Title

Baseline effects of transcranial direct current stimulation on glutamatergic neurotransmission and large-scale network connectivity

Authors

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Keywords: Transcranial direct current stimulation (tDCS), resting-state functional magnetic resonance imaging (rs-fMRI), magnetic resonance spectroscopy (MRS), independent component analysis (ICA), functional network connectivity (FNC), glutamine-glutamate (Glx), parietal lobe.

Abstract:

Transcranial direct current stimulation (tDCS) modulates glutamatergic neurotransmission and can be utilized as a novel treatment intervention for a multitude of populations. However, the exact mechanism by which tDCS modulates the brain's neural architecture, from the micro to macro scales, have yet to be illuminated. Using a within-subjects design, resting-state functional magnetic resonance imaging (rs-fMRI) and proton magnetic resonance spectroscopy (^1H -MRS) were performed immediately before and after the administration of anodal tDCS over right parietal cortex. Group independent component analysis (ICA) was used to decompose fMRI scans into 75 brain networks, from which 12 resting-state networks were identified that had significant voxel-wise functional connectivity to anatomical regions of interest. ^1H -MRS was used to obtain estimates of combined glutamate and glutamine (Glx) concentrations from bilateral intraparietal sulcus. Paired sample t-tests showed significantly increased Glx under the anodal electrode, but not in homologous regions of the contralateral hemisphere. Increases of within-network connectivity were observed within the superior parietal, inferior parietal, left frontal-parietal, salience and cerebellar intrinsic networks, and decreases in connectivity were observed in the anterior cingulate and the basal ganglia ($p < 0.05$, FDR-corrected). Individual differences in Glx concentrations predicted network connectivity in most of these networks. The observed relationships between glutamatergic neurotransmission and network connectivity may be used to guide future tDCS protocols that aim to target and alter neuroplastic mechanisms in healthy individuals as well as those with psychiatric and neurologic disorders.

1. Introduction

Neuroplasticity is essential for brain development and adaptation as it enables the nervous system to reorganize neural pathways based on new experiences. Research is underway to examine ways to harness neuroplasticity in order to promote healing and recovery (see Peled, 2004; 2005; Spedding et al., 2003; and Kays et al., 2012 for review). Transcranial direct current stimulation (tDCS) is a non-invasive brain stimulation technique that modulates the excitability of functional brain networks (Polania et al., 2011; Keeser et al., 2011; Sehm et al., 2012; Peña-Gómez et al., 2012). It is thought to increase the spontaneous firing of cortical neurons near the anodal electrode (with positive polarity) while decreasing it near the cathode (with negative polarity) (Nitsche and Paulus, 2000; Dieckhöfer et al., 2006). By modulating the excitability of glutamatergic pyramidal neurons in the underlying cortex (Radman et al., 2009), tDCS influences neurophysiological mechanisms responsible for neuroplasticity. These mechanisms involve the potentiation of synaptic glutamatergic receptors (Liebetanz et al., 2002; Nitsche and Paulus, 2005), and decreased neurotransmission of γ -aminobutyric acid interneurons (GABA) (Nitsche et al., 2004; Stagg et al., 2009; 2011; also see Medeiros et al., 2012 for review).

In particular, NMDA and AMPA receptors are essential to synaptic plasticity by influencing long-term potentiation and depression (LTP and LTD) across structurally-connected brain regions (Bliss & Collingridge, 1993). These synaptic and neuronal pathways consolidate into stable and long-lasting functional brain networks (Fricke et al., 2011; Venkatakrishnan et al., 2011, 2012). However, the effects of tDCS on glutamate levels and its relation to large-scale network connectivity have yet to be fully elucidated; that is, there must be a better understanding of how tDCS interacts across different scales within the brain's neural architecture by combining

different, yet complementary, imaging modalities (see Hunter et al., 2013 for a review), which was the primary objective of the present study.

1.1. tDCS-Induced Effects on Neurometabolites

Proton magnetic resonance spectroscopy (^1H -MRS) enables quantification of certain neurometabolites within a localized region of the brain (Gruettier, 2001; Steen et al., 2005). This method has been used to examine the effects of tDCS on specific neurometabolites. To date, anodal tDCS has been associated with *increases* in combined glutamine and glutamate (Glx) (Stagg et al., 2009; Clark et al., 2011) and myoinositol concentrations (Rango et al., 2008). Concordantly, *reductions* have been found in GABA concentration with anodal tDCS (Stagg et al., 2009). Consistent with these findings, the activation of metabotropic glutamate receptors, in conjunction with stable long-range intrinsic membrane oscillations, has been shown to entrain local and distributed GABAergic interneurons (Whittington et al., 1995). Together, the observed changes in glutamatergic and GABAergic activity may translate to subsequent alterations in both local and distributed processing—influenced by both excitatory and inhibitory signaling pathways—in functional brain networks.

1.2. tDCS-Induced Effects on Network-Based Connectivity

Functional magnetic resonance imaging (fMRI) is a noninvasive technique for acquiring dynamic changes in blood oxygenation, measured as the blood-oxygenation-level dependent (BOLD) signal. While at rest, spontaneous fluctuations in the BOLD signal (0.10-0.15 Hz) show high correlations across structurally connected and functionally related brain regions (Biswal et al., 1995; Skudlarski et al., 2008; see Fox et al., 2007 for review). These fluctuations reflect a stable, intrinsic organization of the brain that maintains and reinforces established synaptic connections that support cognitive and behavioral functions (see Fox et al., 2007 & Van den

Heuvel and Pol, 2010 for reviews). The most commonly observed intrinsic network is the default-mode network (DMN), which links precuneus and posterior cingulate cortex (PCC) with bilateral inferior parietal and medial frontal cortices, with the highest activations observed in the posterior regions (Raichle, 2001; Greicius et al., 2003). Independent component analysis (ICA) can be used to decompose resting-state fMRI (rs-fMRI) signals into functionally related “groups” of voxels that comprise functionally connected brain networks (Erhardt et al., 2011). The strength of ICA is its ability to resolve data into maximally independent sources, thereby revealing the dynamics of intrinsic networks (McKeown, 1997; Calhoun et al., 2001; Beckmann et al., 2005; Calhoun et al., 2011; 2012).

A recent study of anodal (2.0 mA) tDCS over the dorsolateral prefrontal cortex (DLPFC), with the cathode placed over contralateral supraorbital area, resulted in increased intrinsic functional connectivity *within* the frontal node of the DMN and the left frontal-parietal network (Keeser et al., 2011). Similarly, Peña-Gómez et al. (2012) found that this same tDCS montage produced a redistribution of ICA-generated functional network connectivity (FNC), a measure of the temporal relationship among ICA components. Increase FNC between networks that overlapped with the site of stimulation and with superior parietal networks (comprising task-related circuits) was observed; whereas a decrease in FNC was found between networks that comprise the DMN (Peña-Gómez et al., 2012). These results suggest that anodal tDCS over DLPFC may enhance the flexible balance between brain networks by enhancing network connectivity for cognitive demands while reducing its anti-correlated DMN activity.

