only delivered for approximately 30 s.

Preprocessing and statistical analysis of fMRI data

In brief, as described previously (Lohmann et al. 2010; Taubert et al. 2011), preprocessing of fMRI data was performed using LIPSIA (Lohmann et al. 2001) and included motion correction, bandpass filtering (1/90 – 1/10 Hz) and spatial smoothing (6 mm full width half maximum (FWHM) smoothing). Preprocessed data sets were registered into standard MNI152 (Montreal Neurological Institute) brain space and re-sampled to an isotropic voxel grid with a resolution of 3x3x3 mm. Eigenvector-centrality mapping (ECM) (Lohmann et al. 2010) was used to map changes in network architecture induced by tDCS. ECM is a graph-based method that aims to map the central hubs of functional connectivity networks. ECM specifically weights nodes based on their degree of connection within the network. It does so by counting both the number and the quality of connections so that a node with view connections to some high-ranking other nodes may outrank one with a larger number of mediocre contacts. Google’s “PageRank” algorithm is a variant of eigenvector centrality. Compared to other centrality measures, ECM is computationally fast and does not depend on a preselected set of nodes (Zuo et al. 2012). This measure may be applied to all voxels in the brain thereby avoiding any selection bias. Here, we performed voxel-wise analyses of rs-fMRI data. This requires in our study a large region-of-interest of ~63000 voxels covering the whole brain including the cerebellum, rendering other centrality measures, such as betweenness centrality, computationally intractable (Lohmann et al. 2010). ECM enabled us to obtain whole brain centrality maps and use them in a manner similar
to contrast maps obtained in standard regression analyses. Furthermore, ECM does not depend on a pre-specified threshold for correlation values and captures small-world characteristics of the human brain in contrast to other measures like e.g. degree centrality (Bonacich 2007; Lohmann et al. 2010). One of the strengths of ECM in comparison to other related analysis techniques (such as independent component analysis (ICA)) is that ECM is capturing the centrality of each voxel in a given network while methods such as ICA rather identify sub-networks on a whole brain level. Thus, only voxels changing their network belonging would be identified with ICA analyses. Based on this knowledge, we decided to use ECM instead of ICA in the present study.

Changes in eigenvector centrality of functional connectivity will be described as “eigenvector centrality changes” or “centrality changes in functional connectivity” for the sake of simplicity throughout the text.

After preprocessing, single-subject eigenvector centrality maps were computed for each condition (bilateral, unilateral and sham SM1 stimulation) and each scanning block (baseline, block 1-5). Subsequently, ECM maps were used for group level analysis using general linear regression. Z-maps were thresholded at $z > 3.3$ on a voxel-level. Furthermore, corrections for multiple comparisons were implemented at the cluster level using alphasim (cluster-significance $p < 0.05$, corrected), which is a cluster-size based Monte Carlo simulation (Forman et al. 1995).

Changes in eigenvector centrality during stimulation were analyzed as follows: Z-maps of the stimulation period (e.g. average of block 1, 2 and 3, please refer to Figure 1B) were contrasted against baseline for each condition separately and compared to sham, in line with a recently published study (Keeser et al. 2011): (bilateral $>$ baseline) $>$ (sham $>$ baseline); (unilateral $>$ baseline) $>$ (sham $>$ baseline).
In a next step, we performed an additional linear regression analysis (see above) comparing differences in eigenvector centrality between bilateral and unilateral tDCS over SM1 during stimulation (block 1-3): (bilateral > baseline) > (unilateral > baseline); (unilateral > baseline) > (bilateral > baseline).

The after-effects of the stimulation on eigenvector centrality were analyzed in a similar way by averaging block 4 and 5 contrasted with baseline for both conditions (bi- and unilateral tDCS over SM1) relative to sham. Subsequently, we performed stepwise comparisons for each block (block 1-5) against baseline compared to sham in order to detect potential dynamic changes in eigenvector centrality over time.

