Modulating Human Cortical Plasticity via Transcranial Direct Current Stimulation:

Basic & Clinical Applications

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GENERAL ABSTRACT

As humans we have a unique ability to study, and even to modify the makeup of our own existence. The concept of changing oneself has always intrigued me, and it was what initially piqued my interest in the study of the human brain. In my estimation, the brain was where most of our “existence” derived from (I’ve changed my mind about that somewhat since then), and therefore learning about it, and how to modify it, would be quite an interesting undertaking. My passion for this topic led me to work with Dr. Kelvin Lim, who at the time was building momentum for studying the clinical potential of non-invasive neuromodulation. Over the course of 5 years working with Kelvin, I was able to learn a significant amount regarding neuromodulation, research and science as a whole. This dissertation describes two of my main projects. These studies focus on researching the basic and clinical applications of transcranial direct current stimulation (tDCS) as a means to modulate human brain plasticity.

The first project, described in chapter II, was a basic science study which aimed to investigate how tDCS interacts with functional brain state. Previous literature has reported on the ability of tDCS to modulate plasticity, both in humans and in animal models. However, given the non-focal nature of tDCS, there is an open debate as to how specific outcomes (physiological or behavioral) are achieved. Recently, a hypothesis has been proposed that active brain networks or populations of neurons are preferentially susceptible to the influence of electric fields over inactive networks or groups of cells. This ‘activity-selectivity’ hypothesis has not been thoroughly tested in studies using physiological measures. In this

study I use a novel electrophysiological paradigm to investigate the impact of tDCS on plasticity in the auditory cortex. The unique features of the paradigm allowed me to analyze stimulus specific effects of tDCS, making it possible to test the 'activity-selectivity' hypothesis using a novel physiological measure.

The third chapter of the thesis describes a clinical trial where we used tDCS in combination with cognitive training to treat impaired executive functions in children with fetal alcohol spectrum disorders (FASD). Exposure to alcohol in the womb impairs neuroplasticity in the developing brain and often leads to severe cognitive deficits later in life. Cognitive training is one of a few treatment options for these deficits, however treatment times are long and difficult to complete. Research has shown that pairing cognitive training with tDCS enhances efficacy and can allow for a shorter intervention. However, tDCS has not been tried in children with FASD and it is not clear if it would be tolerated or efficacious in this population. With this in mind, we conducted a first of its kind clinical trial in children with FASD to test the tolerability and feasibility of tDCS augmented cognitive training and its effects on executive functioning.

In sum, this dissertation describes two of my major studies which describe the characteristics and the use of tDCS in both a basic and clinical setting. I believe that the findings generated by these studies will make a significant and positive effect on the field of tDCS and its use in the clinic.

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Chapter I: General Introduction

1.1 Brain Plasticity and Long-Term Potentiation

Neuronal plasticity is a crucial property of the brain which allows it to be shaped by experience. It is this property which allows us to learn and recall patterns, predict and obtain reward, and guides response selection for adaptive behavior (Cooke and Bliss, 2006; Ganguly and Poo, 2013). The ability to make and break connections is essential during development, when nascent circuits are refined by selective pruning, and throughout life, in ubiquitous processes such as learning and memory (Cooke and Bliss, 2006; Kolb and Gibb, 2011). Plasticity is manifest at all scales of brain activity, from the microscale alterations in synaptic connections at the cellular and subcellular level, to mesoscale changes in long-range and local connections amongst groups of neuron, all the way to the macroscale dynamics at the level of entire brain regions.

The concept of neuronal plasticity at the cellular level was first articulated by Ramon y Cajal, who postulated that alterations in synaptic connections had the potential to serve as a substrate for memory (Cajal, 1913). Physiological evidence for these ideas did not emerge for several decades however, with the first demonstration of short-term synaptic plasticity occurring with the discovery of post-tetanic potentiation in the frog neuromuscular junction (Feng, 1941). Our current framework for understanding neural plasticity was postulated shortly thereafter by the psychologist Donald Hebb, who famously hypothesized the existence of cellular mechanisms which strengthen synapses whose pre and post-synaptic activation is correlated:

> "When an axon of a cell A is near enough to excite cell B or repeatedly or consistently takes part in firing it, some growth or metabolic change takes place in one or both cells such that A's efficiency, as one of the cells firing B, is increased" (Hebb and O, 1949)

Hebb's postulate however, was well ahead of its time, and it was not until the discovery of long term potentiation (LTP) in the hippocampus (Bliss and Lømo, 1973), followed by the discovery of long term depression (LTD) in the cerebellum (Ito and Kano, 1982), that his hypotheses could be experimentally validated. The discovery of LTP/D relied on novel in vitro electrophysiological techniques which allowed recording from tissue slice preparations. At first, these paradigms involved the placement of recording electrodes extracellularly in postsynaptic cells (most commonly the pyramidal cells of the dentate gyrus in the hippocampus), while electrically stimulating an afferent pathway to evoke excitatory postsynaptic potentials (EPSPs). To induce plasticity, experimenters used brief trains of high-frequency electrical stimuli (electrical tetanus), delivered at approximately 100Hz. This manner of electrical stimulation led to a persistent enhancement in EPSP amplitude (or slope), a response that came to be known as LTP. In later experiments, more advanced intracellular techniques were used to pair postsynaptic depolarization with simultaneous afferent stimulation. These studies demonstrated at the single cell level that correlation between pre and post synaptic activity is necessary for induction of LTP/D (Dan and Poo, 2004; Markram et al., 1997).

LTP has several crucial properties which make it an attractive candidate to serve as the substrate for learning and memory. First, it results in a persistent modulation of neural activity, lasting for hours and sometimes even inducing permanent structural alterations in cellular morphology (Bosch et al., 2014). Second, synaptic LTP is input-specific, meaning that only coincidentally active synapses are modulated, sparing any effects on neighboring, non-active, synapses on the same cell (Cooke and Bliss, 2006; Hao et al., 2018). Because cortical neurons often receive thousands of connections, this property greatly magnifies the information storage capabilities of the brain. Lastly, the associative property of LTP states that a weak input, which on its own does not induce LTP, can become potentiated if associated with a strong input. This property is especially interesting as it provides a mechanism by which we can associate events or objects in the outside world (Cooke and Bliss, 2006).

The properties of LTP, primarily input-specificity and associativity, require a cellular mechanism which can detect coincident synaptic firing. This mechanism is predominantly provided by the action of the N-methyl-D-aspartate receptor (NMDAR). The NMDAR is an ionotropic glutamatergic receptor which binds glutamate, and is non-selectively permeable to positive cations. The unique dual gating kinetics of this receptor-channel complex suggest that it is ideally suited to function as a cellular coincidence detector. In order for ions to flow through the NMDAR, two events must occur within a tight temporo-spatial window. First, presynaptic glutamate released following afferent action potential must bind to postsynaptic NMDARs. Ligand binding causes a conformational change in

receptor shape, but due to a unique voltage-gated property of the channel, the ion pore itself remains blocked (Liu and Zhang, 2000). Voltage dependence of the NMDAR is conferred by the intricate structure of the channel subunits, which bind extracellular Mg++ and Zn++ ions that block current flow at resting membrane potentials. Only when the dual action of ligand binding and cell depolarization (through the binding of glutamate to α- amino-3-hydroxy-5-methylisoxazole-propionate receptors (AMPAR) for example) occurs, are positive cations allowed to flow through the channel pore. This dual activation property thus allows the NMDAR to act as an integrator of multiple coincident events at the synaptic level (Cooke and Bliss, 2006). The functional activation of NMDARs leads to a rise in intracellular Ca++ levels, triggering a plethora of cellular cascades that serve to change the functional and/or structural state of the cell.

Amongst the many cascades that are triggered by Ca++ influx through the active NMDAR, activation of cyclic adenosine monophosphate (cAMP) and the Ca++/calmodulin-dependent protein kinase II (CaMKII) are arguably the most consequential in respect to plasticity. CAMP levels are regulated primarily by cyclases and phosphodiesterases, which enhance or dampen cAMP activity respectively. Following NMDAR opening, Ca++ influx increases adenyl cyclase activity and thereby enhances cAMP signaling (Waltereit and Weller, 2003). CAMP in turn triggers activation of protein kinases which can be translocated to the nucleus where they act to phosphorylate various transcription factors that modulate gene expression (Plátenník et al., 2000). CaMKII activation is regulated in a similar, calcium dependent manner, and can lead to the phosphorylation

AMPARs (enhancing their conductance) or the insertion of additional AMPARs into the membrane, further facilitating NMDAR activation (Barria et al., 1997). Though the effects of the above-mentioned cascades is primarily postsynaptic, there is ample evidence to suggest that retrograde messenger systems allow for presynaptic modifications to also occur as a result of LTP induction (Castillo, 2012).

Though LTP, and its cousin LTD, are the most extensively studied demonstrations of neural plasticity, there are certainly many additional forms of plasticity that exist in the brain. For example, changes in intrinsic excitability (Desai et al., 1999) and the alteration in the dynamics of brain wide circuits (Polanía et al., 2012) represent forms of plasticity that do not directly rely on LTP/D mechanisms. Even more distinct still are newly discovered mechanisms which rely on non-neuronal components of the brain to modulate plasticity (Parri and Crunelli, 2007). It is almost certain that many different forms of plasticity exist which have not yet been characterized, and it stands as a testament to the dynamic and malleable nature of the brain that decades of research has only uncovered the proverbial tip of the iceberg when it comes to the nature of the plastic brain.

1.2 Impaired Brain Plasticity Has Serious Consequences

Plasticity plays a fundamentally important role in the dynamic functioning of the brain and is required for healthy brain function. When the integrity of the mechanisms which support plasticity become disrupted, significant neurological disorders often emerge. A prime example of this phenomenon is schizophrenia

(SZ), a highly debilitating psychiatric disorder which impacts approximately 2.6 million adults in the United States (Carter, 2015). Pharmacological studies in healthy volunteers show that when NMDAR activity is impaired (via administration of NMDAR antagonists), psychotic symptoms and cognitive deficits, mimicking those found in SZ, can be induced (Javitt and Zukin, 1991; Kapur, 2003). Important support for linking abnormal plasticity with SZ has emerged from recent studies utilizing genome-wide allelic association. These studies have revealed that six different genes whose expression levels were abnormal in SZ were intimately related to glutamatergic transmission, specifically NMDAR-dependent signaling (Harrison and Weinberger, 2005). Similar findings in allelic association have been revealed in other psychiatric disorders as well, most notably in depression and bipolar disorder (Heim and Binder, 2012; Soeiro-de-Souza et al., 2012).

There is another condition, relevant to this dissertation, where disrupted plasticity is implicated as having a direct impact, in fetal alcohol spectrum disorders (FASD). Individuals with FADS present with a wide variety of behavioral and cognitive impairments which are directly correlated with severity of prenatal alcohol exposure (PAE) (Streissguth et al., 1989). It has been well established that alcohol exposure at the prenatal stage can have disastrous effects on the developing brain, a stage at which nascent brain circuits are both being formed and pruned in a highly complex and sensitive manner. Evidence from animal models informs us that LTP is disrupted in response to PAE, leading to behavioral deficits later in life (Clements et al., 2005; Izumi et al., 2005). Disruptions in plasticity during development contribute to the impaired formation of brain circuits at the macro

level, as seen in imaging studies of adolescents with FASD who present with hypoactive dorsolateral prefrontal cortex (DLPFC) activity during executive functioning tasks (Astley et al., 2009; Nunez et al., 2011). The DLPFC is an area of the prefrontal cortex which is crucial to executive functioning, including working memory, cognitive flexibility and decision making. As evidenced in FASD, disruptions in plasticity at the cellular level can have emergent effects at the level of brain circuits and behavior.

Taken together, it is clear that impairments in brain plasticity have dramatically negative effects on health and quality of life. Therefore, interventions that can serve to enhance or modulate human brain plasticity have great therapeutic potential.

1.3 Transcranial Direct Current Stimulation: History

Transcranial direct current stimulation (tDCS) is a promising method for non-invasively modulating human brain plasticity. Though the adaptation of this methodology as research and clinical tool only took place at the turn of the century, the application of electrical currents to the brain has quite a lengthy history. In fact, we have records from ancient times concerning a certain roman physician Scribonius Largus (during the time of Emperor Claudius) who described placing a live electric eel over the scalp in an effort to cure headaches (Hermann Baas, 1889). Galen of Pergamum and Pliny the Elder were two other great medical researchers of the Roman times who reported similar experiments (Kellaway,

1946). We even have records from the 11th century of a Muslim physician, Ibn-Sidah, who used an electric catfish for the treatment of epilepsy (Kellaway, 1946).

The discovery of electricity and the subsequent invention of the battery in the 19th century allowed the investigation of direct current stimulation in a more controlled manner. Famous researchers such as Giovanni Galvani and Alessandro Volta understood that electrical stimulation of varying duration and intensity could evoke significant physiological effects (Zago et al., 2008). Indeed, the first systematic clinical applications of direct current stimulation date back to this time, when Giovanni Aldini (Galvani's cousin) used electrical currents to treat depression (Arndt, 1870). Over the course of the next two centuries, many other researchers used electrical currents for the treatment of psychiatric disorders with varying results (Zago et al., 2008).

The advent of more advanced electrophysiological techniques throughout the 20th century facilitated the study of the effects of electrical currents on brain activity. Studies in animal models were (and continue to be) fundamental in answering important questions which could not be directly probed in humans. Amongst the most impactful initial discoveries in these models were made by Bindman and colleagues who demonstrated polarity specific modulation of evoked potentials and spontaneous spiking in the rat cortex (Bindman et al., 1962, 1964). Similar findings were reported in cats, where alterations in spontaneous activity in the motor cortex were enhanced by anodal (positive terminal) stimulation and decreased by cathodal stimulation (negative terminal) (Purpura and Mcmurtry, 1965).

It wasn't until the turn of the current century however, that tDCS was truly appreciated as an important form of non-invasive brain stimulation. Landmark studies conducted by Priori (Priori et al., 1998), and followed up by Nitsche and Paulus (Nitsche and Paulus, 2000), showed for the first time in human subjects that low-intensity currents could be delivered non-invasively to induce polarity-specific changes in cortical excitability. These studies utilized transcranial magnetic stimulation (TMS) triggered motor evoked potentials (MEPs) as a proxy to probe the effects of tDCS on cortical excitability, finding that anodal stimulation enhanced excitability while cathodal stimulation had opposite effects. These seminal studies were followed by a large number of investigations, both in humans and animal models, which contributed to our understanding regarding the mechanisms underlying tDCS effects on brain plasticity (Fritsch et al., 2010; Kuo et al., 2008; Monte-Silva et al., 2013; Nitsche et al., 2003a), as well as the impact of different stimulation parameters (electrode location, stimulation duration etc.) on tDCS effects (Dmochowski et al., 2011; Peterchev et al., 2012; Radman et al., 2009).

1.4 Transcranial Direct Current Stimulation: State of the Art

The state of the art in tDCS involves the placement of electrodes on the scalp and the application of a low level of direct current (between 0.5-2 mA) to the electrodes. Most commonly, 2 electrodes are used with tDCS, the positive terminal known as the anode and negative terminal termed the cathode. The applied electrical current enters the brain via the anode and passes out of the brain at the

cathode. Because the induced electric fields are greatest underneath the electrodes (Ruffini et al., 2013), some degree of targeting can be achieved by controlling the placement of the electrodes on the scalp (Peterchev et al., 2012). With the traditional two electrode setup, the electrodes can be referred to as the active and the return electrodes. The active electrode (can be anode or cathode, depending on what type of stimulation is being applied) is placed over the nominal brain target, while the return electrode is placed over another cranial or extracephalic region (usually a relatively electrically inert location such as the supraorbital bone is used for the return electrode) (Nasseri et al., 2015). In the case of more advanced tDCS montages (referred to as high definition tDCS (HD-tDCS)), multiple return electrodes are placed in a ring orientation around a single active electrode. This manner of electrode placement is thought to better focalize current spread (Dmochowski et al., 2011).

In addition to electrode montage and current intensity, there are a variety of other stimulation related parameters that likely influence tDCS effects. Two important ones are electrode size and duration of stimulation. The size of electrodes influences the current density that is produced by tDCS, with smaller electrodes producing a higher density of current compared to larger ones and also providing a more focal stimulation zone (Laakso et al., 2019). However, some studies suggest that despite reduced current density, larger electrodes are better able to induce changes in cortical excitability (Ho et al., 2016), suggesting that there is a non-linear relationship between current density and physiological effects induced. Duration of stimulation is another parameter which seems to have

significant effects on tDCS outcomes. Studies have shown that longer stimulation duration (>10min) are required to induce long lasting physiological changes (Nitsche and Paulus, 2000; Nitsche et al., 2008), though as in the case of current density, the relationship between physiological modulation and stimulation duration is not always linear (Mosayebi et al., 2018).

1.5 Transcranial Direct Current Stimulation: Mechanism of Action

TDCS differs from other forms of non-invasive brain stimulation modalities such as TMS and electroconvulsive therapy in that it does not induce neuronal firing by suprathreshold membrane depolarization. Instead, tDCS relies on modulating spontaneous neuronal network activity in a subthreshold manner (Brunoni et al., 2012; Nitsche et al., 2008). At the cellular level, the primary mechanism of action brought about by tDCS is a polarity-dependant modulation in resting membrane potential (Bikson et al., 2004; Brunoni et al., 2012). Generally, it is accepted that anodal stimulation enhances cortical excitability by elevating the resting membrane potential closer to the action potential threshold, whereas cathodal stimulation has opposite effects (Bikson et al., 2004; Nitsche and Paulus, 2000). Data from animal models showing polarity specific modulation in spontaneous firing rates and altered sensitivity to afferent input supports this framework of thought (Bikson et al., 2004; Purpura and Mcmurtry, 1965). This primary polarization mechanism is thought to underlie acute effects of tDCS on cortical excitability in humans (Nitsche and Paulus, 2000).

However, tDCS elicits long-term effects which can last up to an hour after stimulation (Nitsche and Paulus, 2001; Nitsche et al., 2003b). Therefore, the mechanisms of action cannot be solely attributed to modulation of neuronal membrane potential. Indeed, recent work in animal models has expanded our understanding of how tDCS interacts with endogenous brain activity to modulate plasticity and learning on a long-term basis. A seminal study by Fritsch and colleagues demonstrated that DCS can boost LTP in the mouse motor cortex (Fritsch et al., 2010). Importantly, in this study they showed that in order for DCS to boost LTP, it had to be paired with an ongoing depolarizing stimulus. This finding reinforces the fact that tDCS is a subthreshold modulator of neuronal activity and cannot, on its own, produce de novo plastic changes. In addition to showing that DCS, when coupled with ongoing activity, could induce LTP, this paper also showed that tDCS led to improved learning and motor skill acquisition, demonstrating a functional manifestation of plasticity enhancement (Fritsch et al., 2010; Podda et al., 2016). These findings have been robustly replicated in several other studies, both in the motor cortex and in the hippocampus, showing that DCS can boost LTP and learning in a polarity specific and NMDAR dependent manner (Kronberg et al., 2017, 2019; Podda et al., 2016; Ranieri et al., 2012). In humans as well, it has been demonstrated, primarily in the motor cortex, that repeated tDCS application can induce LTP-like, NMDAR dependent enhancements in cortical excitability (Monte-Silva et al., 2013; Nitsche et al., 2003a). In addition to its effects on the neuronal activity, there is emerging evidence that tDCS may also impact the non-neuronal components of the brain. Recent studies have highlighted

the role of astrocytes in mediating the plasticity changes brought about by tDCS (Monai and Hirase, 2018).

In conclusion, the mechanisms of action of tDCS remain to be completely elucidated. Better understanding how tDCS modulates brain plasticity in humans will allow the rational development of best use practices which can inform how we use this technology for therapeutic purposes.

1.6 Transcranial Direct Current Stimulation: Open Questions

In the last 10 years, the use of tDCS in research and clinical studies has grown substantially. As a research tool, tDCS has been used to probe a wide range of motor, cognitive and perceptual processes, including memory, learning, and a host of other executive functions. Due to its low cost, safety and tolerability, tDCS is also an attractive tool to use in clinical trials treating various neuropsychiatric disorders. Over the last decade, such trials have been carried out in major depressive disorder, chronic pain, and schizophrenia to name a few (Jahshan et al., 2017; Pal et al., 2015). Though results from these first-generation studies have been, on the whole, promising, issues regarding heterogeneity of outcomes and limited reproducibility of findings (Mervis et al., 2017; Nilsson et al., 2017; Vercammen et al., 2011) have led some to question the efficacy of tDCS as an effective neuromodulatory tool.

