ABSTRACT

Title of Dissertation: UNDERSTANDING NEUROPLASTIC EFFECTS OF TRANSCRANIAL DIRECT CURRENT STIMULATION THROUGH ANALYSIS OF DYNAMICS OF LARGE-SCALE BRAIN NETWORKS

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Intrinsic adult neuroplasticity plays a critical role in learning and memory as well as mediating functional recovery from brain lesions like stroke and traumatic brain injuries. Extrinsic strategies to aid favorable modulation of neuroplasticity act as important adjunctive tools of neurorehabilitation. Transcranial direct current stimulation (tDCS) is an example of a non-invasive technique that can successfully induce neuroplastic changes in the human brain, although the underlying mechanisms are not completely understood. In this regard, characterization of neuroplastic changes in large-scale brain networks is a functional and necessary step towards non-invasively understanding neuroplastic modulation mediated by tDCS in humans. This dissertation, thus, aimed to understand the effects of tDCS, on large-scale brain network dynamics recorded through magnetoencephalography (MEG) through three specific aims that will provide novel insights into the mechanism(s) through which plastic changes are promoted by tDCS, specifically in the context motor learning. This dissertation pursued a systematic investigation of these changes in whole-head cortical dynamics using both model-free and model-based analysis techniques. Two experiments were conducted to

dissociate between network changes mediated by tDCS at rest as well as when coupled with a task in order to determine optimal conditions for using tDCS for clinical purposes. Results from Study 1 using model-free analysis showed that a specific fronto-parietal network at rest was modulated up to a period of 30 minutes outlasting the duration of the stimulation. Further model-based analysis of this fronto-parietal network showed that these differences were driven by network activity primarily involving high frequency gamma band connectivity to and from the supplementary motor area to associated regions (left primary motor cortex (stimulated region), left prefrontal and parietal cortices). Results from Study 2 showed that the tDCS exerts highly polarity-specific effects on the impact of oscillatory network connectivity, within the functionally relevant fronto-parietal network, on behavioral changes associated with motor learning. These results advance our understanding of neuroplasticity mediated by tDCS and thus, have implications in the clinical use of tDCS for enhancing efficacy of neurorehabilitation in patients with stroke and traumatic brain injury.

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DEDICATION

This dissertation is dedicated to my husband, Ashwin and

my parents, Venkatakrishnan & Poornima.

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CHAPTER 1: INTRODUCTION

Neurorehabilitation is the mainstay in the long-term treatment of functional disabilities in patients suffering from disorder like stroke and traumatic brain injury. Improving efficiency of rehabilitative approaches can thus significantly improve quality of life in these patients while concomitantly reducing costs of healthcare provision. In this context, techniques that help harness and augment intrinsic neuroplasticity, i.e., the nervous system's ability to modify itself in response to new experiences, in the recovering brain act as important adjuncts of neurorehabilitation in the clinical setting. Non-invasive cortical stimulation techniques such as transcranial direct current stimulation (tDCS) is a pain-free, minimal risk method to functionally modulate neuroplasticity in humans, thus acting as a potentially important adjunctive tool for neurorehabilitation (Dimyan & Cohen, 2011; Hummel & Cohen, 2006). The widespread use of this technique, thus, raises an important question about the underlying neural mechanisms mediating the observed neuroplastic changes.

Previous research has shown that anodal (positive polarity) tDCS increases cortical excitability while cathodal (negative polarity) tDCS depresses cortical excitability in general (M. Nitsche et al., 2008a). Specifically, anodal tDCS over sensorimotor cortical regions has been shown to improve motor cortical excitability, as evidenced by increased amplitudes of motor evoked potentials as well as enhanced somatosensory processing (Matsunaga, Nitsche, Tsuji, & Rothwell, 2004). Further, coupling anodal tDCS to the motor cortex with a behavioral motor learning task has been shown to enhance performance improvements (Antal et al., 2004; M. A. Nitsche et al., 2003; Reis et al.,

2009). While there is sufficient evidence to support the neuroplastic modulation induced by tDCS, there is surprisingly less research about the putative mechanism of action of tDCS. Recently, anodal tDCS has been shown to induce an activity-dependent brain-derived neurotrophic factor associated synaptic plasticity in rodent models (Fritsch et al., 2010). In humans, magnetic-resonance spectroscopy suggests that anodal tDCS acts by decreasing local gamma-amino-butyric acid (GABA) levels while cathodal tDCS leads to correlated reduction in local glutamate and GABA levels (Stagg et al., 2009). These findings provide some insight about how tDCS produces its neuroplastic effects; however, it is difficult to draw inferences about specific neuroplasticity induced by tDCS in stimulated brain networks.

Studying brain network activity, an emergent property of interactions of basic neuronal processes and functions, is an important step in understanding the brain's functional and computational processes to make more holistic interpretations about neurophysiological basis of behavior. In this regard, an approach combining tDCS and neuroimaging of large-scale brain networks is a promising approach to study neurophysiological network interactions resulting from local neuronal excitability that is modulated by tDCS, to provide a deeper understanding of neuroplastic effects of tDCS. Importantly, this approach also helps develop potential neural biomarkers of plastic reorganization in the brain that can have far-reaching impact on administering and monitoring of neurorehabilitative interventions in patients. There is some recent evidence of network-level changes induced by anodal tDCS over motor cortex, compared to sham, at rest in electroencephalographic (EEG) brain activity (Polanía,

Nitsche, & Paulus, 2010) as well as hemodynamic brain responses recorded through functional magnetic resonance imaging (fMRI) (Polanía, Paulus, Antal, & Nitsche, 2011) and positron emission tomography (PET) (Lang et al., 2005). These studies demonstrate that direct current stimulation over a focal brain region produces changes in widespread brain activity, attributed in part to the alterations of interactions/connectivity between different neural regions that comprise a large-scale functional brain network. However, a comprehensive account of brain network modulation by tDCS is still lacking, specifically to compare its effects on brain activity at rest versus during motor task performance that is necessary to understand the nature of neuroplastic modulation induced by tDCS.

Specifically, it is important to determine whether the behavioral effects of tDCS are merely associated with enhanced cortical network activation or in fact, cortical network reorganization wherein previously inactive/less active nodes of the network and their interactions with other nodes become more significantly activated (O'Shea, Johansen-Berg, Trief, Göbel, & Rushworth, 2007). This is an important step towards developing more effective applications for therapeutic purposes in patients with traumatic brain injury and stroke to produce favorable biases in cortical network organization for improving functional motor performance.

The primary focus of this dissertation, thus, is to systematically determine polarity-specific effects of tDCS on large-scale brain network dynamics with the aim of explaining neuroplastic mechanisms modified by tDCS through three specific aims outlined below. This involves application of model-free as well as model-based

analytical approaches to whole head brain dynamics to provide a rigorous characterization of these effects on cortical network organization and dynamics. Specifically, these effects will be studied in relation to motor cortical stimulation in the context of changes induced in somatomotor networks necessary for motor learning.

Specific Aims

Specific Aim 1. To identify resting oscillatory brain network dynamics affected by motor cortical tDCS using a robust model-free analytical approach.

In this context, a method combining independent component analysis (ICA) and statistical clustering is a robust, blind and data-driven approach to describe large-scale brain network activity changes. This technique is very useful to identify functional networks associated with neural processes and describe their oscillatory dynamics. Here, neurologically healthy adults will be tested, before and up to 30 min after end of tDCS, to precisely identify specific networks modulated by tDCS and establish the temporal profile of persistence of these effects in terms of their electrophysiological dynamics recorded by magnetoencephalography (MEG). MEG is particularly useful in this context owing to its high temporal and spatial solution, high signal-to-noise ratio, as well as the practical advantages of minimal set-up time necessary for temporal constraints imposed by this experimental design.

Hypothesis 1: Given that previous studies have identified sensorimotor networks to be characterized by oscillatory activity in alpha (8-12 Hz) and beta (13-30 Hz) (Mantini, Perrucci, del Gratta, Romani, & Corbetta, 2007), it is hypothesized that brain networks

recruiting parieto-motor regions characterized spectrally by changes in alpha and beta band power after real tDCS relative to sham would be identified. Additionally, high frequency gamma band activity (> 40 Hz) may be altered by tDCS since it is reflective of engagement of local neuronal circuitry/processes. Since behavioral effects of tDCS have been expected to last at least 30 min to an hour after stimulation (M. Nitsche, Cohen, Wassermann, Priori, Lang, Antal, Paulus, Hummel, Boggio, & Fregni, 2008a), it was hypothesized that network dynamics would remain altered at least up to 30 min as studied here.

Specific Aim 2. To determine effects of motor cortical tDCS on interactions within specific motor network(s) identified in SA1 using a dynamic connectivity-based analytical approach.

The robust model-free method in SA 1 will help provide an unbiased identification of networks modulated by real tDCS as compared to sham. However, one limitation of this method is that it does not identify changes in within network interactions, like changes in connectivity between different regions involved in that network. Thus, it is important to quantify these interactions within the identified network modulated by tDCS as it can be useful as a marker of extent of neuroplastic alterations induced by the tDCS, which can then be related to behavioral changes observed with tDCS. In this context, structural equation modeling (SEM) is an extremely robust method that can be applied to a connectivity model specified between different brain regions involved in any given network (Büchel & Friston, 2000; Rowe, 2010; Rowe

et al., 2002; Sharma, Baron, & Rowe, 2009). However, due to inherent temporal correlation within neural activity time series, Granger Causality estimation based on multivariate regression (Chen et al., 2011) will be applied to study interactions within networks.

Hypothesis 2: Based on SA 1, it is expected that a parieto-motor network will be modulated by motor cortical tDCS relative to sham. Thus, the nodes (i.e., brain regions obtained by transforming MEG sensor data to anatomical brain region through an inverse solution) within a specific left parieto-frontal motor network (Chen et al., 2011; Sharma et al., 2009) will be subjected to data-driven Granger Causality Analysis to estimate path coefficients of network connectivity. It is expected that anodal tDCS will specifically increase strength of network interactions of primary motor cortex (M1) with premotor and supplementary motor cortical regions, (Sharma et al., 2009) while cathodal tDCS is expected to decrease these interactions. Specifically, this connectivity is expected to be occurring in high frequency beta and gamma (broad-band) oscillatory bands (akin to coherence). It is also likely that long-range interactions of premotor and supplementary cortical regions with parietal and prefrontal cortex may be altered in the alpha frequency band by real tDCS (anodal and cathodal) compared to sham.

Specific Aim 3. To determine the influence of changes in brain network organization (identified in SA1 & 2) induced by motor cortical tDCS on learning to adapt motor performance to a novel sensorimotor context (i.e., visuomotor adaptation).

The effect of specific network interactions i.e., cortical reorganization, modulated by tDCS on performance changes associated with motor learning is not known. Here, it is important to use a behavioral learning task that is robust enough to induce performance changes within reasonably short practice duration of about 30 minutes (the hypothesized duration of persistence of effects of tDCS on brain network dynamics). Thus, to establish this relationship between brain network correlates and performance changes, three groups of neurologically healthy adults will participate in a visuomotor adaptation task requiring them to adapt their hand movements to altered screen-cursor relationships after receiving anodal tDCS, cathodal tDCS or sham over left M1 respectively. Recently, anodal tDCS over M1 has been shown to improve retention of such learning (Galea, Vazquez, Pasricha, Orban de Xivry, & Celnik, 2010), but the underlying brain network changes are not known.

Hypothesis 3.1: Anodal tDCS is expected to significantly accelerate learning (increase rate of learning) compared to Cathodal and Sham tDCS.

Hypothesis 3.2: It is expected that the strengthened network interactions of M1 with premotor cortical regions will correlate behaviorally with higher magnitude of learning in subjects receiving anodal tDCS compared to those receiving sham. Additionally, in this group of subjects, enhanced fronto-parietal interactions is expected to correlate with faster rate of learning. Cathodal tDCS could produce the opposite effects or no differences compared to sham. Alternatively, anodal tDCS could produce reorganization in this parieto-frontal network without altering learning performance, suggesting that its

mechanism of action may in part be related to inducing biases in nodes of stimulated networks.

Significance

This research program is the first to provide a comprehensive, systematic account of evidence for neuroplastic alterations induced by tDCS on large-scale brain network characteristics and their relationship to behavioral beneficial effects seen with tDCS. This is an important step towards understanding the mechanisms of neuroplastic modulation caused by tDCS in order for appropriate clinical application in patients for neurorehabilitation. It will help indentify behavioral windows of opportunity after application of tDCS for therapeutic interventions in patients that will help maximize the benefit of stimulation as an adjunctive therapeutic tool. Further, the novelty and innovation of this research lies in the methodical investigation of brain network characteristics underlying neuroplasticity. This will help identify potential non-invasive neural biomarkers of neural plasticity, which can be extremely useful in predicting and monitoring patient response to tDCS as well its efficacy as a treatment. More generally, this will impact developing more objective and quantitative clinical outcome measures in patients that can be used for early diagnosis and progressive patient evaluation in neurological disorders; this will greatly help facilitate effective neurorehabilitation and enhance functional quality of life in these patients.

Five additional chapters are included in this dissertation proposal. The second chapter presents a review of the relevant literature. The third through fifth chapters

present details of the three specific aims respectively. The final chapter presents a brief general discussion of the scope of this research.

CHAPTER 2: REVIEW OF LITERATURE

Combining transcranial direct current stimulation and neuroimaging: novel insights in understanding neuroplasticity. **(Venkatakrishnan & Sandrini, 2012)**

[A part of this literature review has been published as cited above].

Abstract

In recent years, non-invasive brain stimulation techniques like transcranial direct current stimulation (tDCS) have gained immense popularity owing to their effects on modulating cortical activity and consequently motor and cognitive performance. However, the neurophysiology underlying such neuroplastic changes is less understood. This article critically evaluates the contemporary approach of combined tDCS and neuroimaging as a means to provide novel insights in understanding the neurophysiological and neuroplastic processes modulated by this brain stimulation technique. We end by briefly suggesting further lines of inquiry.

External application of direct electrical current to the head is one of the oldest techniques used to modulate cortical excitability. The noninvasive version of direct current application to the scalp surface is more commonly known as transcranial direct current stimulation (tDCS). tDCS modulates cortical excitability by constantly applying weak electrical current over time to increase (positive polarity) or decrease (negative polarity) cortical excitability (Nitsche et al. 2008). This technique has recently been reintroduced in neuroscience research by virtue of its potential for both the

investigation of causal brain-behavior relationships and for the rehabilitation of many diseases. It is worth mentioning here that progress in neuroscience often depends on the convergence of evidence from multiple methods. Since every single technique has its own limitations, there is a clear theoretical advantage in combining different approaches. In the last decade, combined transcranial magnetic stimulation (TMS)-neuroimaging studies have greatly stimulated research in understanding neurophysiological and neuroplastic effects induced by noninvasive brain stimulation (Siebner et al. 2009). In this article, however, we critically evaluate the emergence of the approach to combine tDCS with neuroimaging techniques to understand tDCS-induced neurophysiological effects on whole brain functional networks. Because the majority of evidence has been gained recently from modulation of the primary motor cortex (M1) in healthy subjects, we concentrate on these studies and end by discussing future research directions.

Overview of tDCS administration

tDCS is typically applied through a bipolar electrode montage. The electrodes, covered by a sponge soaked in a conducting solution like saline or tap water, are attached to the subject's scalp. The anode is the positively charged electrode, and the cathode is the negatively charged electrode. The primary stimulation parameters that are controlled by the experimenter include: *1*) electrode size, *2*) intensity of stimulation, *3*) duration of stimulation, and *4*) electrode montage. Typically, large electrode sizes (5 x 5 cm or 5 x 7 cm) are used to maintain a low current density such that the skin sensation of the

electrical stimulation is bearable and also to avoid local skin burns. The intensity of electrical current used most commonly range between 1 and 2 mA, with the latter being more common in montages with one electrode being placed on an extra-cephalic location like the arm. Coupled with the large electrode surface area, these low intensities allow maintenance of an optimal, safe current density between 24 and 29 μA/cm2 (Nitsche et al. 2008). When applied for several minutes, tDCS produces lasting effects in the human cortex. The duration of the excitability changes induced by tDCS depends on stimulation duration. These are stable for up to about an hour if tDCS is applied for 9 –13 min (Nitsche et al. 2008). Finally, the electrode montage for tDCS administration is determined based on the region being stimulated. Most commonly, for the modulation of the left M1, the active electrode is placed over the representational field of the right hand localized using suprathreshold TMS pulses, whereas the reference electrode is generally placed on the contralateral supraorbital region (Nitsche et al. 2008).