As an additional analysis step, differences in eigenvector centrality between stimulation conditions at baseline were analyzed by contrasting the baseline block of each condition (sham, unilateral, bilateral tDCS over SM1) with each other: baseline (sham) vs. baseline unilateral; baseline sham vs. baseline bilateral; baseline unilateral vs. baseline bilateral.

Results

Differences in Eigenvector Centrality between Conditions at Baseline

First, we performed baseline comparisons between conditions (bilateral, unilateral and sham tDCS over SM1). This analysis revealed difference in eigenvector centrality in several subcortical and cortical areas between the 3 stimulation conditions (Figure 2).
Changes in eigenvector centrality during bi- and unilateral SM1 stimulation

20 minutes of bilateral tDCS over SM1 (blocks 1-3) resulted in increased eigenvector centrality in networks that included motor-related regions such as right M1, dorsal premotor cortex (PMd) and bilateral SMA when compared to sham stimulation. Furthermore, also prefrontal regions were modulated, such as right superior frontal gyrus (SFG), inferior frontal gyrus (IFG) and left middle frontal gyrus (MFG).

In contrast, during unilateral tDCS, only left fronto-temporal and bilateral parietal areas showed a significantly increased centrality in functional connectivity as compared to sham stimulation. Furthermore, we found an increase within the right cerebellum (lobule VIIa), ipsilateral to the site of stimulation (p<0.001, corrected; please see Figure 3 and Table 1). Interestingly, no change in eigenvector centrality was found in the cortical area below the stimulating tDCS electrode: the right SM1.

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Differential effects on eigenvector centrality during bi- and unilateral SM1 stimulation

A direct comparison between eigenvector centrality changes during 20 minutes of bi- and unilateral tDCS over SM1 (block 1-3) revealed differential effects between both stimulation types. Bilateral tDCS over SM1 resulted in significantly larger eigenvector centrality changes predominantly in primary and secondary motor areas (including right M1, PMd and left SMA/pre-SMA), bilateral prefrontal areas (SFG) and subcortical regions as compared to unilateral tDCS over SM1. On the other hand, unilateral as compared to bilateral tDCS over SM1
resulted in significantly larger increases in right prefrontal (SFG), left parieto-temporal and subcortical areas including the globus pallidum (p<0.001, corrected; please see Figure 4, for details see Table 2).

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After bilateral tDCS over SM1 (block 4-5), we observed an increase of centrality in functional connectivity in motor-related brain regions such as right M1, PMd as well as bilateral SMA. Since these regions also showed a significant modulation during stimulation, this result indicates that the increase in eigenvector centrality in these regions persisted for at least 15 minutes after termination of stimulation. Furthermore, we observed additional alterations in bilateral prefrontal areas that developed after the stimulation period (p<0.001, corrected; see also Table 3 and Figure 5).

After unilateral tDCS (block 4-5) we observed an increase of eigenvector centrality within the right prefrontal cortex, left middle temporal lobe, right fusiform and middle temporal gyrus as well as bilateral cerebellum (p<0.001, corrected). Please see Table 3 for a detailed list of all clusters as well as Figure 5.

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Step-wise comparison of stimulation-induced connectivity changes over time versus baseline
We further assessed changes in eigenvector centrality in each single scanning block (see Figure 1) as compared to baseline which in turn enabled us to continuously track changes over time, e.g. during (block 1-3) as well as after (block 4-5) tDCS (for an overview please refer to Figure 6 A and B). As described above, bilateral and unilateral tDCS over SM1 resulted in a differential modulation of neuronal networks both during and after stimulation. Moreover, we observed diverse, non-linear patterns of changes in centrality of functional connectivity within different brain areas over time (see Figure 6). For example, bilateral SM1 tDCS condition resulted in a significant change in eigenvector centrality within the cluster of right M1 during the first stimulation block as compared to baseline. This effect decreased in block 2 and 3 and subsequently increased again in block 4 and 5 (after stimulation). In contrast, we observed a different pattern over time within right SFG. Here, a steady increase in eigenvector centrality during bilateral SM1 tDCS (block1-3) was followed by a decrease after termination of stimulation. Similar diverse patterns are observed in other brain areas for uni- as well as bilateral SM1 tDCS as shown in Figure 6 A and B. In general, these results disclose a temporally and spatially dispersed non-linear pattern of tDCS-induced centrality changes of whole brain functional connectivity for both conditions.

