Assessment of Transcranial Direct Current Stimulation (tDCS) on MMN-Indexed Auditory Sensory Processing

by

Danielle Impey

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ABSTRACT

Transcranial direct current stimulation (tDCS) is a non-invasive form of brain stimulation which uses a very weak constant current to temporarily excite or inhibit activity in the brain area of interest via electrodes placed on the scalp, depending on the polarity and strength of the current. Presently, tDCS is being used as a tool to investigate frontal cognition in healthy controls and to improve symptoms in neurological and psychiatric patients. Relatively little research has been conducted with respect to tDCS and the auditory cortex (AC). The primary aim of this thesis was to elucidate the effects of tDCS on auditory sensory discrimination, assessed with the mismatch negativity (MMN) event-related potential (ERP). In the first pilot study, healthy participants were assessed in a randomized, double-blind, sham-controlled design, in which participants received anodal tDCS over the primary AC (2 mA for 20 minutes) in one session and 'sham' stimulation (i.e. no stimulation) in the other. Pitch MMN was found to be enhanced after receiving anodal tDCS, with the effects being evidenced in individuals with relatively low (vs. high) baseline amplitudes. No significant effects were seen with sham stimulation. A second study examined the separate and interacting effects of anodal and cathodal tDCS on MMN measures. MMN was assessed pre- and post-tDCS (2 mA, 20 minutes) in 2 separate sessions, one involving sham stimulation, followed by anodal stimulation, and one involving cathodal stimulation, followed by anodal stimulation. Only anodal tDCS over the AC increased pitch MMN in baseline-stratified groups, and while cathodal tDCS decreased MMN, subsequent anodal stimulation did not significantly alter MMNs. As evidence has shown that tDCS lasting effects may be dependent on N-methyl-D-aspartate (NMDA) receptor activity, a pharmacological study investigated the use of dextromethorphan (DMO), an NMDA antagonist, to assess possible modulation of tDCS' effects on both MMN and working memory (WM)

performance. The study involved four test sessions that compared pre- and post-anodal tDCS over the AC and sham stimulation with both DMO (50 mL) and placebo administration. MMN amplitude increases were only seen with anodal tDCS with placebo administration, not with sham stimulation, nor with DMO administration. In the sham condition, DMO decreased MMN amplitudes. Anodal tDCS improved WM performance in the active drug condition. Findings from this study contribute to the understanding of underlying neurobiological mechanisms mediating tDCS-sensory and memory improvements. As cognitive impairment has been proposed to be the core feature of schizophrenia disorder (Sz) and MMN is a putative biomarker of Sz, a pilot study was conducted to assess the effects of pre- and post-tDCS on MMN measures in 12 Sz patients, as well as WM performance. Temporal, frontal and sham tDCS were applied in separate sessions. Results demonstrated a trend for pitch MMNs to increase with anodal temporal tDCS, which was significant in a subgroup of Sz individuals with auditory hallucinations, who had low MMNs at baseline. Anodal frontal tDCS significantly increased WM performance, which was found to positively correlate with MMN-tDCS effects. The findings contribute to our understanding of tDCS effects for MMN-indexed sensory discrimination and WM performance in healthy participants and individuals with Sz disorder and may have implications for treatment of sensory processing deficits in neuropsychiatric illness.

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LIST OF MANUSCRIPTS

1. **Impey, D**., & Knott, V. (2015). Effect of transcranial direct current stimulation (tDCS) on MMN-indexed auditory discrimination: a pilot study. *Journal of Neural Transmission*, *122*(8), 1175-1185.

2. **Impey, D.,** de la Salle, S. & Knott, V. (2016). Assessment of Anodal and Cathodal Transcranial Direct Current Stimulation (tDCS) on MMN-Indexed Auditory Sensory Memory. *Brain and Cognition*, 105, 46-54.

3. **Impey, D.,** de la Salle, S., Baddeley, A., & Knott, V. (2016). Effects of an NMDA Antagonist on the Auditory Mismatch Negativity (MMN) Response to Transcranial Direct Current Stimulation. *Journal of Psychopharmacology* (Accepted).

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LIST OF ABBREVIATIONS

AC auditory cortex

AHs auditory hallucinations

ANOVA analysis of variance

CADS Clinician Administered Dissociative Symptom Scale

dB decibel

DLPFC dorsolateral prefrontal cortex

DMO dextromethorphan

DSM-IV Diagnostic and Statistical Manuel of Mental Disorders-IV

EEG electroencephalography

ERP event-related potential

FIGS Family Interview for Genetic Studies

GABA gamma-amino butyric acid

HE high efficiency individuals

HEOG horizontal electro-oculographic

Hz hertz

ISI interstimulus interval

LE low discriminating efficiency individuals

LTD long term depression

LTP long term potentiation

M mean

mA milliamps

MAO monoamine oxidase

min minutes

MMN mismatch negativity

MRS magnetic resonance spectroscopy

ms milliseconds

NMDA N-methyl-d-aspartate

NMDAR N-methyl-D-aspartate receptor

NSERC Natural Sciences and Engineering Research Council

p probability

PANSS Positive and Negative Syndrome Scale

PCP Phencyclidine

PSYRATS Psychotic Symptom Ratings Scales

RT reaction time

s seconds

SCID-NP Structured Clinical Interview for DSM-IV-R Non-Patient edition

SE standard error

SOA stimulus onset asynchrony

SPL sound pressure level

SPSS statistical package for social sciences

Sz schizophrenia

tDCS transcranial Direct Current Stimulation

TPA temporal-parietal area

VEOG vertical electro-oculographic

WM working memory

µV micro volt

THESIS FORMAT AND AUTHORSHIP

In accordance with the guidelines set forth by the Faculty of Graduate and Postdoctoral Studies, this dissertation is presented as a collection of manuscripts. This thesis includes four research papers examining the effects of tDCS on the MMN ERP. The first two papers examine the effects of anodal and cathodal tDCS on MMN in healthy participants, the third paper examines the effect of an NMDA antagonist on tDCS-MMN effects and the last paper investigates tDCS-MMN effects in individuals with schizophrenia. A general introduction precedes the research papers and provides a review of relevant literature on tDCS, ERPs and MMN, as well as the major objectives of the thesis. The four research papers follow the general introduction, reproduced as they appear in the published article or submitted manuscript, and each includes their own introduction, methodology, results, and discussion sections. The authors include Danielle Impey, Dr. Verner Knott, Sara De La Salle, Ashley Baddeley and Dr. Alain Labelle. Danielle Impey was implicated in the conceptualization of the research comprising this thesis work, carried out all experiments, conducted analyses and prepared all manuscripts. Dr. Verner Knott was the principal investigator of the research comprising this thesis work and assisted with the analysis, writing and editing of the manuscripts. Sara De La Salle and Ashley Baddeley were research assistants who helped with data collection. Dr. Alain Labelle was the primary physician involved in the clinical study. The thesis concludes with a general discussion, including a summary of all results and overall discussion of thesis findings.

Chapter 1: Introduction

1.1. Overview

Non-invasive brain stimulation techniques provide means to alter neuronal activity in specific brain regions and at a network level (Pascual-Leone et al., 2005). When first discovered, they allowed for stimulation-induced changes in the brain's state to demonstrate the involvement of specific brain areas in particular functions (Jasper and Penfield, 1954). Currently, these brain stimulation technologies are promising cognitive enhancing techniques being investigated in healthy participants, and a variety of neuropsychiatric disorders with cognitive impairments, including, recently, in schizophrenia (Sz; Demirtas-Tatlidede et al., 2013). Transcranial direct current stimulation (tDCS) is a non-invasive and cost effective procedure of cortical stimulation, in which weak direct currents are used to induce changes in excitability of targeted brain regions. Depending on the polarity of the stimulation, tDCS can result in reversible increases (anodal tDCS) or decreases (cathodal tDCS) in cortical excitability in circumscribed stimulated brain areas underneath the active electrode (Gandiga et al., 2006; Nitsche et al., 2003a; 2008). tDCS has been most frequently investigated with respect to its effects on motor and visual cortex activity in healthy volunteers and in neuropsychiatric patients (Fregni et al., 2006, Ferruci et al, 2008; Hill, Fitzgerald & Hoy, 2015; Jo et al., 2009; Nitsche et al., 2003a). Very few studies have investigated the effects of brain stimulation over the auditory cortex and only recently, tDCS has increasingly been used to assess and modulate cognitive function (Brunoni et al., 2014; Brunelin et al., 2012; Mondino et al., 2015a; Vercammen et al., 2011). The event-related potential (ERP) technique has been a particularly useful method for examining sensory and cognitive function, as well as the neuropharmacological mechanisms underlying these processes, due to its high

temporal sensitivity (Luck, 2012). Currently, tDCS effects have been largely unexplored with ERP methodology and the underlying neurobiological mechanisms are still unclear.

1.2. tDCS

Transcranial direct current stimulation is a non-invasive and non-convulsive method to temporarily increase or decrease cortical excitability in targeted, localized brain regions. Stimulation is administrated by applying a weak (~1-2 milliamps; mA), constant current, delivered through a 9-volt constant current regulator, to the scalp via two electrodes: the anode and the cathode (Nitsche et al., 2005; 2008). The electrodes can be used as the active or reference electrode depending on the bipolar placement and size of the electrodes, which are typically superimposed on saline-soaked sponges to increase the conductivity of the current and to reduce any skin irritation that may be experienced during stimulation (Nitsche et al., 2005; 2008). The low constant current, flowing from the smaller, active electrode to the larger, reference electrode (Neuling et al., 2012), is typically applied for 10-30 minutes, and the effects of a single stimulation can persist for up to one hour post-stimulation (Nitsche & Paulus, 2001). In clinical research studies with tDCS, repeated, daily stimulations are needed to produce long lasting effects (lasting weeks; Brunelin et al., 2012). Stimulation effects are compared to 'sham' stimulation, a control condition where the device is applied but no current is administered (Gandiga, Hummel & Cohen, 2006). The moderating factors affecting the effects of tDCS include current strength and polarity, electrode size, stimulation duration, and position of the electrodes on the scalp (McKinley et al., 2012; Nitsche et al. 2008). These factors mediate the relationship between the induced intracerebral flow of current from the electrodes and alterations of local neuronal activity, which allows for the investigation of the relationships between the modulated neural activity and cognitive processes or behaviour.

The majority of tDCS studies have focused on motor cortex function, the first target for tDCS investigation (Nitsche and Paulus, 2000; 2001). These tDCS studies established that tDCS-induced prolonged effects are not only polarity-specific, with anodal stimulation typically having an excitatory effect by depolarizing neuronal membrane potentials in the region beneath the anodal electrode, and cathodal stimulation generally having the opposite effect through a process of hyperpolarization of neuronal membrane potentials beneath the cathode electrode (Nitsche et al., 2007), but the strength and endurance of the after-effects depend on current intensity, duration and electrode placement (Nitsche and Paulus 2000; Nitsche et al. 2008). A simple relation between polarity and cognitive or behavioural modification has been recently challenged as evidence has shown tDCS can both depolarize and hyperpolarize within the same gyrus and different types of neurons are differently affected depending on their structural features and orientation (Reato et al., 2013; Radman et al. 2009). Also, for performance improvement, it is hypothesized that there is an inverted U-shaped dose-response relationship wherein if arousal increases beyond the optimal levels due to external stimulation, performance will begin to deteriorate. This means that enhanced cortical excitability does not necessarily always increase performance (Heimrath et al., 2016) and therefore, it is important to consider baseline functioning. However, current densities studies have shown that with bipolar placement of the anode and cathode, peak current densities occur over the targeted stimulation region (Neuling et al., 2012) and pharmacological studies have also elucidated some of the mechanisms of action underlying tDCS' effects.

As the immediate and long-term (‘after-effects’) of anodal stimulation are no longer present with sodium and calcium channel blockers (Nitsche, Fricke et al., 2003), which are necessary for neurotransmission, pharmacological studies suggest that the immediate short-

lasting effects of tDCS are generated by polarity-specific shifts of the cell's resting membrane potential, with anodal stimulation causing a depolarization of the resting potential (and an increase in spontaneous firing rates), while cathodal stimulation has the opposite effect, causing a hyperpolarization of the resting-membrane potential (and a decrease in spontaneous firing rates). As seen with animal data (Rohan et al. 2015), tDCS changes in excitability and motoric functions continue post-stimulation if tDCS is applied for several minutes, and the 'after-effects', or excitability that continues post-stimulation, can remain stable for an hour or more if tDCS is applied for nine minutes or longer over the motor cortex (Nitsche et al, 2003a; 2004).

Induced brain plasticity in the form of 'after-effects' depend on membrane potential changes and modulations of N-methyl-D-aspartate (NMDA) receptor activity, which is a glutamate receptor crucial to synaptic plasticity and memory function (Castellano, Cestari & Ciamei, 2001; Tang et al., 1999), among other neurotransmitters (Liebetanz et al. 2002; Nitsche et al. 2003a). The NMDA receptor (NMDAR) system has been implicated in the mechanisms of tDCS as NMDA antagonists have been found to prevent motor evoked after-effects, while NMDA agonists prolong motor after-effects by several hours (Liebetanz et al. 2002; Nitsche et al. 2004). Dextromethorphan (DMO), a NMDA-receptor antagonist, induces suppression of the after-effects of both anodal and cathodal stimulation, while carbamazepine (a sodium channel blocker) and flunarizine (a calcium channel blocker) eliminate only the anodal effects (Liebetanz et al. 2002; Nitsche, Fricke et al. 2003). D-cycloserine, a partial NMDA agonist, selectively potentiates the effects of anodal tDCS with increased excitability of motor regions (Nitsche et al. 2004). These pharmacological studies targeting the motor cortex demonstrate the vital role of NMDA receptor activity on the after-effects of tDCS. Also, a magnetic resonance spectroscopy (MRS) study investigating the neuronal mechanisms underlying tDCS' influence on learning

found significantly higher combined glutamate and glutamine levels beneath the stimulating electrode, further suggesting that glutamatergic activity is related to the mechanisms of action for tDCS (Clark et al., 2011). Although these tDCS modulations are dependent on the NMDAR system, other systems vital to brain plasticity including GABAergic interneurons have also been implicated in tDCS effects, as well as dopaminergic, serotonergic and cholinergic activity (Medeiros et al., 2012; Stagg and Nitsche, 2011).

1.3. tDCS and Cognition

Changes in neuronal activity with tDCS have implications not only for motor functions, but also for sensory-perceptual processing (Costa et al., 2015) and cognitive function, including attention, memory and executive functions (Brunoni and Vanderhasselt, 2014). As mentioned, tDCS is a promising research tool for cognitive enhancement and may be used as a method to elucidate the neurobiological mechanisms underlying these perceptual and cognitive processes. It is imperative to study sensory and cognitive function as deficits in these areas can demonstrate significant limitations in an individual's ability to learn and function and they are currently considered to be the core feature and treatment target in psychiatric illness (Gold, 2004; Green et al, 2004).

Anodal stimulation (~1-2 mA) applied to the dorsolateral prefrontal cortex (DLPFC; for 15-30 mins) has improved performance on a variety of cognitive tasks (Fregni et al., 2005; Gandiga, Hummel & Cohen, 2006; Hecht, Walsh, & Lavidor, 2010; Zaehle et al, 2011). The most robust effects are seen with working memory (WM), which refers to the brain function responsible for providing temporary storage and manipulation of information necessary to complete learning tasks (Baddeley, 1992). Measured through WM paradigms such as the N-back tasks and the Sternberg task, anodal stimulation of the DLPFC has increased performance accuracy and

reaction times (Andrews et al., 2011; Gladwin et al., 2012; Teo et al., 2011). Fregni et al. (2005) found that anodal tDCS enhanced accuracy in a 3-back WM letter task and Ohn et al. (2008) found an increase in correct responses based on the same paradigm starting 20 minutes post-stimulation. In clinical populations, beneficial effects of anodal stimulation applied over the left DLPFC have been reported for n-back WM task performance and probabilistic association learning in schizophrenic patients (Hoy et al., 2014; Vercammen et al., 2011). tDCS has also been found to improve WM, attentional performance and information processing in patients with depression (Fregni et al., 2006; Oliveira et al., 2013; Wolkenstein and Plewnia, 2013), visual recognition memory performance in Alzheimer's disease (Boggio et al., 2009), amelioration of memory deficits in Parkinson's disease (Boggio et al., 2006), and improved response accuracy in a Go/NoGo task and verbal 2-back WM task in patients with post-stroke cognitive decline (Kang et al., 2009; Jo et al., 2009). Cathodal tDCS stimulation with the same parameters has also been found to transiently decrease memory performance in healthy controls and Alzheimer's patients (Ferruci et al., 2008), but unlike in motor response, cathodal stimulation has failed to show consistent modulation in cognitive tasks (Fregni et al., 2005; Hecht et al., 2010). However, cathodal tDCS over the primary and secondary auditory cortex has been found to negatively modulate acoustic pitch discrimination and auditory memory performance (Mathys et al. 2010; Vines et al. 2006). Very few tDCS studies have focused on information processing in the auditory cortex and only one investigator had previously assessed the effect of anodal tDCS on auditory discriminability, or the ability to differentiate between dissimilar acoustic sounds or sound patterns (Chen et al. 2014a; 2014b), which motivates the current set of studies.

1.4. Event-related potentials

Sensory and cognitive processes can be assessed through the use of electroencephalography (EEG), an objective and non-invasive measure of electrical brain activity. Electrodes placed on the scalp allow for the measurement of EEG-derived, transient electrical brain potentials elicited by specific external or internal events (stimuli, responses, etc.; Luck, 2011). These measurements, known as event-related potentials (ERPs), directly reflect changes in neuronal activity involved in processing of sensory input. Employing a signal averaging procedure, time-locked EEG epochs associated with multiple presentations of stimuli are averaged to yield an ERP waveform consisting of a sequence of multiple positive (P) and negative (N) voltage peaks or components, each corresponding to a distinct cognitive process, with the amplitude of the peak reflecting the strength of the process, while the latency reflects its timing or speed of processing (Luck, 2011; Braff and Light, 2004). ERPs are typically categorized into early and late components, according to the time when they reach maximal amplitude. First, early latency (< 200 ms) components, referred to as "exogenous" or sensory components, are mostly affected by the physical features of stimuli (e.g., intensity, frequency) and are generally not affected by psychological processes. In contrast, longer latency ERPs are referred to as "endogenous" or cognitive ERPs and are affected mainly by the significance or psychological relevance of the stimulus.

The high temporal resolution afforded by ERPs (1 ms) allows for the investigation of early sensory processes through to later, attention-dependent, higher-order processes and they are relatively inexpensive, allowing for large, multi-site studies to be carried out (Hecht et al., 2010). Several ERP components have analogs in animal models allowing for translational research (Woodman, 2012) and many ERPs are supported by decades of research establishing their sensitivity and test-retest reliability (Bramon et al, 2004). Several ERP components, including

the early P50, the mid-latency mismatch negativity (MMN) and the P300 component, have been frequently investigated as neurophysiological measures of brain processes, but the MMN component is unique as it is associated with neurocognitive and psychosocial functioning (Light, Swerdlow & Braff, 2007) and it allows for measurement without behavioural response, rendering this component well suited for both clinical and translational studies (Javitt et al., 2008)

1.5. Mismatch Negativity (MMN)

The mismatch negativity (MMN), a negative-going ERP component which peaks in amplitude at frontal scalp sites between 100-250 ms post-stimulus onset, corresponds to an automatic response in the brain to any discriminable change in a repetitive sound stimulus (Näätänen, 1995; Näätänen, Jacobsen, & Winkler, 2005). MMN is typically elicited within an auditory oddball paradigm involving frequent presentations (i.e., 80%) of "standard" stimuli interrupted by rare "deviant" stimuli that differ in some physical characteristic such as intensity, duration, or frequency. MMN is also elicited by acoustic rule violations, such as with abstract deviants, omission of an expected sound, or a change in acoustic patterns (Näätänen, Paavilainen, Rinne, & Alho, 2007). Elicited even in the absence of directed attention, MMN is thought to reflect a predominantly pre-attentive (pre-conscious) process of detecting a "mismatch" between the deviant and the features of the standard stimuli stored as a sensory-memory trace (Näätänen, Paavilainen, & Reinikainen, 1989; Näätänen et al., 2009; 2012). According to the theory, a sensory memory trace is formed in response to the repetitive, standard stimuli and the auditory MMN is elicited automatically when an acoustic feature is detected which deviates from the standard memory trace, representing an index of sensory-memory updating (Näätänen et al. 2005; Näätänen et al., 2011). Operationally, MMN is obtained by subtracting the standard from the deviant waveform to obtain an index of pre-attentive acoustic

discrimination. The MMN response is only observed following presentation of a deviant stimuli or rule violation that is mismatched with the current sensory memory trace, and is therefore separate from obligatory electrical brain responses to incoming stimuli, such as the temporally overlapping N100 (N1) ERP component (Näätänen, 1995; Näätänen et al., 2005; Näätänen et al., 2011).

Multiple deviant types can be presented within the same oddball paradigm (e.g., frequency, intensity, and duration deviants), yielding MMNs that vary in amplitude, latency, and cortical source (Näätänen et al., 2004). MMN, a marker of automatic auditory change detection, is generated primarily by neuronal populations in the bilateral temporal (auditory) cortex, , but it also receives contributions from frontal brain regions, which may reflect mechanisms involved in involuntary attention switching in response to the change in stimuli (Alho, 1995; Giard et al., 1990; Näätänen, 1995; Näätänen et al. 2007; Näätänen & Kähkönen, 2009; Näätänen, Kujala, & Winkler, 2011; Opitz et al. 2002; Shalgi & Deouell, 2007). MMN is also a reliable measure which can be used as a sensitive index of central auditory system plasticity (Näätänen et al., 2008). When the magnitude of the discriminable change is made smaller, the MMN is attenuated in amplitude, eventually subsiding at around the discrimination threshold. These discrimination thresholds can be separately determined for each different auditory attribute and the localization of the specific acoustic sources has supported differentiation of cortical generators for each acoustic or deviant feature (Giard et al 1990; Garrido 2009). Although behavioural studies have shown improvement of auditory processing and pitch discrimination with bilateral tDCS (Mathys et al., 2010), functional asymmetry studies have shown that auditory stimuli produces greater activation in the left Heschl's gyrus, the location of the primary auditory cortex (Devlin et al., 2003), which is more tuned for temporal resolution, demonstrating a left hemispheric dominance

for processing rapid acoustic information of non-speech sounds (Heimrath et al., 2014), while the right cortical areas may be more amenable to spectral (pitch) resolution (Zatorre and Belin, 2001; Poeppel, 2003; Heimrath et al., 2016). However, as Chen et al. (2014b) failed to show significant tDCS-MMN effects after stimulating the right fronto-temporal cortical network and Vines and colleagues (2006) found that cathodal tDCS exclusively decreased pitch memory processes when stimulating over the left temporal lobe, the current set of studies will attempt to modulate MMN with tDCS over the left auditory cortex. Importantly, the MMN amplitudes have been shown to positively correlate with cognitive and psychosocial functioning, such that larger MMNs are indicative of higher cognitive and memory function (Light, Swerdlow & Braff, 2007; Näätänen, 1995). Thus, MMN is an objective neural measure of sensory discrimination, which has great utility for the assessment of sensory and cognitive function in humans (Näätänen et al., 2007).

Concerning the underlying pharmacology of MMN, findings have consistently suggested that pharmacological inhibition of NMDA receptors results in the attenuation of MMN (Heekeren et al., 2008; Javitt et al., 1996; Rosburg & Kreitschmann-Andermahr, 2015, Tikhonravov et al., 2008; Umbricht et al. 2002). Animal models have shown NMDA receptors to have a crucial role in memory and have been found to play a key role in auditory sensory discrimination and MMN response, as NMDA antagonists were found to diminish MMN generation (Gil-da-Costa et al. 2013; Javitt et al. 1996; Tikhonravov, 2008). In humans, findings have also suggested that pharmacological inhibition of NMDA receptors results in the attenuation of MMN, results which were not seen with dopaminergic or serotonergic manipulations (Heekeren et al. 2008; Leung et al., 2007; Umbricht et al. 2002; Umbricht and Krljes, 2005). Specifically, the NMDA antagonist ketamine, a dissociative anesthetic, reduces MMN amplitude in human participants and has been used to model impaired cognition in healthy controls (Rosburg and Kreitschmann-Andermahr

2015; Umbricht et al. 2002). Following from this, MMN has been designated a reliable biomarker for the detection of pro-cognitive treatment effects in pre-clinical trials (Butler et al. 2012).

1.6. Biomarkers of mental health

There is currently a lack of reliable physiological markers for brain function and mental illness and thus, the identification of biologically-biased markers and subsequent targeting of genetically determined endophenotypes has been suggested as an effective approach in developing novel treatments for mental impairments (Braff & Light, 2005; Gottesman & Gould, 2003). Endophenotypes are defined as a subset of biomarkers which meet the following criteria: 1) they are associated with an illness in the population and exhibit deficits in patients; 2) they are stable over time; 3) they are primarily state-independent and manifest whether or not the illness is active; 4) their related deficits are present (often to a lesser degree) in genetically related family members (at a higher rate than in the generable population); and 5) they are heritable (Gottesman & Gould, 2003; Light et al, 2012). Biomarkers and endophenotypes can represent a specific brain dysfunction or illness, but an alternative interpretation is that a biomarker may actually indicate intact neural resources, the functions of which are attenuated in illness (Light et al, 2015). Using this perspective, it follows that improvements in a given biomarker through pharmacological manipulation, or neurostimulation, could indicate that these attenuated neural functions can be recovered and that the corresponding pharmacological or treatment approach should be pursued as a target for therapeutic intervention.

1.7. Schizophrenia

Despite advances in psychiatric neuroscience, there is still a lack of single laboratory test to guide diagnoses, treatment and outcome response in mental illness (Light and Swerdlow, 2015). Currently, there is an imperative search for reliable biomarkers to predict and monitor treatment response in schizophrenia (Sz), which presents as a devastating psychiatric disorder with a large burden of illness (Rossler et al., 2005). The disorder is characterized by a multitude of clinically significant symptoms, including positive symptoms, such as auditory or visual hallucinations (altered perception), delusions (false beliefs), disorganized speech, and/or negative symptoms, such as catatonic behaviour (lack of movement) and flattening of affect (lack of emotional expression), which must persist for a minimum of 6 months (APA, 2013). Furthermore, a key diagnostic criterion of Sz is the presence of a disturbance in one or more areas of daily functioning within an individual (APA, 2013). These disturbances are associated with deficits ranging from abnormalities in elementary sensory processing to impairments in complex cognitive tasks (Green, 2000). In fact, cognitive impairment, characterized by deficits in perception, attention, memory and executive functions, has been proposed to be the core feature of Sz, correlating with negative symptoms and functional outcome (Elvevag & Goldberg, 2000; Green, 1996; 2000). Cognitive deficits appear before evidence of psychosis and tend to also be present in unaffected relatives of Sz patients (Heydebrand, 2006) as well as in first episode Sz patients (Snitz, MacDonald, and Carter, 2006) and impairments are often better predicted by the extent of a patient's cognitive deficits than by their clinical symptoms (Keefe & Harvey, 2012). For these reasons, core cognitive deficits are being explored as potential biomarker or endophenotype for Sz, and may be valuable targets for diagnostics and treatment as there is currently an unmet need to effectively treat cognitive impairments in Sz (Elvevag & Goldberg, 2000; Green, 1996; 2000; Goff, Hill & Barch, 2011; Gur et al, 2007; Hill et al, 2010).

Impairments in early stages of sensory processing, such as deficits in the early registration, temporary storage, and manipulation of sensory information, are thought to contribute to higher order cognitive impairments in Sz and are highly correlated with poor functional ability (Javitt, 2009). Sensory disturbances are particularly evident in the auditory modality, as shown with behavioural demonstrations of early auditory processing dysfunction in schizophrenia tone matching (Javitt, Shelley, & Ritter, 2000; Jonsson & Sjostedt, 1973; Rabinowicz, Silipo, Goldman, & Javitt, 2000) and speech perception tasks (Hoffman et al., 1999; McKay, Headlam, & Copolov, 2000; Vercammen, DeHaan, & Alaman, 2008). Impairments in sensory information processing limit the ability of patients to make cognitive improvements. As mentioned, unfortunately there is no approved treatment for sensory impairments and cognitive symptoms in Sz (Gray & Roth, 2007); however, the search for clinically useful neurophysiological biomarkers such as the MMN ERP is promising (Light and Swerdlow, 2015; Näätänen et al., 2015; Turetsky et al., 2007).

1.8. MMN and Schizophrenia

MMN amplitude deficiency has been consistently observed in patients with Sz (Näätänen & Kähkönen, 2009; Näätänen et al., 2012) and this deficit has a large effect size that is reliable over time (Light & Braff, 2005). Moreover, multiple studies have found similar patterns of MMN amplitude deficiency among clinically unaffected biological first-degree relatives of Sz patients (Jessen et al., 2001; Michie et al, 2002; Turetsky et al., 2007), and among children at risk for the development of Sz (Bar-Haim et al., 2003; Schreiber et al., 1992). Relatedly, neuroimaging research has associated the reduction of MMN with the atrophy of grey matter in specific brain regions (e.g., Heschl's Gyrus), suggesting a neurobiological etiology for diminished MMN amplitudes (Salisbury et al., 2007). Thus, deficient MMN amplitude is conceived to be a

biomarker or endophenotype of Sz (Javitt et al., 2008; Light et al., 2012; Light and Swerdlow, 2015; Turetsky et al., 2007). Recently, MMN deficiency has also been described as a breakthrough biomarker in predicting psychosis onset (Näätänen et al., 2015). As with sensory and cognitive symptoms, numerous studies have established the lack of efficacy of antipsychotic treatments in ameliorating the attenuation of MMN in patients with Sz *(*Korostenskaja et al., 2005; Umbricht et al., 1998; 1999*)* and thus there is a continued search for effective drug discovery targeting MMN deficits (Javitt et al., 2008).