Furthermore, a recent rs-fMRI study placed the anode over the right angular gyrus, with the cathode over contralateral supraorbital region (Clemens et al., 2012). Increases in ICA-generated functional connectivity in the cerebellum, medial occipital, sensorimotor, right frontal

parietal, and superior frontal gyrus were observed, while decreases were found in the right putamen and lateral occipital areas. Furthermore, active tDCS has also contributed to both inter-hemispheric (Sehm et al., 2012; 2013; Park et al., 2013) and corticostriatal functional connectivity (Polania et al., 2012; Clemens et al., 2014). Overall, studies that combine fMRI and tDCS show widespread changes in functional connectivity at both local and distant brain regions relative to the area of stimulation (Polania et al., 2011; Keeser et al., 201; Amadi et al., 2014).

1.3. Established Relationships Between Imaging Modalities

In order to examine the relationship between neurotransmission (either excitatory or inhibitory) and the variability of functional brain responses, there are a number of studies that combined ^1H -MRS with fMRI hemodynamic responses (Donahue et al., 2010; Muthukumaraswamy et al., 2012; Falkenberg et al., 2012), task-related negative BOLD responses (Walter et al., 2009; Northoff et al., 2007), resting-state functional connectivity (Horn et al., 2010; Kapogiannis et al., 2013) and effective connectivity (Duncan et al., 2011). The results from these multi-modal imaging studies demonstrate that increases in glutamate concentrations predict increases in BOLD activity, whereas increases in GABA predict decreases in BOLD activity. For instance, increases in glutamatergic concentrations in the precuneus predicted increases in ICA-generated intrinsic network connectivity within the precuneus node of the DMN, while decreases in GABA predicted overall increases in DMN activity (Kapogiannis et al., 2012).

1.4. Study Objectives & Rationale

In summary, tDCS may alter changes in the BOLD signal by modulating neuronal and glial metabolic demands, perhaps related to the glutamate/glutamine cycle (Bonvento et al., 2002). Furthermore, given that Glx was increased by tDCS in our previous study (Clark et al.,

2011), and that there is a strong relationship between glutamate and functional connectivity in other studies that have not used tDCS (Horn et al., 2010; Kapogiannis et al., 2013), the objective of the current study was to examine whether tDCS-evoked changes in Glx may predict variation in functional connectivity *within* and *between* both *local* and *distributed* intrinsic brain networks.

To preferentially modulate excitatory signaling in large-scale functional connectivity, the present study positioned the anode over the right superior parietal cortex, with an extracephalic cathode tDCS montage. By removing the direct influence of the cathode on cortical activity, this montage allows for a more precise evaluation of anodal tDCS on cortical excitability. The parietal cortex is an essential network hub that supports many different attentional processes, including endogenous attentional control and spatial orienting of attention (Corbetta et al., 2008; Vandenberghe and Gillebert, 2009). Moreover, recent tDCS studies have shown that right parietal stimulation produces significant increases in visual-spatial processing (Sparing et al., 2009), covert visual orienting (Bologini et al., 2010), learning in a complex visual detection task (Clark et al., 2012), attentional selection (Moos et al., 2012) and perceived position (Wright & Krekelberg, 2013).

1.5. Hypotheses

Based on the literature summarized above, we hypothesized that tDCS would increase functional connectivity *within* networks located near the right parietal cortex (the targeted area of anodal stimulation), while decreasing functional connectivity within networks in the medial regions of the DMN (Keeser et al., 2011; Peña-Gómez et al., 2012), which could be tested using ICA to assess the network connectivity before and after tDCS. We also hypothesized that tDCS would increase FNC *between* network pairs that contain circuits supporting attention-related functions, as tDCS to this region has been found to enhance attentive cognitive functions. Given

that previous studies found that anodal tDCS enhanced the balance between brain networks, partly by decreasing FNC between DMN and subcortical functional circuits (Peña-Gómez et al. 2012; Polania et al., 2012; Clemens et al., 2014), we also hypothesized that FNC between the DMN and subcortical networks would be decreased after tDCS. Lastly, given that tDCS enhances voxel-wise functional connectivity via glutamatergic signaling pathways, we expected that tDCS-related increases in Glx post-tDCS would relate to increases within and between networks.

2. Results

2.1. Group Independent Component Analysis

Following Allen et al. (2011), a high-dimensional model-order ICA was performed using Group ICA fMRI Toolbox (GIFTv3.0a; <http://mialab.mrn.org/software/gift/>). Prior to the first and second PCA data reductions (1st at $c = 100$; final at $c = 75$), which were computed on each subjects' dataset before doing the group PCA, the time-series at each voxel were scaled to percent signal change using the intensity normalization function in GIFT. This option was selected based on a recent study which showed that this pre-processing method maximizes the detectability of individual differences in functional connectivity measures at the group-level (Khullar et al., 2011; Erhardt et al., 2011). The infomax ICA algorithm, which incorporates nonlinearities in the transfer function to capture higher-order moments in the BOLD resting-state fluctuations (Bell and Sejnowski, 1995), was then used to detect the independent source components in the data. ICA was repeated 15 times using ICASSO toolbox in order to estimate the algorithmic and statistical reliability of the ICA decomposition (Himberg, Hyvärinen, and Esposito, 2004). Back-reconstruction was then run using group ICA (GICA3), with component

spatial maps of functional connectivity scaled to represent the original data units in percent signal change.

2.2. *Identified Networks of Interest*

All 12 networks are displayed in Figure 1. Three networks were identified with aggregate spatial maps that overlapped with parietal regions near the area of stimulation and 1H-MRS voxels. These parietal networks included a bilateral superior parietal lobule (SPL) network, a bilateral inferior parietal network and a bilateral superior parietal sulcus (SPS) network. When taken together, 3 networks captured the full extent of the DMN along the anterior-posterior and inferior-superior axes (Raichle et al., 2001; Allen et al., 2011): a precuneus network, a PCC and bilateral angular gyrus network, and an ACC network. Three additional networks were identified with distributed spatial maps that overlapped with well-known attention-related networks, which included a left-lateralized frontal-parietal network, which showed the highest activation in the parietal regions and a smaller cluster in the right posterior parietal ($k=241$) and PCC ($k=299$) cortices. There was also a right-lateralized frontal-parietal network, which showed the highest voxel-wise functional connectivity in the right lateral frontal regions. A network that displayed the highest functional connectivity in bilateral insulae and dorsal ACC, comprising the well-known “salience” network was also included (Seeley et al., 2007; Menon et al., 2010; Bonnelle et al., 2012; Ham et al., 2013; Uddin et al., 2013). There was one frontal component, which comprised bilateral anterior prefrontal cortex (PFC), with additional voxels observed in the thalamus ($k=160$). Lastly, two sub-cortical networks were found: one represented by the putamen and palladium—labeled the basal ganglia network (BG), and the other consisted of the left cerebellum.

