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The Use of Transcranial Direct Current Stimulation for Long-Term Learning

DISSERTATION

submitted in partial satisfaction of the requirements
for the degree of

DOCTOR OF PHILOSOPHY

in Psychology

by

Jacky Wo Hay Au

Dissertation Committee:
Associate Professor Susanne Jaeggi, Chair
Professor Ramesh Srinivasan
Professor Jeffrey Krichmar

2019

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DEDICATION

To all the people who stood by me on this journey,

For all their unconditional love and support,

For having faith in me even when I faltered,

But mostly for teaching me that it’s ok to have imaginary friends.

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ABSTRACT OF THE DISSERTATION

The Use of Transcranial Direct Current Stimulation for Long-Term Learning

By

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Doctor of Philosophy in Psychology

University of California, Irvine, 2019

Associate Professor Susanne Jaeggi, Chair

Transcranial Direct Current Stimulation (tDCS) is a non-invasive means of electrical brain stimulation that can influence the neural activity of the underlying cortex, and is becoming a popular means of cognitive enhancement. However, some meta-analyses arrive at inconclusive results, and the efficacy of tDCS is controversial, especially during the earlier years of this dissertation work. One reason that could account for the low reliability between some studies is the possibility of delayed effects that may not occur until some hours or days after a session. Evidence for this phenomenon has been accruing with longitudinal studies and is thought to relate to downstream effects of the stimulation on promoting LTP and LTP-like plasticity within task-relevant neurons. The primary aim of this thesis is to explore the conditions under which such long-term changes can occur, and the extent to which they can influence human learning. To do so, three empirical studies were conducted. First, we established that tDCS could improve cognitive function during a week-long period of working memory (WM) training, with follow-up effects lasting up to a year. Our second study investigated the neural underpinnings of these longitudinal effects by using EEG to show greater evoked responses to a visual flicker roughly 24 hours after tDCS. Finally, we repeated our WM training design, but manipulated the stimulation timing window (before, during, or after task performance), in order to optimize our

protocol to manifest greater long-term effects. However, we unexpectedly found that tDCS actually *impaired* performance relative to sham, particularly when applied before or after training. Post-hoc analyses looking at the combined data from our first and third experiments revealed an interesting baseline-dependency that may reconcile our discrepant results. We found that tDCS was only effective for individuals who started off with either low or high WM ability, but ineffective or possibly even detrimental for individuals starting off with more average ability levels. Overall, this thesis argues for a promising outlook for the use of tDCS for increasing long-term learning, but cautions that the strength and direction of effects can vary wildly depending on a variety of individual difference and other factors.

OVERALL INTRODUCTION

The present dissertation details a line of work examining the use of transcranial direct current stimulation (tDCS), a form of noninvasive electrical brain stimulation, for augmenting human cognitive performance and learning. The concept of using electricity to modify brain function is not a novel one, but rather has a long history dating as far back as the first century, AD, where electrical fish were reportedly placed around patients' heads to treat headaches (Priori 2003). Although methods have improved considerably since these early days, the mechanisms of effect still remain somewhat of a black box. The most commonly cited mechanism of modern-day tDCS is that it is able to selectively increase or decrease the resting membrane potential of underlying neurons in a polarity-dependent manner, thereby altering the probability of action potential generation, with after-effects lasting up to an hour after stimulation (Nitsche and Paulus 2000). Importantly, and in contrast to more conventional neurostimulation methods such as transcranial magnetic stimulation (TMS), tDCS operates on a subthreshold level. That is, the electric field it generates in the brain is too weak to elicit action potentials directly, but merely modulates the spontaneous rate of neural firing of pre-existing brain activity. Thus, tDCS is usually paired with a behavioral task to elicit task-specific brain activity upon which tDCS can then act. This subthreshold modulation grants tDCS a fairly specific level of precision, despite the broad swaths of cortical areas through which the current shunts, as task-unspecific activity is less likely to be modulated to any significant degree due to the weakness of the electric field (Bikson, Rahman, and Rahman 2013). This specificity, combined with the favorable tolerability and safety profile of the technology (Bikson et al. 2016) has generated a lot of interest and excitement among researchers to use tDCS for performance enhancement and skill learning across a broad range of behavioral tasks.

However, initial enthusiasm was quickly tempered by small effect sizes, replicability issues, and theoretical concerns by prominent scientists (e.g., Jared Cooney Horvath, Forte, & Carter, 2015a, 2015b; Underwood, 2016). Let's take the working memory (WM) field for example, which is the subject of this dissertation. Despite initial reports of WM enhancement through the use of tDCS over the prefontal cortex (Fregni et al. 2005), later meta-analyses failed to find reliable effects, and where effects did exist, they tended to be small (Horvath et al. 2015b; Mancuso et al. 2016). Furthermore, it is known that a significant amount of current is shunted across the scalp and cerebrospinal fluid, and it has been questioned whether the small amount of current that does reach the desired cortical region is sufficient to manifest any measurable behavioral change (Underwood 2016).

It was in the midst of this controversy that my colleagues and I undertook our first empirical study examining the actions of tDCS on WM in 2014 (published in 2016; see Chapter 2). At the time, the majority of related studies in the field used single-session designs to evaluate the immediate effects of tDCS on WM. In a departure from these studies, we used a longitudinal training design. Given the complex pattern of successes and failures in the literature, we reasoned that perhaps there would be cumulative effects over time that would manifest more readily with a longitudinal design rather than within a single session. Moreover, there was already some evidence then that cognitive training studies, with multiple sessions, were more successful (Elmasry, Loo, and Martin 2015) than single-session studies (Brunoni and Vanderhasselt 2014).

This thesis synthesizes the results of several experiments which collectively suggest that even in the absence of immediately observable effects, tDCS may still continue to work offline to produce long-lasting effects on human brain and behavioral function. Thus, single-session

designs which do not account for these delayed effects may underestimate the true impact of tDCS. Chapter 1 discusses the theoretical rationale for how tDCS can influence LTP and LTP-like plasticity even after a stimulation session has ended, and thus produce long-lasting effects by interacting with the consolidation process. Chapter 2 then describes our first empirical study that uses tDCS to increase learning during WM training. Not only was performance enhanced with tDCS during the week-long training, but the performance gap gradually increased over the course of training, remaining intact even 1 year later, with no additional stimulation in the interim. This suggests that the effects of tDCS can be impressively durable and long-lasting if it interacts with learning and consolidation. Moreover, we found important individual differences in baseline WM ability that moderate receptivity to tDCS enhancement in that those with lower baseline ability showed stronger tDCS-related enhancement. Chapter 3 follows up this study by examining the electrophysiological underpinnings of these longitudinal effects using EEG to measure the evoked response to flickering stimuli during a WM and attention task. Although tDCS increased the measured EEG response to the visual flicker, this was only apparent after a time delay (i.e., when the flicker was presented again the next day without stimulation) and not immediately after stimulation. Consistent with Chapter 2, we see that the strongest effects of tDCS (that we measured in our studies), arise from increased electrophysiological activity in the hours or days *after* stimulation, suggesting interactions with mechanisms of LTP and LTP-like plasticity triggered by the behavioral task. And once again, consistent with Chapter 2, we further find that the responsivity to tDCS is strongest among individuals who start off with lower baseline activation in response to the visual flicker. Chapter 4 seeks to optimize the consolidation-like effects of tDCS on WM training by comparing the effects of stimulating either before, during, or after each training session. However, in contrast to Chapter 2, we failed to

detect any overall enhancement from tDCS, though we did demonstrate instances in which tDCS can actually *impair* performance relative to sham. Finally, Chapter 5 describes a short series of post-hoc analyses combining data from Chapters 2 and 4 that offers a plausible reconciliation of our contrasting results by demonstrating a non-linear dependency of tDCS enhancement on baseline ability such that tDCS is only effective at the extreme ends, whereas individuals starting out with more average ability may not experience any benefit, or in some cases may actually perform *worse*.

CHAPTER 1: Optimizing Transcranial Direct Current Stimulation Protocols to Promote Long-Term Learning

Overview

The following chapter is a theoretical review of the long-term effects of transcranial direct current stimulation (tDCS) and how processes related to consolidation may play a role in evincing these effects. Chronologically, this chapter was actually written and published (Au et al. 2017) after the empirical study described in Chapter 2, and some of the content was inspired by the results of Chapter 2, but it is included here in the beginning because it nicely summarizes the main theme throughout this dissertation relating to the consolidation-like properties of tDCS effects on learning.

Herein, we review the literature describing the nature of tDCS-enhanced consolidation, and argue that some of the mixed results among the single-session studies that currently dominate the extant literature may be explained by a failure to take advantage of these potentially powerful offline effects that occur after a stimulation session. Accordingly, we further contend that the full potential of tDCS cannot be truly realized without a longitudinal design which allows for tDCS to act directly upon learning by promoting consolidation between sessions. Finally, we review preliminary evidence that these consolidation-like effects can be even further enhanced via strategically spaced out stimulation sessions, which take advantage of a long-held tenet in the literature that distributed learning produces better outcomes than massed learning. We conclude by proposing potential study designs to encourage the use of tDCS as more than merely a method to promote temporary enhancement, but also a technique to enhance long-term learning.

Introduction

Transcranial direct current stimulation (tDCS) is a non-invasive form of brain stimulation that sends weak direct currents through the scalp and into the underlying cortex. tDCS is thought to alter the resting membrane potential of target neurons in a polarity-dependent manner such that the anode increases while the cathode decreases cortical excitability (Nitsche et al. 2003). Behaviorally, this has been shown to manifest in increased motor-evoked potentials (MEP; Horvath, Forte, & Carter, 2015a), improved motor functioning (Hashemirad et al. 2016), enhanced working memory (WM) performance (Mancuso et al. 2016), as well as a plethora of other cognitive, physical, and emotional changes (Utz et al. 2010).

However, recent work has raised questions about the mechanisms and effects of tDCS. For example, since most of the current delivered at the scalp gets shunted away by skin, skull, and cerebrospinal fluid before entering the brain, the electric field generated in the brain is orders of magnitude below that typically delivered by other methods such as TMS (Ruohonen and Karhu 2012). It has consequently been argued that the effects of tDCS are too weak to have any meaningful impact on membrane potential (Underwood 2016), furthering existing controversy surrounding the reliability of tDCS (Antal et al. 2015; Chhatbar and Feng 2015; Horvath et al. 2015b; Price and Hamilton 2015). However, this argument has not been empirically evaluated since direct evidence for membrane polarization is based on intracellular recordings in animal models where current is applied directly to cortical slices rather than transcranially (Purpura and McMurtry 1965). Nevertheless, most meta-analyses converge on small overall effects within healthy young adults, which become larger and more robust when studies with lower-performing populations such as clinical patients and the elderly are included (Dedoncker et al. 2016; Hill, Fitzgerald, and Hoy 2016; Hsu et al. 2015; Mancuso et al. 2016). Therefore, it is imperative to develop a

stronger theoretical understanding to account for the diverse effects and to optimize protocols for greater reliability and more meaningful results.

An interesting epiphenomenon within the tDCS literature is that occasionally effects are observed some time after (hours to months), but not immediately during, stimulation. This suggests that changes in membrane potential as a result of tDCS are not exclusively driving behavioral improvements since the polarizing effects of tDCS should have largely washed out by the time these delayed effects emerge. Rather, what these studies suggest is that tDCS may play a role in consolidation. Although the effects of tDCS on neural membrane potential have been argued to be small and inconsequential, its effects on glial cells, which comprise ~50% of brain cells and are sensitive to much smaller depolarizations than neurons, are estimated to fall within a biologically meaningful range (Ruohonen and Karhu 2012). Moreover, glial cells, which secrete many of the same molecules and transmitters as neurons such as calcium and glutamate, play a direct role in learning and synaptic plasticity, thereby providing a tenable mechanism for tDCS to act upon consolidation (Ben Achour and Pascual 2010; Gibbs, Hutchinson, and Hertz 2008; Monai and Hirase 2018). Harnessing these latent effects could help mitigate the low reliability found among some studies, particularly the single-session studies that dominate the extant literature, which are not designed to capture delayed consolidation effects. Here, we overview the neurobiological foundation of tDCS' putative effect on consolidation, and then review the experimental evidence in support of this phenomenon. Finally, we discuss the optimization of protocols to capitalize on these effects.

Neurobiology of Consolidation and tDCS

Consolidation refers to a process by which learning becomes increasingly resistant to interference over time, and operates on two different time scales: a fast-acting synaptic

consolidation on the order of minutes to hours that strengthens local synaptic transmission, and a slow-acting system consolidation on the order of days to weeks or years that anchors the memory trace into a long-term store across distributed brain regions (Born and Wilhelm 2012). Support for this model comes primarily from studies of declarative memory, but other memory domains such as those involved in skill learning are also thought to operate similarly (Dudai, Karni, and Born 2015). The administration of tDCS may enhance consolidation at both of these stages.

Synaptic consolidation involves gene expression changes and protein synthesis that result in higher levels of plasticity-related proteins at recently-active synapses (Frey and Morris 1997; Steward et al. 1998). The after-effects of tDCS are known to be dependent on this ongoing protein synthesis that occurs during the stimulation period, as well as on increased functioning of NMDA receptors which facilitate synaptic transmission. Administration of both protein inhibitors in animal models and NMDA receptor antagonists in humans are able to nullify these after-effects (Gartside 1968; Nitsche et al. 2003). This suggests that online tDCS interacts with the brain's normal plastic response, which allows for the continued manifestation of effects offline. In fact, tDCS has been demonstrated to upregulate the expression of BDNF, an important protein involved in long-term potentiation (LTP) and memory (Podda et al. 2016), and alter the balance of GABAergic and glutamatergic transmission, which can modulate cortical excitability and facilitate or obstruct activity-dependent LTP (Krause, Márquez-Ruiz, and Kadosh 2013). See Stagg & Nitsche (2011) for a more thorough account of the cascade of cellular and molecular modifications that arise from tDCS.

Furthermore, in the minutes to hours following a learning event, cells which were engaged by the event undergo patterned reactivation (Diba and Buzsáki 2007; Foster and Wilson 2006). Such "replay" has been observed both during slow-wave sleep (Wilson and McNaughton

1994) and awake rest (Foster and Wilson 2006; Karlsson and Frank 2009) and is thought to play a prominent role in system consolidation (Sirota and Buzsáki 2005). In fact, disruption of neural replay has been demonstrated to prevent consolidation (Genzel and Robertson 2015). Therefore, it is conceivable that tDCS, with its putative effects on cortical excitability (which may operate via glial-neuron interactions rather than neural stimulation directly), could enhance neural replay and therefore enhance consolidation. Though a direct demonstration is lacking, several studies have applied tDCS during waking rest at time periods when replay is thought to occur (Javadi and Cheng 2013; Sandrini et al. 2014; Tecchio et al. 2010) and subsequently demonstrated greater consolidation. Similarly, the application of slow-oscillating tDCS during slow-wave sleep has been shown to enhance declarative memory (Barham et al. 2016). These enhancements are also accompanied by an increase in slow-oscillating waves (<1Hz), a neural frequency band which is temporally associated with the onset of neural replay (Genzel and Robertson 2015).

Experimental Evidence

A number of animal studies have measured polarity-dependent cellular changes in LTP both *in vitro* (Ranieri et al. 2012; Ruohonen and Karhu 2012) and *in vivo* (Podda et al. 2016; Rohan et al. 2015) as a function of direct-current stimulation. Analogously, human experiments have demonstrated on a behavioral level that tDCS over multiple days can lead to cumulative effects between sessions, suggesting the existence of continued offline processes between each bout of stimulation. These offline between-session effects have even been observed in some studies to be greater than the online within-session effects. For example, extending the classic MEP paradigm (an assessment of motor excitability) over five days led to step-wise increases in baseline amplitude each day (Alonzo et al. 2012; Gálvez et al. 2013). However, despite the impressive between-session effects in these studies, the within-session responsivity to tDCS was

comparable across most days and in fact, did not even always differ significantly from baseline in every session (Horvath et al. 2016).

Similarly, pairing tDCS with a behavioral task has also been shown to improve performance offline, leading to performance increases between consecutive sessions. For example, Reis et al. (2009, 2015) demonstrated greater skill learning using a visuomotor task with concurrent tDCS over motor cortex. Importantly, the respective contributions of online and offline effects were systematically evaluated by starting stimulation only after the first block of training each day. This allowed comparing the offline improvement between this first block and the last block of the previous day, with the online performance gains within a session. Their analysis suggested that the majority of learning occurred offline[1] (Prichard et al. 2014). Furthermore, our previous work in the cognitive domain (Au et al., 2016) showed similar effects using a WM intervention combined with online tDCS. As with motor skill, we also observed a higher rate of learning in our stimulated group, relative to sham. Although we did not systematically evaluate the relative contributions of online and offline learning, several lines of evidence suggest a substantial role of offline consolidation in our study. First, there was no hint of a between-group difference after the first training session, suggesting a minimal or non-existent role of online stimulation, at least for the first session. However, differences became increasingly pronounced over the course of training, after offline consolidation had a chance to occur. Furthermore, we demonstrated that the strongest between-session effects occurred after a weekend break, in accordance with predictions from the learning-consolidation literature (Ebbinghaus 1885), and also demonstrated long-term maintenance of training effects up to a year

[1]In both studies, the first session was the only one that showed within-session effects, suggesting that online effects may saturate early on in an intervention, or may not be reliable. Additionally, we note that a similar paradigm (Prichard et al. 2014) with a different motor task showed predominantly online, but not offline, effects, suggesting some task-specificity in the degree of consolidation

later (Benjamin Katz et al. 2017), suggesting that increased consolidation led to stronger long-term retention.

Altogether, we note that although limitations can sometimes exist in the immediate manifestation of online effects at the behavioral level, a complementary mechanism occurs offline that can further consolidate learning gains beyond the stimulation period. In further support of this notion, delayed effects have been demonstrated even in the absence of immediate effects. For example, there have been rapid consolidation effects where group differences only begin to emerge in the minutes or hours after stimulation, or become stronger/more robust with time (Clark et al. 2012; Ehsani et al. 2016; Hoy et al. 2014; Hsu et al. 2015; Javadi and Cheng 2013; Penolazzi, Pastore, and Mondini 2013; Reis et al. 2015). Similarly, overnight consolidation has been enhanced when performance is measured the next day, despite a lack of group differences on day one (Koyama et al. 2015; Martin et al. 2014), and even cognitive training studies that failed to show immediate tDCS-related enhancements have still demonstrated greater tDCS-related retention a couple months later (Jones, Stephens, et al. 2015; Martin et al. 2013; Stephens and Berryhill 2016).

Optimizing tDCS Protocols

With the many degrees of freedom that tDCS protocols offer, a pertinent goal is to hone in on the parameters that can optimize the benefits derived from electrical stimulation. Based on the evidence discussed herein, we make recommendations for future research to explore with respect to three important parameters: 1) timing of stimulation, 2) number of sessions, and 3) spacing of sessions.

Timing of Stimulation

One issue that should be given careful consideration in future studies is the timing of stimulation - that is, whether it should be done concurrent with a task or offline. Online stimulation seems to be theoretically preferable to offline stimulation in that it potentiates task-relevant networks rather than resting state networks, a distinction which may prove crucial for later consolidation of task performance. In line with this hypothesis, online and offline stimulation were directly contrasted with a WM task, and while both methods led to similar immediate performance, only the online condition promoted greater performance the next day (Martin et al. 2014). Furthermore, as discussed earlier, many studies using online stimulation have successfully demonstrated greater learning consolidation after a delay, but no study to our knowledge has done the same with offline stimulation prior to task performance. The potential delayed benefits of online, rather than offline, stimulation then is an important consideration given that meta-analyses suggest both forms of stimulation provide comparable immediate benefits[2] (Dedoncker et al. 2016; Hill et al. 2016).

[2]Hill et al. (2016) actually found a significant tDCS effect only with offline stimulation, but not online, in healthy young adults. However, the effect sizes are comparable and not different from each other. Also, sample sizes are 3-6 times greater in the offline studies compared to online, thus biasing interpretations based on significance alone. We interpret the data to suggest no difference between online and offline stimulation.

Although offline stimulation in the traditional sense (i.e., before task performance) may arguably not interact with consolidative processes, offline stimulation *after* task performance may present a more viable route as it can coincide directly with consolidation or reconsolidation periods. For example, stimulation immediately after, but not during, training of a finger-tapping task enhanced subsequent performance 30min later, presumably by facilitating early consolidation of procedural memory (Tecchio et al. 2010). Similarly, reactivation of a previously learned word list hours or a day later led to better retention if stimulation was administered directly during this reactivation/reconsolidation period (Javadi and Cheng 2013; Sandrini et al. 2014). Furthermore, stimulation during sleep seems especially fruitful for declarative memory enhancement if timed during the appropriate consolidation period during slow-wave sleep (Barham et al. 2016). Therefore, it is possible that while online stimulation may promote LTP-related protein synthesis at the synapse (Gartside 1968), offline stimulation after task completion or during sleep may directly enhance learning-associated neural replay and system consolidation for long-term retention. Future research should systematically evaluate the mechanisms and relative efficacy of online and offline (after task) stimulation.

Number of Sessions

We have made the argument that the greater potential of tDCS may lie in its role in augmenting consolidation, rather than the online enhancement of membrane excitability which in of itself may arguably produce smaller or less reliable effects. Accordingly, we recommend future studies contain multiple sessions in order to capitalize on these delayed effects. These additional sessions may be stimulated or unstimulated, as either approach should allow consolidation effects from the previous session to manifest. However, we note that additional

stimulation sessions, as commonly done in cognitive or motor training studies, may be particularly effective as these sessions offer the opportunity to simultaneously boost reactivation and neural replay from previous learning (e.g., Javadi & Cheng, 2013; Sandrini et al., 2014), as well as potentiate learning from the current session.

Spacing of Stimulation

If studies are to incorporate multiple sessions, a relevant question arises concerning the optimal spacing of these sessions in order to maximize consolidation and performance on subsequent sessions. Many intervention studies employ once-daily schedules, mostly out of convenience and convention rather than any empirically-derived model. However, there is no reason to assume that this is the most optimal schedule. For example, several lines of evidence indicate that even shorter spacing protocols might be beneficial (reviewed in Goldsworthy, Pitcher, & Ridding, 2015). Monte-Silva et al. (2010) demonstrated that two bouts of cathodal stimulation separated by 20min resulted in greater and more prolonged MEP depression than a continuous bout of cathodal stimulation for the same total duration. A shorter break of 3min produced similar, but more muted effects, while longer breaks of 3 or 24h were detrimental. Similar effects were replicated with anodal stimulation, in which breaks of 3 or 20min were effective in prolonging and enhancing MEP amplitudes even into the next day, while breaks of 3 or 24h showed similar suppression of tDCS effects (Monte-Silva et al. 2013). The short-term spacing of stimulation is thought to induce meta-plastic processes in the cortex, so-called because the first round of stimulation alters the plastic response from the second. Such an approach is also supported by animal models. Repeated trains of high-frequency electrical stimulation, usually spaced apart by intervals of less than an hour, are capable of inducing late-

phase LTP that lasts for periods of weeks or longer, whereas a single train of stimulation is capable only of inducing early-phase LTP lasting hours or less (Goldsworthy et al. 2015). What appears to be critical to induce meta-plasticity with tDCS is to initiate the second round of stimulation during the after-effects of the first, a window of time which typically lasts up to an hour after stimulation (Nitsche et al. 2003). Behaviorally, meta-plastic protocols have also accentuated or prolonged effects on both motor learning (Bastani and Jaberzadeh 2014; Christova, Rafolt, and Gallasch 2015) and WM (Carvalho et al. 2015). In all these cases, it appears that inter-session breaks of approximately 20-30min produce stronger effects than much shorter (3-5min) or much longer (3-24h) breaks, suggesting the existence of a non-linear optimum.

Despite the evidence in support of short, within-day spacing intervals, a number of successful intervention studies have been carried out using a once-daily approach (Alonzo et al. 2012; Au et al. 2016; Reis et al. 2009, 2015; Stephens and Berryhill 2016). Moreover, our own work demonstrated with a WM intervention that participants showed the greatest improvement after a weekend break (Au et al. 2016), suggesting in addition to the meta-plasticity protocols that longer spacing intervals of several days can actually be beneficial for consolidation as well. How do we reconcile these disparate results? One thing to consider is that any consolidation effect that arises is naturally an interaction between the effects of electrical stimulation on brain tissue and task-related neural activation, both of which individually are likely to involve different spacing parameters that optimize LTP-induction. Although current evidence suggests that about half an hour may be an optimum spacing interval for tDCS to synergistically engage meta-plastic processes, consolidation of task-related activity (independent of tDCS) may require longer intervals. Moreover, this consolidation is also likely to be task specific. For example, one meta-

analysis summarizing the cumulative spacing research over the past century suggests that tasks with higher mental but lower physical complexity require longer spacing intervals than tasks with higher physical but lower mental complexity (Donovan and Radosevich 1999). This should be given careful consideration when piloting and designing intervention studies, as this suggests that relying on the parameters of MEP experiments to guide research, as is commonly done, may underestimate the optimum spacing interval required for more complex motor learning tasks, and even more so for cognitive skill learning. For instance, cognitive training studies (without tDCS) have systematically evaluated this phenomenon, finding that once-daily training promoted greater learning and transfer than shorter spacing schedules that involved multiple training sessions per day (Arthur et al. 2010; Wang, Zhou, and Shah 2014). Moreover, it has been demonstrated that the optimal spacing interval increases in correspondence with longer retention intervals. For example, Cepeda et al. (2008) systematically varied the retention interval of a declarative memory task as well as the time gap between two study sessions. They found that a study gap of one day produced optimal retention when test sessions were separated by one week, whereas a gap of three weeks was optimal for retention one year later. Therefore, for the purposes of using cognitive or motor training to build lasting skills, it may be beneficial to space sessions out across days or longer.

Although the relative efficacy of short-term spacing on the order of minutes or longer-term spacing on the order of days should be systematically evaluated in future research, one enticing avenue we propose is to combine the benefits of meta-plasticity with longer-term spacing of learning. This can be accomplished by spacing sessions out over a couple days, but using a repeated, meta-plastic protocol within each session. This may optimize both the delivery of electrical stimulation as well as the timing of task learning for long-term retention.

Conclusion and Future Directions

We have reviewed the role of tDCS in promoting consolidation of learning and argued that in some cases, this mechanism may present a stronger and more reliable source of the tDCS effect than the commonly touted online effects on neural excitability. This is not to suggest that such online effects are weak or non-existent. On the contrary, many studies have documented worthwhile immediate enhancements both on the level of behavior and functional brain connectivity (Fregni et al. 2005; Keeser et al. 2011; Lindenberg et al. 2013; Meinzer et al. 2013; Polanía, Nitsche, and Paulus 2011; Price and Hamilton 2015). Rather we point out that these online effects can have impressive downstream consequences that should be explored in order to gain a more comprehensive understanding of tDCS-induced cognitive enhancement.

Accordingly, we urge future research to include multiple sessions in order to capitalize on these consolidation effects, and to systematically evaluate the parameters that lead to these effects. For example, stimulation during task as well as directly during consolidation or reconsolidation periods afterwards both appear beneficial, but their relative efficacy is unknown. Also, the optimal spacing schedule of stimulation is unknown. Short spacing intervals on the order of minutes appear to effectively engage meta-plastic processes, but there is also evidence to suggest that longer spacing intervals of greater than a day might also be beneficial, especially for more complex cognitive tasks. As a final note, we caution that the evidence we have presented herein stems largely from healthy young adult populations and a few animal studies. The extent to which these lessons can be extrapolated to clinical studies and vulnerable populations is unknown and should be approached with care (e.g., Perceval, Flöel, & Meinzer, 2016).

CHAPTER 2: Enhancing Working Memory Training with Transcranial Direct Current Stimulation

Overview

The following is a two-part chapter consisting of two separate published works based on the same dataset (Au et al. 2016; Benjamin Katz et al. 2017). Though my colleagues and I collectively had ample expertise with conducting WM training interventions, this was our first foray into the tDCS world. Although there is a lot of controversy surrounding the practical relevance of WM training and the transfer of trained skills onto cognition more generally, there are predictable learning effects on the trained task itself and similar laboratory tasks (Au et al. 2015; Schwaighofer, Fischer, and Bühner 2015). Moreover, the enhancement of WM with tDCS is a popular topic of research on which there is no shortage of published guidance (Brunoni and Vanderhasselt 2014; Mancuso et al. 2016). Therefore, this seemed to us like a good vehicle through which to test and understand the effects of tDCS on learning during an intensive cognitive task. Based on our theoretical understanding of tDCS which we outlined in Chapter 1, we were interested to see whether this technology could increase the effect size and durability of longitudinal learning effects, and facilitate the transfer of trained cognitive skills to other untrained tasks. In part A (Au et al. 2016), we randomized sixty-two participants to receive either right prefrontal, left prefrontal, or sham stimulation with concurrent visuospatial WM training over the course of seven training sessions. Results showed that tDCS enhanced training performance, which was strikingly preserved several months after training completion. Furthermore, we observed stronger effects when tDCS was spaced over a weekend break relative to consecutive daily training, and we also demonstrated selective transfer in the right prefrontal group to non-trained

tasks of visual and spatial WM. These findings shed light on how tDCS may be leveraged as a tool to enhance performance on WM-intensive learning tasks.