Attenuation of MMN is greater in clinical populations with severe chronic psychopathology and studies have shown that MMN deficits in Sz depend on deviant-specific features and individual factors, such as age (Näätänen & Kähkönen, 2009; Näätänen et al., 2012; Todd et al., 2008). Duration deviants yield the most impaired MMN amplitudes throughout the course of Sz (Umbricht & Krljes, 2005). Specifically, MMN amplitudes associated with duration and intensity deviants are highly attenuated in patients early in the course of Sz, while frequency and duration are more attenuated in patients later in the course of the disorder (Todd et al., 2008; Umbricht & Krljes, 2005).

MMN deficits in this population have been significantly correlated not only with poor functional outcomes in Sz (Kawokubo et al., 2007; Näätänen et al., 2012), but with negative symptoms (Javitt et al., 2000), positive symptoms (Fisher et al., 2011) and hallucination severity (Fisher et al., 2011; 2012). Fisher, Labelle & Knott (2008) found a significant difference between duration MMN amplitudes in patients who experience auditory hallucinations (AHs) and those who do not. These MMN differences based on hallucination experience and other symptoms are important to note as several interindividual differences at baseline (meaning prior to treatment or at the starting point used for comparisons) have been found to have a significant impact on

neurostimulation study effects (Li et al., 2015; Vercammen et al., 2011). Relatedly, recent studies have reported that fronto-temporal tDCS may be used as a treatment for the reduction of auditory hallucinations in Sz (Andrade, 2013; Brunelin et al., 2012; Ferrucci et al., 2014; Mondino et al., 2015a; 2015b; Nawani et al., 2014). Active tDCS (20 minutes, 2 mA) administered to patients over 5 consecutive days, with the cathode over tempo-parietal areas and the anode over left frontal cortex, was shown to reduce hallucination severity and other clinical symptoms as measured by the Positive and Negative Syndrome Scale (PANSS; Brunelin et al., 2012).

1.9. The NMDA system, Sz, MMN and tDCS

Following research over the past two decades, conceptual models of the mechanisms of Sz have largely shifted from dysfunctions in frontal and limbic brain dopaminergic systems, which are thought to underlie clinical symptoms of Sz, to dysfunctions in glutamate signaling (Coyle, Tsai & Goff, 2003; Coyle & Tsai, 2004; Javitt, 2010; Javitt et al., 2012). Current theories of Sz suggest that because NMDA receptors are widespread throughout the brain, including regions related to dopamine neurotransmission, impaired NMDA-based mechanisms may be more closely linked than dopamine to the etiology of Sz symptoms and better explain the scope of symptoms in Sz (Javitt, 2010; Moghaddam & Javitt, 2012). Post-mortem studies in individuals with Sz have consistently found decreased NMDA receptors and abnormal levels of glutamate and of D-amino acids (i.e., D-aspartate, D-serine), which mediate NMDA receptor transmission (Blanke & VanDongen, 2009; Purves et al., 2001; Wolosker et al., 2008), implicating NMDA hypofunction as an important factor in the symptomology of Sz (Errico et al., 2013; Hashimoto et al., 2003; Kerwin & Meldrum, 1990; Kerwin et al., 1988; Meador-Woodruff & Healy, 2000; Merritt, McGuire, & Egerton, 2013).

Many pharmacological studies have examined the implications of NMDA receptor dysfunction in relation to sensory and cognitive processing deficits associated with Sz (Javitt, 2010; 2012). In early studies with healthy volunteers, phencyclidine (PCP), an NMDA receptor antagonist, was shown to induce states of psychosis that closely resembled those that are characteristic of Sz (Javitt & Zukin, 1991) and recently, in studies employing sub-anaesthetic doses of NMDAR antagonist ketamine, Sz-like symptoms and cognitive deficits were repeatedly observed with acute receptor blockade (Javitt et al., 2012; Moghaddam and Javitt, 2011). This is in contrast to dopamine agonists, such as amphetamines, which could emulate positive symptoms but not negative or cognitive symptoms (Javitt & Zukin, 1991), as well as the failure of first and second generation antipsychotics to ameliorate cognitive symptoms (Hill et al, 2010). Thus, much of the current research in Sz has focused on NMDA receptor hypofunction in key areas such as the PFC and hippocampus (Gilmour et al, 2012).

NMDA receptor hypofunction is also thought to underlie MMN deficits in Sz disorder and has been prioritized as a reasonable molecular target in the development of pharmacological treatments for Sz cognition (Javitt, 2000b; Javitt et al., 2012; Nagai et al., 2013; Umbricht et al., 2000*).* Reduced MMN is proposed to be a putative "translatable" biomarker of glutamatergic dysfunction in Sz, seen in both humans and animal models (Javitt et al., 1996; 2012; Light & Näätänen, 2013; Näätänen et al., 2015a; 2015b; Umbricht et al., 2000*).* Thus, MMN amplitude has been designated as a reliable marker for the detection of pro-cognitive treatment effects in early phase clinical trials and novel treatment interventions (Butler et al., 2012; Javitt et al., 2008; Light & Näätänen, 2013; Light & Swerdlow, 2014; Light et al., 2012; Näätänen et al., 2015a; 2015b; Turetsky et al., 2015).

As previously mentioned, similar to the relationship between Sz and MMN, the NMDA receptor system has been implicated in the mechanism of action of tDCS based on the ability of tDCS to alter cell membrane potentials and firing rates (Nitsche & Paulus, 2011). Inhibition of NMDA receptors has been consistently found to eliminate both immediate and after-effects of tDCS, suggesting that tDCS operates through glutamatergic neuroplasticity mechanisms (Liebetanz et al., 2002; Nitsche, Fricke et al., 2003). Thus, it is suggested that MMN, tDCS, and the cognitive symptoms of Sz all operate to some degree through the NMDA receptor system. Accordingly, it is a viable measure of the neural effects of tDCS on MMN and auditory sensory discrimination, the results of which may have potential applications to clinical populations with Sz. A recent review and meta-analysis reported conclusive cognitive improvements with tDCS and suggested the use of non-invasive brain stimulation techniques for cognitive deficits in psychiatric patients, including schizophrenia (Brunoni and Vanderhasselt, 2014).

1.10. General Objectives and Overall Hypothesis

Although there is an increasing interest in the cognitive modulating actions of tDCS, there have been very few studies assessing the effects of tDCS on neurophysiological measures of pre-attentive auditory processing. Zaehle et al. (2011) found that anodal tDCS over the temporal cortex increased amplitudes of the auditory P50 ERP, while cathodal tDCS over temporal-parietal areas (TPA) induced larger N100 amplitudes, indicating increases in early sensory registration. However, in a study by Chen et al. (2014a), which assessed tDCS over the right frontal cortex, MMN to pitch deviants were reduced with anodal stimulation, while duration MMNs were unaffected by either anodal or cathodal tDCS. These paradoxical (decrease in pitch MMN) and negative findings (with duration MMN) with excitatory anodal stimulation in these studies could be due to differences in the positioning of stimulating electrodes on scalp areas

remote from the auditory cortex, the use of chosen stimulus parameters, or to baseline MMN amplitude values, as the specific influence of these factors on tDCS effects is currently being explored (Li et al., 2015). Both the TPA and the DLPFC have been consistently associated with auditory processing; stimulation of the TPA is applied to modulate activity of the primary auditory cortex and auditory association areas (Shekhawat et al., 2015), whereas the DLPFC plays a role in auditory memory and auditory attention (Bodner et al., 1996; Alain et al., 1998; Voisin et al., 2006) and through a top-down modulation of acoustic processing, exerts inhibitory control of input to primary auditory regions (Knight et al., 1989; Mitchell et al., 2005). The initial pilot studies discussed here assessed tDCS-MMN effects over the auditory cortex, but as auditory MMN also receives contributions from frontal areas and we were also interested in tDCS-memory performance effects, stimulation over the frontal cortex was also investigated.

The present set of studies in healthy volunteers investigated both the effects of anodal and cathodal tDCS on MMN-indexed auditory discrimination, assessing several stimulus parameters. An NMDA-based pharmacological study was also included to elucidate neurobiological mechanisms underlying tDCS effects on perceptual and cognitive processes. Finally, a clinical pilot study was completed with individuals with chronic schizophrenia to assess the possible effects of tDCS on auditory discrimination impairments in this population. tDCS effects were primarily investigated pre- and post-stimulation in the left temporal scalp region overlying the auditory cortex and also in the left frontal scalp region overlying the DLPFC, compared to 'sham' stimulation (i.e. no stimulation). Based on work which has shown that pharmacological modulation of MMN is baseline-dependant (generally increasing ERPs in individuals with low baseline amplitudes, and decreasing ERPs in individuals with high baseline amplitudes; Knott et al., 2013; 2014a; 2014b; 2015) and that tDCS response may also be baseline-function dependant

(Li et al., 2015; Vercammen et al., 2011), we examined the effects of tDCS on MMN in stratified groups differing in baseline response/amplitude. This stratification strategy was implemented as, recently, the utility and importance of individual biomarkers for the development of more effective, personalized treatment has been highlighted, specifically including stratification of baseline samples for the preclinical assessment of cognitive enhancing agents (Butler et al., 2012; Green et al., 2009; Frank & Hargreaves, 2003). Several interindividual differences at baseline, defined as the initial time point used for comparisons, have been found to have a significant impact on tDCS effects (Li et al., 2015) and therefore tDCS effects will be investigated in regards to baseline response, in both healthy and psychiatric participants. It was generally hypothesized that MMN would be enhanced after receiving anodal tDCS stimulation, particularly in individuals with low MMN response at baseline, and that cathodal tDCS would decrease MMN, particularly in individuals with relatively high MMN at baseline. For pharmacological investigations, it was hypothesized that the NMDA receptor antagonist would decrease MMN, and moreover, that they would prevent or abolish the expected anodal tDCS-induced MMN increases. Finally, in the pilot study with schizophrenia patients, it was hypothesized that anodal tDCS could be used a technique to improve MMN-indexed auditory discrimination deficits that occur in this population.

Chapter 2: Effect of Transcranial Direct Current Stimulation (tDCS) on MMN-Indexed Auditory Discrimination: a Pilot Study (Study I)

2.1. Title Page

Effect of Transcranial Direct Current Stimulation (tDCS) on MMN-Indexed Auditory Discrimination: a Pilot Study

Authors:

Danielle Impey[1,2] (danielle.impey@theroyal.ca) and Verner Knott[1,2] (verner.knott@theroyal.ca)

1. School of Psychology, University of Ottawa, Ottawa, Canada

2. University of Ottawa Institute of Mental Health Research, Ottawa, Canada

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2.2. Abstract

Membrane potentials and brain plasticity are basic modes of cerebral information processing. Both can be externally (non-invasively) modulated by weak transcranial direct current stimulation (tDCS). Polarity-dependent tDCS-induced reversible circumscribed increases and decreases in cortical excitability and functional changes have been observed following stimulation of motor and visual cortices but relatively little research has been conducted with respect to the auditory cortex. The aim of this pilot study was to examine the effects of tDCS on auditory sensory discrimination in healthy participants (N=12) assessed with the mismatch negativity (MMN) brain event-related potential (ERP). In a randomized, double-blind, sham-controlled design, in which participants received anodal tDCS over the primary auditory cortex (2 mA for 20 minutes) in one session and 'sham' stimulation (i.e. no stimulation except initial ramp-up for 30 seconds) in the other session. MMN elicited by changes in auditory pitch were found to be enhanced after receiving anodal tDCS compared to 'sham' stimulation, with the effects being evidenced in individuals with relatively reduced (vs. increased) baseline amplitudes and with relatively small (vs. large) pitch deviants. Additional studies are needed to further explore relationships between tDCS-related parameters, auditory stimulus features and individual differences prior to assessing the utility of this tool for treating auditory processing deficits in psychiatric and/or neurological disorders.

2.3. Introduction

Transcranial direct current stimulation (tDCS) is a non-invasive procedure of cortical stimulation, in which weak direct currents are used to induce changes in cortical excitability. Depending on the polarity of the stimulation, tDCS can result in reversible increases (anodal tDCS) or decreases (cathodal tDCS) in excitability in circumscribed stimulated brain regions. Although this allows for the investigation of the relationships between modulated neural activity and behavior (Nitsche et al. 2003a; Nitsche et al. 2008), a simple relation between polarity and behavioural changes has been challenged in non-motor areas because recent evidence has shown tDCS can both de- and hyperpolarize within the same gyrus (Reato et al., 2013) and different types of neurons are differentially modulated depending on their morphology and axonal orientation (Radman, Ramos et al. 2009). With this limitation in mind, tDCS has been most frequently investigated with respect to its effects on the motor and visual cortex in healthy volunteers (Nitsche et al. 2003a; Nitsche et al. 2008), and both neurological (Jo et al. 2009) and psychiatric (Fregni et al. 2006a; Ferrucci et al. 2008) patients, with anodal tDCS generally increasing cortical excitability and function and cathodal tDCS generally decreasing excitability and performance. As seen with animal data, tDCS changes in excitability and motoric functions outlast the stimulation if tDCS is applied for several minutes, and the "after-effects" can remain stable for an hour or more if tDCS is applied longer (Nitsche et al. 2004; Nitsche and Paulus 2008).

Recently, tDCS has increasingly been used to investigate and modulate cognitive processes. A series of experiments have found that with anodal tDCS stimulation using a very weak current (~1-2 mA) over the dorsolateral prefrontal cortex (DLPFC) for 15-30 minutes, there is improved performance on several working memory and word recognition memory tasks

(e.g. the n-back and the Sternberg task) in healthy volunteers (Fregni et al. 2005), and patients with Alzheimer's disease (Ferrucci et al. 2008; Boggio et al. 2008) and depression (Fregni et al. 2006a). By reversing the polarity of the current, cathodal tDCS with similar duration and intensity parameters has been found to decrease performance on a memory task in healthy participants and Alzheimer's patients (Ferrucci et al. 2008). Although recent evident suggests that tDCS has less consistency in polarity effects with cognitive tasks compared to motor functions, all of these studies are randomized, double-blind, sham-controlled studies, which demonstrates the potential of a short treatment of tDCS in modulating cognitive functions.

In the majority of studies, which have focused on motor cortex excitability, tDCS-induced prolonged effects are not only polarity-specific (anodal vs. cathodal), but the strength and endurance of the after-effects depends on current intensity and stimulation duration (Nitsche et al. 2008). A minimum of 0.6 mA current intensity and a minimum stimulation duration of 3 minutes was required to induce significant after-effects with regards to motor evoked potentials and increasing current intensity or duration led to prolonged and larger after-effects (Nitsche and Paulus, 2000). With regards to working memory modulation, a current of 1-2 mA for 10 to 20 minutes applied over the frontal cortex has been found to significantly modulate mnemonic performance, with effects continuing after stimulation (Nitsche et al. 2008). Concerning the safety of tDCS, a stimulation intensity of up to 2 mA and a duration of about 30 minutes has been observed to be safe in over 200 studies with human participants (Nitsche et al. 2008; Iyer et al. 2000), with the observed adverse effects being minor and consisting of light itching beneath the electrodes or mild headache during stimulation (Fregni et al. 2006b), which makes this technique ideal for quick, non-invasive neuromodulation.

Pharmacological studies suggest that the immediate short-lasting functional effects observed during tDCS are generated by polarity-specific shifts of the cell's resting membrane potential, with anodal stimulation causing a depolarization of the resting potential (and an increase in spontaneous firing rates) in the region beneath the anodal electrode, while cathodal stimulation has the opposite effect, causing a hyperpolarization of the resting-membrane potential (and a decrease in spontaneous firing rates) (Liebetanz et al. 2002; Nitsche et al. 2003a). However, polarity-specific changes may be an over simplistic explanation of tDCS mechanisms, as recent evidence has found that stimulation can both de- and hyperpolarize within the same gyrus (Reato et al., 2013) and different neuron morphology and axonal orientation are differentially sensitive to polarization (Radman, Ramos et al. 2009). Induced brain plasticity in the form of after-effects depends not only on membrane potential changes but also on modulations of N-methyl-D-aspartate (NMDA) receptor-efficacy, a glutamate receptor implicated in synaptic plasticity and memory function, among others. Specifically, NMDA antagonists have been found to prevent functional after-effects while NMDA agonists prolong motor potential after-effects over several hours (Liebetanz et al. 2002; Nitsche et al. 2003a; 2004). Although tDCS modulations are dependent on the NMDA receptor system, GABAergic interneurons have also been found to play a role and the aftereffects of anodal tDCS can also be modulated by catecholamines, acetylcholine and serotonin (Stagg and Nitsche 2011).

Although the effects of tDCS on motor and visual cortices are relatively well known within this literature and studies are increasingly targeting frontal lobe functions with tDCS, very few tDCS studies have focused on information processing in the auditory cortex. tDCS over the primary and secondary auditory cortex have been found to negatively modulate pitch discrimination and pitch memory performance with cathodal tDCS (Mathys et al. 2010; Vines et

al. 2006), and only one study to date has assessed the effect of anodal tDCS on auditory discriminability (Chen et al. 2014). Event-related potentials (ERPs) provide an objective, non-invasive neural measure of information processing and ERP components are frequently used to investigate early pre-attentive (e.g. P50 index of sensory gating; mismatch negativity [MMN] index of sensory discrimination; P3a index of novelty detection) and higher order (e.g. P3b index of attentional allocation and processing speed) auditory processes in healthy volunteers and clinical populations (for review see Braff and Light 2004). The MMN, an early (~120-250ms) frontal-maximum negative ERP component is a measure of pre-attentive auditory discrimination mediated by a comparison process within sensory memory (Näätänen et al. 2005; Näätänen et al. 2007), which retains transient representations of auditory stimulus features (e.g. pitch, intensity, duration), as brief "echoic" traces. MMN is most often measured within an 'oddball' paradigm involving repeated presentations of a "standard" stimulus intermixed with rare presentations of "deviant" stimuli, wherein a neural representation is formed automatically for the acoustic features of the repetitive (standard) stimuli and the MMN is elicited automatically (at ~120-250ms) through a memory based comparator process when an acoustic feature is detected which deviates from features comprising the standard trace (the deviant; Näätänen et al. 2005). MMN amplitude is associated with higher-order cognitive and psychosocial functioning in healthy adults, as indexed by performance on a standardized memory task, and overall ratings of psychosocial functioning (Light and Braff 2005). The MMN is generated primarily in the temporal (auditory) cortex but it also receives some contribution from frontal brain regions (Näätänen et al. 2007). Similar to tDCS, NMDA receptors have also been found to play a key role in MMN generation as NMDA antagonists have been found to block MMN generation in

animal studies (Javitt et al. 1996) and diminish MMN amplitudes in humans (Umbricht et al. 2000).

2.3.1. Objectives and Hypothesis

Although there is an increasing interest on the cognitive modulating actions of tDCS, there have been very few studies assessing tDCS effects on early, pre-attentive auditory processing. A recent study (Zaehle et al. 2001) investigating tDCS-induced effects on auditory evoked potentials after anodal, cathodal and sham stimulation found anodal tDCS to increase auditory P50 amplitudes, while cathodal tDCS induced larger N100 amplitudes, indicating increases in early sensory processing. However, in a study by Chen et al. (2014) which assessed MMN after anodal, cathodal and sham stimulation over the right frontal cortex, MMN to pitch deviants were reduced with anodal stimulation, while duration MMNs were unaffected by either anodal or cathodal tDCS. These paradoxical (decrease in pitch MMN) and negative findings (with duration MMN) with excitatory anodal stimulation could be due to the positioning of stimulating electrodes on scalp areas remote from the auditory cortex, or the use of stimulus deviant parameters which were difficult to detect. As previous behavioral studies applying tDCS over temporal regions have shown enhanced working memory with anodal stimulation, as well as modulation of pitch discrimination with cathodal stimulation (Mathys et al. 2010; Vines et al. 2006), the current pilot study in healthy volunteers assessed MMN-indexed auditory discrimination of small and large pitch deviants during anodal (2 mA for 20 minutes) and ‘sham’ tDCS (i.e. no stimulation) of the temporal scalp region overlying the primary auditory cortex. Based on the assumption that anodal stimulation increases NMDA neurotransmission, among others, and on evidence that MMN is generated in the auditory cortex and is NMDA dependant,

it was hypothesized that MMN would be enhanced after receiving anodal tDCS (vs. sham) stimulation, and that this would be observed with low and high pitch deviants.

Ceiling effects may have contributed to negative MMN findings in the previous study (Chen et al. 2014) as optimal MMN generation in healthy participants may show limited further amplitude increases with anodal tDCS. As part of this pilot study, we were interested in obtaining preliminary information on individual differences in auditory response to tDCS. This direction is based on independent work in our laboratory which has shown the pharmacological enhancement and diminishment of sensory (MMN, P50 habituation) and cognitive ERP (e.g. P300)-indexed processes are baseline-dependant (generally increasing and decreasing ERP components in individuals with small and large baseline amplitudes, respectively) (Knott et al., 2013; Knott et al., 2014a; Knott et al., 2014b). In this present study, it was also predicted that enhanced auditory discrimination with tDCS would be more apparent in individuals with reduced baseline MMN amplitudes (vs. high baseline individuals). In so far as our pilot study was specifically focused on MMNs elicited by pitch deviants, we also hypothesized that tDCS enhanced detection of pitch changes would be most evident in the hardest to discriminate condition (i.e., with small [vs. large] pitch deviant).

2.4. Materials and Methods

2.4.1. Participants

This pilot study was conducted with a sample of N=12, which is typical of the majority of tDCS investigations. Healthy, right-handed male controls between the ages of 18-30 were recruited from the local community (primarily from universities via word of mouth). All participants underwent a questionnaire screen for medical history, an interview using both the

Structured Clinical Interview for DSM-IV Non-Patient (SCID-NP; First et al. 1995) Edition and the Family Interview for Genetic Studies (FIGS; Maxwell 1992), to assess personal and family psychiatric history, respectively. Exclusion criteria included the following: 1) past or current psychiatric diagnosis (including substance abuse of any kind) of Axis I or Axis II disorders; 2) any past or present pharmacotherapy, psychotherapy, or counseling, 3) first degree family member receiving treatment for a DSM-IV disorder; 4) prior head injury with loss of consciousness or recent neurosurgery; 5) any neurological diagnosis (including epilepsy); 6) any current medical illness (transient colds or allergies excepted) or prior medical conditions with possible central nervous system sequelae (i.e., pulmonary, endocrinological, cardiac, metabolic, most systemic illnesses); 7) consumption of more than 2 alcohol drinks and 5 cups of coffee on average per day during the past month, 8) metallic implants inside the brain or any electrical medical device (e.g. pacemaker) in the body, and 9) current daily use of any prescriptive or over the counter medications. For inclusion, all participants were required to be between the ages of 18-40 years of age and non-smokers (i.e., smoking a lifetime total of < 100 cigarettes, with no smoking or nicotine use in the past year) as nicotinic cholinergic agents are known to influence tDCS (Kuo et al. 2007) and MMN (Engeland et al. 2002). All participants signed an informed consent form and were compensated $50 (CAD) for study participation. The study was approved by the Research Ethics Board of the Royal Ottawa Mental Health Care Centre.

2.4.2. Design

Participants were assessed in a randomized, repeated-measures, double-blind, cross over design requiring them to attend two test sessions (~ 2-5 days apart), one involving anodal tDCS administration, and one involving sham tDCS administration. Half (randomly selected) of the

participants received anodal tDCS in their first session and sham tDCS in their second session. The remaining half received stimulations in the reverse order.

2.4.3. Procedure

Test sessions occurred in the morning (9 a.m.-12 p.m.) following overnight abstinence from any drugs, alcohol, caffeine, food and medications. Verbal confirmation of abstinence was followed by EEG electrode placement, MMN assessment at baseline, sham/anodal tDCS and MMN re-assessment immediately post-stimulation. During stimulation, participants were asked to sit back, relax and watch a movie with subtitles during MMN recording. At the end of the study session, participants were required to complete an adverse events questionnaire regarding possible side effects of tDCS.

2.4.4. tDCS

Although scalp positioning of stimulating electrodes is considered critical for tDCS effects, the majority of studies have utilized a bipolar placement with both anodal and cathodal electrodes being placed on difference scalp regions. This preliminary study did not attempt to vary electrode placement but for consistency, used the identical placement reported in the three previous auditory studies (Mathys et al. 2010; Vines et al. 2006; Zaehle et al. 2001). Conductive saline-soaked rubber electrodes super-imposed on sponge plates were placed on the scalp overlying the left auditory cortex (anodal electrode) and on the contralateral forehead (reference/cathodal electrode) above the orbit as described previously (Mathys et al. 2010; Boggio et al. 2008). Specifically, the anodal electrode was positioned between C5 and T7 sites (parallel to the left Sylvan fissure) of the 10-10 international system for EEG electrode placement, scalp sites that closely overlap Brodmann Areas 41 and 42 of the primary and secondary auditory cortex, respectively.

Stimulation was applied using a battery-driven constant-current regulator (Oasis Pro, Edmonton). In both anodal and sham tDCS, the DC current was initially increased in a ramp-like fashion over 10 s until reaching 2 mA and was similarly decreased at the end of stimulation (Nitsche et al. 2003a). In anodal tDCS, stimulation was maintained for a total of 20 min to extend 'after-effects'; in sham, it was turned off after 30 s. These sham parameters were chosen based on previous reports that perceived sensations on the skin, such as tingling (during 10 seconds fade in/out of the 30 s sham tDCS) produce no after-effects and re-create the same sensations experienced with anodal stimulation (Gandiga et al. 2006), however, some subjects may still be able to distinguish between real and sham stimulation (O'Connell, Cossar et al. 2012) and thus it is important to assess the effectiveness of blinding with post hoc questioning of participants (Nitsche et al. 2008).

2.4.5. Stimuli

Four blocks of 600 auditory stimuli (70 dB [SPL]) per block were presented to the right ear (through headphones) in an oddball sequence involving frequent (P = 0.85) presentations (stimulus onset asynchrony = 300 ms) of a standard auditory stimulus (1000 Hz, duration = 100 ms) that was randomly inter-mixed with two rare (P = 0.15) deviant stimuli varying in pitch (small pitch deviant: 1050 Hz, 100 ms, P = 0.075; large pitch deviant: 1200 Hz, 100 ms, P = 0.075). Deviant stimuli were presented in a pseudo-random fashion such that deviants never occurred in succession.

2.4.6. Recordings

ERPs were derived with tin electrodes placed on 6 scalp sites (F_z, F_3, F_4, C_z, C_3, C_4) and on left (LM) and right (RM) mastoids, activity from which was referenced to a nose electrode. An electrode on a mid-forehead site served as ground and bipolar recordings of horizontal

(HEOG) and vertical (VEOG) electro-oculogram activity were taken from electrodes over supra-/sub-orbital and external canthi sites, respectively. All electrode impedances were kept below 5 kΩ. Electrical activity was recorded using BrainVision Recorder® software and a BrainVision V-8 Amp® amplifier (Brain Products, GmbH) with a bandpass filter setting of 0.1-100 Hz and a digitization rate of 500 Hz. Data was stored for off-line analysis using BrainVision Analyzer® software (Brain Products, GmbH). For each stimulus, electrical epochs of 400 ms duration (beginning 100 ms prior to stimulus onset) were ocular corrected, digitally filtered (0.1-8 Hz), and baseline corrected (relative to the pre-stimulus segment), and only epochs with EEG voltages below 100μV were used for final ERP averages. MMN 'difference waveforms' were derived by point-by-point digital subtraction of the averaged standard stimulus values from average values elicited by the deviant stimuli. Based on grand averaged waveforms, the primary MMN endpoint was assessed by quantifying the peak negative amplitude (relative to averaged pre-stimulus values) within a latency window of 120-250 ms at the frontal midline recoding site (Fz), the region typically exhibiting the maximal MMN amplitude. Considered as a secondary endpoint, MMN amplitude in the same latency window was also assessed at the left and right mastoid sites, where the MMN component is found to be inverted in polarity, and as such, can also be used to aid in the identification of frontal MMN.

2.4.7. Statistics

The primary analysis was conducted on MMN difference waveform values derived from frontal (Fz) scalp sites, which exhibited maximum MMN amplitudes. The Statistical Package for Social Sciences (SPSS) was used for statistical analysis. Active tDCS and sham stimulation effects on MMN were compared in separate repeated measures analysis of variance (ANOVA) for each deviant, containing within-subject factors: treatment (2 levels; anodal stimulation, sham

stimulation) and time (2 levels; pre- and post-stimulation), as well as a between-subjects subgroup factor (2 levels; low and high efficiency groups). Participants were segmented into two groups using a median split based on MMN baseline amplitudes, which resulted in a relatively low (LE: n = 6) and high efficiency (HE: n = 6) discrimination groups for each separate deviant. In order to begin exploring localization effects and specificity of any observed fontal effects, a secondary set of complementary ANOVAs for each deviant, with the same factors as above, were carried out on mastoid MMNs (Tp9 and Tp10), which may more closely reflect auditory processing of the temporal cortex. Greenhouse-Geisser significant effects ($p < .05$) are reported and a priori planned comparisons were followed up, using Bonferroni corrections, to investigate study hypothesis regarding stimulation differences and group differences.