PLACE FIGURE 1 ABOUT HERE

2.3. *Changes in Neurometabolite Concentrations*

As reported in the larger sample in Clark et al., 2011, paired-sample t-tests showed that there was a statistically significant increase in the Glx obtained in the right parietal cortex after anodal stimulation, $t(8) = 2.61, p = 0.03$, but not the left, $t(8) = 1.41, p = 0.20$.

2.4. *Paired T-Tests of Voxel-Wise Functional Connectivity Before and After tDCS:*

Table 1 displays summary statistics for all the statistically significant cluster- and peak-level differences. For the bilateral SPL network, the right-hemisphere premotor cortex exhibited one of the largest significant increases in functional connectivity (see Table 1 and Figure 2). For the bilateral inferior parietal network, the largest increases were primarily observed in the left hemisphere with only a trend-level increase in the right IPS ($p = 0.06$, FDR-corrected; see Table 1). The ACC network also exhibited robust decreases in functional connectivity after tDCS in ACC proper and medial frontal gyrus (see Table 1 and Figure 3). No significant differences were observed in the other 3 networks/nodes that comprise the DMN.

Of the three distributed intrinsic networks, the left frontal-parietal network exhibited the largest increases in functional connectivity, specifically in the left inferior parietal lobe, extending to the upper division of lateral occipital cortex (see Table 1 and supplemental material Figure 1). For the “salience” network, increases in functional connectivity were observed in the right insula, extending to the IFG (see Table 1 and supplemental material Figure 2). No statistically significant differences were observed in the right frontal-parietal network; however, given the hypothesized increases in this network, SPMs for both pre- and post-tDCS voxel-wise functional connectivity are displayed in supplemental material Figure 3. Results show widespread, albeit sub-threshold differences, in the right IFG ($p = 0.004$, uncorrected) and right lateral posterior parietal cortex ($p = 0.059$, uncorrected).

For intrinsic networks comprised primarily of frontal regions, only the bilateral anterior PFC network displayed significantly increased functional connectivity after tDCS, specifically within left MFG and right anterior PFC and superior frontal gyrus. Both subcortical intrinsic networks displayed significant differences: the BG displayed the largest decreases, specifically in bilateral putamen. The left cerebellum network exhibited increases within Crus I and II (see Table 1).

****PLACE TABLE 1 ABOUT HERE****

2.5. *Relationships Between Within-Network Functional Connectivity and Glx*

2.5.1. *Pre-tDCS Measures:*

All statistically significant relationships between Glx and voxel-wise functional connectivity are displayed in Table 2. As reported in studies by others, all relationships between Glx and functional connectivity were positive; that is, greater Glx predicted greater *within-network* functional connectivity. Within the MRS-mask in the salience network, right Glx predicted right SPL functional connectivity. No other significant relationships were observed in the pre-tDCS measures; however, given the previously observed relationships between glutamate and functional connectivity within the precuneus (Kapogiannis et al., 2013), we imposed an even less stringent significance threshold ($p < 0.004$, uncorrected) and found relationships between Glx and functional connectivity in the ipsilateral region of the precuneus network ($p < 0.003$, uncorrected)

2.5.2. *Post-tDCS measures:*

The strongest post-tDCS relationships were observed between left Glx and functional connectivity in the right precuneus within the bilateral SPL network (See Figure 2). Within the precuneus network, only left Glx predicted functional connectivity in the left precuneus

(See Table 2). Within the ACC network, right Glx predicted functional connectivity in the right precuneus. Furthermore, when the network-mask was applied, right Glx predicted right medial frontal functional connectivity only (see Table 2 and Figure 3).

****PLACE TABLE 2 AND FIGURES 2 - 3 ABOUT HERE****

Significant relationships were observed between left Glx and inferior-parietal functional connectivity in the left frontal-parietal network (see Table 2). In addition, given the cluster of significant functional connectivity in the right parietal cortex within this network ($k=241$)—i.e., the targeted region of stimulation—an additional mask was computed from this cluster. A significant relationship was observed between right Glx and right inferior parietal functional connectivity in the left frontal-parietal network (see Table 2).

For the salience network, right Glx predicted left inferior parietal functional connectivity. Lastly, the BG network displayed the second largest effects, where left Glx predicted left precuneus functional connectivity within this network.

****PLACE FIGURES 4-6 ABOUT HERE****

2.6. *Paired T-Tests on FNC:*

Group means and SDs for the FNC pairs of interest are displayed in Table 3 (the full FNC map is displayed in supplementary material, Fig. 5). For the parietal networks, there was a significant increase in FNC between the left frontal-parietal and bilateral inferior parietal networks, as expected. For the networks that comprise the DMN, the FNC between the ACC and precuneus networks displayed the largest increase in FNC.

For FNC between nodes that comprise distributed networks, there was a decrease in FNC between the salience and ACC networks, from being significantly anti-correlated to zero-correlation after tDCS (see Table 3). For the FNC among subcortical networks and all

other structurally connected brain regions, there was a significant increase in FNC between the cerebellum and the precuneus network, and between the cerebellum and the left frontal-parietal network. There was also a decrease in FNC between the cerebellum and the BG network (see Table 3).

****PLACE TABLE 3 ABOUT HERE****

2.7. *Relationships Between Glx and FNC,*

2.7.1. *Post-tDCS Measures:*

All statistically significant correlations between Glx estimates and FNC are displayed in Table 3. The strongest post-tDCS Glx relationship was observed between left Glx and FNC between the bilateral SPL and precuneus networks. Left Glx was also correlated with FNC between bilateral inferior parietal and bilateral anterior PFC networks. Interestingly, right Glx was positively correlated with FNC between the salience and ACC networks, while it was negatively correlated with FNC between the salience and BG networks.

****PLACE TABLE 3 ABOUT HERE****

3. **Discussion**

By combining ^1H -MRS and rs-fMRI, the present study investigated how tDCS interacts with different levels of the brain's neural architecture. The observed after-effects of tDCS included increases in Glx in right parietal cortex, underneath the site of anodal stimulation, which predicted increases in network connectivity in the precuneus for several different networks. This may indicate that the precuneus acts as an intermediate node that modulates glutamatergic signaling in other pathways, including bilateral SPL, ACC, salience, left frontal-parietal, and BG networks. As discussed below, cross-hemispheric connectivity, specifically within the bilateral inferior parietal network, may be more readily influenced by

inhibitory signaling pathways. While the majority of the results support our hypotheses, we also encountered additional, unexpected findings, discussed below in terms of their potential systems-level contributions to behavior, which may inform future hypotheses for the most optimal cortical targets for enhancing cognitive functions in healthy and clinical populations.