Physiological effects of tDCS

Early investigations of the physiological effects of tDCS and recent computational models of current induction in the cortical tissue by tDCS suggest that short-term polarization with tDCS can change membrane ex- citability rather than actually induce action potentials. In this regard, anodal tDCS increases membrane excitability (i.e., increases resting membrane potentials) while cathodal tDCS hyperpolarizes membrane potentials. It is thus important to recognize that tDCS can mediate almost immediate

changes in membrane excitability, which impacts the response of the involved neural circuit to any incoming inputs, and also me- diate activity-dependent changes in synaptic transmission properties when coupled with some behavioral training (Nitsche et al. 2008). Recently, it was demonstrated that anodal tDCS mediates its physiological effects by long-term synaptic potentiation (LTP) due to activity-dependent release of brain-derived neurotrophic factor (BDNF) (Fritsch et al. 2010). Since BDNF is important for mediating translation and transcription associated with protein synthesis for LTP, it is understandable that offline memory consolidation and long-term retention can be specifically improved by anodal tDCS.

It is also important to highlight the potential interaction between physiological effects of tDCS and homeostatic neural plastic mechanisms because cortical excitability modulation by tDCS may produce some modification in the threshold for LTP and long-term synaptic depression. Synapses that are at a higher level of excitation, likely to occur with conditioning by anodal tDCS, can have a higher threshold for LTP, thus making subsequent LTP induction less probable. On the contrary, synapses at a lower level of baseline excitability, likely to occur with conditioning by cathodal tDCS, can have a lower threshold for LTP, thus making subsequent LTP induction more probable (this is in accordance with the Bienenstock- Cooper-Munroe rule). This form of homeostatic plasticity is an essential mechanism for neurons to prevent an uncontrolled increase in synaptic effectiveness. Moreover, it has important implications regarding the application of tDCS to facilitate neuroplasticity, as related to motor learning, because it

deter- mines appropriate modulation of cortical excitability of brain regions involved in the given functional task performance.

Polarity-specific effects of tDCS over the motor cortex on specific neurotransmitters were recently investigated using magnetic resonance spectroscopy (MRS) (Stagg et al. 2009). MRS is an imaging technique that allows the estimation of different neurotransmitter concentration such as gamma amino butyric acid (GABA), glutamate, etc. These results showed that 10 min of anodal tDCS over left M1 specifically reduced GABA levels in the cortex while cathodal tDCS reduced glutamate levels coupled with correlated decreases in GABA levels. Recently, Stagg et al. (2011) also demonstrated that changes in GABA concentration elicited by anodal tDCS over M1 correlates with the amount of motor learning across individuals: the amount of decrease in GABA concentration due to tDCS was positively correlated with the amount of motor learning in a serial reaction time task (SRTT). Interestingly, when the subjects performed the SRTT in a functional magnetic resonance imaging (fMRI) scanner, GABA responsive- ness also correlated with the decreases in blood oxygenation-dependent (BOLD) signal in the left M1 (contralateral to performing hand) during the task. Thus, these findings high- light the role of GABAergic modulation by anodal tDCS in facilitating motor learning and are suggestive of a possible relevance of GABA in LTP-like synaptic plasticity in human motor learning. Future studies must investigate the relevance of this GABAergic modulation in motor learning following cathodal tDCS since both anodal and cathodal tDCS modulation decreased GABA concentration in the original study conducted by Stagg et al. (2009).

An emerging theme from the aforementioned introduction is that modulation of seemingly "localized" cortical excitability over M1 results in modulation of complex motor performance suggesting that tDCS affects more widespread brain networks, thus inducing plasticity in behavior.

What can brain network analysis inform about neuroplasticity?

The study of brain networks involves examination and quan- tifying properties of interactions between the interconnected components of neural circuits, at multiple spatial scales like from the microscale level of networks of a group of synapses or neurons to the macroscale level of networks of various populations of neurons to the networks in the whole brain. Primarily, brain networks are studied based on the hypothesis that most of the complex cognitive behaviors exhibited by animals emerge from the intricate organization of and interaction between the basic neuronal elements, i.e., neurons over and above the functions and properties of the component neurons themselves (Sporns 2010). In fact, in this multiscale brain network architecture, the interaction between networks at different scales plays an integral role in determining global function. In humans, noninvasive neuroimaging techniques offer the best possible means to study brain networks and their properties in humans in present times. By tracking neural activity in real time, directly or indirectly, neuroimaging methods help provide a complex spatiotemporal description of plastic reorganization in humans, which occurs as a result of experience and also following insults or injuries to the nervous system. Particularly, it involves detailed investigations of connectivity between

different regions of the brain, or in other words, study of dynamics of networks involving regions that are “connected” functionally and/or structurally.

Thus, studying brain networks may serve as an invaluable tool to study underlying neuroplastic processes influenced by noninvasive brain stimulation techniques such as tDCS. In this context, regional cerebral blood flow (rCBF), BOLD signals and oscillatory dynamics through magnetoencephalography, and electroencephalography (EEG), coupled with large-scale brain network analysis, can help identify noninvasive neural markers of neuroplasticity in the human brain. The timing of tDCS relative to neuroimaging defines which questions can be tackled using a combined tDCS neuroimaging approach. When tDCS is applied “offline” before neuroimaging, these techniques can map the spatio-temporal pattern of functional reorganization induced in the brain by tDCS or the lasting functional impact of tDCS on rest- or task-related neural activity at the systems level. Particularly, this might also help clarify some heterogeneous evidence about homeostatic neural plasticity modulated by tDCS. This offline approach, in which tDCS and neuroimaging are separated in time, is also technically easier to implement than the “online” approach, in which tDCS and neuroimaging overlap in time with tDCS having the possibility to adversely affect data acquisition during neuroimaging. However, this online approach is the only means to use tDCS to test how cortical modulation instantaneously modifies the activity and connectivity within the modulated neural circuits or networks.

Neuroplastic effects of tDCS on brain networks

While there is sufficient evidence to support the significant influence of tDCS in inducing neuroplastic changes reflected in observable behavioral changes, the exact mechanism of action of tDCS in producing this neuromodulation is not completely clear. Thus, recent efforts to combine tDCS and neuroimaging in experimental paradigms have been undertaken to provide a more methodical characterization of neuroplastic modulation by tDCS through the use of brain network analysis techniques. Baudewig et al. (2001) examined sensorimotor brain activation before and after 5 min of tDCS over left M1, during performance of a sequential finger-opposition task. This work was the first attempt to detect tDCS-induced modulations of brain activity via changes of the BOLD MRI response to a well-defined functional challenge. The authors found that increased excitability associated with anodal tDCS occurred with in- creased activation in sensorimotor cortical regions while cath- odal tDCS led to decreased activation in the same regions. However, the first study to track changes at rest as well as task-related (finger movements) brain activation used positron emission tomography (Lang et al. 2005). The authors studied rCBF changes after application of 20 min of tDCS over left M1. It was found that real tDCS, i.e., both anodal and cathodal, increased rCBF in M1, sensorimotor cortex, frontal cortical regions compared with sham, and these effects persisted for up to 50 min after end of tDCS. Additionally, anodal tDCS also increased rCBF in subcortical brain regions compared with cathodal tDCS. These findings were the first to show experimentally that the presumed local tDCS application over M1 may produce long-lasting neuroplastic alterations in more widespread brain networks over and beyond stimulated M1 cortical regions.

However, methods to analyze and quantify brain network dynamics were less developed at that time, and recent techno- logical advances have facilitated more detailed computations of network dynamics through analysis of complex neuroimaging data sets of high dimensionality and volume. Thus, the application of characterization of brain network dynamics to study neuroplastic modulation by tDCS is a recent development. In this regard, graph theoretical analyses have been applied to characterize brain network changes in EEG (Polanía et al. 2010) as well as fMRI (Polanía et al. 2011) following 10 min of anodal tDCS over left M1. Graph theory is a mathematical approach to quantify the cost of information transfer and processing in a defined brain network by calculating amount of interconnectivity (edges) between different brain regions (nodes) as well as length of connections within that network.

The results showed that 10 min of anodal tDCS modulated high-frequency oscillatory activity in beta (15–30 Hz) and gamma (60–90 Hz) in the functional EEG synchronization-based connectivity metric during performance of a simple finger-tapping motor task not only in electrodes over the stimulated motor cortex, but also in bilateral frontal, parietal, and premotor cortical regions compared with sham stimulation. On the other hand, with the higher spatial resolution of fMRI, it was possible to show that 10 min of anodal tDCS actually increased short range connections from M1 to premotor and parietal cortical regions, while concomitantly increasing inter-connectedness in prefrontal cortex in resting brain dynamics (Polania et al. 2011).

Interestingly, recent studies also compared changes in fMRI during simultaneous tDCS application during data acquisition in the MRI scanner (“online” approach, Kwon et al.

2008; Antal et al. 2011). In the first study (Kwon et al., 2008), anodal tDCS was applied over left M1 during grasp-release hand movements using 4 x 21-s stimulation phases (resting-tDCS-tDCS-tDCS-tDCS). No cortical activation was detected in any of the stimulation phases except the fourth tDCS phase. Activation was found not only under the electrode but also in the left supplementary motor cortex and the right posterior parietal cortex. However, in this study, cathodal stimulation was not applied. Therefore, in another study (Antal et al. 2011), the authors addressed the question as to whether anodal and cathodal tDCS result in differential BOLD fMRI signal changes during a rest condition as well as the polarity-specific effects of tDCS on the brain network activated by a voluntary finger-tapping task. Although specific brain network analytical approaches were not used here, neither anodal nor cathodal tDCS over the M1 for 20-s stimulation duration induced a detectable BOLD signal change. However, compared with a voluntary finger-tapping task without stimulation, anodal tDCS during finger tapping resulted in a decrease in the BOLD response in the supplementary motor area (SMA). Cathodal stimulation did not result in significant change in BOLD response in the SMA, although a tendency toward decreased activity could be seen. In the control experiment, in which the electrodes were placed over left and right occipito-temporo-parietal junction, neither cathodal nor anodal stimulation resulted in a significant change of BOLD signal during finger tapping in any brain area including SMA, premotor cortex, and M1.

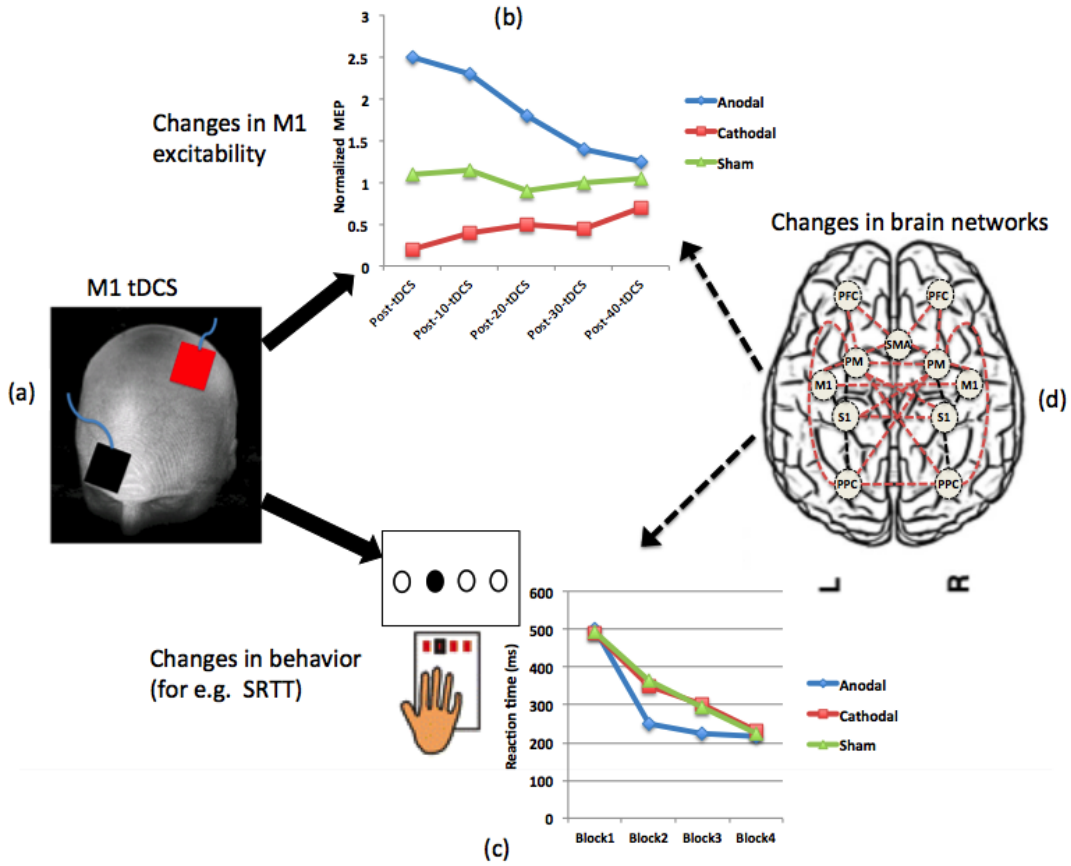

Fig. 1. *A*: a schematic summary of transcranial direct current stimulation (tDCS) effects over M1 (bipolar electrode montage with one electrode over M1, and other over contralateral supraorbital region). *B*: M1 tDCS mediates polarity-specific changes in cortical excitability (shown as motor-evoked potentials, i.e., MEPs expressed as a ratio to baseline) that outlasts duration of stimulation up to 40 min. *C* also leads to changes in behavior such as motor learning on a serial reaction time task (SRTT). Anodal tDCS over M1 improves motor performance on SRTT more than cathodal and sham tDCS (i.e., greater decrease in reaction time in the early blocks). *D*: representative cortical brain networks with interacting regional nodes that are likely modulated by tDCS over M1. PFC, prefrontal cortex; SMA, supplementary motor area; PM, premotor cortex; S1, primary somato- sensory cortex; M1, primary motor cortex; PPC, posterior parietal cortex. Complex brain network dynamics recorded through neuroimaging could help identify and quantify widespread changes in brain activity and functional connectivity within brain networks to provide more novel insights into neuroplastic mechanisms modulated by tDCS. Note: data shown in graphs are representative of previously published results (Nitsche et al. 2008).

Together, these findings provide further support for the notion that tDCS applied over a specific cortical region, like M1, induces widespread changes of cerebral activity at cortical and subcortical levels and alters functional connectivity be- tween this cortex and motor association cortices.

Future directions

While these recent studies provide some insight about potential network-level neuroplastic modulation by localized application of tDCS over M1, it is evident that our understanding of neuroplastic alterations induced by tDCS is far from complete. Future studies are needed to systematically investigate the polarity-specific changes in brain network dynamics induced by tDCS to provide a plausible mechanistic account of neuroplasticity and explain behavioral neurophysiological changes that are modulated by tDCS (see Fig. 1). Furthermore, this approach can help examine homeostatic plasticity induced by tDCS at the level of brain networks, an important phenomenon for the application of tDCS in treating diseases known to have pathological altered cortical excitability (i.e., stroke). An important point to be noted here is that the analytical approach used to describe and quantify brain dynamics significantly influences the inferences that can be drawn from the data. Most of the recent studies described above, including graph theoretical analysis, used connectivity-model based analyses to describe changes in brain network dynamics. However, using model-free, data-driven approaches in the future, like independent component analysis, may also be important to initially identify specific networks modulated by tDCS in an unbiased manner.

Moreover, brain network analytical approaches are useful tools to noninvasively evaluate not only the functional changes induced by tDCS but also to track the effects of other non-invasive stimulation methods that have also been reported to produce neuroplastic alterations in the human brain (e.g., transcranial random noise stimulation, transcranial alternating current stimulation, and TMS). All these approaches will have important implications in future clinical applications of brain stimulation as well as help define noninvasive markers of neuroplasticity.

In summary, such combined approaches to study and quantify neurophysiological processes associated with neuroplasticity are critical to help identify, monitor, and potentiate neuroplasticity that is crucial for functional recovery in patients suffering from brain lesions like stroke and traumatic brain injury.

Methods to study changes in brain networks

Following from the discussion above, it is intuitive that the study of brain networks involves detailed investigations of connectivity between different regions of the brain, or in other words, study of dynamics of networks involving regions that are "connected" functionally and/or structurally. The methods used to study these network properties depend to a large extent on the type of the neural data they are applied to. Here, a few of the methods used to study human brain network properties will be discussed, in the context of the data they are applied to and their relative advantages and disadvantages. Also, relevant contemporary research evidence employing these methods in the study of neural plasticity will be discussed.