2.5. Results

2.5.1. Frontal MMN amplitudes to Large (1200 Hz) Auditory Deviant

There was no main effect of treatment, $F[1,10]= .10$, $p > .05$, time, $F[1,10]= .80$, $p > .05$, and interactions between the factors were also non-significant, $F[1,10]= .18$, $p > .05$. Planned comparisons comparing pre- and post-stimulation amplitudes for each treatment within the two groups failed to show any significant MMN alterations with either active or sham stimulation.

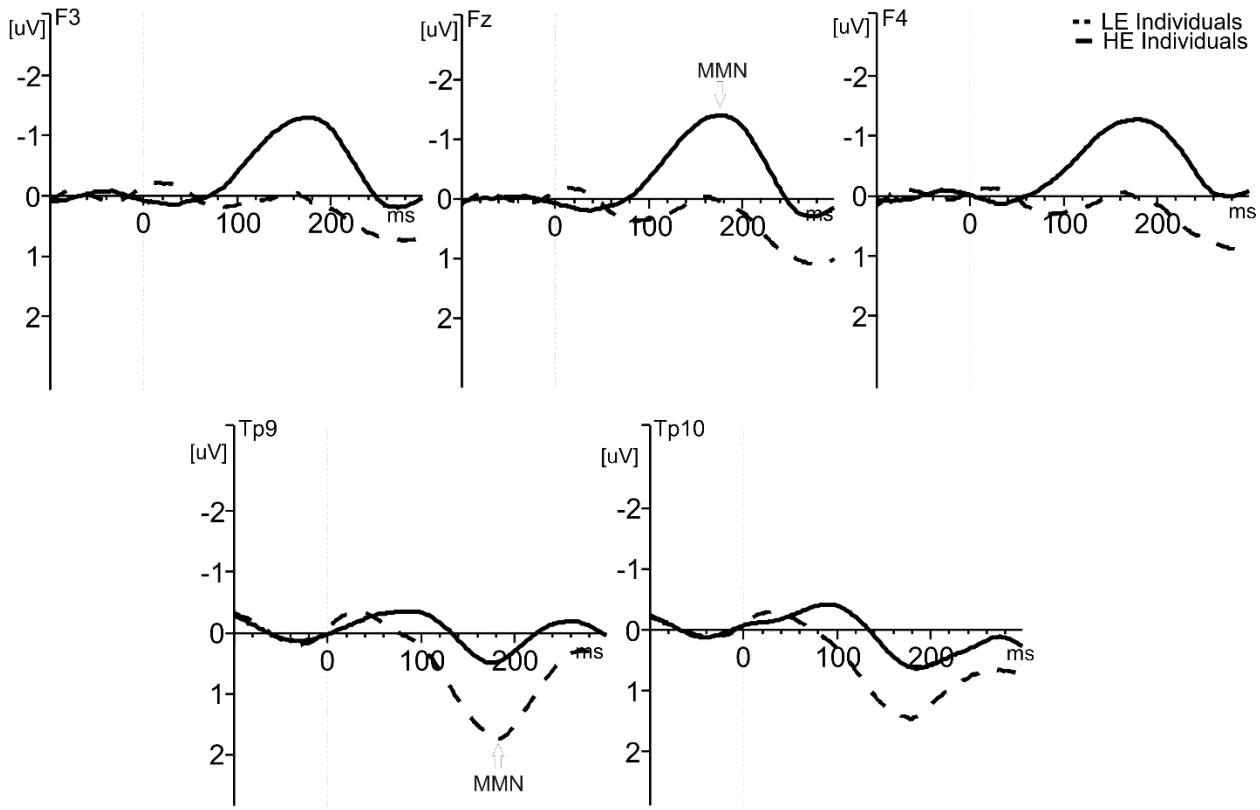

Figure 2.1. Grand averaged MMN waveforms at 5 sites for the low (1050Hz) pitch deviant for baseline sessions (average of both sessions) comparing LE (low discriminating efficiency) individuals and HE (high efficiency) individuals.

2.5.2 Frontal MMN amplitudes to Small (1050 Hz) Auditory Deviant

Grand averaged MMN waveforms for LE and HE individuals at baseline are shown in Figure 1. There was no significant main effect of treatment, $F[1,10]= 1.91$, $p > .05$, or significant treatment x time interaction, $F[1,10]= .88$, $p > .05$. There was a significant time effect, $F[1,10]= 7.39$, $p = .02$, and follow up showed that MMN amplitudes were larger post-stimulation (M = -1.82 µV, SE = .34) compared to pre-stimulation (M = -.82 µV, SE = .13). A priori planned comparisons of the group x time x treatment interaction, $F[1,10]= .76$, $p > .05$, revealed a significant difference across groups, p = .02, between MMN at baseline (M = -.77 µV, SE = .27) and MMN post-stimulation (M = -1.9 µV, SE = .51) for the DC session only. There were no significant increases between pre- and post-stimulation for the sham session. Pre-stimulation

(baseline) MMN amplitudes were similar for DC (*M* = -.77 µV, *SE* = .27) and sham sessions (*M* = -.96 µV, *SE* = .23).

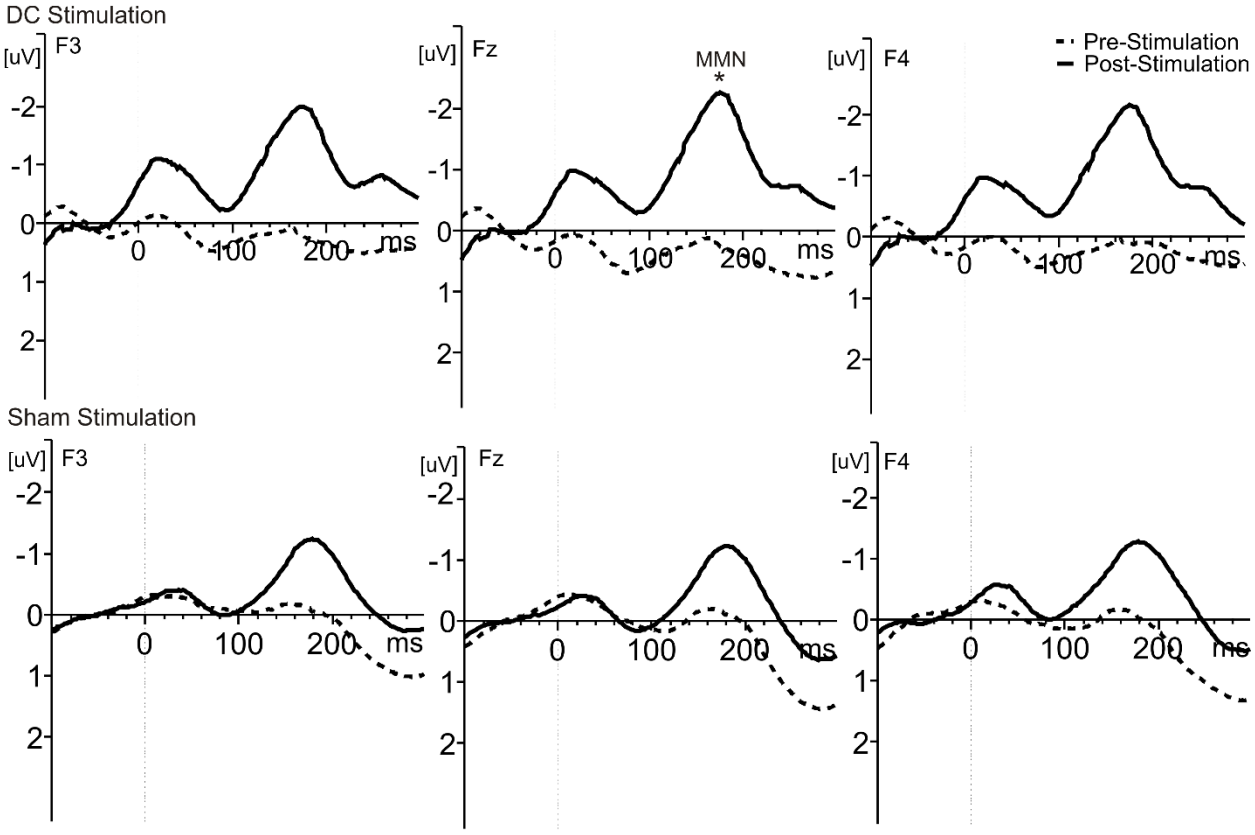

Figure 2.2. Grand averaged frontal MMN low pitch (1050 Hz) deviant waveforms during pre- and post-anodal DC and sham in the LE group. LE individuals displayed significantly greater (* = $p < 0.5$) MMN amplitudes after DC stimulation (vs. baseline), which was not seen for sham stimulation.

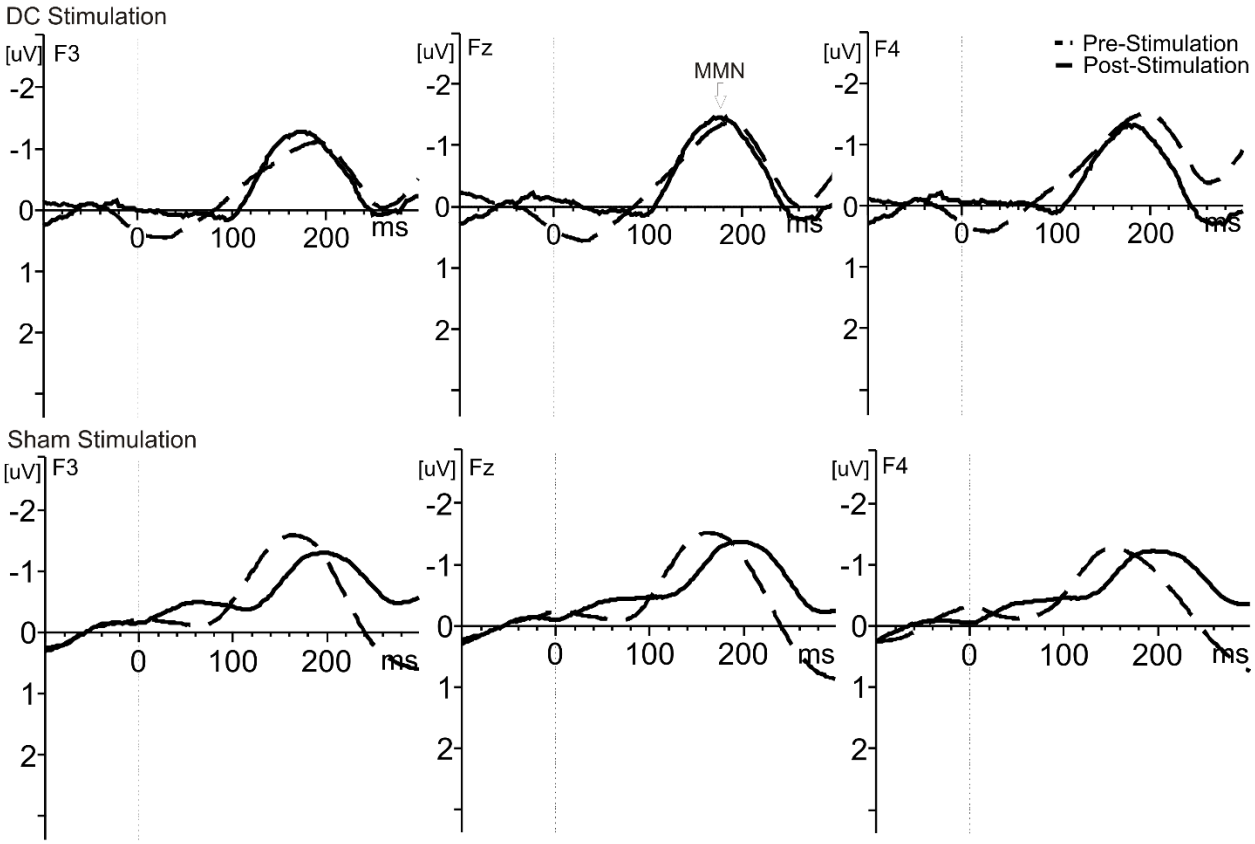

Figure 2.3. Grand averaged frontal MMN low pitch (1050 Hz) waveforms during anodal DC and sham in the HE (high discriminating efficiency) group.

Grand averaged MMN waveforms displaying pre- and post-stimulation in the LE individuals are shown in Figure 2, and the HE group are shown in Figure 3. Regarding group variations, there was a significant time x group interaction, F[1,10]= 5.88, p = .04, and follow-up comparisons showed that groups were significantly different, p = .000, at baseline (*MD* = -1.59 µV, *SE* = .27), as intended, but not post-stimulation, p < .05. Planned comparisons revealed this was due to a significant increase in MMN, *p* = .004, from baseline (*M* = -.021 µV, *SE* = .37) to post-stimulation (*M* = -2.27 µV, *SE* = .72) in the LE group with DC stimulation only. This MMN enhancement was not seen in HE individuals with either DC or sham stimulation. Grand averaged MMN waveforms pre- and post-DC stimulation for LE and HE individuals are shown in Figure 4.

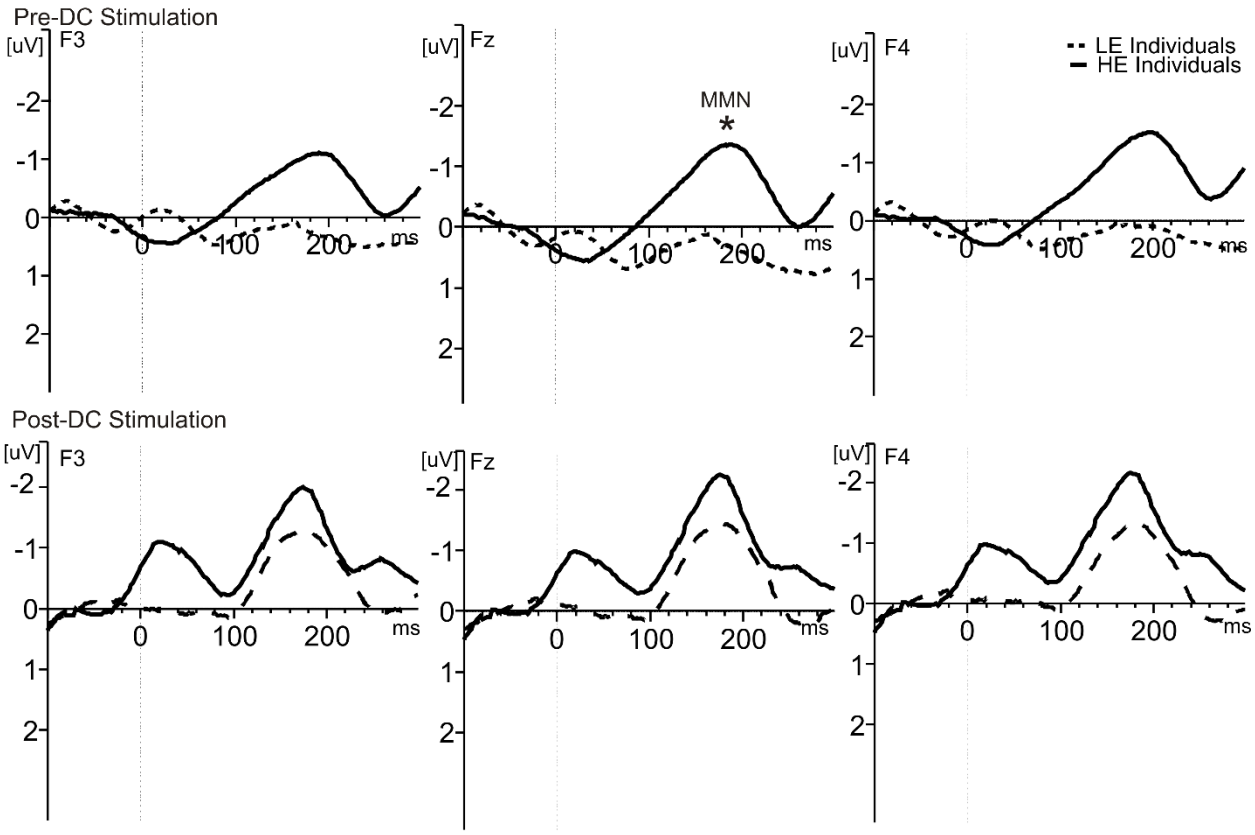

Figure 2.4. Grand averaged frontal MMN low pitch (1050 Hz) deviant waveforms during pre- and post-DC stimulation in LE and HE individuals. LE and HE individuals significantly differed at baseline (* = $p < 0.5$), but not after DC stimulation.

2.5.3. Mastoid MMN amplitudes to Large (1200 Hz) Auditory Deviant

There was no main effect of treatment, $F[1,10]= .46$, $p > .05$, time, $F[1,10]= .41$, $p > .05$, or significant interaction, $F[1,10]= 1.11$, $p > .05$. Planned comparisons comparing pre- and post-stimulation amplitudes for each treatment within the two groups failed to show any significant MMN alterations with either DC or sham stimulation.

2.5.4. Mastoid MMN amplitudes to Small (1050 Hz) Auditory Deviant

There was no main effect of treatment, $F[1,10]= 1.42$, $p > .05$, time, $F[1,10]= 2.31$, $p > .05$ and there were no significant interaction, $F[1,10]= .99$, $p > .05$. Planned comparisons

comparing pre- and post- stimulation for each treatment did not reveal any significant differences with tDCS.

2.5.5. tDCS effects questionnaire

Participants reported mild adverse events related to the tDCS stimulation, including itching, tingling or heating sensation beneath the electrodes. Some participants also reported drowsiness, while others reported alertness after stimulation. Side effects were not related to session condition and participants did not accurately guess post hoc which stimulation they received.

2.6. Discussion

To the best of our knowledge, this is one of the first studies to report electrophysiological evidence of increased cortical activation with transcranial direct current stimulation (tDCS) over the auditory cortex and the first to demonstrate a significant enhancement of MMN-indexed auditory discrimination with anodal tDCS. This randomized, repeated-measures, sham-controlled study showed an increase in MMN amplitudes to a small pitch deviant after a 20 minute tDCS treatment. Importantly, this enhancement was not seen with sham treatment (i.e. no current), which indicates that anodal stimulation of the temporal auditory-adjacent cortex provides a quick, non-invasive method of enhancing auditory sensory processing. These results support previous literature that has shown increased cortical functioning following anodal stimulation of frontal, motor and visual cortices (Nitsche et al. 2008).

Anodal tDCS over the temporal cortex was found to significantly enhance MMN to a small frequency (1050 Hz) deviant immediately after DC stimulation but not with sham ‘stimulation’, which supports our hypothesis that tDCS can improve auditory discriminability.

Although some studies have found that subjects may be able to distinguish between real and 'sham' stimulation (O'Connell, Cossar et al. 2012) , our tDCS questionnaire results confirmed the effectiveness of blinding in this study. Important to note, MMN at baseline (pre-stimulation) did not differ between treatments, which means significant improvements cannot be attributed to differing baseline measurements between sessions. Moreover, when stratified into groups based on baseline MMN, it was found that although groups differed at baseline, as intended, they were no longer significantly different post-stimulation. The a priori planned comparison demonstrated that this was due to participants with initially low discriminability (i.e., LE individuals exhibiting low baseline MMN amplitudes) showing significant MMN enhancement with tDCS, while participants with initially high discriminability (i.e., HE individuals exhibiting high baseline MMN amplitudes) did not. This suggests that individuals with initially reduced deviance detection ability (i.e., LE participants) seem to benefit the most from DC treatment, and that near-ceiling effects may prevent acoustic deviance detection from benefitting with tDCS, as shown in HE participants. It is recommended that future studies expand on our pilot results with a larger sample to verify tDCS modulations in stratified groups.

These results agree with previous pharmacological work in our laboratory which has shown that acute modulation of MMN with the cognitive enhancer nicotine, which increases glutamatergic activity, is baseline-dependant, generally increasing MMN in individuals with small baseline MMN amplitudes, while reducing MMNs in those with high baseline amplitudes (Knott et al., 2014b). Together, these observations provide support for individualized treatment with tDCS as individuals with relatively low MMN amplitudes, which may reflect different NMDA neurotransmission, benefit the most from this technique. The MMN amplitude increase found in this study, which continued post-stimulation, also shows support for possible

involvement of NMDA receptor changes as pharmacologically enhanced NDMA receptor activation prolongs tDCS changes in cortical excitability (Nitsche et al. 2003b; 2004), among other mechanisms, and NDMA activity has been shown to be involved in MMN generation (Javitt et al 1996; Umbricht et al. 2000). Given the role of putative glutamatergic activity in MMN generation, additional pharmacological research is warranted, which, as has been conducted in relation to the motor system, would investigate the ability of NMDA receptor agonists to prolong the after-effects of tDCS on auditory change detection processes probed with the MMN (Nitsche et al. 2003a; 2004; 2008; Liebetanz et al. 2002). Findings from the present study provide tentative support for anodal tDCS's potential effectiveness for deficient auditory deviance detection and allows for future studies investigating the use of this technique in populations with impaired pre-attentive auditory processing, such as in schizophrenia patients, who exhibit robust MMN deficits and behavioural evidence of deficient sound discrimination (Light and Braff 2005).

Anodal tDCS only increased MMN amplitudes elicited by small (Δ 50 Hz) compared to large pitch changes (Δ 200 Hz). This implies that the greater the difficulty in detecting deviant changes, the greater the modulating effect of tDCS. Although, it is possible that the larger (200 Hz) pitch increment was relatively easy to detect (vs. 50 Hz increment) and created a 'ceiling' effect that did not allow further MMN enhancement, this requires verification with a range of frequency deviants, both increments and decrements, as well with variations in other acoustic features as are utilized in the 'optimal' paradigm (Näätänen et al. 2004) such as tone duration, intensity and location. Also, a fixed and relatively short stimulus onset asynchrony (300 ms SOA) was used in the current oddball paradigm. MMN elicitation requires both a memory trace formation (for the standard stimulus) and a comparison of active memory traces for deviance

detection (Näätänen et al. 1989; Näätänen et al. 2011). tDCS may act to modulate either or both processes. The current SOA was of relatively short duration, which allowed for very little if any decay in the standard sensory memory trace prior to its comparison to the deviant stimulus. In future studies, the effects on memory trace formation or decay may be studied by varying SOA to determine whether or not enhancing effects of anodal tDCS on early auditory discrimination are more evident under stimulus conditions promoting weak sensory memory traces.

Although it is presumed that increased excitability of the auditory cortex by anodal tDCS (vs. sham) was responsible for the increases in MMN amplitudes, evidence from this pilot study for this regional effect is not conclusive. The MMN was recorded from both frontal sites, which exhibited the largest amplitudes across the scalp, and from mastoids, as contributions to MMN are thought to be derived from temporal and frontal cortex generators (Näätänen et al. 2007). It is currently unknown if tDCS affects the initial temporal or later frontal MMN generators, or both sources. Results demonstrated that tDCS effects were significant at frontal sites only and were not seen at mastoid sites. However, tDCS changes in temporal generators of MMN may still be contributing to frontal MMN improvements with anodal tDCS. MMN receives contribution from a bilateral supratemporal process at the auditory cortices and a predominantly right-hemispheric frontal process (Giard et al. 1990; Näätänen et al. 2007). Activity in the auditory sources is associated with the establishment of memory traces and comparison with stimulus-specific features (pre-perceptual deviance detection) and the specific location of the these sources slightly differs depending on the physical feature eliciting the MMN, suggesting memory traces to various auditory features are located in different locations (Giard et al. 1990; Näätänen et al. 2007; Shalgi and Deouell 2007). Source activity and the MMN in the frontal lobe is generally thought to mediate an involuntary attention switch caused by the detected acoustic change,

however a review of the literature on the frontal generator in MMN generation could not confirm a direct role (Deouell 2007), but did support the existence of MMN generators outside the main temporal ones, with possible locations in the inferior frontal and possibly also medial frontal cortex. Neuroimaging studies have found signal changes in the right frontal cortex to be larger for smaller deviants, suggesting that frontal MMN generators are activated when temporal auditory change detection mechanisms have difficulty in discriminating unattended acoustic stimuli (Opitz et al. 2002). Whereas the mastoid MMN receives contributions from auditory-cortex generators only, the frontally recorded MMN is composed of contributions from both the auditory and frontal cortices (Näätänen et al. 2012). Taken together, these MMN generator findings could help explain our significant results at frontal sites only for the more difficult to detect low pitch deviants, the MMNs of which were increased where only the auditory cortex was activated with tDCS. These present observations suggest that anodal tDCS over the auditory cortex may activate frontal MMN generators under low discriminability conditions, however studies comparing tDCS effects at temporal and frontal cortical sites are required to verify this selective action.

Although anodal tDCS over the auditory cortex increased MMN in the low MMN individuals, we cannot establish a direct causal relationship between anodal stimulation and sensory discrimination ability in this study because polarity-specific changes in non-motor areas may be an over simplistic explanation of tDCS mechanisms, as evidenced by computational models and animal studies (Reato et al., 2013; Radman, Ramos et al. 2009). De Berker, Bikson and Bestmann (2013) argue that conclusions based on tDCS are limited by poorly understood mechanisms, specifically the localization of currents and expected functional changes. They suggest that individualized current modeling and computational neurostimulation be used to

interpret results, specifically taking into account anatomical variation and the dynamics of polarity and orientation. Improving and demonstrating focality of tDCS like Kuo and colleagues (2013) with the use of high definition electrode arrays and providing conclusions based on realistic explanations of how tDCS works is also recommended. It is also suggested that future studies examine the effects of reference electrode location. Our active electrode was placed over the left temporal cortex, specifically positioned between C5 and T7 sites, scalp sites that closely overlap Brodmann auditory areas, and the reference electrode was placed above the right orbit. These target areas were selected based on other studies which have found significant effects with auditory stimuli (Mathys et al. 2010; Vines et al. 2006) and the reference over the contralateral orbit has been used in many studies as an effective reference point (Boggio et al. 2008; Mathys et al. 2010; Nitsche et al. 2007). Although our MMN changes with anodal tDCS may have involved contributions from the reference/cathodal electrode, it has been shown that current density (current strength/electrode size) determines the efficacy of tDCS, and that reducing the relative size of the stimulation (vs. reference) electrode, as was done in our present study, increases focality of the active electrode and renders the larger reference electrode functionally inefficient (Nitsche et al. 2007). Other studies investigating tDCS effects on the auditory cortex (Mathys et al. 2010; Vines et al. 2006) have assessed the contributing effect of the cathodal electrode position using deferring cathodal electrode placement (for example placing the reference electrode over the motor cortex or occipital lobe) to verify that electrode placement over the temporal cortex was essential for auditory processing changes. However, the inability to localize currents in this study remains a limitation.

In conclusion, this preliminary study provided tentative supportive evidence that anodal (vs. sham) tDCS over the auditory cortex can increase pre-attentive auditory discrimination in

healthy controls as measured by the brain-based MMN ERP. Future studies are needed to verify these effects and to continue to characterize tDCS protocols and deviant parameters for optimal increases in discrimination, which can then be applied for intervention in clinical populations with auditory processing deficits.

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Ethical approval: "All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards."

Chapter 3: Assessment of Anodal and Cathodal Transcranial Direct Current Stimulation (tDCS) on MMN-Indexed Auditory Sensory Processing (Study 2)

3.1. Title Page

Assessment of Anodal and Cathodal Transcranial Direct Current Stimulation (tDCS) on MMN-Indexed Auditory Sensory Processing

Authors:

Danielle Impey[a,b] (danielle.impey@theroyal.ca), Sara de la Salle[a,b] (sara.delasalle@theroyal.ca) and Verner Knott[a,b] (verner.knott@theroyal.ca)

a. School of Psychology, University of Ottawa, Ottawa, Canada
b. University of Ottawa Institute of Mental Health Research, Ottawa, Canada

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3.2. Abstract

Transcranial direct current stimulation (tDCS) is a non-invasive form of brain stimulation which uses a very weak constant current to temporarily excite (anodal stimulation) or inhibit (cathodal stimulation) activity in the brain area of interest via small electrodes placed on the scalp. Currently, tDCS of the frontal cortex is being used as a tool to investigate cognition in healthy controls and to improve symptoms in neurological and psychiatric patients. tDCS has been found to facilitate cognitive performance on measures of attention, memory, and frontal-executive functions. Recently, a short session of anodal tDCS over the temporal lobe has been shown to increase auditory sensory processing as indexed by the Mismatch Negativity (MMN) event-related potential (ERP). This preliminary pilot study examined the separate and interacting effects of both anodal and cathodal tDCS on MMN-indexed auditory pitch discrimination. In a randomized, double blind design, the MMN was assessed before (baseline) and after tDCS (2 mA, 20 minutes) in 2 separate sessions, one involving 'sham' stimulation (the device is turned off), followed by anodal stimulation (to temporarily excite cortical activity locally), and one involving cathodal stimulation (to temporarily decrease cortical activity locally), followed by anodal stimulation. Results demonstrated that anodal tDCS over the temporal cortex increased MMN-indexed auditory detection of pitch deviance, and while cathodal tDCS decreased auditory discrimination in baseline-stratified groups, subsequent anodal stimulation did not significantly alter MMN amplitudes. These findings strengthen the position that tDCS effects on cognition extend to the neural processing of sensory input and raise the possibility that this neuromodulatory technique may be useful for investigating sensory processing deficits in clinical populations.