Several factors contribute to the above-mentioned issues with tDCS. Not least of which is the reality that the biological mechanisms which govern tDCS effects in humans is not yet well understood. Compounding this fact is that, as with

all neuromodulation modalities, the parameter space for tDCS is huge, encompassing a wide range of both stimulation related and subject specific factors. Given the large parameter space, it becomes increasingly important to develop research based, best use protocols which can serve to standardize tDCS interventions across various research and more importantly clinical settings. Another, as yet unanswered, crucial question as it pertains to tDCS is how to best achieve specificity of outcomes. In other words, how can we best target the brain regions we are seeking to modulate, while sparing the regions which are not germane to the intervention? Achieving targeting is especially difficult with tDCS, primarily due to two specific characteristics. First is the fact that tDCS produces a subthreshold level stimulation and does not directly cause neurons to fire (Datta et al., 2009; Ruffini et al., 2013). Second, it has been well established that the current spread from tDCS electrodes is diffuse and spreads to large parts of the cortex (Bikson et al., 2012; Datta et al., 2009; Neuling et al., 2012), making it difficult to achieve anatomical specificity. One approach to achieve better targeting is to manipulate stimulation related parameters such as electrode placement, number and size to focalize current flow (HD-tDCS). However, despite these efforts, current modeling studies continue to show that tDCS electrical fields spread to non-target cortical regions.

One factor that may significantly influence the specificity and efficacy of tDCS effects is the state of the brain at the time of stimulation. The idea that specific concurrent brain activity influences tDCS outcomes is known as the ‘activity-selectivity’ hypothesis (Bikson et al., 2013). This hypothesis posits that

tDCS will preferentially modulate neuronal networks which are concurrently active while sparing those networks which are relatively inactive. Activity-selectivity thus makes the assumption that there is some characteristic feature of the activated network (may be a population of neurons or a functionally connected set of brain regions) which makes it preferentially sensitive to the effects of externally applied electric fields. Though the mechanisms of activity selectivity are still under investigation, behavioral studies (as well as several clinical trials) have already begun to combine tDCS with concurrent task performance to improve treatment outcomes. These initial studies provide some promising evidence to corroborate the activity-selectivity hypothesis, there is still a paucity of physiological evidence for this model. To date, there have only been two studies which have physiologically examined the activity-selectivity model of tDCS effects, and these studies have produced somewhat conflicting results. Further investigation of this hypothesis is warranted in order to better understand the contribution of brain state to tDCS outcomes.

The body of work that encompasses this dissertation is aimed at investigating the effects of tDCS on human brain plasticity, both from a physiological and a behavioral perspective. The first chapter of the thesis describes an elegant electrophysiological study which was conducted in a cohort of healthy individuals. This study utilized a novel event related potential (ERP) paradigm to assess the effects of tDCS on human cortical plasticity. More specifically, this paradigm facilitated the investigation of the activity-selectivity

model of tDCS effects as it allowed the study of tDCS effects in a stimulus-specific manner.

The second chapter describes a clinical trial which combined tDCS with cognitive training to improve cognitive deficits in adolescents with fetal alcohol spectrum disorders (FASD). FASD is highly associated with disrupted plasticity, which often manifests as cognitive deficits across several domains. We sought to leverage the activity-selective properties of tDCS by combining stimulation with specific cognitive training aimed at facilitating executive functions. The primary goal of the study was to demonstrate feasibility and tolerability in an adolescent population, as well as to assess whether tDCS could boost gains from cognitive training compared to training alone.

The final chapter concludes the dissertation and offers reflections on the works described herein and offers some ideas for future research.

CHAPTER 2: Transcranial Direct Current Stimulation (tDCS) Elicits Stimulus-Specific Enhancement of Cortical Plasticity

2.1 ABSTRACT

Deficits in neural plasticity underlie many psychiatric disorders. TDCS is a promising method for enhancing plasticity. Understanding how tDCS interacts with brain state is necessary for rational development of this tool. I used an ERP-based paradigm to assess stimulus-specific effects of tDCS on cortical plasticity in humans. Two pure tones were used as stimuli, with only the target tone being used for plasticity induction. I investigated whether anodal tDCS directed toward the auditory cortex would induce plasticity as measured by change in auditory N100 amplitude. Active tDCS significantly modulated plasticity in the target tone compared to a sham but had no effect on the control tone. The tDCS related modulation was absent 30 min after stimulation. Our results indicate that tDCS can modulate cortical plasticity in the auditory cortex in a stimulus-specific manner. These findings bolster the idea what tDCS can be an effective tool for enhancing cortical plasticity that may be applied for therapeutic purposes.

2.2 INTRODUCTION

Experience dependent plasticity refers to the brain's ability to dynamically shift functional or structural states in response to internal or external events. This property enables us to learn, make predictions and guides response selection for adaptive behavior (Cooke and Bliss, 2006; Ganguly and Poo, 2013). Due to its fundamental role in brain dynamics, maladaptive neuroplasticity often leads to debilitating conditions (Johnston, 2004; Kays et al., 2012). Disrupted plasticity is thought to play a role in the pathophysiology of several psychiatric disorders, including schizophrenia, bipolar disorder and major depressive disorder (Elvsåshagen et al., 2012; Normann et al., 2007; Stephan et al., 2006). Given the implication of disrupted plasticity in psychiatric disease, tools which can modulate plasticity have great clinical potential (Thickbroom and Mastaglia, 2009).

Non-invasive neuromodulation via transcranial direct current stimulation (tDCS) is a promising method for modulating plasticity. With tDCS, a low-intensity direct current is applied using two or more electrodes placed in a specific orientation over the scalp. The current enters the brain via the positively charged anode, and flows towards the negatively charged cathode, leading to polarity specific changes in neuronal excitability. Polarity-dependent modulation of cortical excitability was first demonstrated in animal studies which measured enhanced neuronal firing rates after anodal tDCS and decreased firing rates after cathodal tDCS (Bindman et al., 1962, 1964; Gartside, 1968). These findings were later extended to the human brain, primarily by studying the effects of tDCS applied to

the motor cortex (M1), using transcranial magnetic stimulation (TMS) evoked motor evoked potentials (MEP) as a proxy for cortical excitability (Nitsche and Paulus, 2000, 2001; Nitsche et al., 2003). More recent investigations using modern electrophysiology have demonstrated the capacity of externally applied currents to modulate classical models of Hebbian plasticity such as Long Term Potentiation and Depression (LTP/D) (Kronberg et al., 2017; Podda et al., 2016; Ranieri et al., 2012). The ability of tDCS to interact with these mechanisms, which are thought to serve as a substrate for learning and memory, has been used to explain the positive effects of tDCS on motor learning and cognitive enhancement in both animal and human models (Fritsch et al., 2010; Jahshan et al., 2017; Reis and Fritsch, 2011).

Due to its safety and tolerability (Woods et al., 2016), the use of tDCS has grown substantially. Over the last decade, tDCS has been used to modulate a wide range of motor and cognitive processes, as well as to treat various psychiatric disorders (Mervis et al., 2017; Reinhart and Nguyen, 2019; Reis and Fritsch, 2011). Despite the promise however, tDCS-induced effects appear to be mediated by a large number of both stimulation and subject specific factors, often resulting in highly variable responses (Brunoni et al., 2012; Laakso et al., 2019; Li et al., 2015; Vorobiova et al., 2019). The highly complex parameter space of tDCS presents a challenge when seeking to develop best-use practices and highlights the need to improve on our still rudimentary understanding of the biological mechanisms supporting tDCS-related brain changes.

A crucial question when it comes to the rational development of tDCS is how to achieve specificity (Bikson et al., 2013). TDCS induces a low electrical field in the brain, producing only a subthreshold level of membrane polarization which is diffused across wide brain areas (Radman et al., 2009; Ruffini et al., 2013), making it difficult to achieve anatomical targeting (Bikson et al., 2012; Datta et al., 2009; Neuling et al., 2012). Though modern techniques (e.g. HD-tDCS) allow for a more focal intervention, computational models show that current still spreads to large parts of the cortex (Datta et al., 2009; Dmochowski et al., 2011; Dasilva et al., 2012). The low spatial resolution of tDCS contrasts with its focal effects on cognitive performance (Jacobson et al., 2012; Nitsche and Paulus, 2011) and electrophysiological measures (Keeser et al., 2011; Zaehle et al., 2011), indicating that controlling stimulation parameters alone cannot fully explain how this specificity is achieved.

An important factor shaping tDCS's specificity may be the state of the brain at the time of stimulation. Indeed, several studies aiming to facilitate cognitive or motor learning have applied tDCS during tasks to leverage a potential synergistic relationship between externally applied electric fields and endogenous patterns of brain activity (Martin et al., 2014; Nienow et al., 2016; Reis and Fritsch, 2011). Promising findings from such studies corroborate a recently proposed 'activity-selectivity' hypothesis, which states that tDCS preferentially modulates active over inactive neural populations (Bikson et al., 2013; Fertonani and Miniussi, 2016). However, direct physiological evidence for this model remains limited.

Much of the investigation examining the state-dependent effects of tDCS has been conducted in the motor cortex, demonstrating that modulation of MEPs via tDCS is sensitive to brain state (Antal et al., 2007; Bortoletto et al., 2015). It is important to consider the limitations of MEP-dependent findings, however. TMS triggered MEPs are an indirect measure of brain activity, and the measured signal can be several synapses removed from the actual locus of tDCS effects (Auriat et al., 2015), making direct interpretations regarding neural changes less certain. In addition, reliance on MEPs limits investigations to the motor cortex, reducing the generalizability of these findings to other regions of the brain.

To the best of our knowledge, only two studies have examined the physiology of the 'activity-selectivity' model outside of the motor cortex (Hill et al., 2018; Pisoni et al., 2017). These studies utilized electroencephalography (EEG) recordings to investigate tDCS modulation of brain activity. Due to its high temporal resolution, EEG can be an ideal method to probe neuromodulatory changes brought about by tDCS (Miniussi et al., 2012). Pisoni and colleagues (Pisoni et al., 2017) delivered anodal tDCS over the left inferior frontal gyrus during a verbal-fluency task, using TMS-evoked potentials (TEPs) to probe changes in cortical excitability. They found that the amplitude of TEPs was increased after anodal tDCS, but only in specific task related brain regions. Hill and colleagues (Hill et al., 2018) expanded on these findings by comparing the effects of tDCS when paired with a cognitive task, when applied at rest, or when only the cognitive task was performed. This study used event-related potentials (ERP) and resting state electroencephalography (RS-EEG) in addition to TEPs to assess changes in brain

activity. In contrast to Pisoni et al, no activity selectivity was observed when analyzing TEPs and RS-EEG. However, changes in ERP amplitudes were observed only in the tDCS + Task condition, providing only limited evidence for the 'activity-selectivity' model.

A clearer understanding of the role that brain state plays in shaping tDCS outcomes is crucial in informing the rational use of tDCS. To better investigate the contribution of brain state to tDCS effects, it would be ideal to utilize paradigms that directly probe the plasticity mechanisms which are modulated by tDCS. Research in animals has shown that tDCS is able to modify synaptic efficacy and learning through modulation of LTP (Podda et al., 2016; Ranieri et al., 2012). Though our ability to investigate LTP in humans is limited, it has recently been demonstrated that high-frequency, repetitive presentation of sensory stimuli, or sensory tetanus, can provide a naturalistic method for inducing LTP-like plasticity in the human cortex (Clapp et al., 2006, 2012; Cooke and Bear, 2010). Studies in rodents show that sensory tetanus can lead to enhanced sensory evoked potentials in the cortex, similar to the manner in which high frequency electrical stimulation leads to enhanced synaptic efficacy in slice demonstrations of LTP (Clapp et al., 2012). The enhancement in sensory evoked potentials induced by high frequency sensory presentation displays the critical features of LTP, including persistence, input specificity, and N-Methyl-D-aspartate receptor (NMDAR) dependence (Clapp et al., 2006; Cooke and Bear, 2010). In humans, the effects of sensory tetanus can be observed noninvasively in the EEG as modulations in specific components of sensory ERPs. Indeed, paradigms using sensory tetanus

have been used to induce persistent potentiation of both visual and auditory ERP components in humans (Clapp et al., 2005, 2012; Lei et al., 2017; Teyler et al., 2005). These sensory tetanus paradigms provide a valuable window into the mechanisms thought to underlie neural plasticity and are therefore a promising tool with which to probe the effects of tDCS in humans.

To better evaluate the 'activity-selectivity' hypothesis of tDCS, I carried out a crossover study featuring 2 sessions, with participants undergoing both active and sham tDCS on separate days. Each session utilized an auditory sensory tetanus paradigm which was designed to induce LTP-like plasticity in the auditory cortex in a stimulus specific manner (Clapp et al., 2005; Mears and Spencer, 2012). Previous investigations using variants of this paradigm have shown that the N100 component (a negative deflecting potential peaking approximately 100ms post stimulus presentation) is potentiated following a short bout of sensory tetanus (Clapp et al., 2005; Lei et al., 2017; Teo et al., 2014). This potentiation has been shown to be persistent (Clapp et al., 2005; Lei et al., 2017), stimulus-specific (Mears and Spencer, 2012) and localized to the auditory cortex (Zaehle et al., 2007).

I employed a modified variant of the paradigm, utilizing two pure tones of differing pitch as stimuli. The auditory tones were presented to the participants in a random order at a slow and variable rate in three recording blocks (Fig. 1A). The baseline block featured the presentation of each tone 150 times and was followed immediately by the sensory tetanus block. For sensory tetanus, one of the two tones were pseudo-randomly selected (designated as the target tone) and

presented at a high rate (~13Hz) for a brief period of time. The tone not presented during sensory tetanus was designated as the control tone for that session. The recording for timepoint 1 started after sensory tetanus and was identical in structure to the baseline block. After the completion of timepoint 1, participants were asked to sit in silence or read quietly until 30min had elapsed since the end of sensory tetanus. At that point, the recording for timepoint 2 began. The timepoint 2 block was similar to timepoint 1 in all respects except that it featured the presentation of only half the number of stimuli (90 of each tone). This was done to minimize participant fatigue. Amplitude change of the N100 component relative to baseline was used as a means to quantify plasticity.

To investigate the impact of tDCS on plasticity, anodal stimulation was applied bilaterally to the auditory cortex, simultaneous with sensory tetanus (Fig. 1A and B). The stimulation target was informed by imaging studies which have localized the site of plasticity induction from sensory tetanus to the primary auditory cortex (Chen et al., 2011; Zaehle et al., 2007). Because tDCS electric fields were present only during the presentation of one of the tones, it allowed us to selectively modulate the neural signal associated with the processing of that stimulus alone. By comparing changes in the N100 amplitude of the two tones, I was able to analyze whether tDCS had any modulatory effect on plasticity, and whether this effect was general, or specific to the stimulus presented during sensory tetanus. I predicted that active tDCS would enhance plasticity compared to sham (Fritsch et al., 2010; Kronberg et al., 2017; Pisoni et al., 2017). Further, in accordance with the ‘activity-selectivity’ model of tDCS, I predicted that tDCS effects would be

restricted to the stimulus presented during sensory tetanus (Bikson et al., 2013; Hill et al., 2018; Pisoni et al., 2017).

2.3 METHODS

Participants

22 healthy adults (8 females) completed the study. Our sample size was based on a power calculation derived from similar reports (Clapp et al., 2005; Zaehle et al., 2011). Individuals who reported a history of neurological illness or had participated in a past neuromodulation study were not enrolled. The mean age of our sample was 24.9 years (range 19-42, s.d = 5.6), 20 of the participants were right-handed. Handedness was assessed using the Edinburgh Handedness Inventory (Oldfield, 1971); The hearing threshold of all participants was assessed using the Bekesy Threshold Test (Presentation, Neurobehavioral Systems, Version 19.0). All participants had auditory thresholds <25 dB in both ears. Prior to the start of the study, participants were informed of the study procedures and signed an informed consent form. The study was approved by the institutional review board at the University of Minnesota.

Experimental Design

Study participation involved a crossover design, with each subject undergoing two experimental sessions. The sessions were separated by at least

one day (mean = 8.7 days, s.d = 7.9). Each session involved EEG recording during the auditory sensory tetanus paradigm as well as application of tDCS. Study sessions were identical except for the nature of tDCS applied, either active or sham. The order of active and sham tDCS treatment was counterbalanced across participants, and participants were blind to treatment condition.

Auditory Sensory Tetanus Paradigm & EEG Recording

The auditory sensory tetanus paradigm used in this study presented two pure tones in three recording blocks: baseline (BB), timepoint 1 (T1) and timepoint 2 (T2). A sensory tetanus block featured the presentation of one of the two tones at a high rate for a brief period of time (Fig. 2.1A). Sinusoidal tones of 1900 and 3000 Hz were used as stimuli (50ms duration). Tones were constructed using a sine wave function at 44,000 samples/sec (Neurobehavioral Systems, Version 19.0) and were delivered binaurally at an intensity level of 70dB through a pair of insert headphones (ER-3C, Etymotic Research).

During the BB, each tone was presented 150 times in a random distribution with an ISI jittered between 1800 and 2600ms, lasting ~12min. For sensory tetanus, one of the two tones were presented 4000 times at a rate of 13.3Hz (duration: 5min). The tone selected for sensory tetanus was designated the target tone (TT) while the other tone served as the control tone (CT). The identity of the TT was pseudo-randomly determined and was counterbalanced between participants, with each subject receiving the same TT across sessions. Immediately after sensory tetanus, participants were asked to sit in silence for

45sec to allow aural ringing to dissipate. T1 was identical in nature to the BB and commenced after sensory tetanus. T2 started 30min after the end of sensory tetanus in order to assess persistence of any stimulation effects across time. To reduce participant fatigue, each tone was presented only 90 times in T2. Participants were instructed to either sit in silence or quietly read during the time between T1 and T2.

During the paradigm, participants were seated in front of a 27" computer monitor (1920x1080 resolution, 60Hz refresh) in a dimly lit, electrically shielded room. During recording, participants were instructed to remain still, limit eye blinks and focus their gaze on a white fixation cross in the center of the screen. EEG was recorded using the Starstim8 tDCS/EEG system (Neuroelectrics, Barcelona, Spain). This system provides 8 channels which were placed at Fz, Cz, Pz, Oz, F3, F4, T7 and T8 (10-20 electrode placement system). Impedances were maintained below 10kΩ for the duration of the recording. EEG signal was sampled at 500Hz, analog band passed between 0.1-100Hz and referenced to the right earlobe.

TDCS Administration and Electrical Field Modeling.

TDCS was targeted to the primary auditory cortex bilaterally with anodes at T7 and T8 and with return electrodes positioned at Fp1 and Fp2. Previous studies targeting the primary auditory cortex have used similar montages (Rahimi et al., 2019; Royal et al., 2018; Zaehle et al., 2011). Stimulation was delivered using 3.14cm2 PiStim electrodes (Neuroelectrics, Barcelona, Spain). In the active condition, stimulation was delivered at 1mA per anode for a duration of 5min, with

a current density of 0.318mA/cm2. Importantly, the timing of stimulation was such that it coincided with sensory tetanus. In the sham condition, the current was held constant for 30sec, but was then ramped down to 0 mA over the next 10sec.

I used electric field estimation (SimNIBS 2.1.1, (Thielscher et al., 2015)) to simulate tDCS current flow from our montage. Electric-field models were based on the extended MNI head model derived from 152 structural MRIs taken from normal participants. The parameters used for model simulation mimicked those used in the study. Induced electrical fields were visualized using Gmsh (Geuzaine and Remacle, 2009) (Fig. 2.1B).

Event Related potentials (ERP) Processing

EEG data were preprocessed and analyzed in MATLAB R2018b (MathWorks, Inc., MA) using the EEGLAB toolbox (Delorme and Makeig, 2004) and the ERPlab toolbox extension (http://erpinfo.org/erplab). Raw EEG was down-sampled to 250samples/sec and digitally filtered using a bandpass of 0.1-20Hz and a roll-off of 12dB/octave. Data were segmented with respect to event markers into 800ms epochs extending from 200ms pre-stimulus to 600ms post-stimulus and the mean of the pre-stimulus interval was used as a 0-microvolt baseline. A Moving Window Peak-to-Peak function was used to detect and mark individual epochs for rejection. Data files that produced >25% rejected trials were excluded from further analysis. Using the grand average ERP from all participants, the N100 component was identified (time window: 75-108ms) and the four electrodes with the highest N100 amplitudes were selected for further analysis (Fz, Cz, F3, F4).

Single session N100 mean amplitudes and latencies were calculated over an averaged signal encompassing the 4 fronto-central electrodes. To identify a time-window for calculating N100 mean amplitudes, a custom MATLAB script was used to determine fractional peak latencies where the amplitude of the N100 dropped to 75% of its peak. These fractional latency values were then used as a time-window to calculate the mean amplitude of the N100 component for each ERP. The latency of the local peak identified within the window was used as the peak latency measure. N100 difference values (Δ-values) for amplitude and latency were calculated within each session by subtracting baseline condition values from T1 and T2 condition values. Amplitude and latency measures for the P50 and the P200 components were derived in the same manner as for the N100.