3.1. *Hypothesized Increases in Parietal Network Connectivity*

As expected, tDCS increased functional connectivity within the right hemisphere of the bilateral SPL network; however, the premotor location of this effect was somewhat unexpected. Anatomical studies have confirmed that most of the parietal afferent connections to the dorsal premotor cortex originate from SPL (see Wise et al., 1997 for review). It is therefore possible that parietal stimulation specifically increased the synchronization of these afferent signaling pathways from superior parietal to premotor cortices. This increase may translate to an enhancement of attention to visual processing and the execution of movements (Rushworth et al., 2001; Molenberghs et al., 2007; Moos et al., 2012).

With regard to the bilateral SPL network's relation to glutamatergic activity, right and left Glx concentrations predicted increased functional connectivity in the right precuneus only. This relationship was the strongest in comparison to the other networks, which indicates that tDCS-induced alterations in glutamatergic signaling are localized within the area of stimulation, but also extends to nearby, structurally connected brain regions. The precuneus in particular is thought to be a central core in the cortical anatomical network (Powers et al., 2013), which exhibits heterogeneous functional connectivity with the default-mode, motor, visual, and attentional systems (White et al., 2010; Allen et al., 2011). Given that left Glx also predicted functional connectivity in the right precuneus and the left SPL, which exceeded the FWE-corrected threshold, it is possible that this brain structure acts as an intermediate

node that modulates inter-hemispheric communication via micro-scale alterations in glutamatergic neurotransmission. Indeed, we also observed that only after tDCS, left Glx was positively correlated with the FNC between the bilateral SPL and precuneus network, further demonstrating that tDCS may influence the degree to which two networks communicate between each other via glutamatergic processing.

3.1.1. Increased Inter-Hemispheric Network Connectivity in the Parietal Lobe

The present study found that anodal stimulation produced a redistribution of parietal inter-hemispheric functional connectivity. This is an important finding because tDCS-induced increases in inter-hemispheric functional connectivity are thought to be largely dependent on the contributions of the cathode electrode (Sehm et al., 2012; 2013; Park et al., 2013). In the present study, network connectivity was increased predominantly in the left lateral inferior parietal regions of the bilateral inferior parietal and the left frontal-parietal networks. The enhancement of functional connectivity in the left hemisphere by right hemisphere excitatory stimulation is consistent with the role of transcallosal excitation (Yazgan et al., 1995; see Bloom & Hynd, 2005 for review).

However, given that Glx did not predict functional connectivity within the bilateral inferior parietal network, the observed tDCS-induced increases in cross-hemispheric network connectivity may be more readily influenced by other neurotransmitter pathways, such as GABA. Inferior parietal structures are thought to have an inhibitory influence on one another through transcallosal fiber connections (Kinsbourne, 1993; Sparing et al., 2009). Thus, the current results for the bilateral inferior parietal network should be considered in line with the recent TMS and tDCS studies that targeted the inferior parietal lobe (e.g., Dambeck et al., 2006; Bologini et al., 2010; Moos et al., 2012), which were all in support of the inhibitory

hemispheric rivalry hypothesis (Kinsbourne, 1993). In this view, the two hemispheres have a mutual, reciprocal inhibitory influence on one another. It predicts that stimulation over the parietal cortex should result in trans-hemispheric release of inhibition to the contralateral hemisphere that will in turn result in hyperactivity in the ipsilateral hemisphere (see Fecteau et al., 2006 for review). Thus, during stimulation, the right hemisphere would exert more of an inhibitory influence on the left via GABAergic signaling. Indeed, it has been found that inhibitory network oscillations occur in response to the activation of metabotropic glutamate receptors, which may have implications on large-scale functional connectivity and ultimately cognition (Whittington et al., 1995). For the present study, however, it remains unclear which micro-scale signaling pathways drove the enhancement of within- and between-network connectivity between the bilateral inferior parietal and left frontal-parietal networks. Given that V1 BOLD signal variations have been linked to baseline GABA levels (Donahue et al., 2007), further research is needed address any involvement of GABAergic signaling pathways that may influence network connectivity between inferior parietal networks.

3.2. *Expected Decreases Within Nodes of the DMN*

As expected, tDCS produced a significant decrease in functional connectivity within the ACC network, which was specifically observed in the right medial frontal gyrus. This finding is consistent with the general hypothesis that tDCS may enhance the flexible balance between putative functional brain networks by suppressing functional connectivity within the DMN. In addition, bilateral Glx concentrations predicted bilateral precuneus and right medial frontal gyrus functional connectivity within the ACC network, which highlights the potential role of glutamatergic signaling in decreasing functional connectivity in more distant brain regions (Enzi et al., 2012). To this end, we also observed that the FNC between the ACC and

precuneus was significantly increased after tDCS. This finding is of interest given the concurrent decrease in ACC functional connectivity, which suggests that parietal stimulation may reduce attention directed to the ACC (e.g., internal visceral signals), while enhancing more attentive (tonic monitoring) states with the environment via enhanced communication with precuneus network (Lin et al., 2011; Vogt and Derbyshire, 2009).

3.3. *Expected Increases in the Distributed Salience Network*

Prior to the administration of tDCS, there was a statistically significant relationship between right Glx and right SPL functional connectivity within the salience network. This association is consistent with the hypothesis that tonic excitatory neurotransmission is necessary in order to readily engage other networks when attending to stimuli (Eichler et al., 2008). This is indeed consistent with salience network's active role in switching between the default-mode and task-related states of brain connectivity (Seeley et al., 2007; Menon and Uddin, 2010; Bonnelle et al., 2012; Ham et al., 2013).

After anodal stimulation, increased functional connectivity in the right insula extending contiguously to IFG were also observed. Together, the increased functional connectivity in the right insula and IFG may establish an enhancement of the casual outflow from the right insula and IFG to other nodes that anchor the salience network for further attentional engagement. Across stimulus modalities, the right anterior insula plays a causal role in enabling the recruitment of contextually relevant brain regions (Sridharan et al. 2008)—thereby activating task-related networks and deactivating the DMN. Thus, by enhancing the network activity within right anterior insula and IFG, parietal stimulation may improve cognitive performance by enhancing the initial phase of information processing, specifically as it relates to integrating external stimuli with internal homeostatic context

(Seeley et al., 2007; Singer et al., 2009; Menon and Uddin, 2010; Seth et al., 2011).