3.3. Introduction

Brain stimulation is a current research tool for elucidating the neurobiological mechanisms underlying cognitive processes and a possible treatment for cognitive dysfunction, including deficits in memory and executive function. Transcranial direct current stimulation (tDCS) is a non-invasive and cost-effective method to temporarily increase or decrease cortical excitability in targeted, localized brain regions. Stimulation is administrated by applying a weak (~1-2 mA), constant current to the scalp via two electrodes: the anode and the cathode. The electrodes can be used as the active or reference electrode depending on the bipolar placement and size of the electrodes, which are typically superimposed on saline-soaked sponges to increase the conductivity of the current and to reduce any skin irritation that may be experienced during stimulation (Nitsche et al., 2005; Nitsche et al., 2008). The low constant current, flowing from the active to the reference electrode, with peak current densities over the targeted region (Neuling et al., 2012), is typically applied for 10-30 minutes, and the effects of a single stimulation can persist for up to one hour post-stimulation (Nitsche & Paulus, 2001). In clinical research studies with tDCS, repeated stimulations are needed to produce long lasting effects (lasting weeks; Brunelin et al., 2012).The moderating factors affecting the effects of tDCS include current strength and polarity, electrode size, stimulation duration, and position of the electrodes on the scalp (McKinley et al., 2012; Nitsche et al. 2008). These factors mediate the relationship between the induced intracerebral flow of current from the electrodes and alterations of local neuronal activity, which allows for the investigation of the relationships between the modulated neural activity and cognitive processes or behaviour. As the relationship between tDCS and auditory sensory functioning is not well established, the current study will investigate the effects of 20 minutes of both active anodal and cathodal tDCS over scalp sites overlapping

the primary auditory cortex, using a constant current of 2 mA, compared to 'sham' stimulation (where the electrodes are set up on the scalp, but no current is applied).

The majority of tDCS studies have focused on motor cortex function, the first target for tDCS investigation (Nitsche and Paulus, 2000; 2001). These tDCS studies established that tDCS-induced prolonged effects are not only polarity-specific, with anodal stimulation typically having an excitatory effect by depolarizing neuronal membrane potentials in the region beneath the anodal electrode, and cathodal stimulation generally having the opposite effect through a process of hyperpolarization beneath the cathode electrode (Nitsche et al., 2007), but the strength and endurance of the after-effects depend on current intensity, duration and electrode placement (Nitsche and Paulus 2000; Nitsche et al. 2008). A simple relation between polarity and behavioural modification has been recently challenged as evidence has shown tDCS can both depolarize and hyperpolarize within the same gyrus and different types of neurons are differently affected depending on their structural features and orientation (Reato et al., 2013; Radman et al. 2009). However, pharmacological studies have elucidated some of the mechanisms of action underlying tDCS effects. The immediate and long-term 'after-effects' of anodal stimulation are no longer present with sodium and calcium channel blockers, while blocking glutamate receptors via N-methyl-D-aspartate receptor (NMDAR) antagonists reduces the after-effects, regardless of current polarity (Nitsche, Fricke et al., 2003). Specifically, NMDAR antagonists have been found to prevent functional 'after-effects' while NMDA agonists can prolong motor potential after-effects over several hours (Liebetanz et al. 2002; Nitsche et al. 2003b; 2004). Although tDCS modulations are dependent on the NMDAR system, which is implicated in synaptic plasticity and memory function, GABAergic interneurons also play a role, as well as

dopaminergic, serotonergic and cholinergic activity (Medeiros et al., 2012; Stagg and Nitsche, 2011).

Changes in neuronal activity with tDCS have implications not only for motor behaviours, but also for cognition. Anodal stimulation applied to the prefrontal cortex has improved performance on a variety of cognitive tasks, when compared to 'sham' stimulation (Fregni et al., 2005; Hecht, Walsh, & Lavidor, 2010; Zaehle et al, 2011). The most robust effects are seen with working memory (WM) tasks (as measured through n-back tasks and the Sternberg task), where anodal stimulation has increased performance and reaction times (Andrews et al., 2011; Gladwin et al., 2012; Teo et al., 2011). Fregni et al. (2005) found that anodal tDCS (1 mA) enhanced accuracy in a 3-back letter task and Ohn et al. (2008) found an increase in correct responses based on the same paradigm starting 20 minutes after the beginning of active stimulation. In clinical populations, beneficial effects of anodal stimulation applied over the left DLPFC have been reported for working memory, attentional performances, and information processing in patients with depression (Fregni et al., 2006; Oliveira et al., 2013; Wolkenstein and Plewnia, 2013), probabilistic association learning in a subset of schizophrenic patients (Vercammen et al., 2011), visual recognition memory performance in Alzheimer's disease (Boggio et al., 2009), amelioration of memory deficits in Parkinson's disease (Boggio et al., 2006), and improved response accuracy in a Go/NoGo task and verbal 2-back WM task in patients with post-stroke cognitive decline (Kang et al., 2009; Jo et al., 2009). Unlike motor performance, cathodal stimulation has failed to show consistent significant modulation in cognitive tasks, either decreasing or having little impact on performance on similar tasks (Fregni et al., 2005; Hecht et al., 2010). However, cathodal tDCS over the primary and secondary auditory cortex has been found to negatively modulate pitch discrimination and pitch memory

performance (Mathys et al. 2010; Vines et al. 2006). Very few tDCS studies have focused on information processing in the auditory cortex and only two investigators to date have assessed the effect of anodal tDCS on auditory discriminability (Impey and Knott., 2015; Chen et al. 2014a; 2014b), which motivates the current investigation.

Event-related potentials (ERPs) provide an objective, non-invasive neural measure of information processing and ERP components are frequently used to investigate early pre-attentive auditory processes, including sensory gating (indexed by the P50), sensory discrimination (indexed by the mismatch negativity [MMN]) and novelty detection (measured by P3a), as well as higher order processes such as attentional allocation and processing speed (measured by P3b) in healthy volunteers and clinical populations (for review see Braff and Light, 2004). The MMN, an early (~120-250ms) frontal-maximum negative-going ERP component, is a measure of pre-attentive auditory discrimination mediated by a comparison process within sensory memory (Näätänen et al. 2005; Näätänen et al. 2007). MMN is usually elicited when a sequence of repetitive, 'standard' stimuli are interrupted with a rare, deviant 'oddball' stimulus. MMN is elicited automatically when an acoustic feature is detected in brief 'echoic' memory, which deviates from feature memory traces comprising the standard stimulus (Näätänen et al. 2005). Typically, MMN amplitudes increase and latencies shorten with larger deviance and shorter stimulus onset asynchrony (SOA). The MMN is generated primarily in the temporal (auditory) cortex, which is a marker of automatic auditory change, but it also receives contributions from frontal brain regions, which reflect mechanisms involved in involuntary attention switching in response to the change in stimuli (Näätänen et al. 2007; Opitz et al. 2002; Shalgi & Deouell, 2007). MMN amplitude is associated with higher-order cognitive and psychosocial functioning in healthy adults, as indexed by performance on a standardized

memory task, and overall ratings of psychosocial functioning (Light and Braff 2005), and has been found to be a biomarker of deficient sensory processing in populations with cognitive dysfunction, such as in schizophrenia (Turetsky et al., 2007; Umbricht and Krljes, 2005). Similar to tDCS, NMDAR activity has also been found to play a key role in MMN generation as antagonists have been found to block MMN generation in animal studies (Javitt et al. 1996) and diminish MMN amplitudes in humans (Umbricht et al. 2000). As MMN amplitude is a direct brain measure of auditory sensory discrimination, which correlates with more complex cognitive functioning, investigations into tDCS effects on MMN alteration are warranted and results can be used to determine the utility of the MMN as a quick and reliable biomarker (Butler et al., 2012; Green et al., 2009) of tDCS treatment effects on auditory cognition.

Although there is an increasing interest in the cognitive modulating actions of tDCS, there have been very few studies assessing tDCS effects on early, pre-attentive auditory processing. Zaehle et al. (2001) found that anodal tDCS increased amplitudes of the auditory P50 (an early positivity at ~50 ms) ERP, while cathodal tDCS induced larger N1 (an early negativity at ~ 100 ms) amplitudes, indicating increases in early sensory registration. However, in a study by Chen et al. (2014a), which assessed tDCS over the right frontal cortex, MMN to pitch deviants were reduced with anodal stimulation, while duration MMNs were unaffected by either anodal or cathodal tDCS. These paradoxical (decrease in pitch MMN) and negative findings (with duration MMN) with excitatory anodal stimulation in these studies could be due to differences in the positioning of stimulating electrodes and on scalp areas remote from the auditory cortex, the use of specific stimulus parameters, or to baseline MMN amplitude values.

The present pilot study in healthy volunteers assessed MMN-indexed auditory discrimination of small and large pitch deviants and varying SOA before and after either a 20

minute application of anodal, cathodal or 'sham' stimulation (i.e. no stimulation) of the temporal scalp region overlying the auditory cortex. Based on work which has shown that pharmacological (Knott et al., 2013; 2014a; 2014b; 2015) and tDCS modulation of MMN (Impey and Knott, 2015) is baseline-dependant (generally increasing ERP components in individuals with small baseline amplitudes, and decreasing ERPs in individuals with large baseline amplitudes), we examined the effects of acute tDCS on pitch MMN in stratified groups differing in baseline response/amplitude. This stratification strategy was implemented as, recently, the utility and importance of individual biomarkers for the development of more effective, personalized treatment has been highlighted, specifically including stratification of baseline samples for the preclinical assessment of cognitive enhancing agents (Butler et al., 2012; Green et al., 2009; Frank & Hargreaves, 2003). The effect of anodal stimulation was examined in both baseline subgroups, which followed either 'sham' or cathodal stimulation – the assumption being that possible cathodal-induced MMN effects would moderate MMN alterations induced with anodal tDCS. Specifically, we examined the effect of tDCS on MMN-indexed auditory discrimination in two separate sessions, one including a baseline MMN assessment, followed by MMN assessments after 'sham' stimulation and then again after anodal stimulation, to confirm the enhancing effects of anodal enhancement, compared to sham, found in our first pilot study (Impey and Knott, 2015). The second session included a baseline assessment, followed by cathodal stimulation and then anodal stimulation to investigate the effects of cathodal stimulation on MMN, and further, to assess the potential for anodal stimulation to reverse possible induced MMN amplitude alterations related to cathodal stimulation. This second session included the use of both cathodal and anodal stimulation as we wanted to investigate whether anodal tDCS could reverse the cortical excitability decrease produced by cathodal tDCS,

specifically in regards to auditory MMN response. It was generally hypothesized that MMN would be enhanced after receiving anodal tDCS stimulation, particularly in individuals with low MMN response at baseline, and under more difficult deviance detection conditions (smaller pitch deviant, longer SOA) and that the effects would be altered when cathodal (vs. sham) preceded anodal tDCS, which was expected to decrease MMN, particularly in individuals with relatively high MMN at baseline.

3.4. Materials and Methods

3.4.1. Participants

Healthy, medication-free, right-handed male volunteers between the ages of 18-35 were recruited from the local community. This pilot study was conducted with a sample of N=12, which is typical of the majority of tDCS studies (Chen et al., 2014a; 2014b; Kang et al., 2009; Vines, Schnider & Schlaug, 2006; Zaehle et al., 2011). Participants were screened using a questionnaire for medical history, and were interviewed using both the Structured Clinical Interview for DSM-IV Non-Patient (SCID-NP; First et al. 1995) Edition and the Family Interview for Genetic Studies (FIGS; Maxwell, 1992), to assess personal and family psychiatric history, respectively. Exclusion criteria included past or current psychiatric diagnosis (including any Axis I or Axis II disorders, or substance abuse of any kind), first degree family member receiving treatment for a DSM-IV disorder, prior head injury with loss of consciousness or recent neurosurgery (< 6 months), any neurological diagnosis (including epilepsy) or prior medical conditions with possible central nervous system sequelae, current daily use of any prescriptive or over the counter medications, significant hearing loss, or metallic implants inside the brain or any electrical medical device (e.g. pacemaker) in the body. For inclusion, all participants were required to be non-smokers (i.e., smoking a lifetime total of < 100 cigarettes,

with no smoking or nicotine use in the past year) as nicotinic cholinergic agents are known to influence tDCS (Kuo et al. 2007) and MMN (Engeland et al. 2002). All participants signed an informed consent form and were compensated $100 (CAD) for study participation. The study was approved by the Research Ethics Board of the *Royal Ottawa* Mental Health Care Centre.

3.4.2. Design

Participants were assessed in a randomized, repeated-measures, double-blind, crossover design requiring them to attend two test sessions (~2-5 days apart), each involving a series of MMN assessments, first during baseline and then in response to two different tDCS conditions. One treatment session (baseline, sham, anodal series) included a baseline MMN recording, 'sham' administration and an MMN recording and finally anodal tDCS administration and a final MMN recording. This session was designed to measure MMN changes to anodal stimulation following sham tDCS. The second treatment session (baseline, cathodal, anodal series) included a baseline MMN recording, cathodal tDCS administration and an MMN recording and finally anodal tDCS administration and a final MMN recording. This session was designed to measure MMN response to anodal stimulation following cathodal tDCS. Half (randomly selected) of the participants were assigned the sham/anodal stimulation in their first session and cathodal/anodal stimulation in their second session. For the remaining half, the sessions were reversed in order.

3.4.3. Procedure

Test sessions occurred in the morning (9 am-12 pm) following overnight abstinence from food, caffeine or alcohol. Verbal confirmation of abstinence was followed by EEG electrode placement, MMN assessment at baseline, and either 1) sham stimulation (20 mins), MMN recording and anodal stimulation (20 mins) followed by an MMN recording, or 2) cathodal stimulation (20 mins), MMN recording, and anodal stimulation (20 mins) followed by an MMN

recording. During stimulation, participants were asked to sit back and relax in a semi-reclined chair. During MMN recording, participants watched a silent movie with subtitles while the MMN tones played through headphones. Participants were asked to complete an adverse events questionnaire regarding any side effects experienced during each stimulation.

3.4.4. tDCS

This study used identical placement reported in previous tDCS-auditory cortex studies (Zaehle et al. 2001; Mathys et al. 2010; Impey and Knott, 2015). Conductive saline-soaked rubber electrodes super-imposed on sponge plates were placed on the scalp overlying the left auditory cortex (active electrode, 4.4 x 4.4 cm) and on the contralateral forehead above the orbit (reference electrode, 5.1 x 10.2 cm) as described previously (Impey and Knott, 2015; Fregni et al. 2006; Mathys et al. 2010; Vines et al. 2006). Specifically, the active stimulating electrode (either anode or cathode) was positioned between C5 and T7 sites (parallel to the left Sylvan fissure) of the 10-10 international system for EEG electrode placement, scalp sites that closely overlap Brodmann Areas 41 and 42 of the primary and secondary auditory cortex. Note that recordings were completed immediately before and after stimulation, not during tDCS.

Stimulation was applied using a battery-driven constant-current regulator (Oasis Pro, Edmonton). For each stimulation, the DC current was initially increased in a ramp-like fashion over 10 s until reaching 2 mA and was similarly decreased at the end of stimulation (as in Nitsche et al. 2003a). With active tDCS, stimulation was maintained for a total of 20 minutes; for sham, it was turned off after 30 s. These sham parameters were chosen based on previous reports that perceived sensations on the skin, such as tingling (during 10 seconds fade in/out of sham stimulation) produce no after-effects and re-create the same sensations experienced with anodal stimulation (Nitsche et al., 2007; Gandiga et al. 2006), however, some subjects may still be able

to distinguish between real and sham stimulation (O'Connell et al. 2012) and thus it is important to assess the effectiveness of blinding with post hoc questioning of participants (Nitsche et al. 2008).

3.4.5. Stimuli

Four blocks of 600 auditory stimuli (70 dB [SPL]) per block were presented (through headphones) in an oddball sequence involving frequent (p = 0.85) presentations of a standard auditory stimulus (1000 Hz, 100 ms duration) that was randomly inter-mixed with two rare (p = 0.15) deviant stimuli varying in pitch (small pitch deviant: 1050 Hz, 100 ms, p = 0.075; large pitch deviant: 1200 Hz, 100 ms, p = 0.075). Deviant stimuli were presented in a pseudo-random fashion such that deviants never occurred in succession. The stimulus onset asynchrony alternated between 300 ms and 500 ms every second block. Stimuli were presented to the right ear only as functional asymmetry studies have shown that auditory stimuli produces greater activation in the left Heschl's gyrus, the location of the primary auditory cortex (Devlin et al., 2003), especially for processing of rapidly changing acoustic information (Heimrath et al., 2014).

3.4.6. Recordings

Electrical activity was recorded using BrainVision Recorder® software and a BrainVision V-8 Amp® amplifier (Brain Products, GmbH). Participants were seated in a sound-attenuated chamber. ERPs were derived with tin electrodes placed on 6 scalp sites (F_z, F_3, F_4, C_z, C_3, C_4, P_z, O_z), referenced to a nose electrode and with additional electrodes placed on the mid-forehead to serve as ground, and on orbital ridges and external canthi to monitor vertical (VEOG) and horizontal (HEOG) electro-oculographic activity. Bandpass filters were set at 0.1 – 100 Hz and electrical activity was sampled at 500 Hz. Electrode impedance was kept below 5 kΩ. EEG was analyzed off-line with BrainVision Analyzer® software (Brain Products, GmbH).

Raw EEG for each session was digitally filtered (0.1-8 Hz) and ocular corrected (Gratton, Coles, & Donchin, 1983). For each stimulus, electrical epochs of 400 ms duration (beginning 100 ms prior to stimulus onset) were segmented, a baseline correction was applied (relative to the pre-stimulus segment), and only epochs with EEG voltages below 100μV were used for final ERP averages. MMN 'difference waveforms' were derived by point-by-point digital subtraction of the averaged standard stimulus values from average values elicited by the deviant stimuli. Based on grand averaged waveforms, the primary MMN endpoint was assessed by quantifying the peak negative amplitude (relative to averaged pre-stimulus values) within a latency window of 120-250 ms at the frontal midline recording site (Fz), the region typically exhibiting the maximal MMN amplitude.

3.4.7. Statistics

The primary analysis was conducted with Statistical Package for Social Sciences (SPSS) using MMN difference waveform values derived from frontal (Fz) scalp sites. As sensory discrimination improvements with pharmacological (Knott et al., 2013; Knott et al., 2014a; 2014b) and tDCS treatment (Impey and Knott; 2015) may be baseline-dependant, participants (n = 12) were segmented into two groups using a median split based on MMN baseline amplitudes, which resulted in a relatively low and high discrimination efficiency groups for each separate deviant and SOA. As baseline MMN values between sessions significantly differed, stimulation treatment effects on MMN amplitudes were compared in separate repeated measures analysis of variance (ANOVA) for each deviant type (small 1050 Hz deviant; large 1200 Hz deviant) and SOA block (300 ms; 500 ms) and included a within-subject treatment factor, with 3 levels (baseline; sham; anodal), as well as a between-subjects subgroup factor (2 levels; low and high baseline groups). The second ANOVAs contained the same factors except the 3 levels for the

treatment factor were baseline, cathodal and anodal. MMN latencies, as well as N1 amplitudes for the standard stimuli, were analyzed in similar ANOVAs with the same factors. Greenhouse-Geisser significant effects ($p < .05$) are reported and *a priori* planned comparisons were followed up, using Bonferroni corrections, to investigate study hypothesis regarding stimulation differences and group differences.

3.5. Results

3.5.1. Baseline, Sham and Anodal Treatment Series

Table 1. ANOVA values for the Baseline, Sham and Anodal Treatment Series

MMN type	Effect or Interaction	*df*	*F*	*P*
1050 Hz pitch deviant with 300 ms SOA	Treatment	[2, 18]	= 1.88	.18
	Group	[1, 10]	= 3.72	.08
	Treatment x Group	[2, 18]	= 2.66	.10
1050 Hz pitch deviant with 500 ms SOA	Treatment	[2, 17]	= 0.38	.65
	Group	[1, 10]	= 0.81	.39
	Treatment x Group	[2, 17]	= 5.06	.02*
1200 Hz pitch deviant with 300 ms SOA	Treatment	[2, 13]	= 2.74	.12
	Group	[1, 10]	= 5.88	.04*
	Treatment x Group	[2, 13]	= 0.76	.43
1200 Hz pitch deviant with 500 ms SOA	Treatment	[2, 19]	= 0.48	.61
	Group	[1, 10]	= 9.34	.01*
	Treatment x Group	[2, 19]	= 4.05	.04*

*Denotes a significant effect, $p < .05$

ANOVA values for main effects and interactions are listed in Table 1. Grand averaged MMN amplitudes for each condition by group and treatment are shown in Figure 1a. Baseline stratified groups statistically differed across MMN conditions, with the high baseline group displaying increased MMN amplitudes compared to the low baseline group, except for the small MMN deviant (Δ50 Hz, 500 ms stimulus interval). tDCS treatment effects for the small MMN deviant in the low MMN baseline group are shown in Figure 1b. There was no main effect of treatment, but treatment comparisons showed that, anodal stimulation increased MMN amplitudes (M= -2.38 μV, SE= .23) compared to baseline (M= -1.57 μV, SE= .29), $p = .01$, and compared to 'sham' stimulation (M= -1.26 μV, SE= .63), $p = .07$, for the larger 1200 Hz deviant (300 ms SOA). Significant interaction effects between treatment and group revealed that this significant tDCS-specific enhancement was seen in the low baseline group in all MMN conditions, where MMN amplitude significantly, $p < .05$, increased after anodal stimulation compared to MMN at baseline, and compared to 'sham' in both pitch MMN conditions, $p < .07$. There were no significant MMN differences after the 'sham' stimulation, in either group, as hypothesized. Stratified groups differed at baseline, $p = .01$, in all 4 MMN conditions, but were no longer significantly different after stimulation, suggesting the MMN subgroups were differentially affected by anodal tDCS, as hypothesized.

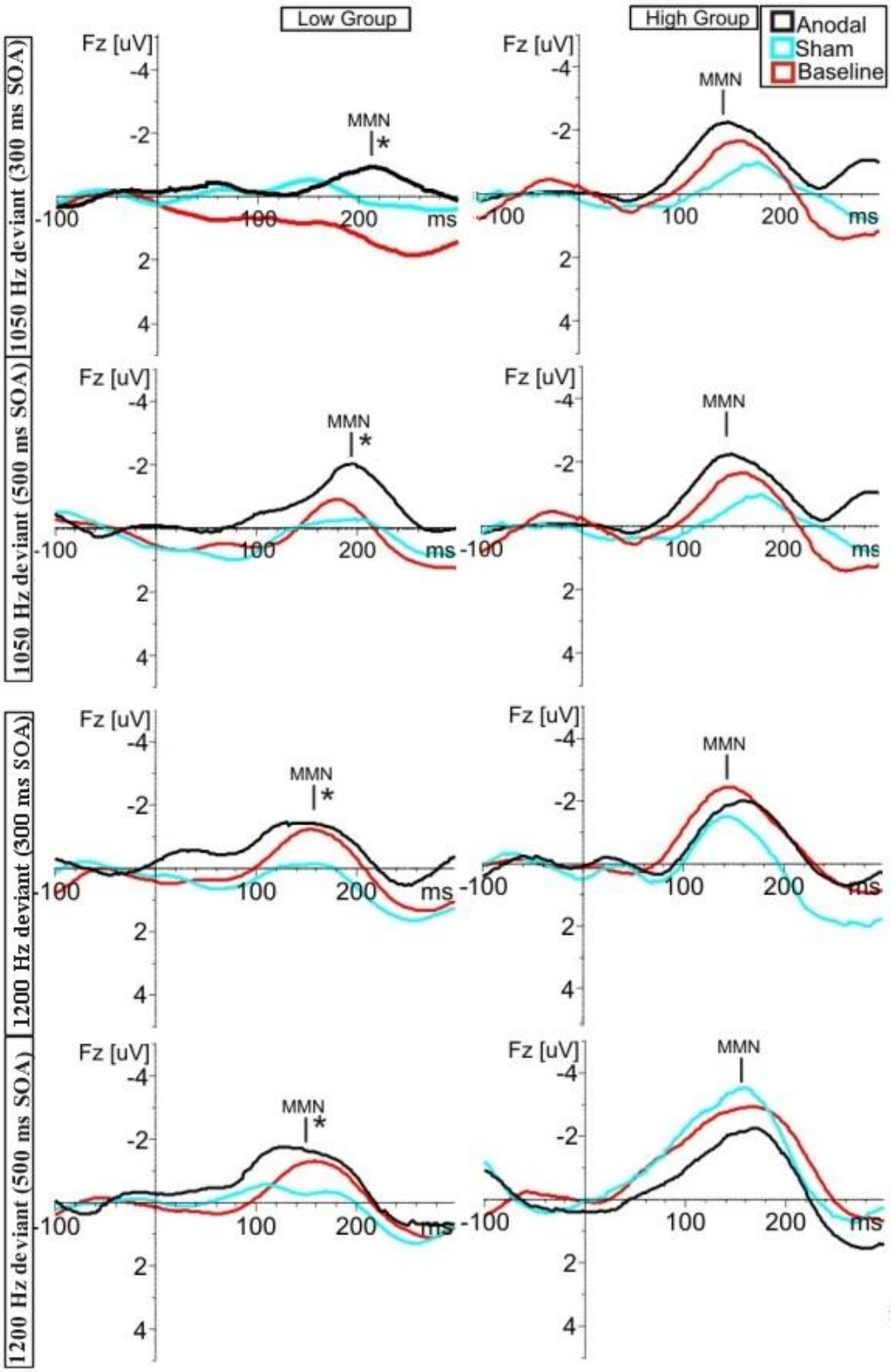

Figure 3.1a. Grand averaged difference waveforms for the baseline-sham-anodal treatment series for each MMN condition displaying MMN amplitudes for each treatment, separated by baseline groups.

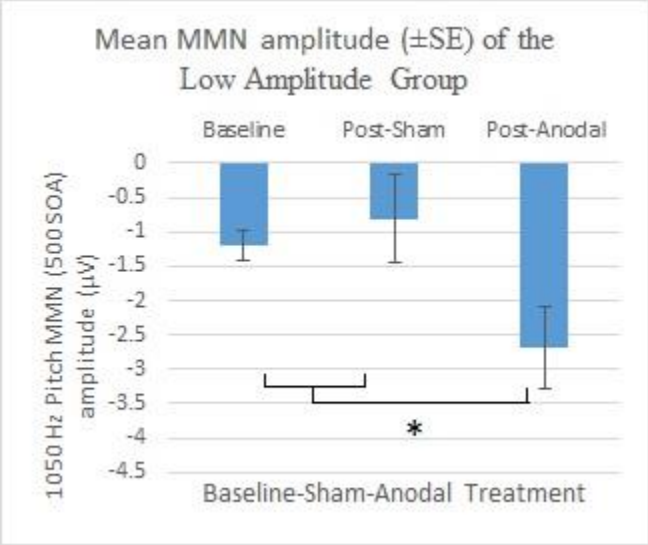

Figure 3.1b. Bar graph depicting the small pitch (Δ50 Hz, 500 ms SOA) mean MMN amplitudes (± SE) for the low baseline MMN individuals in the baseline-sham-anodal treatment series. *p < .05

3.5.2. Baseline, Cathodal and Anodal Treatment Series

Table 2. ANOVA values for the Baseline, Cathodal and Anodal Treatment Series

MMN type	Effect or Interaction	*df*	*F*	*P*
1050 Hz pitch deviant with 300 ms SOA	Treatment	[2, 14] =	0.01	.99
	Group	[1, 10] =	0.06	.81
	Treatment x Group	[2, 14] =	5.30	.03*
1050 Hz pitch deviant with 500 ms SOA	Treatment	[2, 14] =	4.96	.03*
	Group	[1, 10] =	6.69	.03*
	Treatment x Group	[2, 14] =	1.64	.23
1200 Hz pitch deviant with 300 ms SOA	Treatment	[2, 14] =	0.09	.85
	Group	[1, 10] =	1.08	.32
	Treatment x Group	[2, 14] =	4.37	.04*
1200 Hz pitch deviant	Treatment	[2, 15] =	1.15	.33

with 500 ms SOA	Group	[1, 10] = 1.19	.30
	Treatment x Group	[2, 15] = 1.84	.20

*Denotes a significant effect, $p < .05$

ANOVA values and significant effects are listed in Table 2. Grand averaged MMN amplitudes for each condition by group and treatment are shown in Figure 2a. There was a significant main effect of treatment and group for the small (Δ50 Hz, 500 ms stimulus interval) MMN deviant. tDCS treatment effects for the small MMN deviant in the high MMN baseline group are shown in Figure 2b. Stratified baseline groups were significantly different, with the high baseline group displaying increased MMN amplitudes compared to the low baseline group. Across all 4 MMN conditions, stratified groups were significantly different at baseline, $p = .01$, but were no longer different post-stimulation. The significant main effect of treatment showed that cathodal stimulation (M=-1.82 μV, SE=.37) significantly decreased MMN amplitudes, $p = .05$, compared to baseline (M= -2.69 μV, SE= .21), as hypothesized, as well as anodal stimulation (M= -.63 μV, SE= .68), $p = .02$. There was a significant main interaction between treatment and group for both pitch deviants (Δ50 Hz and Δ200 Hz, 300 ms stimulus interval). Follow up comparisons showed that in the high group, cathodal stimulation decreased MMN, compared to baseline, $p < .05$, as hypothesized. This significant reduction was not altered after anodal stimulation, contrary to study hypothesis. In the low group, following cathodal tDCS, anodal stimulation increased MMN amplitudes, $p < .05$, compared to baseline, with both pitch deviants in the 300 ms SOA condition only.