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**Discussion**

Here we provide novel evidence that tDCS over SM1 is capable of modulating functional whole-brain resting-state network connectivity during as well as after stimulation (Zheng et al. 2011). The experimental setup with concurrent tDCS and fMRI allowed us to continuously track tDCS-
induced effects on resting-state functional connectivity over time. We showed that bilateral tDCS over SM1 resulted in widespread connectivity changes such as in primary and secondary motor as well as prefrontal cortex. In contrast, unilateral tDCS over SM1 predominantly modulated functional connectivity in prefrontal, parietal and cerebellar areas. Furthermore we observed for both stimulation types differential effects not only during but also after tDCS that persisted for at least 15 minutes. The time course of changes in functional connectivity in the respective brain areas was non-linear and temporally dispersed.

The combination of non-invasive brain stimulation and modern neuroimaging techniques enables investigation of not only local but also global effects of tDCS on brain networks, e.g. by combining non-invasive brain stimulation and fMRI or EEG measurements (Bestmann et al. 2008; Kirimoto et al. 2011; Neuling et al. 2012). However, until now, only a small number of studies have investigated the effect of non-invasive brain stimulation protocols such as repetitive (r)TMS, (e.g. (van der Werf et al. 2010)) on resting-state networks. Even less is known regarding the effects of tDCS on resting-state functional connectivity. In a first proof-of-concept on 5 healthy subjects, the technical feasibility of concurrent tDCS and rs-fMRI measurements could be demonstrated (Alon et al. 2011). Data analysis was restricted to both SM1 (ROI approach) showing that a decrease in functional connectivity from the right to left SM1 was induced by 7 minutes of anodal tDCS delivered over right SM1. In the present study, we used ECM analysis to identify changes in functional connectivity on a whole brain level. The use of a centrality measure such as ECM is based on the assumption, that important brain regions (hubs) interact with many other regions and facilitate integrative processes (Rubinov and Sporns 2010). The neurobiological interpretation of this measure is that nodes with a high value are interacting
functionally with many other nodes in the network. Thus, changes in centrality represent reorganizational processes within this functional network architecture.

In contrast to other centrality measures such as betweenness or degree centrality, ECM is parameter-free, computationally fast and does not depend on prior assumptions (a priori information) (Lohmann et al. 2010). Previous studies commonly used an anatomical template of 90 regions-of-interest (Achard et al. 2006; He et al. 2009). However, we aimed to perform voxel-wise analysis with our functional data. This required a large region-of-interest of ~63000 voxels in our study which makes measures such as betweenness centrality computationally intractable. The computational speed of ECM enabled us to obtain whole brain centrality maps and use them in a manner similar to contrast maps obtained in standard regression analyses. Furthermore, in contrast to degree centrality, ECM does not depend on a pre-specified threshold for correlation values and captures small-world characteristics of the human brain which degree centrality does not (Bonacich 2007; Lohmann et al. 2010). This method has been used before to detect reorganizational processes in functional connectivity induced by complex motor skill learning (Taubert et al. 2011). In our study, the use of ECM was motivated by findings of concurrent fMRI-TMS experiments showing that non-invasive brain stimulation over SM1 does not only modify the BOLD signal locally within the stimulated or adjacent cortical regions but also in remote interconnected brain areas (Bestmann et al. 2004).