3.3.1 Increased Functional Connectivity in Frontal Networks

Functional connectivity was significantly increased after tDCS within the bilateral anterior PFC network, specifically in right BA10. This brain region is consistently engaged when subjects are instructed to learn new behavioral routines (Koechlin et al., 2002; Strange et al., 2001) and when the amount of uncertainty (i.e., entropy) is increased in regard to multiple options that subjects are simultaneously tracking (Yoshida & Ishii, 2006). After tDCS, the FNC between this network and the bilateral inferior parietal network was significantly correlated with left Glx concentrations. Taken together, tDCS may contribute to learning as it relates to maintaining and switching back and forth between multiple behavioral alternatives in search of optimal behavior (Koechlin et al., 2002; Yoshida & Ishii, 2006; Andersson et al., 2009). However, this hypothesis has to be investigated in the context of problem-solving before and after tDCS.

3.4. Expected Decreases in the Basal Ganglia

Anodal tDCS decreased functional connectivity in bilateral putamen. This finding is consistent with the previously observed intrinsic connectivity between the putamen and parietal cortex (Martino et al., 2008; Cao et al., 2009). To this end, given that the putamen consists primarily of spiny neurons which receive direct input from glutamatergic signaling, tDCS may also influence the inhibitory medium spiny neurons in this brain structure (Garcia et al., 2010; Girault, 2012). However, the putamen and the cortex have no monosynaptic anatomical connections, and so further research is needed to identify the mediating structures and signaling pathways by which tDCS operates.

Furthermore, it has been previously shown that anodal tDCS over M1 (cathode

contralateral frontopolar region) resulted in a reduction in functional connectivity between the left caudate nucleus and the PCC node of the DMN (Polania et al., 2012). The authors suggested that this reduction, or “switch” in activation patterns, was due to the motor-related loop being activated by tDCS. To this end, given the Glx relationships with FNC between the BG and salience networks, further research is needed to test the extent that these two networks engage and disengage one another and whether a “switch” in hemispheric reactivity influences hierarchical attention-processing.

3.5. *Exploratory Analysis of the Cerebellum*

The cerebellum plays an important role in the planning, initiation, stability, organization, and long-term memory of movements (Schmahmann, 1998; Imamizu et al., 2000; Schmahmann et al., 2006; Miall et al., 2007). Parietal stimulation increased network connectivity in left Crus 1 and 2, with no observed relationships with Glx. In a previous rs-fMRI study, the human cerebellum was analyzed using both lobular anatomically-driven and self-organizing map-driven approaches (Bernard et al., 2012). This study found that subdivisions of Crus I and II were correlated with distinct regions of prefrontal, temporal and parietal cortices; furthermore, subdivisions of Crus I contained multiple smaller functional clusters that may reflect the heterogeneity of cognitive processing. Thus, it may be the case that tDCS over the parietal lobe enhanced intra-cerebellar processing, which may further enhance local processing of information related to cognition.

Moreover, the FNC between the cerebellum and precuneus and FNC between the cerebellum and left frontal-parietal networks were all significantly increased after stimulation. Given that there are no monosynaptic connections between the cerebrum and cerebellum (Buckner et al., 2011), the current FNC results provide further evidence for the cerebellum's

indirect influence on intrinsically coupled cerebral association areas, such as the parietal cortex (Habas et al. 2009; O'Reilly et al. 2010). Furthermore, given that the cerebellum exerts an overall inhibitory tone over the cortex during rest—specifically with M1 (Galea et al., 2009), the observed increases in FNC might result from a complex relationship between GABAergic and glutamatergic signaling.

Stimulation also resulted in a significant decrease in FNC between the BG and cerebellum networks. The BG and cerebellum are thought to form multi-synaptic loops with the cerebral cortex (Haber, 2003; Bostan et al., 2010), with the anterior parietal regions playing an integrative role that mediates activity to and from the BG and to the frontal lobe. Clower et al. (2005) demonstrated that the anterior inferior parietal cortex (in the cebus monkey) is the target of both cerebellar and BG output. Thus, from a computational perspective, the BG and cerebellum have been viewed as segregated modules that implement different learning algorithms, with the BG supporting reinforcement learning and the cerebellum supporting supervised learning (Doya et al., 2001; Houk, 2005).

3.6. Clinical Applications and Further Considerations

Understanding the mechanisms by which micro and macro changes occur after tDCS could help to increase its effectiveness in altering neuroplastic mechanisms in order promote healing and recovery (Peled, 2004; 2005; Spedding et al., 2003; Kays et al., 2012; Kuo et al., 2013). For instance, increasing both the glutamatergic neurotransmission and functional connectivity that is otherwise impaired in schizophrenia (Friston, 1998; Stephan et al., 2006; Williamson and Allman, 2012; Szulc et al., 2013), could lead to effective therapies that combine the synergistic effects of cognitive training, psychopharmacology and stimulation protocols. However, medication and other factors should always be taken into consideration when tailoring

stimulation protocols for clinical populations, as the present results may differ as a nonlinear function of dose.

Accordingly, there is a similar issue of nonlinearity with duration of stimulation and homeostatic plasticity. That is, the effects of tDCS on neuroplasticity depends upon the recent history of tDCS-induced excitation, such that repeated stimulation (with short intervals) has resulted in a reversal of excitability (Fricke et al., 2011), similarly for increasing intensity from 1mA to 2mA (Batsikadze et al., 2013). More specifically, Batsikadze et al. (2013) found that compared to 1mA anodal stimulation over motor cortex for 20 minutes, 2mA induced after-effects with a delay, which may have occurred from several reasons, including a transient homeostatic counter-regulation, alterations of intracellular calcium, and/or that deeper cortical layers were affected by 2mA but not 1 mA current. Thus, the observed results with increased functional connectivity in the opposite hemisphere of stimulation may have been a result of this time-dependent homeostatic balance governing the response to tDCS, which should be considered if a particular clinical population is already exhibiting preexisting (baseline) perturbations in the underlying cortex.

3.7. *Limitations*

As mentioned above, a limiting factor of the current study is its inability to track the *real time* influence of tDCS on glutamatergic signaling on large-scale functional connectivity. Future studies are needed to assess the extent that the intrinsic inter-hemispheric balance is maintained *during* cortical polarization. Identifying any differences in this timing may be necessary to reveal the optimal strengthening of the synaptic efficacy resulting in observable changes in both micro-level signaling and network-level interactions.

Another limitation is that the ^1H -MRS sequence did not measure GABA concentrations. Future studies are needed to test whether there are differential relationships between Glx and GABA across different partitions of the inferior parietal, BG, ACC and cerebellum networks. Another potential limitation is the small sample size, which may have reduced statistical power of finding other differences or relationships in the current sample.

Although there was no sham tDCS group to compare the results with, the within-subjects design of our study effectively categorized the “comparison” group as the same participant’s baseline scan, which can be a more sensitive and powerful design in capturing and accounting for important individual differences in network fluctuations. Moreover, previous rs-fMRI studies have shown that sham tDCS does not have a significant effect on large-scale intrinsic network dynamics (Peña-Gómez et al., 2012; Park et al., 2013). In addition, future studies should include several types of cognitive tasks to probe the functional relevance of the present findings. Lastly, given the many different parameters that impact the effects of tDCS (e.g., electrode position, size, duration, intensity, and others), future studies are needed to compare the present results with other stimulation protocols.