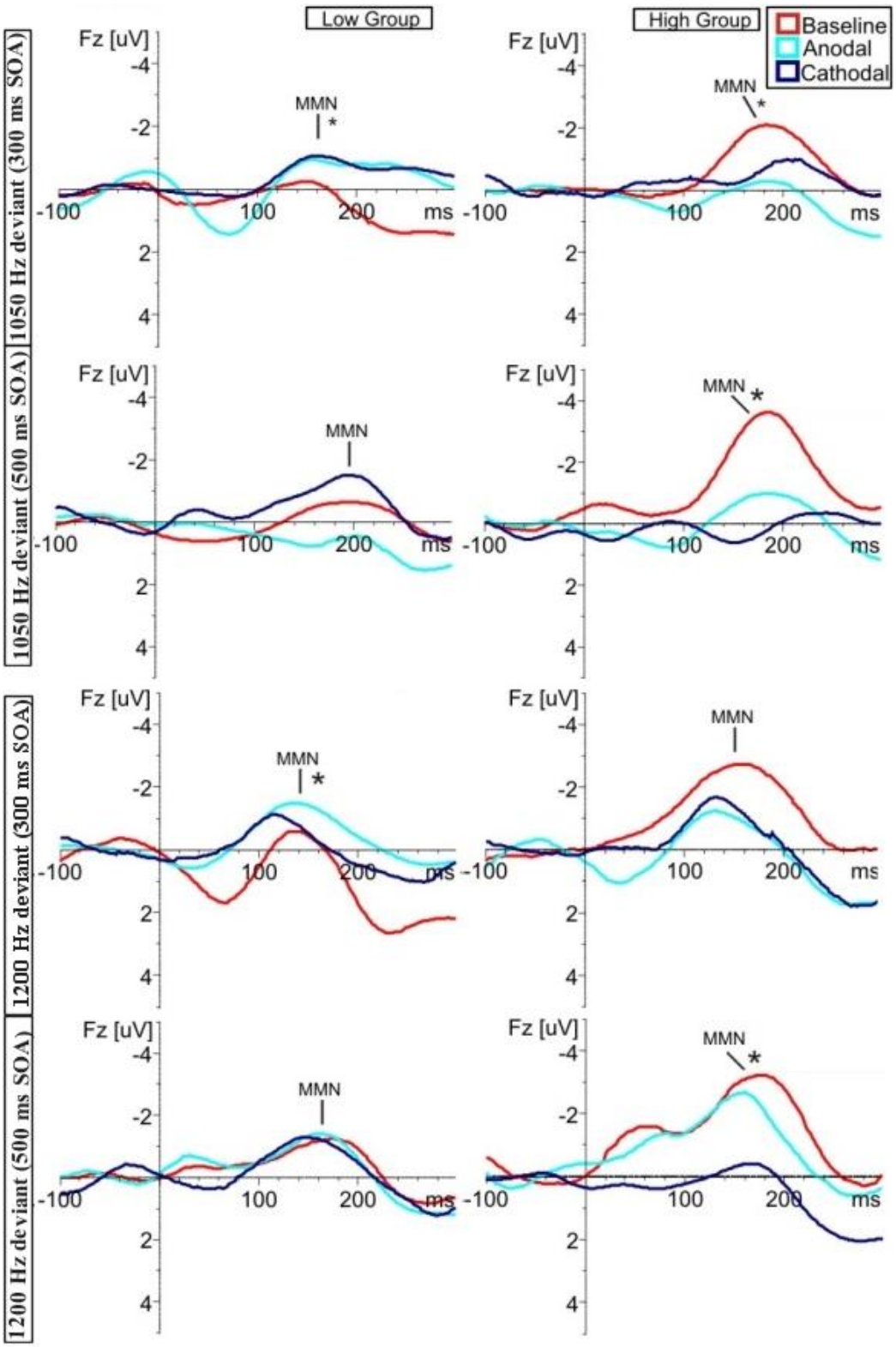

Figure 3.2. Grand averaged difference waveforms for the baseline-cathodal-anodal treatment series for each MMN condition displaying MMN amplitudes for each treatment by group, separated by baseline groups.

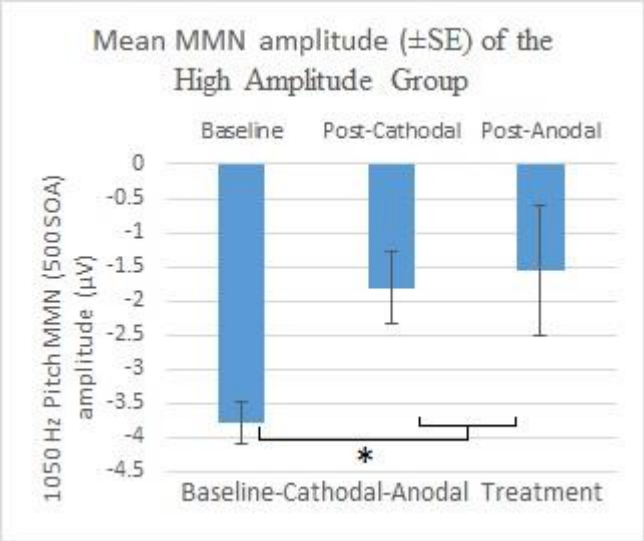

Figure 3.2b. Bar graph depicting the small pitch (Δ50 Hz, 500 ms SOA) mean MMN amplitudes (± SE) for the high baseline MMN individuals in the baseline-cathodal-anodal treatment series. *$p < .05$

3.5.3. MMN latencies

There was no significant effect of treatment on any MMN latencies.

3.5.4. N1 amplitudes

There were no significant differences in N1 amplitudes between treatments.

3.5.5. tDCS-related symptoms

On a scale of 1-5, 1 being no symptoms and 5 being severe symptoms, the most commonly reported was 2 - mild symptoms (e.g. slight tingling). Other side effects reported included drowsiness (n = 3), whereas others reported feeling more excited and clear headed (n =2). Two participants reported headaches after their session.

3.6. Discussion

This pilot study assessed the effect of anodal tDCS over the temporal cortex on MMN-indexed auditory sensory discrimination, comparing differences in stratified groups varying in

baseline deviance detection and examining the moderating effects of pre-treatment with cathodal or 'sham' stimulation. The results demonstrated that anodal stimulation (2 mA, 20 minutes), when preceded by 'sham' stimulation, increased MMN amplitudes for both small, 1050 Hz, and large, 1200 Hz, deviants, and with varying SOA, 300 ms and 500 ms. This significant MMN increase was particularly seen in the low group, who exhibited relatively small MMN amplitudes (i.e., diminished deviance detection efficiency) at baseline, but was also seen across the whole sample for specific deviance parameters (large pitch change, short SOA). These MMN changes were not observed after sham stimulation, as hypothesized, which demonstrates that sham is an effective control condition and that significant effects are due to active current stimulation. For the treatment session involving cathodal stimulation, cathodal tDCS (2 mA, 20 minutes) over the temporal cortex significantly reduced MMN amplitudes, compared to baseline assessment, specifically in the relatively high MMN baseline group, but these decreases were seen across the whole sample with the small (Δ50 Hz) deviant. Anodal stimulation, when preceded by cathodal stimulation, did not result in enhancement or "normalization" of MMN amplitudes as hypothesized. These results demonstrate that anodal stimulation of the auditory cortex can enhance MMN amplitudes, whereas cathodal stimulation can significantly decrease MMN and may prevent the enhancing effects of follow-up anodal tDCS.

The results of the study replicate findings from our previous study (Impey and Knott, 2015) and confirmed our hypothesis that anodal stimulation over the auditory cortex can enhance auditory discrimination. Our hypothesis that cathodal stimulation can decrease MMN-indexed sensory discrimination was also confirmed. In concordance with previous baseline-dependent MMN findings in our laboratory (Knott et al., 2014b; 2015), our investigation indicated that, for each MMN deviant and SOA condition, the stratified groups based on auditory deviance

detection at baseline (relatively low and high groups) demonstrated significantly different MMN amplitudes and were differentially affected by active tDCS, depending on polarity of the stimulation. Following with the study hypothesis, stratified groups were not significantly modulated by 'sham' stimulation. Anodal stimulation was found to increase MMN, particularly in the low group, which may have allowed, or facilitated improvements in auditory discrimination, whereas cathodal stimulation decreased MMN, but not in the low group, presumably due to a floor effect specific to this group. These results confirm findings from other studies (Impey and Knott, 2015; Vercammen et al., 2011) that it is important to consider baseline sensory abilities when administering direct current stimulation for either enhancement, or reduction, of sensory or cognitive function. Our planned manipulation to reverse MMN amplitudes alterations with anodal tDCS after an induced deficient state with cathodal stimulation was not successful in the present study. Cathodal stimulation appears to have prevented or blocked the enhancing effect of anodal stimulation, as tDCS-MMN increases were observed, but not following cathodal stimulation. It is difficult to interpret these results as changes in cortical excitability following stimulation peak around 20 minutes post-stimulation (if applied for 20 minutes) in sensory and cognitive domains, which is when anodal stimulation was administered, and therefore is impossible to confirm if anodal stimulation did or did not enhance pitch discrimination from a deficient state, based on our design. However, the current study sought to assess whether anodal tDCS could reverse the cortical excitability decrease induced by cathodal tDCS, specifically in regards to the auditory MMN response, as this type of manipulation has not been previously investigated. Taken together, the significant modulation of both anodal and cathodal stimulation on MMN amplitudes found in this study does support the use of tDCS as an effective neuromodulation technique for auditory discrimination function.

The results of our study with anodal tDCS are largely supported by the literature which has shown enhancement of memory-based processing. Anodal stimulation applied to the prefrontal cortex has improved performance on a variety of cognitive tasks, when compared to 'sham' stimulation (Fregni et al., 2005; Hecht, Walsh, & Lavidor, 2010), with the most robust effects seen with WM tasks, with anodal stimulation increasing performance and reaction times (Andrews et al., 2011; Teo et al., 2011; Gladwin et al., 2012; Fregni et al. 2005). The few studies that have investigated the ability of tDCS to modulate auditory processing (Mathys, Loui, Zheng, & Schlaug, 2010; Vines, Schnider, & Schlaug, 2006) have demonstrated a detrimental effect of cathodal tDCS on frequency discrimination, but they found no conclusive results about the effects of anodal tDCS on auditory discrimination. Previously, two groups of researchers have investigated the effects of tDCS on auditory MMN. Contrary to all other research showing cognitive enhancement with excitatory anodal tDCS, Chen and colleagues (2014a) found right frontal anodal tDCS to reduce the amplitude of frequency MMN, while neither frequency nor duration MMNs were affected by cathodal stimulation. As previously mentioned, these confounding results could be due to the positioning of stimulating electrodes on scalp areas remote from the auditory cortex, or other methodological limitations, including measurement presentation and parameters. In the present pilot study, tDCS-MMN alterations were seen with each deviant and SOA, except for the large 1200 Hz deviant with the longer 500 ms SOA. Activity in the temporal-auditory sources of MMN generation is associated with the establishment of memory traces and comparison with stimulus-specific features and the specific location of these sources slightly differs depending on the physical feature eliciting the MMN, suggesting memory traces to various auditory features are differentiated (Giard et al. 1990; Näätänen et al. 2007; Shalgi and Deouell 2007). These differences in auditory sources may

explain differential effects of tDCS on established memory traces (depending on length and duration) and different pitch features (relatively easy or hard acoustic discrimination) found in the present study, and in the previous pilot study from our laboratory (Impey and Knott, 2015).

The influence of tDCS treatment on MMN amplitudes as exhibited by the present findings is likely reflective of MMN's high reliance on the glutamatergic neurotransmitter system and cortical NMDA receptors. Javitt et al.'s (1996) research in primates demonstrated that NMDAR antagonists decreased MMN generation through neurophysiological mechanisms: cortical NMDARs play a critical role in evaluating the familiarity of stimuli and MMN generation is representative of the selective current flow through unrestricted NMDA channels. NMDAR antagonists have also been found to diminish MMN amplitudes in humans (Umbricht et al. 2000). tDCS is also thought to work through NMDA receptor activation, as well as shifts in the cell's resting membrane potential, as NMDA antagonists have blocked the "after" effects of tDCS, regardless of the polarity of stimulation (Nitsche, Fricke et al, 2003). A study investigating the neuronal mechanisms of how tDCS influences learning using magnetic resonance spectroscopy (MRS) found significantly higher combined glutamate and glutamine levels beneath the stimulating electrode, indicating that glutamatergic activity may be related to the mechanisms of action for tDCS (Clark, Coffman, Trumbo & Gasparovic, 2011). Additional pharmacological research is warranted, which, as has been conducted in relation to the motor system, would investigate the ability of NMDA receptor agonists to prolong the after-effects of tDCS on auditory change detection processes probed with the MMN. As both MMN generation and tDCS effects are heavily influenced by glutamatergic transmission, among other mechanisms, for clinical populations who have glutamatergic dysfunction, such as patients with schizophrenia, who show abnormal brain glutamate levels (Merritt, McGuire, & Egerton, 2013)

and deficient MMN generation, these findings may have particular relevance for understanding the etiology of sensory impairment in this disorder, as well as its treatment.

The current study had a number of limitations that warrant mention and discussion. The primary limitation of this study was the small sample size of participants. Although the vast majority of tDCS studies are conducted with a sample of 12 (Chen et al., 2014a; 2014b; Kang et al., 2009; Vines, Schnider, Schlaug, 2006; Zaehle et al., 2011), because the analyses examined stratified groups of low- and high-baseline MMN individuals, it is likely that the study was statistically underpowered. However, our results showed significant effects of tDCS, even with low statistical power and as previous studies have shown MMN modulations to be baseline-dependant (Knott et al., 2014b), we could not ignore this as a factor for the present study and strongly suggest assessing baseline-dependency effects in future studies. A second limitation of the present study is the incomplete design of the study and the use of sham stimulation as a control condition, only in the baseline, sham, anodal treatment series. Sham parameters were chosen based on previous reports that perceived sensations on the skin, such as tingling (during 10 seconds fade in/out of the 30 s sham tDCS) produce no after-effects and re-create the same sensations experienced with anodal stimulation (Gandiga et al. 2006). Although some studies have found that subjects may be able to distinguish between real and 'sham' stimulation (O'Connell, Cossar et al. 2012) , our tDCS questionnaire results confirmed the effectiveness of blinding in this study. As the present study did not include current modeling or imaging to confirm current densities, the inability to localize currents in this study remains a limitation. However, current modelling studies have shown that the low constant current, flowing from the active to the reference electrode, does have peak current density over the targeted region (Neuling et al., 2012) and that by adjusting the current density (with both the current strength and

electrode size), we can optimize the efficacy of tDCS to reach targeted brain regions (Nitsche et al., 2008). Although we cannot establish a direct causal relationship between stimulation and auditory discrimination ability in this study, our significant results do indicate a robust modulation of MMN, with both anodal stimulation (vs. sham) and cathodal stimulation, compared to baseline.

The pilot findings showed that anodal tDCS over the temporal cortex improved MMN-indexed auditory discrimination, compared to sham stimulation, particularly in individuals with relatively low sensory discriminability, and demonstrated reduced MMN amplitudes with cathodal tDCS, compared to baseline assessment, particularly in individuals with relatively high discriminability. This study underscores the importance of the use of neurophysiological markers based on baseline response for the preclinical assessment of tDCS, among other cognitive enhancing agents. Overall, the findings provide tentative supportive evidence that active tDCS of the auditory cortex can significantly modulate pre-attentive auditory discrimination in healthy controls as measured by the brain-based MMN ERP. Future studies are needed to continue to characterize tDCS protocols for optimal increases in sensory functioning, and to investigate anodal tDCS as a treatment for auditory discrimination deficits in clinical populations.

3.7. Acknowledgments and Declarations

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Conflict Of Interest: None of the authors have any financial interests or conflict of interests to report.

Ethical approval: "All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards."

Chapter 4: Effects of an NMDA Antagonist on the Auditory Mismatch Negativity (MMN) Response to Transcranial Direct Current Stimulation (Study 3)

4.1. Title Page

Effects of an NMDA Antagonist on the Auditory Mismatch Negativity (MMN) Response to Transcranial Direct Current Stimulation

Authors:
Danielle Impey[1,2] (danielle.impey@theroyal.ca), Sara de la Salle[1,2] (sara.delasalle@theroyal.ca), Ashley Baddeley [1] (Ashley.Baddeley@theroyal.ca) and Verner Knott[1,2] (verner.knott@theroyal.ca)

1 Clinical Neuroelectrophysiology & Cognitive Research Laboratory, University of Ottawa Institute of Mental Health Research, Ottawa, Canada
2 School of Psychology, University of Ottawa, Canada

Keywords: transcranial direct current stimulation (tDCS); auditory discrimination; event-related potentials (ERPs); mismatch negativity (MMN); NMDA antagonist, dextromethorphan (DMO)

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Psychopharmacology.

4.2. Abstract

Transcranial direct current stimulation (tDCS) is a non-invasive form of brain stimulation which uses a weak constant current to temporarily alter cortical excitability and activity. tDCS-induced increases in neuronal excitability and performance improvements have been observed following anodal stimulation of brain regions associated with visual and motor functions, but relatively little research has been conducted with respect to auditory processing. Recently, pilot study results indicate that anodal tDCS can increase auditory deviance detection, whereas cathodal tDCS decreases auditory processing, as measured by a brain-based event-related potential (ERP), mismatch negativity (MMN). As evidence has shown that tDCS lasting effects may be dependent on N-methyl-D-aspartate (NMDA) receptor activity, the current study investigated the use of dextromethorphan (DMO), an NMDA antagonist, to assess possible modulation of tDCS' effects on both MMN and working memory performance. The study, conducted in 12 healthy volunteers, involved four laboratory test sessions within a randomized, placebo and sham-controlled crossover design that compared pre- and post-anodal tDCS over the auditory cortex (2 mA for 20 minutes to temporarily excite cortical activity locally) and 'sham' stimulation (i.e. device is turned off) during both DMO (50 mL) and placebo administration. Anodal tDCS increased MMN amplitudes with placebo administration. Significant increases were not seen with 'sham' stimulation, nor with anodal stimulation during DMO administration. With sham stimulation, i.e. no stimulation, DMO decreased MMN amplitudes. Findings from this study contribute to the understanding of underlying neurobiological mechanisms mediating tDCS-sensory and memory improvements.

4.3. Introduction

Transcranial direct current stimulation (tDCS) is a non-invasive procedure of cortical stimulation, in which a weak direct current is used to induce changes in cortical excitability of targeted brain regions. Depending on the polarity of the stimulation, tDCS can result in reversible increases (anodal tDCS) or decreases (cathodal tDCS) in cortical excitability in circumscribed stimulated regions, which allows for the investigation of the relationship between brain activity and behaviour (Gandiga et al. 2006; Nitsche et al. 2003a). As tDCS can alter cortical activity, this technique has been investigated as a tool to modify perceptual and behavioural functions, most frequently in the motor and prefrontal cortex (Nitsche et al. 2003a; Nitsche et al. 2008). Moreover, some studies have shown beneficial clinical effects in neurological and psychiatric patients (i.e., motor function recovery post-stroke and anti-depressant effects; Fregni et al. 2006; 2008; Hill, Fitzgerald & Hoy, 2015; Jo et al. 2009). Recently, tDCS has increasingly been used to investigate cognitive function. Improvement in performance on working memory tasks in healthy volunteers and neuropsychiatric populations have been reported with 1-2 milliamps (mA) of anodal stimulation over the dorsolateral prefrontal cortex (DLPFC) for 15-30 minutes (Boggio et al. 2006; 2008; Brunoni, Vanderhasselt, 2014; Ferrucci et al. 2008; Fregni et al. 2005; 2006; Hill Fitzgerald and Hoy 2015). Cathodal tDCS stimulation with the same parameters transiently decreased performance on a memory task in healthy participants and Alzheimer's patients (Ferruci et al. 2008). These studies were conducted within randomized, double-blind, sham-controlled (i.e. no stimulation) designs, which demonstrates the potential effectiveness of a very short treatment of tDCS for cognitive improvement or modulation, without any severe adverse events (Iyer et al. 2005; Nitsche et al. 2003b; Utz et al. 2010). The current study will assess tDCS effects on auditory processing, an

understudied area in the field of neurostimulation, and will attempt to replicate associated higher-order tDCS-working memory effects found in previous studies. Moreover, the primary objective of this study is to investigate the effects of a glutamatergic agent on these tDCS-sensory and memory alterations as stimulation effects may be dependent on glutamatergic activity.

Pharmacological studies suggest that the immediate short-lasting effects of tDCS are generated by polarity-specific shifts of the cell's resting membrane potential, with anodal stimulation causing a depolarization of the resting potential (and an increase in spontaneous firing rates), while cathodal stimulation has the opposite effect, causing a hyperpolarization of the resting-membrane potential (and a decrease in spontaneous firing rates). However, induced brain plasticity in the form of 'after-effects', or excitability that continues post-stimulation, depend on membrane potential changes and modulations of N-methyl-D-aspartate (NMDA) receptor activity, which is a voltage sensitive glutamate receptor crucial for experience-dependant plasticity and short-term modulations of synaptic efficacy, among other neurotransmitters (Liebetanz et al. 2002; Nitsche et al. 2003a). As seen with animal data (Rohan et al. 2015), tDCS changes in excitability and motoric/visual functions continue post-stimulation if tDCS is applied for several minutes, and the 'after-effects' can remain stable for an hour or more if tDCS is applied for nine minutes or longer over the motor cortex (Nitsche et al, 2003a; 2004). NMDA antagonists have been found to prevent motor evoked after-effects, while NMDA agonists prolong motor after-effects by several hours (Liebetanz et al. 2002; Nitsche et al. 2004). Dextromethorphan (DMO), a NMDA-receptor antagonist, induces suppression of the after-effects of both anodal and cathodal stimulation, while carbamazepine (CBZ; a sodium channel blocker) and flunarizine (a calcium channel blocker) eliminate only the anodal effects (Liebetanz et al. 2002; Nitsche, Fricke et al. 2003). D-cycloserine, a partial NMDA agonist, selectively

potentiates the effects of anodal tDCS with increased excitability of motor regions (Nitsche et al. 2004). These pharmacological studies targeting the motor cortex demonstrate the vital role of NMDA receptor activity on the 'after-effects' of tDCS.

Although the effects of tDCS on motor and visual cortices are relatively well known, very few tDCS studies have focused on the auditory cortex. Event-related potentials (ERPs) are a robust, powerful and non-invasive neural measure of cognitive function and ERP components are frequently used to investigate early pre-attentive auditory processes in healthy participants and clinical populations (for review see Braff & Light, 2004). The mismatch negativity (MMN) ERP component is a measure of pre-attentive deviance detection and can be used to indirectly assess auditory sensory memory function (Näätänen et al. 2005), which refers to the ability of the brain to retain (in brief 'echoic' memory) transient representation of physical (e.g. pitch) or abstract features of stimuli, representing an early sensory-memory trace formation. The MMN can be reliably and quickly obtained, with no participant task requirements, within a conventional 'oddball' paradigm involving repeated presentations of a 'standard' auditory stimulus intermixed with presentations of rare 'deviant' stimuli. According to the theory, a sensory memory trace is formed in response to the repetitive standard stimuli and the MMN is elicited automatically when a deviant is detected which deviates from the standard memory trace (Näätänen et al. 2005). In this sense, MMN may reflect a regularity violation or predictive coding of auditory stimuli (Näätänen et al. 2011; Winkler 2007). Operationally, MMN is obtained by subtracting the standard from the deviant waveform to obtain an index of pre-attentive auditory discrimination. MMN amplitude is significantly correlated with cognitive and psychosocial functioning in healthy adults (Light Swerdlow and Braff 2007). Consistently, findings have suggested that pharmacological inhibition of NMDA receptors results in the

attenuation of MMN (Heekeren et al. 2008; Javitt et al. 1996; Umbricht et al. 2002). Animal models have shown NMDA receptors to have a crucial role in memory and have been found to play a key role in auditory sensory discrimination and MMN generation, as NMDA antagonists were found to diminish MMN generation (Javitt et al. 1996; Gil-da-Costa et al. 2013). In humans, findings have suggested that pharmacological inhibition of NMDA receptors results in the attenuation of MMN (Heekeren et al. 2008; Umbricht et al. 2002). Specifically, the NMDA antagonist ketamine attenuates MMN amplitude in human participants and has been used to model impaired cognition in healthy controls (Rosburg and Kreitschmann-Andermahr 2015; Umbricht et al. 2002). Following from this, NMDA receptor hypofunction has been prioritized as a reasonable molecular target in the development of pharmacological treatments for cognition in psychiatric disorders, such as schizophrenia (Brunelin et al. 2012; Javitt 2000; Javitt et al. 2008; 2012; Nagai et al. 2013), and MMN has been designated a reliable biomarker for the detection of pro-cognitive treatment effects in pre-clinical trials (Butler et al. 2012).

4.3.1. Objectives and Hypotheses

Although there have been some investigations of cognitive modulation by tDCS, there have been very few studies assessing cognitive changes associated with tDCS effects on auditory functions or the underlying neurobiological mechanisms. Recently, pilot study results from our laboratory demonstrated that anodal tDCS over the auditory cortex can increase auditory sensory processing (Impey and Knott 2015), whereas cathodal tDCS decreases auditory processing (Impey et al. 2016). Although other studies have investigated MMN-tDCS effects (Chen et al, 2014; Heimrath et al., 2015), this was the first study to our knowledge to show support for anodal tDCS's ability to enhance MMN-indexed sensory discrimination. As research has shown that tDCS lasting effects may be NMDA-dependent (Liebetanz et al. 2002; Nitsche et al. 2003a;

2004), the current study investigated the use of an NMDA antagonist, DMO, to assess possible modulation of tDCS' effects on MMN-indexed deviance detection, which is also known to be NMDA function dependent (Javitt et al. 1996). As tDCS is a possible treatment for auditory sensory processing deficits in psychiatric and neurological disorders, it is important to investigate and understand the underlying neurobiological mechanisms. As previous research has been conducted on the motor cortex, this is the first study to propose investigation of the pharmacological modulation of tDCS's effects on the auditory cortex. The current study assessed MMN amplitude in healthy volunteers during double-blind sessions involving randomized administration of anodal tDCS and 'sham' stimulation with DMO (vs. placebo). Across treatment conditions, and more specifically in the placebo condition, it is expected that MMN will be enhanced after receiving anodal tDCS (compared to pre-stimulation assessment), and that this effect will not be observed with 'sham' stimulation. In the treatment condition, the DMO drug is expected to block the expected enhancing effect of active tDCS (vs. pre-stimulation MMN), and in the 'sham' condition, i.e. no stimulation, DMO is expected to decrease MMN amplitudes (vs. pre-stimulation). As MMN is associated with higher-order working memory function (Javitt et al., 1995), a secondary, exploratory objective of this study will also confirm the enhancing effects of anodal tDCS (vs. sham) on working memory performance (Fregni et al. 2005; Keeser et al. 2011; Teo et al. 2011) and possible alterations with the NMDA antagonist, DMO.

4.4. Method

4.4.1. Participants

Twelve (12) healthy, right-handed male participants between the ages of 18-35 were recruited from the local community (primarily from universities via word of mouth). All

participants underwent a medical screening interview for study inclusion, a psychiatric interview using the Structured Clinical Interview for DSM-IV Non-Patient Edition (SCID-NP) and the Family Interview for Genetic Studies (FIGS). Exclusion criteria included the following: 1) past or current psychiatric diagnosis of Axis I or Axis II disorders; 2) current use of psychotropic or central nervous system medication, including monoamine oxidase (MAO) inhibitors (as drug interactions with DMO have been noted; Sjoqvist 1965); 3) first degree family member receiving treatment for a DSM-IV disorder; 4) prior head injury with loss of consciousness, or recent neurosurgery; 5) any neurological diagnosis (including epilepsy); 6) any current medical illness (transient colds or allergies excepted) or prior medical conditions with possible central nervous system sequelae (i.e., pulmonary, endocrinological, cardiac, metabolic, most systemic illnesses); 7) clinical history of EEG or neuroimaging scans; 8) prior treatment for substance abuse of any kind; 9) consumption of more than 2 alcohol drinks and 5 cups of coffee on average per day during the past month; 10) metallic implants or any electrical device (e.g., pacemaker) in the body. For inclusion, all participants were non-smokers (i.e., smoking a lifetime total of <100 cigarettes, with no smoking or nicotine use in the past year) as cholinergic agents are known to influence tDCS (Kuo et al. 2007). All participants had normal hearing as assessed via audiometric screening. All participants signed an informed consent form. The study was approved by the Research Ethics Board of the *Royal Ottawa* Mental Health Care Centre and has been performed in accordance with the ethical standards from the 1964 Declaration of Helsinki.

4.4.2. Design

Participants were assessed in a randomized, repeated-measures, double-blind design requiring them to attend 4 test sessions, 2-5 days apart, two involving anodal tDCS administration (one with placebo treatment, one with DMO treatment) and two involving sham

tDCS administration (one with placebo treatment and one with DMO treatment). The session order was pseudo-randomized and counter balanced.