Part B (Benjamin Katz et al. 2017) re-analyzes the same dataset but includes a new, longer-term follow-up to assess later performance, and additional participants were added so that the Sham condition was better powered. We were particularly interested in individual difference factors within our sample of participants that may moderate tDCS efficacy. We assessed baseline cognitive ability, gender, training site, and motivation level and found significant interactions between both baseline ability and motivation with condition (Active or Sham) in models predicting training gain. Also, the improvements in the Active condition versus Sham condition appear to be stable even as long as a year following the original intervention.

Part A: Training and Transfer Effects

Introduction

Working memory (WM) is a fundamental cognitive ability that is limited in capacity and supports complex thought. It is highly predictive of academic and professional success (Alloway and Alloway 2009; Gathercole et al. 2004), and thus, interventions to improve WM are highly sought. Training of WM typically leads to substantial improvements on the trained task, and has also been shown by many studies to enhance various aspects of cognitive functioning, from improving performance on non-trained WM and executive function tasks (Melby-Lervåg and Hulme 2013; Schwaighofer et al. 2015) to broader tests such as those indexing fluid intelligence (see Au et al., 2015; Karbach & Verhaeghen, 2014; Weicker, Villringer, & Thöne-Otto, 2016 for recent meta-analyses). However, obtaining reliable results often requires extensive training on the order of weeks or even months, thereby rendering participant compliance difficult and research costs high. These practical constraints have often led to underpowered studies (Bogg and Lasecki 2015) and inconsistent results in the literature. Therefore, the field would benefit from a catalyst to intensify or expedite the effects of WM training. Herein, we evaluated the efficacy of transcranial direct current stimulation (tDCS) to boost the effects of training on both trained and untrained measures of WM and executive function over a short period of 7 days. In contrast with previous investigations, the design of the current study included both a long-term follow-up as well as a training schedule that permitted us to explore the impact of spacing on training performance. Thus the present research not only adds to the growing literature in support of the effects of tDCS on WM, but it also offers novel insights with regard to the cumulative efficacy of multi-session stimulation, the effects of inter-session spacing, and the long-term durability of stimulation-enhanced training.

The use of tDCS for cognitive enhancement has sparked great interest over the past decade. tDCS is commonly thought to modify cortical excitability by altering the relative ionic distribution across neural membranes. If so, this can lead to polarity-specific increases or decreases in the resting membrane potential of neurons lying underneath the anodal or cathodal electrodes, respectively (Stagg and Nitsche 2011). Moreover, this can directly affect brain plasticity by making relevant networks more or less likely to fire in concert (Keeser et al. 2011), and it seems to modulate long-term potentiation (LTP)-like plasticity at the synapse via alterations of GABAergic and glutamatergic neurotransmission (Ardolino et al. 2005; Nitsche et al. 2005; Stagg and Nitsche 2011; Wagner, Valero-Cabre, and Pascual-Leone 2007).

Many studies have now been conducted that evaluate the use of tDCS to augment WM performance, the majority of which specifically use the n-back task as we do in the present report (c.f., Brunoni & Vanderhasselt, 2014). Despite mixed initial results (Brunoni and Vanderhasselt 2014; Horvath et al. 2015b; Tremblay et al. 2014), recent meta-analyses confirm reliable net effects of tDCS on WM performance (Hill et al. 2016; Summers, Kang, and Cauraugh 2016). Importantly, it is worth noting that the precise parameters under which tDCS may most optimally exert its benefits are not well understood, and consequently there is much methodological heterogeneity among studies (c.f., Jared C. Horvath, Carter, & Forte, 2014). In other words, it is likely that a more thorough mechanistic understanding of optimal stimulation conditions might lead to even larger effects in future studies.

For example, most extant studies employed single-session designs. However, converging evidence from the motor cortex indicates that the effects of tDCS can accumulate over consecutive daily sessions, such that gains are greater in later versus earlier sessions (Hashemirad et al. 2016). This has been demonstrated both by enhanced excitability (Alonzo et al. 2012; Ho et al. 2016) as

well as enhanced motor learning (Boggio et al. 2007; Reis et al. 2009). It has been documented that offline effects, which refer to enhancements present immediately *after* stimulation, are related to LTP-like consolidation (Stagg and Nitsche 2011), which presents a viable mechanism to explain how tDCS effects may accumulate over consecutive sessions. Though direct evaluations of single relative to multiple sessions of stimulation have not been evaluated with cognitive tasks, proof-of-concept has been demonstrated whereby anodal stimulation over left dorsolateral prefrontal cortex (DLPFC) during a WM task led to enhanced performance the next day (Martin et al. 2014). It is plausible, therefore, that the single-session designs prevalent in the extant literature mask the potential of tDCS to enhance learning and consolidation between sessions.

Additionally, the effects of tDCS seem to be site specific (Bikson et al. 2013), a particularly important consideration in cognitive studies, which target behaviors involving a functional network of multiple brain regions. This renders the choice of stimulation site an important matter. For WM, the DLPFC has proven itself to be a prime target (Tremblay et al. 2014). However, the DLPFC itself is functionally lateralized such that the left hemisphere tends to mediate verbal WM performance while the right mediates visuospatial WM performance (Smith, Jonides, and Koeppe 1996; Wager and Smith 2003). The existing literature does a good job of addressing half the equation, with the majority of studies targeting left DLPFC using a verbal WM task, as modeled after the seminal work by Fregni et al. (2005). To lend credence to the specificity of this montage, Kim et al. (2014) showed that greater behavioral improvements in verbal WM correlated with greater current density over the left DLPFC, using a tDCS set-up similar to the one used in the present report. However, there is a relative dearth of studies using visuospatial WM tasks or right DLPFC stimulation, and direct evaluations of interactions between WM domain and hemisphere are even more rare.

The motivation for the present study was to evaluate the efficacy and durability of multi-session tDCS on visuospatial WM-training performance, with an emphasis on possible interactions between the spatial nature of the training and the laterality of DLPFC stimulation. This was of particular interest to us in light of previous training research demonstrating enhanced transfer effects to visuospatial relative to verbal tasks (Buschkuehl et al. 2008; Jaeggi et al. 2014; Schneiders et al. 2011). We hypothesized a generalized effect of tDCS on improving spatial WM performance, with cumulative gains resulting in a steeper rate of improvement in the stimulated group. Due to the functional lateralization of the DLPFC, we expected the strongest advantage to be in the right DLPFC group. Additionally, our seven-day training schedule, which excluded training on weekends, afforded us a natural opportunity to explore spacing effects in our design, which have been previously reported to positively impact outcomes both in terms of motor excitability with tDCS as well as cognitive training without tDCS (Wang et al. 2014). We predicted that greater training gains would be observed when stimulation was spaced apart by a weekend break compared with consecutive daily sessions. Furthermore, although our principal aims in this study centered on the effects of tDCS on WM training, an auxiliary goal was to assess transfer effects onto untrained visual and verbal WM tasks. Given the brevity of our seven-day training schedule and the fact that our Sham tDCS group also received WM training, we did not expect very pronounced transfer differences between groups. However, to the extent that we found transfer at all, we predicted a selective advantage of right DLPFC stimulation in augmenting performance on visual WM measures. Finally, but no less significantly, we evaluated the durability of training gains at a follow-up session several months after conclusion of training. If tDCS is to have a substantial impact on cognitive training, it is important to demonstrate that the effects of the stimulation are not ephemeral but are long-lasting. In this regard, to anticipate our results, we

find a striking preservation of the effect of stimulation months after the stimulation and training have ceased.

Materials and Methods

Participants

Right-handed individuals between the ages of 18 and 35 were recruited from the campuses of the University of California, Irvine and the University of Michigan, Ann Arbor. Participants were excluded if they had any history of psychological or neurological disorders (including seizures and strokes), previous cognitive training or neurostimulation, past or present drug/alcohol abuse, or if they were taking any medications that would affect attention or memory. Eighty-one individuals were deemed eligible and were recruited to participate. Eleven voluntarily withdrew after consent due to scheduling difficulties, two were excluded for falling asleep during the experiment, four were excluded due to computer errors during data collection, and two were excluded as outliers based on their training data (see Results below). Ultimately 62 healthy, college-aged participants, split evenly between universities, were included in the final sample. All research procedures were approved by the Institutional Review Boards at both universities and each participant provided informed consent.

General Procedure

We used a between-subjects pretest-posttest intervention design and randomized participants into one of three intervention groups (Figure 2a.1). Twenty received active tDCS over the right DLPFC (Active Right), 20 received active tDCS over the left DLPFC (Active Left), and 22 received sham stimulation over either the right or left DLPFC (Sham). All groups received seven days of visuospatial n-back training concurrently with either Active or Sham stimulation. In order to preserve the integrity of blinding, participants were not *a priori* informed about the

existence of a sham group. All participants were simply told that the aim of the study was to investigate the effects of electrical stimulation over the prefrontal cortex to enhance WM training.

During the intervention period, participants attended 7 daily training plus stimulation (or training plus sham) sessions, excluding weekends. Each session lasted approximately 45 minutes, including set-up and clean-up. Duration of stimulation, including sham stimulation, was fixed at 25 minutes. If participants finished the training task early, they were asked to sit quietly until stimulation discontinued. Immediately before stimulation, participants were asked to rate their level of motivation for the study on a 1-10 scale, with 10 being very highly motivated. Upon the conclusion of stimulation, participants were asked to indicate any possible symptoms or side effects they experienced. Immediately before and after stimulation, participants rated their level of alertness on a 1-10 scale, with 10 being most alert. The average alertness rating during each session was used as the dependent variable. All study procedures from pre- to post-test were concluded within 2 business weeks for all participants, with no more than one intervening weekend. Upon conclusion of the study, participants were debriefed about the existence of a sham group and were asked to guess their condition.

All participants were invited back for a follow-up session to examine the stability of training and transfer effects following a long break from the intervention. Forty-one participants returned for the follow-up (14 Active Right, 13 Active Left, and 14 Sham). The mean delay between the final training session and the follow-up was 221 days (range: 97 – 393; SD = 82). During this follow-up session, participants completed a single session of the trained n-back task (without tDCS) as well as the Backward Block Tapping task.

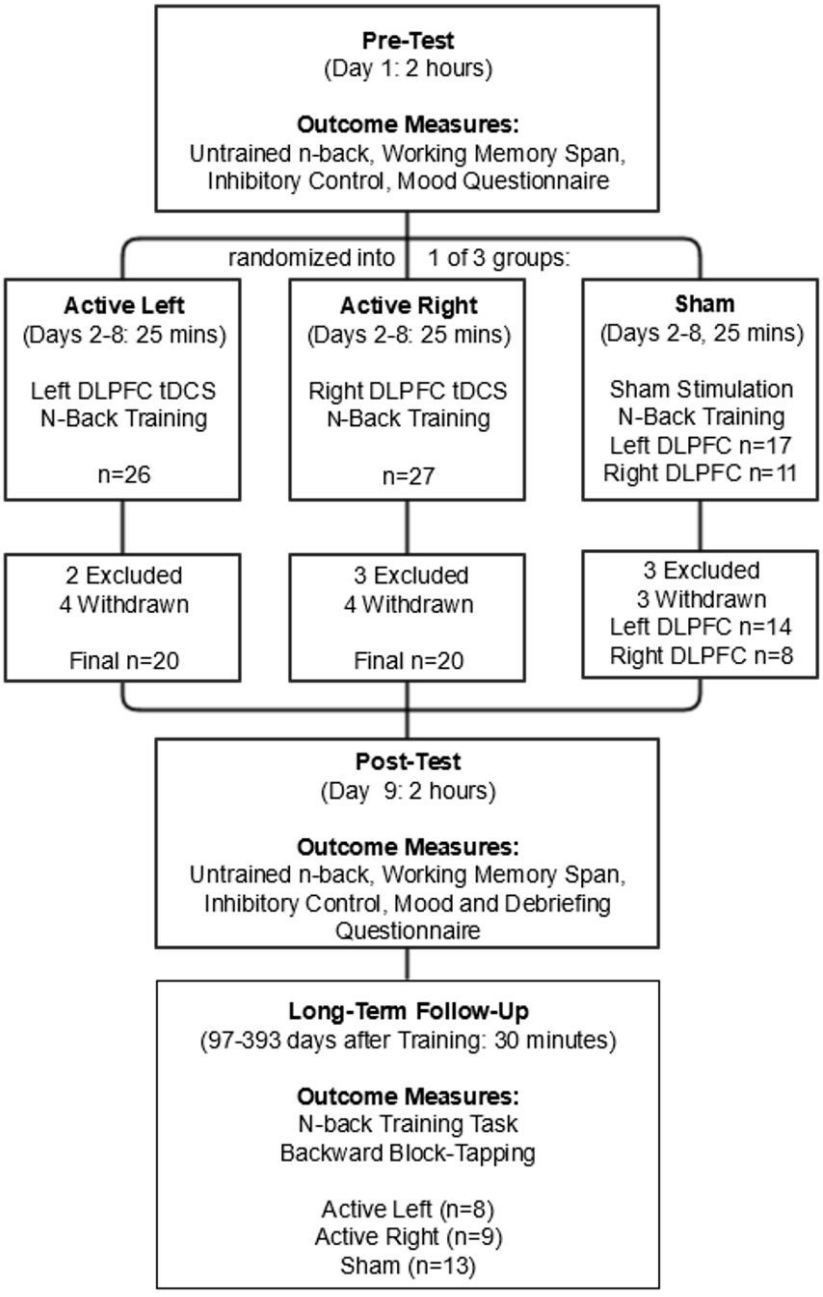


Figure 2a.1: Overall Flow Chart of Study Design and Attrition Rate
DLPFC=Dorsolateral prefrontal cortex; tDCS = transcranial direct current stimulation

Working Memory Training

The training task used was a computerized adaptive version of the visuospatial n-back task used previously (Buschkuehl et al. 2014; Jaeggi et al. 2010, 2014) (see Fig 2a.2). A series of blue squares was displayed, each in one of eight possible spatial locations. Participants were asked to indicate whether the current square was in the same position as the square presented *n* trials ago by responding with the letter "a" to targets and "l" to non-targets, using a standard computer keyboard. The difficulty of the task adapted continuously based on the trainee's performance. Each stimulus was presented for 500ms followed by a blank screen for 2,500ms. A training session consisted of 15 blocks, each with 20+n trials where 6 were targets and 14+n were non-targets. Training duration for one session typically lasted between 20-25 minutes. Accuracy rates of 70% and 90% (inclusive) were used as cut-offs to decrease and increase the level of *n* in the next block, respectively. For the first three training sessions, participants started at a 1-back level, and for the last four and the follow-up session, they started at 2-back. Training performance per session (i.e., the dependent variable) was operationalized as the average n-back level of the last 12 out of 15 blocks. The first three blocks of each session were treated as warm-up blocks and not considered in the analyses.

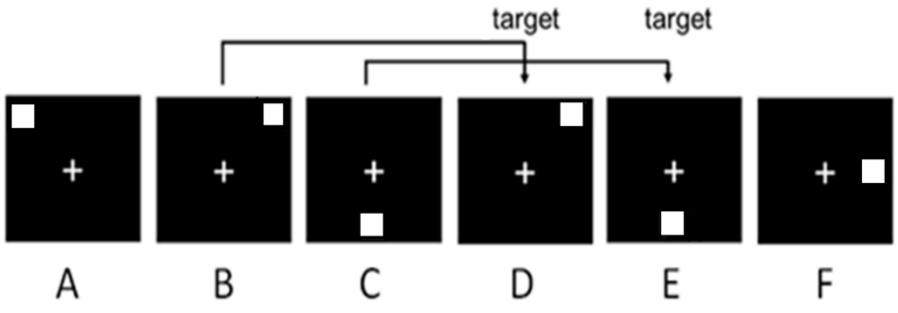


Figure 2a.2: Visualization of the Training Task
A 2-back condition is demonstrated. Trials D and E each match the stimulus presented 2-back ago. All other trials are non-target trials. During training, n-level adapted continuously to participants' fluctuating performance.

Transcranial Direct Current Stimulation

Stimulation was administered via a Soterix Medical 1x1 Low-Intensity tDCS device (Model 1300A) using 5x7cm sponge electrodes placed horizontally on the head. The anode was placed over either right or left DLPFC (sites F4 and F3 in the international 10-20 EEG system) and the cathode was placed over the contralateral supraorbital area (sites Fp1 or Fp2). Sponges were securely fastened to the head using 5" wide Velcro straps that covered the sponges entirely in order to prevent flaring out of sponge edges that can occur with narrower straps, leading to non-uniform skin contact (c.f., Jared C. Horvath et al., 2014). Additionally, the anodal sponge was laterally shifted away from the cathode by approximately three centimeters such that the edge (and not the center) of the sponge lay directly over the target, a set-up that has been suggested to maximize the peak current density underneath the target site (Faria, Hallett, and Miranda 2011). Stimulation lasted 25 minutes, with a current intensity of 2mA, which ramped up and down for the first and last 15 seconds of stimulation. Sham tDCS was set up in exactly the same way, except the current was shut off in-between the 15-second ramping periods at the beginning and end of each session.

Transfer Measures

Pre- and post-testing consisted of outcomes that assessed the generalization of training gains onto untrained variants of the n-back, WM span, and inhibitory control tasks. Each cognitive measure consisted of a short practice round before the actual test; these measures were divided into verbal and visual variants in order to assess interactions of these measures with stimulation site. Additionally, affect was assessed via questionnaire. Pre and post-test sessions lasted approximately 2 hours each, and were administered one day immediately before and after the intervention period.

Affect Rating:

We used the 60-item Positive and Negative Affect Schedule (PANAS; Watson, Clark, & Tellegen, 1988) to assess mood and emotional experience along two dominant dimensions that consistently emerge across studies of affective structure, General Positive Affect (GPA) and General Negative Affect (GNA). Each dimension is measured from the responses to 10 items, and each item is rated on a 1-5 scale in order of increasing valence. Participants were asked to base all responses on emotional experiences within the past week. DLPFC stimulation has been reported to modulate affective symptoms and emotional regulation (Feeser et al. 2014; Shiozawa et al. 2014), which in turn can interact with WM function. Therefore, it is plausible that our training design, whether via overlapping or distinct pathways, could modulate both WM performance as well as affective experience.

Untrained N-Back

In order to evaluate near-transfer training gains, we evaluated performance on untrained variants of the trained visuospatial n-back task, consisting of both an auditory-verbal and a non-spatial visual n-back task. Our previous research with the same training task has demonstrated positive transfer effects both within and across modalities to untrained n-back variants (Buschkuehl et al. 2014; Jaeggi et al. 2010).

In the auditory n-back task, participants were required to process a continuous stream of spoken letters presented through headphones. Difficulty varied sequentially from 2- through 4-back, with three blocks at each level. The visual n-back task consisted of colored and textured circles presented in the center of the screen. Difficulty increased from 2- to 3-back, with 9 blocks at each level. In both n-back variants, stimuli were presented for three-second intervals, with 500ms of presentation and a 2,500ms inter-trial interval, and each block contained 20+n trials.

The primary dependent variable of interest was hit rate minus false alarm rate (Snodgrass and Corwin 1988).

Working Memory Span

Meta-analyses demonstrate robust immediate effects of WM training on a variety of simple and complex span measures (Melby-Lervåg and Hulme 2013; Schwaighofer et al. 2015; Weicker et al. 2016). Similarly, tDCS has been reported to improve performance in Digit Span (Martin et al. 2013; Park et al. 2014) as well as Operation and Symmetry Spans (Richmond et al. 2014). We therefore tested whether our short training regimen could also elicit similar transfer effects.

To measure auditory/verbal span, we administered the Digit-Span task, as per the standardized administration rules used in the WAIS-IV (Wechsler 2008). Trained examiners read aloud a series of digits at a rate of 1 per second and participants were asked to repeat them back verbally in either forward or backward order. Span length increased from three to nine digits in the Forward condition and three to eight digits in the Backward condition, with two trials at each span. Testing was discontinued if a participant missed both trials of a particular span, and the primary dependent variable was the total number of trials correctly repeated.

The Block-Tapping task (Schellig 1993) is a visual analogue of the Digit Span. In our computerized version, nine white squares were displayed and participants were required to reproduce a sequence of positions presented at a rate of 1 per second by clicking in either the given or the backward order. In both the Forward and Backward conditions, span lengths increased from three to nine or until a participant made three consecutive errors. The primary dependent variable was the total number of trials correctly reproduced.

We used parallel-test versions at pre- and post-test for both WM span tasks.

Inhibitory Control

WM and inhibitory control are closely related, sharing neural substrates in the prefrontal cortex (Nee et al. 2013). Therefore, training the former may improve the latter, supported by our previous research (Hsu et al. 2013; Jaeggi et al. 2011; Zhang et al. 2014). In order to assess possible enhancing effects of tDCS, we assessed inhibitory control in two ways. First, we embedded lure trials into our visual n-back task; these were identical to target stimuli except that they were presented in the wrong position (corresponding to trials n±1 back). These lures comprised 33% of total trials and indexed the participant's ability to inhibit inappropriate, but salient, distracters. Performance was measured as the percentage correct among lure trials.

Additionally, we employed the AX-CPT task (Cohen et al. 1999) to directly measure inhibitory control. The task consisted of a continuous stream of letters presented visually on a computer screen for 300ms each with an inter-stimulus interval of 1,000ms. Participants responded to each letter via a button press, but had to make a target response for 70% of trials in which the letter "X" followed the letter "A". While this happened on the majority of "A" trials, creating pre-potent response tendencies, participants had to make a "non-target" response for a small percentage (10%) of trials when "A" was followed by another letter ("AY" trials). The remaining 20% of trials were filler trials. The primary dependent variable was the percentage accuracy during "AY" trials and participants completed 13 blocks of 60 trials each.

Analytical Approach

Statistical analyses were conducted using STATA version 13 (StataCorp 2013). Baseline characteristics between conditions were compared using one-way analyses of variance (ANOVA) for continuous variables and χ^2 tests for categorical variables. To evaluate training effects between groups, as well as potential confounds such as level of alertness and motivation, we ran 3x7 mixed-

design ANOVAs with the between-subjects factor, Condition (Active Right, Active Left, Sham) and the within-subjects factor, Session (1-7). Significant interactions were followed with planned Helmert contrasts to evaluate pairwise differences in gain scores (Session 7 minus Session 1, or Follow-up Session minus Session 1) for the following groups: Combined Active (Active Right and Active Left) vs. Sham in order to evaluate global effects of tDCS, and Active Right vs. Active Left in order to assess potential laterality-dependent effects of stimulation. An additional analysis was run on gains between Sessions 3 and 4, where by the nature of our 9-day design, approximately half our participants experienced an intervening weekend and approximately half trained on consecutive weekdays (Thursday and Friday). This allowed us to evaluate potential effects of spacing on training (Wang et al. 2014).

To evaluate transfer effects, we used analyses of covariance (ANCOVA) to test each post-test or follow-up measure against the factor, Condition, using pretest performance as a covariate. Significant effects were followed up with planned Helmert contrasts to assess pairwise differences in the adjusted post-test means among the Combined Active vs. Sham and Active Right vs. Active Left contrasts.

Planned contrasts were evaluated with one-tailed tests when hypotheses were directional (i.e., Combined Active > Sham and Active Right > Active Left in the context of a visual measure). Two-tailed tests were used for Active Right vs. Active Left contrasts in the context of an auditory or verbal outcome measure, due to the lack of a directed hypothesis.

Results

Outlier Analysis

Outliers were removed from the dataset based on average training performance over all 7 sessions, using a criterion of 2 SD for the combined Active group and the Sham group, separately. This resulted in the identification of two low-performing outliers (1 Active Right and 1 Active Left), both of whom averaged below a 2-back level across all 7 training sessions and whose data were excluded from all subsequent analyses. Additionally, one participant's data (from the Sham group) were lost on the Auditory n-back and AX-CPT tasks due to computer errors. All other data from this participant were included in analyses.

Baseline Characteristics

Demographic and baseline characteristics are presented in Table 2a.1. One-way ANOVAs with the factor, Condition, were calculated separately for each dependent variable: Years of Education, Age, and Baseline n-back Composite (a composite representing the average performance on the untrained Auditory and Visual n-back measures to index baseline WM abilities related to n-back performance). No differences were found between groups on any baseline measure (p's > .27). Additionally, a χ^2 test was run on gender (% women), revealing no significant differences (p=.78).

Table 2a.1: Demographic and baseline information of the three groups.

	Active Right	Active Left	Sham	*p*-value
Years of Education	15.05 (1.88)	15.25 (2.15)	14.32 (1.09)	0.27
Age (years)	20.91 (2.34)	21.55 (2.86)	20.52 (1.93)	0.57
% Women	65	55	64	0.78
Pretest n-back Composite (P_R[α])	0.60 (0.12)	0.65 (0.16)	0.59 (0.16)	0.40

Note: Parentheses are standard deviations. P-values are calculated from one-way ANOVAs for continuous variables and χ^2 test for categorical variables (% Women)

[α] P_R is calculated as percentage hits minus percentage false alarms.

Training Gains

We next sought to test the effects of tDCS on training using a 3x7 mixed ANOVA with the factors, Condition and Session[1]. We found a main effect of Session: $F(6, 354) = 28.65$, $p<.001$, $\eta_p^2 = .33$, a marginal effect of Condition: $F(2, 59) = 2.57$, $p = .08$, $\eta_p^2 = .09$, and importantly, a significant Session x Condition interaction: $F(12, 354) = 2.04$, $p=.02$, $\eta_p^2 = 0.06$.

Planned comparisons (Helmert contrasts) revealed that this interaction was driven by larger gains (Session 7 minus Session 1) in the Combined Active group relative to Sham: $t(60) = 2.86$, $p = .002$ (one-tailed), d = .77, indicating that tDCS was effective in augmenting WM training (see Fig 2a.3). Notably, the contrast between Active Right and Active Left was not significant ($t(38) = -.61$, $p = .27$, one-tailed, d = .20), suggesting that both stimulation groups benefited equally.

Additionally, we verified the homogeneity of participants in the Sham group by comparing the training performance of those who received a left DLPFC montage vs. a right DLPFC montage. Since no current was run through these participants (except for the brief ramp up and down), we expected no differences. This was confirmed with a 2x7 ANOVA with the between-subjects factor, Condition (Sham Left, Sham Right) and the within-subjects factor, Session (1-7), There was a main effect of Session: $F(6,120) = 3.50$, $p = .003$, $\eta_p^2 = .15$, indicating that WM training was equally successful for both groups. However, there was no main effect of Condition: $F(1,20) = .62$, $p = .44$, $\eta_p^2 = .03$), and no Session X Condition interaction: $F(6,120) = 1.24$, $p = .29$, $\eta_p^2 = .06$, confirming the homogeneity of the Sham group.

[1] A separate analysis was run using Site as an additional factor. There was a main effect (p=.04) indicating University of Michigan students outperformed University of California students. This may be associated with demographic differences (e.g., SAT and ACT average) between the two universities (U.S. Department of Education, Institute of Education Sciences [IES], 2014). Critically, however, there was no Session x Site x Condition interaction, indicating similar patterns of improvement across both universities. Due to the lack of this triple interaction, and in order to prevent loss of power by further reducing our sample size, site analyses were not probed further and are not reported.



Figure 2a.3: Training Data across Seven Sessions
Both the Active Left and Active Right groups show significantly greater gains than the Sham group, but comparable gains relative to each other. Y-axis represents average n-back level achieved per session. Error bars represent standard errors.

We also carried out a post-hoc analysis to examine the effect of spacing between training sessions. By design, study visits were constrained to fit into two consecutive weeks, with one intervening weekend. Therefore, most participants had to start the study on either a Monday or Tuesday in order to finish the 9-day study by the following Thursday or Friday. This naturally created a Monday and Tuesday cohort of participants, whose 3rd and 4th sessions respectively fell either on Thursday and Friday (Consecutive group) or on Friday and Monday (Spaced group; See Figure 2a.4a). We therefore compared gain scores over these sessions for both the Consecutive and Spaced groups, separately for Active and Sham participants, to evaluate the effect of a two-day break on training performance. Some participants voluntarily were tested on weekends and were excluded from these analyses because their schedules did not fit the definition of the Consecutive or Spaced group. Since our previous analysis showed that both Active Right and Active Left benefited similarly from the training, we combined the two groups

for this analysis in order to increase power. Among Active participants, we found larger gains in the Spaced group (n = 16) compared to the Consecutive group (n = 15; see Fig. 2a.4): $t(29) = 2.25$, p = .01, one-tailed, d = .82. This pattern was not observed among Sham participants (n = 15 and n=7 for Spaced and Consecutive, respectively): $t(20) = .07$, p = .95, d = .03 (Figure 2a.4b).