Other graph-theoretical analyses have been previously applied to investigate tDCS-induced neuroplastic changes. Using EEG (Polania et al. 2010) as well as fMRI (Polania et al. 2011), it has been demonstrated that tDCS evokes intra- and interhemispheric connectivity changes after 10 minutes of stimulation over left M1. These effects were not only seen over the stimulated M1
but also in bilateral frontal, parietal and premotor cortical regions (Polania et al. 2010). Furthermore, with the help of the higher spatial resolution in fMRI, it was demonstrated that 10 min of anodal tDCS over left SM1 increased short range connections from M1 to premotor and parietal cortical regions, while concomitantly increasing interconnectedness in prefrontal cortex in resting brain dynamics (Polania et al. 2011). Compared to our study, there are some essential differences regarding the stimulation setup: Polania and colleagues used unilateral anodal tDCS of the left SM1, while in our study anodal tDCS was applied over the right SM1. Furthermore, the stimulation duration differed remarkably between aforementioned studies (10 minutes in the study of Polania vs. 20 minutes in our study). Despite methodological differences in these studies, tDCS in general seems to modulate widespread changes not only in local but also distant brain areas.

Here, we further extend previous work on tDCS-induced brain network changes by investigating two important issues: First, we provide novel evidence regarding dynamic on-line effects on large-scale networks during 20 minutes of tDCS. We chose this timescale to be consistent with relevant studies investigating behavioral effects of tDCS, e.g. (Reis et al. 2009; Vines et al. 2008)). Second, we provide evidence regarding differential tDCS effects induced by uni- and bilateral tDCS over SM1.

Effects of tDCS on functional connectivity during stimulation

During bilateral tDCS over SM1, we detected increases within a cluster covering right M1/ PMd, e.g. the cortical area under the anodal electrode. Since a tDCS-related change in right M1/ PMd was only elicited by bilateral (right anodal and left cathodal) and not unilateral (only right
anodal) SM1 stimulation, it seems likely that not only the local facilitatory effect of the right anodal stimulation but also the additional cathodal (inhibitory) stimulation of the left SM1 contributed to this effect. As shown previously, cathodal tDCS leads to a decrease in corticospinal excitability most likely through a hyperpolarization of the resting membrane potential, and/or through a modification of synaptic efficacy (Nitsche et al. 2005; Stagg and Nitsche 2011). Thus it is tempting to speculate that down-regulation of left SM1 by cathodal tDCS results in a disinhibition of the interhemispheric inhibitory drive from the left to the right M1, which in turn causes the observed increase of eigenvector centrality of the homologous SM1. Alternatively, as shown recently in the somatosensory domain, a modulation of interhemispheric inhibitory interactions between primary somatosensory cortices might as well account for this effect (Ragert et al. 2011).

Since all subjects were right-handed, we cannot rule out that this effect might be due to a modulation of lateralized interhemispheric interactions between both M1 (Baumer et al. 2007). To elucidate this, further studies should investigate the effects of a converse stimulation setup.

Nonetheless our results raise the hypothesis that the previously reported superior effects of bilateral as compared to unilateral right SM1 stimulation (Vines et al. 2008) are at least partly mediated by a modulation of functional connectivity in the right primary motor cortex.

Apart from eigenvector centrality changes in right SM1, bilateral tDCS over SM1 resulted in significant changes in secondary motor areas such as the premotor cortex (right PMd) and SMA. Previous animal and human studies showed that both areas are tightly interconnected with the stimulated SM1 (Civardi et al. 2001; Strick et al. 1998). Therefore, bilateral tDCS over SM1 might also result in changes within interconnected brain areas which are reflected by ECM.
Similarly, remote effects of non-invasive brain stimulation in SMA have been successfully
identified using concurrent TMS over M1 and fMRI measurements (Bestmann et al. 2008).

In contrast to bilateral tDCS over SM1, no online-changes in eigenvector centrality were found
in SM1 or premotor areas during unilateral tDCS over SM1. The absence of unilateral tDCS-
effects in these areas was surprising and certainly requires further investigation. One important
aspect in our experimental design is the fact that in the unilateral SM1 tDCS condition, the right,
non-dominant motor cortex was target area of anodal tDCS. This stimulation setup is consistent
with the study by Vines and colleagues (Vines et al. 2008) that compared the effects of uni- and
bilateral tDCS over the motor cortex on motor performance. With our experimental design we
cannot rule out, that different effects on functional connectivity would be observed when tDCS
would be applied over M1 of the dominant (left) hemisphere (Nitsche et al. 2003c), an issue that
requires further investigation. In this vein, a recent study suggests, that the dominance of the
targeted motor cortex does differentially contribute to stimulation induced after-effects (Schade
et al. 2012). Another important point with respect to this experimental condition pertains to the
attachment of the cathodal electrode over the left supraorbital region. Using our – well
established - stimulation setup it is not unlikely, that also a modulation of frontal activity by this
electrode contributes significantly to changes in functional connectivity that we observed on
whole-brain level.