4.4.3. Drug

Dextromethorphan (DMO), a non-competitive NMDA antagonist, is a psychoactive drug which is most often used as a cough suppressant. It is one of the main ingredients in many over the counter cough suppressants, including generic Life Brand Clear Cough Syrup DM (Trillium Healthcare Products Inc, Brockville, ON), which has high dose of DMO (15 mg/5 ml) with no other major additives. DMO is well absorbed from the gastrointestinal tract with maximum serum level, as well as peak plasma levels occurring at ~2.5 hours (Barnhart et al. 1979; Silvasti et al. 1987). Based on these findings and on studies which have used DMO as an NMDA antagonist in tDCS studies (Liebetanz et al. 2002; Nitsche, Fricke et al. 2003), each participant received a dose of either 50 ml Hydrobromide DMO (equivalent to 150 mg of DMO) or a placebo (non-medical, no-sugar syrup) 2 hours prior to active tDCS, so that peak treatment effects would be attained during tDCS-MMN assessment. Each dose was given to participants in no-sugar cranberry juice and they wore a nose plug to minimize olfactory/gustatory cues. Effects with low doses of DMO include possible drowsiness or dizziness, which are symptoms previously reported with equivalent doses (Nitsche, Fricke et al. 2003). In very high doses, DMO can lead to hallucinations, dissociation and nausea, typically seen with NMDA antagonists. For safety reasons, adverse events were monitored by the Adverse Events Questionnaire, and the Clinician Administered Dissociative Symptom Scale [CADS; Bremmer et al. 1998], a questionnaire which measures possible dissociative symptoms with items that assess perception changes. Vital signs were also monitored.

4.4.4. Procedures

Test sessions occurred in the morning (9-11 a.m.) following overnight abstinence from drugs (including medications, alcohol, nicotine and caffeine) and food. EEG electrode placement and baseline auditory MMN assessment was completed. Two hours prior to anodal or sham tDCS, each subject received a dose of either 50 ml DMO or a placebo, with 100 ml of no-sugar cranberry juice. tDCS was applied for 20 minutes (anodal or sham depending on the testing session randomization) and post-stimulation MMN assessment immediately followed. The double-blind procedure was maintained with the help of an assistant who turned off the tDCS with sham sessions. Administration of the 1- and 2-back working memory tasks always followed. Adverse events were monitored following peak assessment time and at the end of the session by the Adverse Events Questionnaire and Checklist of DC symptoms, a Likert-scale which required participants to rate the severity of symptoms from 1 (No symptoms) to 5 (Severe symptoms).

4.4.5. tDCS

Conductive saline-soaked rubber electrodes super-imposed on sponge plates were placed on the scalp overlying the left auditory cortex (active electrode, 4.4 x 4.4 cm) and on the contralateral forehead above the orbit (reference electrode, 5.1 x 10.2 cm) as described previously (Impey and Knott, 2015; Mathys et al. 2010; Vines et al. 2006). Specifically, the anodal electrode was positioned between C5 and T7 sites of the 10-10 international system for EEG electrode placement, scalp sites that closely overlap Brodmann Areas 41 and 42 of the primary and secondary auditory cortex, respectively. Stimulation was applied using a battery-driven constant-current regulator (Oasis Pro, Edmonton). In both active tDCS session and sham tDCS sessions, the DC current was initially increased in a ramp-like fashion over 10 s until

reaching 2 mA and was similarly decreased at the end of stimulation (Nitsche et al. 2003a). In active tDCS, stimulation was maintained for a total of 20 min; in sham, it was turned off after 30 s. These sham parameters are chosen based on previous reports that perceived sensations on the skin, such as tingling (during 10 seconds fade in/out of the 30 s sham tDCS) produce no after-effects and re-create the same sensations experienced with active stimulation, thus serving as an ideal control condition (Nitsche et al. 2003a; Gandiga et al. 2005). tDCS has been shown to be applied repeatedly (i.e., daily), for up to 30 minutes without any adverse reactions (Iyer et al. 2005; Nitsche et al. 2005).

4.4.6. MMN Paradigm

Participants were seated in a recording chamber and were instructed to ignore the sounds and watch a silent movie with subtitles. Four blocks of 600 auditory stimuli (70db [SPL]) per block were presented to the right ear only, through headphones, in an oddball sequence involving frequent (P = 0.85) presentations (stimulus onset asynchrony = 300 ms) of a standard stimulus (1000 Hz, duration = 50 ms) that was randomly inter-mixed with two rare (P = 0.075 each) deviant stimuli varying in pitch (1050 Hz, 50 ms, P = 0.075) or duration (1000 Hz, 100 ms, P = 0.075).

4.4.7. ERP Acquisition and Processing

ERPs were derived with 32 scalp electrode positions based on the 10-20 EEG system, referenced to a nose site and with additional electrodes placed around orbital regions to monitor vertical (VEOG) and horizontal electro-oculographic activity (HEOG). BrainVision Recorder software (Brain Products, GmbH) was used to collect data. Amplifier bandpass filter settings was 0.1 – 100 Hz and digital sampling was 500 Hz. Electrode impedance was kept below 5 kΩ. EEG was analyzed off-line with BrainVision Analyzer software (Brain Products, GmbH). Electrical

activity was filtered using 0.1-30 Hz filter settings, ocular corrected with the Gratton and Coles algorithm (Gratton et al. 1983), and stimulus-locked epochs (600 ms) were baseline corrected (-100 ms relative to the pre-stimulus segment), and only epochs with voltages below 100 μV were used for final ERP averaging for each stimulus type. MMN peak amplitude was measured from digital 'subtraction waveforms' (i.e., deviant minus standard), and was selected as the most negative peak between 120-250 ms from stimulus onset at the frontal Fz site, the region typically exhibiting the maximum MMN amplitude (Näätänen 2007). To distinguish MMN effects from the early N1 response, the same procedures were applied to the N1 ERP, which was measured from the standard waveform and selected as the most negative peak between 70-120 ms at Fz.

4.4.8. Working Memory Task

To measure working memory performance, the 1 and 2-back tasks were administered from the computerized CogState Research Memory Battery (CogState Limited 2009). Participants were required to click a mouse to determine if they have recognized the same playing card which appeared either 1 card back (1-back task), or 2 cards back (2-back task). There were 32 trials for each task with a maximum stimuli duration of 3500 ms and an interstimulus interval (ISI) that varied between 1500 ms and 2500 ms. Feedback for correct and incorrect responses was indicated to the participant with respective sounds presented through speakers in the testing chamber. Results were analyzed by assessing the accuracy of performance, using the arcsine transformation of the square root of the proportion of correction responses (higher score = better performance) and speed of processing, using reaction time (RT) for accurate responses.

4.4.9. Statistics

MMN amplitude data was analyzed in a repeated measures analysis of variance (ANOVA) containing a time factor (2 levels: pre- and post-stimulation), stimulation factor (2 levels: anodal and sham stimulation) factor, and a drug factor (2 levels: DMO and placebo). Separate ANOVAs were completed for duration and pitch MMNs at frontal site Fz. *A priori* planned pairwise comparisons of time (pre- and post-stimulation) for each stimulation treatment were investigated to follow up on study hypothesis. As previous work has shown that modulation of MMN amplitude and tDCS effects may be dependent on baseline functioning, with pharmacological interventions and neurostimulation effects (Impey and Knott 2015; Knott et al. 2014; 2015; Li et al., 2015), post-hoc comparisons were also investigated regarding baseline-dependant MMN groups. Using the same median-split strategy, participants were stratified into 2 groups based on baseline (placebo) MMN recordings, to assess possible treatment differences in relatively low vs. high baseline MMN individuals in our sample. Separate ANOVAs with the same factors were completed for the N1 ERP and for Working Memory performance on the 1- and 2-back. Greenhouse-Geisser significant effects ($p < .05$) were determined and *a priori* planned comparisons were followed up, using Bonferroni-adjusted corrections, to investigate study hypothesis.

4.5. Results

4.5.1. Pitch MMN amplitudes

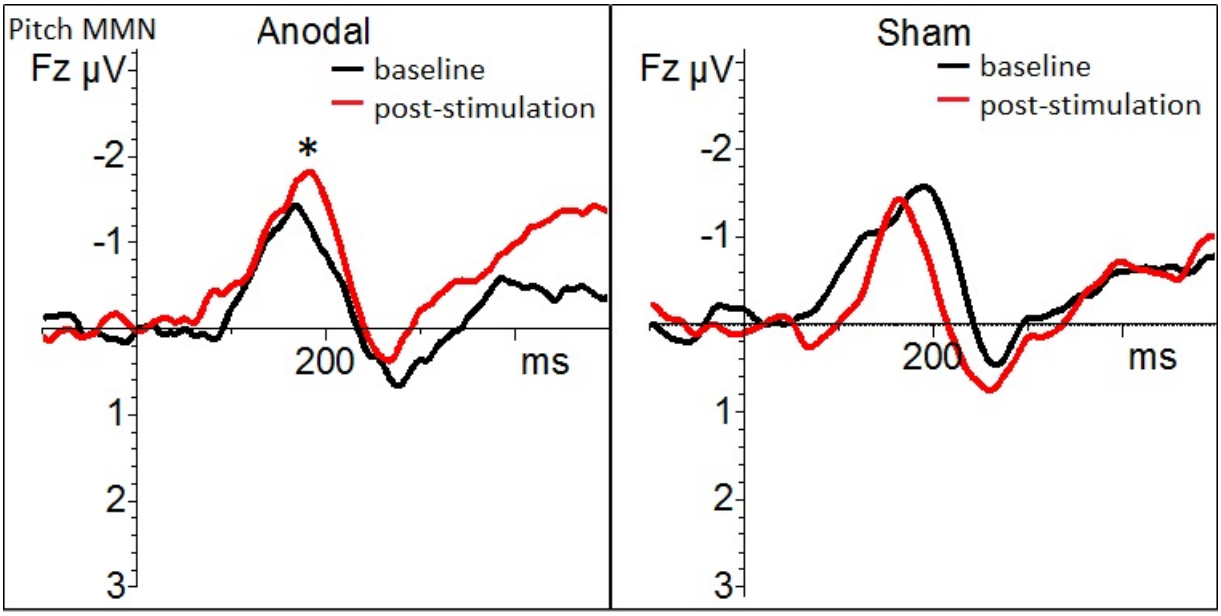

Figure 4.1. Grand averaged difference waveforms for pitch deviants displaying MMN amplitudes at baseline and post-stimulation. There was a significant (*p < .05) increase with anodal tDCS

There was no significant main effect of tDCS treatment (tx), time (pre and post), or drug (DMO and placebo) at frontal Fz. To follow up on study hypothesis, planned pairwise comparisons of tx and time demonstrated that MMN post-stimulation (M = -2.10 μV, SE ± .12) was greater, p = .02, than MMN at baseline (M= -1.54 μV, SE ± .19) for anodal stimulation. These differences were not seen post-'sham' stimulation, as shown in Figure 1. Planned comparisons of tx, time and drug interactions revealed that post-stimulation MMN (M = -2.10 μV, SE ± .14) was significantly greater, p = .03, than pre-stimulus baseline (M = -1.50 μV, SE ± .20) for active tDCS sessions only, during placebo administration, as hypothesized. This effect was not seen with DMO administration, or with either DMO or placebo after sham tDCS. MMN amplitudes for each condition are shown in Figure 2.

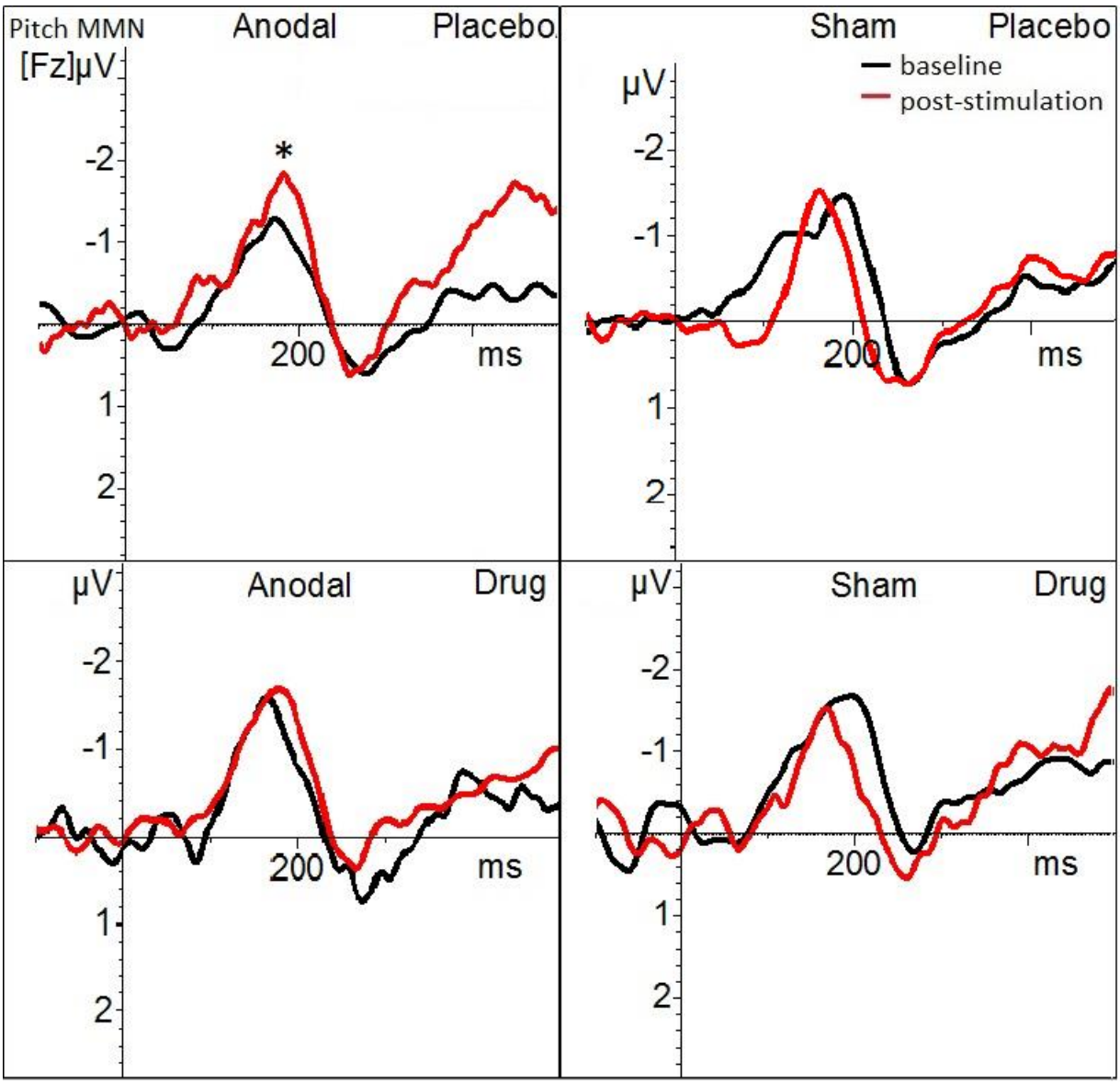

Figure 4.2. Grand averaged difference waveforms for pitch deviants displaying MMN amplitudes at baseline and post-stimulation for placebo and DMO drug administration. There was a significant (*p < .05) increase with anodal tDCS during placebo sessions

With respect to comparisons involving MMN-stratified subgroups, there was a main effect of group, $F(1,10) = 5.80$, $p = .04$, which showed that MMN in the high baseline group (M = -2.20 μV, SE ± .21) was significantly greater than the low baseline group (M = -1.48 μV, SE ± .21). Post-hoc comparisons of group, treatment, drug and time revealed that in high group receiving 'sham' stimulation during the DMO drug session, MMN post-stimulation (M = -1.73

μV, SE ± .33) was reduced, p = .03, compared to MMN at baseline (M = -3.21 μV, SE ± .41). The opposite was seen in low baseline individuals: With anodal tDCS, for placebo administration only, MMN amplitude post-stimulation (M = -2.00 μV, SE ± .27) was greater, p = .02, than baseline assessment (M = -1.09 μV, SE ± .33).

4.5.2. Duration MMN amplitudes

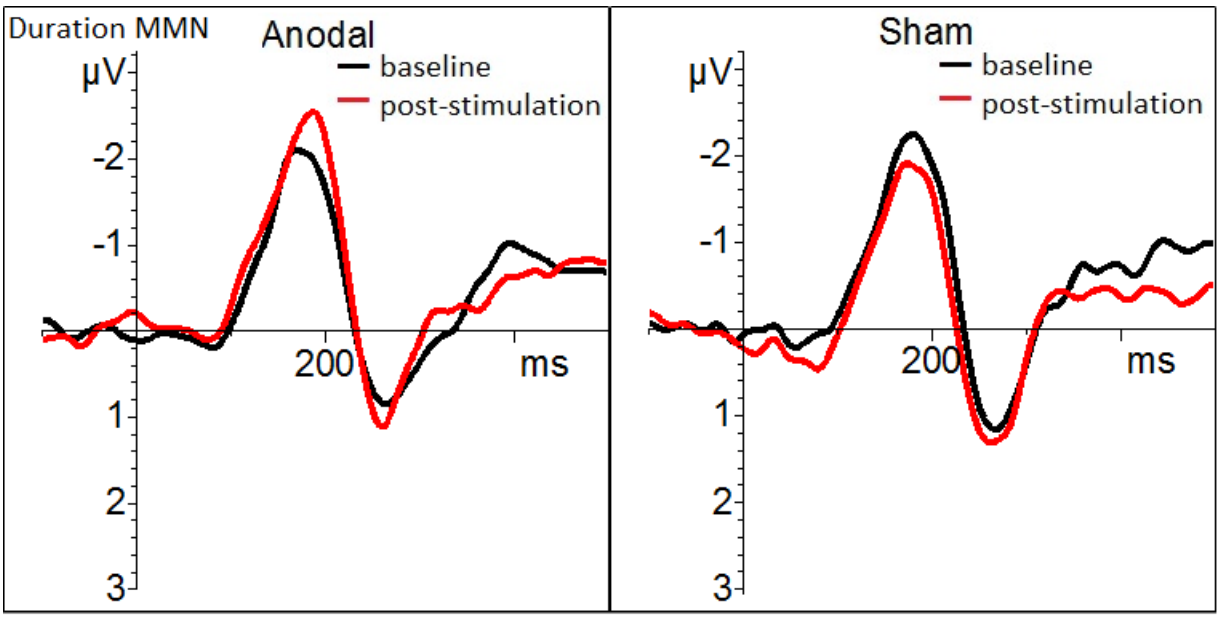

Figure 4.3. Grand averaged difference waveforms at Fz for duration deviants displaying MMN amplitudes at baseline and post-stimulation.

Although there was no main effect of treatment (tx), there was a significant interaction effect involving tx and time, F(1,10) = 9.80, p = .01, and a significant 3-way interaction between drug, time and group, F(1,10) = 4.97, p = .05. Follow-up comparisons of tx and time demonstrated that for anodal tDCS, there was a trend, p = .07, which showed that MMN was increased post-active tDCS (M = -2.88 μV, SE ± .18) vs. pre-tDCS baseline (M = -2.50 μV, SE ± .15), as shown in Figure 3. Planned comparisons of tx, drug and time showed that the enhancing effect of active tDCS was significant, p = .03, during placebo sessions only, as predicted. The treatment and time comparisons also showed that post-'sham' stimulation (M = -2.25 μV, SE ±

.16), MMN amplitudes were reduced, p = .03, compared to pre-sham MMN (M = -2.94 µV, SE ± .22). Planned comparisons of tx, drug and time showed that the significant decrease was only significant during the DMO drug session, p = .04, as hypothesized. MMN amplitude for each condition are shown in Figure 4.

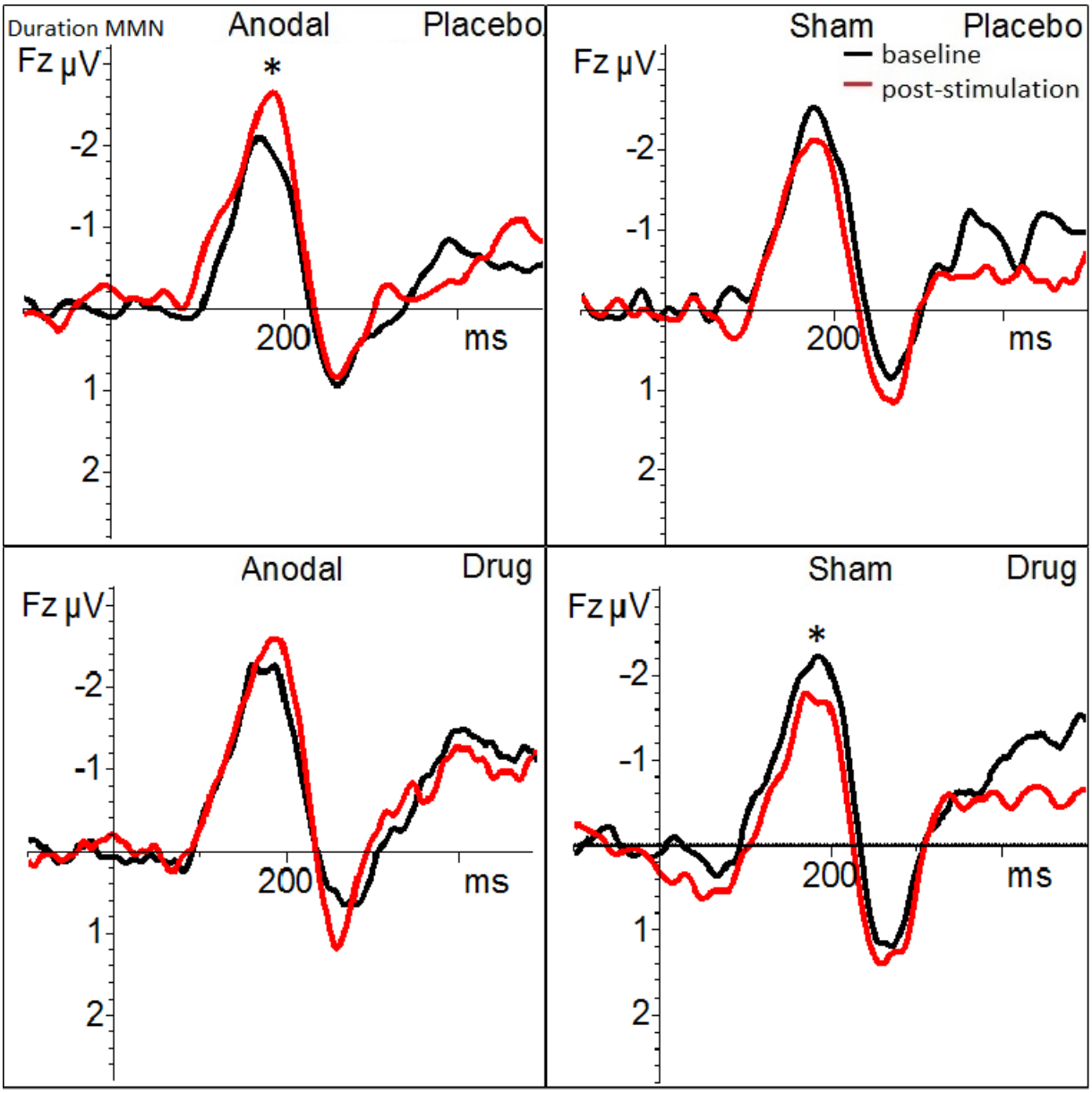

Figure 4.4. Grand averaged difference waveforms for duration deviants displaying MMN amplitudes at baseline and post-stimulation for placebo and DMO administration. There was a significant (*p < .05) increase with anodal tDCS during placebo sessions and a significant decrease with sham stimulation during active drug sessions

In relation to subgroup analyses, there was a significant effect of group, F(1,10) = 33.96, p = .0001, showing greater MMNs in the high group (M = -3.17 μV, SE ± .13) than the low group (M = -2.15 μV, SE ± .13), as expected. Post-hoc comparisons of the group, treatment, drug and time interaction revealed that in the high baseline group, with DMO drug administration only, MMN post-'sham' stimulation, i.e. no stimulation (M = -3.80 μV, SE ± .22) was significantly reduced, *p* = .02, compared to baseline MMN (M = -2.71 μV, SE ± .36). In the low baseline group, post-anodal tDCS (M = -2.86 μV, SE ± .52) demonstrated a trend, *p* = .08, for larger MMN amplitudes, compared to pre-tDCS baseline (M = -2.09 μV, SE ± .13) during placebo sessions only. This enhancing effect was not seen post-sham stimulation, nor during active tDCS with DMO drug administration.

4.5.3. MMN latencies

There was no significant main effects or interactions of drug, treatment or time on pitch or duration MMN latencies.

4.5.4. N1 amplitude and latency

There was no significant effect of time or treatment on N1 amplitudes for the standard stimuli and there were no significant interactions effects. There was a main effect of drug, F(1,10)=12.48, *p* = .01, which showed that placebo produced smaller N1 amplitudes (M= -.32 μV, SE ± .16) compared to DMO (M= -.52 μV, SE ± .16), when collapsed across time and treatment conditions. There were no main effects of drug or treatment, or any interaction effects on N1 latency. There was a significant effect of time, F(1,10)=5.13, *p* = .05, which showed that N1 latencies were slightly shorter at baseline (M= 96.86 ms, SE ± 2.84) compared to post-

stimulation (M= 100.55 ms, SE ± 2.61). Follow-up of the 3-way interaction showed that this effect was only significant, p = .05, for the sham session with dextromethorphan administration.

4.5.5. Working Memory Performance

Working Memory performance was superior for the 1-back task (M= 1.2, SE= .07) compared to 2-back task (M=1.1, SE=.06), p = .03. Follow-up comparisons of the significant drug and treatment interaction, F(1,8)=5.04, p = .05, showed that in the 2-back condition, anodal stimulation increased performance, p < .01, (M=1.16, SE=.10) compared to sham (M=1.03, SE=.13) during DMO administration only. There was also a trend, p = .06, which showed that anodal stimulation also increased performance in the 1-back condition (M=1.22, SE= .14), compared to sham (M=1.13, SE= .13), for the DMO session only. There was no significant effects on reaction time.

4.5.6. DC Checklist

Anodal stimulation produced slightly more symptoms (M = 2.2, SE = .14) compared to sham stimulation (M = 1.8, SE = .14). However, on average, both stimulations were rated as 2 - Mild symptoms (i.e. slight tingling). Nearly all participants guessed that they had received active stimulation (vs. sham), during both active and sham sessions. The most frequently reported symptom was a tingling/itching sensation during both sessions.

4.5.7. Drug symptom results

The active drug produced more reported symptoms (M = 2.3, SE = .22) compared to placebo (M = 1.3, SE = .17), p = .003. On average, dextromethorphan symptoms were rated as 2 - Mild symptoms (i.e. light-headed), whereas placebo symptoms were rated as 1 - No symptoms. Some participants were able to correctly guess (66% accuracy) whether they had placebo

administration, compared to active drug administration. The most common drug-related symptoms were drowsiness/light-headedness, dizziness and nausea, however drowsiness and dizziness symptoms were also reported with placebo administration.

4.6. Discussion

This study is the first to investigate the ability of an NMDA antagonist to modulate MMN-indexed auditory discrimination after anodal tDCS of the auditory cortex, in a placebo and sham-controlled, double-blind, randomized, repeated measures design. For both pitch and duration MMN, 20 minutes of anodal tDCS (2 mA) increased MMN amplitudes at frontal regions, compared to baseline (pre-stimulation) assessment, during the placebo condition. These increases were not seen with 'sham' stimulation, i.e. no stimulation, confirming that active anodal tDCS directed at the auditory cortex enhanced MMN-indexed auditory discrimination. Specifically, these increases were seen in individuals with relatively low MMN amplitudes at baseline. MMN amplitude increases post-stimulation (compared to pre-stimulation assessment) were not observed for active dextromethorphan (DMO) drug sessions, which suggests that the administration of the NMDA antagonist prevented the enhancing after-effects of anodal tDCS that were seen with the placebo condition, as predicted. Further, MMN amplitudes at frontal regions were reduced after DMO drug administration with the 'sham' stimulation condition. These reductions were not seen with the placebo, which suggests that NMDA antagonism significantly reduced MMN amplitudes in the sham condition, as hypothesized, specifically in the high baseline MMN amplitude group. Thus, anodal stimulation increased MMN, an effect which was moderated by DMO treatment, and independent of stimulation, DMO significantly reduced MMN. Working memory (WM) performance was also improved after anodal tDCS, but not with the control 'sham' tDCS condition, which confirms reports of WM improvements with

tDCS. Together, the results of this study validates our hypothesis that anodal stimulation of the auditory cortex can enhance associated sensory and memory processing, and that these effects are dependant in part on glutamatergic neurotransmission.

The enhancement of frontal MMN amplitudes with anodal tDCS over auditory regions observed in this study replicates the significant results found in our previous pilot studies examining the effects of anodal tDCS (Impey and Knott 2015; Impey, De La Salle and Knott, 2016). Both frontal pitch MMN and duration MMN, which appear to have different neural sources (Giard et al 1990; Paavilainen et al., 1991; Schairer et al. 2001), were significantly moderated by anodal tDCS. Anodal stimulation improved the MMN component at frontal regions, where the MMN amplitude is maximal. MMN is caused by two separated yet connected generators in the temporal and frontal cortex, the latter of which is involved in a cognitive, comparator-based mechanism and the attention switching response to relevant stimuli (Giard et al 1990; Garrido 2009; Näätänen 2007). Current results suggest that anodal tDCS improved the frontal comparator-based response. Moreover, significant MMN response to tDCS was separate from participants' N1 ERP response, suggesting that tDCS does not alter sensory registration, as indexed by N1, but rather the formation of memory traces or their comparison in sensory memory (Näätänen 1995; Näätänen et al. 2005; 2007; 2011). This study also replicated the negative findings with sham stimulation, which shows strong support that MMN enhancements seen with anodal tDCS can be attributed to the 20 minutes of 2 mA of direct current, which has been found to increase cortical excitability (Nitsche et al. 2003a). Sham stimulation included the exact set-up as active stimulation, including the first 30 seconds of the current ramping up, but the current was turned off for the rest of the 20 minutes, in a double-blind design. Self-reports of tDCS effects showed that adverse events were relatively minor and that participants could not

accurately differentiate active and sham stimulations, confirming that tDCS is an efficacious treatment technique with a reliable control condition.