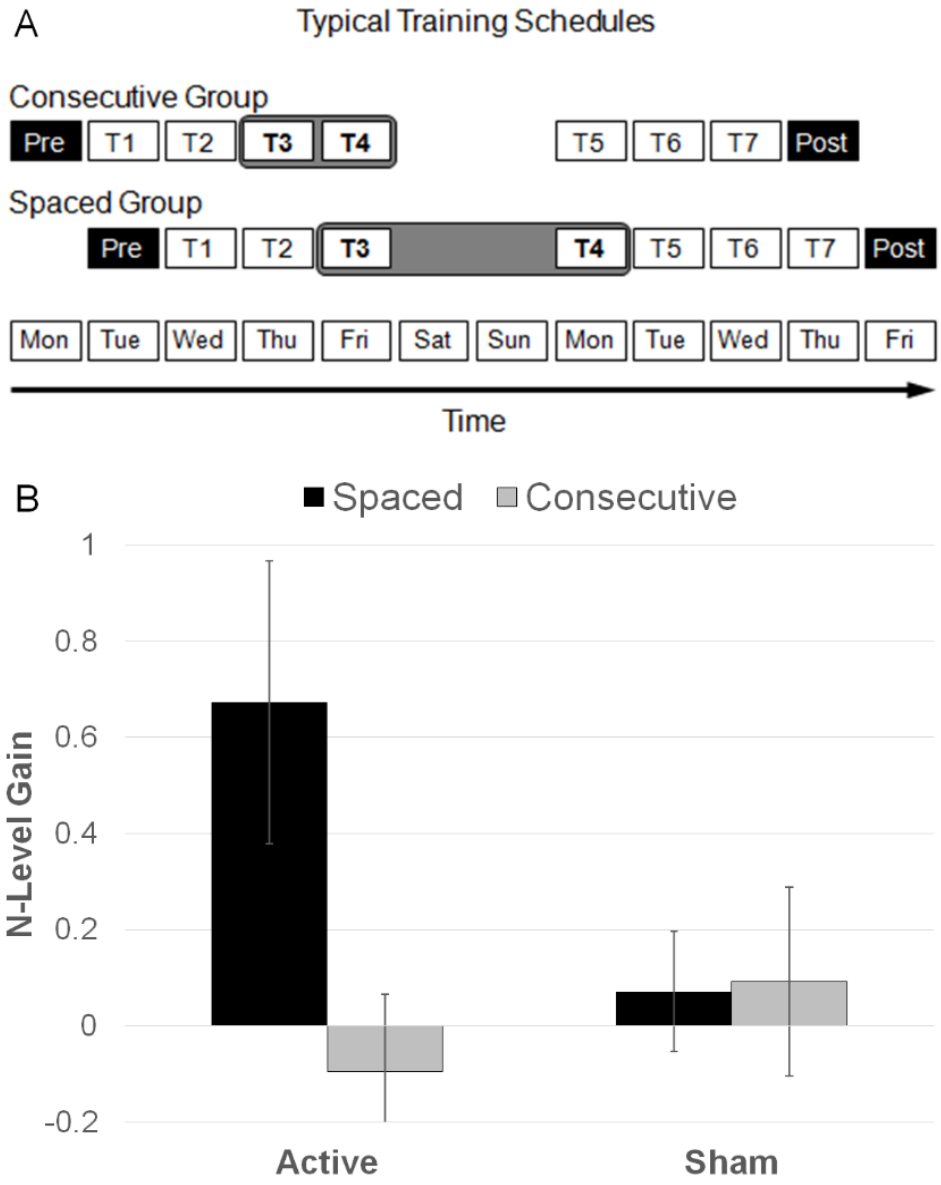


Figure 2a.4: Gain Score Analyses Between Sessions Three and Four
A) Typical training schedules show that sessions 3 and 4 are separated by a weekend in the Spaced group, but occur on consecutive weekdays in the Consecutive group. B) Active participants in the Spaced group (mean ± SD: .67 ± 1.18) have higher gain scores between these sessions than those in the Consecutive (mean ± SD: -.09 ± .62) group. No differences emerge in the Sham group (Spaced mean ± SD: .07 ± .33; Consecutive mean ± SD: .09 ± .76). Error bars represent standard errors.

Alertness and Motivation

In order to rule out certain confounding influences on training, we conducted separate 3x7 ANOVAs for both motivation and alertness, which were assessed by self-report during each training session. For motivation, there was no main effect of Condition, $F(2,349) = .05$, $p = .95$, $\eta_p^2 = .00$, nor of Session, $F(6, 349) = 1.780$, $p = .10$, $\eta_p^2 = .03$, and no Session x Condition interaction, $F(12,349) = 1.39$, $p = .17$, $\eta_p^2 = .05$. For alertness, there was no main effect of Condition, $F(2,349) = .53$, $p = .59$, $\eta_p^2 = .02$, nor of Session, $F(6, 349) = 1.20$, $p = .30$, $\eta_p^2 = .02$, and no Session x Condition interaction, $F(12,349) = 1.34$, $p = .19$, $\eta_p^2 = .05$.

Side Effects and Blinding

Pairwise t-tests showed no significant differences between the Combined Active and Sham groups on any self-reported side effects (i.e., headache, neck pain, scalp pain, tingling, itchiness, hotness, skin redness, sleepiness, trouble concentrating, acute mood changes, nervousness, or changes in visual perception; all ps > .12). Furthermore, after debriefing participants about the existence of a sham condition, participants were unable to reliably guess their condition, with the majority of participants believing they were in the Active condition (83% of Active Right, 65% of Active Left, and 68% of Sham participants; $\chi^2 = 1.72$, $p = .42$). Confidence ratings about condition, made on a -10 to +10 scale also were not significantly different. Negative and positive values represented sham and active guesses, respectively and higher magnitudes indicated higher confidence (mean ± SD: Active Right, 5.00 ± 5.96; Active Left, 2.88 ± 7.41; Sham, 2.95 ± 7.00; $F(2, 59) = 0.56$; $p = 0.57$.)

Transfer Measures

Despite the short timeframe of our 7-day intervention, a duration usually too short to manifest convincing transfer onto untrained outcomes (Jaeggi et al. 2008), we carried out a

provisional analysis to evaluate the potential for tDCS to enhance transfer effects over and above sham WM training. Means, standard deviations, p-values, re-test reliabilities, and effect sizes are presented in Table 2a.2. ANCOVA statistics are presented in Table 2a.3. Significance thresholds were not corrected for multiple comparisons in this provisional analysis, and therefore results should be interpreted as preliminary.

Significant differences were observed for the Visual n-back, Backward Block-Tapping, and Forward Digit Span tasks (see Fig 2a.5 and Table 2a.3). Planned contrasts showed that the adjusted post-test means of the Combined Active group were significantly greater than those of the Sham group in Visual n-back: $t(60) = 2.59$, $p<.01$ (one-tailed), $d = .70$, but the effect was only marginal in Backward Block-Tapping: $t(60) = 1.61$, $p =. 06$ (one-tailed), $d = .43$, and absent in the Forward Digit Span: $t(60) = .86$, $p = .23$ (one-tailed).

However, the Active Right vs Active Left contrasts revealed significant differences in all three tests: Visual n-back: $t(38) = 2.38$, $p = .01$ (one-tailed), $d = .77$; Backward Block-Tapping: $t(38) = 2.26$, $p = .01$ (one-tailed), $d = .73$; Forward Digit Span: $t(38) =- 2.46$, $p =. 02$, $d = -.80$. The first two tasks favored Active Right while the Forward Digit Span favored Active Left. In all three tasks, the non-favored stimulation group performed comparably to the Sham group (Fig 2a.5 and Table 2a.2), thus obscuring effects in the Combined Active vs. Sham analysis. Of note, Active Right also outperformed Sham ($t(40) = 2.53$, $p = .01$ (one-tailed), $d = .80$) in the Backward Block-Tapping task, but Active Left did not improve significantly relative to Sham in the Forward Digit Span ($t(40) = 1.49$, $p = .07$ (one-tailed), $d = .48$).

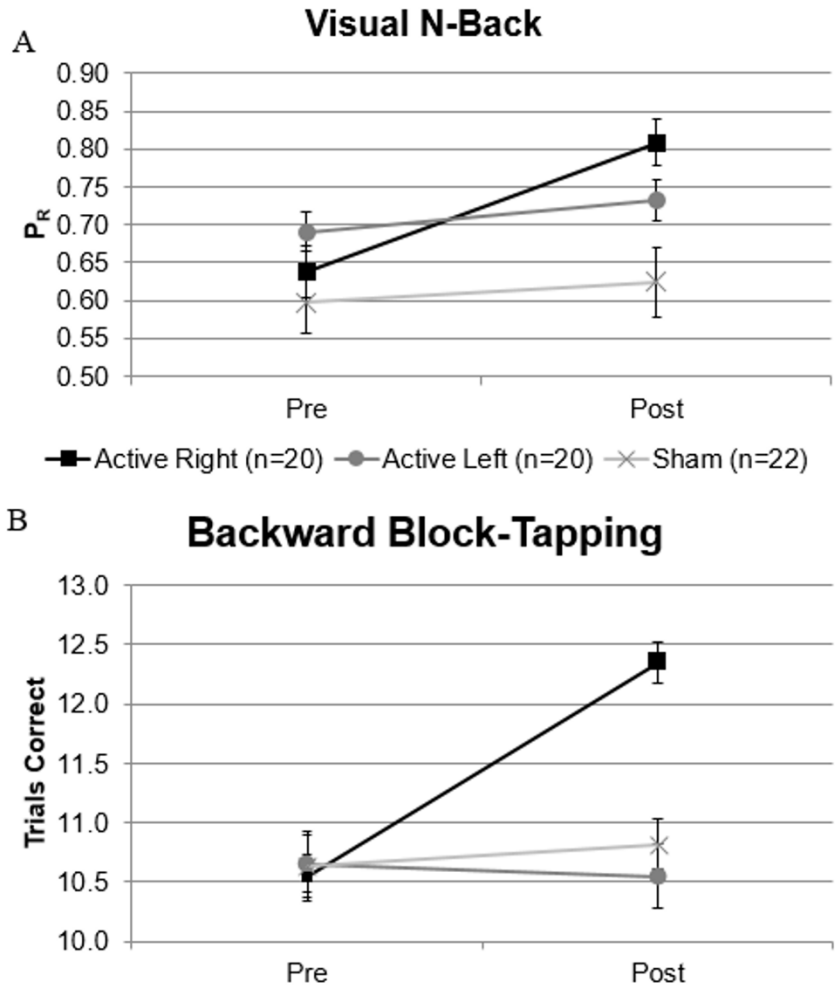


Figure 2a.5: Transfer to Visual Working Memory
A) Y-axis is measured as P_R , the hit rate minus false alarm rate. B) Y-axis refers to the total number of correct trials. Maximum possible score is 21. Both transfer measures show significant improvement in the Active Right condition, compared to both the Sham and Active Left conditions.

Table 2a.2: Descriptive Data of Training and Transfer Measures

	Active Right (N=20)				Active Left (N=20)				Sham (N=22)*			
	Pre	Post	r	d	Pre	Post	r	d	Pre	Post	r	d
Trained N-Back												
N-Level	3.68 (.80)	5.28 (1.39)	.56	1.30	4.07 (1.17)	5.48 (1.35)	.66	1.10	3.77 (1.21)	4.52 (1.77)	.75	.45
Untrained N-Back (P_R)												
Auditory N-Back	.57 (.12)	.69 (.13)	.18	1.01	.61 (.17)	.72 (.16)	.69	.64	.58 (.15)	.66 (.16)	.59	.53
Visual N-Back	.64 (.16)	.81 (.14)	.44	1.14	.69 (.17)	.73 (.19)	.79	.23	.60 (.20)	.62 (.22)	.60	.13
Forward Digit Span	8.90 (1.97)	8.60 (2.33)	.34	-.14	9.15 (2.56)	10.30 (2.47)	.80	.46	8.41 (2.38)	8.60 (2.63)	.64	.07
Backward Digit Span	5.90 (2.02)	6.20 (2.42)	.76	.13	7.35 (2.64)	7.35 (2.98)	.62	.00	5.36 (2.11)	5.41 (1.74)	.66	.02
Forward Block-Tapping	12.70 (2.40)	12.80 (2.09)	.27	.04	12.25 (2.02)	12.90 (2.83)	.38	.26	12.45 (3.10)	12.91 (2.56)	.55	.16
Backward Block-Tapping	10.55 (2.26)	12.35 (1.95)	.45	.85	10.65 (3.03)	10.55 (2.91)	.23	-.03	10.64 (3.13)	10.82 (2.13)	.53	.07
Inhibitory Control (% Accuracy)												
Visual Lures	.78 (.12)	.91 (.14)	.43	.98	.84 (.11)	.92 (.05)	.24	.95	.72 (.17)	.83 (.16)	.81	.62
AX-CPT	.68 (.20)	.66 (.14)	.45	-.09	.64 (.19)	.61 (.18)	.70	-.17	.60 (.17)	.61 (.22)	.70	.07
PANAS Scales												
Positive Affect	31.61 (5.90)	29.83 (6.42)	.68	-.29	31.47 (10.45)	30.39 (9.87)	.85	-.11	32.41 (6.40)	28.14 (5.55)	.43	-.71
Negative Affect	15.78 (4.73)	14.78 (2.96)	.52	-.24	17.26 (6.93)	17.32 (6.11)	.81	.01	18.59 (6.42)	17.23 (5.94)	.52	-.22

Note: Values in parentheses are standard deviations. r = correlation between pre- and post-test. For the trained n-back, pre and post refer to Session 1 and Session 7, respectively. *All n's for Sham = 22 except for Auditory n-back and AX-CPT where n=21.

Cohen's d effect sizes for correlated samples were calculated as: $(Mean_{Post} - Mean_{Pre}) / \frac{\sqrt{SD^2_{Pre} + SD^2_{Post} - 2r * SD_{Pre} * SD_{Post})}}{\sqrt{2(1-r)}}$.

Table 2a.3: ANCOVA Results of Transfer Measures

	Condition			Active vs. Sham			Right vs. Left		
Outcome Measure	*F*(2,58)	*p*	η_p^2	*t(60)*	*p*	*d*	*t(38)*	*p*	*d*
Auditory n-back	.57[α]	.57	.02	1.07	.15	.29	.01	.99[┼]	.00
Visual n-back	**6.33**	**<.01**	**.18**	**2.59**	**<.01**	.70	**2.38**	**.01**	.77
Forward Digit Span	**3.38**	**.04**	**.10**	.86	.20	.23	**-2.46**	**.03**[┼]	-.80
Backward Digit Span	.47	.63	.02	.95	.17	.26	-.22	.83[┼]	-.07
Forward Block-Tapping	.61	.54	.02	.51	.31	.14	-.98	.17	-.32
Backward Block-Tapping	**8.74**	**<.01**	**.12**	1.61	.06	.43	**2.26**	**.01**	.73
Visual Lures	1.26	.15	.04	1.44	.08	.39	.60	.28	.19
AX-CPT[α]	.28	.75	.01	.35	.36	.09	.66	.51[┼]	.21
Positive Affect[β]	1.74	.19	.06	**1.82**	**.04**	.49	.38	.71[┼]	.12
Negative Affect[β]	.81	.45	.03	.04	.48	.01	1.27	.21[┼]	.41

Note. Significant effects are bolded. Planned contrasts are reported for all outcomes in this table, but interpretations in the text are only based on outcomes where the Condition factor is significant. Cohen's d effect sizes, which represent adjusted post-test means between groups, differ from the within-group effect sizes reported in Table 2a.2.
[α] Within-Subject *df* = 52, [┼]two-tailed test, otherwise one-tailed

Follow-up Effects

Due to the promising effects we demonstrated on the trained n-back task and Backward Block-Tapping, we decided to conduct a follow-up analysis to assess the long-term stability of training and transfer effects. Approximately three months after the last participant completed the study, we invited all participants to return to the laboratory to complete an abbreviated battery consisting of just those tasks for which we observed the strongest effects immediately after training completion. 14 Active Right, 13 Active Left, and 14 Sham participants returned.

Although the time lag between the end of training and the follow-up assessment was variable among participants, ranging from 97 – 393 days, there was no significant difference between groups: Combined Active (mean ± SD; 207.48 ± 79.11), Sham (mean ± SD; 246.43 ± 82.78), t = 1.47, p = .15. An ANCOVA revealed a significant effect in favor of the Combined Active group on the trained n-back task by comparing gain scores from the 1st to the 8th (follow-up) session, controlling for time lag (see Fig 2a.6): $F(2, 37) = 4.03$, $p = .03$, $\eta_p^2 = .18$, $d = 1.04$. No effect was observed on the time lag covariate: $F(1, 37) = 0.001$, $p = .99$, $\eta_p^2 < .001$ and similarly

to the original seven training sessions, no effect was observed between Active Left and Active Right groups: $F(1, 24) = .04$, p=.84, $\eta_p^2 = .002$, d = .16; time lag covariate: $F(1, 24) = .27$, p=.61, $\eta_p^2 = .01$.

No effects were observed for follow-up scores on the Backward Block-Tapping task controlling for pretest performance and time lag: $F(1, 37) = .08$, p = .78, $\eta_p^2 = .002$; pretest covariate: $F(1, 37) = 17.19$, p <.001, $\eta_p^2 = .32$; time lag covariate: $F(1, 37) = 0.001$, p = .99, $\eta_p^2 < .001$.



Figure 2a.6: Follow-up Results
Plot of gain from baseline in Active versus Sham group at follow-up. The Active group maintained significantly greater performance relative to the Sham group at follow-up. Error bars represent standard error.

Discussion

The primary finding of this study is that tDCS was successful in enhancing the WM training performance of healthy, young adults and is potentially a useful tool to supplement n-back training interventions. These enhancements were more pronounced when training sessions were spaced apart by a weekend, and the enhanced effect due to stimulation was maintained for several months after training completion. Furthermore, our results cannot be explained by circumstantial factors such as baseline demographics, level of alertness, motivation, or mood, which were well-matched between groups, and all participants were led to believe they received active stimulation (i.e., they were blind to the existence of a sham group). Even after being debriefed upon conclusion of the study, participants could not reliably distinguish their condition, with no significant differences in their guesses or reported levels of confidence about stimulation condition. Moreover, there was also no significant difference between the Combined Active and Sham groups on self-reported side effects.

Although WM enhancement with tDCS has been extensively explored before (Jantz, Katz, & Reuter-Lorenz, in press), yielding inconsistent results across studies that nevertheless aggregate into a small to moderate positive net effect (Hill et al. 2016; Summers et al. 2016), the present study contributes to a nascent literature exploring the use of tDCS in multi-session training paradigms. These studies are an important departure from previous single-session experiments in that they allow for the potential of tDCS effects on between-session learning to manifest. In contrast to previous WM training studies (Martin et al. 2014; Richmond et al. 2014), we provide the first evidence that tDCS can enhance the rate of learning between training sessions. Our ANOVA showed a significant Condition x Session interaction supporting a steeper rate of improvement in the combined Active group relative to Sham. This is in contrast to Martin et al.

(2014) who failed to find significant differences between groups when baseline performance was controlled. And although Richmond et al. (2014) were more successful in demonstrating group differences in verbal WM training after left DLPFC stimulation, they found only a main effect but no interaction, thereby demonstrating an enhanced (upwards-shifted) learning curve with no difference in learning rate.

Several key differences may explain these discrepant results. First, Martin et al. (2014) used a more difficult dual n-back task that typically shows shallower improvement curves relative to single n-back (Jaeggi et al. 2010). This may have restricted their ability to discriminate a differential learning rate relative to sham controls. Richmond et al. (2014), on the other hand, chose to stimulate mostly offline, with only 5 minutes of overlap between stimulation and commencement of training, arguing that the effects of tDCS typically last well beyond the stimulation period itself. Although this argument is supported by some previous research, we note that online and offline effects likely operate via different mechanisms (Stagg and Nitsche 2011), and that the nature of cognitive activity during stimulation may influence the later effects of tDCS (Bikson et al. 2013; Gill, Shah-Basak, and Hamilton 2015). For example, it is thought that online effects operate mainly via membrane depolarization while offline effects are thought to rely on a combination of membrane depolarization and LTP-like plasticity (Stagg and Nitsche 2011). Therefore, online stimulation which promotes targeted activation of task-relevant regions may also selectively facilitate later LTP-like plasticity in neuronal populations of interest. In fact, reports confirm online stimulation is superior to offline both in increasing cortical perfusion during stimulation (Stagg et al. 2013) as well as enhancing between-session consolidation of learning (Martin et al. 2014). Therefore, online stimulation may have played an important role in manifesting between-session learning effects in our study that Richmond et al. (2014) failed to

detect. These effects are not unusual in light of evidence from motor cortex demonstrating cumulative effects from daily stimulation (Alonzo et al. 2012; Boggio et al. 2007; Reis et al. 2009). Accordingly, we point out that there are no differences between our Active and Sham groups on Training Day 1 but differences gradually became more pronounced each day until reaching significance midweek (see Fig. 2a.3). Additionally, our results suggest that these differences are durable and manifest at follow-up even several months post-training; the average follow-up time was seven to eight months after the initial intervention. Coupled with our finding of spacing effects after a weekend break from training, our study implicates an important role for tDCS in learning and consolidation.

Furthermore, an interesting finding in our data is that although tDCS provided a general benefit on the training task, irrespective of stimulated hemisphere, our transfer results suggested selective improvement by right DLPFC stimulation on tasks with a visual and/or spatial component. This latter finding is in line with our hypothesis, and fits with evidence of a left/right hemispheric dissociation for verbal and visual WM function, respectively. Moreover, we also found modest evidence for a left DLPFC advantage on the Forward Digit Span task (pre to post gains: d=.46), although this result should be interpreted with caution: while the contrast against Active Right was significant, the contrast against Sham was not. However, this does provide a nice complement to the finding by Richmond et al. (2014) that left DLPFC stimulation enhanced verbal WM training, but not spatial. Although these results in combination are suggestive of a role for tDCS in strategically targeting the functional neuroanatomy of the brain (Bikson et al. 2013), this argument is severely hampered by the lack of functional specificity in our training data, where both Active tDCS groups improved comparably despite the visuospatial nature of the training. Future studies therefore should seek to further elucidate a potential dissociation between left and

right prefrontal stimulation in terms of verbal and visual WM tasks, using well-designed visual and verbal test batteries that can verify this effect at the level of latent constructs. Until then, our transfer results should be interpreted as preliminary.

Limitations

Although our study lends great support to the efficacy of tDCS in enhancing learning during WM training, the lack of functional specificity in the training data cannot rule out the alternative hypothesis that stimulation effects are general and not related to the DLPFC. For example, there is a large literature on tDCS-induced motor effects (Hashemirad et al. 2016; Summers et al. 2016), and it is known that tDCS induces wide-spread perfusion changes across the brain (Stagg et al. 2013), which may inadvertently excite motor areas that can confound improved cognitive performance with increased motoric priming/readiness. While we cannot definitively exclude this possibility, previous research has shown that stimulation of the DLPFC but not motor cortex improves WM performance (Boggio et al. 2007; Fregni et al. 2005), and also that multi-session stimulation to the DLPFC over two weeks can improve cognitive, but not motor, skills in Parkinsonian patients (Doruk et al. 2014). Together, this suggests that the effects of stimulation to prefrontal and motor cortices operate fairly independently of one another.

Additionally, we point out that our finding of sustained effects is limited by potential self-selection biases with regard to the individuals who were willing to come back again for a follow-up months after completing the initial study. Furthermore, due to the post-hoc nature of the follow-up, the sham participants had already been un-blinded. However, since neither group received tDCS at follow-up, and expectations of sustained effects after such a long interval (in most cases, over half a year) were likely muted, we argue that placebo effects at follow-up may not have played a very substantial role. Nevertheless, future studies should test the permanence of tDCS learning

effects more rigorously by implementing a more standardized follow-up protocol for all participants.

Another limitation of our study concerns our provisional transfer results. Only three of the eight comparisons among our cognitive transfer measures revealed significant effects between groups (Visual N-back, Backwards Block-Tapping, and Forwards Digit Span). Although all of our measures were theoretically grounded in previous literature, our results are not immune to issues of multiple comparisons. Nevertheless, the percentage of significant results (3/8 = 38%) exceeds the false discovery rate, which assumes that 5% of our transfer measures would suffer from Type I errors based on our threshold for significance testing ($p<.05$). Moreover, our findings of improvement selectively on the visual n-back and Block Tapping tasks in the Active Right group are theoretically justified in that they align with both our hypothesis of selective visual benefits as well as previous WM training results which have unanimously demonstrated transfer to either an n-back or WM span task as a result of tDCS (Jones, Stephens, et al. 2015; Martin et al. 2013; Park et al. 2014; Richmond et al. 2014).

An important caveat to our visual WM transfer findings is that we did not detect any effects in the Forward version of Block Tapping, despite our findings with the Backward version. This is not necessarily surprising in that the Forward and Backward versions do not share identical properties. The latter tends to be more difficult and participants perform worse, as evidenced both in our study (Table 2a.2) as well as others (Monaco et al. 2013). Backward Block-Tapping may therefore involve more central executive resources in addition to short-term retention of visuospatial information (Vandierendonck et al. 2004). Consequently, it may share more overlapping properties with the trained n-back task than the forward version. Moreover, selective

tDCS effects on only the backward version of Block-Tapping have been reported before (Wu et al. 2014).

Conclusion

This study successfully demonstrated that tDCS can durably enhance the performance curve of n-back training studies, and therefore is a promising adjunctive tool to use in WM training interventions. Moreover, this enhancement was even more pronounced when stimulation sessions were separated by a weekend break, and we also exhibited some preliminary success in demonstrating selective transfer with right DLPFC stimulation on measures of visual or spatial WM. These results are meaningful amidst the controversy surrounding the efficacy of WM training, particularly with respect to far transfer, which is often found in meta-analyses (Au et al. 2015; Karbach and Verhaeghen 2014; Schwaighofer et al. 2015; Weicker et al. 2016) but not consistently among primary studies. However, given the small meta-analytic effects on domains such as attention, reasoning, and executive functioning, individual studies are often underpowered with respect to far transfer (Bogg and Lasecki 2015). Since recruiting and maintaining large samples over an extended period of time can be logistically challenging for many training studies, the prospect that tDCS might strengthen these effects would go a long way toward overcoming these issues and allow more reliable investigations into the true benefits and limitations of cognitive training interventions. Finally, since most n-back training studies employ considerably more than seven sessions, a time period too short to allow most participants to reach their individual ceilings, an enticing open question is whether tDCS merely facilitates reaching ceiling more quickly, or whether it can actually raise this ceiling relative to sham with more training sessions.

Part B: Individual Differences and Long-term Consequences of tDCS-augmented Cognitive Training

Introduction

Given the importance of working memory (WM) for success in a wide variety of real-life contexts, including school (Alloway and Alloway 2009) and work (Higgins et al. 2007), it is unsurprising that a variety of WM interventions have been proposed in recent years. Transcranial direct current stimulation (tDCS) and cognitive training are two cognitive enhancement techniques that have recently been used together to improve WM, with promising, but by no means conclusive, results. A recent meta-analysis from Mancuso et al. (2016) suggests that dorsolateral prefrontal cortex (DLPFC) stimulation during training results in a small but significant enhancement effect, which survives corrections for publication bias. Recent research from our own laboratory (Au et al. 2016) provides further evidence that DLPFC stimulation (both right and left) enhances performance on a widely used *n*-back training task over the course of seven sessions, relative to a Sham stimulation condition. While these initial findings do provide some preliminary support for the use of tDCS to enhance learning of WM-intensive tasks, we note considerable heterogeneity in the literature. For example, a similarly designed n-back/tDCS training study failed to find an effect of tDCS after correcting for baseline differences (Martin et al. 2013), and the ten tDCS/WM training studies covered in the Mancuso et al. (2016) meta-analysis differ substantially in the magnitude of their effects, with Hedges' g values ranging from 0.074 to 0.565. A variety of factors, including differences in stimulation intensity, density, location, and other parameters, as well as the design and implementation of the cognitive training paradigm, may explain the disparities in the strength of these effects (see Au et al., 2016

for a brief discussion). However, one additional possibility is that individual differences among participants – including motivation, gender, and baseline ability, among many factors – may play important roles. These factors may influence the outcome of the combined intervention in their own right, but they may also be associated with other individual-difference characteristics that influence performance (for example, different geographic training locations may be confounded with educational background). While extant research does suggest that individual differences play a significant role in both tDCS interventions (Krause and Cohen Kadosh 2014) and cognitive training interventions (Jaeggi et al. 2014; Katz et al. 2014) by themselves, these factors have rarely been investigated directly in studies that combine both interventions.

Baseline performance and other individual difference factors in tDCS

Studies by Wiethoff, Hamada, and Rothwell (2014) and Lopez-Alonso et al. (2014) have found that even in tDCS experiments that successfully demonstrate an effect on cognition overall, less than half of the participants demonstrate improved performance. This suggests that a considerable proportion of participants in each study may not be responding to the treatment. Additionally, recent work using cadavers has raised controversy about the previously dominant neural explanation for tDCS-related cognitive enhancement (Underwood 2016). While the consensus thus far has been that anodal stimulation causes depolarization of the resting membrane potential, facilitating the production of action potentials, Buzsáki's work with cadavers questions the amount of current that actually reaches the cortex. Thus it is possible that certain individual physical characteristics could have a larger effect than expected previously. For example, even something as seemingly minor as hair thickness may impact electrode contact and further reduce the amount of current passing through the scalp and skull. However, several individual-difference factors have been studied in conjunction with tDCS prior to Buzsáki's

provocative findings. Kraus and Cohen Kadosh (2014) suggested that age, gender, and neuronal factors, namely regional cortical excitability, may influence the effectiveness of transcranial electrical stimulation. For example, it has been proposed that an optimal balance of excitation/inhibition in different cortical regions promotes optimal cognitive functioning. Therefore, tDCS may exert different and sometimes contradictory effects in populations that vary with respect to this balance, such as those with ADHD or depression (Krause et al. 2013). Furthermore, genetic factors (Brunoni et al. 2013; Plewnia et al. 2013) and anatomical differences that impact the electric field generated by tDCS (Kim et al. 2014) may also influence the response to stimulation.