Fatigue and Blinding

Prior to the start of study procedures, participants completed a questionnaire designed to assess the level of fatigue ranked on a scale of 0 (alert) to 3 (very tired). The questionnaires were completed again at the end of T2. To assess the effectiveness of the blind, participants were asked to guess which treatment they had received at the end of each session.

Statistical Analysis

ERP Amplitude and Latency Analysis

All statistical analyses were carried out in R software. I analyzed ERP component Δ-values using a hierarchical linear modeling (HLM) approach. Raw amplitude was also modeled (see Fig. S2.1). In the final model, treatment (Active/Sham), tone (TT/CT), and timepoint (T1/T2) were considered as main effects, as well as an interaction between treatment x tone and between treatment x timepoint. Random effects were included in the HLM to account for the repeated measures across timepoints for each subject within tone and within treatment. A timepoint x tone fixed effect interaction was also considered but the interaction was not significant, nor did it improve model fit. Time between sessions (in days), treatment order, pitch (whether the TT was the high or low pitch tone) and change in fatigue were included as covariates, but dropped from the final model since they did not sufficiently improve model fit (according to the akaike information criterion (AIC) (Akaike, 1974)).

Planned contrasts were used to further explore significant main effects and interactions in our model. I focused on two contrasts, (1) comparing active vs. sham Δ-values for the TT and (2) for the CT. These contrasts were applied at both timepoints; bonferroni-holm correction was used to account for multiple comparisons.

Analysis of Fatigue and Blinding Efficacy

An HLM was used to analyze change in fatigue. I tested for the main effects of treatment and time as well as their interaction.

To assess the effectiveness of our blind, I categorized participant responses as either “correct” or “incorrect” after each session. A general linear model was used to assess any significant effect of treatment on blinding.

2.4 RESULTS

Baseline Amplitude and Latency

Comparisons were first performed to assess differences in baseline amplitude and latency for the major mid-latency ERP components (P50, N100, P200). Separate models were used for each component, taking into consideration the main effects of treatment (active/sham) and tone (target tone/control tone). No significant baseline differences were found between any of the conditions for any ERP component ($p>.05$ for all comparisons), indicating consistent amplitudes and latencies prior to sensory tetanus and tDCS.

Amplitude and Latency Modulation Across Time

Sensory Tetanus Induces Stimulus-Specific Plasticity in the N100

I modeled N100 amplitude Δ-values (difference from baseline at each timepoint) using a hierarchical linear modeling approach. The model revealed a strongly significant effect of tone ($t_{21}=5.53$, $p<.001$), indicating that N100 amplitude was differentially modulated depending on tone identity. Irrespective of tDCS, the target tone N100 was potentiated compared to baseline, whereas the control tone N100 did not significantly differ from baseline (Table 2.2 & Fig. S2.1). This finding

reinforces the idea that sensory tetanus using auditory tones can be used to induce a stimulus-specific modulation of cortical plasticity in the human brain (Clapp et al., 2012; Mears and Spencer, 2012).

TDCS Modulates Plasticity in an Activity-Selective Manner

Having established a stimulus-specific potentiation in the target tone ERP, next I sought to ascertain whether tDCS modulated the induced plasticity and whether tDCS effects were general (observed across both tones), or specific to the tone presented during stimulation. Our model revealed a significant main effect of treatment (t_{42}=2.84, p<.01), and a significant interaction between treatment and tone (t_{42}=-2.62, p<.01). These results confirm our hypothesis that tDCS can modulate the level of plasticity induced in the auditory cortex and that tDCS modulation is highly sensitive to brain state during stimulation (Table 2.2 & Fig. S2.1).

A significant main effect of timepoint (t_{86}=3.30, p<.01) revealed that N100 amplitude modulation was dependent on time from stimulation. To examine the effects of tDCS across time, I analyzed N100 Δ-values at each timepoint using planned contrasts. At timepoint 1, contrasts revealed that active tDCS resulted in a greater potentiation in target tone N100 amplitude compared to sham (p=.02), while having no discernible effect on the control tone amplitude (p=.61) (Fig. 2.2). These findings at timepoint 1 were corroborated by a post-hoc analysis of baseline corrected grand average ERP waveforms, where paired t-tests were used to identify timepoints at which active and sham ERP waves significantly differed (Fig.

2.3). Significant differences between active and sham conditions were identified only in the target tone ERP, in a time region corresponding to the N100 peak (Fig. 2.3B).

The recording for timepoint 2 was conducted 30min post tDCS/sensory tetanus to assess the persistence of any plasticity changes across time. At this timepoint, I no longer observed a tDCS effect (Fig. 2.4), with no significant difference between active and sham conditions in the target tone ($p=.19$) or in the control tone ($p=.20$).

No significant effects of treatment, tone or timepoint were revealed for N100 latency.

No Amplitude and Latency Modulation in Secondary ERP Components

I also sought to assess whether other prominent components of the auditory ERP (P50, P200) were impacted by sensory tetanus and tDCS. No significant effects or interactions were revealed for P50 amplitude or latency (Supplementary Table 2.3 & 2.4). A significant main effect of timepoint ($t_{86}=2.75$, $p<.01$) was observed for P200 amplitude, indicating increased amplitudes over time. This enhancement occurred irrespective of tone or treatment however, as no main effects or interactions were found in respect to these factors (Supplementary Table 2.1). No significant main effects or interactions were found for P200 latency (Supplementary Table 2.2).

Fatigue and Participant Blinding

There was no significant effect of tDCS on fatigue (t_{63}=2.75, p=.71), with both active and sham groups becoming more fatigued over time (t_{63}=5.27, p<.001). TDCS did not have a significant effect on the ability to guess treatment condition correctly (t_{21}=-0.961, p=.33) as participants were close to chance when guessing treatment condition (59% accuracy).

2.5 DISCUSSION

I used a unique ERP-based plasticity paradigm to explore whether tDCS could be used to modulate human cortical plasticity in a stimulus-specific manner. Our manipulation allowed us to ascertain whether tDCS effects would be altered by the functional state of the brain at the time of stimulation, as postulated by the 'activity-selectivity' hypothesis (Bikson et al., 2013). The current results provide strong physiological evidence that anodal tDCS can modulate cortical plasticity and that these effects are sensitive to brain-state (i.e. what stimulus the brain is processing during tDCS). I also find that the effect of tDCS on plasticity degrades over time.

Induction of Stimulus-Specific Plasticity via Sensory Tetanus

My sensory tetanus paradigm was designed to induce plasticity in the auditory cortex (Chen et al., 2011; Krumbholz et al., 2003; Zaehle et al., 2007). Previous reports have demonstrated that sensory tetanus with auditory stimuli modulates the sensory ERP, leading to a persistent and stimulus-specific

potentiation in the amplitude of the N100 component (Clapp et al., 2005; Lei et al., 2017; Mears and Spencer, 2012). The N100 component is a prominent mid-latency sensory evoked potential, deflecting approximately 100ms after stimulus onset, and is evoked in response to a wide array of sensory stimuli (Näätänen and Picton, 1987). Though the functional implications of modulating the N100 are still unclear, the presence of this potential across diverse stimulation conditions and sensory modalities suggests that the N100 is a general electrophysiological marker of cortical activation, indexing the brain's response to a particular input (Du et al., 2017; Näätänen and Picton, 1987). In the case of auditory stimulation, the cortical sources for the N100 have been localized to the superior and middle temporal gyri (Chen et al., 2011; Ford et al., 2016), resulting in a dipole which is best observed in the EEG over fronto-central electrodes.

Irrespective of tDCS, I showed that N100 amplitude was potentiated in response to the target tone following sensory tetanus. This potentiation was not present in the control tone (Fig. S2.1), reinforcing the idea that sensory tetanus can induce stimulus-specific plastic changes within the human cortex. Though I can only speculate as to the mechanism of these plastic changes, it seems plausible that presenting auditory tones at a rapid rate activates synapses within the auditory system in a manner similar to what is seen in cellular studies of LTP, where high frequency electrical stimulation is used to induce plasticity in neuronal tissue. Animal studies have shown that plasticity can be driven by persistent exposure to a sensory stimulus and can lead to stimulus-specific and NMDAR dependent changes in the neocortex of rats and mice (Clapp et al., 2006; Cooke

and Bear, 2010). These studies demonstrate that sensory tetanus can indeed induce neuronal changes which feature the cardinal properties of Hebbian plasticity. Nevertheless, because I recorded non-invasively with scalp electrodes, I cannot be certain that I are inducing the same sort of neuronal modifications as in the previously mentioned animal studies. Further investigation using more spatially precise methods would serve to greatly complement my current findings.

Stimulus-Specific Effects of tDCS on Plasticity

TDCS modulated sensory tetanus induced plasticity in a stimulus-specific manner. I observed a greater degree of potentiation in the target tone N100 under active tDCS compared to sham, bolstering the idea that tDCS can modulate plasticity in the human cortex. Further, I found that the effects of tDCS were restricted to the target tone, with the control tone ERP showing no stimulation dependent modulation. This indicates that tDCS did not cause a general enhancement of cortical excitability, but rather an alteration in cortical reactivity that was dependent on stimulus-specific brain responses.

A plausible explanation for the observed stimulus-specific effects can be derived from the fact that tDCS modulation is strictly dependent on endogenous brain activity (Bindman et al., 1964; Fritsch et al., 2010; Kronberg et al., 2017; Rahman et al., 2017). Thus, if a neuronal population is not active concurrent with tDCS, no plastic change should occur. Studies in animals investigating effects of tDCS on plasticity in the M1 support this view (Fritsch et al., 2010). For instance, application of DCS alone to M1 slice did not modulate synaptic efficacy as

measured by field excitatory postsynaptic potentials. However, when applied concurrently with synaptic input (via afferent stimulation), DCS resulted in a robust change in synaptic efficacy. Furthermore, at the cellular level, DCS is known to modulate the level of potentiation in a specific pathway, but only if that pathway is co-active with stimulation (Kronberg et al., 2017; Ranieri et al., 2012). TDCS modulation of plasticity may be mediated by changing membrane potential and removal of the Mg2+ block (Stagg and Nitsche, 2011), but because tDCS fields are subthreshold (Ruffini et al., 2013), only those neuronal populations activated during tDCS would experience this potentiation.

In the context of my experiment, I was activating a population of neurons responsible for processing a specific stimulus, and then selectively exposing that population to tDCS. The N100 is tonotopically distributed in the auditory cortex (Woods et al., 1993; Yamamoto et al., 1992), and it has been shown that distinct populations of neurons are responsible for processing auditory stimuli of differing pitch (Bitterman et al., 2008). I can then posit that those groups of neurons which were active during tDCS (those involved in processing the target tone) were preferentially modulated. These results provide the most robust physiological evidence for the 'activity-selectivity' model to date, demonstrating the ability of tDCS to selectively modulate a neuronal signal associated with processing a specific input (Bikson et al., 2013; Fritsch et al., 2010; Rahman et al., 2017).

It is important to note that while I achieved plasticity modulation, this effect was time-dependent, with tDCS effects no longer detectable 30min post stimulation. Several different factors may have contributed to this finding. One such

factor is related to stimulation parameters. Previous studies using MEPs have demonstrated that long stimulation durations (>10min) are required to elicit long term psychological changes from tDCS (Mosayebi et al., 2018; Nitsche and Paulus, 2000). Given my short bout of tDCS (~5min), it is not altogether unexpected to see effects fade over time.

The lack of tDCS effects at timepoint 2 could also be ascribed to the design of the experiment. As noted, ERPs from timepoint 2 were constructed using only half the number of trials used for baseline and timepoint 1. A smaller number of trials reduces my signal to noise ratio, thus making interpretations regarding timepoint 2 in respect to baseline and timepoint 1 less certain.

Alternately, a physiological explanation for reduced effects at timepoint 2 may be related to the slow rate of stimulus presentation in the recording blocks. As mentioned previously, it is the high rate of stimulus presentation during sensory tetanus that leads to plasticity induction. High frequency inputs result in a tight temporal correlation between spikes of pre- and postsynaptic neurons, leading to potentiated postsynaptic functioning. Conversely, low frequency inputs can have the opposite effect, leading to de-correlation and a reduced level of postsynaptic activity (Gerstner et al., 1996). Given this mechanism, it is plausible that the reduced effects at timepoint 2 were a result of an active depotentiation due to the repeated slow presentation of stimuli, rather than merely a passive decay of plasticity over time. This interpretation is supported by findings from related studies. First, in a study utilizing a visual variant of the sensory tetanus paradigm, Tyler et al. only found significant potentiation after 1 hour if early post-tetanus

recording blocks were withheld (Teyler et al., 2005). Second, it has been shown that LTP induced by high frequency electrical stimulation of surgically resected human neocortex can be actively de-potentiated via low frequency electrical stimulation (Chen et al., 1996).

Limitations

One limitation of this study is that the spread of current from my montage is diffuse, reaching cortical regions outside of my nominal target (Fig. 1B). Thus, off target effects could potentially confound my interpretations. However, unless concurrently activated, it is unlikely that those off target regions were significantly modulated (Pisoni et al., 2017).

As discussed previously, in order to reduce participant fatigue, I utilized a reduced number of trials for recording timepoint 2. Due to the difference in the number of trials used to construct ERPs at baseline and timepoint 1 compared to timepoint 2, I exercise some caution in interpreting timepoint 2 results. Nevertheless, given the prominence of the N100, I believe that the number of trials used for timepoint 2 (90) are usually sufficient to accurately characterize this component (Luck, 2014).

Lastly, it is important to note that I did not include any form of behavioral task to assess functional implications of my manipulation, as I felt this was outside the scope of the present study. It would be interesting for future investigations to include a perceptual discrimination task to determine whether modulating the sensory ERP has some form of functional relevance (i.e. reaction time, sensitivity).

Conclusions

I demonstrate that tDCS can modulate a physiological marker of cortical plasticity. Further, I show stimulus-specific modulation, demonstrating that pairing tDCS with a targeted brain-state is a crucial factor in eliciting tDCS effects. These findings support the 'activity-selectivity' hypothesis (Bikson et al., 2013), confirming in humans what has been found in animal models. Together this body of work represents a solid theoretical framework which can aid in the rational advancement of tDCS. The important translational step provided by this study further emphasizes the importance of combining tDCS with concurrent, and specifically targeted brain network activation in order to improve outcomes from tDCS interventions. This research is thus especially informative for future clinical studies which seek to effectively optimize tDCS interventions for remediation of deficits in a variety of brain disorders.

TABLES & FIGURES

Age (yrs)	24.9 ± 5.6
Gender (M/F)	14/8
Handedness (R/L)	20/2
Time Between Sessions (days)	8.8 ± 8.2

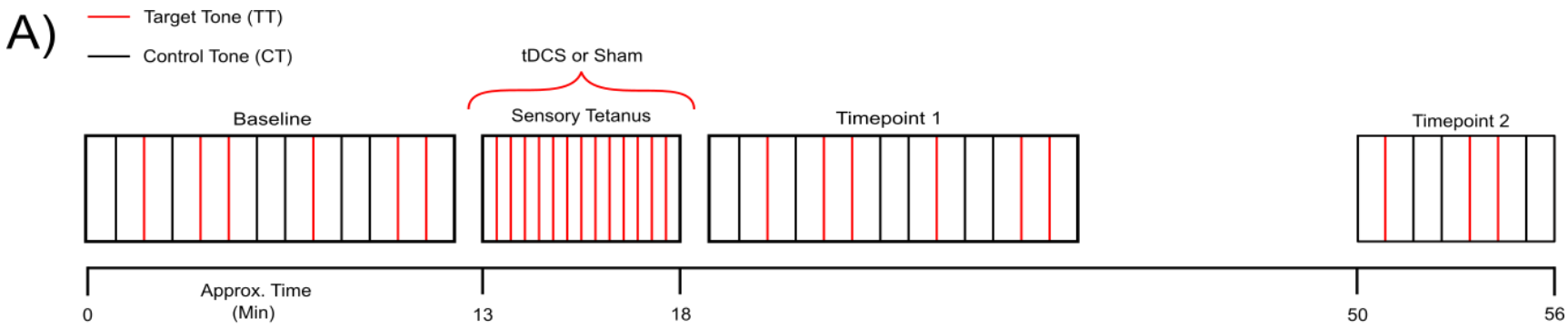
Table 2.1: Subject demographic information

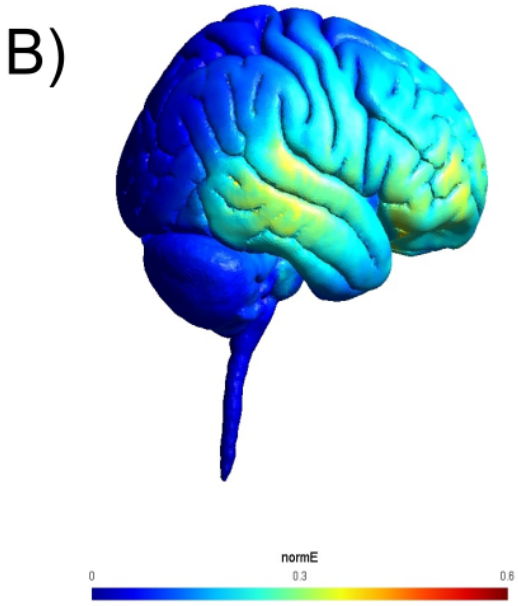
	Baseline					Timepoint 1					Timepoint 2				
	Target Tone		Control Tone			Target Tone		Control Tone			Target Tone		Control Tone		
	M	SD	M	SD		M	SD	M	SD		M	SD	M	SD	
ACTIVE tDCS															
Amplitude (μV)	-3.92	1.97	-4.39	2.26		-5.02	2.05	-4.32	1.89		-4.70	2.15	-3.60	1.59	
Latency (ms)	98.4	14.5	97.5	15.5		96.4	11.0	95.8	10.8		92.2	14	91.6	14.1	
SHAM tDCS															
Amplitude	-4.38	2.01	-4.21	2.59		-4.73	2.10	-4.27	2.35		-4.71	2.08	-3.85	2.09	
Latency	91.6	13.4	93.3	15.3		91.1	12.1	91.2	14.5		89.8	11.1	89.6	13	

Table 2.1: N100 Amplitude and Latency: Mean and standard deviation for the Amplitude (microvolts) and latency (milliseconds) values of the N100 component at each timepoint. Amplitude and latency from individual subject grand average waveforms are averaged together to produce these values.

	Δ Timepoint 1					Δ Timepoint 2				
	Target Tone		**Control Tone**			**Target Tone**		**Control Tone**		
	M	SD	M	SD		M	SD	M	SD	
ACTIVE tDCS										
Amplitude (μV)	-1.10	1.18	0.073	1.07		-0.778	1.22	0.797	1.31	
SHAM tDCS										
Amplitude	-0.354	0.534	-0.060	0.814		-0.333	0.661	0.361	1.32	

Table 2.3: N100 Amplitude Δ-values: Difference values for the N100 component across the two timepoints. Difference values for each subject were computed by subtracting baseline amplitude from amplitude at T1 and T2. The mean and standard deviation were calculated at each timepoint across conditions using individual subject difference values


A)
Target Tone (TT)
Control Tone (CT)
tDCS or Sham
Baseline
Sensory Tetanus
Timepoint 1
Timepoint 2
Approx. Time
(Min)
0
13
18
50
56


B)
normE
0
0.3
0.6

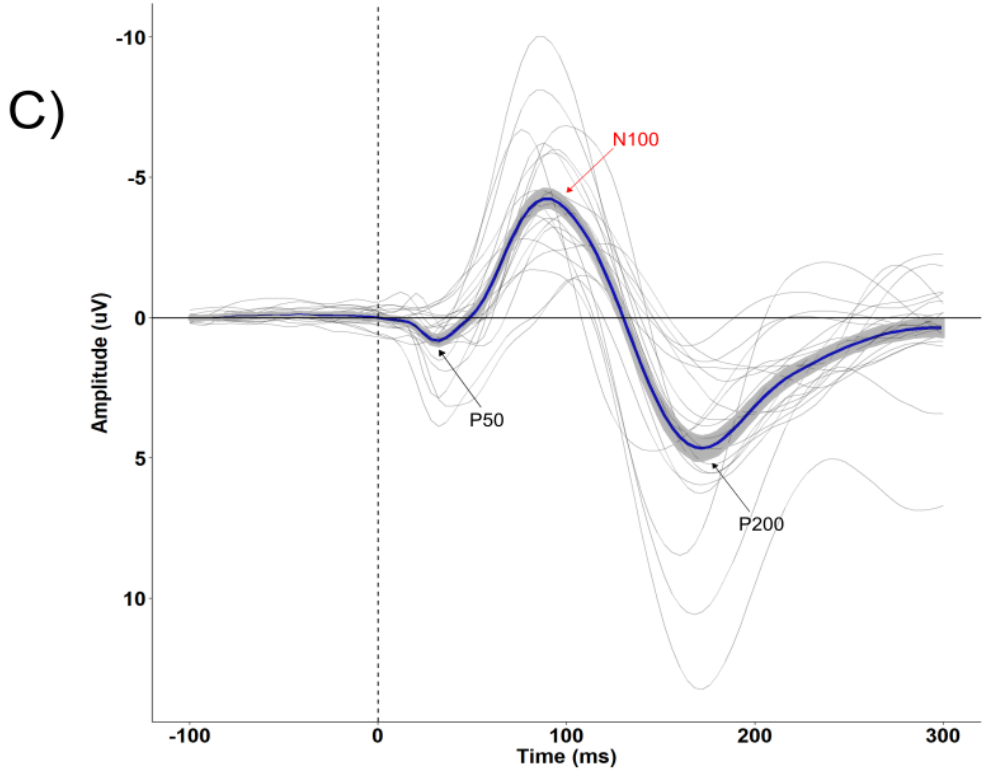

C)
N100
P50
P200
Amplitude (uV)
-10
-5
0
5
10
-100
0
100
200
300
Time (ms)

Figure 2.1A) Schematic depicting the timeline of events in a single study session involving the auditory ST paradigm and tDCS. **B)** Finite-element model of the normalized electric field produced in the brain by my tDCS montage. **C)** Grand average ERP and topographical map showing voltage distribution across the scalp. The grand average ERP shown in blue is collapsed across all subjects and conditions, prominent ERP components are labeled. Thin grey traces are derived from single subject grand average ERPs.