A) Blind Model-free Analysis

Independent Component Analysis

Independent component analysis (ICA) is a blind source separation technique that allows decomposing complex data into their maximally independent, underlying contributing source signals (here presumed to originate from specific functional brain networks or neural generators) through a linear demixing process by minimizing mutual information among these sources. In the context of neural data, ICA has been successfully applied to hemodynamic data recorded through fMRI to identify spatially independent, temporally overlapping patterns of brain activity (Albert, Robertson, & Miall, 2009; Esposito et al., 2005; Mantini et al., 2007; van de Ven et al., 2005). Herein, each pattern or component separated by the algorithm is representative of a brain network with functionally inter-connected regions. The application of this technique to fMRI data has revealed stable patterns of activity or brain networks that are activated not only during specific task performance, but also during awake, alert, resting states. Additionally, this method combined with statistical clustering has also been applied to electrophysiological brain data to decompose it into spatially overlapping, temporally independent brain networks with the objective of studying their complex oscillatory dynamics (Contreras-Vidal & Kerick, 2004). The main advantage of using this method is that it allows blind, data-driven separation of contributing brain networks from complex data mixtures, thus allowing more objective and unbiased exploration of brain network dynamics during functionally relevant behavioral states. However, an inherent disadvantage of this method is that it cannot track dynamic interactions or connectivity

changes among brain regions within identified networks. Nevertheless, it serves as an excellent first pass to identify brain networks engaged in specific cognitive and behavioral processing, which can then be studied in greater detail using other analytical methods. Interestingly, application of this method to fMRI resting brain data showed that a cerebellar motor network was engaged significantly more strongly after adaptive learning of novel screen-cursor relationships as compared to before learning (Albert et al., 2009). This study was the first to show that resting brain network activity could be a very useful to study neuroplastic events occurring in the human brain.

B) Connectivity Analysis

As the name suggests, this group of analysis techniques are used to study functional and dynamic interactions between different regions or component nodes of a given brain network. These techniques are different from the previous class of model-free analysis in that by definition, they assume some kind of model based on measures of connectivity. For the sake of simplicity, these will be discussed under two main categories, namely methods that allow studying causal interactions and those that do not.

Causal Connectivity Analysis

A number of analytical models are useful to study directional relationships in brain network dynamics and can be particularly useful in understanding and elaborating the neural processing involved in specific behaviors. The methods that fall under this

classification include *Dynamic causal modeling (DCM), Granger causality modeling, Structural equation modeling (SEM) and Graph theoretical analysis*. These methods are widely applicable both to fMRI as well as electrophysiological brain dynamics and compute causal connectivity metrics in a network based on different underlying assumptions. DCM and Granger causal measures can explore both linear and non-linear dependencies in the data while SEM and Graph theoretical metrics primarily explore linear relationships in the data (Rowe, 2010) (Note that graph theory metrics can also be computed on networks built with non-linear connectivity measures). Graph theory principles find its roots in the fact that brain networks show properties of scale-invariance, critical dynamics and complex architecture (or small worldness), which helps, maximize global efficiency by reducing cost of information transfer and communication. The main advantage of these methods obviously is the ability to test causal connectivity between nodes of relevant brain networks. Particularly, with SEM and Graph theoretical analyses, it is also possible to build whole network models and estimate connectivity parameters as they change with respect to specific behaviors. This offers a robust approach to tie together brain network dynamics to behavioral measures in humans. There is no specific demerit of these methods, however, as with any model-based analytical approaches, appropriate model definitions on the part of the experimenter are crucial to drawing meaningful interpretations of the results of such computationally intense analyses.

As expected, these analytical approaches, specifically graph theoretical analysis, are gaining popularity to examine changes in brain networks, particularly in the context

of dynamic reorganization in neurological disorders and neural plasticity following learning (Bassett & Bullmore, 2009; E. Bullmore & Sporns, 2009; Rowe, 2010); a few of these important findings are discussed here. Modification of functional connectivity in dorsal attention, default and visual resting state networks (fMRI) in response to training a visual perceptual learning task has been recently demonstrated (Lewis, Baldassarre, Committeri, Luca Romani, & Corbetta, 2009). Here, Granger causaslity connectivity changes between frontal eye field and visual cortex reflecting top-down attentional processing and bottom-up visual processing in the frontal regions following training also correlated with amount of behavioral learning. Specifically, greater changes in GC measures within this network correlated with worse behavioral performance, supporting the notion that network organization can be an excellent predictor of behavior. Recently, it has also been shown using graph theory analysis that functional brain networks (MEG) show active reorganization depending on the behavioral state – active performance of a simple motor task leads to increased long-range fronto-parietal connectivity in beta frequency bands (15-30 Hz) brain networks as compared to rest (Bassett, Meyer-Lindenberg, Achard, Duke, & Bullmore, 2006). Interestingly, lesser cost-efficiency of information transfer in this higher frequency oscillatory activity also correlates with more impaired working memory in patients with schizophrenia as compared to healthy controls (Bassett et al., 2009). These findings, thus, emphasize the usefulness of studying brain networks to understand differences in underlying neural processes in various behavioral states.

Importantly, these brain network characteristics may also serve as important neural markers of functional recovery in patients with neurological disorders, as well as markers of active neuroplastic reorganization. This has been validated by the finding that motor network organization in recovering stroke patients showed lesser small-world organization, with less optimal efficiency that correlated with improving function over time (Wang et al., 2010). Further, this network also showed greater within node processing in the ipsilesional motor cortex and contralesional cerebellum, with active changes in functional connectivity to these regions over time, and specifically these network changes correlated with clinical measures of motor function. Together these results suggest that studying and characterizing brain networks using causal connectivity measures in humans can significantly improve our understanding of functional and adaptive neuroplastic reorganization in humans.

Non-Causal Connectivity Analysis

These approaches involve using connectivity analyses using simple linear correlation and partial least squares techniques (McIntosh & Lobaugh, 2004). Particularly, simple correlation analysis between different regions based on the fMRI blood oxygen level dependent (BOLD) signal time series, termed functional connectivity or fcMRI, has been widely used to study brain networks both at rest and during behavior (Fox & Raichle, 2007). This is a widely used technique with the limitation that it requires identifying a priori, seed regions and the caveat is that the functional connectivity analysis depends on identification of the seed. Using this technique, the signature of any functional brain

network is simply the spatial map of the correlated regions seen in both humans (Bressler & Menon, 2010; Dosenbach et al., 2007; 2007; Fox & Raichle, 2007; Mantini et al., 2007) as well as in animals (van Meer et al., 2010). In this regard, fcMRI has been studied widely in the context of resting state networks because these are functional networks that are activated during task performance (Dosenbach et al., 2007; Greicius & Menon, 2004) and also are modified by training/learning (Albert et al., 2009; Lewis et al., 2009). Other non-causal connectivity measures that are mainly computed on oscillatory brain dynamics include measures of coherence in specific frequency bands of brain activity (Brookes et al., 2011). The principle of applying this technique is similar to that fcMRI except that it is applied to oscillatory brain activity and is very useful in describing electrophysiological properties of brain networks, which can provide more insight about underlying functional neural processes.

Given that these resting state networks (RSN) are known to be modifiable, it is not surprising that brain lesions can also alter their dynamics. To this effect, (Carter et al., 2010) elegantly demonstrated that reduced fcMRI patterns, especially interhemispheric connectivity in the somatomotor RSN significantly correlated with upper extremity impairment after stroke. This connectivity was actually not affected by an actual structural lesion, which suggests that functional connectivity stems from reasons other than mere structural connections. On the other hand, (van Meer et al., 2010) corroborate this evidence in an animal stroke model, wherein recovery of function correlates significantly with recovery of this interhemispheric connectivity in the sensorimotor RSN. These findings provide further support for the hypothesis that

learning/training are likely to be traced through changes in RSN activity. This is intuitive considering that a lot of motor skill learning literature attributes consolidation to offline mechanisms, including those occurring in sleep (n.d.). These findings have strong implications in designing and developing neurorehabilitation regimes. For one, it is possible to track the functional reorganization in the brain following lesions and this can be used to test the efficacy of a particular regime. Further, it may also be important in determining optimal time windows to start/accelerate rehabilitation protocols depending on the state of reorganization.

In summary, the study of brain networks is an important tool to understand neural processes underlying cognition and behavior as well as for tracking and understanding dynamic neuroplastic reorganization in the brain that constantly occurs with experience and also following lesions to the brain. This section discussed an overview of some of the methods used in these analyses, and more details of relevant methodology that is used in this dissertation will be discussed later (Chapters 3, 4, 5).

CHAPTER 3: SPECIFIC AIM 1

To identify resting oscillatory brain network dynamics affected by motor cortical tDCS using a robust model-free analytical approach.

[This work has been published: (Venkatakrishnan, Contreras-Vidal, Sandrini, & Cohen, 2011)]

Introduction

Noninvasive cortical stimulation techniques such as transcranial direct current stimulation (tDCS) have been widely used to modulate cortical excitability, particularly in the motor cortex, to promote plasticity and augment functional recovery along with functional rehabilitative techniques (M. Nitsche, Cohen, Wassermann, Priori, Lang, Antal, Paulus, Hummel, Boggio, & Fregni, 2008b). However, the mechanisms underlying neuroplastic changes induced by tDCS are poorly understood. One approach to gain insight into these mechanisms is the investigation of changes induced by tDCS on resting brain activity, which is known to show neuroplastic modulation after motor learning (Albert et al., 2009). Recently, evidence of changes in resting brain activity immediately after tDCS has been shown in fMRI and EEG using connectivity-based model driven analysis (Polanía et al., 2010; 2011). However, resting oscillatory dynamics post-tDCS were less differentiable relative to sham (Polanía et al., 2010). Thus, here we sought to use blind, data-driven analysis of whole-head brain activity as it may provide unbiased insights that advance the understanding of mechanisms affected by tDCS.

In this context, a method combining independent component analysis (ICA) and

statistical clustering is a rigorous, blind approach that allows separation of surface summed cortical activity into underlying functional network function associated with specific spectral signatures. Tracking changes in these networks in terms of their spectral characteristics can be very useful to describe functional neural processes engaged/modified by a specific experimental intervention. Previously, this technique as applied to electroencephalographic (EEG) data recorded during performance of a visuomotor learning task was useful to identify functional oscillatory networks associated with neural processes of motor learning (Contreras-Vidal & Kerick, 2004). Here we applied this method to whole-head MEG activity to identify the temporal profile of changes induced in oscillatory network dynamics up to 30 minutes after tDCS.

Methods

Experimental procedure and Data acquisition

Twelve right-handed (6 females), neurologically healthy adults (23-40 yrs, mean age 27.2 $\pm$ 5.7 yrs) participated in this study after providing informed consent as approved by the Institutional Review Board (IRB) at the National Institute of Neurological Disorders and Stroke. Neuromagnetic data were recorded at 600 Hz with a bandwidth of 0-150 Hz using a CTF 275 MEG system (CTF Systems, Inc., Canada) composed of a whole-head array of 275 radial 1st order gradiometer/SQUID channels housed in a magnetically shielded room (Vacuumschmelze, Germany). Synthetic 3rd gradient balancing was used to remove background noise on-line. Participants, blind to type of stimulation, participated in 3 sessions –anodal/cathodal/sham tDCS, at least 24 hours

apart, with the order of stimulation pseudo-randomized and balanced. Target region for stimulation i.e., contralateral/left M1 was determined by transcranial magnetic stimulation targeting the optimal scalp position to elicit motor evoked potentials of the right abductor pollicis brevis. A Phoresor II Auto (model PM850, IOMED, Salt Lake City, UT) device was used to apply tDCS over M1 using a bipolar montage with the cephalic reference electrode over the right supraorbital area. The stimulation was delivered by 25 cm^2 conducting electrodes covered by saline-soaked sponge, at an intensity of 1 mA (current density 0.04 mA/cm^2; total charge 0.048 C/cm^2) for 20 min in the anodal and cathodal tDCS sessions and for up to 20 seconds in the sham session according to a previously described method (Gandiga, Hummel, & Cohen, 2006). Rest MEG recordings were performed in 5 blocks of 5 minutes each (see fig. 1), 1 before (Pre) and 4 after stimulation, allowing measurement of changes up to 35 minutes post-tDCS. During the recording, participants were instructed to stay completely still and relaxed with their eyes closed.

Fig. 1. A) Experimental timeline showing Pre and Post-tDCS rest MEG recordings. Recordings were performed in 5 min intervals, immediately after tDCS (Post-Imm), and at 10, 20, and 30 minutes after tDCS (Post-10, Post-20, and Post-30 respectively). B) Bipolar electrode montage for anodal tDCS application over left M1 is shown; red represents anode and black represents cathode. These polarities were reversed for cathodal tDCS application.

Data Pre-processing

Data from each rest block (3 min, excluding the first and last minute of recording) per subject were demeaned and band-pass filtered between 0.15-150 Hz using a 4^{th} order, zero-phase, Butterworth filter and notch-filtered at 60 and 120 Hz using a 2^{nd} order Chebyshev-type1 filter to remove line noise.

Independent Component Analysis and Clustering

Each rest block of data was subjected to an extended Infomax independent component analysis (ICA) to decompose it into spatially overlapping, temporally independent components. All analyses were performed using custom written programs employing the EEGLAB toolbox (Delorme & Makeig, 2004) in MATLAB 7.11 (The Mathworks, Inc, Natwick, MA).

Component clustering was performed in 3 consecutive steps. *Step 1*: K-means clustering algorithm was used to partition consistent patterns of activation across subjects for each stimulation condition within each block. The algorithm was iteratively optimized to extract K mutually exclusive clusters by minimizing the sum of squared Euclidean distances of each object in the cluster from its centroid. Features used for clustering include (1) scalp component map (2) power in functional rhythms (delta:1-4 Hz, theta: 4-8 Hz, alpha: 8-13 Hz, beta: 13-30 Hz, low gamma: 30-50 Hz, high gamma: 70-100 Hz) computed by integrating power spectral density (PSD) obtained using multitaper method, between frequency intervals (3) component kurtosis and (4) component entropy (281 features total). Clusters with artifacts were identified by visual inspection

of cluster mean scalp map and kurtosis values and were excluded from further analyses. *Step 2*: K-means centroids from step 1 were hierarchical clustered based on Euclidean distance inconsistencies (thresholded at 0.9) to link changing clusters across time. Cophenetic correlation coefficients were further computed between clustering decision and data structure to assess the quality of classification suggested by clustering. *Step 3*: This step was performed to test the null hypothesis that no differences existed between the 3 stimulation conditions. Clusters identified from step 2 for each stimulation condition were subjected to hierarchical clustering (as in step 2), first within each block, and next across blocks. Cophenetic correlation coefficients were computed similar to Step 2. Finally, spectral characteristics of identified clusters (representative of networks) were compared based on 95% confidence intervals of bootstrapped distributions (n=100000) of mean power in each frequency band.

Results

The blind ICA decomposition and clustering method identified 3-5 functional clusters for each stimulation condition at all times of measurement, retaining over 90% of artifact-free data. Cophenetic coefficients computed for both steps of hierarchical clustering were greater than 0.9 indicating correct clustering. All 3 stimulation conditions were represented across 4 functional networks identified pre-tDCS by step 3 clustering.

However, this method identified an effect of real tDCS relative to sham in a left parieto-motor network characterized by a progressive decrease in alpha and increase in gamma band power, starting immediately and lasting up to the Post-20 block after

stimulation (Fig. 2). This network appeared only following tDCS in Post-Imm and also transiently engaged frontal regions in the Post-10 block after tDCS (Fig. 3). Using this approach, no differences between anodal and cathodal tDCS were found. No differences between real tDCS and sham were identifiable in the last block i.e., Post-30 minutes following stimulation.

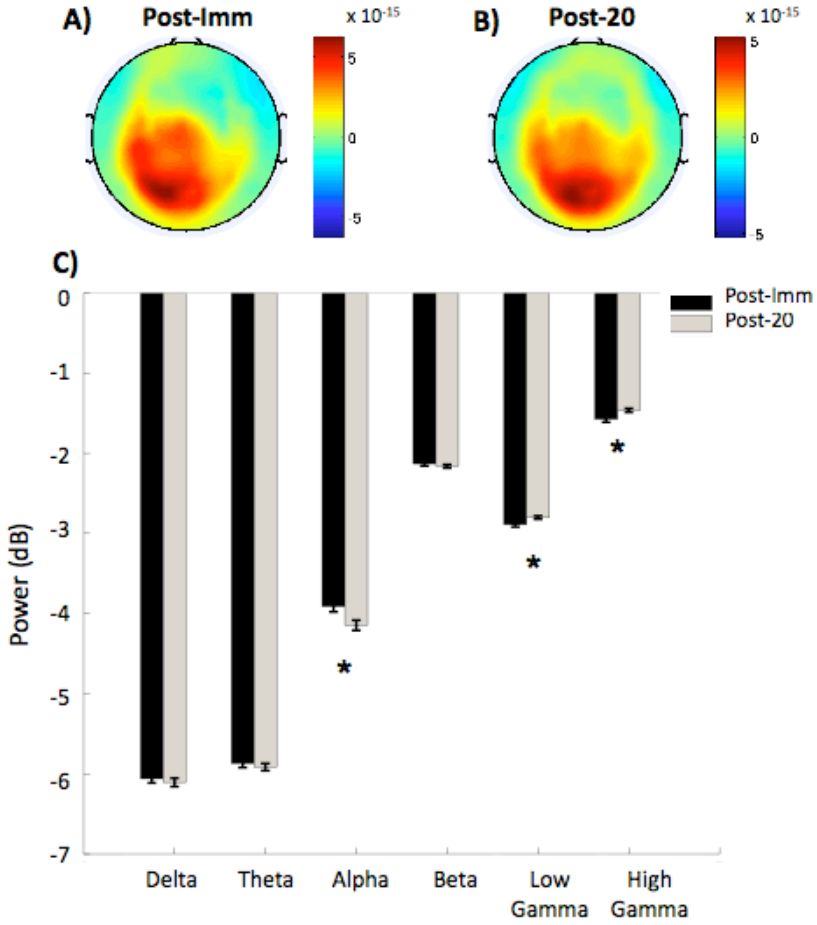

Fig. 2. Real tDCS engaged a parieto-motor network (A) immediately and (B) 20 minutes after stimulation respectively. (Topoplots of cluster means are shown, with activation in femtotesla.) (C) The spectral signature of this network showed a progressive decrease in alpha power and increase in low and high gamma power. Error bars represent 95% confidence intervals. Significant differences in power are indicated by * ($p < 0.05$).