Apart from the divergent results in SM1 and secondary motor areas, we observed changes in
centrality in prefrontal areas during both uni- and bilateral tDCS over SM1. Studies in primates
suggested that the prefrontal cortex is involved in motor control such as context-dependent
movement selection (Matsumoto et al. 2003), supported by anatomical findings in macaques
showing multisynaptic connections between prefrontal and premotor/motor cortex (Miyachi et al. 2005). Nevertheless, it still remains elusive whether centrality changes in prefrontal areas are directly related to a modulation of SM1 or rather reflect a general effect of tDCS on the resting-state network *per se*. Furthermore, as discussed above, in the unilateral SM1 tDCS condition, it might be that the “reference” electrode attached to the contralateral supraorbital region also contributed at least partially to centrality changes within prefrontal areas that are in close spatial proximity to the electrode. At least for the bilateral SM1 tDCS condition it is reasonable to assume, that tDCS is capable of modulating the connectivity not only within adjacent, but also remote brain areas such as the prefrontal cortex.

Interestingly, only unilateral facilitatory stimulation over right SM1, but not bilateral tDCS with facilitatory right and inhibitory left SM1 stimulation induced an increase of centrality within the ipsilateral right cerebellum. Only recently it could be demonstrated that tDCS applied over the cerebellum modulates the overall inhibitory tone that exerts the cerebellum over the motor cortex (Galea et al. 2009). In our study it is tempting to speculate that the increase in centrality of the cerebellum during ipsilateral facilitation of SM1 might be mediated via facilitatory cerebrocerebellar interactions (Kelly and Strick 2003).

What is the potential meaning of the present study for future applications of tDCS in patient populations such as chronic stroke? The present data on healthy individuals certainly do not allow to speculate whether one or the other setup might be more efficient in motor rehabilitation (Hummel et al., 2005; Lindenberg et al., 2010), but might help to generate hypotheses for future studies. Given that we observed very different patterns of changes depending on the stimulation setup, it might be that patients who differ e.g. in their lesion location might also differentially
benefit from one stimulation setup or the other. Future studies need to address these questions in patient populations in order to identify benchmarks for the establishment of individualized adjuvant tDCS protocols in motor rehabilitation.

It remains noteworthy that a considerable part of the areas modulated by tDCS is not specifically related to motor planning or execution. Furthermore, in the unilateral SM1 stimulation condition we did not detect changes in eigenvector centrality within the stimulated cortex. Our results of changes in remote regions induced by tDCS are in line with previous studies combining TMS and fMRI or PET. Using this methodological approach, changes in activation (BOLD signal) in remote but interconnected regions have consistently been observed, even in the absence of significant changes in activity at the stimulation site (Bestmann et al. 2003; Bestmann et al. 2004; Bohning et al. 1999; Denslow et al. 2005). However, we applied well-established stimulation parameters (electrode size; stimulation intensity; stimulation duration; e.g. (Vines et al. 2008)). Thus we are confident that our results are, even though not specific to the motor system, specific to the tDCS conditions that were applied: bilateral and unilateral tDCS over primary sensorimotor cortices.