3.8. *Conclusions*

The present study provides evidence that the “excitatory” after-effects of anodal tDCS on glutamatergic signaling and network connectivity contribute to local, cross-hemispheric and subcortical alterations. The observed after-effects of tDCS include increases in glutamatergic signaling that originate from the parietal site of stimulation, with the precuneus possibly acting as an intermediate node that modulates glutamatergic signaling in other pathways that include bilateral SPL, ACC, salience, left frontal-parietal, and BG networks. Cross-hemispheric connectivity, specifically for the bilateral inferior parietal network, may be more readily

influenced by inhibitory signaling pathways. Overall, the importance of investigating potential interactions across these levels of analyses could inform future hypotheses for the most optimal cortical targets for healthy and clinical populations.

4. Experimental procedure

4.1 Participants

Data from the 11 right-handed volunteers reported in Clark et al. (2011) were included in the present study. However, two participants were excluded due to missing post-tDCS rs-fMRI data. Data from the remaining 9 subjects (mean age = 22, SD = 3 years old; 6 Female) were analyzed. All participants reported English as their primary language, with no history of head injuries or concussions, current or previous history of mental, neurological, alcohol or drug abuse disorders, current prescription medication affecting CNS function, or hearing/visual impairments. Subjects were also excluded for metal implants, claustrophobia and pregnancy, as these are contraindications for MRI.

4.2. Procedure and tDCS Administration

After screening and consent, pre-tDCS ^1H -MRS and rs-fMRI scans were obtained, which lasted a total of 2.1 hours on average. Immediately after, participants were removed from the scanner and tDCS was applied for 30 minutes while participants sat in a sound-attenuated room. An Iomed Phoresor PM850 was connected to square-shaped, 11 cm², saline-soaked sponge electrodes. The anode was placed over 10–20 electrode site P4, with a current of 2.0 mA delivered for 30 minutes. The cathode was placed over the contralateral upper arm. To better control for activation state of the brain (Silvanto et al., 2008), participants were asked to rest with their eyes open through stimulation. This ensured the detection of tDCS-induced neuroplastic changes by removing any possible “spill over” effects related to performing a cognitive task

(Clemens et al., 2014). In order to reduce the length of our experimental procedure, no sham condition was used; moreover, other rs-fMRI studies have shown that sham tDCS does not have a significant effect on large-scale intrinsic network dynamics (Peña-Gómez et al., 2012; Park et al., 2013).

Within 5-10 minutes after tDCS, post-tDCS ^1H -MRS and rs-fMRI scans were obtained, with the ^1H -MRS collected at the same voxel locations (left and right) as the pre-tDCS voxel placements. The same ^1H -MRS and rs-fMRI parameters were used for both pre- and post-tDCS sequences.

4.3. ^1H -MRS Data Collection & Preprocessing

The same ^1H -MRS data and analysis previously reported in Clark et al., 2011 were used here for a subset of subjects that also received rs-fMRI testing. All fMRI and MRS scans were obtained on a 3 T Siemens TIM Trio MRI system with a 12-channel radiofrequency head coil. All parameter details for the ^1H -MRS sequence can found in Clark et al., 2011. In summary, A PRESS (point-resolved spectroscopy) sequence was collected (TR/TE = 1.5 s/40 ms, voxel size = 20 mm $\times$ 20 mm $\times$ 20 mm, averages = 192), with an unsuppressed water signal used as a concentration reference and eddy current correction in post-processing. ^1H -MRS spectra were analyzed using LCModel (Provencher et al., 2001). Metabolite concentrations were estimated as previously reported (Gasparovic et al., 2009). Only Glx estimates with a Cramer–Rao lower bound. Glx estimates from two participants that did not meet this criterion. However, in order to include these subjects in further analyses, these 2 data points were interpolated using SPSS 21 (IBM SPSS Statistics for Windows, Armonk, NY: IBM Corp.), where missing values were replaced with the mean of their designated group (i.e., from right pre- or left post-measures).

4.4. fMRI Data Collection & Processing

High-resolution T2*-weighted functional images were acquired using a gradient-echo EPI sequence with TE=29ms, TR=2s, flip angle=75°, slice thickness=3.5mm. Resting-state scans were five minutes in duration, and participants were instructed to keep their eyes open, relax and attend to a cross hair on the center of the screen.

Preprocessing was completed using SPM 5 (Wellcome Department of Imaging Neuroscience, London, UK. <http://www.fil.ion.ucl.ac.uk/spm>), within MATLAB (Mathworks Inc., Sherbon MA). The first three EPI volumes were discarded to remove T1 equilibration effects. Realignment was then completed using INRIalign and slice-timing correction was applied, with the middle slice used as the reference frame. Data were then spatially normalized into the standard Montreal Neurological Institute (MNI) space, resliced to 3 mm × 3 mm × 3 mm voxels, and smoothed using a Gaussian kernel with a full-width at half-maximum of 10 mm. No subjects were excluded due to excessive head motion, defined as more than 3.125 mm of translation in any plane or more than 5 degrees of rotation in any plane (Gusnard et al., 2001). Pre- and post-tDCS measures did not significantly differ in any of the six head position parameters assessed here (i.e., translational displacements along X, Y, and Z axes and rotational displacements of pitch, yaw, and roll; all p 's > 0.40).

4.4.1. Network Identification

Please see Supplementary Information for details regarding the exclusion of networks not of interest. In summary, 63/75 components were excluded, and the remaining 12 components were included in all further analysis based off their anatomical regions that comprise each network and are described in detail in the results section.

To ensure that the topological false-discovery rate (FDR) procedures based on both spatial extent and peak values were utilized (Chumbley et al., 2009; Chumbly et al., 2010), all

spatial comparisons on ICA-derived statistical parametric maps (i.e., output images from GIFT) were conducted in SPM 8. First, to test the significance of individual component voxel-wise functional connectivity (difference from zero) for both pre- and post-tDCS fMRI measurements, one-sample t-tests were computed at each voxel across all subjects and sessions. The resulting significant clusters within each component (FDR-corrected, $p < 0.01$) were then saved as a spatial mask to limit the number of voxel-wise comparisons in subsequent analyses.

4.5. *Comparing Within-Network Functional Connectivity*

To assess whether the voxel-wise functional connectivity was altered after tDCS, paired-sample t-tests were computed on each voxel within component spatial maps. Head motion (Euclidian linear translation) was included as the only covariate. Model parameters were estimated based on restricted maximum likelihoods, which is the classical estimation method used in SPM. Pair-wise differences are reported only for effects that exceed FDR-correction ($p < 0.05$), with a cluster voxel extent threshold of $k > 10$.