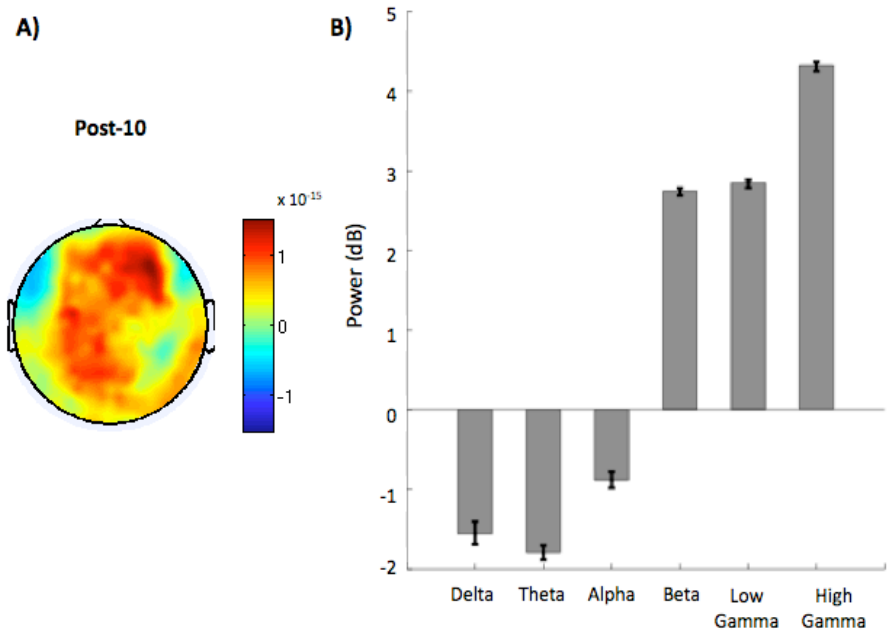

Fig. 3. A) Real tDCS also caused the parieto-motor network to transiently engage frontal cortical regions. This network was hierarchically clustered different from the parieto-motor network in fig. 2 due to different topology. B) The spectral signature of this network is also characterized by higher power in higher frequencies, namely, beta, low, and high gamma. Error bars represent 95% confidence intervals.

Discussion

The temporal profile of neuroplastic changes in large-scale oscillatory network dynamics induced by tDCS is presently unknown. Here, we show that real tDCS over M1 produces changes in resting cortical dynamics in a related parieto-motor network indicative of within-network enhanced local cortical processing. Importantly, we show that these network changes are persistent for up to 25 min post-tDCS. These findings are important in advancing our understanding of the mechanisms mediating lasting effects of non-invasive cortical stimulation over M1 and its influence over a distributed fronto-parietal motor network.

General Considerations

We demonstrate for the first time the feasibility of using a data-driven method combining ICA and statistical clustering to study neuroplastic changes in cortical network dynamics affected by tDCS, both in terms of altered regional activity and cortical dynamics. The identified network here comprises neural regions that are functionally and structurally interconnected and relevant to motor control and learning. Surprisingly, our method failed to find differences between anodal and cathodal polarities of tDCS. Several factors could account for absence of polarity-specific effects. Since anodal and cathodal polarities of stimulation are directed over the same, relatively large cortical area (M1), cortical activity detected by MEG, within folds in underlying cortical gyri, may be insensitive (i.e., cancel out) to subtle differences in polarity-specific activation of underlying neural populations. Indeed, recent evidence from magnetic resonance spectroscopy showing localized reduction in γ-amino-butyric acid i.e., GABAergic activity following both anodal and cathodal tDCS may support the lack of difference in cortical oscillatory dynamics observed here (Stagg et al., 2009). This is also consistent with a non-polarity specific increase in regional cerebral blood flow found with positron emission tomography in stimulated M1, right frontal pole, right primary sensorimotor cortex after tDCS relative to sham (Lang et al., 2005).

Clinical Implications

tDCS is rapidly gaining popularity as an adjunct for neurorehabilitation of motor and cognitive impairments (Floel & Cohen, 2010; Hummel et al., 2010; Miniussi et al., 2008;

Sparing et al., 2009). Thus, clearer understanding of functional changes induced in specific networks engaged by tDCS could lead to a more principled application of this technique. If network changes as identified here are shown to parallel behavioral improvements induced by tDCS, it is conceivable that in the future, individual analysis of the changes in cortical dynamics induced by tDCS could predict the magnitude of behavioral effects, an issue of potential clinical relevance. Such neural biomarkers could contribute to effective rehabilitation strategies by allowing direct monitoring of patient response to treatment.

Conclusion

In summary, these results document a strong effect of motor cortical tDCS in enhancing local cortical processing in a specific fronto-parietal motor network. This finding has implications for the understanding of mechanisms underlying tDCS effects on cortical function and for optimizing its use to augment neuroplasticity in patients with brain lesions like traumatic brain injury (TBI) and stroke for neurorehabilitation.

CHAPTER 4: SPECIFIC AIM 2

To determine effects of motor cortical tDCS on interactions within specific motor network(s) identified in SA1 using a dynamic connectivity-based analytical approach.

Introduction

Studying brain network dynamics to understand neuroplastic effects of tDCS is a relatively new approach. Recently, graph theoretical analyses have been applied to characterize brain network changes in EEG (Polanía et al., 2010) as well as fMRI (Polanía et al., 2011) following 10 minutes of anodal tDCS over M1. Interestingly, it was seen that functional connectivity in fMRI activity at rest and high frequency oscillatory activity synchronization in EEG during finger movement was modulated not only in the stimulated motor cortical regions, but also in bilateral frontal, parietal and premotor cortical regions compared to sham stimulation. A caveat in using graph theoretical analysis as in these experiments is that it is more of an exploratory approach to identify changes in connectivity within networks, and is subject to biases when thresholding is performed on obtained connectivity strengths across different regions. Particularly, when the network modulated by tDCS is not clearly identified, it may be more difficult to test dynamic interactions within that network.

In this regard, we have previously identified that anodal tDCS over M1 modulates a frontoparietal network that outlasts the stimulation duration for up to 30 minutes using data-driven independent component analysis and clustering (SA1) of resting MEG data. However, the changes in interactions, or connectivity, between the regions involved in

that network are not clearly understood yet. More importantly, the differences in temporal dynamics (profile) between anodal and cathodal tDCS in these network interactions occurring after tDCS are not known. Here, we sought to apply a new method to study connectivity changes within this fronto-parietal network and also characterize their temporal profile after the end of tDCS. In this context, structural equation modeling (SEM), a method widely used in the social sciences, is a very powerful method to study functional connectivity within a given network and has been widely applied to fMRI based functional connectivity (Büchel & Friston, 2000; Rowe, 2010; Rowe et al., 2002; Sharma et al., 2009). An important advantage of such a method is that it can study and identify causal connectivity strengths, while allowing validation of models with hypothesized connectivity within a given network. An important advantage over current graph theoretical analysis is that it also can estimate direction of connectivity i.e., positive or negative rather than absolute magnitude of connectivity strength. However, a limitation of the application of this method directly to neuroimaging data is that it is assumes lack of temporal correlation within time series, which is violated in neural data. In this regard, multivariate vector autoregression modeling methods such as “Granger Causality” estimation, are useful to account for temporal correlations within neuroimaging time series (Moratti, Saugar, & Strange, 2011). Hence, here this method was applied to MEG anatomical source localized data with the objective of describe the time-course (up to 30 minutes after the end of tDCS) of oscillatory network interactions modulated by motor cortical tDCS within a fronto-parietal motor network shown in fig. 1. This network has been identified to be of clinical

relevance in motor execution in stroke patients (Sharma et al., 2009). Sharma et al., (2009) identified and studied a bilateral (and symmetrical) fronto-parietal network. In this case, however, the unilateral, left-sided component fronto-parietal network was modeled and analyzed as the tDCS was delivered only to the left primary motor cortex (M1) and also owing to the relevance of the left side of this network to right-handed reaching and pointing motor behavior (Doyon et al., 2009; Shadmehr & Wise, 2005). This analytical method, one form of Time Series Inference (TSI), comes with the advantage that it naturally accommodates stochastic processes, and thus is well suited to the ubiquitous variability that is found in neural time series data. Furthermore, under proper conditions, TSI methods can be effectively used to relate neural activity to cognitive function (Bressler & Seth, 2011). Here we chose to apply this method, for the first time, to study the effects of non-invasive tDCS on brain network activity.

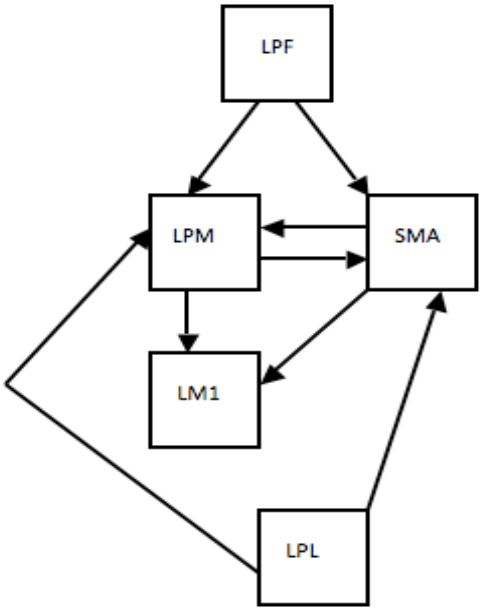

Fig. 1. Structural connectivity model for Granger Causality (or TSI) analysis of hypothesized causal connectivity between regions of a bilateral fronto-parietal motor network. L: Left; PF: Prefrontal cortex (Brodmann area 10); PM: Ventral Premotor cortex; M1: Primary motor cortex; PL: Parietal cortex (Brodmann area 7).

Methods

Experimental procedure and Data acquisition

The experimental procedure and data acquisition was the same as in specific aim 1 (the experimental timeline is shown again in fig. 2). Neuromagnetic data thus obtained were subjected to connectivity analysis as described in the following sections.

Fig. 2. A) Experimental timeline showing Pre and Post-tDCS rest MEG recordings. Recordings were performed in 5 min intervals, immediately after tDCS (Post-Imm), and at 10, 20, and 30 minutes after tDCS (Post-10, Post-20, and Post-30 respectively). B) Bipolar electrode montage for anodal tDCS application over left M1 is shown; red represents anode and black represents cathode. These polarities were reversed for cathodal tDCS application.

Data Pre-processing and Synthetic Aperture Magnetometric (SAM) Analysis

Raw neuromagnetic data were band-pass filtered between 0.15-150 Hz using a zero-phase FIR filter and power line noise was removed using notch filter (60 Hz and higher harmonics). The data were then transformed into three-dimensional estimates of source power using synthetic aperture magnetometry (SAM) (Vrba & Robinson, 2001) based on co-registration with structural brain images obtained for each individual subject. [T1-weighted structural brain images (Magnetization Prepared Rapid Gradient

Echo - MPRAGE) were acquired separately for each subject with repetition time i.e., TE/TR = 2.672s /6.256s, 0.9375x0.9375mm^2 in-plane resolution, with 198 slices at 1mm thickness per volume per volume, Acquisition Matrix = 256x256, FOV = 240x240mm]. Each voxel within the cortex is associated with a beamformer (275 x 1 vector), a unique set of weighting factors that SAM generates from the recorded magnetic field at each sensor, to generate a volumetric representation of brain activity. The filter output at each voxel is a virtual channel, a linear combination of the measurements over time for that specific brain location. SAM determines optimal spatial filters based on a minimum-variance beamformer that estimates current dipole power changes in a voxel within particular time windows and frequency bands and the optimal orientation of the dipole was estimated using the vector-based approach (Rutter et al., 2009). A dipole spacing of 7 mm, corresponding to a cubic voxel width of 7 mm, was used for each dataset i.e. per block of recording before and after tDCS. Single-state SAM imaging to estimate the power source distribution in the brain and normalization was accomplished via a constant noise estimate based on the very high frequency power (>100 Hz) (i.e., Estimated Power = Raw Source Power/Constant Noise Estimate) as in (Rutter et al., 2009). Further, the time-frequency representation of power across the 180 seconds of the SAM filter output at each virtual channel was computed by convolution with a sliding Hamming window (200 millisecond wide) with 75 % overlap in order to yield a 3D time series (3601 data points) of activation for each voxel of anatomical source of neuromagnetic activity with an optimal time- and spectral-resolution. The SAM covariance matrix was calculated in the frequency bands relevant to sensorimotor

processing as described in the literature (and these were also identified to be modulated in specific aim 1) i.e., alpha: 8-13 Hz, beta: 13-30 Hz, low gamma: 30-50 Hz, high gamma: 70-100 Hz.

Connectivity analysis using Granger Causality

The network shown in fig. 1, relevant to motor function (Sharma et al., 2009), is based on anatomical connectivity based on the CoCoMac database (www.cocomac.org). It was subjected to Granger Causality multivariate regression analysis using programs written in the R statistical computing environment that adopts a data-driven approach for estimation of the lagged effects of temporal correlation within neural time series. Vector auto-regressive modeling (VAR), as applied to neuroimaging data, is a primarily data-driven approach to estimate temporal (or lagged) inter-regional effects within a network. Model order was determined to be 1 for these data (based on the AIC criterion). The main difference between VAR and Structural equation modeling (SEM) is that the latter models instantaneous relationships between regions in a network, and importantly allows testing hypothesis-driven models of connectivity and estimating effective connectivity strengths, known as path coefficients. Given that neither of these methods individually optimally represents the true state of brain network dynamics, Structural Vector Autoregression i.e., SVAR would be an excellent approach to combine the strengths of these two approaches to estimate network connectivity in a hypothesis-driven manner. However, since this is a relatively new approach, it is not yet to set up to deal with neural networks involving bidirectional interactions, such as those found

between premotor and supplementary motor areas (LPM and SMA, respectively) in our network of interest. Therefore, given the superior temporal resolution of MEG data (compared to fMRI), TSI or Granger causality estimation was the most suitable method to estimate network interactions in oscillatory brain activity, in a manner that optimally models neural time series, in order to provide valuable insights into neural mechanisms/processes that may be modified by changing network interactions. Thus, here this method was applied to test an anatomical model of connectivity of oscillatory dynamics in a bilateral fronto-parietal network relevant to motor function with the objective of characterizing the temporal profile of neuroplastic modulation induced by motor cortical tDCS compared to sham.

Table 1. Tailarach coordinates of Regions of Interest (ROI) in Left Frontoparietal Network

	X	Y	Z
L M1	-37	-21	58
L PMv	-50	5	22
L PFC	-24	56	6
SMA	-2	-7	55
L PC	-16	-60	48

The anatomical regions' time series were obtained from single 7 mm cubic voxels around the Talairach coordinates specified for M1, PMv, SMA based on a meta-analysis of the brain maps of sensorimotor cortical activation (See Table 1) (Mayka, Corcos, Leurgans, & Vaillancourt, 2006) and for PF and parietal cortical regions based on coordinates of Brodmann areas specified by (Sharma et al., 2009) from a standard Talairach atlas. These analyses were performed using functions in AFNI 2010 (Cox, 1996). The model estimation was performed at the individual subject level for each

frequency band of interest (alpha, beta, low gamma and high gamma), within each block of standardized rest data recording for every session of tDCS (Anodal/Cathodal/Sham). Owing to plausible day-to-day differences in power within a subject, the estimated path coefficients, or effective connectivity measures, for post-tDCS blocks of recording were subsequently baseline corrected i.e., normalized (Pre-tDCS effective connectivity (EC) for each path was subtracted from corresponding paths in each of the 4 Post-tDCS blocks) for each frequency band per subject. From a practical perspective, absolute effective connectivity measures are also not very informative, since these may vary across various neuroimaging methods and also across nature of data used for modeling (namely, spectral power, phase locking values, etc). Thus, it is evident that while considering effects of an intervention such as tDCS on brain network activity, normalized effective connectivity offers a better platform to study and describe the emergent changes in network characteristics following the tDCS.