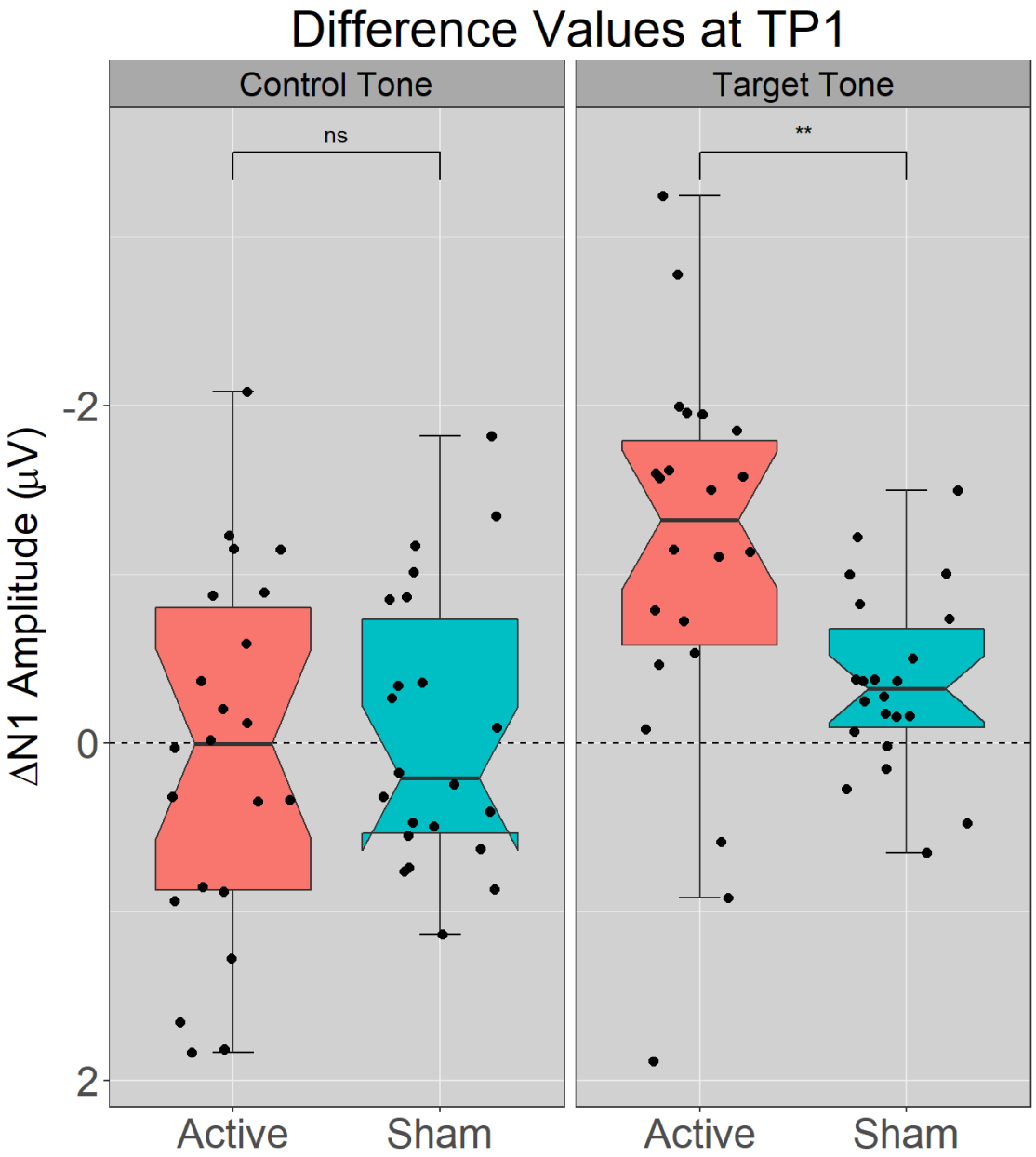
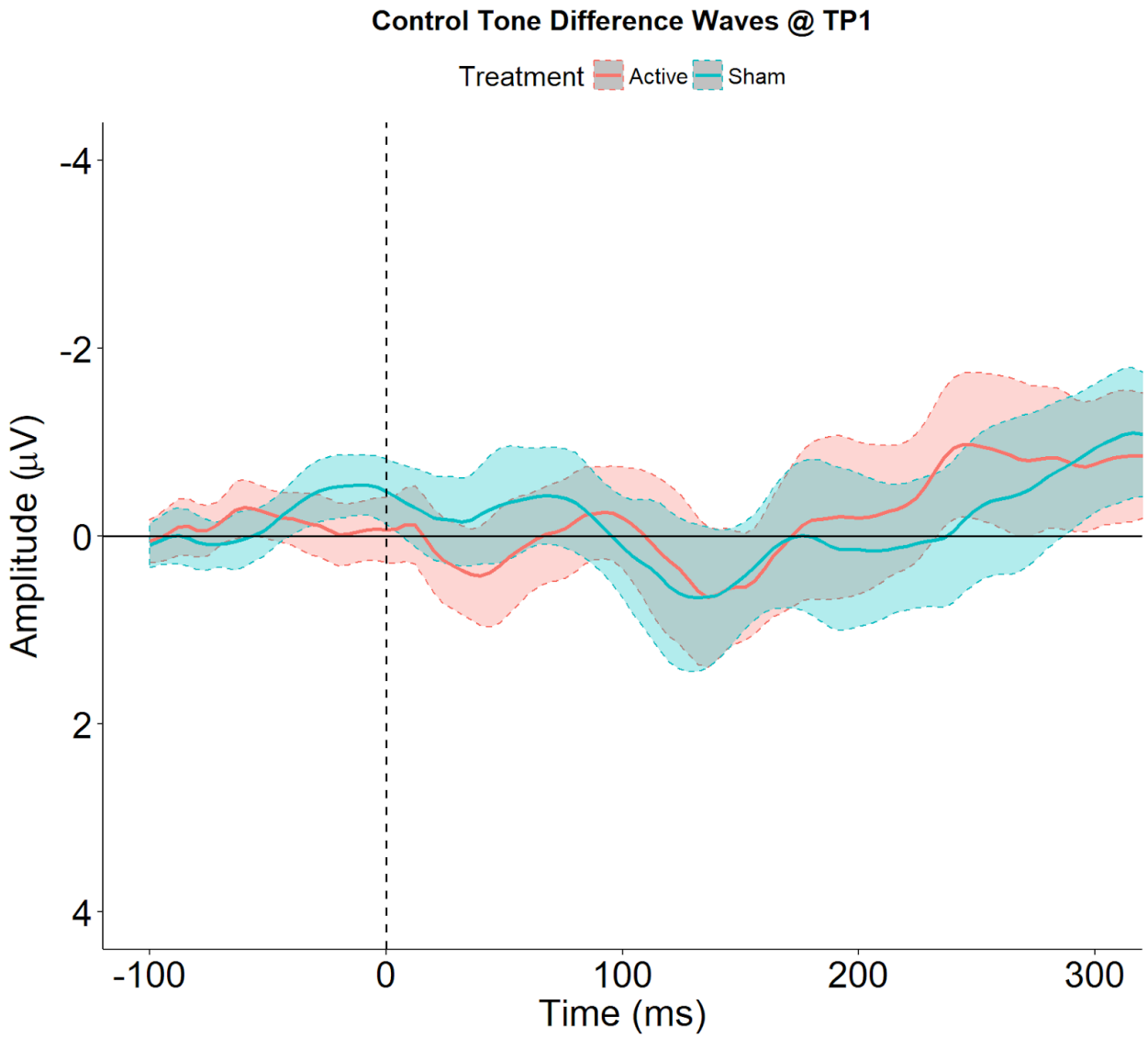

Difference Values at TP1
Control Tone
Target Tone
ns
**
ΔN1 Amplitude (μV)
-2
0
2
Active
Sham
Active
Sham

Figure 2.2: Box plots displaying N100 amplitude difference values at timepoint 1 (T1). Difference values were computed by subtracting N100 amplitude at baseline from the amplitude at T1. Control Tone difference values are shown in the left-hand panel, right hand panel displayed Target Tone (TT) difference values. Post-hoc contrasts revealed significant differences between treatment groups only in the TT.

A)


Control Tone Difference Waves @ TP1
Treatment
Active
Sham
Amplitude (μV)
-4
-2
0
2
4
-100
0
100
200
300
Time (ms)

B)

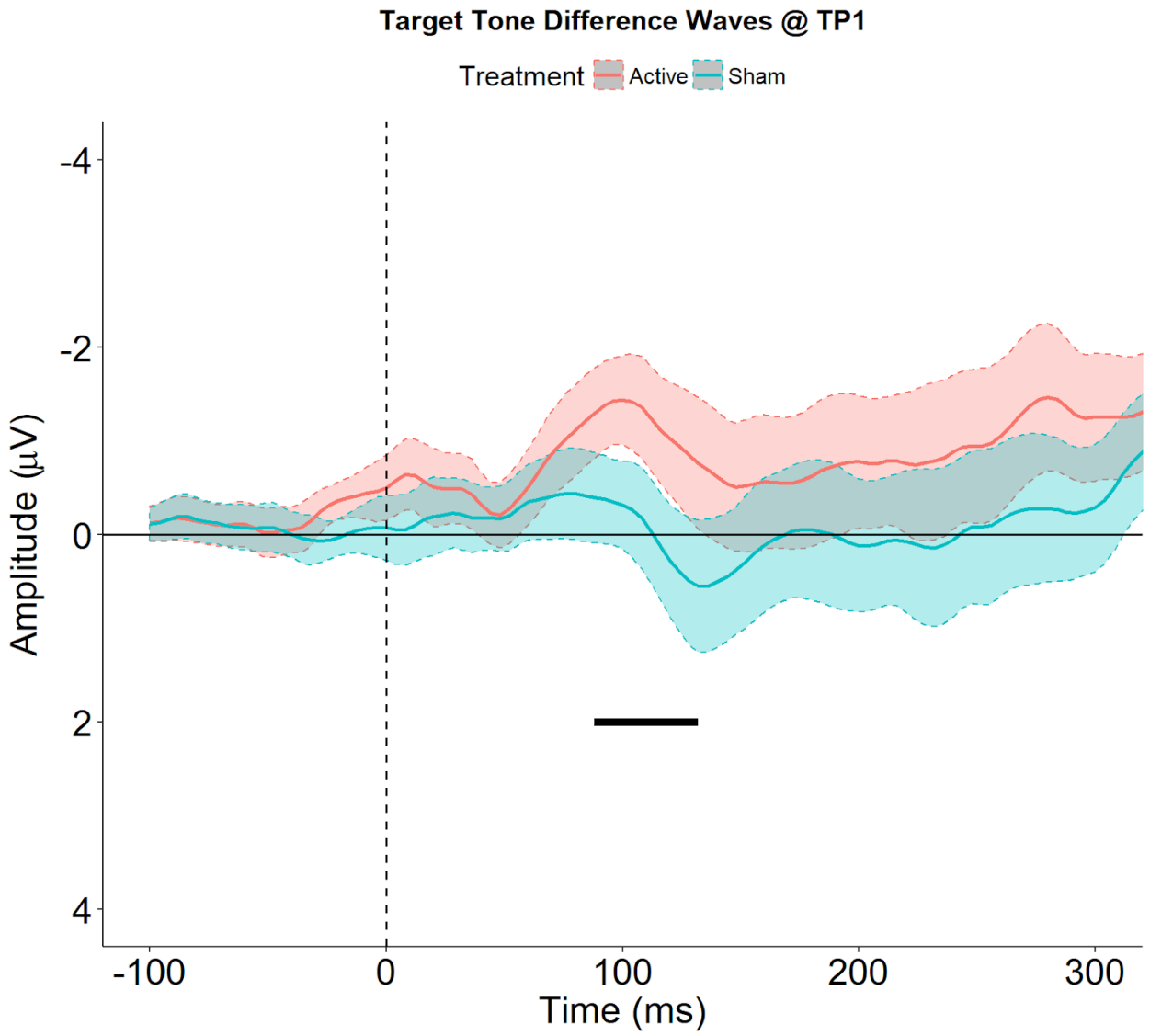

Target Tone Difference Waves @ TP1
Treatment
Active
Sham
Amplitude (μV)
-4
-2
0
2
4
-100
0
100
200
300
Time (ms)

Figure 2.3: Grand average ERP plots showing difference waveforms at timepoint 1 (T1) for the Control **(A)** and Target Tones (TT) **(B)**. Difference waves were constructed by subtracting baseline waveform from the waveform at T1. Transparent ribbon around the ERPs represents within-subject 95% confidence interval. Solid black bar underneath the waveforms (seen in panel B) indicates timepoints at which the waveforms differed significantly from each other on a paired t-test (FDR corrected for multiple comparisons). Significant differences across treatment conditions were observed only between TT ERPs, specifically in the time window where the N100 is most prominent.

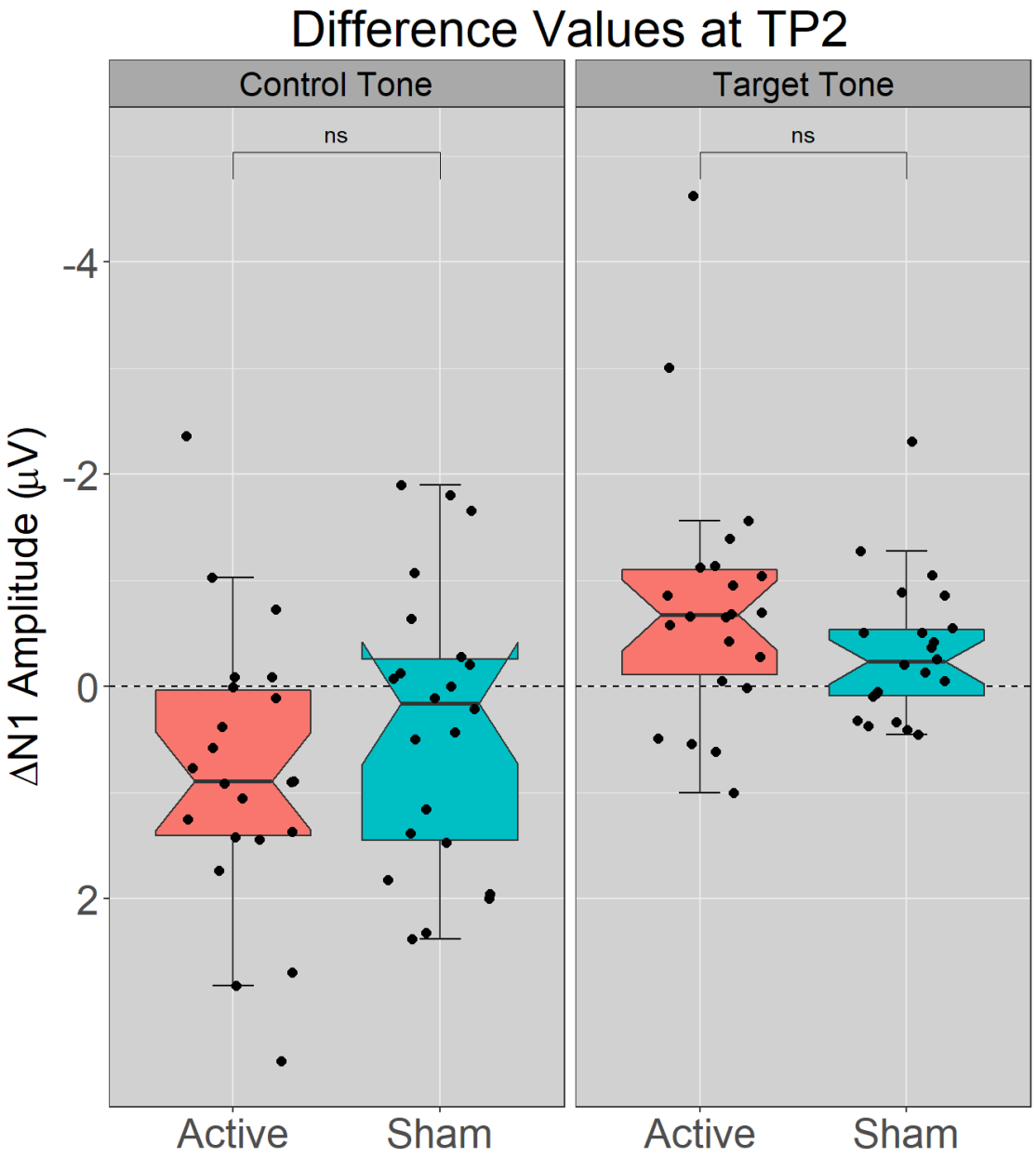
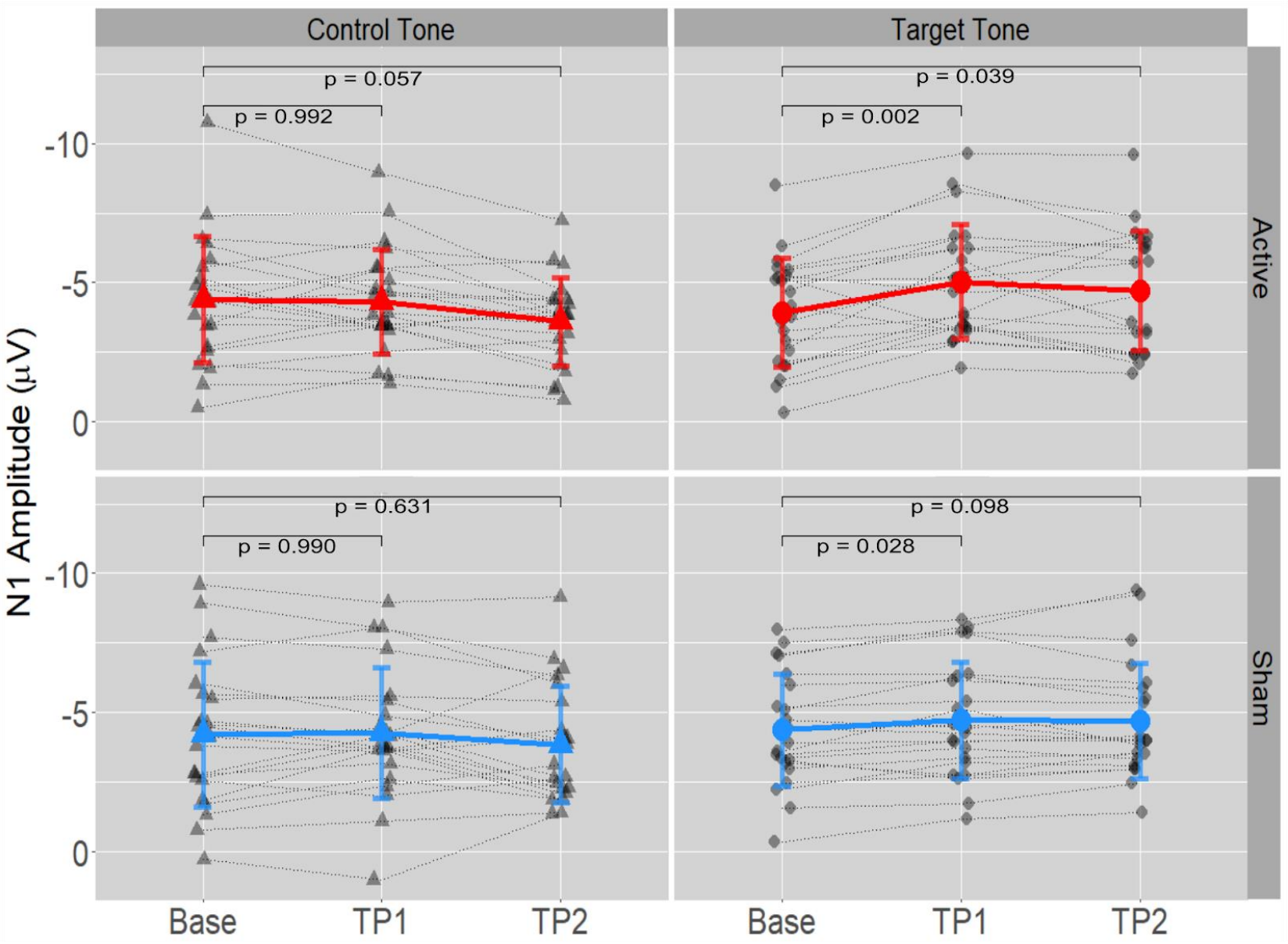

Difference Values at TP2
Control Tone
Target Tone
ns
ns
ΔN1 Amplitude (μV)
-4
-2
0
2
Active
Sham
Active
Sham

Figure 2.4: Box plots displaying N100 amplitude difference values at timepoint 2 (T2). Difference values were computed by subtracting N100 amplitude at baseline from the amplitude at T2. Control Tone difference values are shown in the left-hand panel, right hand panel displayed Target Tone difference values. Post-hoc contrasts revealed no significant differences across treatment groups in either tone.