The enhancing effect of anodal tDCS was only observed during the placebo condition. Dextromethorphan, an NMDA antagonist, blocked the enhancing effect of anodal tDCS as no significant tDCS-related increases were seen during the active drug condition. These results are similar to other studies which have investigated the effect of NMDA antagonist on motor evoked potential changes after active tDCS over motor regions (Nitsche, Fricke et al. 2003). The current findings are the first to be investigated with the auditory cortex. These results suggest that NMDA receptor function is critical to cortical excitability changes induced by active tDCS that last beyond the stimulation administration. Specifically, the current study demonstrated that tDCS-MMN changes were moderated by NMDA receptor activity. Results also showed that MMN alterations were independently moderated by the NMDA antagonist as MMN amplitudes were significantly reduced after sham stimulation (i.e. no current) with dextromethorphan administration. These MMN amplitude decreases were not seen with placebo**.** These significant MMN reductions present with NMDA antagonist treatment replicate findings of other NMDA-MMN investigations, such as the use of ketamine protocols (Rosburg and Kreitschmann-Andermahr 2015; Umbricht et al. 2002) and data from animal work (Javitt et al. 1996; Tikhonravov et al. 2008), which have shown significant modulation of MMN with glutamatergic agents. A magnetic resonance spectroscopy (MRS) study investigating the neuronal mechanisms of how tDCS influences learning found significantly higher combined glutamate and glutamine levels beneath the stimulating electrode, suggesting that glutamatergic activity is related to the mechanisms of action for tDCS (Clark, Coffman, Trumbo & Gasparovic, 2011). Together, these

results demonstrate that both anodal tDCS effects and tDCS-MMN amplitude generation is significantly moderated by glutamatergic neurotransmission.

The results of this study also confirmed the utility of anodal tDCS for improving working memory performance on the 1-back and 2-back memory tasks seen in other studies when tDCS was administered over the prefrontal cortex (Fregni et al. 2005; Hill et al. 2015; Keeser et al. 2011; Teo et al. 2011). The tDCS-memory performance improvements in this study were only seen in the active drug condition, when sensory discrimination was attenuated due to NMDA antagonist effects. As working memory accuracy in the current study was very high, it is possible ceiling effects prevented anodal stimulation from further increasing performance during placebo administration. The drug-related reduction in sensory processing during active DMO sessions may have allowed for the enhancing effects of anodal tDCS on memory performance. This finding agrees with decades of research which have found that the NMDA receptor system has been implicated in many cognitive processes, including working memory (Rezvani, 2006). Specifically, NMDA antagonists, such as ketamine, impair WM performance (Rezvani, 2006). These findings may have treatment implications for populations with memory impairments due to a dysfunctional glutamate system, such as individuals with schizophrenia (Javitt 2010). In fact, MMN-indexed sensory processing has been found to reflect dysfunctional working memory in schizophrenia (Javitt et al., 1995) and recent investigations have found some benefits of tDCS on cognitive function in these patients (Brunoni and Vanderhasselt, 2014).

The current study had a number of limitations that warrant mention. The primary limitation of this study was the relatively small sample size of participants. However, the vast majority of tDCS studies are conducted with a sample of 12 (Chen et al. 2014; Heimrath et al., 2015; Vines et al. 2006; Zaehle et al. 2011) and our pilot study results showed significant effects

of anodal tDCS, even with the possibility of low statistical power. Secondly, as the present study only included auditory cortex stimulation, and did not include current modeling or imaging to confirm current densities, the inability to localize specific cortical sources in this study remains a limitation. However, current modelling studies have shown that the low constant current, flowing from the active to the reference electrode, does have peak current density over the targeted region (Neuling et al. 2012) and that by adjusting the current density (with both the current strength and electrode size), we can optimize the efficacy of tDCS to reach targeted brain regions (Nitsche et al. 2008). Recommended tDCS parameters were used in this study and our significant results do indicate a robust modulation of both MMN amplitudes and working memory performance. However, a current of 2 mA was used in this study, similar to other protocols (Nitsche et al. 2003a; 2008), while other working memory investigations have found improvements with 1 mA, which may be more optimal (Hoy et al. 2013). Although beyond the scope of this study, the use of network analyses (i.e., dynamic causal modelling - DCM) to study the effects of NMDAR manipulation on the plasticity induced by oddball stimuli and related WM paradigms may be of interest in future studies. These studies measure changes in neuronal effective connectivity underlying MMN and may have implications for disorders with NMDA receptor hypofunction (Garrido et al. 2008; Moran et al. 2011; Ranlund et al. 2016; Wacongne 2016).

A last limitation of our study to discuss is the use of a single dose of dextromethorphan hydrobromide (50 mL), delivered in a no-sugar cranberry syrup to mask the taste, odour and texture. This dose was chosen based on previous studies which have found significant tDCS effect's on motor-evoked response, which used an equivalent dose of DMO (Liebetanz et al. 2002; Nitsche, Fricke et al. 2003). The 50 mL dose was sufficient to produce significant

attenuations of tDCS effects on MMN amplitudes at peak drug absorption; however, there were some reported drug-related adverse events. Sedation effects and vertigo were reported with both the active drug and with placebo, but symptoms were more significant for DMO than placebo administration and these could have affected current results. However, the primary objective of this study was to assess the effects of an NMDA antagonist at doses which have shown significant effects on tDCS-related modulations in motor regions. As this has now been demonstrated with auditory processing, future studies could consider controlling for sedation effects by comparing DMO effects with a comparator drug such as an anxiolytic agent, for example. Importantly, MMN amplitude, our primary measure, is relatively immune to state, and is still intact in drowsy, sleeping and certain comatose individuals (Morlet and Fischer 2014). Future studies could consider assessing the effect of different doses, or different NMDA antagonist and agonists on tDCS-sensory discrimination effects. It is also suggested to investigate other neurotransmitters on tDCS-auditory processing as catecholamines, serotonin, acetylcholine and GABA, among other neurotransmitters, have been implicated in tDCS modulatory effects.

4.6.1. Conclusions

tDCS is a brain stimulation technique which has been found to increase sensory and memory function and has been proposed as a possible cognitive treatment for some neurological and psychiatric disorders. Sensory processing deficits as reflected in impaired MMN ERPs are strongly linked to functional and cognitive performance in healthy participants and patients with auditory processing dysfunction, such as in schizophrenia disorder. MMN has been established as a reliable biomarker for the detection of pro-cognitive treatment effects in pre-clinical trials (Butler et al. 2012). Given that MMN dysfunction is mediated in part via aberrant NMDA

receptor signaling, and that tDCS 'after effects' are thought to be NMDA-dependent, this study demonstrated the first evidence of NMDA modulation of tDCS' effects on MMN-indexed auditory discrimination and also confirmed associated higher-order working memory performance enhancement with anodal tDCS over the auditory cortex. These findings also underscore the importance of the use of neurophysiological markers based on baseline response for the preclinical assessment of tDCS treatment. This study helps contribute to our knowledge on the pharmacological basis of tDCS effects on auditory sensory processing and WM and these findings have implication for disorders associated with sensory deficits involving NMDA dysfunction.

4.7. Acknowledgements and Declarations

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Declaration of Conflicting Interests: The authors declare that there is no conflict of interest.

Chapter 5: Effects of Transcranial Direct Current Stimulation on the Auditory Mismatch Negativity Response and Working Memory Performance in Schizophrenia: A Pilot Study (Study 4)

5.1. Title Page

Effects of Transcranial Direct Current Stimulation on the Auditory Mismatch Negativity Response and Working Memory Performance in Schizophrenia: A Pilot Study

Authors:
Danielle Impey[1,2] (danielle.impey@theroyal.ca), Ashley Baddeley [1] (Ashley.Baddeley@theroyal.ca), Alain Labelle (alain.labelle@theroyal.ca)[3] and Verner Knott[1,2] (verner.knott@theroyal.ca)

1 Clinical Neuroelectrophysiology & Cognitive Research Laboratory, University of Ottawa Institute of Mental Health Research, Ottawa, Canada
2 School of Psychology, University of Ottawa, Canada
3 Schizophrenia Program, The Royal, Ottawa, Canada

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Psychophysiology and revisions have been requested which are included in the present thesis.

5.2. Abstract

Cognitive impairment has been proposed to be the core feature of schizophrenia (Sz). Transcranial direct current stimulation (tDCS) is a non-invasive form of brain stimulation which can improve cognitive function in healthy participants and in psychiatric patients with cognitive deficits. tDCS has been shown to improve cognition and hallucination symptoms in schizophrenia (Sz), a disorder also associated with marked sensory processing deficits. Recent findings in healthy controls demonstrate that anodal tDCS increases auditory deviance detection, as measured by the brain-based event-related potential (ERP), mismatch negativity (MMN), which is a putative biomarker of Sz that has been proposed as a target for pre-clinical treatment of Sz cognition. This pilot study conducted a randomized, double-blind assessment of the effects of pre- and post-tDCS on MMN-indexed auditory discrimination in 12 Sz patients, moderated by auditory hallucination (AH) presence, as well as working memory performance. Assessments were conducted in three sessions involving temporal and frontal lobe anodal stimulation (to transiently excite local brain activity), and one control session involving 'sham' stimulation (meaning with the device turned off, i.e. no stimulation). Results demonstrated a trend for pitch MMN amplitude to increase with anodal temporal tDCS, which was significant in a subgroup of Sz individuals with AHs. Anodal frontal tDCS significantly increased WM performance on the 2-back task, which was found to positively correlate with MMN-tDCS effects. The findings contribute to our understanding of tDCS effects for sensory processing deficits and working memory performance in Sz and may have implications for psychiatric disorders with sensory deficits.

5.3. Introduction

Cognitive impairment, characterized by deficits in perception, attention, memory and executive functions, has been proposed to be the core feature of schizophrenia (Sz), with deficits correlating with negative symptoms and functional outcome, as well as being a treatment target (Elvevag & Goldberg, 2000; Green, 1996; 2000). Antipsychotic medications are relatively ineffective for these impairments and there is no approved treatment for cognitive symptoms in Sz (Gray & Roth, 2007). Non-invasive brain stimulation techniques provide means to alter neuronal activity in specific brain regions and at a network level (Pascual-Leone et al., 2005) and are promising cognitive enhancing techniques being investigated in a variety of neuropsychiatric disorders, including Sz (Demirtas-Tatlidede et al., 2013). Transcranial direct current stimulation (tDCS) is a non-invasive procedure of cortical stimulation, in which weak direct currents are used to induce changes in excitability of targeted brain regions. Depending on the polarity of the stimulation, tDCS can result in reversible increases (anodal tDCS) or decreases (cathodal tDCS) in cortical excitability in circumscribed stimulated brain area underneath the active electrode (Gandiga et al., 2006; Nitsche et al., 2003a; 2008). tDCS has been most frequently investigated with respect to its effects on the motor and visual cortex in healthy volunteers and in neuropsychiatric patients (Fregni et al., 2006, Ferruci et al, 2008; Hill, Fitzgerald & Hoy, 2015; Jo et al., 2009; Nitsche et al., 2003a). Recently, tDCS has increasingly been used to improve cognitive function and it is currently being investigated as a concurrent treatment to alleviate positive symptoms (e.g. auditory hallucinations) of schizophrenia (Brunoni et al., 2014; Brunelin et al., 2012; Mondino et al., 2015a; Vercammen et al., 2011).

The tDCS technique has increasingly been used to modulate sensory-perceptual processing (Costa et al., 2015) and cognitive function (Brunoni and Vanderhasselt, 2014).

Several reports have found that with anodal tDCS stimulation of 1-2 mA, delivered through a constant current regulator with electrode pads placed over scalp areas overlapping the dorsolateral prefrontal cortex (DLPFC) for 15-30 minutes, there is improved performance on several working memory and word recognition memory tasks in healthy participants (Boggio et al., 2008; Fregni et al., 2005), Alzheimer's patients (Boggio et al., 2006; Ferrucci et al., 2008) and patients with depression (Fregni et al., 2006; Kuo et al., 2014). Cathodal tDCS stimulation with the same parameters has also been found to transiently decrease memory performance in healthy controls and Alzheimer's patients (Ferruci et al., 2008). In individuals with Sz, anodal tDCS has been administered over the prefrontal cortex to successfully increase excitability and probabilistic association learning performance in a subset of patients based on baseline performance (Vercammen et al., 2011) and Hoy and colleagues (2014) found the same tDCS effects on a working memory task after 2 mA stimulation. A recent review and meta-analysis found cognitive improvements with tDCS and suggested the use of non-invasive brain stimulation techniques for WM deficits in psychiatric patients, including Sz (Brunoni and Vanderhasselt, 2014). The majority of these studies are randomized, double-blind, sham-controlled studies which demonstrates the effectiveness of a very short treatment of tDCS for cognitive improvement or modulation.

Recent pharmacological studies suggest that the immediate short-lasting effects of tDCS are generated by polarity-specific shifts of the cell's resting membrane potential, with anodal stimulation causing a depolarization of the resting potential and an increase in spontaneous firing rates while cathodal stimulation causes a hyperpolarization of the resting-membrane potential and a decrease in spontaneous firing rates (Nitsche et al., 2008). However, the polarity-specific changes may be an over simplistic explanation of tDCS mechanisms (see Nitsche et al., 2015).

tDCS 'after-effects', a form of induced brain plasticity, depend on membrane potential changes as well as modulations of N-methyl-D-aspartate (NMDA) receptor function, among other neurotransmitter systems: NMDA antagonists act to prevent functional after-effects and NMDA agonists prolong after-effects by several hours (Liebetanz et al., 2004; Nitsche et al., 2004; 2008). Treatment interventions targeting the glutamate system and NMDA receptor function in particular have been proposed for Sz (Javitt, 2004) as NMDA receptor hypofunction is considered to be one of the main underlying causes of cognitive symptoms in Sz (Javitt, 1996; 2007; 2010).

Brain based event-related potentials (ERPs) can be used as an objective measure of sensory and cognitive processing. They are a robust, powerful and non-invasive index of neural function and ERP components are frequently used to investigate early pre-attentive and attention-dependent auditory processes in healthy controls and clinical populations (e.g. P50 index of sensory gating; mismatch negativity [MMN] index of change detection, P3a index of novelty detection; P3b index of attentional allocation and processing speed; for review see Braff & Light, 2004). The auditory MMN ERP component, generated primarily in the auditory cortex, with contributions from the prefrontal cortex (Alho, 1995; Opitz et al., 2002), is an index of pre-attentive processing and acoustic change detection (i.e. sensory discrimination; Näätänen et al., 2005; 2009; 2012). It is often elicited in a passive 'oddball' paradigm, wherein presentations of a 'standard', repetitive stimulus are intermixed with rare presentations of 'deviant' stimuli. In healthy individuals, it is hypothesized that a brief 'echoic' memory trace is formed automatically from the repetitive (standard) stimuli and the MMN is elicited automatically when an acoustic feature is detected in sensory memory which deviates from the standard memory trace (Näätänen et al., 2005). Both human and animal models have shown NMDA receptor function to have a

crucial role in memory and have been found to play a key role in auditory sensory discrimination and MMN generation (Gil-da-Costa et al. 2013; Heekeren et al. 2008; Javitt et al. 1996; Umbricht et al. 2002). Specifically, the NMDA antagonist ketamine attenuates MMN amplitude in human participants and has been used to model impaired cognition in healthy controls (Rosburg and Kreitschmann-Andermahr 2015; Umbricht et al. 2002).

As the MMN is automatically obtained without needing any behavioural response from patients, data collection is quick and non-invasive and thus relatively easy to obtain in psychiatric patient populations. Deficits in MMN generation to pitch and duration deviants are a robust feature in chronic Sz (Näätänen et al., 2015a; 2015b; Näätänen and Kahkonen, 2009; Javitt et al., 1995; Umbricht and Krljes, 2005), which are thought to result from hypofunctional NMDA receptor activity in patients (Javitt et al., 1996). As such, MMN amplitudes have been proposed as a biomarker of glutamatergic dysfunction in Sz and a target for novel treatment interventions (Butler et al., 2012; Javitt et al., 2008; Light & Näätänen, 2013; Light & Swerdlow, 2014; Light et al., 2012; Näätänen et al., 2015a; 2015b; Turetsky et al., 2015). MMN deficits in this population have been significantly correlated with negative symptoms (Javitt et al., 2000), positive symptoms (Fisher et al., 2011), duration of illness (Umbricht and Krjles, 2005) and functional outcome status in Sz (Kawokubo et al., 2007). Hallucination severity has also been found to correlate with MMN amplitudes (Fisher et al., 2011; 2012) and there is a significant difference between MMN in patients who experience auditory hallucinations (AHs) and those who do not (Fisher, Labelle & Knott, 2008). These MMN differences based on hallucination experience will be explored in the current study as several interindividual differences at baseline have been found to have a significant impact on tDCS effects (Li et al., 2015). AHs are a core symptom of Sz and may be associated with abnormal hyperactivity in the left temporo-parietal

junction and abnormal connectivity between frontal and temporal areas (Hoffman & Hampson, 2011; Sommer et al., 2012). Recent studies have reported that fronto-temporal tDCS may be used a treatment for the reduction of auditory hallucinations (Andrade, 2013; Brunelin et al., 2012; Ferrucci et al., 2014; Mondino et al., 2015a; 2015b; Nawani et al., 2014). Active tDCS (20 minutes, 2 mA) administered to patients over 5 consecutive days, with the cathode over tempo-parietal areas and the anode over left frontal cortex, was shown to reduce hallucination severity and other clinical symptoms as measured by the Positive and Negative Syndrome Scale (PANSS; Brunelin et al., 2012).

5.3.1. Objectives and Hypotheses

Reduced MMN amplitude is a putative biomarker of NMDA receptor hypofunction in Sz and diminished sensory processing is thought to contribute to cognitive impairment in the disorder (Javitt et al., 1996; Näätänen et al., 2015a; 2015b). As recent pharmacological studies have shown that tDCS-induced effects may be NMDA receptor dependant (Liebetanz et al., 2004; Nitsche et al., 2004; 2008) and as NMDA receptors have been a proposed target for Sz cognition, this study will assess the use of tDCS applied over the auditory cortex to improve MMN-indexed auditory change detection in Sz. Pilot results investigating tDCS-MMN effects have shown that anodal tDCS over the temporal cortex increases MMN amplitudes and cathodal stimulation decreases MMN, in a stratified subset of healthy participants (Impey & Knott, 2015; Impey et al. 2016). Heimrath and colleagues (2015) also found that MMN was significantly enhanced by anodal tDCS over the left auditory cortex. Contrary to research showing enhancement with excitatory anodal tDCS, two studies which applied anodal tDCS over the prefrontal cortex found that MMN to specific deviants was significantly decreased (Chen et al., 2014; Weigl et al., 2016). These results could be due to the positioning of stimulating electrodes

on scalp areas remote from the auditory cortex. Knechtel et al. (2014) also investigated frontal tDCS-MMN effects in individuals with Sz but failed to find any significant changes. In the current study, anodal tDCS treatment (2 mA, 20 minutes) will be compared to pre-stimulus baseline response (vs. 'sham' stimulation [i.e. no current]) in a repeated measures, sham-controlled, design. It is expected that anodal tDCS over the left temporal cortex will improve MMN-indexed sensory discrimination, compared to pre-tDCS baseline and 'sham' stimulation.

As previous tDCS studies with Sz patients have found significant cognitive effects, including working memory (WM) benefits, with stimulation of the prefrontal cortex (Brunelin et al., 2012; Vercammen et al., 2011), a secondary objective of this study will include a frontal cortex stimulation session to examine the localization of tDCS effects for WM performance. It is hypothesized that tDCS over the left frontal cortex will enhance working memory accuracy and reaction time in patients. We also investigated the mediating relationship between the presence of auditory hallucinations and tDCS-MMN effects. We expect that Sz participants with AHs to have reduced MMNs and therefore, based on previous studies which showed that low MMN responders at baseline may be pre-disposed to respond to tDCS (Impey & Knott, 2015; Impey et al. 2016), we hypothesize that MMN in patients with AHs will increase with anodal tDCS. Finally, we explored the relationship between MMN and WM measures, and as MMN has been found to reflect dysfunction in WM in Sz (Javitt et al., 1995), we expect to find a positive correlation between measures.

5.3. Methods

5.4.1. Participants

Twelve (12) participants (10 male) between the ages of 18-60 (47.5 years ± 12.37) were recruited from the Outpatient Schizophrenia Program of the Royal Ottawa Mental Health Centre (ROMHC). For inclusion into the study, participants were required to have a primary diagnosis of Sz according to DSM-IV criteria (APA, 2013) and were required to be clinically stable for at least the 3 month period prior to testing (as indicated by no significant change in symptoms or medications by their primary physician). Participants were excluded if they met any of the following criteria: co-morbid psychiatric disorder, current history of drug/alcohol dependence, history of head injury resulting in loss of consciousness, diagnosis of epilepsy or any other neurologic disorder, metallic implants or any electrical device (e.g., pacemaker) in the body, or abnormal audiometric assessment (i.e., thresholds for pure tones >25 dB [SPL]). Participants continued their prescribed atypical anti-psychotic medication, including 7 participants on Clozapine and the others on a combination (i.e. Clozapine, Ziprasidone; Quetiapine, Paliperidone; Clozapine, Risperidone; Quetiapine; Clozapine, Quetiapine). Participants were required to remain on their current dose and type of medication throughout the study. Additionally, 8 out of the 12 participants had a current history of auditory hallucinations (AHs), confirmed by both clinical history and the Positive and Negative Syndrome Scale (PANSS) for Sz (Kay et al., 1989), using a cut-off score of ≥ 3 (mild or greater hallucinatory experiences), and the AH subscale of the Psychotic Symptom Rating Scale (PSYRATS; Haddock et al., 1997). Participants were compensated $70 (CAD) for their time and effort. Written consent was obtained by participants prior to commencement of the study. The study was approved by the Royal Ottawa Health Care Group Research Ethics Board and has been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki.

5.4.2. Design

Participants were assessed in a randomized, repeated-measures, double-blind design requiring them to attend three test sessions (~ 2-5 days apart), one involving anodal tDCS administration over the left temporal cortex, one involving anodal tDCS administration over the left frontal cortex and one involving 'sham' tDCS administration (i.e. no current). Session order was randomized as to ensure there were no session order effects.

5.4.3. Procedures

Test sessions occurred between either 9-11 a.m. or 1-3 p.m consistently for each participant. Participants arrived at the lab following at least 2 hour abstinence from food, caffeine and any drugs (excluding their anti-psychotic medication). EEG electrode placement was followed in a fixed order: MMN assessment at baseline, tDCS stimulation for 20 minutes (either temporal or frontal anodal stimulation [2 mA] or sham stimulation [no current] depending on the testing session randomization), and MMN assessment immediately post-stimulation. Participants completed the 1- and 2-back working memory task directly after post-stimulation MMN assessment in each session. PSYRATS hallucination ratings and adverse events ratings, consisting of a Likert-scale which required participants to rate the severity of tDCS symptoms from 1 (No symptoms) to 5 (Severe symptoms), were also taken at the end of each session.

5.4.4. tDCS

Conductive saline-soaked rubber electrodes super-imposed on sponge plates were placed on the scalp overlying the left auditory cortex or left frontal cortex (anodal electrode**,** 4.4 x 4.4 cm) and on the contralateral forehead (reference/cathodal electrode, 5.1 x 10.2 cm) above the orbit as described previously (Brunelin et al., 2012; Impey & Knott, 2015; Nitsche et al., 2005;

Vercammen et al., 2011). Specifically, for the temporal and 'sham' stimulation, the anodal electrode was positioned between C5 and T7 sites (approximately parallel to the left Sylvan fissure) of the 10-10 international system for EEG electrode placement, scalp sites that closely overlap Brodmann Areas 41 and 42 of the primary and secondary auditory cortex, respectively. For frontal stimulation, the anodal electrode was positioned over the left DLPFC at electrode site F3 of the 10-10 international system, as described previously in clinical tDCS studies (Brunelin et al., 2012; Vercammen et al., 2011). The reference electrode was placed on the contralateral forehead. Stimulation was applied using a battery-driven constant-current regulator (Oasis Pro, Edmonton). In both active and sham tDCS sessions, the DC current was initially increased in a ramp-like fashion over 10 s until reaching 2 mA and was similarly decreased at the end of stimulation (Nitsche et al., 2003a). During stimulation, participants were required to sit back and relax in a semi-reclined position. In active tDCS, stimulation was maintained for a total of 20 min; in sham, it was turned off after 30 s. Another experimenter turned off the device for sham sessions, maintaining the blind. These sham parameters were chosen based on previous reports that perceived sensations on the skin, such as tingling (during 10 seconds fade in/out of the 30 s sham tDCS) produce no after-effects and re-create the same sensations experienced with active stimulation, thus serving as an ideal control condition (Nitsche et al., 2003a; Gandiga et al., 2005). tDCS has been shown to be applied repeatedly (i.e., daily), for up to 30 minutes without any adverse reactions (Iyer et al., 2005; Nitsche et al., 2005).

5.4.5. MMN Paradigm

Participants were seated in a recording chamber and were instructed to ignore the sounds and watch a silent movie with subtitles. Four blocks of 600 auditory stimuli (70db [SPL]) per block were presented to the right ear, through headphones, in an oddball sequence involving

frequent (P = 0.85) presentations (stimulus onset asynchrony = 300 ms) of a standard stimulus (1000 Hz, duration = 50 ms) that was randomly inter-mixed with two rare (P = 0.075 each) deviant stimuli varying in pitch (1050 Hz, 50 ms, P = 0.075) or duration (1000 Hz, 100 ms, P = 0.075).

5.4.6. ERP Acquisition and Processing

ERPs were derived with 32 scalp electrode positions based on the 10-20 EEG system, referenced to a nose site and with additional electrodes placed around orbital regions to monitor vertical (VEOG) and horizontal electro-oculographic activity (HEOG). BrainVision Recorder software (Brain Products, GmbH) was used to collect data. Amplifier bandpass filter settings was 0.1 – 100 Hz and digital sampling was 500 Hz. Electrode impedance was kept below 5 kΩ. EEG was analyzed off-line with BrainVision Analyzer software (Brain Products, GmbH). Electrical activity was filtered using 0.1-30 Hz filter settings, ocular corrected with the Gratton and Coles algorithm (Gratton et al. 1983), segmented (600 ms epochs) and baseline corrected (-100 ms relative to the pre-stimulus segment), and only resulting epochs with voltages below 100 μV were used for final ERP averaging. Separate averages were created for standard and deviant stimuli. MMN peak amplitude was measured from digital 'subtraction waveforms', i.e., deviant minus standard, and was selected as the most negative peak between 120-250 ms at the frontal Fz site, the region typically exhibiting the maximum MMN amplitude.

5.4.7. Working Memory Task

To assess working memory performance, the 1- and 2-back tasks were administered from the computerized CogState Research Memory Battery (CogState Limited 2009). Participants were required to click a mouse to determine if they have recognized the same playing card which appeared either 1 card back (1-back task), or 2 cards back (2-back task). There were 32 trials for

each task with a maximum stimuli duration of 3500 ms and an interstimulus interval (ISI) that varied between 1500 ms and 2500 ms. Feedback for correct and incorrect responses was indicated to the participant with respective sounds presented through speakers in the testing chamber. Results were analyzed by assessing the accuracy of performance, using the arcsine transformation of the square root of the proportion of correction responses (higher score = better performance) and speed of processing, using reaction time (RT) for accurate responses.

5.4.8. Statistics

tDCS effects on duration and pitch MMN amplitudes and latencies were analyzed in separate repeated measures analysis of variance (ANOVA) containing a treatment factor (3 levels: active temporal, active frontal and sham stimulation) and time factor (2 levels: pre and post tDCS). As previous work has shown that MMN-tDCS effects may be dependent on such factors as baseline function and the presence of auditory hallucinations (Fisher et al., 2008; 2012; 2013; Impey and Knott, 2015; Li et al., 2015), planned comparisons were also investigated regarding hallucination groups (AH present = 8 vs. AH not present = 4), based on clinical history, PANSS score and presence of AHs as determined on the PSYRATS subscale. Separate ANOVAs were completed for WM accuracy and speed of processing on the N-back tasks containing a similar treatment factor (3 levels) and task factor (2 levels) and for tDCS-related adverse events. For both MMN and WM analysis, significant effects ($p < .05$) were followed up with Bonferroni-adjusted planned pairwise comparisons to follow up on study hypothesis. Pearson r correlations were also used for MMN amplitudes and tDCS-WM effects to investigate relationships between tDCS-induced MMN and WM changes.