In addition to these physiological characteristics, it is also possible that psychological characteristics, such as baseline cognitive ability, may influence the outcome of stimulation. Several studies have demonstrated selective tDCS benefits among individuals with low, but not high, baseline WM abilities (Gözenman and Berryhill 2016; Heinen et al. 2016; Tseng et al. 2012), and meta-analyses tend to report stronger effect sizes in clinical or older adult populations compared to the higher-performing young adult population (Dedoncker et al. 2016; Hill et al. 2016; Hsu et al. 2015; Summers et al. 2016). Moreover, the evidence extends beyond the WM domain. Individuals with poorer motor coordination (McCambridge et al. 2011; Uehara, Coxon, and Byblow 2015), postural control (Zhou et al. 2015), visual acuity (Reinhart et al. 2016), and attention (London and Slagter 2015; Sikström et al. 2016) all showed improvement in the relevant domains while their higher-performing peers did not. However, it should be noted that these low-baseline effects are not found universally. One group of researchers has repeatedly found an advantage for high-baseline individuals on WM performance during parietal stimulation (Berryhill and Jones 2012; Jones and Berryhill 2012; Jones, Gözenman, and

Berryhill 2015), which has been replicated by others (Learmonth et al. 2015). Another group examining lateralized attention bias found both high- and low-baseline advantages in two separate experiments, but the direction of this advantage depended critically on stimulation intensity (Benwell et al. 2015). Therefore, there is no consensus on the influence of baseline performance at present. Also, there are likely even more nuanced issues to consider, such as the brain region stimulated and task-specific optimum levels of neural activity. Thus, there is considerable value in studying tDCS effects in the context of baseline ability, as well as other individual difference factors.

Baseline performance and other individual difference factors in working memory training

Some research has also been done to examine the effects of individual difference factors in the outcome of WM training by itself, unaided by tDCS. For example, baseline performance has also been studied in this context, and much like in the tDCS literature, there is also evidence that baseline WM abilities could impact training performance in two possible directions. Some have suggested that individuals with a lower baseline score should have more room to improve at the trained task during the intervention; for example, Zinke and colleagues have demonstrated this through two studies with older adults (Zinke et al. 2012, 2014). Others have posited that individuals with higher baseline WM performance are better prepared to take advantage of the intervention and thus improve more throughout the training (Lövdén et al. 2010). There is not yet consensus regarding the impact of baseline performance for the outcome of cognitive training; it also remains possible that ceiling effects and differences in the design of the training intervention itself may also influence the relationship between starting WM ability and level of improvement in any individual study.

A variety of other individual-difference factors have also been discussed in the context of cognitive training. For example, motivation to complete a task may influence how receptive one is to a training intervention (Jaeggi et al. 2011, 2014). Many interventions include game-like elements that may influence participant motivation as well as their performance on the task (Katz et al. 2014; Prins et al. 2011). Additionally, many training studies provide considerable monetary remuneration for participation, and it is possible that this payment may undermine motivation and thus impact overall performance (Au et al. 2015). As mentioned earlier, the study location (Au et al. 2015) may influence the outcome of training, although it is difficult to identify which element of geographic location, including cultural factors, actually may play a role in performance. Age has also been studied extensively as a factor that may determine performance on cognitive training tasks. In general, older individuals seem to improve less on untrained tasks administered at pre- and post-test, as well as on the training task itself (Borella et al. 2013; Brehmer, Westerberg, and Bäckman 2012; Schmiedek 2010; Zinke et al. 2014). Although one meta-analysis found no differences in transfer improvements as a function of age (Karbach and Verhaeghen 2014), another meta-analysis with a larger range of ages found that younger adults improved more on untrained tasks than older adults (Wass, Scerif, and Johnson 2012). These age-related disparities make some sense given well-established differences in age-related WM performance (Park et al. 2002) and theoretical perspective on cognitive plasticity and aging (Lövdén et al. 2010). However, it remains unknown whether age-related differences in cognitive training performance are due to differences in baseline performance or other factors related to aging. Finally, traits such as conscientiousness and neuroticism (Studer-Luethi et al. 2012; Studer-Luethi, Bauer, and Perrig 2016) may also impact the outcome of training, while other factors, such as gender, have been found to influence the outcome of training in some studies

(Söderqvist et al. 2012) but not others (Klingberg et al. 2005). It remains possible that a number of other factors that have been largely unexplored (Segretin et al. 2014) may play a role, at least in some interventions.

Given the relevance of individual-difference factors to the outcome of cognitive training and tDCS independently, a salient question is how these individual-difference factors influence combined interventions featuring both tDCS and WM training together. It is possible, and perhaps even likely, that there are interactions between these two interventions such that some individual-difference factors matter more than others, particularly in the outcome of a combined intervention. For example, in light of the evidence that baseline cognitive ability impacts both the amount one is able to improve during a training intervention and the participant's response to tDCS, it is possible that it will play a much larger role in a combined intervention. The relative paucity of tDCS-augmented cognitive training studies means that it is unsurprising that these factors have not yet been explored in combined interventions. However, given the possibility that they may play a substantial role in the outcomes of such interventions, there is considerable impetus for studying them. Thus, the present paper uses a recently published dataset of tDCS and WM training data to evaluate the influence of individual differences including baseline performance, motivation, gender, and geographic training location on WM training performance.

As illustrated above, individual differences are one topic of relevance to improving our understanding of why stimulation-augmented cognitive training may be effective for any individual participant. Another point of significant practical importance is how durable training improvements may be over the weeks and months following the intervention. It would likely not make sense to utilize tDCS/WM interventions in real-world applications if the improvements generated by the stimulation dissipated shortly after the intervention. While research from our

own laboratory suggests that there is durability even several months following the intervention (Au et al. 2016), little extant tDCS work examines the stability of improvements over time, and results from WM training research suggest that washout may be a common occurrence within a short time following a training intervention (Melby-Lervåg and Hulme 2013). By contrast, some studies suggest that improvements following tDCS interventions may remain weeks or even months following the stimulation. Jeon et al. (2012), Jones et al. (2015), and Park et al. (2014) all found continued improvements to WM performance from a week to two months following stimulation. Persistent, long-term changes have also been detected as a function of learning or training in other domains as well, such as motor-skill training (Reis et al. 2009), math training (Looi et al. 2016), and episodic-memory retrieval (Manenti et al. 2016). However, to our knowledge, no other study of combined tDCS and cognitive training has examined whether these follow-up effects are maintained for time periods in excess of two to three months after the intervention. In the present paper we added to the follow-up findings from Au et al. (2016), including new data not previously reported in which participants returned an average of 12 months following the intervention to complete one more session of the WM training (without stimulation).

Methods

Participants

Our dataset comprised largely the same participants as that of Au et al. (2016), which recruited healthy, right-handed individuals between the ages of 18 and 35 as part of a collaborative effort from the campuses of the University of California, Irvine (UCI) and the University of Michigan, Ann Arbor (UM). Six additional individuals completed study procedures subsequent to the previous report, one of whom was excluded as an outlier (see Results), for a total sample size

of 67 in the current dataset. As before, participants were excluded if they had had any history of psychological or neurological disorders (including seizures or strokes), previous cognitive training or neurostimulation, past or present drug/alcohol abuse, or if they were taking any medications that would affect attention or memory. All research procedures were approved by the Institutional Review Boards at both universities, and each participant provided informed consent.

General Procedure

The experiment, an extension of our previous report (Au et al. 2016), consisted of a between-subjects pretest-posttest intervention design in which participants were randomized into one of two groups. Forty received Active tDCS (Active group) over the right or left DLPFC and 27 received Sham stimulation (Sham group) to the same regions in which current was turned off after the first 30 seconds without the participants' knowledge. Our previous report analyzed the right and left DLPFC groups separately in the Active condition, but since we found no differences in the training effect, they are collapsed together in the present report. Both groups completed seven days of visuospatial n-back training concurrently with either Active or Sham stimulation.

After the initial training period, all participants were invited back for two follow-up sessions to examine the stability of training effects. Forty-one participants returned for the first follow-up (27 Active and 14 Sham), as reported previously (Au et al. 2016), and 26 participants returned for the second follow-up in the present study (18 Active and 8 Sham). Due to the long delay, the follow-up visits were marred by substantial attrition rates, but 25 of the 26 participants in the second follow-up also participated in the first follow-up, thereby allowing us to evaluate the longitudinal trajectory of a stable cohort of individuals. The mean delay after the initial training period was 221 days (range: 97 – 393; SD = 82) for the first follow-up and 355 days

(range: 251 - 471; SD=73) for the second follow-up. Maintenance of transfer effects was not evaluated at this second follow-up due to the lack of sustained transfer during the first follow-up.

Working Memory Training

The training task was a computerized adaptive visuospatial n-back task in which a series of blue squares was displayed one at a time, each in one of eight possible spatial locations. Participants were asked to indicate whether the current square was in the same position as the square presented *n* trials ago by responding with the letter "A" to targets and "L" to non-targets, using a standard computer keyboard. The difficulty of the task adapted continuously based on the trainee's performance. The average n-back level at which a participant trained was calculated each day, and the primary dependent variable for analysis was the logarithmic slope of the 7-session training curve. Further details regarding the design of the training task can be found in Au et al. (2016).

Transcranial Direct Current Stimulation

Stimulation was administered via a Soterix Medical 1x1 Low-Intensity tDCS device (Model 1300A) using 5x7cm sponge electrodes placed horizontally on the head. The anode was placed over either right or left DLPFC (sites F4 and F3 in the international 10-20 EEG system) and the cathode was placed over the contralateral supraorbital area (sites Fp1 or Fp2). Stimulation lasted 25 minutes, with a current intensity of 2mA, which ramped up and down for the first and last 30 seconds of stimulation. Sham tDCS was set up in exactly the same way, except that the current was shut off between the 30-second ramping periods at the beginning and end of each session.

Individual Difference Variables

Baseline: Baseline score for each participant was determined by his/her visual n-back score at pre-test, measured as P_r, the proportion of hits minus the proportion of false alarms (Snodgrass and Corwin 1988). The visual n-back task, which required participants to identify whether a series of colored balls matched the color of the items presented *n* before, is similar but not identical to the trained visuospatial n-back, which involved sequential presentation of a square in different spatial locations. In the absence of a true unstimulated baseline of the actual training task, the visual n-back was chosen as the closest reasonable facsimile. Although our pretest battery consisted of four WM tasks – visual n-back, auditory n-back, digit span, and corsi blocks – the latter two are span tests which correlate only weakly with n-back performance (Redick and Lindsey 2013), while the former two are structurally similar to the trained visuospatial n-back. Although our previous report (Au et al. 2016) made the a priori decision to combine these two n-back tests into a composite measure to test for group differences in baseline, we ended up finding strong transfer effects only in the visual, but not auditory, n-back test. This suggests a close link between visual n-back and our visuospatial training task (correlation between pre-test visual n-back score and first visuospatial n-back training session: r = .65). Therefore, it is chosen as the most appropriate index of baseline WM ability in the current study. The average baseline performance on the visual n-back task in the Active group was .66 (SD=.16) and the average score in the Sham group was .62 (SD=.19); the difference between groups was not significant (p = .36).

Motivation: Motivation was assessed before each training session by self-report. Participants were asked to rate their own motivational state on a scale from 1-10, with 10 being the most highly motivated. An average motivation score over all seven sessions was calculated for each participant and used as the dependent variable in analyses. Average motivation scores were

6.1 (SD=1.24) in the Active group and 6.1 (SD=1.01) in the Sham group. We note that although motivation was evaluated in our previous report (Au et al., 2016), our analysis focused on confirming the stability of motivation across groups and time, and we did not previously evaluate motivation as an individual difference factor to predict training outcome as we do in the current report.

Gender: Gender information was collected as part of a standard demographic questionnaire during the consent process. The Active group comprised 60% women, while the Sham group comprised 67% women.

Training Site: 50% of Active participants were recruited on each campus (UCI and UM), while 59% of Sham participants were recruited at UM.

Analytic Approach

Statistical analyses were conducted using IBM SPSS Statistics version 22 and STATA version 13 (StataCorp 2013). To identify the effects of individual-difference variables on training performance, separate regression models were calculated for each variable of interest using parameters of a logarithmic model run on the training data, yielding a 7-session training slope as the outcome variable, with condition, the variable of interest, and their interaction as prediction terms. Note that Au et al. (2016) used a seven-level repeated measures ANOVA to analyze training performance. However, for our current analyses, we required an index of training performance as an outcome variable for the regression models. We opted for individual slopes in order to take into account the entire trajectory of training performance. Individual-difference variables included gender, school site, motivation, and baseline *n*-back performance. All continuous variables were standardized, and thus also mean-centered, while categorical variables remain unstandardized to preserve the inherent structure of the dummy coding and to maintain interpretability. To identify

the effects of the long-term follow-up, a similar method was used as in Au et al. (2016) in which gain on the training task was calculated by subtracting performance in the follow-up session from that of the initial training session. This gain was then used as the dependent variable in an ANCOVA with condition as a between-subjects factor. Due to the post-hoc nature of this follow-up, the time lag between the final session of the initial intervention and the follow-up varied between participants and thus was included as a covariate.

Results

Outlier Analysis

Outliers in the data were evaluated by examining the average training performance across all 7 sessions for each participant, as done previously (Au et al. 2016). Outliers were only examined in the Sham group because no new Active participants were enrolled since our previous report. Using a criterion of 2 SD, we identified one high-performing outlier who trained at an average n-back level of 7.9, almost twice the group average of the remaining Sham participants (mean: 4.19, SD=1.27). However, we also note that the primary findings presented below are not impacted by the presence or removal of this outlier.

Training Performance by Condition (Active vs. Sham)

Because five participants were added to the sample beyond the participants included in Au et al. (2016), and because here we use the parameters of a logarithmic model (slope of training curve) as a measure of training progress (instead of the mixed ANOVAs with training performance for each session as used before), an initial model was calculated to re-establish the difference between the Sham and Active conditions. A standard linear regression was performed between training slope as the outcome variable and condition (Active and Sham) as the predictor variable. The condition factor was found to explain a significant amount of variance in the slope, $F(1,65) =$

11.65, $p < .001$, $R^2 = .15$, R^2 adjusted = .14. Condition significantly predicted slope (Beta = .79, $t(66) = 3.41$, $p = .001$) in that Active participants on average performed .79 standard deviations above Sham participants.

Individual Difference Factors

For each individual difference factor, standard multiple regressions were performed between training slope as the outcome variable and condition, the individual difference, and the interaction between condition and the difference as predictor variables. Regression results are presented in Table 2b.1.

Table 2b.1: Regression Results for Individual Difference Measures

Model	*Variable*	N	*B*	*SE B*	*β*	*p*	*Adj. R^2*
Baseline	Condition	67	1.41	0.34	0.76	.002	0.17
	Baseline WM		0.98	0.49	0.30	.07	
	Cond x baseline		-1.07	0.53	-0.47	.04	
Motivation	Condition	67	-1.63	0.68	0.81	<.001	0.25
	Motivation		-0.31	0.09	-0.64	.001	
	Cond x motivation		0.35	0.11	0.73	.002	
Gender	Condition	67	0.45	0.16	0.78	.004	0.15
	Gender		0.25	0.21	0.44	.25	
	Cond x gender		-0.04	0.27	-0.06	.90	
Site	Condition	67	0.59	0.20	1.04	.004	0.13
	Training site		0.08	0.21	0.15	.69	
	Cond x site		-0.27	0.27	-0.48	.31	

Dummy coding of the categorical variables condition, gender, and training site employed the following references, respectively: sham, female, UCI. Unstandardized coefficients (B) are not mean-centered while standardized coefficients inherently are, and should be interpreted accordingly. For example, in the motivation model, B suggests a Sham advantage of 1.63 in the training slope when motivation is zero, while β suggests a tDCS advantage of .81 standard deviations when motivation is average.

Baseline performance

The model containing condition, baseline *n*-back performance, and the interaction term between condition and baseline performance explained a significant amount of variance in the training slope, $F(3,63) = 5.53$, $p = .002$, $R^2 = .21$, R^2 adjusted = .17. The partial effect of condition was significant (Beta = .76, $t(66) = 3.30$, $p = .002$) with larger slopes in the Active condition compared to Sham, holding baseline constant at the sample mean (i.e., baseline is mean-centered to zero). The partial effect of baseline, referenced to the Sham condition, suggests at the trend level that greater baseline performance is associated with larger slopes in the *absence* of tDCS (Beta = .30, $t(66) = 1.83$, p =.07). Importantly, the interaction term between condition and visual n-back performance at baseline was significant (Beta = -.47, $t(66) = -2.06$, $p = .04$), indicating that each

standard deviation increase in baseline performance reduces the effect of condition by .47 standard deviations. This suggests that tDCS is most effective among low-baseline individuals (Figure 2b.1).

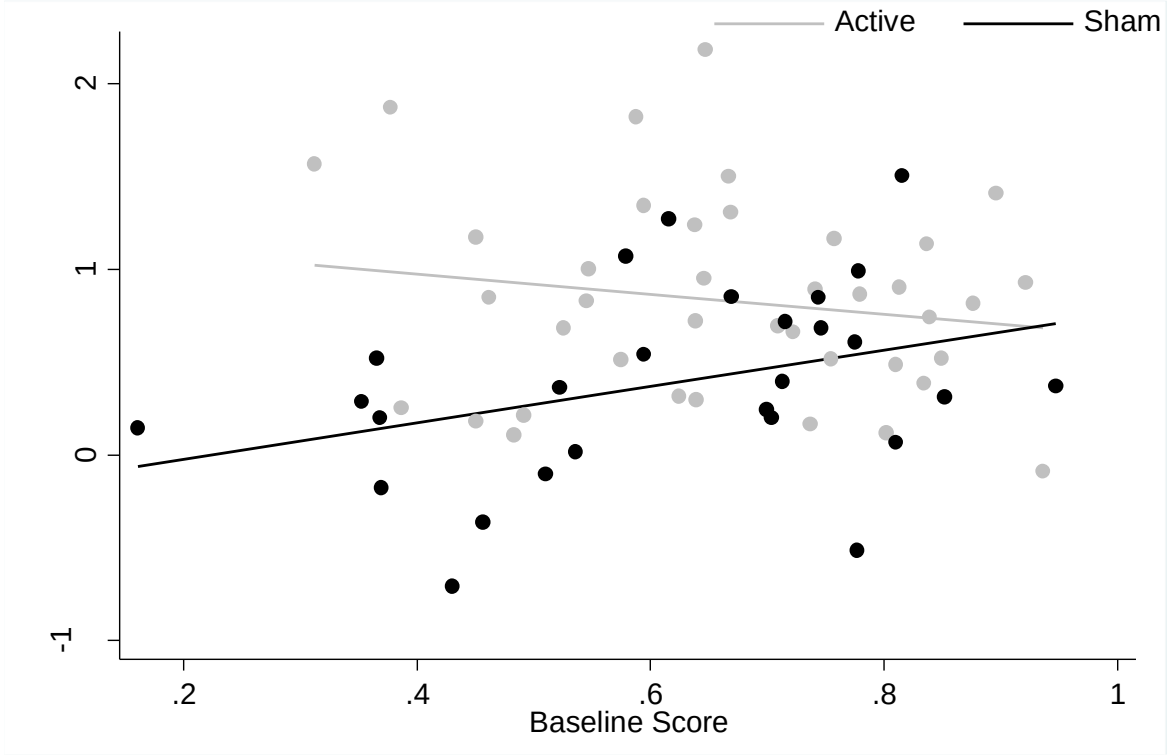


Figure 2b.1: Plot of Baseline Regression Results
Active participants with low baseline scores outperform Sham participants with low baseline scores, but the tDCS advantage gradually disappears with increasing baseline ability. Individuals with high baseline ability all improve similarly on the training task, regardless of condition.

Motivation

The model containing condition, motivation, and the interaction term between condition and motivation also explained a significant amount of the variance in the training slope $F(3,63) = 8.45$, $p < .001$, with an $R^2 = .29$, R^2 adjusted = .25. The partial effect of condition, holding motivation constant at the mean, was significant (Beta = .81, $t(66) = 3.78$, $p < .001$), reiterating the superior performance of the Active condition. However, the partial effect of motivation

referenced to the Sham condition was also significant (Beta = .-64, $t(66) = -3.41$, $p = .001$), as was the interaction term between condition and motivation (Beta = .73, $t(66) = 3.15$, $p = .002$),

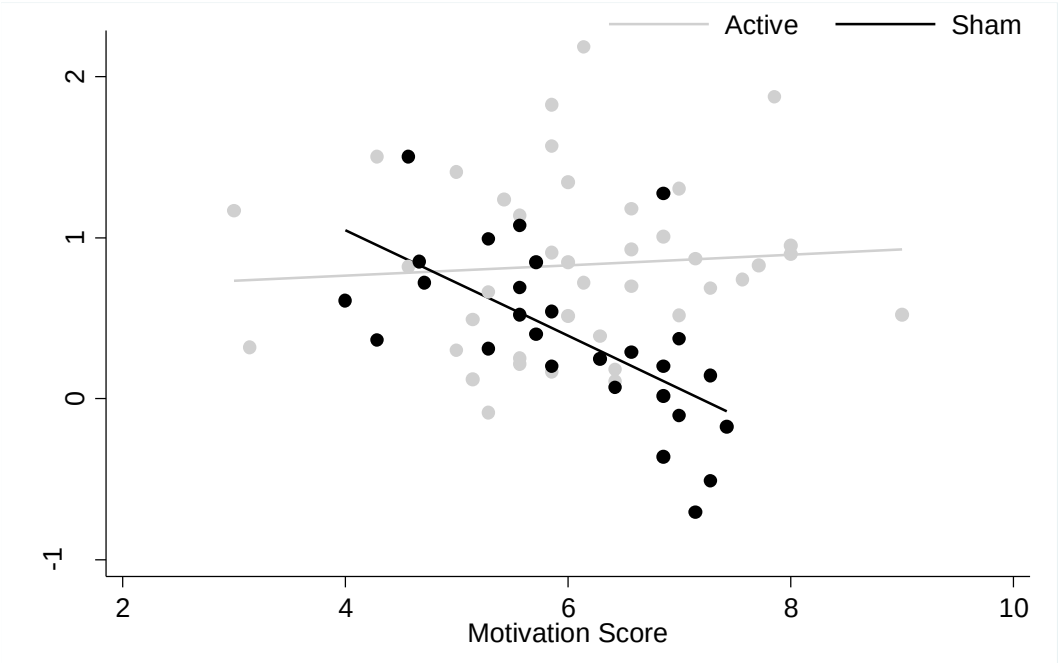


Figure 2b.2: Plot of Motivation Regression Results
Active participants all improve similarly irrespective of motivation, but Sham participants show a paradoxical decrease in performance with increasing motivation.

suggesting somewhat paradoxically that within the sham group, participants with self-reported higher motivation perform worse than participants with lower motivation (Figure 2b.2).

Gender

The model containing condition, gender, and the interaction term between condition and gender explained a significant amount of the variance in the training slope $F(3,63) = 4.93$, $p = .004$, with an $R^2 = .19$, R^2 adjusted = .15. While the partial effect of condition holding gender constant among women was significant (Beta = .78, t(66) = 2.73, p < .01), neither gender (Beta = .44, t(66) = 1.17, p = .25), nor the interaction term between condition and gender (Beta = -.06, t(66) = -.13, p = .90) was significant.

Study Site

The model containing condition, site of training (i.e. UM or UCI), and the interaction term between condition and site also explained a significant amount of the variance in the slope $F(3, 63) = 4.33$, $p = .008$, with an $R^2 = .17$, R^2 adjusted = .13. Again, while condition was a significant predictor (Beta = 1.04, t(66) = 2.98, p = .004), neither training site (Beta = .15, t(66) = .40, p = .69), nor the interaction term between condition and training site (Beta = .48, t(66) = 1.02, p = .31) was significant.

Long-term Follow-up

An ANCOVA was conducted with condition as a factor, time between the intervention and the follow-up as a covariate, and gain on the training task from the first training session to the second follow-up as the dependent variable to evaluate whether an effect of condition remained at the second follow-up that took place, on average, 355 days following the conclusion of the intervention. Condition remained a significant factor for the second follow-up, $F(1,23) = 12.43$, p = .002, with Active participants continuing to outperform Sham participants (Figure 2b.3), while, as in the first follow-up reported in Au et al. (2016), time between the intervention and follow-up was not a significant predictor $F(1,23) = 1.18$, p = .29.

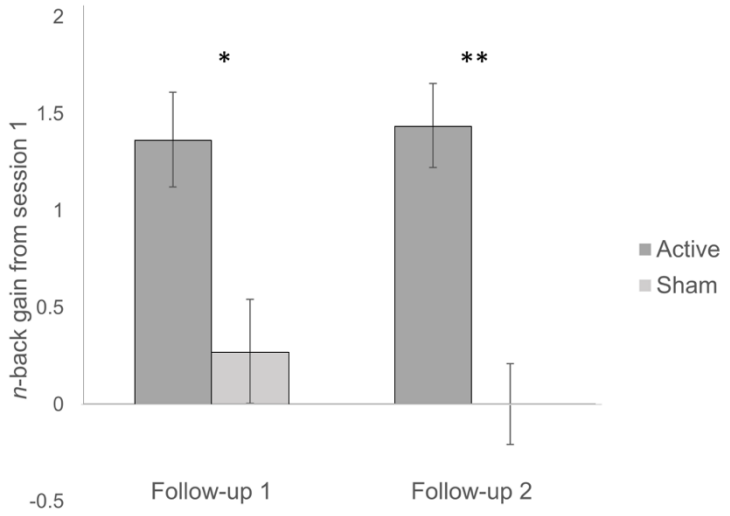


Figure 2b.3: Follow-up Performance
Follow-up 1 represents n-back level gain from first session to the first follow-up for Active and Sham participants reported in Au et al. (2016), Follow-up 2 represents gain from first session to the new second follow-up approximately twelve months following the intervention.

Discussion

Here we present evidence that certain individual-difference factors do have a significant impact on the outcome of combined WM training and tDCS. The effect of baseline was particularly striking. We found a trend suggesting that Sham participants who started with higher baseline ability tended to improve more over the course of training. Though this finding did not reach traditional levels of statistical significance (p=.07), it is nevertheless consistent with previous literature suggesting cognitive training may be more helpful to those who already have strong cognitive abilities (Looi et al. 2016; Lövdén et al. 2012). More importantly, however, the interaction between baseline ability and condition (Active/Sham) was significant (see Figure 2b.1), suggesting that the effects of baseline ability affected Active and Sham participants

differently. Specifically, the advantage of tDCS seemed to increase proportionately with decreasing baseline ability, such that a participant who started off 1 standard deviation below the mean in terms of visual WM ability before training ended up outperforming a comparable Sham participant by .46 standard deviations over the course of training. However, this tDCS advantage declines with increasing baseline ability, and confers little additional advantage to a participant who already performs high at baseline relative to a comparably high-performing peer in the Sham group. Although it is unclear what may mediate this interaction between stimulation and low baseline performance, it may have to do with differences in brain state and baseline cortical excitability between high and low groups (Krause and Cohen Kadosh 2014). For example, it is known that the effects of transcranial magnetic stimulation (TMS) are influenced by the baseline excitability of the targeted cortex (Pasley, Allen, and Freeman 2009; Silvanto et al. 2008) and that lower or more suppressed levels of neural excitability can increase the facilitatory effect of TMS.

We note that this finding of selective tDCS-enhancement among low-baseline individuals is not unique in the literature. For example, a number of studies also suggest a selective tDCS benefit among low-baseline populations, both within the WM domain (Gözenman and Berryhill 2016; Heinen et al. 2016; Minichino et al. 2015; Tseng et al. 2012) as well as in other cognitive domains, such as attention and dual-tasking (London and Slagter 2015; Reinhart et al. 2016; Zhou et al. 2015). However, one critical difference between these studies and ours is that ours is a training study involving multiple sessions of stimulation in conjunction with task performance, rather than just a single session (Looi et al. 2016). Consequently, our results demonstrate enhancements not only to overall WM performance, but more specifically, to the rate of learning (as measured by the slope of improvement) across sessions. This raises the possibility that the

selective effects of stimulation on low-baseline participants may impact not only online performance, but also offline consolidation, an important distinction for the enhancement of long-term learning (Au et al. 2017). Though these offline effects were supported in our previous work by demonstrating especial tDCS benefits when training sessions were spaced apart by a weekend (Au et al. 2016), a possible interaction of baseline performance and weekend consolidation in the present work is difficult to demonstrate due to power issues. For the same reason, the influence of baseline ability on follow-up performance is similarly difficult to evaluate.