Control Tone
Target Tone
p = 0.057
p = 0.992
p = 0.039
p = 0.002
p = 0.631
p = 0.990
p = 0.098
p = 0.028
Active
Sham
N1 Amplitude (μV)
-10
-5
0
Base
TP1
TP2

Supplementary Figure 2.1: N100 amplitude for each subject is plotted in transparent grey datapoints. Larger colored datapoints plot group means with standard error of the mean represented by the error bars. Top panels show data from active tDCS sessions, while bottom panels display data from sham tDCS sessions. Right hand panel corresponds to CT amplitudes whereas the left-hand panel shows TT amplitudes. Paired t-tests were used to compare amplitude at each timepoint to baseline amplitude. P-values reflect a Bonferroni-Holm correction for multiple comparisons.

P200 Amplitude	D_f	t-value	p-value
Treatment	42	1.25	0.218
Tone	21	1.84	0.081
Timepoint	86	2.75	**<0.001**
Treatment x Tone	42	-1.32	0.194
Treatment x Timepoint	86	0.810	0.420

Supplementary Table 2.1: Hierarchical linear model results for P200 amplitude change.

P200 Latency	D_f	t-value	p-value
Treatment	42	0.510	0.612
Tone	21	-1.79	0.089
Timepoint	86	0.576	0.566
Treatment x Tone	42	0.738	0.464
Treatment x Timepoint	86	0.407	0.684

Supplementary Table 2.2: Hierarchical linear model results for P200 latency change.

P50 Amplitude	D_f	t-value	p-value
Treatment	42	0.373	0.711
Tone	21	1.73	0.100
Timepoint	86	-0.113	0.910
Treatment x Tone	42	-1.09	0.281
Treatment x Timepoint	86	0.060	0.952

Supplementary Table 2.3: Hierarchical linear model results for P50 Amplitude

P50 Latency	D_f	t-value	p-value
Treatment	42	0.768	0.446
Tone	21	0.753	0.459
Timepoint	86	-0.044	0.965
Treatment x Tone	42	-1.32	0.194
Treatment x Timepoint	86	-0.031	0.975

Supplementary Table 4: Hierarchical linear model results for P50 Latency

CHAPTER 3: TDCS Augmented Cognitive Training for Executive Disfunction in Fetal Alcohol Spectrum Disorders

3.1 ABSTRACT

We conducted a first-of-its-kind pilot study examining the effects of a cognitive remediation training augmented with tDCS in children and adolescents with FASD. Prenatal alcohol exposure (PAE) has profound detrimental effects on brain development and, as a result, has permanent consequences for cognition, learning, and behavior. Individuals with Fetal Alcohol Spectrum Disorders (FASD) commonly have a range of neurocognitive impairments that directly lead to practical problems with learning, attention, working memory, task planning and execution, and decision making, among other areas of functioning. Despite the profound public health burden posed by FASD, there have been very few treatment studies of any sort in this population. Currently, the most commonly studied treatment for FASD related executive deficits is cognitive training, which involves repeated drilling of exercises targeting the impaired function (i.e. working memory, attention). However, as currently implemented, cognitive training requires many hours and effect sizes from these studies have not been convincing. Therefore, there remains a need for innovative advancements which can enhance the efficacy of this intervention. Targeting the neuronal machinery which is compromised by PAE may be informative for the rational advancement of such therapies. Though the exact mechanism of PAE mediated deficits has not been elucidated, several preclinical studies have demonstrated that neuronal plasticity throughout the brain is negatively affected in FASD. Neuromodulation via transcranial direct current stimulation (tDCS) has been shown to be an effective means of modulating human

brain plasticity and studies have shown that combining tDCS with cognitive training can enhance efficacy and generalization of this intervention. This approach to intervention has not yet been tested in FASD, a condition in which we know there are brain plasticity abnormalities. The results demonstrate that tDCS augment cognitive training, at levels of intensity delivered with adults, is well tolerated and feasible in children with FASD. Further, tDCS led to an improvement on sustained attention compared to sham. No tDCS dependent improvement on working memory was found.

3.2 INTRODUCTION

Fetal alcohol spectrum disorders (FASD) is an umbrella term that encompasses a wide spectrum of effects which can occur in an individual who was exposed to alcohol in the womb. It is the leading cause of mental retardation in the western world, with approximately 40,000 cases reported annually in the United States alone (Abel, 1995). On a worldwide scale, it is estimated that 0.97 births out of a 1000 will be on the FASD spectrum, resulting in numbers that are far higher than that of Down Syndrome, spina bifida and muscular dystrophy combined (Abel, 1995; O'Connor and Paley, 2009).

Persons with FASD can manifest a wide variety of behavioral and neuropsychological problems depending on the timing and extent of prenatal alcohol exposure (PAE). In general, the amount of alcohol consumed is correlated with the severity of outcome (Streissguth et al., 1989). However, pattern of alcohol exposure can often moderate these effects, with binge-like episodes resulting in more severe deficits than chronic exposure (Bailey et al., 2004). Timing of exposure is also important. Alcohol exposure during different periods of fetal development can greatly influence the pattern and severity of structural and functional abnormalities (Guerri et al., 2009). During the first-trimester, alcohol alters the growth of the neural tube and crest, leading to various developmental disorders such as microcephaly, hydrocephaly, and the facial dysmorphology which often characterizes FASD (Miller, 1996; Sulik and Johnston, 1983). During the second trimester, PAE strongly affects the proliferation of glia and neuronal precursors (Luo and Miller, 1998), leading to disordered migration of cortical

neurons (Siegenthaler and Miller, 2004). During the final trimester of human gestation, the brain goes through a period of accelerated growth where neurons are highly susceptible to the apoptotic effects of alcohol exposure (Ikonomidou et al., 2000). Alcohol during this critical period impairs synaptogenesis and may lead to persistent deficits in neuronal plasticity (Ikonomidou et al., 2000).

Given the fundamental role of plasticity in healthy brain function, it has been postulated that disrupted plasticity brought about by PAE may be the primary contributing factor in the cognitive deficits often associated with FASD (Medina, 2011). A growing body of literature supports this claim. In animal models, it has been demonstrated that PAE for as little as one day can persistently disrupt NMDAR function, leading to impaired expression of LTP and LTD (Izumi et al., 2005; Sutherland et al., 1997). It is thought that the behavioral deficits commonly observed in these models are a direct result of disrupted plasticity (Clements et al., 2005; Girard et al., 2000; Marino et al., 2004). For example it has been shown, in several different studies, that FASD leads to impaired performance on the Morris water maze, a hippocampal dependent learning task (Girard et al., 2000; Wozniak et al., 2004). Interestingly, this finding has been directly replicated in human subjects. Hamilton and colleagues (2004) employed a virtual Morris water maze task and were able to demonstrate that children with FASD showed similar impairments in place learning as seen in animal models of the disease (Hamilton et al., 2003). These results indicate that changes in brain plasticity are likely a crucial neural substrate for the cognitive and behavioral deficits that follow prenatal alcohol exposure.

Cognitive deficits make up one of the most common and debilitating symptoms of FASD. PAE is associated with cognitive impairments in a wide range of neurocognitive domains (Mattson et al., 2011), but perhaps most notably with deficits in executive functioning (Kodituwakku, 2009). Executive functions are loosely defined as the "the ability to maintain an appropriate problem-solving set for attainment of a future goal" (Welsh and Pennington, 1988). This complex construct encompasses a variety of higher order cognitive skills, including working memory, response inhibition, and decision making, and necessitates the integration of more basic processes such as perception, attention and motor activity (Pennington and Ozonoff, 1996). The brain circuits that subserve executive functioning are formed by cortico-striatal loops involving projections from the prefrontal cortex to the basal ganglia and thalamic nuclei (Cummings, 1993). These networks have been found to be especially vulnerable to prenatal alcohol exposure (Fryer et al., 2007; Mattson et al., 1996). Children and adults with FASD perform poorly on cognitive tasks which assess executive abilities such as set-shifting, attention and working memory (Olson et al., 1998; Vaurio et al., 2008). In fact, findings from neuroimaging studies have implicated impaired prefrontal connectivity, specifically within the dorsolateral prefrontal cortex (DLPFC), as being highly correlated with decreased performance on tasks which rely on these functions (Infante et al., 2017). Compared to age-matched controls, FASD individuals score >1.5 standard deviations below average on parent questionnaires such as the Behavior Rating Inventory of Executive Functioning (BRIEF) (Rasmussen et al., 2007), performing at levels which are predictive of

poorer social skills and correlated with greater problem behaviors (Schonfeld et al., 2006).

Despite the prevalence and severity of executive functioning impairments in FASD, there have been few treatment studies of any sort in this population. Early stage pharmacological interventions aimed at improving neuronal plasticity have been carried out (Wozniak et al., 2015), but there was no improvement in primary outcome measures, and effect sizes were small. Cognitive training (CT) is another method of improving cognitive functioning and involves repeated drilling of exercises of the impaired function. This method of cognitive remediation has been shown to improve cognitive skills (such as working memory), but with only modest effect sizes and considerable subject effort (Kerns et al., 2010, 2017). An additional limitation of traditional CT is that improvement beyond the trained task(s) is limited and does not transfer well to untrained cognitive domains or measures of daily functioning.

Cognitive training has been tried in FASD. (Kerns et al., 2010) used computer-based attention exercises administered by educational assistants in ten children ages 6-15 years. An average of 16 hours of training was provided over 9 weeks. Significant improvement was noted in several attention measures and there were trend improvements in working memory. In a study that combined subjects with autism spectrum disorder and FASD (Kerns et al., 2017), a game designed to target attention and working memory was used in subjects 6-11 years old. Approximately 12 hours of training was provided over a 12-week period. Significant improvements were noted in attention and working memory. However,

since the two groups were merged, the specific effect for those with FASD could not be determined. In addition, because neither of the two aforementioned studies utilized control groups, it is difficult to assess the true effectiveness of the cognitive training intervention. Novel approaches which can serve to augment the efficacy of currently available treatments are needed.

Neuromodulation, via transcranial direct current stimulation (tDCS), is an effective means of modulating brain plasticity. As has been outlined in previous chapters (see chapter 1), tDCS is a non-invasive method of delivering low intensity electrical current to the brain in a safe and tolerable manner. Studies in animal models show that tDCS can serve to boost synaptic plasticity, leading to improvements in learning and memory (Fritsch et al., 2010). In humans as well, it has been demonstrated that tDCS can boost plasticity in an activity-dependent manner (Monte-Silva et al., 2013; Pisoni et al., 2017) and can enhance a wide range of cognitive and motor functions (Jacobson et al., 2012; Jones et al., 2017; Katz et al., 2017; Saucedo Marquez et al., 2013).

The activity-selective nature of tDCS makes it an especially attractive candidate to pair with CT (Gill et al., 2015; Hill et al., 2018; Pisoni et al., 2017). It has been demonstrated that augmenting CT with concurrent tDCS boosts outcomes and improves transfer to non-trained tasks (Au et al., 2016; Brunoni et al., 2014; Nienow et al., 2016). Targeting the DLPFC with tDCS in conjunction with cognitive training has been tried in various psychiatric disorders, including schizophrenia (Nienow et al., 2016) and depression (Brunoni et al., 2014), resulting in improved effect sizes and enhanced transfer to non-trained tasks. Despite the

relative success of tDCS-paired CT, studies using tDCS in children and adolescents are few, and none have been carried out in an FASD population.

This study is the first to use a randomized controlled design to examine the immediate and long-term effects of a cognitive remediation training program augmented with tDCS targeting the DLPFC in children and adolescents with FASD. Our aims for this study were to: 1) Characterize the feasibility and tolerability of combining CT and tDCS in children ages 10-16 with FASD, and 2) evaluate the potential additive benefits of tDCS to the base CT program.

3.3 METHODS

Participants

Subjects with FASD were recruited from a list provided by the Minnesota Organization on Fetal Alcohol Syndrome. In addition, participants were recruited from two University of Minnesota Medical Center Clinics, the Fetal Alcohol Spectrum Disorders clinic and the International Adoption Clinic. Inclusion criteria for the study necessitated a documented history of heavy prenatal alcohol exposure (self-report, social service record, or adoption records), as well as meeting criteria for an associated FASD diagnosis (FAS or partial FAS). Potential participants with comorbid psychiatric or neurological disorders were excluded from the study. All participants signed informed consent and all study procedures were approved by the internal review board at the University of Minnesota.

44 participants with FASD were enrolled in the study and randomized to receive either active or sham tDCS. The mean age of the sample was 12.7 years old (s.d = 2.05 years). 27 of the subjects were male, 17 were female. There were no significant differences across groups on any demographic or clinical characteristic (Table 3.1).

Study Design

We conducted a randomized, double blind, placebo (sham) controlled clinical trial. Participants were randomly assigned to receive either active (n = 20) or sham tDCS (n = 24). All members of the research team as well as the subjects were blind to treatment assignment.

At the initial study visit, participants underwent a neuroimaging session involving functional and resting state magnetic resonance imaging (MRI). Following the imaging session, the participants completed a baseline cognitive and behavioral assessment featuring several standardized cognitive batteries and parent/guardian self-reports. These are described in detail in the section below. After completing the baseline assessments, the subjects completed the first treatment visit involving cognitive training and tDCS. Each treatment visit was approximately 46 minutes of cognitive training interleaved with two 13-minute blocks of tDCS. The study was made up of 5 such treatment visits, each session being separated by 1 week. After the last treatment visit, a two-hour follow-up visit was completed. The follow-up visit was scheduled within one week of the last

treatment session and involved a final cognitive assessment as well as a neuroimaging session.

Cognitive Training

Cognitive training was delivered using the BrainHQ™ system from Posit Science™. An array of 5 different training tasks were selected. Two of the tasks targeted working memory, two targeted attention and cognitive control and the remaining task was aimed at training processing speed. Each of the 5 training tasks were completed 4 times over the course of a single treatment session, with each treatment session lasting approximately 46 min. The training tasks employed an adaptive difficulty algorithm which adjusted difficulty level depending on how well the participant performed on the task during the previous session.

At the start of each treatment visit, the participants were seated in front of a computer monitor and were asked a series of questions regarding potential tDCS side-effects. Following the tDCS questionnaire, the participants were outfitted with the tDCS cap. At the first treatment session, the participants completed two near-transfer assessment tasks prior to tDCS and cognitive training. In sessions 2-5 the tDCS device was turned on, and after a 30sec pause the participants were instructed to begin cognitive training. The near-transfer tasks were completed at the end of the session at visits 2-5. A research team member was on hand to observe and assist at all times during each treatment session.

Transcranial Direct Current Stimulation

TDCS was targeted at the left DLPFC using a bipolar montage with the anode placed at F3 and the cathode placed over the supraorbital bone at Fp2 (according to the 10-20 electrode placement system) (Fig. 1). This montage has been used in previous studies seeking to target the left DLPFC for enhancing cognitive function (Nienow et al., 2016). TDCS leads were connected to 25cm2 saline soaked sponges which were then placed over the scalp. For each treatment session, the stimulation was initiated 30sec prior to the start of cognitive training and lasted for a period of 13min. At the end of the first 13min period, the tDCS device turned off and remained off for a period of 20min. After the 20min break, the device started up again and completed another 13min period of stimulation. This 13-20-13 tDCS procedure accounts for the effects of tDCS on “metaplastic effects” and has been shown to facilitate tDCS-based interventions (Carvalho et al., 2015; Monte-Silva et al., 2013).

In the active condition, stimulation was delivered at an intensity of 2mA for the duration of the two 13min stimulation periods. In the sham condition, the device ramped up to 2mA over the course of 30sec but then proceeded to ramp back down to 0mA over the next 30sec. This was done in order to facilitate blinding by mimicking the tingling sensations which are often associated with active stimulation.

Prior to the start, and at the end of each treatment session, a questionnaire was completed which assessed the presence and level of any side-effects from tDCS stimulation.

Cognitive & Behavioral Assessments

Two standard cognitive tasks were administered to assess learning and near transfer of any cognitive gains from our training regimen. These tasks examined the cognitive domains which were drilled during training but were selected to test these functions in a different context. The first assessment task was a visuospatial working memory task (WM) adopted from Stromer and colleagues (Störmer et al., 2013). The task asked participants to track and recall the location of an increasing number of objects amidst distractors. The average span of the number of objects recalled over the course of the task was used as the outcome measure. The second assessment task was a continuous performance task (CPT) designed to assess sustained attention. Participants had to press a button in response to a frequent visual stimulus and abstain from doing so when a rare target image was presented. These tasks were first administered at baseline, prior to any cognitive training or tDCS. Following the baseline visit, these tasks were administered at the end of each treatment session, after the training tasks and tDCS had been completed.

Far-transfer of training gains to cognitive domains not directly trained were assessed using subtests from the Delis-Kaplan Executive Function System (DKEFS). We focused on two specific tasks, the trail making task and verbal fluency task. The trail-making task (TMT) consists of three conditions, condition A requires the participant to draw lines to connect circled numbers in a numerical sequence as quickly as possible. In condition B the participant draws lines to connect circled letters, whereas in condition C, the line has to be drawn to connect

numbers and letters in an alternating sequence. The time it takes to complete the sequence across conditions was used as the primary metric of performance. Though this task is very simple, it is thought to reflect a wide range of cognitive processes including attention, set-shifting, abstraction and fluid intelligence (Salthouse, 2011). For the verbal fluency task (VFT), participants were asked to produce as many words as possible within a specific semantic category, or words starting with a specific letter. VFTs are often included in neuropsychological assessments, in clinical practice, and in research. For example, they have been used to support diagnosis of attention-deficit/hyperactivity disorder, and cognitive impairment in a variety of neurodegenerative diseases (Andreou and Trott, 2013; Zhao et al., 2013). Much like the TMT, research has shown that VFT performance involves the recruitment of many executive functions (working memory, inhibition, cognitive flexibility) and is a good index of fluid intelligence (Shao et al., 2014).

In addition to these assessment tests, a standard behavioral questionnaire was given to the parents/guardians to complete. The Behavior Assessment System for Children – 3nd Edition (BASC-3) (Reynolds et al., 2015) is a standardized parent-report questionnaire of typical and atypical child behavior which is commonly utilized in clinical trials.

Data Analysis

BrainHQ Training Data

The 5 training tasks were each analyzed to identify any group differences in performance and learning across the 5 training sessions. The primary measure

of performance analyzed was the “stars awarded” metric. This gives a normalized measure of performance at each level compared to the database of all BrainHQ users. Between 0-5 stars can be awarded each time the task is played depending on performance. The user is awarded 0 stars if their score is <-1.5 standard deviations (SD) from the mean performance of all users on that level, 1 stars if their score is ≥-1.5 SD, 2 stars if ≥-0.5 SD, 3 stars if ≥0.5, 4 stars if ≥1.0 SD, and a maximum of 5 stars if their performance is ≥1.5 SD from the population mean.