Group-level statistical analysis was performed by running a mixed effects two-way repeated measures analysis of variance (ANOVA) with 2 within subject factors, namely, type of stimulation and time (4 blocks) for the biologically extant connections within the left parieto-frontal network (8 that are shown in fig. 1). This analysis was performed for each frequency band of interest that is analyzed, namely, alpha, beta, low and high gamma. Post-hoc pairwise comparisons were performed via Tukey tests (corrected for multiple comparisons). All analyses were performed using custom programs written in Matlab 7.11 (The MathWorks, Inc., Natick, MA) and AFNI (Analysis of Functional Neural Images, [v. AFNI_2011_12_21_1014]). Statistical analyses were

performed using the R-package (v. 2.14.1) and Statistical Package for the Social Sciences (SPSS, 18.0).

Results

There was no significant stimulation x time interaction, interestingly, for any of the 8 hypothesized paths (normalized effective connectivity), in any of the 4 frequency bands tested in this analysis. However, the analysis revealed a main effect for type of stimulation for the following connections: Supplementary motor area to Left premotor cortex (SMA-LPM) ($p<0.0001$) and Supplementary motor area to Left primary motor cortex (SMA-LM1) ($p=0.0098$) in the low gamma band. Additionally, in the high gamma band, a significant main effect was found for the connection between SMA and LPM ($p=0.0404$), as well as Left prefrontal cortex and SMA (LPF-SMA) ($p=0.0440$).

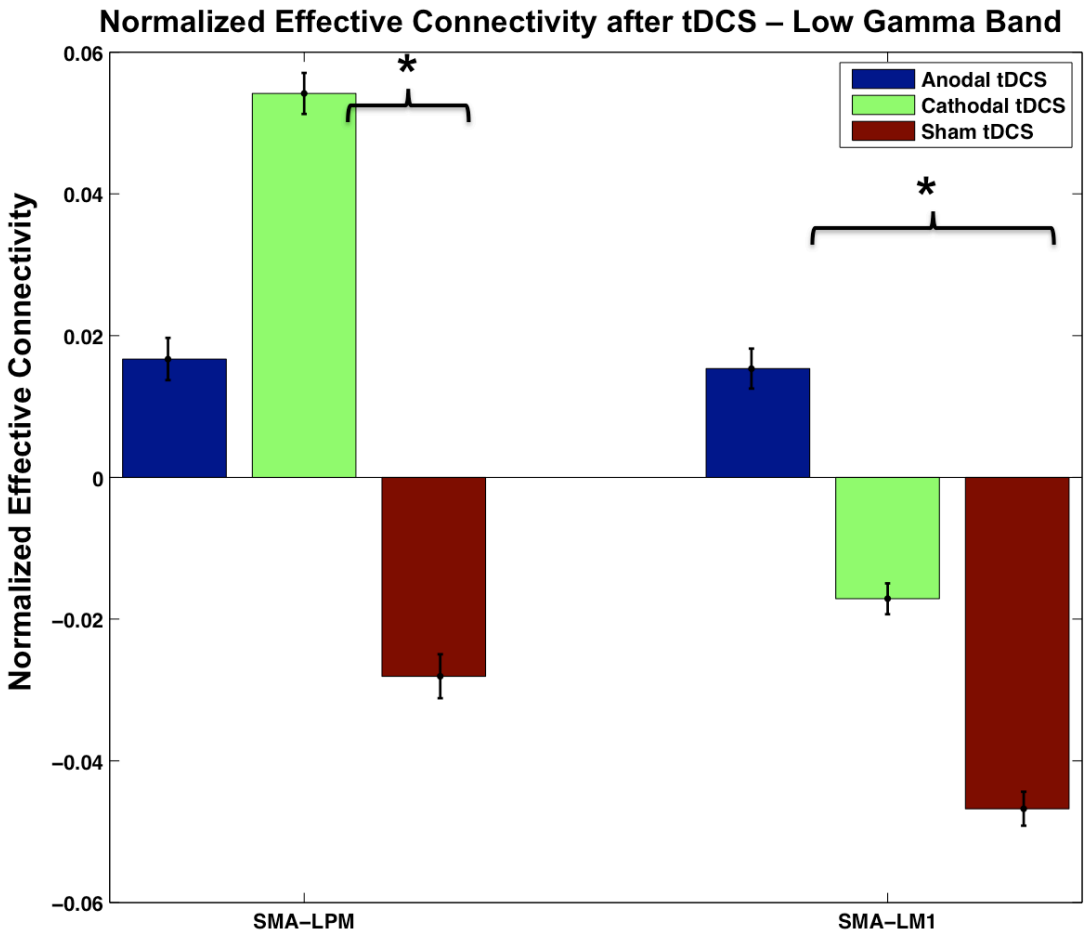

Fig. 3. Changes in normalized effective connectivity following tDCS in low gamma band network activity for the 3 stimulation conditions (main effect - averaged across all 4 blocks; mean ± standard error). * $p<0.05$

Changes in Low Gamma band network connectivity

Post-hoc tests (Tukey adjusted pair-wise comparisons) revealed that a significant difference between Sham and Cathodal-tDCS ($p<0.001$; adjusted p-values are reported for post-hoc comparisons), with greater normalized EC following Cathodal-tDCS in the SMA-LPM normalized effective connectivity in the post-stimulation period (see fig. 3). The difference between Anodal and Cathodal-tDCS groups for this connectivity did not reach significance ($p=0.17$) despite a strong trend, and the difference between the Sham and Anodal-tDCS nearly reached significance ($p=0.08$).

For the SMA-LM1 path, post-hoc tests revealed a significant difference between Sham and Anodal-tDCS ($p=0.01$), with greater normalized EC in this path in the Sham condition compared to Anodal-tDCS. There were no significant differences between Anodal and Cathodal-tDCS or Cathodal and Sham-tDCS. Overall, Anodal-tDCS appeared to produce the least increases in normalized EC across both these paths.

Changes in High Gamma band network connectivity

Post-hoc pairwise comparisons revealed a significantly higher normalized EC in the SMA-LPM path in the Sham-tDCS compared to Anodal-tDCS ($p=0.024$) as well as Sham compared to Cathodal-tDCS ($p=0.0036$) (see fig. 4). However, for the LPF-SMA path, the increase in normalized EC was significantly greater for the Sham compared to Cathodal-tDCS ($p=0.029$). There were no significant differences between Anodal and Cathodal-tDCS effects on either of these paths despite a strong trend.

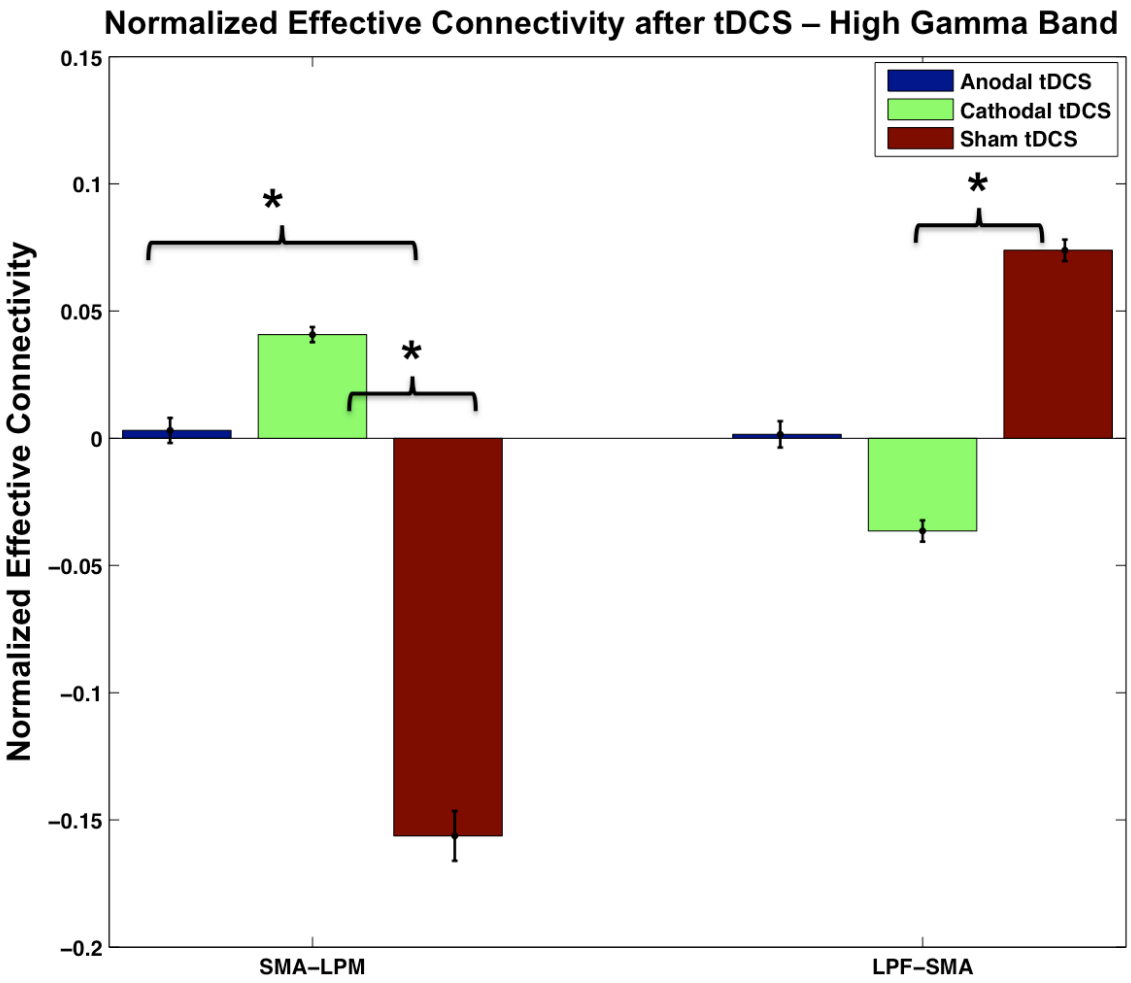

Fig. 4. Changes in normalized effective connectivity following tDCS in high gamma band network activity for the 3 stimulation conditions (main effect - averaged across all 4 blocks; mean ± standard error). * $p<0.05$

Discussion

Hypothesis-based analyses of a left fronto-parietal network activity following tDCS revealed a significant difference between Sham and real (Anodal & Cathodal) tDCS, specifically in high frequency, gamma band oscillatory activity, analogous to results found based on approach adopted in Specific Aim 1 (Venkatakrishnan, Contreras-Vidal, Sandrini, & Cohen, 2011). Furthermore, these changes appear to be driven by network

activity primarily involving the connections to and from the SMA to associated regions (LM1 (stimulated region), LPM as well LPF).

Interestingly, it appears that the increases in normalized EC are much lower in all the 4 paths following Anodal tDCS compared to Cathodal and Sham tDCS. While there were no significant differences between Cathodal and Anodal-tDCS, there appeared to be a trend for greater increases in the former (see figs. 3 & 4). Given that this is resting state recording of spontaneous brain activity over 30 minutes following tDCS, fluctuations are likely expected with subject's state of mind, even in the absence of any intervention, such as during the Sham-tDCS condition. One possible interpretation is that these spontaneous changes or variations in resting state activity could account for injecting higher noise in network activity patterns. In fact, a certain optimal amount of noise may actually favor the ability to quickly transition between different "modes" or oscillatory states of network activity that are "in tune" with different kinds of behavior (Buzsáki, 2006; Buzsáki & Draguhn, 2004; Sejnowski & Paulsen, 2006). However, in the context of modulating a network (or "network priming"), as is attempted with approaches like non-invasive tDCS, reducing noise in the system, and concomitantly increasing signal-to-noise ratio (SNR) could potentially be very favorable to bias behaviors that follow stimulation, in a desirable manner.

Typically, some "perturbations", akin to sensory or motor behavioral stimuli, to the network can actually provide some stability to an oscillatory network that typically fluctuates between critical and stable state dynamics (Buzsáki, 2006). In this case, a possible interpretation of these differences between normalized EC following Anodal

versus Cathodal and Sham tDCS could be that Anodal-tDCS may likely help maintain steady state, or likely some form of homeostasis, in the network over a certain duration following the stimulation, 30 minutes in this case. Thus, it is conceivable that the network could likely have a lower threshold for synaptic plasticity induction following Anodal tDCS, in line with the rules of homeostatic plasticity (Stagg & Nitsche, 2011). Moreover, the changes seen in high frequency gamma band network activity are also consistent with the findings of higher modulation of GABA-ergic neurotransmitter activity in the Cathodal compared to Anodal tDCS (as studied by identifying a region of interest in the stimulated M1 region; Stagg et al., 2009). Together, these could be interpreted as "network priming" induced by Anodal tDCS, which is identified here through oscillatory network connectivity parameters. However, as previously discussed, it is important to recognize that this possible reduction in SNR likely produced by Anodal tDCS may not necessarily serve as "priming" for all kinds of subsequent sensory-motor behavior or interventions. This task-dependence of the effects of network priming may help explain, in part, the controversial evidence on testing of homeostatic plasticity effects of polarity-specific tDCS (Nitsche et al., 2007).

Finally, an important caveat in interpretation of these results is the method used to record and analyze the neural data. Two major neural substrates that influence this fronto-parietal network are the cerebellum and the basal ganglia. Given the limitation of MEG's sensitivity to neural activity of deep-seated neural regions such as the cerebellum and basal ganglia, it is almost impossible to provide a composite depiction of all associated sensory-motor network activity influenced by tDCS given to M1.

Furthermore, this analysis was a priori hypothesis driven to study the fronto-parietal network, based on results obtained through the blind analytical approach involving Independent component analysis and clustering (ICA) in Specific Aim 1. Thus, if additional networks are modulated by M1 tDCS, those are confounding effects that cannot be identified or described, within the scope of this research approach adopted here.

In summary, there appears to be a significant effect of real-tDCS (Anodal & Cathodal) on biasing this left fronto-parietal network, even at rest, compared to Sham. These findings are important because they show for the first time that tDCS specifically influences oscillatory network connectivity within this brain network that is implicated very strongly in cognitive and sensory-motor behavior. Additionally, it also provides for alternative approaches to likely quantify and compare effects of tDCS in patients with various neurological disorders (Schlaug, Renga, & Nair, 2008; Sparing et al., 2009), with the objective of estimating dose-response relationships in them and inform more prudent clinical application of this technique for interventional purposes.

CHAPTER 5: SPECIFIC AIM 3

To determine the influence of changes in brain network organization (identified in SA1 & 2) induced by motor cortical tDCS on learning to adapt motor performance to a novel sensorimotor context (i.e., visuomotor adaptation).

Introduction

The application of neuromodulatory effects of tDCS to augment motor learning is a question of significant practical and clinical importance. Moreover, motor learning is an excellent model of intrinsic neuroplasticity in humans that can be measured indirectly through behavioral changes. Thus, combining tDCS and motor learning allows studying interactions between modulation of intrinsic and extrinsic neuroplasticity. In this context, a common approach has been the application of anodal tDCS concurrent with motor learning in an experimental context (M. Nitsche, Cohen, Wassermann, Priori, Lang, Antal, Paulus, Hummel, Boggio, & Fregni, 2008a; Stagg & Nitsche, 2011; Stagg, Bachtiar, & Johansen-Berg, 2011). Previous research has shown the beneficial effects of anodal motor cortical tDCS over primary motor cortex (M1) in improving visuomotor learning of motor sequences involving finger tapping (Nitsche et al., 2003) as well as sequential modulation of pinch-force (Reis et al., 2009).

Recently, it has been shown that visuomotor adaptation also appears to benefit from neuromodulation by tDCS. In this paradigm, subjects are required to adapt their motor performance levels in novel environments in which visual information about hand movement is altered and dissociated from its kinesthetic perception (Galea et al., 2010;

Hadipour-Niktarash, Lee, Desmond, & Shadmehr, 2007; Hunter, Sacco, Nitsche, & Turner, 2009). Primarily, investigations using this adaptive motor learning paradigm have involved subjects performing fast, straight center-out reaching movements to a target using a robotic manipulandum while unbeknown to the subject, a force perturbation is applied by the robot causing a dissociation in the visual and kinesthetic feedback of the movement. Over the course of several trials (over 30-40 minutes), subjects adapt their motor performance by exerting compensatory forces in the opposite direction, thus reducing their performance errors to reach a behavioral asymptote. When these subjects are tested on trials where the force perturbation is removed, immediately after this training block, they show huge deviation errors in the compensatory direction opposite to that of the force-field (i.e., "after-effects"): the magnitude of these after-effects can be considered akin to magnitude of learning in this paradigm. Additionally, the rate of reduction of errors across trials in the training block is mostly referred to as rate of learning, while the rate of reduction of errors to baseline in the after-effects block is often called rate of deadaptation or retention of the force field. Furthermore, relearning of a given perturbation for a second time is typically faster than initial learning, a phenomenon known as "savings" (Huang, Haith, Mazzoni, & Krakauer, 2011; Krakauer & Mazzoni, 2011; Smith, Ghazizadeh, & Shadmehr, 2006).