While self-reported motivation also had a significant impact on the outcome of training, the finding of a significant interaction between motivation and condition was somewhat puzzling. The nature of the interaction is such that motivation is inversely related to slope in the Sham group only. It is unclear why lower-motivated individuals outperformed higher-motivated individuals in the Sham condition, but one possibility is that lower motivation was also associated with other influential factors, such as higher baseline performance (it is possible that for individuals who performed very well already, the intervention was not as interesting, while those who were aware of pre-existing limitations were more eager to improve their cognitive abilities). In fact, there is a moderately strong inverse correlation between baseline and motivation within the Sham group (r=-.42), suggesting that some of the observed motivation effect simply recapitulates the baseline effect. Nevertheless, we also note that both high and low motivated individuals within the Active group experienced similar improvement during the intervention, suggesting that for those individuals receiving stimulation, motivation was not a major factor that impacted performance. We also note that our motivation measure – a single question asked each day before training – may be less ideal than a more in-depth survey measure

(and such a measure might be better equipped to explain the curious motivation results discussed here). Finally, neither gender, nor site of training, nor the interaction between those variables and condition, predicted the slope of training. Thus, these analyses provide evidence that some individual difference factors, such as baseline WM performance, play a major role in the outcome of combined tDCS and cognitive training, while others do not.

Within the context of the larger corpus of tDCS research, these findings have significant implications for both existing and future studies that combine cognitive training with stimulation. Given the extent to which these factors, including baseline performance in particular, influence the outcome of training, it is possible that these differences may explain why so many participants in any individual study do not benefit from stimulation. Furthermore, it may also explain some of the null findings and even some of the varied outcomes observed among successful studies. At the very least, these findings provide an impetus for examining baseline differences as a covariate of interest in training and stimulation studies. This also means that future studies must be adequately powered to account for these differences and allow for them to be examined.

We also note the continued difference between the Active and Sham conditions approximately a year (on average) following the intervention, extending the medium-term follow-up findings established in Au et al. (2016). This suggests that applying tDCS with cognitive training may not only result in more robust and rapid improvements on the training task, but also that the improved performance on the training task relative to the Sham group may remain stable, even up to a year after the intervention. Importantly, we note that this follow-up examined only training effects, rather than any improvements in transfer tasks. Future work will be needed to establish the extent that transfer gain may also persist at long-term follow-up.

We note that these results must be tempered by certain limitations in our dataset. The baseline measure included here is perhaps less ideal than having the participant complete a session of the training task prior to stimulation, which would give a "true baseline" that might be a better predictor of subsequent performance. Additionally, there was considerable attrition between the initial study and the second follow-up. Finally, although this study was fairly well-powered for a tDCS and training intervention, even more participants would be needed to have better confidence about the individual difference findings presented here. Furthermore, we acknowledge that this study is not powered well enough to examine more than one individual difference factor at a time, and the follow-up sample is too small to examine in the context of the individual-difference factors covered here. Thus it is important to note the preliminary nature of the present analyses.

Despite these limitations, the practical implications of the baseline finding are of particular interest, both for cognitive training studies as well as tDCS-augmented learning more generally. Within cognitive training research, some studies have suggested that it is necessary for participants to demonstrate improvement on the training task in order to achieve transfer gains (e.g., S. M. Jaeggi et al., 2011). TDCS may enable participants with lower starting performance to reach similar gains to their higher-performing peers, thus overriding individual differences in baseline ability, and allowing more to benefit from the intervention. In the context of learning more generally, tDCS may offer a means of helping individuals who might be struggling on a particularly WM-demanding task, such as math, improve more quickly. Preliminary research, albeit with only two sessions, suggests that this may indeed be possible (Looi et al. 2016). Additionally, subsequent work should combine this line of investigation with fMRI or EEG; the combination of physiological or neuroimaging data may allow researchers to better understand how physical characteristics and anatomical differences may impact the flow of current

generated by the stimulation device. Most importantly, these results reinforce the importance of considering individual-differences during the administration of tDCS and training – as well as collecting samples well-powered enough to actually examine them.

CHAPTER 3: TDCS-Induced Plasticity Enhances the Steady-State Visual Evoked Potential

Overview

Chapter 3 focuses on identifying neural correlates for the behavioral effects we observed in Chapter 2. Namely, we wanted to see if we could detect an increase in electrophysiological activity between sessions, even without additional stimulation, which would suggest the existence of an offline mechanism that continues to work even after stimulation has ended. We have hypothesized in previous chapters that this mechanism relates to an increase in LTP-like plasticity in task-relevant networks. Furthermore, we wanted to see if the baseline-dependency we described in Chapter 2B would also occur at the electrophysiological level. To do so, the following chapter evaluated the use of EEG to measure steady-state visual evoked potentials (SSVEP), which provide a neurophysiological marker of task-relevant cortical excitability and thus can be used to index tDCS effects. We also tested for behavioral correlates on a WM task. In a single-blind, within-subjects design, we measured SSVEP and WM performance before and after active or sham tDCS over three consecutive days. We evaluated immediate effects by measuring changes in SSVEP strength and WM performance pre and post-tDCS each day as a function of stimulation condition. Delayed effects of stimulation were also evaluated by examining the dependence of the latter two pre-test sessions on the previous day's stimulation. Although no immediate (within-session) behavioral/SSVEP effects were observed, delayed SSVEP (but not WM) increases were observed the day after active, but not sham, tDCS. In accordance with the literature, these effects occurred in a baseline-dependent manner such that only those starting off with initially low SSVEP measurements responded to tDCS. We conclude that SSVEP appears to be a useful tool to probe the delayed effects of tDCS-induced plasticity. The results presented here were focused on the parietal-occipital area, but SSVEP can potentially be measured throughout the cortex and thus may

be an effective biomarker to evaluate delayed effects of a variety of tDCS montages. At the time of this writing, this chapter is currently under review for publication.

Introduction

Transcranial direct current stimulation (tDCS) is a form of noninvasive brain stimulation that has been increasing in popularity as a means of influencing brain activity and performance across a variety of domains and populations (Aleman et al. 2018; Elsner et al. 2017; Hashemirad et al. 2016; Hsu et al. 2015; Klaus and Schutter 2017; Mancuso et al. 2016; Oldrati and Schutter 2018; Simonsmeier et al. 2017; Summers et al. 2016). Though meta-analyses generally find net positive effects, studies are often criticized for inconsistent results and low replicability (Horvath et al., 2015). At least part of the problem stems from an incomplete mechanistic understanding of how tDCS affects the brain, and under what conditions. There is still a great deal of methodological heterogeneity between studies because researchers don't fully understand the best areas to target for which behaviors, the optimal stimulation parameters, or the target population most likely to respond to intervention.

The use of motor-evoked potentials (MEP) in tDCS research has provided some insight into these issues (Nitsche and Paulus, 2000; Nitsche et al., 2005). Such brain-based excitability measures are useful to study because they can be more direct and robust indices of tDCS-induced excitability, compared to behavioral measures that often involve multiple processes arising from a complex network of brain activity. In fact, it is not uncommon for neurophysiological changes to occur in response to tDCS even in the absence of a measurable behavioral change (Hill et al. 2017; Nikolin et al. 2018; Royal et al. 2018). Thus behavioral studies alone may underestimate the true impact of tDCS. Importantly however, when both do occur, changes in brain activation

can be used to inform changes in behavior, as has been evaluated with MEPs and motor learning (Dumel et al. 2018; Goodwill et al. 2013).

Thus, the use of MEPs in tDCS studies can serve as a proxy for how stimulation might affect behavior, and such studies have deepened our understanding of the effects of various stimulation parameters, such as current intensity, duration, and stimulation intervals (Batsikadze G. et al. 2013; Gálvez et al. 2013; Monte-Silva et al. 2010, 2013). However, this knowledge does not easily extrapolate outside motor function since MEP induction is spatially constrained to montages involving M1, and the effects of tDCS may differ in other cortical regions (Jacobson, Koslowsky, and Lavidor 2012). Moreover, since MEPs are measured by EMG on the hand, they are a measure of corticospinal excitability, and neither a direct measure of tDCS effects on the cortex, nor on higher-level cognition which is a major focus of a substantial body of the tDCS literature.

The goal of the current study, therefore, is to introduce a novel paradigm to probe extra-motor tDCS effects at a basic, neurophysiological level, in order to inform research extending beyond the motor domain. To fulfill this aim, we used steady-state visual evoked potentials (SSVEP), which are induced by the use of flickering stimuli. Although previous work has had some success with potentiating traditional visual evoked potentials (VEP) with tDCS (Accornero et al. 2007; Antal et al. 2004; Sczesny-Kaiser et al. 2016), VEPs also suffer some of the same generalizability issues as MEPs. SSVEPs, on the other hand, have been extensively used in studies across broad visual, perceptual, and cognitive domains over diverse cortical regions (Silberstein et al. 2001, 2011; Srinivasan et al. 2007; Srinivasan, Bibi, and Nunez 2006; Vialatte et al. 2010). Not only are they famously used to study vision and attention in parieto-occipital areas, but they also have a number of cognitive, clinical, and engineering applications that are

not vision-specific. These include being used as markers of arousal and cognitive engagement in studies of working memory, attention, neuropathologies, and brain-computer interfaces (Vialatte et al., 2010). Therefore, being able to modulate the SSVEP signal has broad implications that can extend beyond merely our proposed use as a marker of tDCS efficacy.

For our purposes however, the current study evaluates the sensitivity of this tDCS-SSVEP paradigm to detect electrophysiological changes arising from tDCS during a low-level visual attention task as well as a working memory (WM) task. The visual task (VT) was designed to only minimally engage cognitive and attentional processes in order to measure the effects of tDCS on SSVEP during an approximation of a pure sensory task, whereas the WM task was designed to evaluate the effect of tDCS on SSVEPs in a task involving higher cognitive function. Previous work has shown that SSVEP strength over the parieto-occipital region during the encoding portion of a WM task is predictive of eventual recall (Peterson et al. 2014), which opens the possibility that tDCS-related modulation of SSVEP may also modulate the likelihood of eventual recall. We remained agnostic as to the direction of SSVEP effects, as either an increase or decrease seemed equally likely, given the bilateral nature of our montage (see Methods), as well as the possibility of previously reported bidirectional effects (Monte-Silva et al. 2013; Strube Wolfgang et al. 2016). However, we expected enhancement of behavioral WM performance based on a previous report with the same montage and task (Heinen et al. 2016).

Finally, we were particularly interested in detecting electrophysiological signatures from our SSVEP paradigm relating to several phenomena on which we (and others) have previously reported. First is the capability of tDCS to produce delayed effects, even hours or days after the end of stimulation, a period of time commonly considered to extend beyond the window of any direct after-effects of tDCS (Nitsche and Paulus 2001). This delayed phenomenon is thought to

involve interactions with downstream LTP or LTP-like plasticity that occur after completion of the behavioral task, which can strenghten task-relevant synaptic connections. (reviewed in Au et al., 2017). Second is a frequently reported individual difference factor where tDCS responders tend to start off with lower baseline ability on the metric of interest than non-responders (Benjamin Katz et al. 2017; Li, Uehara, and Hanakawa 2015). Thus, we measured changes in SSVEP and behavioral performance before and after tDCS, and repeated the protocol over the course of three consecutive days. This allowed us to evaluate and compare both immediate effects within a day as well as delayed effects that may not show up until the next day. In instances where tDCS effects were present, we also tested whether low baseline status on Day 1 predicted stronger responsiveness to tDCS.

Materials and Methods

Participants

Twenty-four healthy young adults (16 women, mean age = 23.58, SD = 3.79) recruited from the University of California, Irvine and the surrounding community participated in the present experiment. All participants were right-handed and reported no previous history of neurological or psychiatric disorders, or metal implants. All research procedures were approved by the Institutional

Experimental Design

In our within-subjects design (Figure 3.1), participants were asked to come into the lab over three consecutive days, and were randomly assigned to receive Active and Sham tDCS on the first two days in a counterbalanced order. On Day 3, everybody received Active tDCS. Behavioral performance and SSVEP strength were measured before and after tDCS each day. Review Board and all participants signed informed consent.

This design allowed us to evaluate both the immediate effects post-tDCS, with an opportunity for replication on Day 3, as well as to evaluate potential delayed effects that may still exist at baseline the next day, both of which are commonly reported (Au et al. 2017; Gálvez et al. 2013).

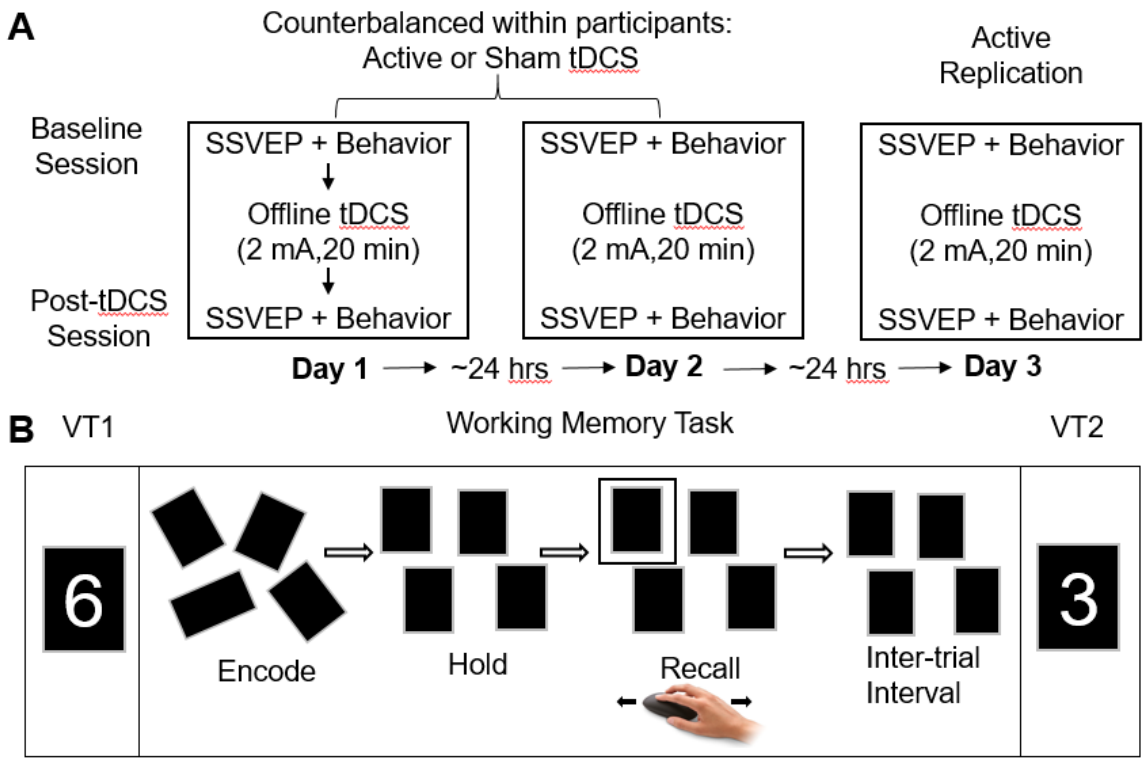


Figure 3.1: Study Design and Behavioral Task.
A) Participants performed the same sequence of tasks each day, with the only difference being whether Active or Sham tDCS is applied. On Day 3, all participants receive Active tDCS to serve as an internal replication. B) Example stimuli from the behavioral tasks are shown. All stimuli flickered at a rate of 5, 15, or 40 hz during the actual task. During the CPT tasks, participants merely had to click whenever an even number appeared. During the WM task, a random rectangle is outlined during the recall phase, and participants must use the mouse to rotate that rectangle left or right in order to recreate the original orientation presented during the encode phase.

Behavioral Task

The behavioral task was programmed in PsychoPy (Peirce 2009) and consisted of a low-level visual task (VT) and a higher-level working memory (WM) task, each lasting approximately 6-7 minutes. The VT was designed to only minimally engage cognitive and attentional processes in order to obtain a measure of the effect of tDCS on SSVEP responses in a sensory task, whereas the WM task was designed to evaluate the effect of tDCS on SSVEPs in a task involving higher cognitive function. Previous work has shown that SSVEP strength over the parieto-occipital region during the encoding portion of a WM task is predictive of eventual recall (Peterson et al. 2014), and therefore we hypothesized that tDCS-related modulation of SSVEP may also modulate the likelihood of eventual recall. All stimuli were presented at eye level for each participant, who was situated in a dark room exactly 50 cm away from an LED monitor set to a 120 hz refresh rate.

The VT was split in half to bookend the WM task. This was done in order to test the time course of potential effects by testing for a difference between the first and second halves of the VT, as it is not known how long the after-effects of stimulation on SSVEP would last. Participants were simply asked to maintain their gaze on a flickering reverse-contrast black rectangle with dimensions 3.5 x 5.5 degrees of visual angle (width x height). Three flicker frequencies were presented (5hz, 15hz, and 40hz), each for two continuous blocks of 25 seconds each in an interleaved manner. Embedded within the rectangles were a series of even or odd numbers which flickered in opposition to the rectangle (i.e., appeared white when rectangle was black, etc.) and appeared for 3 seconds each, with 1 second between presentations. Participants were instructed to click on even numbers, but ignore odd numbers, solely as a means to ensure participants were attending to the rectangle and not gazing away from it.

The WM task consisted of an orientation discrimination paradigm modeled after previous work (Heinen et al. 2016) which showed tDCS-related enhancement using an identical montage. The psychometric properties of this task were desireable for our study because the orientation discrimination task, unlike traditional span-dependent WM tasks, exhibited fairly minimal practice effects across sessions (Heinen, personal communication, Jan 2017), and therefore is more suited for repeated use in our within-subjects design implemented here. The stimuli consisted of four rectangles (each 3.5 x 5.5 deg) presented near the center of the screen in varying orientations ranging from -85 to -5 and 5 to 85 degrees. The task was split into 4 phases lasting 1200 ms each. First was an encoding phase during which the four rectangles were presented on the screen, followed by a hold phase in which all rectangles were reset to neutral orientation (0 degrees) to serve as a visual mask. During the recall phase, one rectangle was randomly selected (outlined by a white box), and the participant's task was to recreate the original orientation of that rectangle precisely by moving the mouse left or right. Although the recall phase was temporally unconstrained (i.e., subjects could take as long as they needed to make a decision), later analysis of the EEG data included only the first 1200 ms in order to stay consistent with the other phases, and trials which lasted less than 1200 ms were omitted from SSVEP power analyses. Finally, there was an inter-trial phase during which the rectangles once again reverted back to neutral orientation, but there were no task demands except to keep the gaze fixated at center and wait for the next trial to begin. Rectangles continuously flickered at 15hz throughout all phases, including the inter-trial phase, and each rectangle was presented at a randomly jittered location within its quadrant that was between 3 to 5 degrees of visual angle away from the center along both the x and y axes, with a resolution of .33 degrees. The dependent variable of interest was precision, as used in Heinen et al. (2016). This was defined as

the inverse of the standard deviation of the margin of error in radians between the orientation of the participant's response and that of the presented stimulus for all accurate trials. However, since participants attended to 4 different rectangles, and since responses could vary continuously within a large range (-85 to +85 degrees), we wanted to reduce the undue influence of wild guesses that could skew our precision metric. Therefore, we excluded trials in which participants were unable to even orient the rectangle in the correct general direction (i.e., clockwise or counterclockwise relative to neutral 0°).

Electroencephalography

EEG was measured using a 128 electrode Hydrocel Geodesic Sensor Net (Electrical Geodesics, Eugene, OR), in accordance with the 10-20 International system. Cz was used as the online reference, but activity was re-referenced offline to the average of all electrodes. 6 electrodes chosen at the periphery of the cap by the ears were connected to photocells in order to ensure accurate stimulus timing during data analysis, thus leaving 122 usable electrodes for EEG acquisition. The EEG signal was hardware-filtered between 1 and 50 hz with a sampling rate of 1000 hz. After acquisition, the EEG data was imported into MATLAB for offline processing and analysis. Linear trends were removed from the data and obvious artifacts, including eye-blinks, muscle movement and other non-cortical sources, were removed via Independent Component Analysis (Bell and Sejnowski 1995). SSVEPs were measured in each participant first by averaging data across all trials, and then calculating the Fourier coefficients of the phase-locked data. Power at each frequency of interest (i.e., 5, 15, and 40) was then normalized by dividing the power at each channel by the average power over all channels (Richard B Silberstein et al., 2001), so that the value at each channel indicated SSVEP strength relative to the rest of the cortex. This value was then averaged across all participants, and a region of interest (ROI) was

defined separately for each frequency by identifying electrodes that were at least 2 standard deviations above the cortical mean. This resulted in ROIs consisting of 7-9 electrodes surrounding the center of the occipital cortex (electrode Oz) for all frequencies.

Transcranial Direct Current Stimulation

tDCS was administered bilaterally over the posterior parietal cortex via a battery-driven stimulator (Oasis Pro, Mind Alive, Canada) at 2 mA for 20 minutes through two saline-soaked 5x7 cm sponge electrodes. Stimulation was applied offline during quiet rest after baseline behavioral and SSVEP measurement each day and the anode was positioned over the right parietal region corresponding to electrode P4 and the cathode was placed contralaterally over P3. This montage was chosen due to a previous report that showed efficacy in enhancing WM performance on an orientation discrimination task similar to the one used in the current study (Heinen et al. 2016). Additionally, current modeling (see Results) suggests this montage effectively targets the broader parieto-occipital region, which is primarily responsible for the SSVEP response (Srinivasan et al. 2006; Vialatte et al. 2010). Both electrode positions were marked under the EEG cap during baseline testing, and were thoroughly cleaned with rubbing alcohol and dried with a hair dryer to remove moisture from the previous EEG session prior to administration of tDCS.

Since the sensations from stimulation at 2 mA can sometimes be discernible from sham (O'Connell et al. 2012), we designed a unique sham protocol to mitigate participant awareness. First, we did not mention the existence of a sham condition during consent so all participants were led to believe they received Active stimulation all 3 days and debriefed afterwards. Second, although Sham stimulation commenced in a traditional fashion, i.e., current was shut off after a 30-second ramp-up period), a brief 1 mA spike was introduced every few minutes, lasting about

10 seconds including ramp-up and ramp-down, in order to periodically re-introduce skin sensation in participants. Third, Sham stimulation was always applied over the frontal cortex (electrodes Fp1 and Fp2), while Active stimulation was always applied over the parietal cortex. Participants were primed to believe that any differences in sensation they experienced were due to the differences in site and sensitivity between skin and scalp, and were told that our hypotheses concerned exploring the differential effects of frontal and parietal stimulation on improving WM performance.

Statistical Analyses

Statistical analyses were conducted using Stata (StataCorp 2013) and JASP (JASP Team 2018). Our primary analyses centered around testing both the immediate and delayed effects of tDCS. For the EEG results, we separately analyzed the SSVEP signal derived from the VT and the WM task. To look at immediate effects of stimulation within a day, we conducted repeated-measures analyses of variance (RM-ANOVA) with the following factors: Session within a day (baseline/post-tDCS), Condition across days (Active/Sham/Active Replication) and Task Segment within a session (blocks 1 and 2 for the VT, or encode, hold, recall, and ITI for the WM task). For the analysis of delayed effects, we were only interested in the baseline measurement each day, which occurs prior to tDCS and is unconfounded by any potential immediate effects. Day 1 consisted of a true baseline where participants were naïve to any form of tDCS, whereas Days 2 and 3 consisted of a roughly 24-hour follow-up after either Active or Sham tDCS. Thus, we ran ANOVAs with the factors: Baseline Session (Day 1, After Active, and After Sham) and Task Segment (blocks 1 and 2 for the VT or encode, hold, recall, and ITI for the WM task). Greenhouse-Geisser corrections were applied whenever assumptions of sphericity were violated. Behavioral analyses were also conducted in the same fashion, but without the Task Segment

factor – i.e., a 2x3 RM-ANOVA to test for immediate effects and a one-way ANOVA across baseline sessions for delayed effects. Finally, where significant tDCS effects were found, we re-ran the relevant ANOVA, but with an additional between-subjects factor, Day 1 Baseline Status (high/low), which was calculated as a median split separating high and low-performers based on their true baseline on Day 1.

Results

Current Modeling

We modeled the electric field intensity generated from our P3-P4 montage using Comets2 (Lee et al. 2017). Results showed that our montage effectively targeted the parietal-occipital region, with peak electric field intensity ranging around .35 V/m centrally positioned

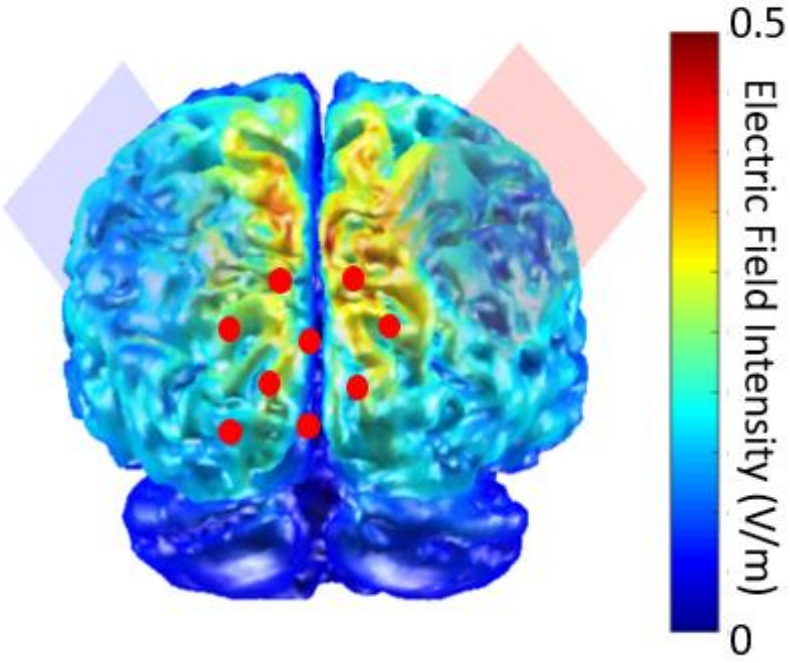


Figure 3.2: Current Modeling and SSVEP ROI.
Computational model of the posterior aspect of the brain shows peak electric field intensity between the anode (right parietal lobe) and cathode (left parietal lobe). Dots represent the 9 electrodes that make up the occipital ROI from which we sampled our 15hz SSVEP signal.

between the electrodes (Figure 3.2). The SSVEP ROI is also exposed to a substantial electric field, ranging around 0.25 V/m.

Effects of tDCS on SSVEP

There were no immediate effects of tDCS on SSVEP power, neither for the VT, Session x Condition: F(2,46)=.130, p=.879, BF10=.074, Session x Condition x Task Segment: F(2,46)=1.92, p=.159, BF10=.149, nor the WM task, Session x Condition, F(2,46)=.052, p=.949, BF10=.039), Session x Condition x Task Segment, F(3.28,75.46)=.542, p=.671, BF10=.013. Despite the lack of significant interactions, however, we observed consistent drops in SSVEP power between sessions for both the VT and WM tasks, irrespective of stimulation condition. There was a marginal main effect of Session for the VT: F(1,23)=3.523, p = .073, BF10=35. 71, d= -.383, and a significant main effect of Session for the WM task, F(1,23)=5.25, p = .031, BF10>1000, d=-.468.

However, we did find evidence for delayed effects with the VT in that participants who received Active stimulation on a given day exhibited greater SSVEP power present during the baseline session the next day, relative to Sham. Our analyses revealed a main effect of Session: $F_{(2, 46)}$=4.408, p=.018, BF_{10}=58.705, but no interaction with Task Segment: $F_{(2, 46)}$=.129, p=.879, BF_{10}=.117, suggesting general effects of tDCS on enhancing the next-day visual flicker response that last throughout the behavioral task, (i.e, present during both VT segments before and after the WM portion of the task). A planned Helmert contrast on Session, comparing the After-Active baseline session to the average of the Day 1 and After-Sham baseline sessions found significantly greater SSVEP power: t(23) = 3.376, p=.003, d=0.69, BF=15.23. No delayed SSVEP effects were detected during the WM task: main effect of Session, $F_{(2, 46)}$=2.036, p=.142, BF_{10}=6.92, Session x Task Segment, $F_{(2, 46)}$=1.512, p=.179, BF_{10}=.017. Despite the lack of

significant effect at the frequentist level, the Bayes Factor suggests a positive effect of Session is 6.92 times more likely than no effect, and similar patterns are observed throughout the WM task as in the VT – namely that SSVEP power is always numerically greater after Active tDCS compared to Day 1 or after Sham. The effect of Session is displayed in Figure 3.3 separately for each task segment, along with individual Bayes Factors.

We re-ran the above analyses on the 5hz and 40hz frequencies, but found no evidence for either immediate or delayed effects (p's >.215, BF_{10}'s<.459). Summary data are presented in Tables 3.1 and 3.2.