Dynamics of stimulation-induced centrality changes

Our study design using concurrent tDCS and high-resolution of fMRI enabled us to continuously track changes in functional connectivity not only during but also after stimulation in order to unravel dynamic processes of tDCS-induced neuroplasticity.
Here, covering both online- and after-effects of stimulation, we provide novel evidence that the pattern of tDCS-induced engagements of different neural networks is temporally dispersed. Previously, it has been suggested that neuroplastic changes after the application of non-invasive brain stimulation protocols do not necessarily appear directly after the stimulation but may arise with a temporal delay. For example, after paired-associative stimulation (PAS), a specific form of non-invasive brain stimulation, functional changes in corticospinal excitability have been reported to appear after an interval of 20 to 30 minutes post-intervention (Missitzi et al. 2011). The authors speculated that only after a latent interval the optimal strengthening of the synaptic efficacy might be consolidated and becomes apparent. Along these lines, it is tempting to translate this observation into the dynamic and diverse temporal onsets of functional resting-state changes as seen in our study. Along these lines, recent neuroimaging studies suggested that other plasticity-inducing interventions like motor sequence learning (Steele and Penhune 2010) or complex motor skill learning (Taubert et al. 2011) may not only result in steadily increasing (linear) brain alterations but at least to some extent also to diverse, non-linear dynamic changes within different brain areas.

Finally, the present study bears some limitations. First, our method (ECM) relies on resting-state measurements of the BOLD signal, thus we do not have a behavioral measure that could prove the relevance of our stimulation protocols. Hence, we cannot directly claim that the effects that we observed are linked to behavioral consequences of tDCS. However, we used an established and frequently tested stimulation setup known to improve motor performance and learning (e.g. (Nitsche et al. 2003c; Vines et al. 2008)). Here we aimed to study changes in functional connectivity elicited by these established stimulation protocols. Therefore, our study should be considered in line with previous studies that explore neurophysiological effects of tDCS in the
absence of a behavioral task (e.g. (Nitsche et al. 2003a; Nitsche and Paulus 2000; Polania et al. 2011; Zheng et al. 2011)). Second, by using rs-fMRI and a data-driven analysis approach (ECM), we certainly cannot claim to provide a complete picture of tDCS-induced connectivity changes. However, the scope of the present study was to obtain a global picture of tDCS-induced functional connectivity changes without hypotheses about special brain regions. To further elucidate the specific involvement of certain brain regions, such as the stimulated M1, more hypothesis-driven approaches should address these issues in future studies. Third, to avoid any potential bias from baseline differences in ECM between conditions (sham, bi- and unilateral tDCS over SM1) we normalized the ECM maps during and after tDCS against baseline. This analysis is in line with a recently published study investigating after-effects of tDCS over the dorsolateral prefrontal cortex. Since we found in an additional analysis that baseline ECM maps were in fact different between conditions, the possibility remains that baseline differences per se might have influenced the tDCS-induced ECM changes. Similar findings have been reported in recent non-invasive brain stimulation studies, a phenomenon known as homeostatic plasticity (Ziemann and Siebner 2008). More specifically, we cannot entirely rule out the possibility that the individual pre-interventional state might have had an impact on subsequent tDCS-induced ECM changes. The impact of homeostatic plasticity on ECM changes should therefore be of interest in future investigations.

In the present study, we did not record respiration and heart beat to model physiological noise. Therefore, we cannot rule out the possibility that these parameters might influence our research findings. However, a previous study investigated the influence of these parameters on resting state fMRI data and highlighted that default-mode network changes cannot be explained by cardiorespiratory processes alone and is likely related to cognitive neuronal processing (van...
Therefore, we are confident that the observed tDCS-induced ECM changes are not contaminated by physiological noise.

Taken together, we demonstrated that tDCS over SM1 induces widespread and dynamic changes in resting-state functional connectivity both during and after stimulation. The pattern of network connectivity changes is temporally and spatially dispersed and critically depends on the stimulation setup (uni- and bilateral tDCS over SM1).
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primate. J Neurosci 23(23):8432-44

stimulation over the motor association cortex induces plastic changes in ipsilateral primary


Figure 1. Experimental setup showing (A) the different electrode montages used and (B) the time course of one experiment. A: For bilateral tDCS over SM1, the anode was mounted over the right, while the cathode was mounted over the homologous left SM1. For unilateral SM1 tDCS, the anode was again placed over the right SM1, while the cathode electrode was mounted over the contralateral supraorbital region. B: During each experimental session, an initial baseline scan was acquired before tDCS application. Subsequently, the respective tDCS (uni-, bilateral or sham SM1 tDCS) was applied for 20 minutes during the next 3 blocks (total of 600 volumes, duration of ~23 min) followed by 2 additional EPI blocks (duration of ~15.3 min) that were acquired directly after tDCS. The same procedure applied for the sham condition except that the tDCS was only applied for approximately 30 s. For details see methods section.