(Hunter et al., 2009) found that anodal tDCS applied over M1, compared to sham, during the training block of this adaptive learning led to increased magnitude of after-effects suggesting that anodal tDCS facilitated acquisition of the novel sensori-motor mapping of the dissociation between the visual and kinesthetic feedback. A

recent study confirmed that anodal tDCS over M1 provided during training improves the retention of the acquired visuomotor mapping i.e., greater persistence of after-effects which are errors in the direction opposite to the one imposed by the training block (Galea et al., 2010). Additionally, this study also showed that application of anodal tDCS over the cerebellum during training increased the rate of learning, wherein the errors in the training block reach the asymptote faster than in the sham stimulation.

While it is known that motor cortical tDCS applied concurrently during adaptive motor learning improves retention of the newly acquired sensory-motor mapping, it is not known whether "offline" motor cortical tDCS can produce any changes in acquisition and/or savings of such learning. Evidently, the neural correlates of such performance changes in terms of cortical network reorganization are also unknown. Specifically, performance in savings following visuomotor adaptation subsequent to tDCS over M1 is unknown, and as are the neural correlates of these behavioral changes (if any). This is an important question, particularly, in the context of motor rehabilitation because tDCS can be a more useful adjunct if effects of a brief bout of stimulation can outlast the stimulation duration and produce functional changes in motor performance. Additionally, rehabilitation sessions are often repeated multiple times, thus, influence of tDCS on re-learning a novel task/goal is equally critical. Finally, understanding of the cortical network dynamics that occur with such neuroplastic reorganization following a brief period of motor cortical tDCS can help guide more appropriate rehabilitation interventions in the period after tDCS.

Thus, the primary objective of this study was to determine the relationship between motor performance changes associated with such adaptive motor learning as well as savings and cortical network reorganization as measured by effective brain network connectivity. Given the established role of the frontoparietal network in mediating sensorimotor adaptive learning (Anguera, Reuter-Lorenz, Willingham, & Seidler, 2009; Doyon et al., 2009; Hikosaka, Nakamura, Sakai, & Nakahara, 2002; Krakauer et al., 2004; Seidler, 2010; Seidler & Noll, 2008), the hypothesized connectivity in the left frontoparietal motor network as in specific aim 2 was examined in greater detail here to achieve this objective.

Methods

Experimental procedure and Data acquisition

Thirty right-handed neurologically healthy adults were recruited to participate in this study after providing informed consent as approved by the Institutional Review Board (IRB) at the National Institute of Neurological Disorders and Stroke. The participants, blinded to type of stimulation, were assigned pseudo-randomly to one of the three tDCS groups - anodal, cathodal or sham (n=10 each). Target region for stimulation i.e., contralateral/left M1 for each subject was determined by transcranial magnetic stimulation targeting the optimal scalp position to elicit motor evoked potentials of the right abductor pollicis brevis. A Phoresor II Auto (model PM850, IOMED, Salt Lake City, UT) device will be used to apply tDCS over M1 using a bipolar montage with the cephalic reference electrode over the right supraorbital area. Given the recent evidence on

intensity-related variations in effects of tDCS i.e., greater intensity associated with stronger changes in brain activity recorded through fMRI (Zheng, Alsop, & Schlaug, 2011), we chose to increase the stimulation intensity from 1 mA to 1.5 mA in this experiment. The stimulation was delivered by 25 cm^2 conducting electrodes covered by saline-soaked sponge, at an intensity of 1.5 mA (current density 0.06 mA/cm^2; total charge 0.072 C/cm^2) for 20 min in the anodal and cathodal tDCS sessions and for up to 20 seconds in the sham session according to a previously described method (Gandiga et al., 2006).

Neuromagnetic data were recorded at 600 Hz with a bandwidth of 0-150 Hz using a CTF 275 MEG system (CTF Systems, Inc., Canada) composed of a whole-head array of 275 radial 1st order gradiometer/SQUID channels housed in a magnetically shielded room (Vacuumschmelze, Germany) with synthetic 3rd gradient balancing to remove background noise on-line. MEG data were recorded as participants performed a center-out screen cursor pointing/reaching task by controlling a joystick: before and after 20 minutes of tDCS. The experimental time-line is shown in fig. 1 below.

Experimental Task

Participants were instructed to perform center-out pointing movements of a screen cursor to one of three randomly appearing peripheral targets (at 30^o, 90^o, 150^o) at 10 cm from the central home-circle by controlling a joystick. Joystick movements were sampled at 100 Hz and displayed in real time on a monitor using LabView 8.6 (National Instruments, Inc.) as the cursor while direct vision of hand movement was occluded.

Subjects performed trials of the task in blocks of 20 trials, wherein order and number of presentation of the 3 targets was pseudo-randomized and balanced. Subjects performed 2 blocks (40 trials) in Pre-exposure/Baseline prior to administration of tDCS, and 1 block of Baseline after administration of tDCS. This was designed to minimize confound due to baseline performance changes, if any induced by tDCS.

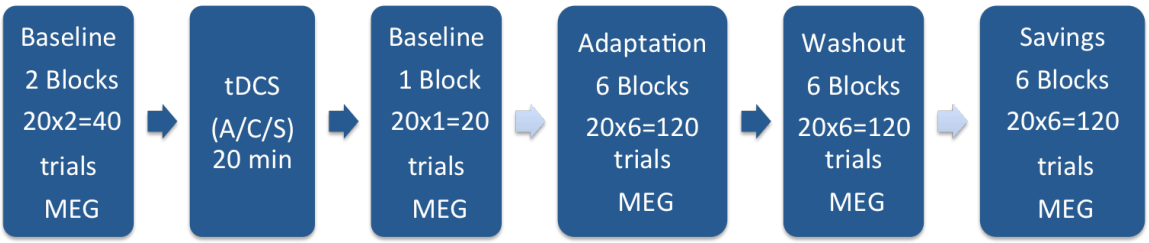

Fig. 1. Experimental timeline showing Pre and Post-tDCS MEG recordings during visuomotor adaptation & savings. Recordings will be performed as shown in the figure during the baseline performance of the task, Pre-exposure wherein screen-cursor relationship is one-to-one, before and after administration of tDCS. After tDCS, MEG recordings will be performed as subjects perform motor adaptation to altered screen-cursor relationships, during Washout (similar to Baseline screen-cursor mapping) and finally during Savings to study effects of tDCS on re-learning the novel screen-cursor mapping.

During exposure to visuomotor distortion phase, screen cursor-joystick movement relationship was rotated clockwise up to 30^{o}. Participants were instructed to perform the movement "as straight and as fast as possible" to hit the target appearing on the screen; the target turned green when the cursor hit it. The movement of the screen cursor was terminated when the subjects hit the circumference of the 10 cm radius circle around the home-circle, and each new trial started with the cursor automatically re-centered to the home-circle. The target appearance was jittered

around a 500 ms interval (randomly) after participants centered the cursor in home-circle and held still for the 500 ms. Each target remained on the screen for about 3.5 seconds after which the trial was aborted; all targets were presented equally in pseudo-random order to avoid any directional biases. The inter-trial interval was jittered between 3 and 5 seconds. Participants performed a total of 21 blocks of 20 trials each, as shown in fig. 1. The task performance is illustrated during pre-exposure, adaptation/savings and washout phases in fig. 2.

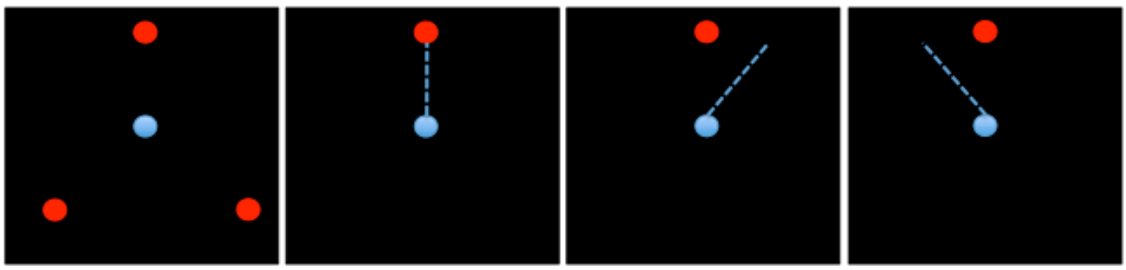

Fig. 2. Panels showing the screen appearance during task performance (left to right): Target positions relative to home-circle (10 cm distance) on screen shown to subject initially; one target appeared at a time and screen cursor motion indicated by a dashed line here is mapped one-to-one in the pre-exposure phase; screen-cursor joystick movement relationship is rotated 30^{o} clockwise during exposure trials forcing subjects to learn novel sensory-motor mapping; finally, the acquisition of this new sensory-motor mapping or "internal model" is reflected by errors in opposite direction of screen-cursor rotation in the post-exposure trials.

Behavioral Data Analysis

Individual trial data were low-pass filtered with cut-off of 10 Hz with a 4^{th} order dual-pass Butterworth filter. For each trial, initial directional error (IDE, in degrees) score was calculated as the angle between a vector from initial position of the joystick to that at peak velocity after movement onset and a vector extending from start position to target. Peak velocity was used to compute IDE since the movement was very small

(Anguera et al., 2007). IDE is thought to represent the state of the internal model of the novel environment and was the primary dependent variable across trials that was used to establish learning rates for each subject based on a single-exponential curve fitted to the data during the exposure phase trials in Adaptation and Savings, given by the general equation, $y = Ae^{-bx} + c$ [where A & c are constants] (Huang et al., 2011).

Neuromagnetic Data Analysis

Neuromagnetic data were epoched into 3100 ms around the point of movement onset – 1100 ms before movement onset and 2000 ms after movement onset to include post-performance neural processing of sensory-motor errors, consistent with previous methodology used in our lab (Contreras-Vidal & Kerick, 2004) for each trial. Data from blocks of 20 trials each were concatenated and de-noised (0.15-150 Hz band-pass filtering, notch filtered to remove power line noise) and submitted to the SAM source localization analysis to compute anatomical source waveforms. The method used was identical to that described in Specific Aim 2, however, a dipole spacing of 5 mm, corresponding to a cubic voxel width of 5 mm, was used for each dataset i.e. per block of 20 trials. The time-frequency representation of power obtained through the Single-state SAM imaging across the 620 seconds of the SAM filter output at each virtual channel was computed by convolution with a sliding Hamming window (100 millisecond wide) with no overlap in order to yield a 3D time series (620 data points) of activation for each voxel of anatomical source of neuromagnetic activity with an optimal time- and spectral-resolution. The SAM covariance matrix was calculated in the frequency bands

relevant to sensorimotor processing as described in the literature (and these were also identified to be modulated in specific aim 1) i.e., alpha: 8-13 Hz, beta: 13-30 Hz, low gamma: 30-50 Hz, high gamma: 70-100 Hz. These were subjected to Granger causality effective connectivity modelling in the same way as described in Chapter 4 (Specific aim 2). As described in Specific Aim 2 Methods, normalized effective connectivity (change from Baseline) was computed for each of the 8 paths at the end of Adaptation and Savings. In order to determine relationship between network connectivity and behavioral measures of motor performance change, the rate of learning during Adaptation and Savings was separately (for each period) regressed in a multiple regression model, on the normalized effective connectivity (EC) of the 8 paths in the last block of Adaptation and Savings, respectively. The last block was chosen because the rate of learning represents the acquisition of the novel sensory-motor mapping as required by the task, which occurs when the performance asymptote is reached (close to zero errors), typically at the last block of Adaptation/Savings. These interactions were specifically examined based on a priori hypothesis given the putative role of the activity of these regions in mediating visuomotor adaptive learning. These effects were examined in alpha, beta, low and high gamma frequency bands.

All analyses were performed using custom programs written in Matlab 7.11 (The MathWorks, Inc., Natick, MA) and AFNI (Analysis of Functional Neural Images, [v. AFNI_2011_12_21_1014]). Statistical analyses were performed using the R-package (v. 2.14.1) and Statistical Package for the Social Sciences (SPSS, 18.0).

Results

Behavioral changes during Adaptation and Savings

The behavioral data showed that all groups (Anodal, Cathodal and Sham tDCS) were able to adapt to the novel visuomotor distortion (see fig. 3). There were no differences in baseline performance of the task, under unaltered screen-cursor conditions, across the groups, before and after tDCS (ANOVA, $p>0.05$). However, as expected, there was a significant difference in the rate of learning during adaptation across groups (one-way ANOVA $p=0.007$). The learning rate was significantly higher in the group receiving Anodal as compared to the one receiving Cathodal tDCS ($p=0.006$). Additionally, the mean rate of learning during adaptation for Anodal tDCS group was higher than Sham as well, but this difference was not significant ($p=0.08$) (see fig. 4). On the other hand, there were no significant differences across the 3 groups in the rate of learning measured during savings (one-way ANOVA, $p=0.364$), although Anodal tDCS group had the highest mean learning rate, and the Cathodal tDCS group had the lowest mean learning rate (See fig. 4).

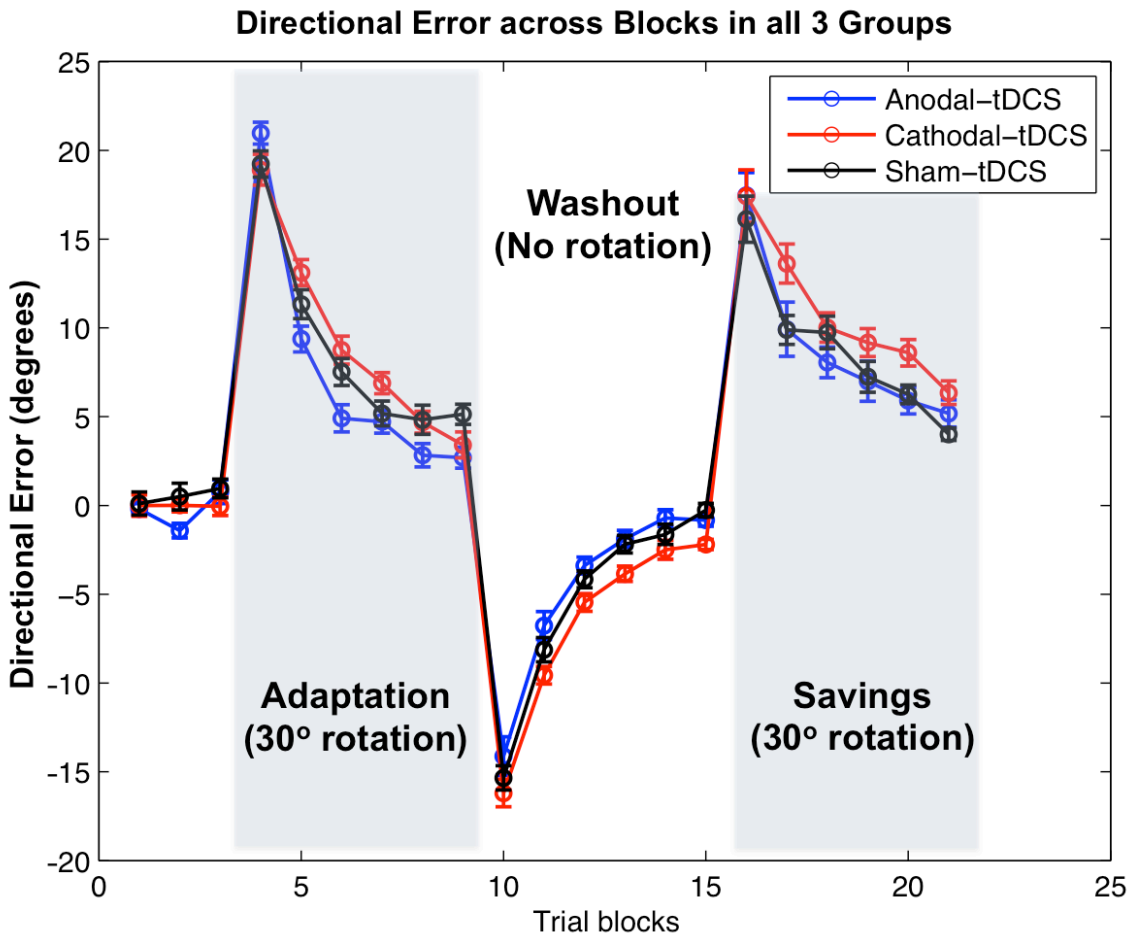

Fig. 3. Changes in mean directional error (± standard error) across subjects over the trial blocks in the experiment. All 3 groups showed increased error immediately after introduction of visuomotor distortion (30o clockwise rotation), as seen in the first block of adaptation and savings, and consequently adapted their motor performance i.e., learned or acquired the novel sensory-motor mapping, as evidenced by reduction in directional error across trial blocks.