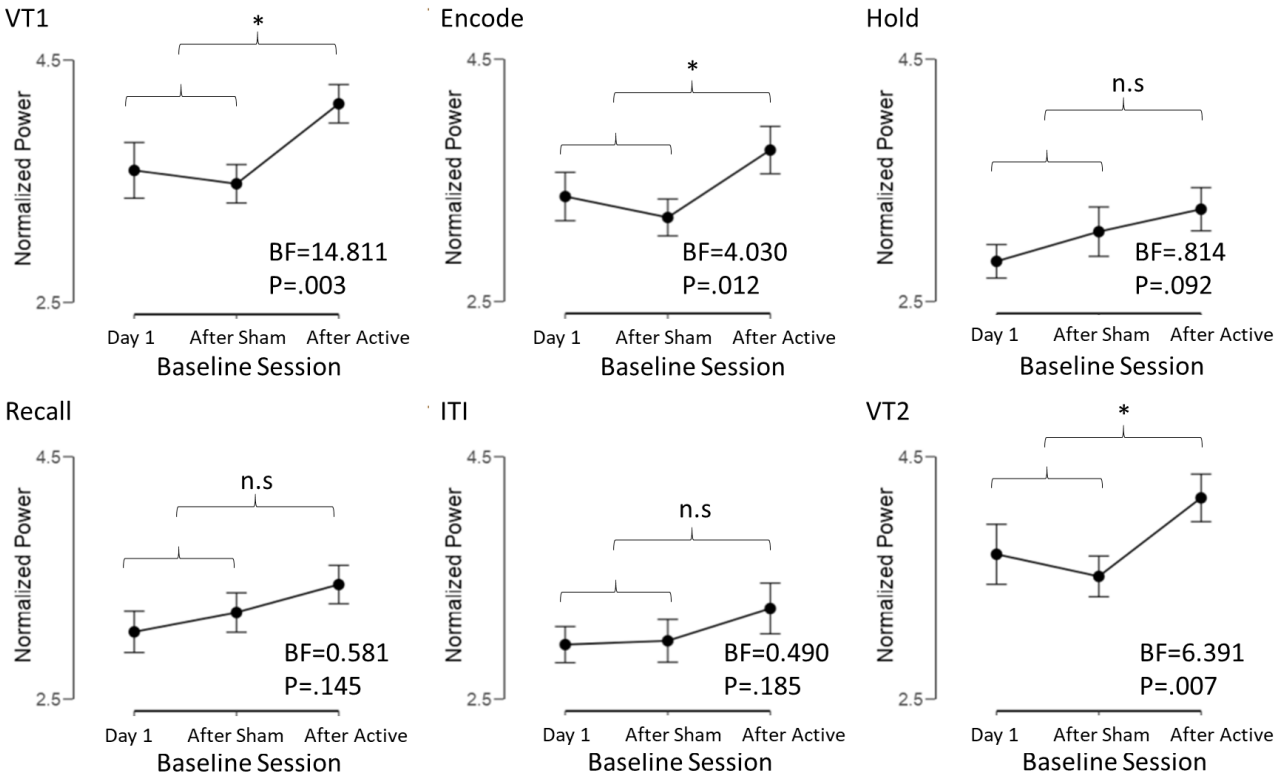


Figure 3.3: Delayed SSVEP Results.
Both sections of the VT and the encoding phase of the WM task all show significant differences at baseline the day after active tDCS compared to the mean of the other two sessions. Other phases of the WM task show the same numerical pattern but are not significant on their own. Error bars represent SEM.

Individual Differences Analysis

To follow up the significant delayed effects we found with tDCS on SSVEP during the VT, we ran an individual differences analysis using Day 1 Baseline as a between-subjects factor and found that only low-baseline individuals were responsive to tDCS. The main effect of Session, irrespective of Day 1 Baseline was still significant: $F_{(2, 44)}$=4.989, p=.011, BF_{10}=54.637, but more importantly, there was also a significant interaction between Session and Day 1 Baseline: $F_{(2, 44)}$=4.032, p=.025, BF_{10}=35.752. Re-running our prevous Helmert contrasts (After Active session compared to mean of Day 1 and After Sham sessions) at each level of Day 1 Baseline shows a significant contrast only among low-baseline individuals, t(23) = 3.400, p=.003, d=1.002, BF=10.03, but not high baseline, t(23) = 0.836, p=.423, d=0.395, BF=0.611. See Figure 3.4.

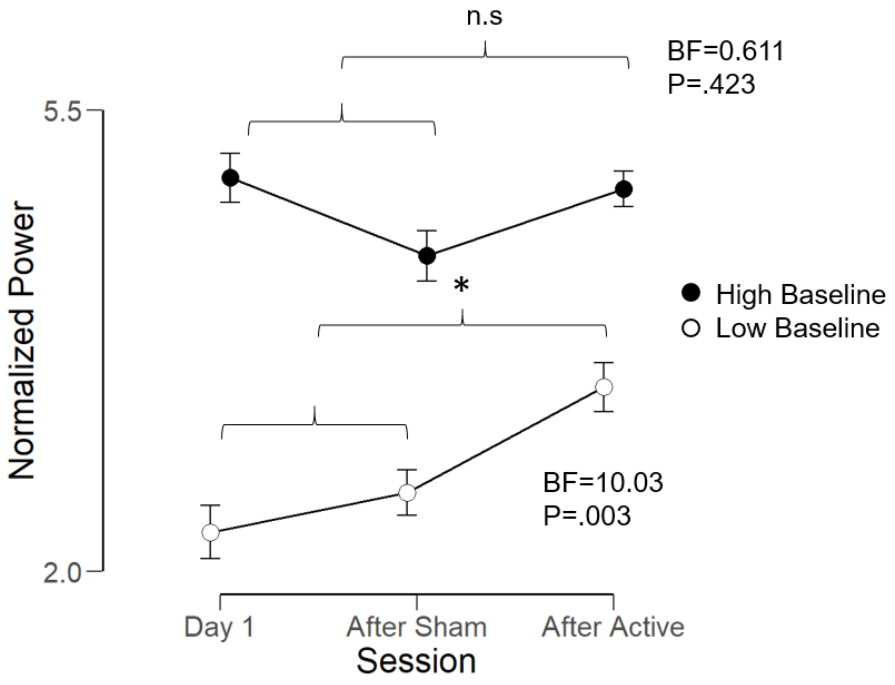


Figure 3.4: Delayed VT SSVEP Results by Baseline.
Individual difference analysis of delayed SSVEP enhancement during the VT shows the effect is only significant among those starting off with lower baseline activation. Error bars represent SEM.

Effects of tDCS on Working Memory

We tested whether WM performance improved over time as a function of stimulation condition, but found no main or interaction effects, neither on an immediate nor on a delayed timescale (p's > .15, BF_{10}'s < 0.576), and thus no evidence of any behavioral effect in our study. See Tables 3.1 and 3.2 for descriptive data.

Table 3.1: Descriptive data of immediate effects.

	Active			Sham			Active Replication		
	Baseline	Post-tDCS	d	Baseline	Post-tDCS	d	Baseline	Post-tDCS	d
VT1 5hz SSVEP	2.711 (±1.305)	2.255 (±1.356)	-0.445	2.654 (±1.281)	2.155 (±1.115)	-0.417	2.649 (±1.262)	2.484 (±1.017)	-0.086
VT1 15hz SSVEP	3.512 (±1.682)	3.265 (±1.654)	-0.217	3.896 (±1.636)	3.374 (±1.809)	-0.337	3.799 (±1.641)	3.323 (±1.41)	-0.360
VT1 40hz SSVEP	1.918 (±0.889)	1.640 (±0.790)	-0.336	2.170 (±1.415)	1.655 (±0.693)	-0.373	2.277 (±1.274)	1.839 (±1.158)	-0.267
VT2 5hz SSVEP	2.745 (±1.224)	2.263 (±0.951)	-0.428	2.876 (±0.980)	2.303 (±1.081)	-0.632	2.649 (±1.282)	2.421 (±1.315)	-0.208
VT2 15hz SSVEP	3.742 (±1.804)	3.358 (±1.663)	-0.342	3.811 (±1.945)	3.655 (±1.922)	-0.089	3.814 (±1.864)	3.357 (±1.407)	-0.307
VT2 40hz SSVEP	1.980 (±1.001)	1.688 (±0.878)	-0.284	2.196 (±1.362)	1.596 (±0.700)	-0.496	2.194 (±1.202)	1.915 (±1.294)	-0.174
Encode SSVEP	3.216 (±1.828)	2.794 (±1.851)	-0.409	3.546 (±1.724)	3.047 (±1.986)	-0.352	3.546 (±1.898)	3.100 (±1.668)	-0.308
Hold SSVEP	2.847 (±1.774)	2.425 (±1.668)	-0.412	3.025 (±1.787)	2.661 (±1.749)	-0.332	3.297 (±1.967)	2.833 (±1.403)	-0.385
Recall SSVEP	3.173 (±1.548)	2.983 (±1.651)	-0.185	3.221 (±1.579)	3.025 (±1.875)	-0.142	3.319 (±1.714)	2.979 (±1.572)	-0.267
ITI SSVEP	2.913 (±1.627)	2.607 (±1.62)	-0.329	3.179 (±1.694)	2.606 (±1.612)	-0.550	3.086 (±1.996)	2.734 (±1.625)	-0.236
WM Precision	5.54 (±1.17)	5.28 (±1.06)	-.246	5.24 (±1.03)	5.47 (±1.04)	0.255	5.500 (±0.82)	5.690 (±1.14)	0.187

Note: Values represent mean (±standard deviation) before and after tDCS during each condition. d=Cohen's d effect size. VT1 and VT2 refer to the visual task conducted before and after the working memory (WM) task, respectively, and ITI = intertrial interval of the WM task, during which task-irrelevant stimuli continued to flicker on the screen.

Table 3.2: Descriptive data of delayed effects.

	Day1	After-Sham	After-Active	d (Active-Day1)	d (Active-Sham)
VT1 5hz SSVEP	2.675 (±1.263)	2.511 (±1.178)	2.760 (±1.170)	0.088	0.262
VT1 15hz SSVEP	3.589 (±1.63)	3.479 (±1.785)	4.139 (±1.475)	0.461	0.724
VT1 40hz SSVEP	2.297 (±1.401)	2.096 (±1.173)	1.972 (±1.045)	-0.249	-0.082
VT2 5hz SSVEP	2.692 (±1.182)	2.586 (±1.321)	2.993 (±0.917)	0.287	0.361
VT2 15hz SSVEP	3.695 (±1.893)	3.512 (±1.951)	4.160 (±1.700)	0.333	0.676
VT2 40hz SSVEP	2.389 (±1.291)	1.962 (±1.085)	2.019 (±1.174)	-0.280	0.037
Encode SSVEP	3.366 (±1.678)	3.193 (±1.813)	3.750 (±1.929)	0.333	0.562
Hold SSVEP	2.831 (±1.701)	3.076 (±1.964)	3.261 (±1.862)	0.432	0.191
Recall SSVEP	3.055 (±1.441)	3.214 (±1.668)	3.445 (±1.703)	0.313	0.225
ITI SSVEP	2.950 (±1.531)	2.981 (±1.802)	3.247 (±1.973)	0.244	0.250
WM Precision	5.164 (±1.084)	5.580 (±1.02)	5.542 (±1.902)	0.408	-0.033

Note. Values represent mean (±standard deviation) of the baseline session of each day. d = Cohen's d effect size, for both the Active/Day 1 contrast and the Active/Sham contrast. VT1 and VT2 refer to the visual task conducted before and after the working memory (WM) task, respectively, and ITI = intertrial interval of the WM task, during which task-irrelevant stimuli continued to flicker on the screen.

Discussion

Our study successfully demonstrates that SSVEP signals can be modulated by tDCS. If this effect is found to be robust with future research, this can open up multiple avenues for future work, including the use of this paradigm to probe the neurophysiological effects of different stimulation parameters or to supplement existing protocols outside of tDCS that use SSVEP to study various phenomena such as cognition, clinical disorders, or to enhance the effectiveness of SSVEP-dependent brain-computer interface technologies (Vialatte et al. 2010). The former goal is particularly pertinent to the tDCS community as there is currently a need for a greater understanding of the basic physiological effects of tDCS in the intact human brain. When selecting the tDCS parameters to use in their studies, many researchers are informed by the seminal work of Nitsche and Paulus (2000) and other MEP studies in the motor cortex, which generally suggest an excitatory/inhibitory function of the anodal/cathodal electrodes, respectively, and that greater current intensities and longer stimulation durations cause stronger effects. Although subsequent work has revealed that these relationships are not so straightforward (e.g., Jacobson et al., 2012; Batsikadze G. et al., 2013; Monte-Silva et al., 2013), no concrete mechanism has been identified that explains the non-linear relationships in stimulation length, intensity, and polarity that are sometimes observed. Therefore, for lack of a compelling alternative, researchers are still largely guided by the conventional wisdom of the early MEP studies. We contend, therefore, that the use of SSVEP to track basic excitability changes in cortical structures outside the motor cortex can go a long way to supplementing existing MEP work in order to deepen our understanding of these complex issues, particularly as there is evidence that tDCS modulation of non-motor areas does not always seem to follow the

same polarity rules as motor functions, particularly with respect to the cathode (Jacobson et al. 2012).

Importantly, the SSVEP signal enhancement we observed with tDCS occurred in ways consistent with predictions from our previous work, demonstrating the applicability of this protocol to studying real phenomena reported in the literature. First, we had predicted there would be delayed effects on SSVEP, showing up the next day, consistent with a previous experiment we ran in which tDCS-related enhancements to WM training performance did not show up on the first day of stimulation, but gradually became more and more evident over the course of a week, with long-term effects lasting up to a year (Au et al. 2016). Such delayed effects are not uncommon with tDCS, and both *in vitro* and *in vivo* animal studies show that brain tissue exposed to electrical fields can increase long-term potentiation (LTP) and production of LTP-related proteins such as BDNF over a time scale of hours to weeks after stimulation (Fritsch et al. 2010; Podda et al. 2016; Ranieri et al. 2012; Rohan et al. 2015), with concomitant behavioral improvements on a maze learning task in mice (Podda et al. 2016). Moreover, these LTP effects are not restricted to the hippocampus but LTP-like plasticity has also been achieved in M1 slices (Fritsch et al. 2010), suggesting that these effects operate similarly in the superficial cortical areas generally targeted in human studies. Indeed, human studies have shown increased motor excitability evident even 24 hours after stimulation, as indexed by stronger MEPs (Gálvez et al. 2013; Monte-Silva et al. 2013). Other studies have also shown behavioral correlates suggestive of similar mechanisms, with long-term enhancements in such domains as working memory (Au et al. 2016; Benjamin Katz et al. 2017; Ruf, Fallgatter, and Plewnia 2017), motor skill learning (Koyama et al. 2015; Reis et al. 2008, 2009, 2015), and episodic memory (Javadi and Cheng 2013; Ladenbauer et al. 2016; Manenti et al. 2016; Sandrini et al. 2014). Therefore,

our findings of increased SSVEP signal strength appearing approximately 24 hours after stimulation is in accordance with phenomena described in the broader literature, and thus we interpret this as indicative of increased LTP-like plasticity occurring in the visual cortex after exposure to the 15hz flicker which allows the relevant SSVEP network to reactivate more strongly the next time it is exposed to the same stimulus.

A second prediction we made was that individuals who started off with relatively lower SSVEP signal strength at baseline on Day 1 would be more responsive to tDCS modulation. Indeed, our moderator analysis showed this to be true (Fig 3.4), where effects are dominated by our low-baseline participants, with no statistically detectable effects among our high-baseline participants. This is consistent with the broader tDCS literature that has documented a low-baseline advantage across a range of behavioral tasks (Li et al. 2015), including our own previous work in which individuals starting with lower WM performance at baseline demonstrated greater tDCS-related improvement during WM training (Benjamin Katz et al. 2017). Since most of the evidence to date for this baseline-dependent responsivity to tDCS comes from behavioral studies, it is not known precisely why tDCS would selectively benefit lower-performing individuals. However, it has been postulated that tDCs interacts with the ongoing excitability levels in the cortex. That is, where excitability is sub-optimal, anodal tDCS may push the brain to a more optimal state, but where it is already optimal, or perhaps even over-excited, anodal tDCS may have no further effect, or may even possibly impair performance through over-excitation (Krause et al., 2013, but see Talsma et al., 2018). The current report lends support to this hypothesis on a neural level by eliciting SSVEPs to directly measure the excitability of the visual cortex.

However, our effects come with a few caveats. First, they are confined to the 15hz frequency, as we did not observe significant modulation at 5hz or 40hz. However, it is important to note that both of these other frequencies were only presented during the VT task which alternated between these three frequencies between blocks, and not the WM task which was constantly presented at only a 15hz flicker. Therefore, participants had less exposure to the 5hz and 40hz flickers and it is possible that greater task engagement with these frequencies or greater statistical power with more participants could also manifest similar effects as we observe at 15hz. However, it is worth noting that the spatial and attentional properties of SSVEP have been shown to differ as a function of flicker frequency (Ding, Sperling, and Srinivasan 2006; Srinivasan et al. 2006); therefore we cannot rule out the possibility that there may be some frequency-specific property to the 15hz SSVEP that makes it more amenable to tDCS modulation.

Secondly, despite the strong delayed effects, we did not observe any changes immediately after stimulation, as is classically displayed with the MEP protocols which were the basis of our design (Nitsche and Paulus 2000). There are several reasons that could account for this. First is the possibility of a competing habituation mechanism. Regardless of stimulation condition, we observed a reliable decrease in SSVEP power on the second session each day. It may be therefore that any immediate excitability changes caused by tDCS are overshadowed by this strong habituation effect. Another possibility however is simply that our stimulation parameters were ineffective at influencing brain activity in the immediate term. For example, a recent study suggests that bilateral montages, such as the one used in our study, do not produce the typical immediate excitability changes that unilateral montages do (Parkin et al. 2018), but

does not preclude the possibility of delayed effects which may occur under different mechanisms.

The lack of behavioral effects in our data further suggests that our stimulation parameters were suboptimal, particularly since Heinen et al. (2016) found immediate tDCS-related improvements on WM precision using a very similar task. We found evidence for neither an immediate nor a delayed effect of tDCS on precision. The absence of delayed effects is not necessarily surprising since we purposefully chose a task that we hoped would minimize learning and practice effects across days. This was important since we used a within-subjects cross-over design. Nevertheless, we still failed to see even an immediate effect of stimulation, despite using an identical stimulation montage as Heinen et al. (2016). However, one critical difference between our designs is the timing of stimulation. Their stimulation occurred online concurrent with task performance, while ours, by necessity, occurred offline since we were not able to apply tDCS concurrently with EEG. This could have substantially influenced results since tDCS can be very sensitive to the ongoing brain activity during and immediately surrounding the stimulation period (Bikson et al. 2013). For example, there are some reports that have documented selective benefits of online stimulation over offline, specifically with regards to delayed effects on cognitive performance (Martin et al. 2014; Oldrati, Colombo, and Antonietti 2018). However, there is no consensus in the broader literature as to which form of stimulation is more effective under what circumstances, and meta-analyses in the WM domain show comparable effect sizes across studies regardless of stimulation timing (Hill et al. 2016; Mancuso et al. 2016). So there could well be other mediating factors that underlie our lack of WM improvement.

Conclusions and Future Directions

We have demonstrated the utility of SSVEP as a neurophysiological marker of the influence of tDCS on the occipital cortex, and its sensitivity to two often-reported phenomena in the tDCS literature: the existence of delayed enhancement arising the next day and a low-baseline dependency. As this is a pioneering study, to our knowledge, of the use off SSVEP to index tDCS effects, we urge replication in future research. However, if our effects are found to be robust, this opens up many potential applications. For example, it can be used as a relatively direct and cost-effective method of parameter optimization in order to test the influence of different montages, current strengths, or electrode sizes prior to embarking on a large behavioral trial, or it can also potentially be used to screen out likely non-responders from responders based on their baseline SSVEP measurements. Moreover, since our effects manifested the day after stimulation, and not immediately, we believe that this technique can be particularly beneficial for longitudinal studies that involve learning over time, as we theorize our delayed effects are indicative of a consolidation-like phenomenon that involves enhanced LTP-like plasticity in the occipital cortex. Furthermore, we reiterate that SSVEP is not spatially constrained to the occipital cortex, thus opening up the possibility that the utility of our paradigm can be applicable to testing a wide range of montages throughout diverse cortical areas. However, this would need to be empirically tested with something other than the bilateral parietal montage used in the present study. Finally, although we did not observe any change in WM performance, we note a number of possibilities for future research that may more successfully demonstrate the behavioral relevance of our paradigm. First, a span-based rather than precision-based WM task may prove more fruitful at showing learning effects over multiple days, as demonstrated before (Au et al. 2016; Jones, Stephens, et al. 2015). Additionally, using a non-WM task, perhaps a

perceptual learning task that is more reliant on the visual networks potentiated by tDCS in the current paradigm, may be more successful in evincing both brain and behavioral effects.

CHAPTER 4: Optimal Timing of Transcranial Direct Current Stimulation for Cognitive Training

Overview

The previous two chapters detailed evidence for long-term effects of tDCS on learning and neural activity that outlast the stimulation period, and identified baseline performance and neural excitability as important individual difference factors that predict responsiveness to tDCS. However, there is still much that is unknown regarding the optimal stimulation parameters that would maximize the effect size of intervention. For example, the timing of tDCS delivery has been shown to be an influential variable that can interact with task learning. The current chapter therefore describes a very similar longitudinal intervention design as that described in Chapter 2, but seeks to determine the optimal stimulation timing by randomizing participants to receive tDCS either online during a training session or offline immediately before or after a training session. We also controlled for baseline ability between groups since we have previously identified this to be an influential variable in tDCS-responsivity. The primary finding described herein is an inferiority of offline stimulation to online, but no advantage of any stimulation condition relative to sham. This unexpected pattern of results prompted us to run some additional post-hoc analyses in an attempt to understand these effects, which are described in Chapter 5. At the time of this writing, this chapter, along with the additional analyses described in Chapter 5 are both currently under review for publication as part of one manuscript.

Introduction

The use of transcranial direct current stimulation (tDCS) to modulate cortical excitability has generated considerable excitement among cognitive intervention researchers due to its ability to influence learning and improve cognitive functions. In fact, we previously demonstrated that tDCS over the dorsolateral prefrontal cortex (DLPFC) can not only improve performance over the course of a 7-day working memory (WM) training intervention, but also that these effects can last up to a year (Au et al. 2016; B. Katz et al. 2017). We previously postulated that our results were driven by the ability of tDCS to facilitate LTP and LTP-like plasticity (Au et al. 2017; Monai and Hirase 2018; Podda et al. 2016) in order to improve learning consolidation during training. Although our results were replicated by an independent team of researchers who conducted a very similar study (Ruf et al. 2017), not all cognitive training studies have been as successful (e.g., Nilsson et al., 2017).

In order for the field to move forward, it is important to understand the nuances that can make tDCS more or less effective in evincing the desired neuromodulatory result. For example, our previous work found that both baseline WM ability and spacing between training sessions were predictive of training gain. Specifically, our results were driven primarily by individuals who performed relatively poorly at baseline, and much of this gain occurred over a weekend break rather than between consecutive weekday sessions. Although our demonstrated spacing effect was novel, and perhaps due to an interaction of tDCS with time-dependent consolidation-like mechanisms, the low-baseline advantage we observed has been documented many times in the literature (reviewed in Li et al., 2015), and even a high-baseline advantage has been reported a few times (e.g., Jones & Berryhill, 2012; Jones et al., 2015), suggesting that individual performance differences might interact with stimulation efficacy. However, one critical

dimension that is not often explored, and to our knowledge has never been directly compared in the context of a longitudinal cognitive training paradigm, is the effect of stimulation timing relative to engagement with cognitive training.

Most studies apply stimulation either before a task to prime the targeted cortical area or during task performance in order to potentiate task-relevant networks. In either case, the putative mechanism of action is a change in neural resting membrane potential that makes task-relevant neurons either more or less likely to fire depending on the electrode polarity (Nitsche and Paulus 2000). Observed concomitant biochemical changes in levels of neurotransmitters and neuromodulators are theorized to increase LTP and LTP-like plasticity and set the stage for offline changes post-stimulation that continue to promote long-term neural rewiring (Stagg and Nitsche 2011). In fact, a number of studies now show that it is possible for tDCS effects to emerge after a delay, usually hours or days, even in the absence of immediately observable effects (Au et al. 2017). To capitalize on the natural consolidation process that occurs after any learning event, a few studies have actually sought to apply stimulation *after* task performance and have demonstrated the ability of tDCS to augment the early consolidation process immediately after learning (Javadi and Cheng 2013; Rumpf et al. 2017; Tecchio et al. 2010).

Although stimulating online during task performance or offline before/after task performance all have been documented to evince positive tDCS effects, relatively few studies have directly compared these stimulation timing conditions to each other. Therefore, their relative efficacy is not well understood. On a meta-analytic level, there is no clear consensus, as both online and offline stimulation have been documented to be more advantageous depending on various parameters such as the task, population, or montage (Dedoncker et al. 2016; Hill et al. 2016; Hsu et al. 2015; Summers et al. 2016). Similarly, the few studies that have directly

compared these timing conditions to each other have sometimes demonstrated an online advantage (Fertonani et al. 2014; Martin et al. 2014; Sriraman, Oishi, and Madhavan 2014; C.J. Stagg et al. 2011)(Fertonani, Brambilla, Cotelli, & Miniussi, 2014; Martin, Liu, Alonzo, Green, & Loo, 2014; Sriraman, Oishi, & Madhavan, 2014;Stagg et al., 2011) and sometimes an offline (before) advantage (Cabral et al. 2015; Giacobbe et al. 2013; Pirulli, Fertonani, and Miniussi 2013). And only a handful of studies have even looked at stimulating *after* task performance, (Cabral et al. 2015; Javadi and Cheng 2013; Rumpf et al. 2017; Tecchio et al. 2010).

The purpose of the current study, therefore, was to explicitly test the relative efficacy of these three timing conditions in the context of WM training, using the same visuospatial n-back training that we previously demonstrated to show strong tDCS effects. We expected to replicate our previous effects that online stimulation would facilitate training performance in a baseline-dependent manner, with greater gains over the weekend compared to consecutive weekdays, but we remained agnostic as to the relative efficacy of stimulating before or after training.

Methods

Participants

Eighty-two neurologically and psychiatrically healthy right-handed participants (mean age: 20.40, SD: 1.68, 63.41% female) were recruited at the University of California, Irvine and University of Michigan campuses. One participant exhibited exceptionally poor performance and regularly failed to advance beyond 1-back even after 1 week of training. Given our population of healthy college undergraduates, this level of performance was considered non-compliant, and heavily skews the data if left in our sample. No other participant in our sample failed to advance beyond 1-back even once during their training. Therefore, this participant was excluded, leaving

81 total participants in our final sample. All research procedures were approved by the Institutional Review Boards at both universities and each participant provided informed consent.

General Procedure

In our between-subjects design, participants were assigned to one of four stimulation conditions (offline pre-training, online, offline post-training, and sham) that were matched on baseline WM performance on the n-back training task. Participants were also constrained to begin their sessions on either a Tuesday or a Wednesday, so that the weekend break fell at a fairly consistent point in training for all participants. This allowed us to control for potential weekend-related disruptions between subjects and also to replicate our previous analysis evaluating the interaction between tDCS and spacing between training sessions (Au et al. 2016). An Excel algorithm was used that minimized the difference between group averages each time a participant was recruited based on his/her average n-back level on Day 1 (obtained without stimulation). Thus, our study design was pseudorandom in order to ensure participants were matched on baseline scores across conditions and across spacing cohorts (Tuesday/Wednesday). This psuedorandomization can sometimes be more effective than true randomization (Shawn Green et al. 2019), and in our case was critical to ensure comparable baseline scores, as our previous analysis demonstrated baseline to be a significant moderator of tDCS effects (B. Katz et al. 2017). All participants completed their WM training during 6 consecutive days (except weekends), but stimulation was *not* delivered on Day 1 in order to establish a comparable baseline between conditions. All participants were asked to come back approximately 1 month after their last training session (within 3-5 weeks) in order to perform the training task one final time without any stimulation in order to test for long-term consolidation effects.

Each training session lasted approximately 1 hour, including set-up and clean-up. Duration of stimulation, including sham stimulation, was fixed at 25 minutes, while the n-back training typically lasted between 18 and 22 minutes. Participants in the offline stimulation conditions (pre- and post-training) were asked to sit quietly and remain alert (i.e., asked not to fall asleep) during stimulation, and simply allow their minds to wander without fixating on any one thing in particular. Otherwise, they were given no task or any further instructions during stimulation. Participants in the online and sham conditions received real or fake stimulation, respectively, concurrently with training, but were asked to sit quietly for 10 minutes beforehand in order to control for total time spent in the lab. This 10-minute time period was chosen instead of the full 25-minutes as done in the offline conditions as a compromise between controlling for quiet time prior to training and keeping participants' restlessness and suspicions to a minimum. Our experimenters noted anecdotally during pilot testing that online and sham participants grew very skeptical and restless during this quiet period of doing nothing, in contrast to the offline participants who received stimulation during this time period. All participants, including sham participants, were told they were receiving active tDCS, and that the purpose of the study was to compare differences in stimulation timing. This was done in accordance with our previous study (Au et al. 2016), and because past research has shown that 2mA of tDCS, as used in our study, can be distinguishable from sham by some participants (O'Connell et al. 2012). Participants were debriefed at the end of their 1-month follow-up session and asked to judge whether they received active or sham stimulation.