Figure 2. Baseline comparisons between conditions (bilateral, unilateral and sham SM1 tDCS). A) Significant clusters of the comparison bilateral > sham are displayed in red, the inverse contrast (sham>bilateral) in blue. B) Significant clusters of the comparison unilateral > sham are displayed in red, the inverse contrast (sham>unilateral) in blue. C) Significant clusters of the comparison bilateral > unilateral are displayed in red, the inverse contrast (unilateral>bilateral) in blue. For the analysis, only the first scanning block (baseline, see Figure 1 of the manuscript) of each condition was used. All clusters are presented on axial slices at a threshold of z>3.3 (p<0.05, corrected on cluster level).

Figure 3. Brain areas that showed a significant increase in eigenvector centrality during (A) bilateral tDCS over SM1 and (B) unilateral tDCS over SM1 as compared to sham. Significant clusters are presented on axial slices at a threshold of z>3.3 (p<0.05, corrected on cluster level). Color bars indicate z-score in a range of 3.3 to 6. Abbreviations: M1/ PMd = primary motor cortex / dorsal premotor cortex; SMA = supplementary motor area; SFG = superior frontal gyrus; MFG = middle frontal gyrus; Ins = posterior Insula; MB = midbrain; IFG = inferior frontal gyrus; Hipp = hippocampus; BS = brainstem; IPC/ OC = inferior parietal / occipital cortex; MTG = middle temporal gyrus; Cb = cerebellum Lobule VIIa hemisphere.

Figure 4. Differential effects of bilateral versus unilateral tDCS over SM1 on eigenvector centrality. Bilateral tDCS results in stronger eigenvector centrality increases in primary and secondary motor areas (including right M1, PMd and left SMA/pre-SMA), bilateral prefrontal areas (SFG) and subcortical regions when directly compared to unilateral SM1 tDCS (bilateral > unilateral tDCS, clusters shown in red). Significant clusters of the inverse contrast (unilateral > bilateral tDCS) are shown in blue. The corrected threshold was set to z>3.3. Color bars indicate z-score in a range of 2.8 to 6. (p<0.05, corrected on cluster-level) Abbreviations: M1 = primary motor cortex; PMd = dorsal premotor cortex; SMA = supplementary motor area; SFG = superior frontal gyrus; OC = occipital cortex; Ins = Insula; Pt = Putamen; OFC = orbitofrontal cortex; Cb
= cerebellum Lobule VIIb Vermis; BS = Brainstem; IPC/ OC = inferior parietal /occipital cortex; S1 = primary somatosensory cortex; STG = superior temporal gyrus; GP = globus pallidum.

**Figure 5.** Brain areas that showed a significant increase in eigenvector centrality after (A) bilateral and (B) unilateral tDCS over SM1 as compared to sham. Significant clusters are presented on axial slices at a threshold of z>3.3 (p<0.05, corrected on cluster level). Please see also Table 3 for a detailed cluster list. Abbreviations: M1/ PMd = primary motor cortex / dorsal premotor cortex; SMA = supplementary motor area; Pre-SMA = presupplementary motor area; SFG = superior frontal gyrus; MFG = middle frontal gyrus; OFC = orbitofrontal cortex; IFG = inferior frontal gyrus; MTG = middle temporal gyrus; ITG = inferior temporal gyrus; TTG = transverse temporal gyrus; CB = cerebellum; PHC = parahippocampal cortex; FG = fusiform gyrus.