4.6. *Assessing Relationships Between Voxel-Wise Functional Connectivity and Glx Concentrations*

To examine the linear relationships between Glx and the functional connectivity within components before and after tDCS, second-level multiple regression analyses were performed within SPM 8. Each subject's spatial maps were entered as dependent variables, separately for pre- and post-tDCS measures, and right and left Glx levels were entered as the predictor variables. As $^1\text{H-MRS}$ voxels were obtained within the right SPS, this analysis was initially conducted only on those voxels within this region, but also included inferior parietal and precuneus (BA7) cortices—given the close proximity with the $^1\text{H-MRS}$ voxel, herein labeled as the *MRS-mask* (See supplementary material, Fig4). If significant relationships were observed

within this MRS-mask, a second, extended regression analysis was performed including all significant voxels within that network, herein labeled the *network-mask*. Results are reported only for effects that exceed peak-voxel threshold of $p < 0.05$ (FWE-corrected). However, given previous glutamate and ICA-generated functional connectivity relationships were observed with moderate effects (Kapogiannis et al., 2013: $t \leq 3.40$, $p \geq 0.004$), we also assessed relationships significant at $p < 0.001$ (uncorrected) in order to capture similar relationships as previous studies that may still be informative for future research.

4.7. *Comparing FNC Among Specific Network Pairs and Their Relation to Glx*

To compute the relationship between the network activation time courses of each of the 14 components, a constrained-lag correlation was performed within the FNC toolbox v.2.3 (Jafri et al., 2008; <http://mialab.mrn.org/software/fnctb/>). First, a band-pass filter was applied (0.012 Hz and 0.12 Hz), and the maximum lag interval was constrained to ± 4 s (to remove the impact of lag differences on the correlation results). Thereafter, a Fisher's z transformation was applied on all pair-wise FNC correlations. Pair-wise t-tests were then computed on FNC for component-pairs of interests. Furthermore, using a one-sample t-test ($\alpha = 0.05$), all further analyses were conducted only on network pairs that resulted in significant FNC in either the pre- or post-tDCS measures. To examine the relationship between Glx and FNC for component pairs of interest, Pearson correlations were computed separately for pre- and post-tDCS measures, and for left- and right-hemisphere Glx.

Acknowledgments:

This work was supported by the Department of Energy (Government contract DE-FG02-99ER62764) and Office of Director of National Intelligence (2014-131270006) awarded to Dr. Vincent Clark, the National Institute of Mental Health, NIH (R21MH097201) awarded to Dr. Vince Calhoun, and the National Academies Ford Pre-Doctoral Fellowship and the National

Science Foundation Graduate Research Fellowship Program (DGE-0903444) awarded to Michael A. Hunter and by R21MH097201 awarded to Dr. Terran Lane.

. The authors would like to thank the participants who enrolled into this study and to the MRI technicians, Diana South, George Malloy and Catherine Smith for their help with data collection and thanks to many others for their support that made this study possible.

Financial Disclosure:

All authors have no conflicts of interest to report.

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Figure Legends:

Figure 1. ICA-generated spatial maps of the 13 networks of interest: Spatial maps are plotted as Z-scores, $Z > 3.0$ and are displayed at the slice showing highest network connectivity. The (x,y,z) MNI coordinates are labeled underneath each component as well as the low-frequency to high-frequency power ratio (LF/HF) for each network. Higher values indicate more power in lower frequency. Color bars correspond to the range of connectivity values derived from a one-sample t-test. Abbreviations: RH, right hemisphere; LH, left hemisphere; PCC, posterior cingulate cortex; ACC, anterior cingulate cortex; SPL, superior parietal lobule; SM, sensorimotor; SPS, superior parietal sulcus.

Figure 2. Statistical parametric maps for the superior parietal lobule network: Differences after tDCS are displayed in red. Voxels exhibiting significant relationships with post-tDCS RH Glx measures are displayed in yellow and LH Glx measures in green (note the Glx relations in precuneus).

Figure 3. Statistical parametric maps for the anterior cingulate network: Statistically significant differences after tDCS are displayed in red. Voxels exhibiting significant relationships with post-tDCS RH Glx measures are displayed in yellow (note the Glx relations in precuneus).

Fig 1.

Group-level ICA-generated Resting-State Networks

Parietal RSNs that overlap with MRS Voxel Placement

RSNs that comprise the Default-Mode Network

Distributed RSNs

Frontal RSNs

Sub-cortical RSNs

Fig 2. Superior Parietal Lobule and SM Network:

Increased network connectivity and its relation to post-tDCS Glx concentrations.

Fig 3: ACC Network:

Decreased network connectivity and relation with post-tDCS Glx concentrations.

= Post < Pre
 $T = 16.5$

12, 39, 6

= RH Glx
 $T = 9.72$

-15, -75, 27

= LH Glx
 $T = 5.57$

6, -45, 60

**Surface-based
effects combined**

Fig. 4: Left Frontal-Parietal Network:

Increased functional connectivity and relations with post-tDCS Glx.

= Post > Pre

$T = 19.9$

= RH Glx

$T = 6.08$

= LH Glx

$T = 10.74$

**Surface-based
effects combined**

-63, -45, 36

48, -63, 39

-9, 63, 30

Fig 5. Salience Network:

Increase network connectivity and its relations to pre- and post-tDCS Glx.

Fig 6. RH Frontal-Parietal Network:
Pre- and post-tDCS network connectivity.

■ **Pre-tDCS** ■ **Post-tDCS** ■ **Overlap**

Table 1. Summary Statistics for paired-sample T-tests on component spatial maps, comparing before and after tDCS.

Component Component Sub-regions	Direction of Effect	Peak-level		Cluster-level	
		<i>T-score*</i>	<i>X Y Z (MNI)</i>	<i># of voxels</i>	<i>FDR p-val.</i>
Parietal (local tDCS) RSNs					
Bilateral SPL Network					
<i>RH SPL and Precentral-Motor</i>	Post > Pre	16.74	36 -30 72	11	0.04
Bilateral Inferior Parietal					
<i>LH IPS</i>	Post > Pre	20.28	-39 -75 36	14	0.04
<i>LH lateral IPS</i>	Post > Pre	17.93	-27 -78 45	15	0.04
<i>RH IPS</i>	Post > Pre	3.66 (u.c.)	24 -84 45	16	0.06
Node within DMN					
ACC Network					
<i>RH ACC</i>	Post < Pre	16.5	12 39 6	47	<0.001
<i>RH medial frontal gyrus</i>	Post < Pre	10.74	12 57 18	19	0.005
Distributed RSNs					
LH Frontal-Parietal					
<i>LH Superior Division of LOC</i>	Post > Pre	10.24	-42 -66 45	28	0.002
<i>LH Inf. Parietal</i>	Post > Pre	19.9	-63 -45 36	20	0.006
"Salience" Network					
<i>Right IFG& Ins</i>	Post > Pre	12.98	48 27 0	23	0.09
Frontal RSN					
Bilateral Anterior PFC Network					
<i>RH aPFC & SFG</i>	Post > Pre	10.41	27 48 36	54	<0.001
<i>LH MFG</i>	Post > Pre	13.05	-36 42 18	14	0.01
Sub-cortical RSNs					
BG Network					
<i>RH Putamen (dorsal)</i>	Post < Pre	21.62	27 -9 -12	242	<0.001
<i>LH Putamen (rostral)</i>	Post < Pre	18.17	-21 0 -15	323	<0.001
LH Cerebellum Network					
<i>LH Crus I</i>	Post > Pre	9.61	-39 -81 -24	19	0.003
<i>LH Crus II</i>	Post > Pre	9.59	-21 -90 -27	14	0.005

*All Peak-level differences were thresholded using $p < 0.05$ (FWE-corrected), unless noted otherwise.