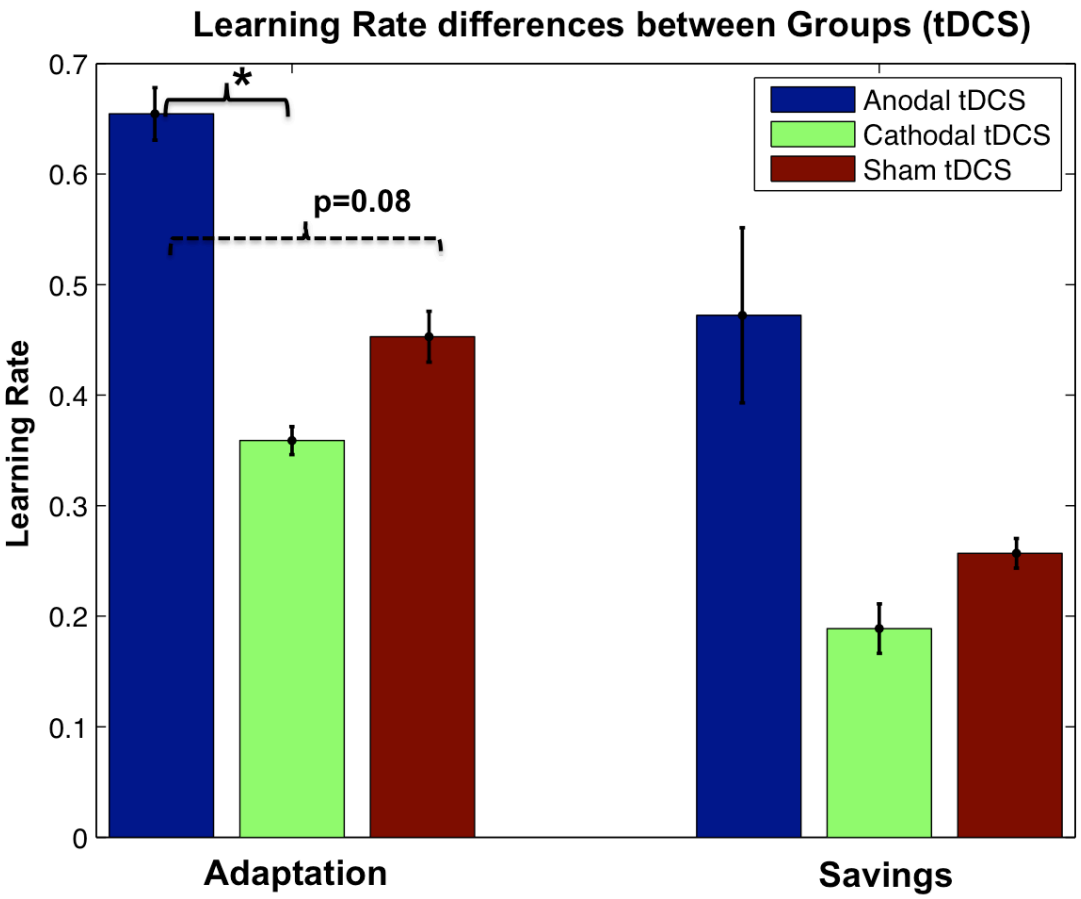

Fig. 4. Differences in learning rates during Adaptation and Savings following tDCS (mean rate ± standard error). Anodal tDCS group shows the fastest learning rate across Adaptation and Savings, while Cathodal tDCS group shows the slowest learning rate. Significant differences are indicated by * $p<0.05$; strong trends are indicated with corresponding p-values.

Fronto-parietal network connectivity changes during Adaptation and Savings

Interestingly, network connectivity that predicted behavioral learning rates was primarily in the alpha, low and high gamma frequency bands, similar to the findings in Specific Aims 1 and 2. Within the scope of this dissertation, the multiple regressions that were significant and/or nearly significant, having at least one path as a significant predictor of learning rates, will only be discussed in detail further. During Adaptation, multiple regressions, with the normalized effective connectivity coefficients as

predictors, were nearly significant for predicting learning rates across groups in the alpha (adjusted R^2=0.65, p=0.19), and high gamma band (adjusted R^2=0.77, p=0.11). However, during Savings, the multiple regressions were highly significant in predicting learning rates in the alpha band (adjusted R^2=0.99, p=0.001) as well as the low gamma band (adjusted R^2=0.96, p=0.0091). In the high gamma band, the multiple regression nearly reached significance (adjusted R^2=0.65, p=0.19). Specifically, the network paths that were significantly engaged within these frequency bands included the connections between SMA-LPM, SMA-LM1, LPF-LPM during Adaptation, and additionally, the connections between LPF-SMA and LPL-SMA during Savings.

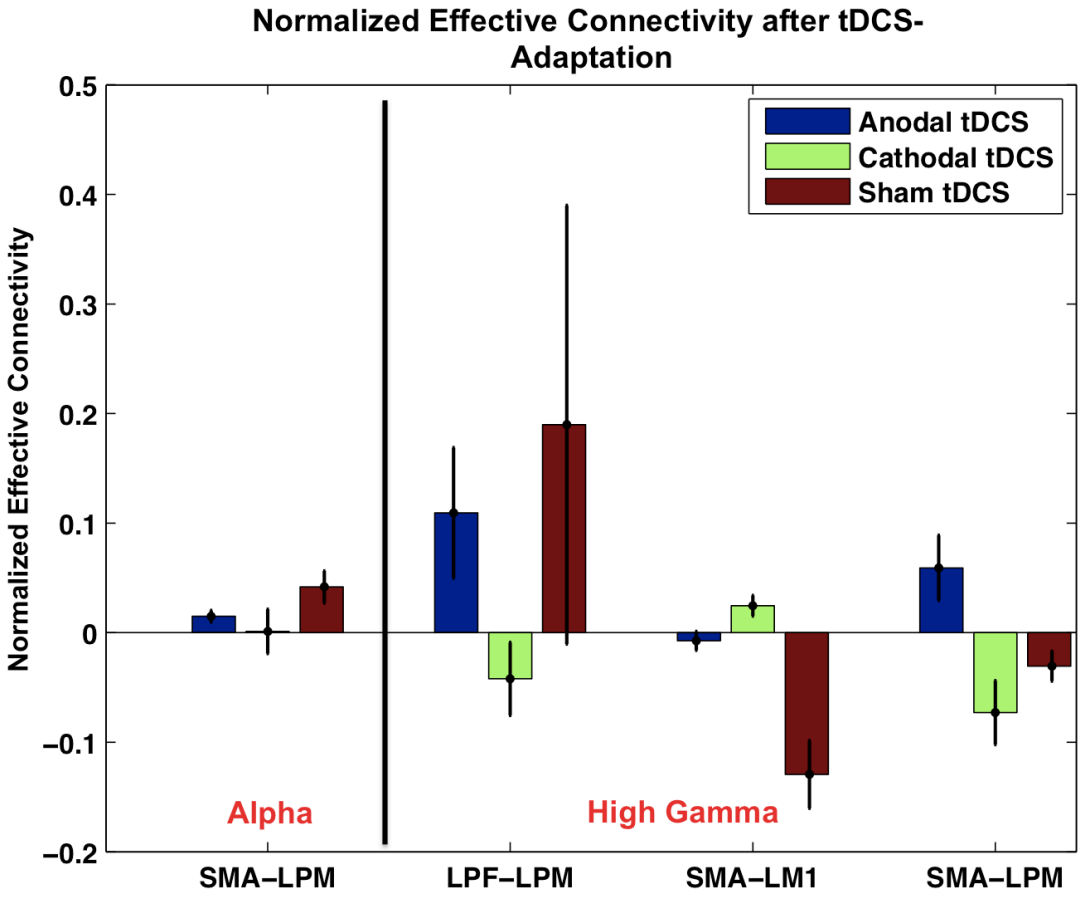

Fig. 5. Normalized effective connectivity following tDCS within the left fronto-parietal network at the end of Adaptation (mean ± standard error).

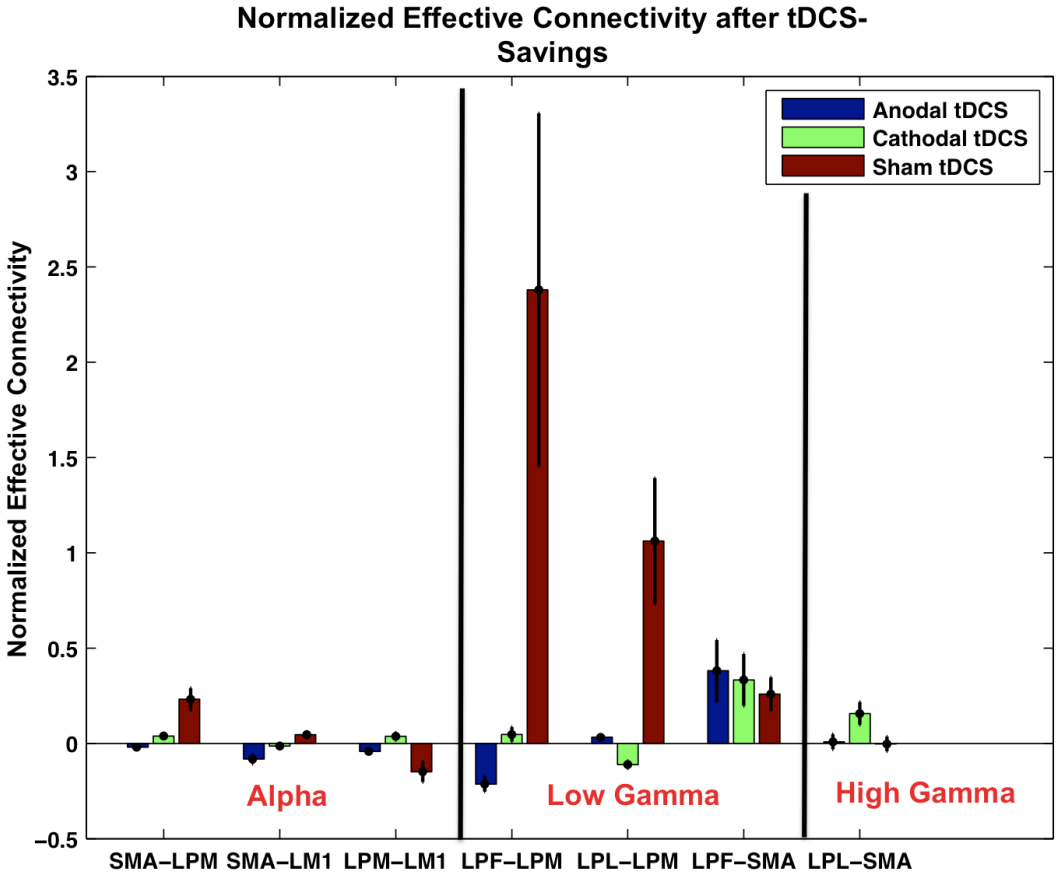

Fig. 6. Normalized effective connectivity following tDCS within the left fronto-parietal network at the end of Savings (mean $\pm$ standard error).

Fig. 5 shows the normalized effective connectivity after tDCS within the paths that were significant predictors, across the 3 stimulation groups in Adaptation, while Fig. 6 shows the normalized EC across the 3 groups after tDCS, in Savings in the identified significant predictors. It is important to note here that no statistical comparisons were made between the normalized ECs across the 3 groups, because this was not directly relevant to the research objective here. In general, Anodal tDCS appeared to have the least normalized connectivity compared to Cathodal and Sham tDCS across alpha and gamma band oscillations, similar to the resting state normalized connectivity patterns

observed in Specific Aim 2. Additionally, normalized connectivity in the Anodal tDCS condition appears to be in the direction opposite to that of Cathodal tDCS (see fig. 5).

Network connectivity in Adaptation

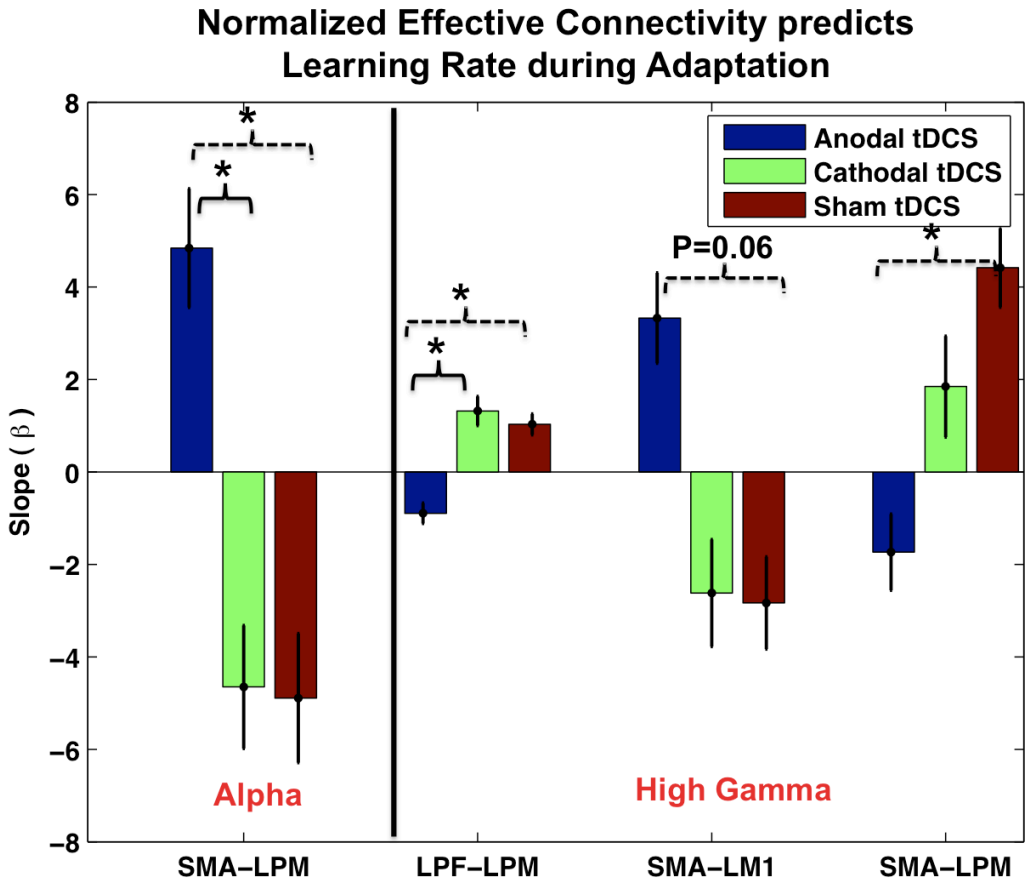

Fig. 7. Relationship between Normalized effective connectivity and learning rate in Adaptation following Anodal tDCS is significantly different from Cathodal and Sham tDCS (Bars show mean slopes ± standard error; significant differences are indicated by * $p<0.05$; strong trends are indicated with corresponding p-values).

The hypothesis-driven analysis was aimed to study the relationship between oscillatory network normalized EC and learning rate in Adaptation as well as Savings. Here, we found that normalized EC for SMA-LPM in alpha band in the Anodal tDCS

group significantly predicts learning rate in Adaptation (increase in normalized EC leads to increase in learning rate; see fig. 7). Interestingly, this relationship between normalized EC and learning rate is reversed for Cathodal and Sham tDCS (difference between slopes is significant at $p<0.05$).

In the high gamma band, a similar pattern was observed for the relationship between SMA-LM1. However, for the LPF-LPM as well as SMA-LPM paths, decreases in normalized EC led to an increase in learning rate for Anodal tDCS group. Again, the relationship for Cathodal and Sham tDCS was in the opposite direction as compared to Anodal tDCS (see fig. 7).

Network connectivity in Savings

While there were no significant differences between learning rates across groups in Savings, interestingly, there were significant differences in the influence of normalized EC within the fronto-parietal network in predicting learning rate across the 3 stimulation groups (see fig. 8). In the alpha band network activity, increases in SMA-LPM and LPM-LM1 path EC led to an increase in learning rate in the Anodal tDCS group. Conversely, increases in these normalized ECs led to a decrease in learning rate in both Cathodal and Sham tDCS. On the other hand, decrease in SMA-LM1 path EC predicted increases in learning rates for the Anodal tDCS group; again, opposite effects were seen for the Cathodal and Sham tDCS - increase in this path led to increase in learning rates.