**Figure 6.** Dynamic progression of changes in eigenvector centrality during (block 1-3) and after (block 4-5) bilateral (A) and unilateral (B) tDCS over SM1 as compared to sham. Color-coded fields represent the z-values resulting from the contrasts of a single block (1 to 5) and the baseline at a threshold of z>3.3. During both conditions (A and B), distributed brain areas are modulated by tDCS. Please note that the pattern of changes for both stimulation types seems to be non-linear and temporally dispersed. The small line plots on green background represent continuous ECM changes throughout the time course of the experiment and includes below threshold z-values. Color bars indicate the z-score in a range of 3.3 to 6.3. Abbreviations (in the order of appearance): M1/ PMd = primary motor cortex / dorsal premotor cortex; SMA = supplementary motor area; Pre-SMA = presupplementary motor area; aSFG = anterior part of superior frontal gyrus; MFG = middle frontal gyrus; OFC = orbitofrontal cortex; IFG = inferior frontal gyrus; MTG = middle temporal gyrus; ITG = inferior temporal gyrus; TTG = transverse temporal gyrus; Hipp = hippocampus; pIns = posterior Insula; MB = midbrain; BS = brainstem; Cb.(Lob.VIIa) = cerebellum, Lobule VIIa; PHC = parahippocampal cortex; FG = fusiform gyrus; IPC/ OC = inferior parietal/ occipital cortex; Cb = cerebellum, Lobule VI.
Table 1: Brain regions that show significant increases in eigenvector centrality during bilateral and unilateral tDCS over SM1 as compared to sham stimulation.

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Abbreviations: H = hemisphere; BA = Brodmann Area; tal = Talairach space; z max = maximum z value; Cl = cluster size; M1 = primary motor cortex; PMd = dorsal premotor cortex; SMA = supplementary motor area; SFG = superior frontal gyrus; MFG = middle frontal gyrus; IFG (p. orbit.) = inferior frontal gyrus, pars orbitalis; IPC = inferior parietal cortex; OC = occipital cortex; MTG = middle temporal gyrus; Cb = cerebellum
Table 2: Differential effects of bilateral and unilateral tDCS over SM1 on eigenvector centrality during stimulation.

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Abbreviations: H = hemisphere; BA = Brodmann Area; tal = Talairach space; z max = maximum z value; Cl = cluster size; M1 = primary motor cortex; PMd = dorsal premotor cortex; SMA = supplementary motor area; SFG = superior frontal gyrus; ACC = anterior cingulate cortex; OFC = orbitofrontal cortex; OC = occipital cortex; BS = brainstem; Cb = Cerebellum; S1 = primary somatosensory cortex; IPC = inferior parietal cortex; STG = superior temporal gyrus; GP = globus pallidum
Table 3: After-effects of bilateral and unilateral tDCS over SM1 on eigenvector centrality.

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<tr>
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<td>-25</td>
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</table>

Abbreviations: H = hemisphere; BA = Brodmann Area; tal = Talairach space; z max = maximum z value; Cl = cluster size; M1 = primary motor cortex; PMd = dorsal premotor cortex; SMA = supplementary motor area; MFG = medial frontag gyrus; OFC = orbitofrontal cortex; IFG (p. orbit.) = inferior frontal gyrus, pars orbitalis; MTG = middle temporal gyrus; TTG = inferior temporal gyrus; Cb = Cerebellum; SFG = superior frontal gyrus; PHC = parahippocampal gyrus; FG = fusiform gyrus; MTG = middle temporal gyrus
### A

<table>
<thead>
<tr>
<th>Left Hemisphere</th>
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#### rs-fMRI during stimulation
- M1/PMd
- SMA
- Pre-SMA
- aSFG
- MFG
- OFC
- IFG
- MTG
- ITG
- TTG
- Hipp
- pLNS
- MB
- BS
- Cb 1

#### rs-fMRI post stimulation

### B

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#### rs-fMRI during stimulation
- SFG
- MTG
- PHC
- FG
- IPC/OC
- Cb 2
- Cb 3

#### rs-fMRI post stimulation

- n.s.
- 4
- 5
- 6
- 8

Legend:
- n.s. = not statistically significant
- 4, 5, 6, 8 = statistically significant levels