Table 2

Table 2. Summary of relationships between left and right hemisphere Glx concentrations and "within" network functional connectivity.

GLX and ICA-spatial Map Correlations:		PRE TDCS				POST TDCS			
Components of Interest:		<i>t</i> -score	Type of mask	Gl _x Hem	Peak Voxel	<i>t</i> -score	Type of mask	Gl _x Hem	Peak Voxel
Component Sub-regions									
Parietal RSNs (local tDCS)									
<i>Bilateral SPL Network</i>									
	<i>RH Precuneus</i>	---	---	---	---	7.26	MRS	RH	6 -72 33
	<i>RH Precuneus</i>	---	---	---	---	20.62**	MRS	LH	9 -45 51
	<i>LH SPL</i>	---	---	---	---	8.27	MRS	LH	-30 -57 54
<i>Bilateral SPS Network</i>									
	<i>LH SPS</i>	---	---	---	---	10.27**	MRS	RH	-12 -42 60
DMN:									
<i>Precuneus Network</i>									
	<i>LH Precun</i>	4.73 (uc)	MRS	LH	-24 -60 63	8.64	MRS	LH	-3 -72 39
	<i>RH Precuneus</i>	3.92 (uc)	MRS	RH	33 -48 63	---	---	---	---
<i>ACC Network</i>									
	<i>LH Precuneus</i>	---	---	---	---	9.72	MRS	RH	-15 -75 27
	<i>RH Precuneus</i>	---	---	---	---	6.84	MRS	RH	9 -63 33
	<i>RH Precuneus</i>	---	---	---	---	5.57	MRS	LH	6 -45 60
	<i>RH medial frotnal gyrus</i>	---	---	---	---	5.75	Network	RH	12 45 24
Distributed RSNs:									
<i>Left Frontal-Parietal Network</i>									
	<i>LH Precuneus</i>	---	---	---	---	10.74**	MRS	LH	-9 -63 30
	<i>RH Inferior Parietal</i>	---	---	---	---	6.08	Network	RH	48 -63 39
<i>"Salience" Network</i>									
	<i>RH SPL</i>	9.6	MRS	RH	33 -51 69	---	---	---	---
	<i>LH precuneus</i>	---	---	---	---	8.35**	MRS	RH	-24 -81 27
Frontal RSN:									
<i>Bilateral Superior Frontal Gyrus</i>									
	<i>RH SPL</i>	---	---	---	---	5.38	MRS	RH	18 -54 72
	<i>RH Frontal eye-fields</i>	---	---	---	---	6.67**	Network	RH	30 33 51
Subcortical RSNs:									
<i>BG Network</i>									
	<i>LH Precun</i>	---	---	---	---	12.16**	MRS	LH	6 -72 42

*All reported T-scores correspond to a p-value < 0.001, uncorrected; ** Corresponds to a T-score $p < 0.05$ (voxel-wise FWE-corrected).

Table 3

Table 3. Summary statistics for paired-sample t-tests on selected FNC pairs and Pearson correlations between Glx and FNC before and after tDCS.

FNC Pairs	Pre-tDCS	Post-tDCS	T-score	Pre Glx & FNC	Post Glx and FNC
Among Parietal nodes (local to anodal tDCS):					
RH frontal-Parietal & Lh frontal-Parietal	0.52 (0.28)	0.56 (0.24)	0.35	0.70* (LH)	---
RH frontal-Parietal & Precuneus	0.24 (0.16)	0.18 (0.40)	-0.46	---	---
Lh frontal-Parietal & Bilateral SPS	-0.27 (0.28)	0.02 (0.25)	2.15*	---	---
Lh frontal-Parietal & Bilateral Inf. Parietal	0.10 (0.39)	0.29 (0.39)	2.02*	---	---
Bilateral SPL & Precuneus	0.29 (0.45)	0.19 (0.43)	-0.72	---	0.76* (LH)
Bilateral SPL & Bilateral Inf. Parietal	0.27 (0.28)	0.19 (0.33)	-0.48	---	---
Bilateral SPS & Precuneus	0.40 (0.28)	0.33 (0.29)	-0.47	---	---
Bilateral SPS & Bilateral Inf. Parietal	0.20 (0.34)	0.42 (0.17)	1.64	---	---
Nodes that comprise DMN:					
ACC & PCC	0.54 (0.21)	0.51 (0.26)	0.34	---	---
ACC & Precuneus	-0.05 (0.28)	0.30 (0.19)	2.44*	---	---
Nodes that comprise distributed networks:					
Superior Frontal Gyrus & Bilateral SPL	0.27 (0.33)	0.04 (0.43)	-2.76*	---	0.65* (LH)
Bilateral aPFC & Bilateral Inf. Parietal	-0.28 (0.30)	-0.15 (0.27)	1.48	---	0.77* (LH)
Salience & ACC	-0.24 (0.32)	0.07 (0.33)	2.02*	---	0.69* (RH)
Salience & BG	0.23 (0.25)	0.25 (0.25)	0.15	-0.84* (LH)	-0.74* (RH)
Among cerebellum network:					
Bilateral SPL	0.53 (0.24)	0.55 (0.19)	0.23	---	---
Bilateral Inf. Parietal	0.14 (0.30)	0.30 (0.25)	1.65	0.66* (LH)	---
Bilateral SPS	0.21 (0.37)	0.31 (0.23)	1.25	---	---
Precuneus	0.53 (0.37)	0.72 (0.16)	1.76*	0.71* (LH)	---
PCC	0.14 (0.25)	0.30 (0.23)	1.3	---	---
LH frontal-Parietal	-0.06 (0.29)	0.15 (0.33)	2.01*	---	---
RH frontal-Parietal	0.23 (0.25)	0.35 (0.22)	1.52	0.76* (LH)	---
BG	0.34 (0.18)	0.02 (0.52)	-1.75*	---	---

All FNC pairs are displayed as Fisher Z. * $p < 0.05$ (uc). LH, left hemisphere; RH, right hemisphere; Inf, inferior.