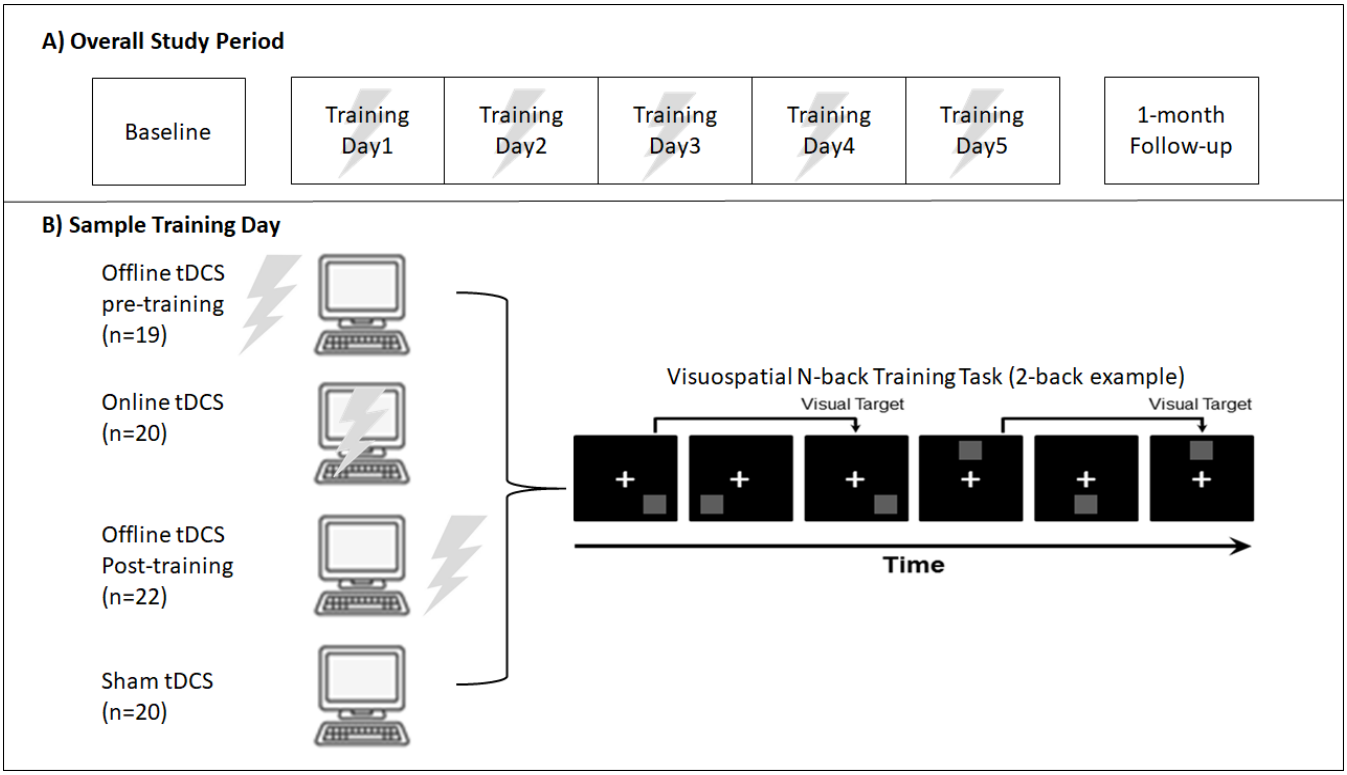


Figure 4.1: Study Design.
(A) Participants came in for a total of seven sessions, the first and last of which did not involve any stimulation. (B) Sample training day consisted of stimulation according to the participant's group assignment as well as training on the visuospatial n-back task. A sample 2-back block is depicted.

Working Memory Training and Transcranial Direct Current Stimulation Set-Up

The training task used was a computerized adaptive version of the visuospatial n-back task that we used previously, and complete task details can be found there (Au et al. 2016). Similarly, our tDCS montage was identical as previously described, with a couple small exceptions. First, we only stimulated over the right DLPFC for all participants, since our previous results found no difference between left and right hemispheric stimulation, but right DLPFC stimulation is more appropriate on theoretical grounds due to the visuospatial nature of our WM task (Smith et al. 1996). Second, rather than doing all the full measurements using the 10-20 international system, we used a shortcut algorithm devised by Beam et al. (2009).

Analytical Approach

Statistical analyses were conducted using STATA version 13 (StataCorp 2013) and Jasp 0.9.1.0 (JASP Team 2018). Since our previous results showed tDCS effects on training to be critically influenced by baseline ability, we included Baseline as a factor in our omnibus ANOVA evaluating training performance: 6x4x2 mixed ANOVA with the within-subjects repeated factor, Session (1-6) and the between-subjects factors, Condition (pre-training, online, post-training, sham) and Baseline (High, Low). Baseline was calculated as a median split based on average n-level during the unstimulated first session. Violations of sphericity were corrected using the Greenhouse-Geisser adjustments when epsilon was below .75 or the Huynh Feldt procedure if epsilon was above .75 (Girden 1992). We ended up using Huynh Feldt for all adjustments in the manuscript. Based on our previous work, we expected significant Session x Condition and Session x Condition x Baseline interactions in favor of the online group outperforming sham, especially among low baseline individuals. Thus, in order to replicate these effects and to compare the relative efficacy of the different stimulation conditions, we planned to follow up our expected interactions by comparing gain scores (session 6 minus session 1) between the online group and each of the other three conditions, both with our overall sample and also separately at each level of Baseline. Follow-up and Weekend effects were also evaluated as done previously, and once again using the online stimulation condition as our Reference group. Follow-up effects were analyzed with independent samples t-tests comparing gain scores between the follow-up session and session 1. Weekend effects were analyzed with paired t-tests comparing gain scores over the weekend and average gain scores during weekdays. Weekends either took place between sessions 3 and 4 for the Wednesday cohort or between 4 and 5 for the Tuesday cohort. Average weekday gains were calculated based on the average gain

between all pairs of consecutive weekdays, with the exception of the first two days because stimulation was not applied on the first session (i.e., average of gain between sessions 2-3, 4-5, and 5-6 in the Wednesday cohort or sessions 2-3, 3-4, and 5-6 in the Tuesday cohort). A paired t-test was then used to evaluate the within-subject differences between weekend and weekday gains for each condition.

Results

Current Modeling

We modeled the electric field intensity generated from our montage using Comets2 (Lee et al. 2017). Results showed that our montage effectively targeted the prefrontal cortex, with peak electric field intensity ranging between .3 - .4 V/m around the dorsolateral and dorsomedial prefrontal cortices (Figure 4.2).

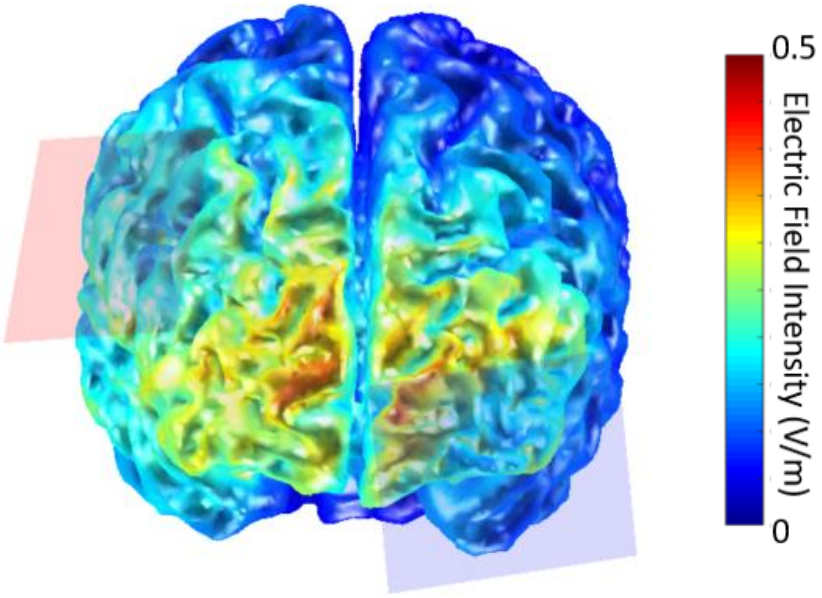


Figure 4.2: Computational Modeling.
Computational modeling of the anterior aspect of the brain shows peak electric field intensity between the anode shown in red (right DLPFC - just 1 cm lateral to position F4) and cathode shown in blue (left supraorbital ridge - position Fp1), with current reaching the entire frontal cortex, and extending into the right motor cortex. Note that the model does not show the polarity of current flow, so the electric field may not necessarily be exerting the same effects over the left and right hemispheres.

Sham Debriefing

Of the 81 participants in our sample, 73 came back for the 1-month follow-up and upon conclusion of the study, were debriefed about the existence of a sham group and asked to guess their condition. 83.93% of participants receiving active tDCS correctly guessed their condition, but 64.71% of sham participants also guessed that they had in fact received active tDCS. The difference was only marginally significant ($\chi 2 = 2.95$, $p = .086$), but it important to note that all participants believed they were receiving active tDCS during training.

Overall Training Effects

We found differences in training performance as a function of tDCS timing: Session x Condition interaction [$F_{(12.933,314.695)}$=1.74, p=.052, $\eta 2_p = .067$]. Overall, online stimulation outperformed both offline conditions: versus offline pre-training [$t_{(73)} = 2.53$, p=.012, d=.592], and versus offline post-training [$t_{(73)}$= 3.79, p<.001, d=.887]. However, contrary to our hypothesis, no difference was observed relative to sham [$t_{(73)}$= 0.02, $p = 0.984$, d=.005]. We also observed a significant three-way interaction with baseline ability: [$F_{(12.933,314.695)}$=2.14, p=.012, $\eta 2_p = .081$] which showed that online stimulation was only superior to the other stimulation conditions among high baseline individuals: versus offline pre-training [$t_{(73)} = 2.15$, p=0.032, d=.503], versus offline post-training [$t_{(73)}$= 3.82, p<.001, d=.894], and was even marginally superior to sham [$t_{(73)}$= 1.95, p=.052, d=.456]. However, there were no differences against other stimulation conditions among low baseline individuals: versus offline pre-training [$t_{(73)}$= 1.44, p=.150, d=.337], versus offline post-training [$t_{(73)}$= 1.43, p=.154, d=.335]. But interestingly, and

again contrary to our hypothesis, the low-baseline sham group was superior to the low-baseline online group [$t_{(73)}$= 1.98, p=.049, d=.463]. See Figure 4.3.

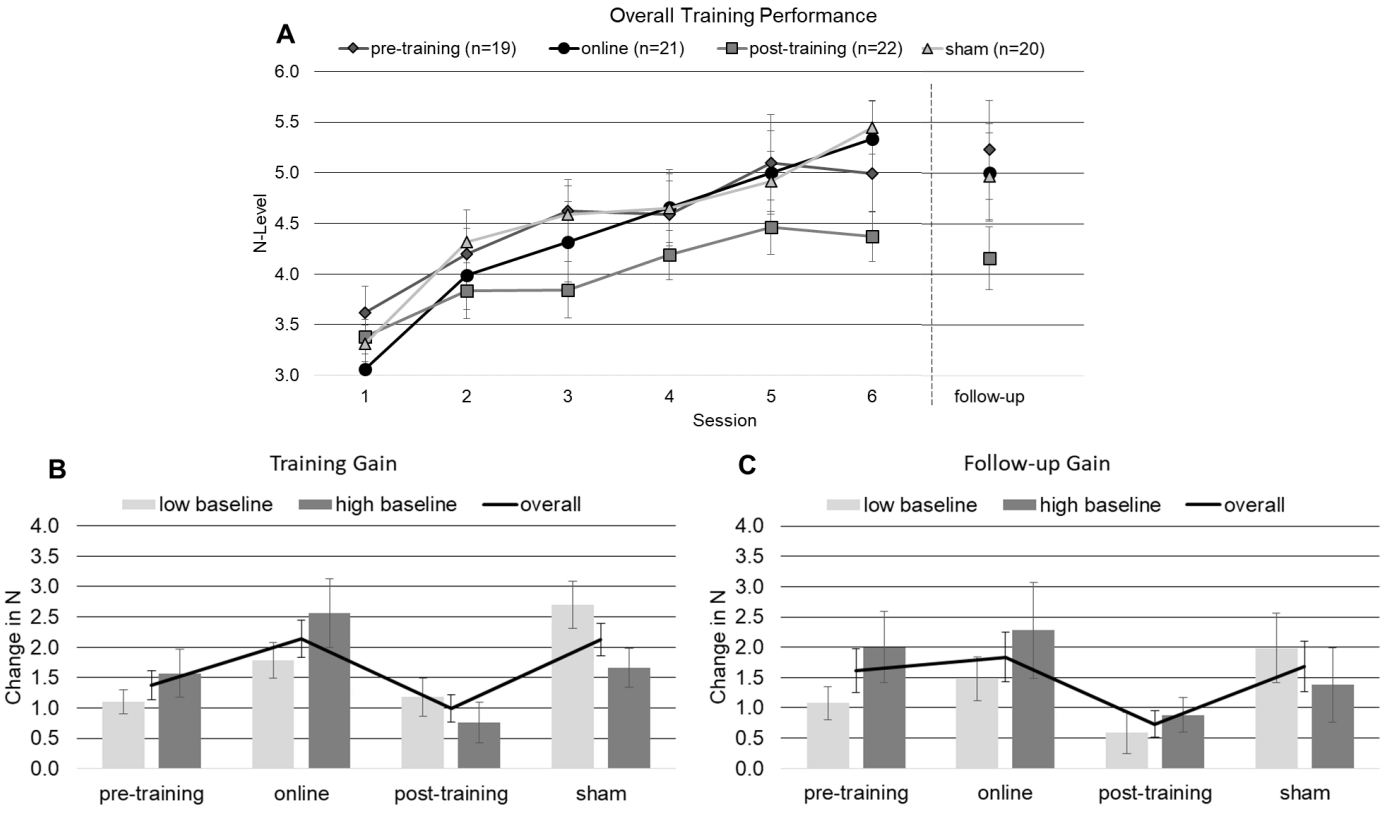


Figure 4.3: Training performance as a function of baseline and group.
All error bars represent SEM. (A) Overall training curves for all groups are shown (B) Change from baseline at the end of the 6-day training shows superiority of online stimulation to both offline conditions, but comparable performance to sham. Among low baseline individuals, all stimulation groups underperformed relative to sham, and among high baseline individuals, online stimulation outperformed all other groups. C) Change from baseline at the end of the 1-month unstimulated follow-up showed overall that online stimulation maintained its superiority to offline post-training. There was no evidence of baseline-dependent differences at follow-up.

Follow-up and Weekend Effects

T-tests of the follow-up data revealed that the advantage of online stimulation over offline post-training maintained at follow-up [$t_{(37)}$=2.073, p=.045, d=.682], but no other planned contrasts were significant, neither overall nor at each level of baseline (p's > 0.313). However, although not part of our planned contrasts, visual examination of Fig 4.3c clearly shows that the offline post-training condition underperforms relative to all conditions at follow-up, and not just online stimulation.

Paired t-tests revealed a significant advantage of weekend gains over weekday gains only in the offline post-training condition [$t_{(21)}$= 2.271, p=.034, d=.484], but not in any of the other conditions (p's > .351; Fig 4.4).

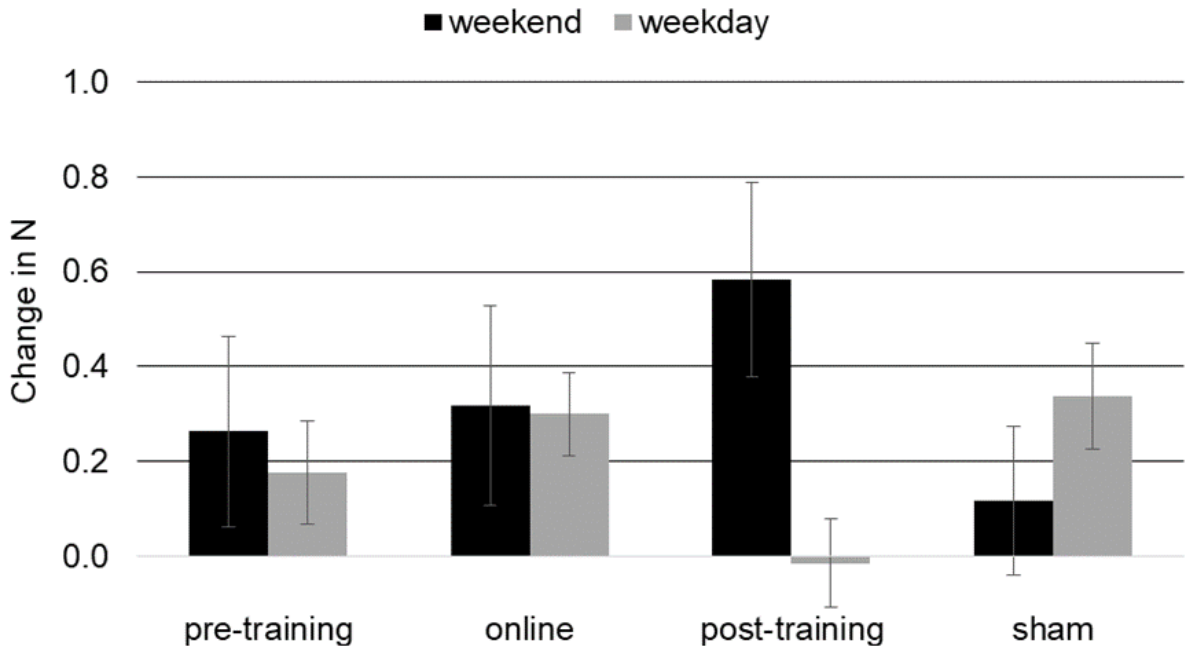


Figure 4.4: Weekend Effect.
In the offline post-training stimulation group, weekend gains between Friday and Monday were significantly greater than gains between average consecutive weekdays (excluding the first training session, which was unstimulated). No differences were found between weekday and weekend sessions in all other groups. Error bars represent standard error of the mean, and are generally smaller in the weekday condition because this consists of aggregated data from

Discussion

Our goal in the current study was to determine the optimal window during which to deliver stimulation in order to most effectively enhance WM training. While we found that stimulating during training was superior to either before or after training, we were unsuccessful in replicating the enhancement over sham stimulation. Therefore, it appears that the superiority of online stimulation in the current study is actually driven by *impaired* performance derived from offline stimulation rather than any enhancement derived from online stimulation. This selective impairment in the offline conditions was maintained at follow-up for the post-training condition, but not the pre-training condition, which caught up to the other groups after 1 month. The possibility of tDCS impairing rather than enhancing performance is not without precedent, and has actually been reported several times in the literature (e.g., Iuculano & Cohen Kadosh, 2013; Rosen et al., 2016; Sarkar et al., 2014; Steenbergen et al., 2016), and likely many more times in the proverbial file drawer. However, this is the first study of which we are aware that documents impairment in a long-term cognitive training protocol, which brings with it important considerations in terms of creating durable plastic changes with tDCS. Nevertheless, there are a few important caveats to discuss. First, both of our hypothesized moderators, spacing between training sessions and baseline WM ability, were significant, though not in the ways we predicted.

First, we previously reported with the same training paradigm that training gains were greatest in the online stimulation condition after a weekend break, as opposed to consecutive weekdays (Au et al. 2016), but were unable to replicate this effect in the current study. This is not necessarily surprising given the lack of an overall enhancement effect of online stimulation; however, what was more surprising was that we did observe this weekend effect in the post-training offline stimulation condition, despite its persistent underperformance throughout the rest

of training, across baseline sub-groups, and into the follow-up session. Although it is not clear whether this weekend effect represents enhanced learning from tDCS over the weekend, or merely a release from the weekday inhibition, either result is interesting in that it suggests tDCS can exert different effects under different conditions, even within the same individual. We speculate more on this in the General Discussion below. However, since this weekend effect is not a robust result and has only been reported once before by us, we cannot rule out the possibility that it is merely an artifact of multiple comparisons, given the four conditions present in the current study and that this effect did not arise in our hypothesized condition.

Secondly, based on our own previous work (B. Katz et al. 2017) as well as reports in the literature (Li et al. 2015), we also expected baseline WM performance to moderate the effects of tDCS, particularly in the online condition, such that tDCS is most effective among low-baseline participants. However, in contrast with our earlier study, our current data show that online tDCS only shows the advantage over sham among our high-baseline subgroup, whereas among the low-baseline performers, not only is the advantage absent, but there is actually a *disadvantage*, where all tDCS conditions underperform relative to sham. Neither of these findings are unique in the literature, as tDCS has both been shown to impair cognition (Iuculano and Cohen Kadosh 2013; Rosen et al. 2016; Sarkar et al. 2014; Steenbergen et al. 2016) as well as selectively benefit high-performers (Jones and Berryhill 2012; Jones, Gözenman, et al. 2015). The different and sometimes conflicting findings in the literature can often be dismissed as a consequence of the methodological heterogeneity between studies – differences in stimulation timing, duration, intensity, and location, or differences in the properties of the task itself. In our case, however, we kept most of the stimulation and task parameters between our two studies identical, but yet still managed to find such disparate results, both in terms of our baseline and weekend effects.

Therefore, we sought to explore these differences further and address them in the next chapter with a series of exploratory post-hoc analyses which combine our datasets together.

CHAPTER 5: Analyses of Combined Dataset

Overview

Here, we follow up on our unexpected results from Chapter 4 where we failed to replicate an overall enhancement effect of online tDCS relative to sham during WM training. To do so, we combined data from Chapters 2 and 4 since both studies used a very similar intervention design. Our post-hoc analyses ended up providing a plausible reconciliation of our discrepant results by suggesting a non-linear baseline-dependency such that both low and high performers on Day 1 end up benefiting from tDCS, but not average performers. In fact, there is some suggestion that tDCS may even *impair* performance in some instances, and it is this complex responder profile that leads to the observed null effect at the group-averaged level. A theoretical account for this pattern of effects is provided which proposes that tDCS is able to nudge cortical excitability levels closer to an individual optimum, but can also push away from that optimum in instances where excitability already starts off close to optimal.

Introduction

Chapters 2 and 4 both reported very similar experiments using tDCS to boost WM training performance over the course of a week, but yet arrived at different results. The 2016 study reported in Chapter 2 found an overall enhancement effect when stimulating online during training, with moderator analyses showing stronger tDCS effects among those who start off with low WM performance at baseline. The 2019 study in Chapter 4 found a null effect of online stimulation compared to sham, but found unexpectedly *impaired* performance among the offline stimulation conditions that received tDCS either before or after each training session. Moreover, moderator analyses showed that online stimulation was more beneficial among *high*-baseline

individuals, rather than low-baseline. Given the identical stimulation and behavioral procedures between both studies, we reasoned that the discrepant results we observed were likely due to individual differences in our participants. Although many factors could be at play here, including genotype, brain anatomy, and baseline cortical excitability (Krause and Cohen Kadosh 2014), we were limited in what we could examine through our behavioral data. However, the first thing we did notice behaviorally was that the sham groups did not appear to be the same between studies – namely, the shams in the 2019 study outperformed the shams from the 2016 study, and in fact seemed to more closely resemble just the high baseline sub-group, especially by the end of training (Fig 5.1).

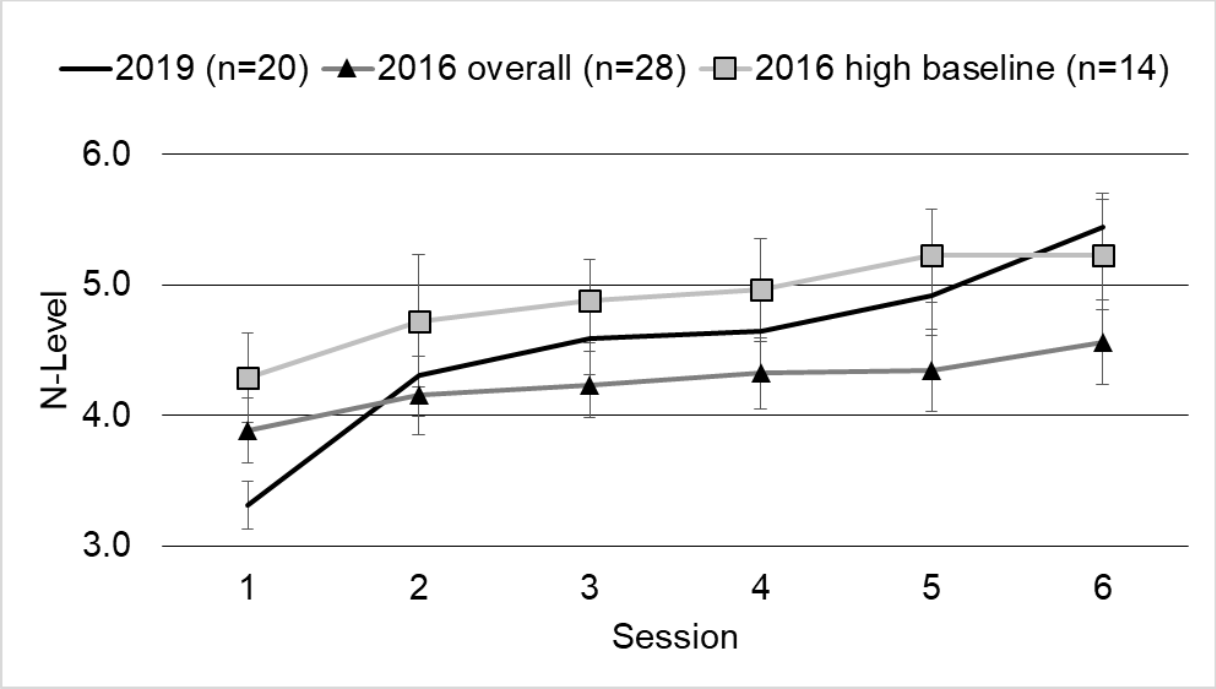


Figure 5.1: Sham Performance Across Studies.
A comparison of the previous (2016) and more recent (2019) sham performance shows clear discrepancies supporting a higher-functioning cohort of participants in the 2019 sample. Note that the differences in starting performance on Day 1 should be interpreted with caution since the 2016 study involved extensive pre-testing prior to the start of training that included exposure to untrained n-back tasks that were similar in nature to the training task. An arguably better comparison of the curves could be made if the 2016 curve were shifted one session to the right since their first training session is more akin to the second session of the 2019 curve. All error bars represent SEM.

Since we carefully matched all our participants on baseline ability between conditions in the 2019 experiment, the higher performance of the sham group also likely indicates that our entire cohort of participants is also higher functioning relative to our 2016 study. We previously demonstrated that tDCS has very little, if any effects among such higher functioning participants (B. Katz et al. 2017). Although this may plausibly explain the lack of any overall facilitation effect in the present experiment, it still does not explain the unexpectedly positive baseline relationship, where within this sample of higher-functioning participants, even higher baseline ability actually predicted greater tDCS efficacy.

If the results of both our studies are true, that there exists both a low-baseline advantage among a relatively lower-performing sample and a high-baseline advantage among a relatively higher-performing one, then we hypothesized that there must exist a non-linear relationship between baseline performance and tDCS efficacy such that tDCS is most effective at the extremes, but has little or perhaps even detrimental effects in between. Such a non-linear baseline dependency would also reconcile some of the differences reported in the literature, where both low (Li et al. 2015) and high (Jones and Berryhill 2012; Jones, Gözenman, et al. 2015) baseline-advantages have been documented, and some studies have also even shown decreased performance relative to sham (Iuculano and Cohen Kadosh 2013; Rosen et al. 2016; Sarkar et al. 2014; Steenbergen et al. 2016). Therefore, given that we have two datasets that use nearly identical stimulation and behavioral procedures but arrive at different conclusions, we are in the unique position to combine our samples in order to produce a dataset with greater baseline variation for a more extensive and better-powered analysis of baseline-dependencies and

possible non-linearities. Moreover, we were unable to replicate the weekend effect with the online stimulation group in the 2019 study and argued this was due to the overall null effect versus sham. Therefore, we hoped to be able to re-establish the existence of this weekend effect within the baseline subgroups that respond positively to tDCS.