In order to gauge performance across sessions for each individual training task, an average number of stars awarded per treatment session was calculated for each participant on each training task (each task was played 4 times per treatment session). To derive an overall measure of performance across all training tasks, we collapsed stars awarded for all tasks, producing a mean number of stars awarded per session across the 5 tasks. A hierarchical linear modeling (HLM) approach was used to assess the main effects of treatment (tDCS) and time across treatment sessions. The interaction between treatment and time was also included in the model.

Assessment Tasks & BASC-3 Analysis

The 2 near-transfer assessment tasks were deployed a total of 5 times, once at baseline, and once at the end of each of the 4 subsequent treatment sessions. For WM, the average span of the number of objects recalled over the course of the task was used as the outcome measure. For the CPT, the metric used was the SD of the average response time (in ms). This metric has been

shown to be indicative of sustained attention, as performers with worse attention tend to have larger SD compared to those who are consistently attentive to the task (Levy et al., 2018). In order to better assess change in performance on these tasks, we derived a baseline adjusted score by subtracting each participant's baseline performance score from the score at each subsequent treatment session. These delta scores (**Δ**-score) were used in our final analysis. An HLM was used to assess the effects of treatment and time, as well as their interaction on task performance.

The TMT and the VFT from the DKEFS were deployed at baseline and again at the follow-up visit. Raw scores on these tests is converted to an age specific standardized score with a mean of 10 and a standard deviation of 3. For each task, a baseline adjusted **Δ**-score was derived for each participant. A general linear model (GLM) was used to analyze these **Δ**-scores to examine differences in change across treatment groups.

We focused our analysis on four specific measures from the BASC-3, internalizing problems, externalizing problems, attention problems and hyperactivity. Internalizing problems is a composite metric which can be characterized as a broad index of inwardly directed distress that reflects internalized problems a child may experience. Externalizing problems is also a composite metric, focusing on external problems which a child may express, such as aggression, isolation or other conduct problems. Attention problems refers to a tendency to be easily distracted and unable to concentrate more than momentarily.

Relatedly, hyperactivity is a metric which reflects a tendency to be overly active, rush through work or activities, and to act without thinking

BASC-3 provides normalized t-scores to assess how a participant's survey results fit in a distribution of impairment across a broad population of all survey takers in the BASC database. On our measures of interest, higher t-scores indicate more impairment. For each measure, a baseline adjusted **Δ**-score was derived for each participant. A general linear model (GLM) was used to analyze these **Δ**-scores to assess change on the BASC-3 measures across treatment groups.

TDCS Related Symptoms

At the start and end of each treatment session, participants were asked to report the presence and severity of 17 different tDCS related symptoms. We utilized two tailed chi-square tests to assess differences in the number of times each symptom was reported across the treatment groups.

3.4 RESULTS

Feasibility and Tolerability

A total of 44 participants were recruited for the study, 20 of these individuals were randomly assigned to the active treatment group whereas 24 were assigned to sham treatment (Fig. 3.1). In the active group, all participants completed the study except for one individual who discontinued due to inability to tolerate tDCS related sensations. In the sham group, a total of 5 individuals did not complete the study. Of the 5, one was unable to tolerate stimulation, two were unable to

complete due to the time commitment, and 2 individuals dropped out and did not provide a reason for discontinuing. In total, 19 individuals in each group completed all 6 study visits.

At the start and end of each treatment session, we conducted a questionnaire assessing the presence of 17 potentially stimulation related side-effects. No significant differences between the treatment groups were found on any of these side-effects ($p>0.10$ for all comparisons; Table 3.2).

BrainHQ Training Tasks

The BrainHQ training battery consisted of 5 separate tasks. We first analyzed performance on each individual task separately in order to identify any differences across groups. There was no significant effect of tDCS on task performance on any of the individual tasks ($p>0.05$ for all comparisons). Next we collapsed training data across all 5 tasks to see if there was an overall effect of tDCS (Fig. 3.2). Again, we did not find an effect of tDCS ($F_{1/33}=.10$, $p=0.75$), or an effect of time ($F_{1/138}=2.77$, $p=0.10$) on overall training task performance. There was also no interaction effect between these two factors on training performance ($F_{1/138}=0.15$, $p=0.70$).

Near-Transfer: Visuospatial Working Memory & CPT

We first compared performance at baseline on the CPT and the WM task (Table 3.3) to examine whether we had a significant pre-study difference on these measures. No significant differences were found at baseline ($p>0.05$).

We analyzed these tasks using baseline adjusted **Δ**-scores. With the WM task we found a significant effect of time ($F_{1/144}$=2.46, p=0.04), with both groups showing improvement, but no significant effect of tDCS ($F_{1/39}$=0.01, p=.91) or an interaction effect ($F_{1/144}$=4.41, p=0.61) on task performance (Fig. 3.3).

For performance on the CPT, we identified a significant effect of tDCS ($F_{1/39}$=4.41, p=0.03), with the active tDCS group performing better over time compared to the sham group (Fig. 3.4). We also observed a non-significant effect of time ($F_{1/144}$=1.36, p=0.24) and a non-significant interaction ($F_{1/144}$=1.46, p=0.22). Contrasts were applied to the model to determine at which visits the groups differed in performance. These contrasts revealed significant differences at visit 3 (p=0.03), visit 4 (p=0.04), and visit 5 (p=0.04). Bonferroni-Holm correction was applied to adjust for multiple comparisons.

To assess the effect size of our intervention on performance, we collapsed the **Δ**-scores across the 4 visits for each participant. We then calculated d' using the collapsed **Δ**-scores from each group. For the WM we found a small effect size (d'=0.05), for the CPT we found a moderate effect size (d'=0.64).

Far-Transfer: Trail Making and Verbal Fluency Tasks

No differences between groups were found when examining baseline performance on these tasks (p>0.05). Baseline adjusted **Δ**-scores were used to assess differences between treatment groups (Table 3.4). For performance on the VFT we found no significant effects of tDCS on either letter VF ($F_{1/36}$=0.07, p=0.80), or category VF ($F_{1/36}$=0.05, p=0.82).

Further, no treatment effect was revealed for TMT performance, whether analyzing number sequencing ($F_{1/36}$=0.06, p=0.80), letter sequencing ($F_{1/36}$=2.75, p=0.11), or combined letter and number sequencing ($F_{1/36}$=0.19, p=0.66).

BASC-3 Parent/Guardian Questionnaires

We focused our analysis on four specific measures from the BASC-3, internalizing problems, externalizing problems, attention problems and hyperactivity. No significant differences were identified between groups ($p<0.05$ for all comparisons) on any of these four measures (Table 3.5).

Correlating CPT Performance with Attention Problems & Hyperactivity

We correlated change on CPT performance with change on related measures from the BASC-3, namely attention problems and hyperactivity. In order to derive a single **Δ**-score for CPT performance, we averaged the **Δ**-scores at each of the 4 visits for each participant. We correlated this overall **Δ**-score from the CPT with the **Δ**-score from the BASC-3 using a linear regression model.

When correlating change in CPT performance with change in the AP metric collapsed across groups, we did not find a significant correlation (t=0.13, p=0.28; Fig. 3.5). Interestingly however, when analyzing the correlation separately for each group there were diverging results. We observed a trend-level significance between change in CPT performance and change in the attention problems metric when analyzing the active tDCS group (t=2.06, p=0.05; Fig. 3.6). Interestingly, we found a highly non-significant correlation when looking at sham tDCS group

(t=0.09, p=0.93; Fig. 3.7). There were no significant correlations between CPT and the hyperactivity metric from the BASC for either group.

3.5 DISCUSSION

We carried out a first of its kind clinical trial utilizing tDCS augmented cognitive training to improve executive functioning in children with FASD. The primary aim for this study was to characterize the feasibility and tolerability of combining non-invasive neuromodulation with cognitive training in a unique and vulnerable population. Secondarily, we sought to evaluate any potential additive benefits associated with combining tDCS with cognitive training compared to cognitive training alone.

Feasibility and Tolerability of TDCS Combined Cognitive Training in FASD

TDCS has been used extensively to treat a variety of psychiatric disorders in adults, such as major depression, stroke and chronic pain (Berlim et al., 2013; Schulz et al., 2013). Its track record of safety and efficacy, as well as its low cost and ease of use, make tDCS an appealing tool to use in children as well. There is however, very limited data regarding the use of tDCS in adolescents and children (Palm et al., 2016), raising concerns over the vulnerability of pediatric populations to a technique that has not been thoroughly tested on the developing brain. Specifically, research with tDCS in children with FASD is lacking, with no reported studies having been carried out to date. This type of research is particularly needed as effective treatment options for this vulnerable population are severely limited.

Results from this study demonstrate for the first time that tDCS treatment over the course of multiple sessions is well tolerated in children and adolescents with FASD. Of the 44 individuals who were enrolled into the study, only 2 discontinued due to inability to tolerate stimulation related side-effects (Fig. 3.1). One of these individuals was in the active treatment group while the other was in the sham group. Furthermore, we did not observe a significant difference in the total number of tDCS related symptoms reported between the two groups ($t=-0.81$, $p=0.43$). This was the case when looking at the symptoms on an individual basis as well, with no significant differences reported on any of the symptoms (Table 3.2).

When discussing tolerability, it is crucial to consider the stimulation parameters which were utilized. With this study we employed stimulation parameters which mimic those used in adult tDCS studies, demonstrating that sacrificing stimulation intensity and duration are not required in order for tDCS to be tolerated in children. This is an important finding due to the fact that some research has suggested the need to reduce stimulation strength in order to make tDCS more tolerable for young adults and children (Palm et al., 2016). However, it is still important to note that we are making statements in regard to tolerability only, and not in regard to how current flow might differ between the brains of adults and children. There is still a great need for modeling studies in children to determine how tDCS currents interact with a smaller head size and a thinner skull.

In terms of feasibility, we were able to demonstrate that 5 one-hour sessions of tDCS combined with cognitive training is possible to achieve in this population,

with only two of the 44 individuals dropping out due to concerns with time commitment. In future studies, it is likely that more sessions will be required to improve effect sizes from this intervention, these results are thus crucial in informing future investigations.

Efficacy of TDCS Combined Cognitive Training in FASD

Cognitive training is a method of improving cognitive ability by repeatedly drilling exercises of the target function. Cognitive training is one of the few interventions that have been studied to treat the executive functioning deficits which are often associated with FASD (Kerns et al., 2010, 2017). However, these studies suffer from small effect sizes and require considerable subject effort. Therefore, novel methods which can improve the efficacy of cognitive training are needed to enhance outcomes from this important intervention. Enhancing plasticity via noninvasive brain stimulation may be an effective means of achieving this goal. By its very nature, cognitive training is heavily reliant on the neuroplastic properties of the brain (Park and Bischof, 2013). Therefore, tools which can serve to promote brain plasticity may be effective as adjunct therapies to improve outcomes (Nienow et al., 2016). In this study, we employed cognitive training from the BrainHQ system, utilizing 5 tasks which targeted a range of executive domains. Two of the tasks targeted working memory, two targeted attention and cognitive control and the remaining task was aimed at training processing speed.

When analyzing the training task data, we did not find any significant differences across groups (Fig. 3.2). This was the case on a task to task basis as

well as on overall performance across all training tasks. A lack of a treatment effect on training tasks may have been due to the fact that the metric we used to analyze the data was not sensitive enough to pick up on small differences in performance. We used a standardized metric which compared the user's performance to the average performance of all users on the BrainHQ database who had completed that level. Depending on how many standard deviations away from that mean the performance was, the user was awarded a certain number of stars (see methods). This manner of assessing performance could have missed small changes that other metrics, such as response time, would have picked up. However, due to the fact that the difficulty of the training tasks changed from session to session, this made it problematic to analyze non-standardized metrics.

When assessing the effectiveness of cognitive training interventions, it is important that outcomes lead to meaningful functional improvements. In order for the trained skills to be deemed valuable, they need to be 1) applied outside of the training environment, and 2) used when solving and coping with real world problems and events. It has been proposed (Mayer, 1975) that the amount of transfer derived from an intervention can be thought of as a continuum, with "near-transfer" located at one end of the continuum (i.e., performance improvements on similar tasks and in the setting similar to training), and "far-transfer" situated at the other end (i.e., performance improvements on different tasks and in dissimilar contexts than those involved in the training). Hence, the extent of the transfer of acquired skills and the generalizability of those skills can serve as an evaluation

tool to assess the usefulness of a cognitive intervention, with the ultimate goal being for skills to generalize from trained to non-trained tasks.

We employed two tasks to examine near-transfer of cognitive improvement across sessions for two relevant cognitive domains, working memory and attention. We found a significant effect of tDCS on CPT performance, with the active treatment group showing improved performance across time compared to the sham group (Fig. 3.4). Interestingly, contrasts revealed that significant differences between the groups did not emerge until the third treatment visit, indicating that the effects of tDCS are potentially additive and require multiple sessions to become evident. Often, many sessions of training are required before improvements are found on near-transfer tasks (Kerns et al., 2010), so these findings are both informative and promising for future clinical studies which seek to utilize tDCS to boost gains from cognitive training.

In addition to finding a significant tDCS effect on CPT performance, we also identified a tDCS dependent correlation between improvement on the CPT and change in the “attention problems” (AP) measure from the BASC-3. This measure describes a person's tendency to be easily distracted and unable to concentrate more than momentarily. The BASC-3 is a standardized rating-scale which is often used in clinical studies to assess the impact of an intervention on improvements in everyday functioning. When correlating change in CPT performance with the AP measure, we found that the strength of the correlation was highly dependent on tDCS. In the active group, this correlation was stronger ($r=0.20$) and at a trend-level significance ($p=.05$; Fig. 3.6), whereas in the sham group the relationship

between these two measures was much weaker (r=0.0005) and non-significant (p=0.93; Fig. 3.7). Though we did not find a significant effect of tDCS on change in AP on a group level, when looking at the active treatment group there was a negative shift compared to baseline of 1.5 t-scores (Table 3.5), indicating a reduced impairment and a response to tDCS in a subset of participants. Given the correlation between improvement in CPT with improvement in AP, it is interesting to speculate whether changes in performance on such relatively simple cognitive tasks can be used as a predictor of treatment response. If this were the case, cognitive tasks such as the CPT could be used at an early stage in longer clinical trials to identify individuals more likely to respond to tDCS treatment. However, more investigation is necessary to elucidate the existence of such a relationship.

We did not find a significant effect of tDCS on performance when analyzing the WM task (Fig. 3.3). This task tested visuospatial working memory in a different context than seen in training. Despite a lack of a tDCS effect, we did find a significant effect of time, indicating that both groups improved on this task over the course of the study. A lack of a tDCS effect on working memory metrics may be due to the design of our cognitive training regimen. The training program consisted of tasks that targeted a wide range of cognitive abilities and was not focused on one particular function. Given the non-focal nature of our training it is perhaps unsurprising that we did not achieve tDCS effects in any one specific functional category. This interpretation is supported by what we know regarding the important contribution of brain state to tDCS specificity and outcomes. It is known that tDCS effects are dependent on both the functional and structural states of the brain, with

only task specific circuits and functions being facilitated (Bortoletto et al., 2015; Hill et al., 2018; Pisoni et al., 2017). With training dispersed across several cognitive domains, it is likely that a number of different brain circuits were activated to varying degrees concurrent with tDCS. It is perhaps the case then, that the effects of tDCS were shared across these circuits and no one circuit received the dose necessary to result in functional enhancement. The one exception to this would be attention (as indexed by the CPT). It could be argued that, unlike the other cognitive functions, sustained attention had to be maintained throughout the course of each session in order to maintain attention to the training at hand. Therefore, it is plausible that the brain circuits which subserve sustained attention were active concurrent with tDCS for a longer period of time and were therefore preferentially modulated.

In this pilot study, we also failed to demonstrate an effect of tDCS on improvements in far-transfer, finding no significant differences on either the VFT or the TMT. Demonstrating far-transfer to untrained tasks and cognitive domains is the gold standard of cognitive training interventions but studies have struggled to find such transfer despite many hours of training (Edwards et al., 2002; Melby-Lervåg et al., 2016). It is the hope that adjunct therapies such as tDCS can facilitate the transfer of training gains to contexts outside of the training environment, and there is some evidence that this can indeed be the case (Nienow et al., 2016; Trumbo et al., 2016). However, the comparatively small dose of stimulation and cognitive training applied in this study may not have been enough to produce detectable changes in this regard.

Conclusions & Limitations

The primary aim of this first of its kind study was to establish tolerability and feasibility of carrying out a tDCS augment cognitive training intervention for treating executive dysfunction in children with FASD. The findings here demonstrate that tDCS, at parameters delivered in adult studies, is well tolerated in children with FASD. We only had a single subject drop out due to inability to tolerate stimulation in the active group and further we do not report any differences on tDCS side-effects across the two groups. Importantly, this clinical trial also provides some promising findings regarding feasibility. We were able to maintain 86% retention across an intense 7-week study which featured long sessions, demanding both on participants and parents/guardians.

This study gives some credence to the idea that tDCS can be used in FASD to facilitate cognitive training. More importantly though, it is informative to future studies which are needed before we can decisively answer questions surrounding efficacy and functional outcomes. As noted, the current investigation was a clinical trial meant to demonstrate feasibility; as such, we did not design the study to deliver the full dose of training and stimulation as is seen commonly in other tDCS/cognitive training studies (Palm et al., 2016; Weickert et al., 2019). Such studies are generally designed to include daily training sessions with concurrent stimulation. Though the extent of the training with these tDCS augmented studies is short in comparison to cognitive training only studies, it is still far more than the once a week session delivered in our study. An additional improvement that future

studies can make would be to narrow the scope of the cognitive training. As was discussed earlier in the section, having multiple brain circuits active concurrent with tDCS may not be the best way to harness the plasticity enhancing effects of this tool. In fact, there is reason to believe (Bikson et al., 2013) that it would be advantageous to narrowly focus the activation of a single network, allowing this population to be maximally influenced by the applied current. Having neuroimaging data across the span of the study would be extremely informative as well in designing studies which seek to target a specific network. Though we collected MRI for this sample, we did not have time to analyze and report those findings here.

Finally, though we demonstrate that tDCS delivered over the DLPFC at a 2mA intensity was well tolerated, much still is unknown how tDCS currents interact with the specific characteristics of a child's head. Smaller head size and a thinner skull no doubt make the models which inform adult studies obsolete for a younger population. Studies which can model current flow and induced electrical fields in realistic children’s head-models would go a long way in improving the rational design of future studies.

CHAPTER III: TABLES & FIGURES

N(%) or mean (SD)	Sham (*n*=19)	Active (*n*=19)	Statistical Test
Age	12.79 (2.10)	12.05 (1.90)	$t(36)=1.134$, $p=.264$
Gender			
Male	10 (52.63%)	12 (63.16%)	$x^2(1)=.432$ $p=.511$
Female	9 (47.37%)	7 (36.84%)	
Racial Categories			
White	11 (57.89%)	9 (47.37%)	$x^2(5)=.3.95$ $p=.557$
Black or African American	2 (10.53%)	2 (10.53%)	
American Indian/Alaska Native	3 (15.79%)	1 (5.26%)	
Asian	1 (5.26%)	0 (0%)	
More than One Race	2 (10.53%)	6 (31.58%)	
Other	0 (0%)	1 (5.26%)	
Ethnicity			
Hispanic	0 (0%)	1 (5.26%)	$x^2(1)=.027$ $p=.311$
Not Hispanic or Latino	19 (100%)	18 (94.74%)	
Alcohol Exposure			
Alcohol Confirmed	19 (100%)	16 (84.21%)	$x^2(1)=3.257$ $p=.071$
Alcohol Suspected	0 (0%)	3 (15.79%)	
Other Drug Exposure			
None	3 (15.79%)	6 (31.58%)	$x^2(1)=1.311$ $p=.519$
Drug Exposure Suspected	5 (26.32%)	4 (21.05%)	
Drug Exposure Confirmed	11(57.89%)	9 (47.37%)	
Dysmorphic Facial Features			
Lip (score 4 or 5)	8 (42.11%)	6 (31.58%)	$x^2(1)=.452$ $p=.501$
Philtrum (score 4 or 5)	11 (57.89%)	10 (52.63%)	$x^2(1)=.106$ $p=.744$
Palpebral Fissure *(≤10th percentile)* [a]	7 (36.84%)	6 (31.58%)	$x^2(1)=.117$ $p=.732$
≥ 2 Facial Features Present	11 (57.89%)	7 (36.84%)	$x^2(1)=1.689$ $p=.194$
Growth Deficiency (≤10th percentile)			
Height	3 (15.79%)	3 (15.79%)	$x^2(1)=.000$ $p=1.000$
Weight	0 (0.00%)	1 (5.29%)	$x^2(1)=1.027$ $p=.311$
Deficient Brain Growth (≤10th percentile) [a]			
Occipital-Frontal Circumference (OFC)	3 (15.79%)	2 (10.53%)	$x^2(1)=.230$ $p=.631$
IOM Diagnostic Category			
FAS	1 (5.29%)	2 (10.53%)	$x^2(5)=2.800$ $p=.247$
Partial FAS	10 (52.63%)	5 (26.32%)	
ARND	8 (42.11%)	12 (63.16%)	

Table 3.1: Participant demographic and clinical characteristics. Mean and standard deviation (or % of sample) are shown for both demographic and clinical indications across our sample. For diagnostic categorization we use the Institute for Medicine (IOM) criteria. Our sample presented with Fetal Alcohol Syndrome (FAS), partial FAS or Alcohol-related neurodevelopmental disorder (ARND). We carried out statistical tests (either two tailed t-test or 2-sided Chi-Square tests) to assess differences across groups on demographic or clinical features.