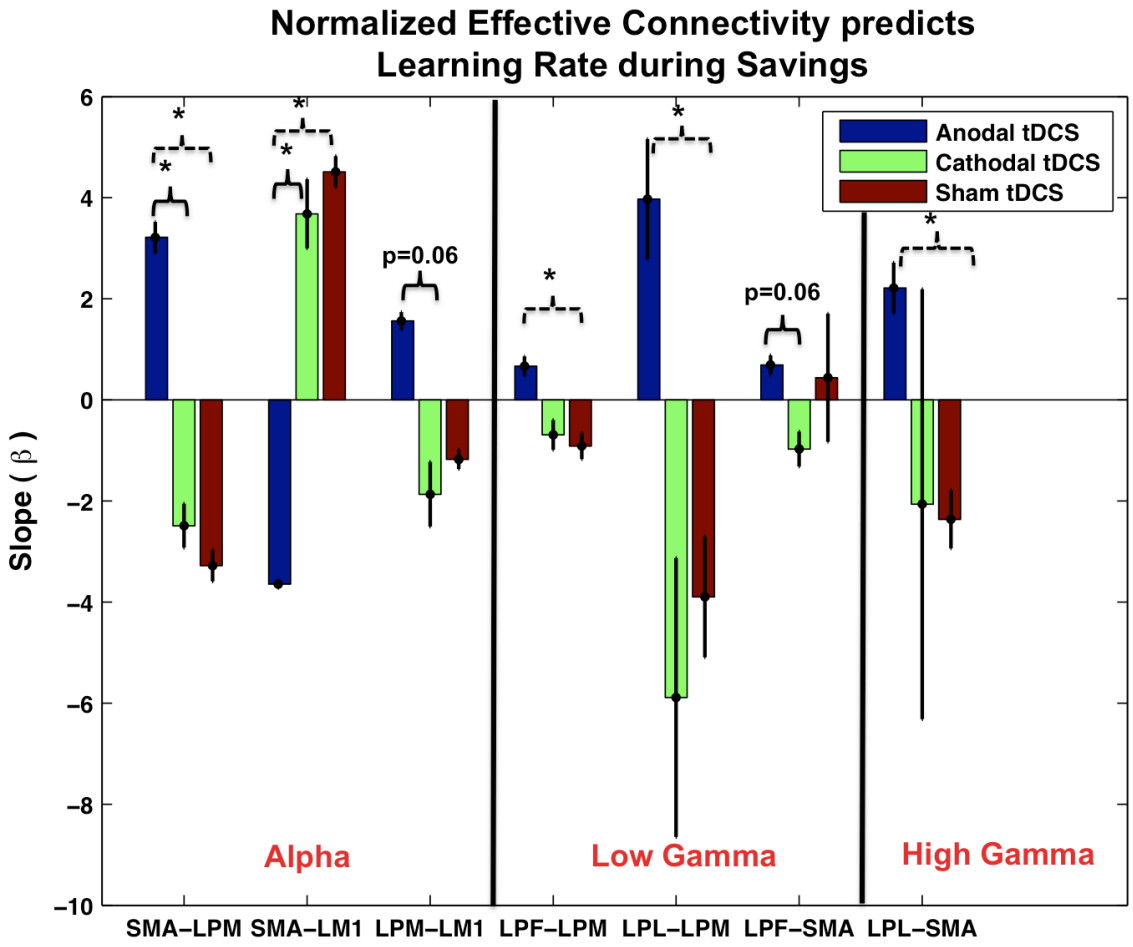

Fig. 8. Relationship between Normalized effective connectivity and learning rate in Savings following Anodal tDCS is significantly different from Cathodal and Sham tDCS (Bars show mean slopes $\pm$ standard error; significant differences are indicated by * $p<0.05$; strong trends are indicated with corresponding p-values).

In the high frequency gamma band network activity, the positive predictors of learning rates included primarily paths between LPF and LPL through their influences on LPM and SMA. In lower gamma band (30-50 Hz), increase in LPF-LPM, LPL-LPM, LPF-SMA normalized ECs led to increases in learning rate in the Anodal tDCS group, while the opposite effects were seen for the Cathodal and Sham tDCS groups (an exception is the LPF-SMA path for the Sham, wherein increases in normalized EC positively predicted

learning rates). In the high gamma band (70-100 Hz), the normalized EC between LPL-SMA also positively predicted learning rate increases; and this was significantly different that Cathodal and Sham tDCS groups, which again showed the opposite relationship between, network activity and learning rates.

Discussion

To summarize, these results show for the first time, the neural correlates, in terms of oscillatory network connectivity that predict behavioral changes of adaptation and savings in visuomotor adaptation following polarity-specific tDCS. In particular, this method also identified polarity-specific differences in neural network activity that were seen in the absence of significant behavioral differences during savings.

Polarity-specific effects of tDCS on Visuomotor Adaptation and Savings

It was found that Anodal tDCS significantly increased the rate of learning compared to Cathodal tDCS in adaptation. Thus, participants performing a behavioral task that involves learning a novel sensory-motor mapping were able to "learn the recalibration" process much faster after Anodal tDCS over left M1, than Cathodal tDCS as well as Sham tDCS (strong trend toward significance). This is the first evidence to suggest that "offline" effects of tDCS may help in speeding (boosting) the process of sensory-motor adaptation to new environments. Functionally, sensory-motor adaptation forms a significant portion of one's motor repertoire, and is therefore applicable to wide range of activities of daily living (for e.g., a stroke patient is constantly challenged with

learning to walk on a level surface with no obstacles and being able to adapt these acquired performance gains to walking on uneven surfaces with many obstacles, as in a home/garden). From the perspective of motor rehabilitation, this is a very significant finding because it can help using Anodal tDCS to "neurally prime" an individual to optimize training within any given session of rehabilitative intervention.

In this regard, a significantly higher rate of learning following Anodal tDCS in savings would have proved even more useful. Savings is akin to practical situations wherein one learns a new behavior, and significantly gains or advances on subsequent repetitions of the same behavioral set. Here we found that Anodal tDCS group still showed a much higher rate of learning in savings compared to Cathodal and Sham tDCS groups, although these differences did not reach statistical significance. One possible reason for this could be the "waning" of the effects of tDCS over time. tDCS was administered for 20 minutes, and savings trials were performed after adaptation and washout, almost 35-40 minutes following the end of stimulation. It is likely that this could have reduced the potency of the stimulation effects in savings. This temporal window also exceeds what we identified previously in Specific Aim 1, wherein effects of tDCS on resting brain network activity was seen up to 25-30 minutes after the end of stimulation.

Polarity-specific effects of tDCS on left fronto-parietal network activity in Adaptation

Previous research has shown the effects of Anodal tDCS on improving sensory-motor adaptation, primarily by studying and comparing behavioral changes seen while learning

to perform visuomotor adaptation (Galea et al., 2010; Hunter et al., 2009). However, we show here for the first time, the polarity-specific relationship between network connectivity and the behavioral changes seen following tDCS.

Previous literature investigating the neural correlates of visuomotor adaptation have shown that that prefrontal cortex (primarily dorsolateral PFC) and bilateral posterior parietal cortical regions, along with the supplementary motor area play a very critical role in mediating the behavioral changes required to perform error-reduction and consequently learning the novel sensory-motor mapping (Anguera et al., 2009; Della-Maggiore, Malfait, Ostry, & Paus, 2004; Doyon et al., 2009; Krakauer et al., 2004; Krakauer & Mazzoni, 2011). Understandably, these regions can likely directly influence these changes by affecting M1 activity as well as indirectly through the premotor cortical region. The premotor cortex, in addition also plays a significant role in visual to motor transformations that are necessary for reaching and pointing, as is typically required by this task (Shadmehr & Wise, 2005).

Here, we find that during adaptation, SMA through strengthening its paths to LPM (alpha) and LM1 (high gamma), directly increases the learning rate, i.e., hastens the process of learning the new environmental context wherein compensatory movements have to be made to offset the externally imposed screen-cursor perturbation. Interestingly, decreasing high frequency gamma band connectivity between LPF to SMA, and SMA to LPM, directly increases learning rate in adaptation. This negative relationship could primarily be explained by two things: 1) the LPF-SMA path suppression could likely be involved in “suppressing” or “inhibiting” the pre-potent

cognitive processes associated with the sensory-motor mapping existing for screen-cursor relationships in the absence of external perturbations (30 deg rotation); 2) the SMA-LM1 path suppression could be directly related to suppressing the physical movement with the unwanted sensory-motor mapping. This is very likely because the role of PFC in inhibiting prior associations to learn new ones has been previously described (Anguera et al., 2009; Gentili, Bradberry, Hatfield, & Contreras-Vidal, 2009; Gentili, Bradberry, Oh, Hatfield, & Vidal, 2011). In addition, the role of the SMA in particularly identifying "sets" or "rules" in the context of motor learning and aiding selection of appropriate behaviors/responses is also sufficiently established (Hikosaka et al., 2002; Nachev, Kennard, & Husain, 2008). In terms of the nature of differences in the network activity across different oscillatory/frequency bands, it is conceivable that connections within and between these regions are likely to exist across multiple scales i.e., short range connections, likely mediated through GABAergic activity, as well as more long-range connections that could lead to oscillations in a lower frequency bandwidth (Buzsáki, 2006). In other words, it can be said that tDCS appears to influence brain networks at multiple scales, and therefore offers the possibility to more widely influence networks in a way that may be suitable or optimal for producing behavioral changes (depending on the polarity of tDCS).

It is interesting to note that, normalized EC for Anodal tDCS at end of adaptation appears to be relatively lower than for Cathodal or Sham tDCS (fig. 5). These findings are similar to those seen for resting state network activity in Specific Aim 2. An argument can be made that, in some form, Anodal tDCS appears to help increase the "gain" in the

system, such that very small changes in network activity can still be very tightly coupled to strongly positive behavioral changes i.e., increased learning rates. This fits very well within the framework of Anodal tDCS's ability to increase signal-to-noise (SNR) in the system. Conversely, Cathodal tDCS appears to lower the gain in the system, and/or also reduces SNR in the system, which may make it much harder to produce behavioral changes in the desired direction. Future research to carefully explore this phenomenon would be immensely help characterizing more clearly the nature of effects of external brain stimulation on a globally wired neural network i.e., the whole brain.

Polarity-specific effects of tDCS on left fronto-parietal network activity in Savings

One of the most interesting findings of this study was polarity-specific change in fronto-parietal network activity in the absence of significant behavioral differences across the 3 stimulation groups in savings. Interestingly, the normalized effective connectivity or EC between SMA-LPM, LPM-LM1 (alpha), LPF-LPM, LPF-SMA, LPL-LPM (low gamma) and LPL-SMA (high gamma) all positively predict increases in learning rate during Savings in the Anodal tDCS group. Furthermore, this relationship is significantly stronger than the opposite relationship between the corresponding paths and learning rates in Cathodal and Sham tDCS. Similar to adaptation, the SMA-LM1 path, in the alpha band in savings (instead of high gamma in adaptation) negatively predicts increases in learning rates. Taken together, these paths, and hence the network appears to "reverberate" in a strong fashion during savings in the Anodal tDCS group compared to Cathodal and Sham tDCS. Potentially, this could imply that when a new behavior is learned in the context of

a *de novo* environment (or sensory-motor mapping is acquired) following stimulation of M1 with Anodal tDCS, the underlying brain network (fronto-parietal in this case) is recruited much more robustly, and likely more optimally than in the absence of tDCS i.e., Sham.

Here, we demonstrate evidence for potential "network priming" that is induced by Anodal tDCS, which has been speculative thus far. If this is indeed the case, this effect of Anodal tDCS may become even more clinically relevant and significant. Particularly, providing non-invasive Anodal tDCS to a given network (by stimulating one or more nodes of that network) during the first session of learning or behavior modification may have strong ramifications in inducing and sustaining similar brain network patterns in subsequent repetitions of the task/behavior. Since this effect was observed here by "offline" administration of Anodal tDCS, it would be necessary to explore this phenomenon in greater detail during concurrent tDCS and neuroimaging ("online"). Nevertheless, this may have significant implications in using Anodal tDCS to boost network robustness or immunity, and consequently produce favorable behavioral changes in the context of cognitive and motor rehabilitation. On the contrary, the other question to be answered is if providing Cathodal tDCS can "weaken" network activity patterns, and therefore be used to extinguish unwanted behavioral patterns such as addiction etc. Overall, this is an important future direction to take in the study of effects of non-invasive brain stimulation since these effects of tDCS may tremendously increase the scope and efficacy of various treatment interventions for a multitude of neurological and psychiatric disorders. As stated previously, in the Discussion in Specific Aim 2, one

limitation of the current approach is that influences of deeper subcortical substrates such as the cerebellum and basal ganglia on this fronto-parietal network activity cannot be identified and studied. Thus, future research must also apply alternative data acquisition and analyses to tease apart the role of these regions in mediating tDCS effects on sensory-motor behavior.

Gamma oscillations and Neuroplasticity

An important pattern that emerges in examining brain network connectivity is that gamma oscillations seem to play an integral role during learning and savings following tDCS. This is particularly interesting because gamma oscillations are postulated to be most crucial as a central timing mechanism for synaptic plasticity by causing synchronous oscillations of multiple cortical areas (Buzsáki, 2006). By means of causing oscillatory activity in discrete active cortical locations/columns, gamma oscillations enable to appropriately time pre-synaptic and post-synaptic activity such that synaptic activity can be suitably modulated. And this phenomenon seems to allow linking of sensory-perceptual binding to neural plasticity by way of synchronous gamma oscillatory activity. As previously demonstrated, since tDCS strongly modulates GABAergic circuits (Stagg et al., 2009), it is possible to conceive the direct influence of tDCS on neuroplasticity. In this regard, it is more interesting to note that Anodal and Cathodal tDCS modulate the balance between GABAergic and Glutamatergic circuits in a polarity-specific manner (Stagg et al., 2009). Therefore, they are likely able to differentially modify gamma oscillations, which could in turn regulate downstream

behavioral neuroplasticity. This study presents the first piece of evidence to show that gamma oscillatory connectivity within a relevant fronto-parietal network is modulated in a polarity-specific manner by tDCS during learning (see Fig. 5, Adaptation). Thus, it is possible to speculate that the mechanism of action of tDCS in terms of modulating neuroplasticity, in part, may be by modulating gamma oscillations directly. This is a significant step forward in understanding neuroplastic effects of non-invasive brain stimulation. Future research needs to explore this in greater detail, to particularly consider brain stimulation techniques that can employ "frequency-modulation" i.e., directly stimulate the oscillatory brain activity in a specific frequency of interest.

In summary, this study identified significant polarity-specific differences in behavioral adaptation induced by tDCS and quantitatively established the underlying neural correlates mediating these differences in terms of oscillatory network connectivity within a left fronto-parietal network. These findings are an important critical step in the direction of elucidating mechanism of action of tDCS in producing complex cognitive-motor behavioral changes.

CHAPTER 6: GENERAL DISCUSSION

Overall, the findings from this research have significantly advanced the understanding of neuroplastic effects of tDCS, which is particularly relevant in the context of motor rehabilitation. Here, a novel method of studying neuroplasticity mediated by tDCS was identified and applied that involved characterization of changes in brain network oscillatory dynamics following administration of tDCS. The cortical dynamics modulated by a brief bout of motor cortical tDCS has been identified to occur in a fronto-parietal motor network using a robust, data-driven network analysis approach. Additionally, the modulation of effective functional connectivity within this network was characterized using a data-driven multivariate connectivity modeling approach. Further, the behavioral changes and their relationship with modulation of such functional reorganization in cortical network connectivity were also examined during adaptive motor learning. Taken together, these findings provide critical insights into the possible nature of neuroplastic effects mediated by tDCS. tDCS, anodal tDCS in particular, appears to favorably modulate network activity in a way that not only optimizes the processing by improving signal-to-noise, but also appears to favor robustness in network activity patterns. Importantly, this dissertation demonstrated, for the first time, that tDCS may exert its polarity-specific neuropalstic effects in part, by directly modifying gamma oscillatory brain network activity. These findings, in the context of extant literature about widespread behavioral effects of tDCS, may help explain how these behavioral changes are produced. Furthermore, these findings may

potentially inform more prudent application of this technique for clinical and therapeutic purposes in the context of cognitive and sensory-motor rehabilitation.

At this point, it is also important to recognize certain limitations inherent to the proposed methodology. As discussed previously, examining electrophysiological cortical dynamics offers an excellent approach to study oscillatory brain networks, but such non-invasive scalp measurement techniques like MEG are less sensitive underlying deeper sub-cortical neural activation of cerebellum and basal ganglia. Since these regions contribute significantly to motor performance and learning, it will be necessary to use other neuroimaging techniques in the future, like fMRI, to study the combined activation of these regions to the proposed network here to provide a more complete account of neuroplastic modulation of a more general motor network by tDCS.

Nevertheless, in the context of the vast literature on the behavioral effects of tDCS, the findings from this research may help significantly advance the knowledge in the field by providing a more comprehensive description of plausible neuroplastic mechanisms modulated by tDCS and discriminate between the polarity-specific effects of tDCS. Importantly, such methods will help identify and describe neuromodulation induced by non-invasive cortical stimulation in general. From a practical perspective, this is critical to help identify, monitor and potentiate neuroplastciity that is crucial for functional recovery in patients suffering from brain lesions like stroke and traumatic brain injury. In fact, in the years to come, methods of characterizing network dynamics and properties using non-invasive neuroimaging techniques may have significant value in advancing our understanding of the computational processes performed by the brain

during human cognition and behavior and more importantly, to explain the tremendous plasticity exhibited by the brain in the face of experience and injury.

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