Methods

Data Combination Procedure

In order to combine our datasets, we first had to derive a common metric for baseline. This was necessary since baseline was measured differently in our two studies. Our 2016 study measured accuracy on untrained n-back measures (a visual and auditory n-back) at pre-test using fixed loads (n-levels), while the present experiment used the average n-level of the first, unstimulated training session which used adaptive loads. Unfortunately, we could not use the first training session of our 2016 study to calculate baseline ability because stimulation was already applied during this session. Therefore, in order to attain a reasonably comparable baseline metric between studies, we calculated the average accuracy scores (hit rate minus false alarm rate) of all 2-back levels that participants completed on their first day in the lab. In our 2016 study, this meant that we selected the 2-back levels for the auditory and visual n-backs during the pre-test session, which consisted of 204 total trials across 12 blocks for each participant. For the 2019 study, we selected all completed 2-back levels of the visuospatial n-back training task during the unstimulated first session. Since the n-level of the training task was adaptive, participants were exposed to different amounts of 2-back blocks. On average, our baseline metric was calculated from 76.10 ± 58.86 (mean ± SD) trials from each participant, ranging from 20 to 200 trials, or 1 to 10 blocks. Only 2-back was selected because this was the only consistent n-back load to which all participants across both studies were exposed during the

first day in the lab, and only the online and sham conditions from the 2019 study were used since our 2016 study did not assess offline stimulation. This left us with an admittedly imperfect metric that conflates together different stimulus modalities, but provides the best possible measure of general n-back ability at baseline given the post-hoc nature of our analyses.

Analytical Approach

In order to test our hypothesis of non-linear effects of baseline on tDCS efficacy, we repeated the same ANOVA analysis conducted in Chapter 4, but with our combined dataset and taking advantage of our increased sample size to use quartile splits instead of median splits for our Baseline factor. This resulted in a 6x4x2 (Session x Baseline x Condition) mixed ANOVA, where the Baseline factor consisted of four levels: 15 online (10 from 2016) and 12 sham (8 from 2016) participants in quartile one, 14 online (9 from 2016) and 11 sham (6 from 2016) participants in quartile two, 16 online (12 from 2016) and 11 sham (6 from 2016) participants in quartile three, and 15 online (9 from 2016) and 14 sham (8 from 2016) participants in quartile four. We then repeated our weekend analyses using paired t-tests to compare weekend gains with average weekday gains within each participant, per condition and per quartile.

Results

Training Effects

Our analysis of overall training effects affirmed that online tDCS still showed superiority over sham with our combined datasest: Session x Condition interaction [$F_{(4.256,425.607)}$=3.230, p=.011, $\eta 2_p$ = .031]. This analysis also suggested that these effects may differ by quartile, with a marginal three-way interaction [$F_{(12.768,425.607)}$=1.581, p=.089, $\eta 2_p$ = .045]. Consistent with this, we also observed a marginal Condition x Baseline interaction [$F_{(3,100)}$=2.636, p=.054, $\eta 2_p$ =

.073], suggesting differences in average training performance (averaged over the Session factor) exist within certain quartiles.

First, we probed the three-way interaction by running the same gain score contrasts used in both our previous studies, and found evidence to support our hypothesized u-shaped function. Training gains (from session 1 to session 6) were significantly greater with online tDCS relative to Sham only in the first [$t_{(100)}$=2.210, p=.027, d=.442] and fourth [$t_{(100)}$=2.950, p=.003, d=.590] quartiles, but there were no significant effects in between (p's>.184). Next, when evaluating the Condition x Baseline interaction, we found that the pattern of effects actually *reversed* in the second quartile, with the sham group outperforming the online group [$t_{(100)}$=-2.085, p=.048, d=.417] across all sessions. No other main effects were found at any other level of Quartile, (p's>.139), with the exception of a marginal effect in favor of tDCS in the first quartile [$t_{(100)}$=1.798, p=.084, d=.360], which is in agreement with the three-way interaction above supporting stronger training gains with tDCS. See Fig 5.2 for a breakdown of training effects by quartile.

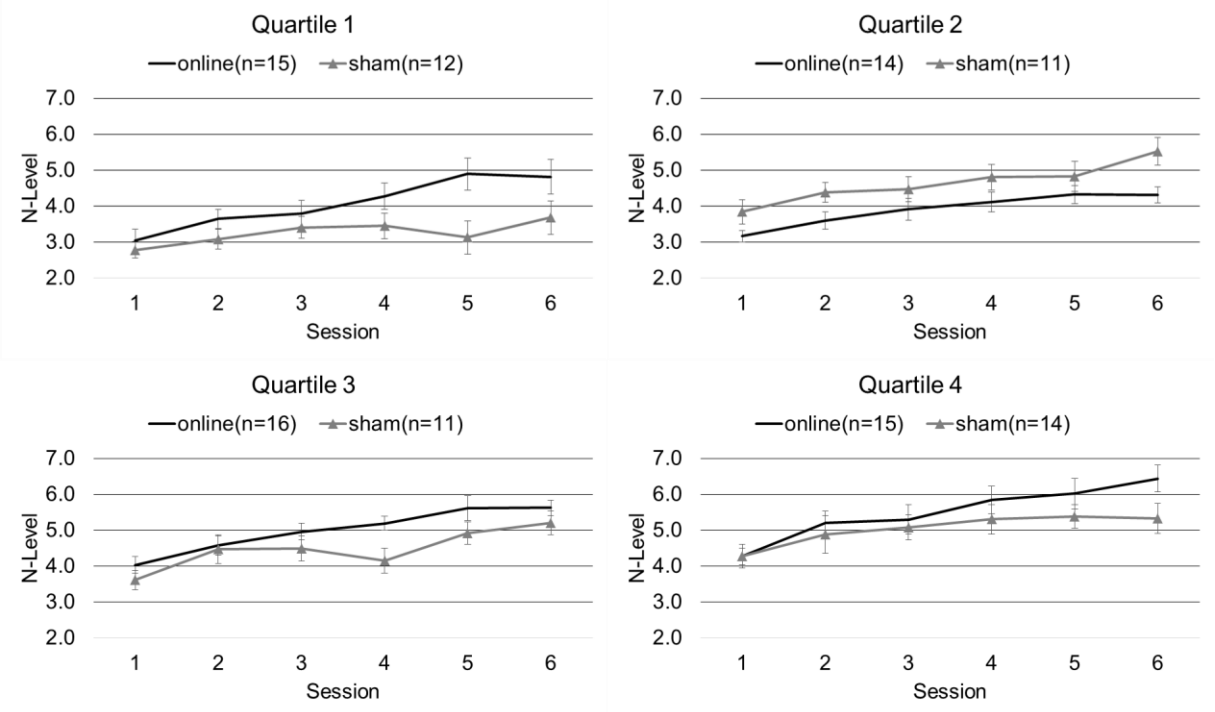


Figure 5.2: Combined Results by Quartile.
A breakdown of our combined results by quartile shows significantly greater training gains in the online stimulation group only in the first and fourth quartiles. Quartile 2 shows a main effect in favor of the sham group. The breakdown of participants from either study is enumerated in the Methods section and is not driven primarily by one study or the other in any quartile. All error bars represent SEM.

Weekend Effects

Paired t-tests confirmed stronger gains over the weekend compared to the average weekday in the first quartile [$t_{(11)}$=2.664, p=.022, d=.769] and a marginal effect in the fourth quartile [$t_{(13)}$=2.122, p=.054, d=.492]. No other quartile showed any significant effects, nor did any of the sham groups in any quartile (p's > .46), although we note that the sham group in the first quartile showed a significant effect in favor of *weekday* gains [$t_{(10)}$=2.789, p=.019, d=.841], and the online group in the third quartile showed a marginal effect also in favor of weekday gains, [$t_{(12)}$=1.835, p=.091, d=.509]. See Figure 5.3.

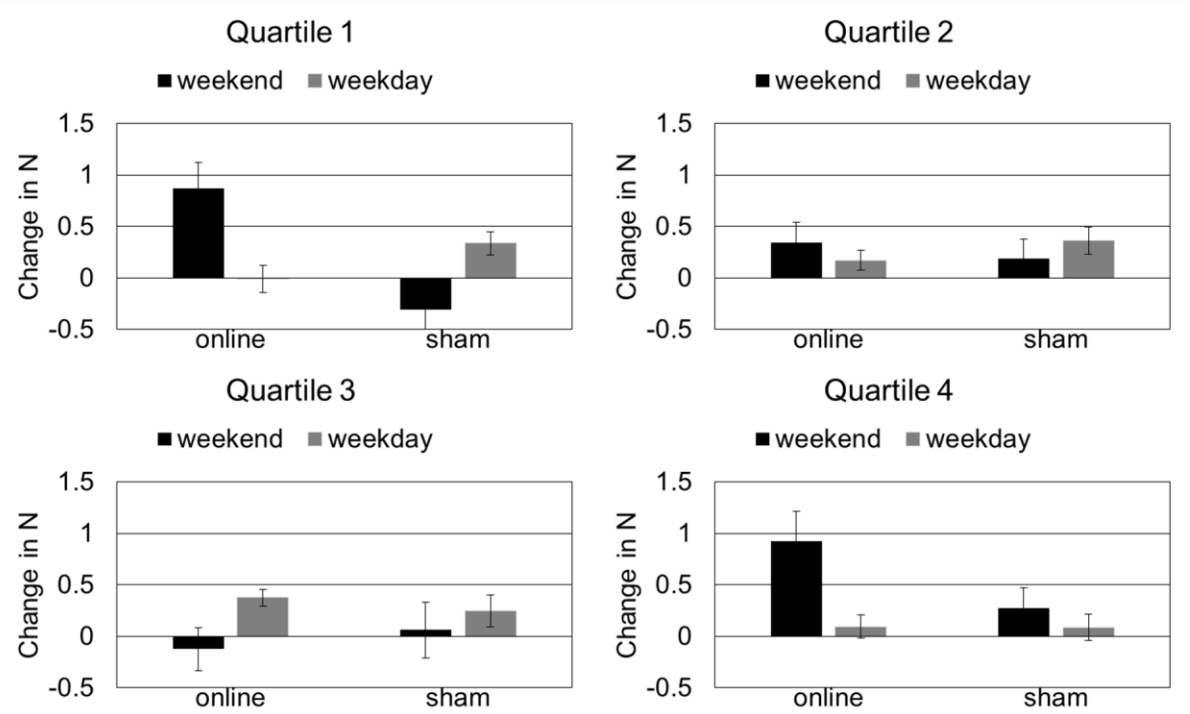


Figure 5.3: Weekend Spacing Effects by Quartile
Re-examining each quartile for weekend effects corroborate the selective efficacy of tDCS only within the first and fourth quartiles. No weekend advantage is observed in the middle quartiles for either group, and in fact, quartile 3 actually shows a significant *weekday* advantage in the tDCS group. All error bars represent SEM.

Discussion

Despite our inability to replicate the advantage of online tDCS over sham stimulation in Chapter 4, the present post-hoc analyses using our combined dataset reveal a more nuanced result that depends heavily on individual differences in a participant's baseline WM ability. Our combined results show that online tDCS is only effective in the extreme baseline groups – the bottom and top quartiles. Not only did these quartiles show significantly greater gains in the tDCS groups by the end of the training, but they also demonstrated the signature Weekend effect, which was not present in the intermediate quartiles. Interestingly, the advantage actually *reversed* in the second quartile so that the sham group outperformed the tDCS group, suggesting

that it is not merely the case that tDCS is only effective for certain people, but it can also be actively disadvantageous for others. This is also in line with the offline tDCS results in Chapter 4 where these conditions seemed to impair performance.

The important question is why tDCS seems to be effective only at the extremes and why it can sometimes be detrimental for more average people. One possible model that may account for this has been proposed by Krause et al. (2013), arguing that the influence of tDCS interacts with an individual's baseline cortical excitation/inhibition (E/I) balance. Specifically, they point out that tDCS has been shown to modulate GABA and glutamate levels, which are respectively the primary inhibitory and excitatory neurotransmitters in the brain (Stagg et al. 2009). Furthermore, fluctuations in these key neurotransmitters, namely the reduction of GABAergic tone induced by anodal tDCS, have been linked to greater learning in the brain (Charlotte J. Stagg, Bachtiar, and Johansen-Berg 2011). Therefore, by altering this E/I balance towards greater excitability, tDCS may improve performance and learning for individuals who start out at a sub-optimum level, but may have little to no effect on people who are already pretty close to their individual optimum. However, the brain is under constant homeostatic control and only operates optimally within a narrow biological range. Many neuropsychiatric conditions such as autism, schizophrenia, or ADHD have abnormally high E/I ratios in the brain, and this imbalance has been proposed to be a key causal factor in impaired information processing and associated social/behavioral/cognitive impairments (Yizhar et al. 2011). Therefore, it is possible that shifting this E/I balance too far to the right may have deleterious effects.

Although our behavioral studies were unable to measure neurotransmitter levels and E/I balance, our baseline WM measures may serve as a rough proxy. Indeed, higher WM performance is linked to higher E/I ratios in the frontal cortex, both at rest (Marsman et al. 2017)

as well as during task performance (Woodcock et al. 2018). Therefore, tDCS may nudge the E/I balance of our low baseline participants closer to their optimum, while for those in the middle quartiles, tDCS either has no effect because they are already performing fairly optimally, or it may even nudge them towards excitotoxicity and impair neural functioning, which may explain the instances in our data in which the sham group seems to outperform the tDCS groups. However, the brain has built-in homeostatic mechanisms that resist too much perturbation. Although a few minutes of anodal or cathodal tDCS over the motor cortex has been famously shown to respectively increase or decrease corticospinal excitability in accordance with electrode polarity (Nitsche and Paulus 2000), these effects can actually reverse in direction under conditions of over-excitation or over-inhibition. For example, increasing the duration of anodal stimulation beyond 26 minutes (Monte-Silva et al. 2013) can *reduce* corticospinal excitability and increasing the intensity of cathodal stimulation to 2 mA (Batsikadze G. et al. 2013) has been shown to *increase* it. Similarly, pre-conditioning the motor cortex with anodal stimulation causes subsequently administered repetitive transcranial magnetic stimulation (rTMS) to *reduce* corticospinal excitability whereas pre-conditioning with cathodal stimulation causes rTMS to *increase* excitability. That is, rTMS acts homeostatically in the direction that opposes the previous manipulation (Siebner et al. 2004). Although speculative, we posit that such homeostatic mechanisms are also at play in our experiments. Whereas, tDCS may nudge E/I towards optimum in low baseline individuals, and beyond optimum or possibly towards excitotoxicity in mid-baseline individuals, we suggest that it nudges high baseline individuals to the point where homeostatic mechanisms start to kick in to bring the balance back towards optimum.

Although our data have demonstrated that the effects of tDCS on different individuals can range from enhancement to null, and even to impairment, we must also explore the possibility that this same variation in effect can happen within the same individual as well. This intra-individual variability has already been reported in the motor cortex (Dyke et al. 2016; Horvath et al. 2016), and we observe in our own data that all instances of tDCS enhancement are completely driven by weekend gains, whereas the average weekday gains in these instances are no different than or sometimes even smaller than those of the respective sham group (see Figs 4.4 and 5.3). So it seems that even within the same individual, tDCS is capable of showing either an enhancement, impairment, or null effect on different days of the training protocol. However, unlike these previous motor studies which identified no reason for the poor intra-individual reliability of tDCS other than the capriciousness of the technology, our results seem to be driven by the spacing interval between sessions. There is strong theoretical grounding to explain the facilitatory effect of greater spacing that we have observed, but explaining the potential detrimental effect of shorter 24-hour spacing intervals (e.g., in the offline post-training stimulation group or 1st quartile of the combined data) would be speculative at best since our study was not designed to explore that. Whatever the reason though, this observation is a critical one to consider when designing future studies, especially for protocols that are 5 days or shorter that may not otherwise involve a weekend break.

Conclusions

Our combined results offer several important insights. First, stimulating offline, whether pre- or post-training, seems to be detrimental to training performance. Second, although we were initially unsuccessful in replicating the advantage of online stimulation over sham, combining our 2019 dataset with the 2016 one in Chapter 2 showed a non-linear relationship where only the

lowest and highest baseline performers benefited from tDCS. Third, we also confirm a relatively new finding, which has only been previously reported by our group so far (Au et al. 2016), that spacing sessions over the time course of days can critically influence the strength and direction of tDCS effects during WM training. Most importantly, we stress that our combined studies demonstrate that the effects of tDCS can be seemingly capricious without a thorough understanding of all the possible moderators that influence the strength and direction of these effects. Unfortunately, the field still lacks a thorough understanding of these possible moderators, but we have gained much ground over the past decade and have begun to identify a few key factors. Our findings, in combination with all the recent findings suggesting factors such as age, genetics, hormones, cortical excitability, and various stimulation parameters, etc. (Krause and Cohen Kadosh 2014), can produce both intra- and inter-individual differences in tDCS responsivity suggest that future studies must be very careful when interpreting null results. The non-linear effects we observed, where participants can not only be responders or non-responders, but also potentially negative-responders, could easily masquerade as an overall null effect even in studies where true effects may actually exist if researchers knew to look for them. In fact, we argue that these nuanced and bidirectional effects of tDCS, at least in part, contribute to the preponderance of null results reported in the literature (e.g., Horvath et al., 2015a, 2015b), especially in the earlier years of brain stimulation research when possible moderating effects were even less understood than they are now.

CHAPTER 6 – Concluding Remarks

Overall Summary

Throughout this dissertation, a series of experiments are described which collectively address a couple major issues in the use of tDCS for cognitive enhancement. First, we addressed the ability of tDCS to produce long-term changes in brain and behavior through consolidation-like mechanisms, and second, we identified an important individual difference factor, baseline ability, that moderates the effectiveness of these tDCS effects. Together, these results emphasize that ostensibly null effects in tDCS studies may actually harbor real effects beneath the surface. A null effect one day may manifest into a significant effect in the following days if a longitudinal design is employed, and a null effect at the group level may manifest into significant effects at subgroup levels if relevant moderators are taken into consideration. Therefore, the conclusions of meta-analyses that suggest the general ineffectiveness of tDCS should be interpreted with caution (Horvath et al. 2015a, 2015b).

Long-Term Effects

Chapter 1 presented a cohesive overview of the theoretical and empirical evidence for how tDCS can capitalize on existing consolidation mechanisms within the brain in order to boost learning and memory. We cited evidence from animal studies that demonstrated the ability of direct current to increase cellular LTP, and also reviewed human studies that showed behavioral correlates consistent with this idea. Even in the absence of effects during or immediately after stimulation, many instances of delayed enhancement across a range of behavioral tasks have been observed hours, days, or even up to a year after the initial stimulation session. In other words, we argued that even if the direct influence of tDCS on neural membrane potential is not strong enough to manifest a behavioral difference immediately, effects can still appear later on

due to increased LTP or LTP-like plasticity that take place upon completion of the behavioral task that can produce stronger task-relevant networks in the future.

Chapter 2 began evaluating these ideas in the context of WM training and found evidence that tDCS improved performance over the course of the 7-day training period compared to sham stimulation. Importantly, performance between groups was almost identical after the first training day, and the gap steadily widened each day until it became significant after Day 4. As we argued in Chapter 1, the direct effects of tDCS on membrane polarization and the probability of action potential generation during stimulation are not always strong enough to be observable on a behavioral level, but effects can become more and more pronounced over time if tDCS interacts with the consolidation process. However, our experimental design forbade us from directly concluding this, as stimulation was delivered each day of training, so it was not known whether tDCS promoted better learning offline between sessions, or whether participants were simply becoming more and more responsive to tDCS during each successive stimulation session. Nevertheless, there were a few clues in our data that were suggestive of an interaction with consolidation. For example, we observed an effect of spacing, where the greatest learning gains were produced over the weekend break, as opposed to consecutive weekdays. This effect only occurred with active stimulation, and not with sham. We interpreted this as an interaction between tDCS and the consolidation process such that more time for consolidation between sessions resulted in more pronounced tDCS effects. Furthermore, these training gains persisted for up to a year at follow-up when re-tested on the same task without stimulation, suggesting impressive durability of learning with tDCS whereas the sham group more or less returned to baseline after 1 year.

Building off these observations, the study reported in Chapter 3 sought to elucidate the electrophysiological underpinnings of these delayed effects, and to directly compare the extent to which immediate and delayed effects of tDCS could be detected. We used EEG to measure cortical excitability over the parietal-occipital area by eliciting steady-state visual evoked potentials (SSVEP). Consistent with the behavioral data in Chapter 2, there were no immediate changes in SSVEP directly after stimulation relative to sham, but there were delayed effects the next day. Importantly, the increased SSVEP strength detected the next day occurred even before additional stimulation was delivered, indicating that the changes that occurred in the neural network responsible for the SSVEP signal occurred offline in between sessions. This observation lent stronger evidence to the interpretations we laid out in Chapters 1 and 2 that the source of these delayed effects is in fact an increase in LTP-like plasticity in task-relevant networks that takes place in the hours or days after a stimulation session.

Chapter 4, however, introduced an additional layer of complexity by demonstrating that these delayed between-session effects of tDCS can also be detrimental as well as beneficial in some cases. Namely, offline stimulation delivered either immediately before or after training, stunted learning by the end of the week-long training relative to both online stimulation and sham. This effect was especially pronounced in the offline post-training condition where group differences remained significant even after 1 month. As we pointed out in Chapter 4, this is not unusual in the tDCS literature, and under different conditions, tDCS can seem to either enhance or dampen brain activity. Unfortunately, it is not clearly known the precise conditions that produce which effect. However, our results suggest that for our specific task and design, stimulating *during* learning is the most promising avenue for observing behavioral enhancement over time, similar to another report also using an n-back WM task like we did (Martin et al.

2014) Accordingly, Chapter 3 involved offline stimulation due to the technological limitations of measuring EEG and applying tDCS at the same time, and also did not demonstrate any behavioral enhancement, despite the presence of cortical effects. However, the study upon which we based our task design in Chapter 3 did use online stimulation, and they did observe behavioral enhancement (Heinen et al. 2016). Finally, the combined results described in Chapter 5 indicate that whenever online stimulation does produce an enhancement effect (in the extreme baseline subgroups), the pattern is the same in that there are no immediate effects of stimulation in the beginning, but differences emerge over time, presumably through an interaction between tDCS with the learning and consolidation process during training.

Baseline Differences as a Moderator of tDCS

The other important theme consistent throughout all experiments described in this dissertation is the importance of individual differences in how people respond to tDCS. Even with the exact same protocol, different people can show different, and sometimes completely opposite, effects. Our first experiment in Chapter 2B showed that individuals who performed more poorly on the WM tasks at pre-test received a greater benefit from tDCS compared to their low-performing sham counterparts whereas high-baseline individuals received less benefit compared to high-baseline shams. Chapter 3 followed this up on a neural level by demonstrating that individuals starting off with lower SSVEP activity on Day 1 ended up experiencing more tDCS-related SSVEP enhancement the next day compared to when they received sham stimulation. Though high-baseline individuals showed a similar numerical pattern, there were no statistically significant changes.

Though the low-baseline advantage was consistent across these two studies, as well as the broader literature (Li et al. 2015), Chapter 4 once again introduced an extra layer of complexity

by actually demonstrating a high-baseline advantage of online stimulation rather than low. However, the striking difference we noticed between this study and our previous one described in Chapter 2 was that the Chapter 4 sham group significantly outperformed the previous one, suggesting cohort differences in the participants who were recruited into the respective studies. Given that a few studies have also documented high-baseline advantages before (Jones and Berryhill 2012; Jones, Gözenman, et al. 2015), we reasoned that it was plausible both high- and low-baseline advantages could co-exist but requires a sample with sufficient variation to detect. By combining our datasets together, as described in Chapter 5, and coming up with a common baseline metric for all participants across both studies, we actually did find evidence for a non-linear baseline-dependency of tDCS such that individuals in both the first and last quartiles of baseline performance showed tDCS-related enhancement over the course of training. Importantly, representation between studies was fairly equal across all four quartiles, with 50-75% of participants coming from the Chapter 2 study among all quartiles. Moreover, the first and last quartiles also showed the weekend effect whereby stronger learning gains are found after a weekend break as opposed to consecutive weekdays among those receiving active tDCS. We first described this effect in Chapter 2 as a signature of the interaction between tDCS with spaced learning and consolidation. The second and third quartiles neither showed evidence of a tDCS benefit during training nor any weekend effects. In fact, there was even evidence that some individuals in these intermediate quartiles were actually impaired by stimulation rather than helped.

Although it is not entirely clear why baseline ability moderates the effects of tDCS, the evidence that it does do so is reliable, not only among our studies, but also in the broader literature (Li et al. 2015). It has been previously theorized that the effects of tDCS on enhancing

cortical excitability can either nudge a sub-optimally excited cortex towards optimum, or nudge an optimally-excited cortex away from optimum (Krause and Cohen Kadosh 2014; Krause et al. 2013). We add to this by suggesting that homeostatic mechanisms can nudge an over-excited cortex back down towards optimum, and provide evidence from the literature of other instances in which this has occurred (see Chapter 5). This model, though still somewhat speculative, accounts for our observed pattern of results, suggesting that low-baseline individuals who benefit from tDCS start off with sub-optimal prefrontal excitation that can be boosted by tDCS. However, more average individuals who start off with near-optimal excitability, may be nudged into excito-toxicity, thereby impairing performance, while high-baseline individuals may be slightly past optimum but still functioning fairly well, and thus the additional excitation caused by tDCS can engage homeostatic mechanisms that bring them back down towards their individual optimum. This simple, admittedly overly-simple, model of our results, is not likely to be completely accurate, particularly as there are many other moderating influences that complicate the effects of tDCS on an individual. Limitations of this model, and our overall series of studies are discussed further below.

Limitations and Future Directions

There are several important lessons to learn from the series of experiments described herein that could inform future research. Arguably the most important one is that tDCS does not affect everybody in the same way. Not only are there responders and non-responders, but there can also be negative responders. It is imperative for future research to identify the factors that promote this varied responder profile, so researchers or clinicians can anticipate ahead of time whether an intervention will help a particular individual or possibly harm them. Throughout this dissertation, we have described one important moderator, baseline ability, and argued that tDCS

interacts with an individual's pre-existing state of cortical excitability by nudging sub-optimal states closer to the optimum, but this also runs the risk of nudging already-optimal states away from this optimum. Although there is theoretical and empirical support for this model from the literature, one important limitation is that our data are mostly behavioral in nature. Thus, we are making the assumption that WM performance is a proxy for cortical excitability. Although the two are generally correlated (Marsman et al. 2017; Woodcock et al. 2018), the correspondence is far from one to one, and a more direct demonstration of our model should involve brain measures in addition to behavioral, similar to our SSVEP experiment described in Chapter 3. Although that experiment did find neural support for tDCS increasing excitability among low-baseline individuals, it was limited in that the sample size was too small to run a proper analysis of middle- and high-baseline individuals as well. Moreover, we were unsuccessful at demonstrating any behavioral effects. A future study with a larger sample size and greater power may be more successful in directly demonstrating our observed non-linear baseline effects at the neural level, and also using a different behavioral task or different stimulation parameters in order to demonstrate brain-behavior correlations.

Also, given the varied responder profile and the evidence for long-term neural/behavioral changes, we must also be wary of the possibility of long-term detriment to cognitive functioning. At the moment, there is no evidence to support that any such detriment would be anything other than task-specific. That is, receiving stimulation during task performance has the potential to decrease learning and mastery on that task in the long-term, but there is no evidence for general detriments to cognitive functioning outside of the specific task learned during stimulation. Nevertheless, there are reports of cognitive/behavioral improvements even in the absence of a behavioral task. For example, the use of tDCS for the treatment of major depression (Meron et

al. 2015) and other psychiatric disorders (Tortella et al. 2015) is often done with stimulation alone in order to alter the resting excitability of the target cortical area. Therefore, the possibility of long-term maladaptive changes to brain function that are task-nonspecific cannot be ruled out and should be a subject of future research.

Furthermore, the ability of tDCS to enhance WM training provides proof of principle that learning and cognitive skills can be improved. But, unless transfer to untrained tasks is demonstrated, there is ultimately little translational utility. Thus, there are two possible routes for future research to improve the translational appeal of this research. Either transfer to untrained cognitive tests could be assessed or the trained behavioral task can be changed to something more socially relevant. In the first case, a pretest-intervention-posttest design similar to that described in Chapter 2 would be appropriate. However, even the outcome measures used in Chapter 2 themselves have little translational utility, as they are just different laboratory tests of WM. An outcome of greater social interest might be a test of fluid intelligence, which has a controversial link with WM training and has been traditionally difficult to reliably elicit via conventional training methods (Au et al. 2015; Jaeggi et al. 2008; Melby-Lervåg and Hulme 2016). However, if a causal link truly exists between training WM and fluid intelligence, then perhaps the use of tDCS as a performance enhancer could tease out this link more reliably, especially if low and high baseline performers are selectively recruited to increase the probability of responding positively to tDCS. In the second case, replacing WM training with another learning task might prove fruitful as well. One such candidate that nicely bridges the gap between a laboratory-controlled task and an ecologically-relevant task could be vocabulary learning. Several such studies have already been conducted demonstrating the efficacy of tDCS on improving vocabulary learning (Javadi and Cheng 2013; Meinzer et al. 2014), but it has not

yet been demonstrated that these effects have consolidation-like properties such as those described in the current dissertation. For example, a spacing manipulation such as the weekend breaks used in Chapters 2 and 4 could be used. Such a study would not only be of great social interest for language learning, but would also potentially provide a strong demonstration of how tDCS might enhance hippocampal-dependent consolidation with word learning. Such a demonstration would greatly enhance our knowledge of how tDCS works, and also address a weakness in the current series of experiments in that the consolidation-like properties described in the preceding chapters arise from learning during a WM training task, which is not necessarily hippocampal-dependent and thus may not directly involve consolidation as it is traditionally conceptualized.

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