Symptom	Sham (n=24)	Active (n=20)	χ2 Value (df=1)	Asymptotic Significance (2-sided)
Headache	4	5	.466	.495
Unusual feelings on the skin of your head	7	8	.570	.450
Neck Pain	0	2	2.514	.113
Tingling	6	6	.138	.711
Itchiness	10	8	.013	.911
Sleepiness	12	12	.440	.507
Difficulty paying attention	5	7	1.104	.293
Unusual feelings, attitudes, or emotions	2	2	.037	.848
Tooth pain	0	1	1.228	.268
Change in hearing	0	1	1.228	.268
Nausea/Sick to Stomach	0	2	2.514	.113
Unusual twitches or movements in muscles	1	0	.853	.356
Dizziness	0	1	1.228	.268
Anxious/Worried/Nervous	1	2	.584	.445
Forgetful	3	3	.058	.810
Difficulty with your balance	2	1	.191	.662
Change in movement in your stronger hand	0	1	1.228	.268

Table 3.2: TDCS related symptoms, number of participants reporting each symptom across the two groups is reported. We used a two-tailed Chi-Square test to assess any differences across.

VISOSPATIAL WORKING MEMORY	**Baseline** (mean ± sd)	**Visit 2**	**Visit 3**	**Visit 4**	**Visit 5**
ACTIVE TDCS	4.32 ± 1.08	4.20 ± 1.34	4.55 ± 1.07	4.21 ± 1.02	4.79 ± 0.851
SHAM TDCS	3.97 ± 1.31	3.89 ± 1.18	3.91 ± 1.31	3.96 ± 1.26	4.22 ± 1.94
CONTINUOUS PERFORMANCE TASK					
ACTIVE TDCS	85.8 ± 4.12	79.2 ± 18.1	77.2 ± 19.2	83.6 ± 8.85	81.0 ± 9.36
SHAM TDCS	77.6 ± 21.8	78.8 ± 18.9	80.2 ± 17.4	85.2 ± 4.87	82.1 ± 12.7

Table 3.3: Mean and standard deviation of performance on the two near-transfer assessment tasks at each visit. The visuospatial working memory task measures average span of the number of objects recalled. The continuous performance tasks metric is average standard deviation of response times (a lower score on this metric indicates better performance). No significant differences in baseline performance were found across groups.

VERBAL FLUENCY (LETTER)	**Baseline** (mean ± sd)	**Follow-Up**	**Δ-Score**	**$F_{1/36}$**	**p-value**	**Cohens *d'***
ACTIVE TDCS	7.32 ± 2.69	7.21 ± 2.05	-0.105 ± 2.05	0.008	0.796	0.084
SHAM TDCS	5.42 ± 2.08	5.47 ± 1.68	0.051 ± 1.68			
VERBAL FLUENCY (CATEGORY)						
ACTIVE TDCS	9.21 ± 3.88	5.53 ± 4.53	-3.68 ± 3.93	0.049	0.826	0.072
SHAM TDCS	7.42 ± 3.81	4.00 ± 3.18	-3.42 ± 3.37			
TRAIL MAKING (NUMBERS)						
ACTIVE TDCS	9.53 ± 3.19	10.6 ± 1.86	1.11 ± 3.20	0.064	0.801	0.082
SHAM TDCS	7.79 ± 3.46	9.16 ± 3.27	1.37 ± 3.20			
TRAIL MAKING (LETTERS)						
ACTIVE TDCS	8.05 ± 3.85	9.11 ± 3.75	1.05 ± 3.29	2.75	0.102	0.534
SHAM TDCS	5.58 ± 3.89	8.63 ± 3.67	-2.95 ± 3.73			
TRAIL MAKING (COMBINED)						
ACTIVE TDCS	9.37 ± 4.90	8.21 ± 5.14	-1.16 ± 6.24	0.197	0.659	0.144
SHAM TDCS	10.0 ± 5.26	7.95 ± 4.96	-2.05 ± 6.18			

Table 3.4: Summary metrics and analysis for the Verbal Fluency (VFT) and the Trail Making Tasks (TMT). Mean and standard deviation scores are shown the for baseline and follow-up visits. Baseline adjusted Δ-scores were calculated on a per subject basis, positive Δ-scores indicate improved performance. An ANOVA was conducted on the Δ-scores to identify differences across groups. Cohens *d'* was computed using the Δ-scores to estimate effect sizes.

EXTERNALIZING PROBLEMS	BASELINE (MEAN ± SD)	FOLLOW-UP	Δ-SCORE	$F_{1/35}$	P-VALUE	COHENS *D'*
ACTIVE TDCS	72.1 ± 17.2	68.1 ± 11.8	-4.00 ± 12.7	2.78	0.101	0.222
SHAM TDCS	73.3 ± 15.2	71.7 ± 13.7	-1.67 ± 7.29			
INTERNALIZING PROBLEMS						
ACTIVE TDCS	58.0 ± 12.8	56.1 ± 10.1	-1.89 ± 5.79	2.50	0.122	0.521
SHAM TDCS	60.6 ± 12.9	61.4 ± 12.4	0.833 ± 4.59			
ATTENTION PROBLEMS						
ACTIVE TDCS	66.9 ± 6.93	65.4 ± 8.60	-1.53 ± 6.26	0.717	0.401	0.278
SHAM TDCS	67.4 ± 7.83	67.6 ± 6.92	0.167 ± 5.88			
HYPERACTIVITY						
ACTIVE TDCS	73.2 ± 13.3	69.7 ± 11.7	-3.47 ± 9.65	0.010	0.912	0.034
SHAM TDCS	73.8 ± 11.7	70.0 ± 11.2	-3.78 ± 8.37			

Table 3.5: Summary metrics and analysis of BASC-3 questionnaire data. Mean and standard deviation scores are shown the for baseline and follow-up visits. Baseline adjusted Δ-scores were calculated on a per subject basis; negative Δ-scores indicate reduced impairment. An ANOVA was conducted on the Δ-scores to identify differences across groups. Cohens *d'* was computed using the Δ-scores to estimate effect size.

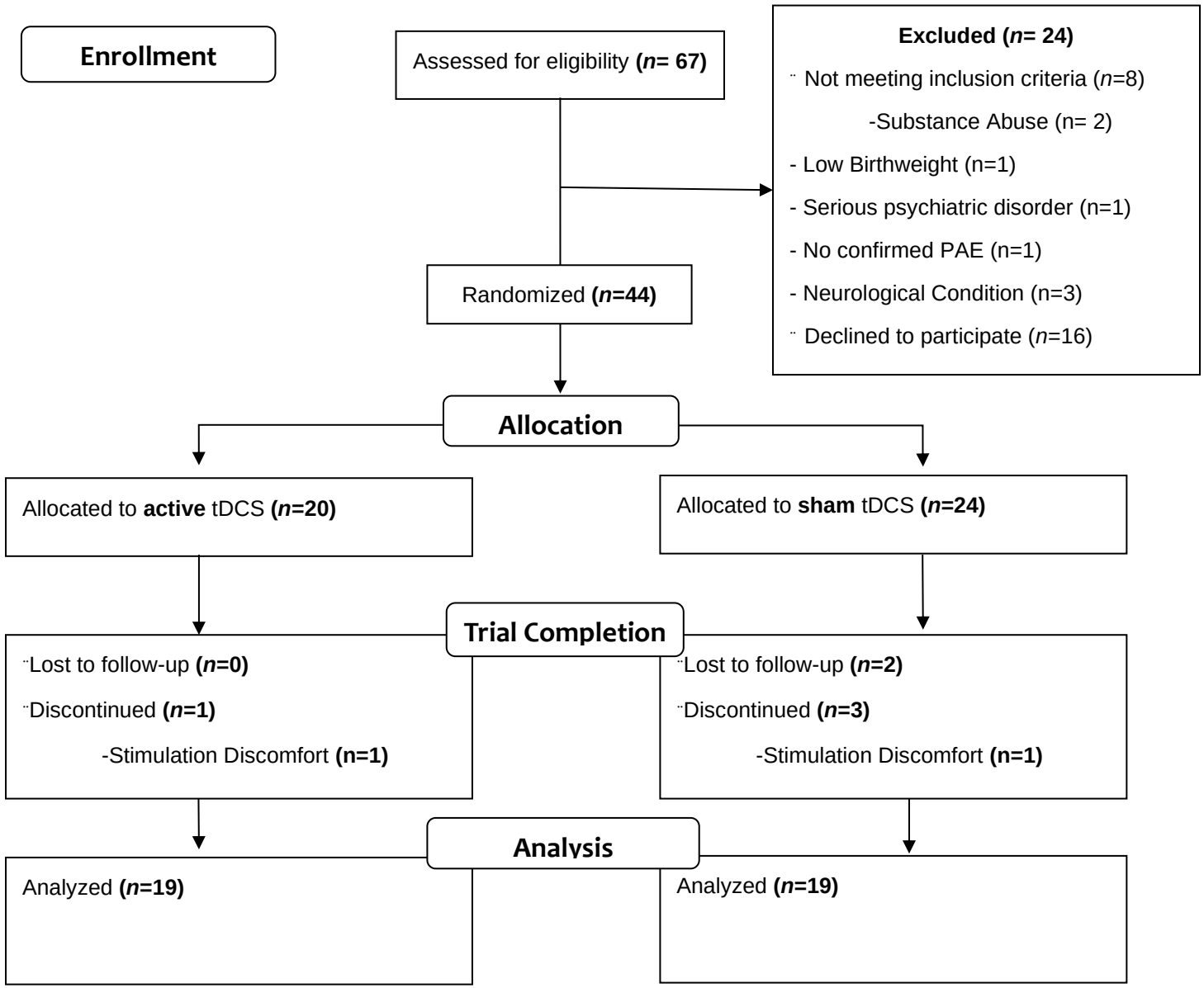

Enrollment
Assessed for eligibility (n= 67)
Excluded (n= 24)
¨ Not meeting inclusion criteria (n=8)
-Substance Abuse (n= 2)
- Low Birthweight (n=1)
- Serious psychiatric disorder (n=1)
- No confirmed PAE (n=1)
- Neurological Condition (n=3)
¨ Declined to participate (n=16)
Randomized (n=44)
Allocation
Allocated to active tDCS (n=20)
Allocated to sham tDCS (n=24)
Trial Completion
¨Lost to follow-up (n=0)
¨Discontinued (n=1)
-Stimulation Discomfort (n=1)
¨Lost to follow-up (n=2)
¨Discontinued (n=3)
-Stimulation Discomfort (n=1)
Analysis
Analyzed (n=19)
Analyzed (n=19)

Figure 3.1: Flow diagram depicting participant progression throughout the study.

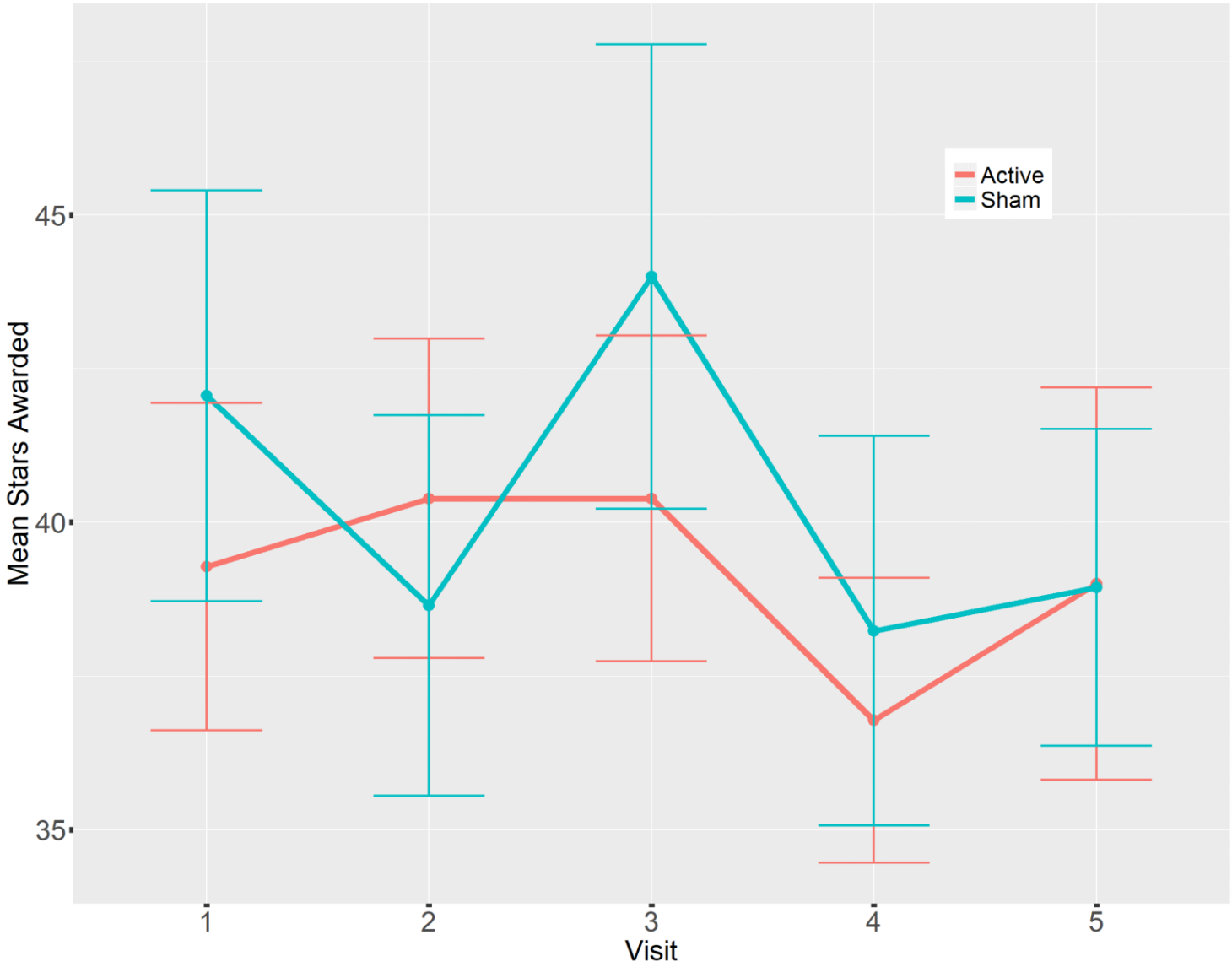

Active
Sham
Mean Stars Awarded
45
40
35
1
2
3
4
5
Visit

Figure 3.2: Performance Across All Training Tasks: Plotting average number of starts earned per treatment visit across all 5 training tasks. Each training task was played a total of 4 times per visit and between 0 and 5 stars were awarded each time a task was completed. Stars were awarded based on how the user's performance compared to all users who had completed that task (at the specific level) in the BrainHQ database. The error bars represent the standard error of the mean.


Visuospatial Working Memory
Objects Recalled (Baseline Adjusted)
0.50
0.25
0.00
-0.25
1
2
3
4
5
Visit
treatment
Active
Sham

Figure 3.3: Visuospatial Working Memory Task: This task was used to assess transfer of training gains to a related cognitive task. Participants were asked to keep track of and recall the location of an increasing number of objects amidst distractors. The primary metric of performance is average span length (# of objects over the course of the task). Here we plot the baseline adjusted average span length across the treatment visits for each group. Error bars represent standard error of the mean.

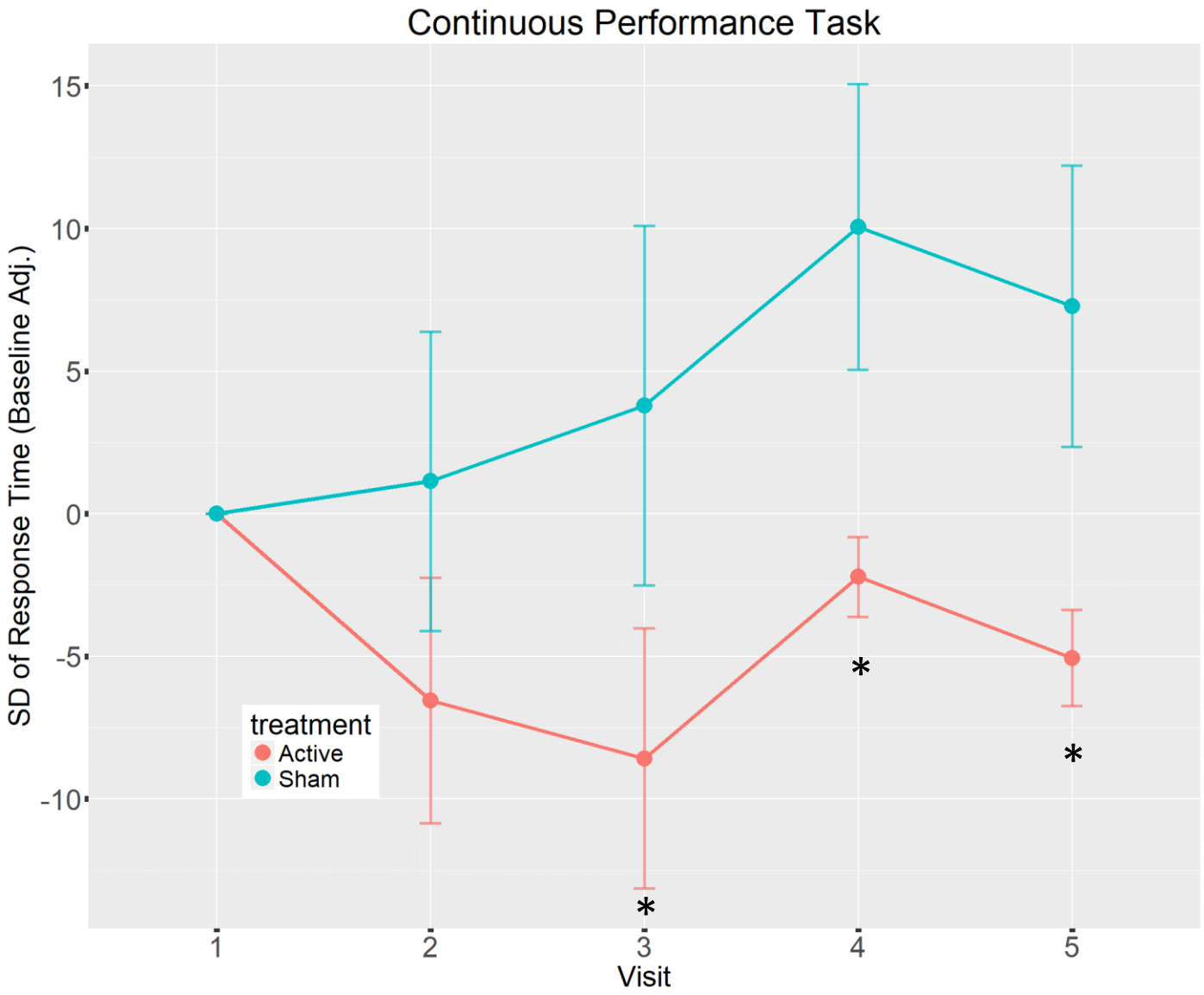

Continuous Performance Task
SD of Response Time (Baseline Adj.)
15
10
5
0
-5
-10
1
2
3
4
5
Visit
treatment
Active
Sham
*
*
*

Figure 3.4: Continuous Performance Task: This task was used to assess transfer of training gains to a related cognitive task. Participants were asked to press a button in response to frequent visual stimuli while inhibiting a button press when presented with a rare target. The primary metric of performance is the standard deviation (SD) of response time, with lower SD indicative of improved performance. Here we plot the baseline adjusted average SD of response time across visits. There was a significant effect of tDCS on performance. Asterisks indicate visits at which performance was significantly different across groups (ANOVA contrasts, Bonferroni-Holm corrected.

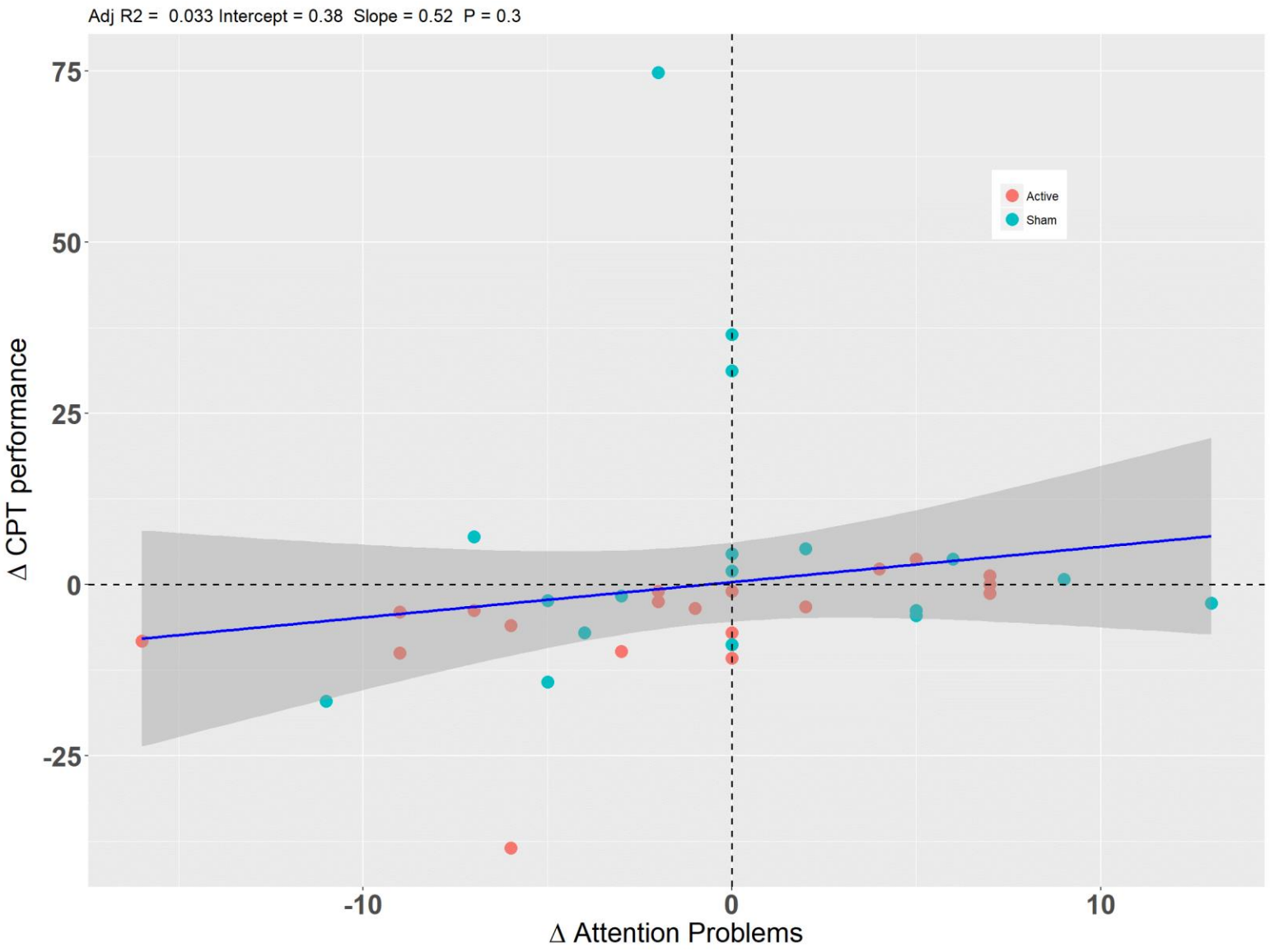

Adj R2 = 0.033 Intercept = 0.38 Slope = 0.52 P = 0.3
75
50
25
0
-25
Δ CPT performance
-10
0
10
Δ Attention Problems
Active
Sham

Figure 3.5: Correlating Change in CPT with Change in the Attention Problems Metric: We correlated average change in CPT performance with change in the attention problems metric from the BASC. When analyzing across both groups be did not observe a significant correlation.

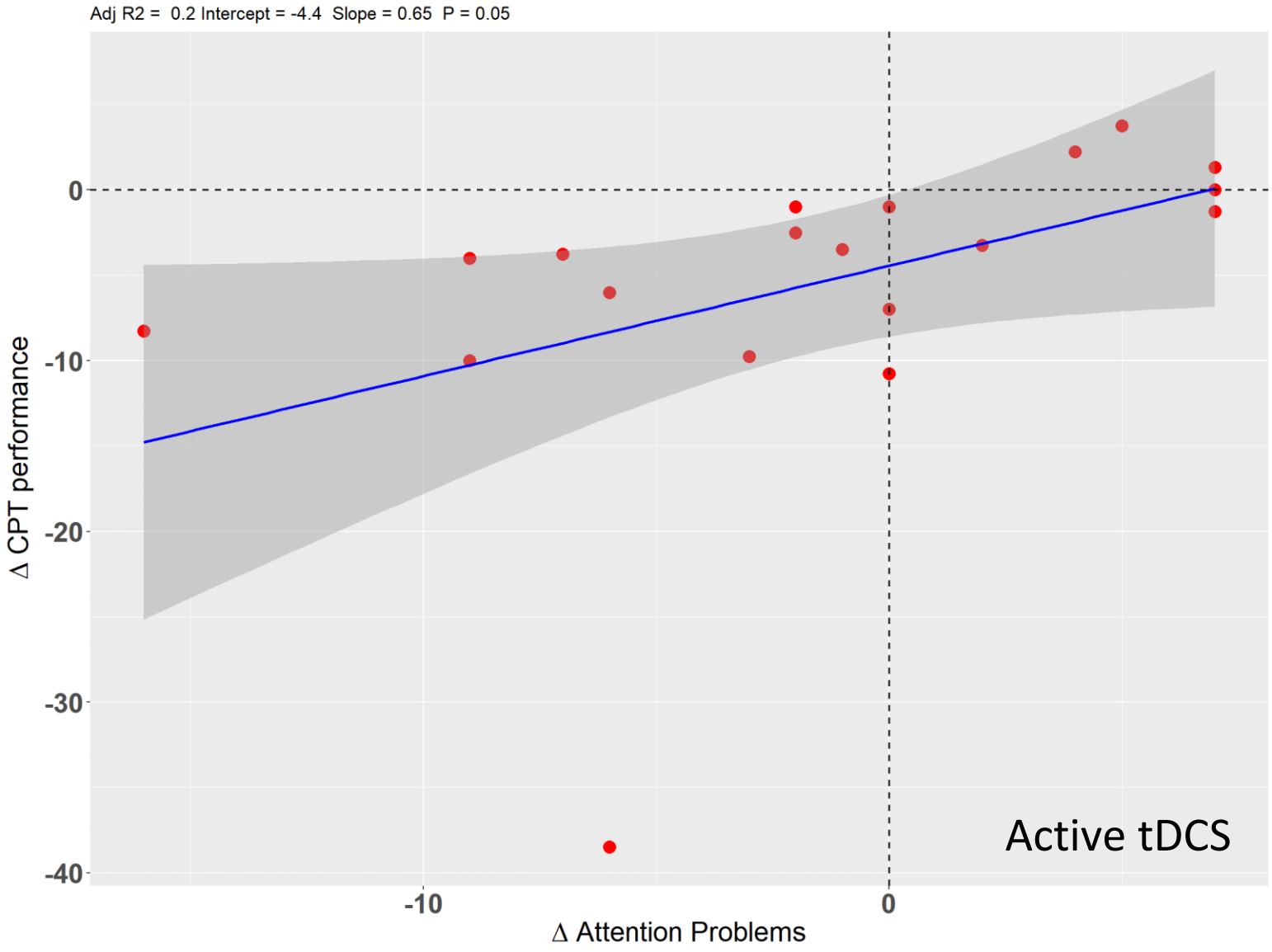

Adj R2 = 0.2 Intercept = -4.4 Slope = 0.65 P = 0.05
0
-10
-20
-30
-40
Δ CPT performance
-10
0
Δ Attention Problems
Active tDCS

Figure 3.6: Correlating Change in CPT with Change in the Attention Problems Metric – Active tDCS Group: We correlated average change in CPT performance with change in the attention problems metric from the BASC. When analyzing the active tDCS group we obtained a trend-level significant correlation.

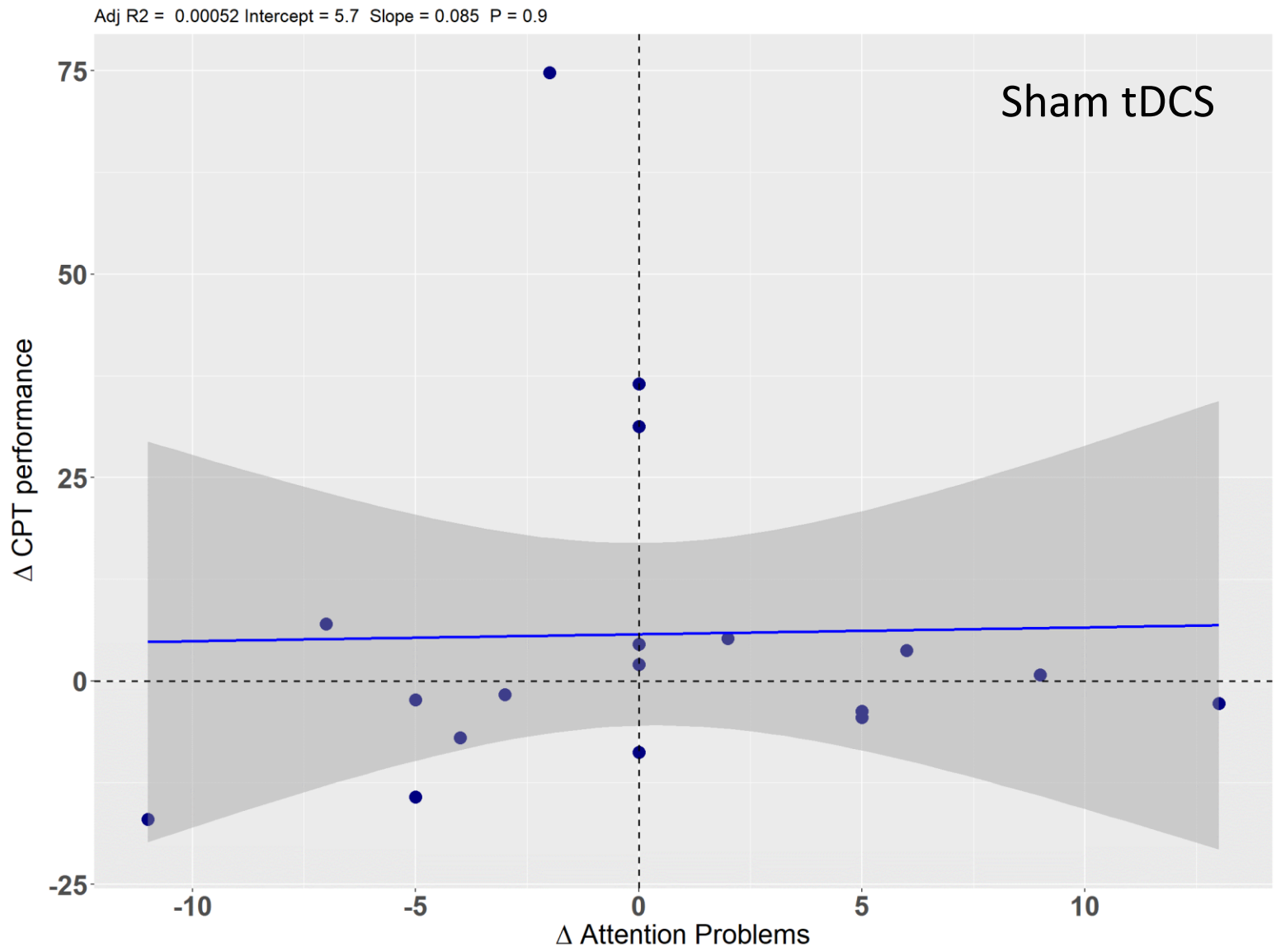

Adj R2 = 0.00052 Intercept = 5.7 Slope = 0.085 P = 0.9
Sham tDCS
75
50
25
0
-25
Δ CPT performance
-10
-5
0
5
10
Δ Attention Problems

Figure 7: Correlating Change in CPT with Change in the Attention Problems Metric – Sham tDCS Group: We correlated average change in CPT performance with change in the attention problems metric from the BASC. When analyzing the active tDCS group we obtained a non-significant correlation.

CHAPTER IV: GENERAL DISCUSSION

General Discussion

The human brain has an almost miraculous ability to dynamically shift its functional and even structural state throughout life. In response to novel input or traumatic injury, the brain can go through complex patterns of reorganization and consolidation, allowing us to learn new skills, make long-term decisions and successfully navigate a chaotic world. Neural plasticity is present all across the wide spectrum of brain activity, from microscale interactions between individual cells/synapses, to the re-patterning of whole brain networks and behaviors at the macroscale end of the spectrum. Plasticity is also important throughout the lifespan. The brain is most plastic in the developmental stages of our life, when new neural connections are made, as old ones are pruned away. It partly this hyperplastic state of the developing brain which makes it so vulnerable to insult from environmental toxins. Even as we mature and age however, plasticity continues to play a fundamental role in overall brain health. Studies in aging populations demonstrate that even as age-related processing deficits (e.g., processing speed, sensory perception) build, the brain can still maintain many of its higher order functions given a healthy, plasticity promoting lifestyle (exercise, social engagement, learning new skills) (Jones et al., 2006; Kramer et al., 2004).

Given the fundamental importance of neuroplasticity to healthy brain function, it is no surprise that when mechanisms which support plasticity are impaired, that severe neurological disorders result. As previously discussed (see Chapter 1), the causal contributors to disorders such as schizophrenia and bipolar disorder have been identified in genes which code for the protein machinery

supporting vital cellular mechanism of plasticity. The implication of disrupted plasticity in these, and other, disorders makes enhancing plasticity a promising therapeutic target to research.

Non-invasive neuromodulation via transcranial direct current stimulation (tDCS) is a promising method by which human brain plasticity can be modulated. Over the course of the last two decades, the use of tDCS in both clinical and research settings has grown substantially due to the low cost and ease of use. Studies have used tDCS to modulate a variety of cognitive, motor, and perceptual functions, while also using it to treat a range of neurological disorders. Despite the prevalence of use in recent times however, there are still a number of unanswered questions and concerns regarding mechanism and efficacy of tDCS.

Of primary interest to the work in this dissertation was the question of specificity of outcomes from tDCS intervention. As described previously, tDCS stimulation results in an induced electrical field in the brain which is widely distributed across many brain regions. Additionally, because tDCS electrical currents are weak, the induced electrical fields are subthreshold and do not directly cause neuronal firing. Given these two properties, it is currently still an open question regarding how to best achieve specific outcomes from such a non-focal and non-specific tool. One factor that may play a fundamental role in shaping the effects of tDCS is the state of the brain at the time of stimulation. Indeed, some evidence had been established already that tDCS effects differ depending on if the participant is engaged on a specific task or passively receiving stimulation (Antal et al., 2007). This preliminary evidence has led some to postulate an ‘activity-

selectivity' hypothesis of tDCS effects. This hypothesis states that tDCS will preferentially modify circuits or brain areas which are simultaneously active with stimulation while sparing those which are relatively inactive in comparison. Though some limited evidence for this postulate exists, physiological evidence is lacking.

The second chapter of this dissertation was focused on evaluating the activity-selectivity hypothesis of tDCS effects using a novel, EEG based plasticity paradigm. Using this paradigm, I was able to show that tDCS can enhance the level of plasticity achieved over the auditory cortex in a stimulus-specific manner. More specifically, I demonstrated that the effects of tDCS were restricted to a population of neurons which were active in processing the auditory stimulus being processed during stimulation. Importantly, no tDCS modulation was observed in response to the control stimulus which was not presented during tDCS. Together, these results provide robust physiological evidence in support of the activity-selectivity hypothesis, demonstrating that the functional state of the brain during stimulation plays a crucial role in determining tDCS outcomes. This finding is especially informative to clinical studies which seek to refine the development of treatment protocols for using tDCS as an intervention. For example, these findings reinforce the idea that tDCS should be paired with concurrent endogenous activation of the targeted brain network, meaning that the participant should be activating the stimulation target endogenously by performing a specific cognitive task.

Despite the interesting results I was able to report here, I think that, given the time, much more work could have been done to expand on these findings. For

example, I would have liked to collect more data as I believe I was underpowered to detect effects at the 30min post-stimulation timepoint. With the current sample size, I had trending effects at this timepoint, but perhaps a few more data points would have pushed me under that magic 0.05 mark. Additionally, I would have liked to assess the functional impact (if any) of my intervention. For example, did the subjects get better at detecting the target tone after tDCS and sensory tetanus? Perhaps running some sort of oddball task could have been a way to answer this interesting question. On the analysis side as well, I think there may be some very interesting findings once I get around to doing a spectral analysis. Event related spectral perturbation (ERSP) would be a perfect complement to my current ERP analysis. That being said however, I am happy with how the project came out and excited about the clinical work I did as well.

Basic research, in animal models or human subjects, is fundamental to the advancement of knowledge and is vital for developing new treatment options. My work investigating the activity-selective properties of tDCS is a good example of such research. On the other hand, it could be argued that clinical research is more focused on refining existing treatments and optimizing outcomes. The study described in the third chapter of this dissertation is a nice demonstration of how basic research findings can both inform and be put into practice in a clinical trial.

One of the most debilitating aspects of fetal alcohol spectrum disorders (FADS) are the associated cognitive deficits. Individuals with FASD frequently present with severe deficits in higher order executive functions, such as working memory, attention and decision making (Fryer et al., 2007; Medina, 2011).

Treatment options to address these deficits are significantly limited. Cognitive training is one of the few interventions that has recently been tried for treating executive dysfunction in FASD (Kerns et al., 2010, 2017). Findings from these studies have been promising, and more research is certainly warranted. Interestingly, it has been suggested that many of the functional abnormalities which are associated with prenatal alcohol exposure are a result of disrupted plasticity (Medina, 2011). Given the importance plasticity plays in learning, it would be ideal to pair cognitive training with a tool which can enhance plasticity (tDCS).

Our study, headed up by Dr. Jeff Wozniak and myself (as well as our research assistant Alyssa Kreuger, who was fantastic), sought to demonstrate the tolerability and feasibility of conducting tDCS augmented cognitive training in children with FASD. We applied tDCS, targeting the dorsolateral prefrontal cortex, simultaneously with cognitive training over the course of 5, once a week treatment visits. We found that tDCS, at levels of intensity used with adults, is well tolerated and safe, with no significant side-effects. We also report that the active tDCS led to improved performance on metrics of sustained attention compared to sham but had no effect on non-trained cognitive domains or behavioral reports.

The main takeaway from this first of its kind pilot study is that tDCS is well tolerated in children with FASD. Given the exploratory nature of this study however, it is perhaps not surprising that we did not show significant change in cognition across our various metrics. Our intervention was limited to once a week session, which is atypical when considering that most tDCS studies in adults feature daily stimulation sessions over the course of multiple weeks. Nevertheless,

findings from our investigation open the door for more studies which can better refine treatment parameters and find the correct dose of training and simulation to achieve optimal outcomes.

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In conclusion to my dissertation I would like to review some of the other projects that I worked on, as well as to reflect on what my goals were coming into the program and what I was able to accomplish. The first couple of years of my PhD, I worked mainly under the supervision of Dr. Brent Nelson, who was an associate faculty in the psychiatry department. We worked together to build a platform agnostic gaming engine which would be used to both develop and deploy cognitive training games in a flexible and cloud-based manner. It was unfortunate that Brent left somewhat abruptly because I think we could have made something pretty cool. Nevertheless, we completed enough of the project to get a conference paper from the IEEE Wireless Health Conference. That was my first conference as well and I had to present 2 posters at once (Brent's and mine) and give a short talk. I got a lot out of that experience. I learned how to get comfortable with coding and much more importantly, I refined my ability to take something I am really bad at and get proficient at it. I learned to be a better learner, a master lesson.

Coming into graduate school, I already had thought a lot about what sort of research I wanted to get into. I was intrigued by the prospect of leveraging modern technology to enhance human brain function. I was specifically interested in non-invasive neuromodulation and had identified Kelvin as a potential mentor. The last 5 years have been eye opening in many ways and it has been really rewarding

getting to work on something that I am passionate about. I know that I can take the lessons learned and apply them wherever my path takes me. I will always be grateful for this time.

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