5.4. Results

5.4.1. Pitch MMN Response

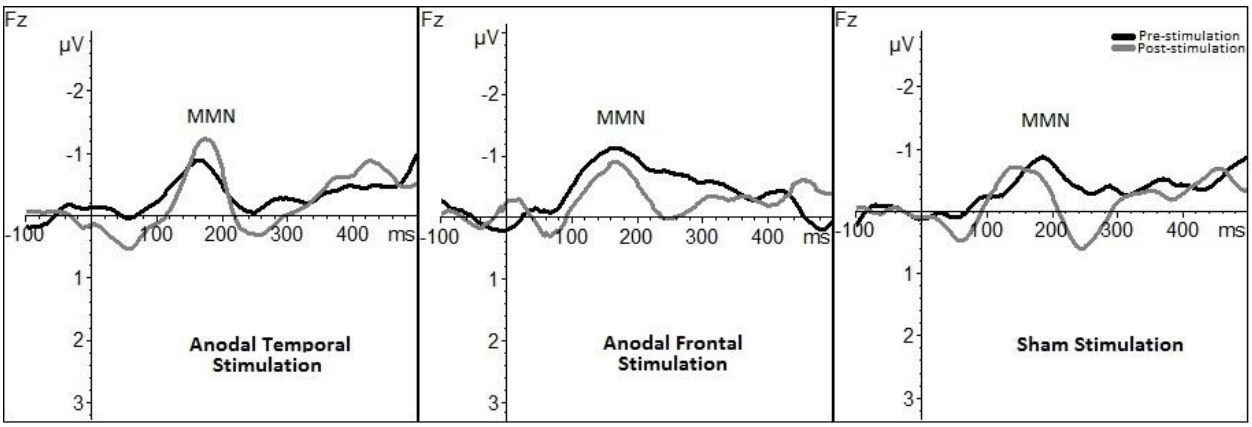

Figure 5.1. Grand averaged pitch MMN amplitudes (n = 12) in response to anodal temporal, frontal and sham tDCS.

Grand averaged pitch MMN amplitudes in response to tDCS are shown in Figure 1. There was no main effect of treatment or time on MMN amplitude. To verify study hypothesis, planned follow-up of the treatment and time interaction, $F(1,15)=3.20$, $p = .08$, revealed that a non-significant trend, $p = .06$, for MMN amplitude post-stimulation over the temporal cortex to be larger (M= -1.98 μV, SE± .340), compared to baseline MMN (M= -1.27 μV, SE± .34). There was no significant change with stimulation of the frontal cortex, or with 'sham' stimulation.

There was no significant effect of treatment or treatment and time interaction for MMN latency.

5.5.2. Duration MMN Response

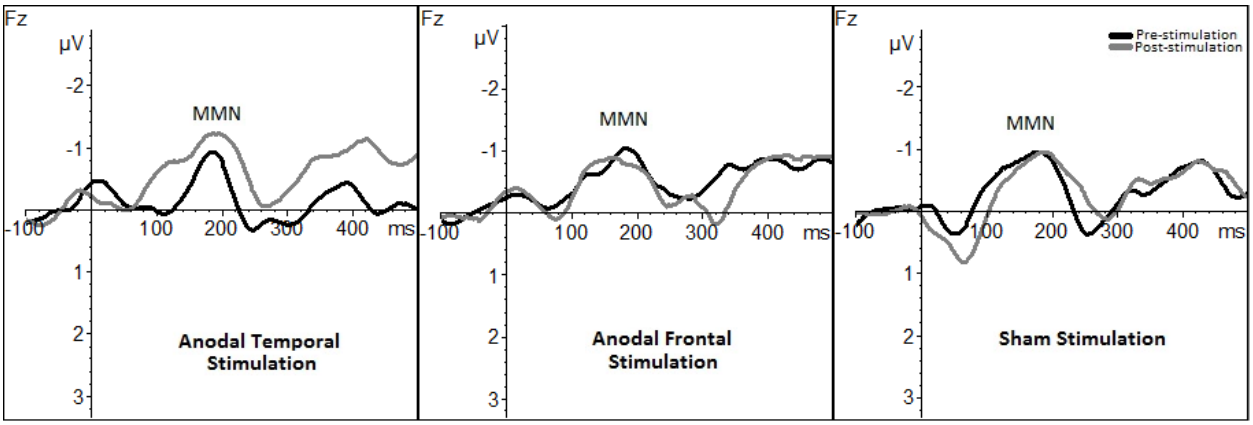

Figure 5.2. Grand averaged duration MMN amplitudes (n = 12) in response to anodal temporal, frontal and sham tDCS.

Grand averaged duration MMN amplitudes in response to tDCS are shown in Figure 2. There was no main effect of treatment, time or main interaction, $F(2,19)=.68$, $p > .05$ on MMN amplitude. Follow-up of study hypothesis with planned comparisons did not reveal any significant modification of MMN after temporal or frontal stimulation, or with 'sham' stimulation.

There was no main effect of treatment or significant treatment and time interaction for MMN latency.

5.5.3. Auditory Hallucination Group and MMN

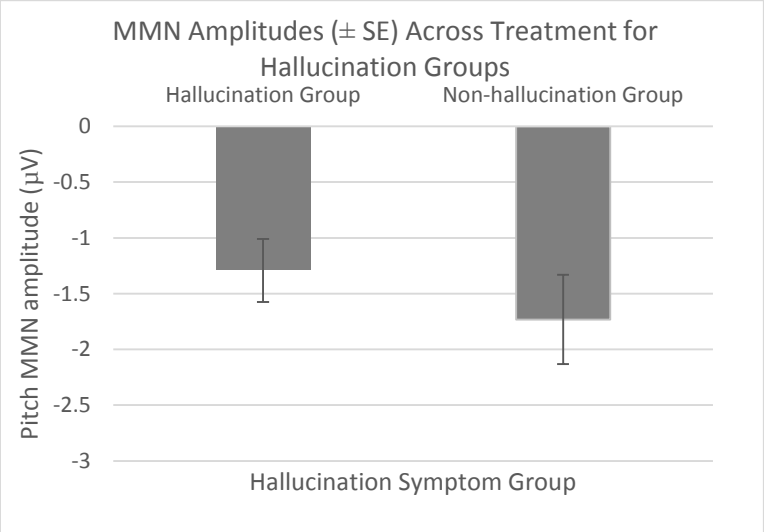

Figure 5.3. Bar graph depicting pitch MMN amplitudes (± S.E.) for Sz participants with auditory hallucinations vs. Sz individuals without auditory hallucinations.

The hallucinating group (n = 8) exhibited a lower overall pitch MMN amplitude (M= -1.29 µV, SE± .28) compared to those who did not experience hallucinations (M= -1.73 µV, SE± .40), shown in Figure 3. Follow-up of group, treatment and time revealed that MMN was significantly improved, $p = .05$, with anodal stimulation over the temporal cortex in the hallucination group only (Mean Difference ± .91 µV, SE ± .42), shown in Figure 4. There was no significant change for frontal stimulation or sham, or in the non-hallucination group.

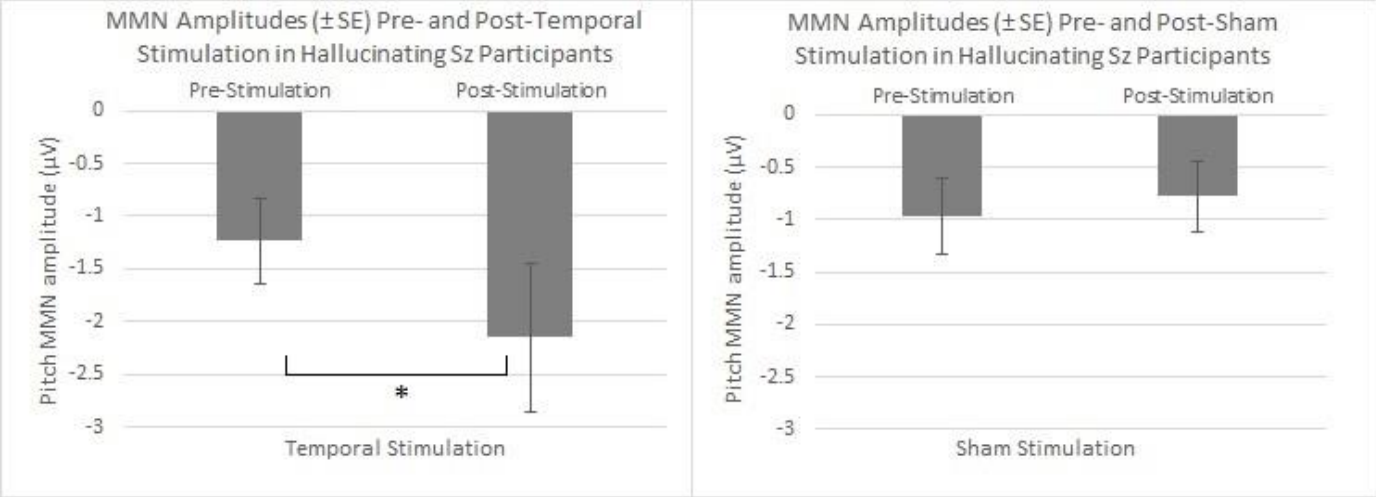

Figure 5.4. Bar graph showing pitch MMN amplitudes (± S.E.) for Sz participants with auditory hallucinations pre- and post-temporal active tDCS and 'sham' tDCS. *p = .05

The hallucination group had lower duration MMN amplitude (M= -1.26 µV, SE± .26) compared to the non-hallucination group (M= -1.80 µV, SE± .37). There were no significant treatment differences among either group.

5.5.4. Working Memory Performance

Task difficulty affected accuracy of performance, F(1,6)=7.53, p = .03, with participants performing better on the 1-back (M =1.09, SE± .09) compared the 2-back WM task (M = .92, SE± .07). Follow up of study hypothesis revealed that for the 2-back task, participants had better accuracy, $p < .05$, after frontal anodal stimulation (M= 1.03, SE± .06), compared to temporal stimulation (M= .83, SE± .08), and sham stimulation (M= .88, SE± .08). These improvements were not seen with the 1-back task.

There was also a significant effect of task difficulty on speed of performance, F(1,6)=5.71, p = .05, with faster reaction times for the 1-back task (M =2.96 $\log_{10}$ ms, SE± .02) compared the 2-back task (M =3.06 $\log_{10}$ ms, SE± .06). Follow up of study hypothesis demonstrated that for the 2-back task only, performance speed was improved, $p < .05$ after

frontal anodal stimulation (M= 3.02 $\log_{10}$ ms, SE± .06) compared to sham stimulation (M= 3.08 $\log_{10}$ ms, SE± .05).

5.5.5. Working Memory and MMN Correlations

There were no significant correlations between performance accuracy and treatment effects for pitch or duration MMN. There was a significant correlation between speed of performance and pitch MMN response to tDCS. The frontal stimulation effect (change score: post-stimulation MMN – baseline MMN) was positively correlated with speed of performance post-frontal stimulation for both the 1-back task, r = .71, p = .01, and the 2-back task, r = .57, p = .04, which indicated that greater frontal tDCS-related changes in pitch MMNs were correlated with a faster response time. This significant correlation was not seen post-temporal stimulation or post-sham stimulation, or for tDCS-induced duration MMN changes.

5.5.6. Adverse Effects

There was no significant difference between reported tDCS-related symptoms for each treatment, $F(2,16) = 0.05$, $p > .05$. Participants reported mild symptoms for all 3 treatment sessions (Mean rating = 2 [Mild symptoms]), which included either no symptoms, tingling/ itching or drowsiness, for both active and sham stimulations. Participants could not distinguish between treatments as they accurately guessed at chance level (50%).

5.6. Discussion

The current study is the first to investigate the effect of anodal transcranial direct current stimulation over both the temporal and frontal cortices on MMN-indexed sensory discrimination and working memory (WM) performance measures in individuals with Sz. As the MMN ERP is a proposed biomarker of the disorder (Light & Näätänen, 2013; Light & Swerdlow, 2014; Light

et al., 2012; Näätänen et al., 2015a; 2015b; Turetsky et al., 2015) and tDCS effects may be dependent on interindividual differences at baseline (Impey & Knott, 2015; Li et al., 2015), we also investigated the relationship between tDCS-MMN changes and WM performance, as well as the relationship between tDCS-MMN effects and the presence of AHs. Anodal tDCS over the temporal cortex resulted only in a non-significant trend for improvement of auditory change detection, as measured by pitch MMN amplitude. The absence of strong stimulation effects may be related to the marked interindividual variability in patient MMN amplitude and/or the considerable heterogeneity in clinical symptoms, including AHs. Interindividual factors, such as baseline cognitive function and physiology, have been found to contribute to the variability of response to tDCS (Li et al., 2015). Our recent work assessing tDCS-MMN effects demonstrated that healthy subjects stratified by low MMN at baseline responded to anodal tDCS, suggesting that individuals with low baseline MMNs may be pre-disposed to best respond to tDCS (Impey & Knott, 2015; Impey, de la Salle, Knott, 2016). When we investigated the presence of AHs on tDCS-induced MMN changes, patients with hallucinations had smaller pitch MMNs (compared to those who did not experience hallucinations) and in these low MMN hallucinators, pitch MMNs were significantly improved with tDCS over temporal brain regions**,** which supports our previous line of work. As for WM performance, tDCS over the left frontal cortex increased performance on the 2-back memory task in patients, both in accuracy and response time. Post-frontal stimulation, response time was correlated with stimulation-induced changes in pitch MMN amplitudes, suggesting a relationship between altered pre-attentive acoustic change detection processes and WM.

Although found not to be significant in our Sz sample, the direction of tDCS-related pitch MMN effects in patients parallel findings of previous healthy volunteer research that has found

pitch MMN, but not duration MMN, improvements with 2 mA of anodal direct current delivered to scalp areas overlapping the auditory cortex (Impey & Knott, 2015). Näätänen and Kähkönen, (2009) suggest that pitch MMN deficits in Sz are more state-related, while duration MMN deficits may be more trait dependant and thus possibly less amenable to change with treatment interventions. However, a recent meta-analysis did not find evidence for this and suggests that the impairments to duration and frequency deviants in Sz differ quantitatively rather than qualitatively (Erickson et al., 2015). Source localization studies have shown that MMN generators for each acoustic deviant feature have different cortical generators (Giard et al 1990; Garrido 2009), which may help explain findings with pitch, but not duration deviants. Current results suggest that tDCS seems to have a variable effect on the different generators. MMN amplitudes were not shown to be increased with frontal stimulation and may be explained by the location of MMN generators, which have been isolated primarily in the auditory cortex, with some contributions from the frontal cortex (Alho, 1995; Näätänen, 1995; Näätänen et al. 2007; Näätänen & Kähkönen, 2009; Näätänen, Kujala, & Winkler, 2011; Opitz et al. 2002; Shalgi & Deouell, 2007). Current findings suggest that tDCS-MMN amplitude increases depend on stimulation in proximity of the temporal (auditory) cortex generators, which play a more critical role in the automaticity of acoustic deviance detection compared to the frontal generators, which are thought to be implicated in attentional orienting to deviance (Garrido 2009; Näätänen 2007; Näätänen & Kähkönen, 2009), which is supported by previous studies investigating temporal cortex stimulation (Heimrath et al., 2015; Impey & Knott, 2015). Importantly, we did not find any changes with sham stimulation, as expected, which indicates that the sham protocol was an effective control condition.

The current study also confirmed that stimulation of the prefrontal cortex can increase working memory performance. Our findings showed an increase in performance accuracy and response speed on the visual 2-back WM task of the CogState Cognitive Battery after frontal stimulation. There were no tDCS-improvements on the 1-back task, which is much less demanding on mnemonic resources and as such ceiling effects may have prevented improvements on the easier WM task, compared to the 2-back task. The existence of ceiling effects on tDCS-neuronal modulation has been previously suggested and may explain the variable effects of tDCS on cognitive performance (Li et al., 2015). Current findings did not show WM improvements after temporal tDCS, or with 'sham' stimulation. This is to be expected as WM enhancement is dependent on frontal executive processes (Wood & Grafman, 2003), which would require direct active stimulation of frontal brain areas, as opposed to temporal brain regions. These positive results agree with other studies that have investigated tDCS-WM effects in healthy volunteers and individuals diagnosed with neuropsychiatric disorders (Boggio et al., 2006; 2008; Brunoni & Vanderhasselt, 2014; Ferrucci et al., 2008; Fregni et al., 2005; 2006; Hoy et al., 2014; Vercammen et al., 2011), including in patients with Sz. With the current findings, there is now additional support for the enhancing effect of frontal anodal tDCS on working memory performance as assessed on the n-back task (Fregni et al. 2005; Hill et al. 2015; Hoy et al., 2014; Keeser et al. 2011; Teo et al. 2011). In the present study, faster response time on the WM task was correlated with a greater frontal tDCS-related MMN change. tDCS has previously been found to be associated with improved accurate reaction times (Hoy et al., 2014; Teo et al. 2011) and the current finding suggests that tDCS-related MMN-indexed pitch processing changes, which are assumed to rely on sensory memory context updating (Näätänen, Kujala, & Winkler, 2011), may be related to speed of processing during the memory task. This finding

suggests a relationship between tDCS-MMN amplitudes changes and working memory performance, which agrees with previous conclusions that MMN is associated with WM deficits (Javitt et al., 1995). This significant correlation is also compatible with previous studies that have found that tDCS effects are dependent on baseline cognitive function, among other factors (Li et al., 2015; Tseng et al., 2012).

The current study also explored the relationship between AHs and the MMN ERP. Positive symptoms, including AHs, have been correlated with MMN-indexed change detection (Fisher et al., 2011; 2012; Youn et al., 2003). AHs are a common feature of Sz and they are associated with abnormal activity in the left temporal-parietal junction and abnormal fronto-temporal connectivity (Hoffman & Hampson, 2011; Sommer et al., 2012). Fronto-temporal tDCS has been shown to effectively reduce AH severity (Andrade, 2013; Brunelin et al., 2012; Ferrucci et al., 2014; Mondino et al., 2015a; 2015b; Nawani et al., 2014). There was an MMN amplitude difference, albeit non-significant, between Sz hallucination groups. MMN amplitudes for both duration and pitch deviants were reduced in patients who experienced hallucinations (vs. those who did not). These results agree with a previous study by Fisher and colleagues (2008) that found significant MMN differences between hallucinating and non-hallucinating groups, which may stem from auditory cortex defects in the hallucination group (Michie et al., 2002). When looking at the effect of tDCS, only individuals who had AHs responded to temporal tDCS. Active temporal tDCS significantly increased MMN amplitudes in patients with AHs. It is suggested that this subset of patients may have had more NMDA receptor (NMDAR) mediated disruption in fronto-temporal function or connectivity which allowed more benefit from the temporal stimulation, which is NMDAR function dependant. Although we cannot support this suggestion within the current study, this is in agreement with dipole studies that have shown that

MMN generation is located in different regions of the auditory cortex but there is also contribution of frontal-lobe activity (Alho, 1995; Javitt et al., 1994), which may be disrupted in this population. More evident, significant anodal tDCS effects in the subset of Sz patients with AHs may be due to their low baseline MMNs. Our recent work assessing tDCS-MMN effects demonstrated that participants stratified by low MMN at baseline responded to anodal tDCS (Impey & Knott, 2015; Impey, de la Salle, Knott, 2016). These findings suggest that individuals with low baseline MMNs, such as the case in the chronic Sz patients with AHs included in this study, may be pre-disposed to best respond to tDCS. Our findings highlight the importance of individual differences on tDCS effects, which has been reported in previous reviews (Li et al., 2015). Future studies may consider interindividual differences, such as hallucination presence or severity, and other factors such as baseline function and anatomy, when developing treatment protocols for Sz patients. The complex relationship between tDCS, cortical excitability and cognitive or clinical symptoms, including AHs, is still fairly novel and warrants further investigation.

The current study had some limitations which warrant discussion. The primary limitation was the relatively small sample size of participants diagnosed with Sz. The goal of this pilot study was to investigate the effects of tDCS in a sample of individuals with Sz. The majority of tDCS investigations are conducted with a sample of 12 (Chen et al. 2014; 2014b; Heimrath et al., 2015; Vines et al. 2006; Zaehle et al. 2011) and during development of this protocol, very few studies had investigated the use of tDCS in Sz patients. We first sought to confirm tDCS effects on MMN and WM in patients, but with low statistical power, we only observed significant modification of tDCS in a subsample of patients who experienced hallucinations. It is possible that with increased statistical power, the tDCS effect trend found in the whole sample could have

shown significant enhancement, as has been shown previously in healthy control participants (Impey and Knott, 2015; Impey, de la Salle, Knott, 2016). Also, the Sz subjects included in this study were chronic Sz patients with mild to severe clinical symptoms. As MMN is correlated with functional impairment, negative symptoms and duration of illness (Javitt et al., 2000; Kawokubo et al., 2007; Umbricht and Krjles, 2005), the clinical severity of these patients may have implications for the generalizability of these tDCS-MMN findings. The low baseline MMNs associated with chronic illness may have pre-disposed these individuals with Sz to respond to tDCS, irrespective of AH presence, however significant MMN enhancement was only seen in individuals who experienced hallucinations. Secondly, patients remained on their current course of atypical anti-psychotics, including clozapine. Although one study found a relationship between clozapine dose and MMN amplitude (Horton et al. 2011), most studies have not found an effect of anti-psychotic medications on MMN amplitudes (Korostenskaja et al., 2005; Umbricht et al., 1998). It is possible that antipsychotic medication may have altered cortical excitability in our sample, but the interaction effects of antipsychotics on tDCS are currently largely unknown (Bruoni et al., 2012). Finally, the inability to localize specific cortical sources in this study remains a limitation as the present study did not include current modeling or imaging to confirm current densities. However, current modelling studies have shown that tDCS does have peak current density over the targeted region (Neuling et al. 2012) and that by adjusting the current density (with both the current strength and electrode size), the efficacy of tDCS can be optimized to reach targeted brain regions (Nitsche et al. 2008). tDCS parameters used in previous studies for cognitive improvement were employed in this study (with regard to electrode size and placement, current intensity and the use of a ‘sham’ control condition) and our results do indicate modulation of both sensory discrimination and working memory performance

with active stimulation. Future studies should continue to use recommended protocols for clinical and research use of tDCS, such as those suggested in the regulatory considerations from a tDCS expert panel (Fregni et al., 2014).

In conclusion, tDCS has been proposed as a possible brain stimulation treatment for numerous neurological and psychiatric disorders, including schizophrenia. Auditory processing deficits as reflected in impaired MMNs are robustly found in individuals with Sz, and are strongly linked to functional and cognitive outcome. Given that MMN dysfunction in Sz is mediated via dysfunctional NMDA receptor signaling and that tDCS 'after effects' are thought to be NMDA dependant, this pilot study provides tentative evidence of tDCS' effects on deficient MMN-indexed sensory discrimination function in individuals with Sz, which may be mediated by individual factors such as the presence of auditory hallucinations and baseline response. The current study also replicated findings that have shown improved working memory performance with frontal tDCS in Sz patients. Present findings support further study of tDCS as a technique for the improvement of auditory sensory processing and WM deficits in this population. Future treatment studies may want to consider interindividual differences, such as baseline response and presence of auditory hallucinations, among other factors, when assessing tDCS effects for cognitive improvement.

5.7. Acknowledgments and Declarations

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Chapter 6: General Discussion

6.1. Overview

The purpose of this thesis is to elucidate the effects of tDCS on auditory sensory processing, as assessed by the MMN ERP. In two initial pilot studies, both anodal and cathodal tDCS effects on MMN were investigated, assessing different deviant type and stimulus onset timing on possible tDCS-MMN effects. An NMDAR-based pharmacological study was completed to elucidate neurobiological mechanisms underlying the tDCS effects on sensory and cognitive processes. Finally, a clinical pilot study was completed with individuals with Sz to assess the effects of tDCS on MMN and WM impairments in this population. tDCS effects were investigated pre- and post-stimulation from temporal scalp regions overlying the left auditory cortex and also in frontal scalp regions overlying the left dorsolateral prefrontal cortex, in a sham-controlled (i.e. no stimulation), repeated measures, double-blind design. Based on work which has shown that pharmacological modulation of MMN is baseline-dependent (generally increasing ERPs in individuals with low baseline amplitudes, and decreasing ERPs in individuals with high baseline amplitudes; Knott et al., 2013; 2014a; 2014b; 2015) and that tDCS response may also be baseline-function dependent (Li et al., 2015; Vercammen et al., 2011), we examined the effects of tDCS on MMN in stratified groups differing in baseline response in both healthy controls and patients with Sz.

6.2. Summary of results

To the best of our knowledge, the initial pilot study included in the current thesis is one of the first to report electrophysiological evidence of increased cortical activation with tDCS over the auditory cortex (AC) and the first to demonstrate a significant enhancement of MMN-indexed auditory processing with anodal tDCS. Specifically, anodal tDCS (2 mA) applied over

the left temporal cortex for 20 minutes significantly enhanced MMN at frontal sites to a small pitch (Δ50 Hz) deviant immediately after active stimulation but not with sham stimulation, as hypothesized. The second pilot study replicated the anodal stimulation MMN enhancement effects, particularly seen in the low group, who exhibited relatively small MMN amplitudes (i.e., diminished deviance detection efficiency) at baseline, and extended significant findings to variations in pitch deviants and stimulus onset asynchronicity. When reversing the polarity of stimulation, cathodal tDCS over the AC significantly reduced MMN amplitudes at frontal sites, compared to baseline assessment, and prevented the enhancing effects of follow-up anodal tDCS.

In the pharmacological investigation, anodal tDCS increased MMN for both pitch and duration deviants in the placebo condition, but these tDCS-induced increases were not observed after dextromethorphan (DMO) administration, which suggests that the NMDAR antagonist may have prevented the enhancing after-effects of anodal tDCS. In the sham condition, MMN amplitudes were significantly reduced after DMO administration, which suggests that NMDA antagonism significantly reduced MMN amplitudes independently of tDCS effects and prevented MMN enhancement in the active anodal condition, indicating that tDCS-MMN effects are dependent, in part, on glutamatergic neurotransmission. Finally, in the clinical pilot study, anodal tDCS over the AC significantly improved pitch deviance detection in a subgroup of Sz patients with auditory hallucinations (AHs), who had reduced pitch MMNs (compared to individuals who did not experience hallucinations). In both the NMDAR study with healthy participants and the clinical study with patients, working memory (WM) measures were also improved with anodal tDCS, but not with sham stimulation, which confirms reports of WM improvements with anodal tDCS. In patients with Sz, response time was correlated with tDCS-induced changes in pitch

MMN, suggesting a relationship between MMN-indexed sensory processing and WM performance.

6.3. Stratification Strategy

Important to note, in the pilot studies, MMN at baseline, defined as the initial time point used for comparisons pre-stimulation, did not differ between stimulation treatments, which indicates significant improvements cannot be attributed to differing baseline measurements between sessions. Moreover, when stratified into groups based on baseline MMN, it was found that although groups differed at baseline, as intended, they were no longer significantly different post-stimulation. Participants with initially low discriminability (i.e., individuals exhibiting low baseline MMN amplitudes) showed significant MMN enhancement with anodal tDCS, while participants with initially high discriminability (i.e., individuals exhibiting high baseline MMN amplitudes) did not. This suggests that individuals with initially reduced deviance detection ability (i.e., participants with low baseline MMNs) seem to benefit from anodal tDCS and that near-ceiling effects may prevent participants with initially high MMNs from further increasing with anodal tDCS. Notably, the modulatory effects of tDCS observed in the current set of studies were not seen with sham stimulation, which indicates that tDCS-MMN effects were due to active stimulation, and not simply regression or time effects. This stratification strategy was implemented as, recently, the utility and importance of individual biomarkers for the development of more effective, personalized treatment has been highlighted, specifically including stratification of baseline samples for the preclinical assessment of cognitive enhancing agents (Butler et al., 2012; Green et al., 2009; Frank & Hargreaves, 2003). Several interindividual differences at baseline have been found to have a significant impact on tDCS effects (Li et al., 2015).

It is important to consider the non-linear relationship between stimulation effect and response. For neural reactivity, it is hypothesized that there is an inverted U-shaped dose-response relationship wherein if reactivity increases beyond the optimal levels, e.g. due to external stimulation from tDCS, neuronal reactivity will begin to deteriorate. Therefore, enhanced cortical excitability does not necessarily increase response if already performing at optimal levels (Heimrath et al., 2016). Further, it can be hypothesized that if you have a deficient state of auditory processing, an enhancement of AC-related excitability will results in improvement of perceptual processing (Heimrath et al., 2016). Such an inverted U-shape relation has been shown for the influences of auditory tDCS on acoustic processing (Heimrath et al., 2014) and for the influence of psychotropic drugs of tDCS effects (Monte-Silva et al., 2013). Thus it is vital to consider baseline function or response to determine if anodal or cathodal stimulation will be effective in increasing or decreasing function to an optimal level, which explains some variations in tDCS polarity effects.

6.4. Interpretation of tDCS-MMN effects

It is presumed that increased excitability of the auditory cortex by anodal tDCS was responsible for the increases in MMN amplitude. Anodal stimulation was found to increase MMN, particularly in the low group, which may have allowed, or facilitated improvements in auditory discrimination, whereas cathodal stimulation decreased MMN, but not in the low group, presumably due to a floor effect specific to this group. Anodal tDCS effects agree with a study by Heimrath and colleagues (2015) that also found increased MMN amplitudes with temporal tDCS. Cathodal tDCS effects on MMN-indexed auditory discrimination are mixed. Contrary to research showing enhancement with anodal tDCS, Chen and colleagues (2014a) found right frontal anodal tDCS to reduce the amplitude of frequency MMN, while neither frequency nor

duration MMNs were affected by cathodal stimulation. These confounding results (i.e. MMN decreases with anodal stimulation) could be due to the positioning of stimulating electrodes on scalp areas remote from the auditory cortex, or baseline deviance detection that were at optimal functioning. Findings from the current studies support the conclusion that to increase or decrease MMN amplitude, stimulation needs to be applied to the scalp over the auditory cortex. The planned manipulation to reverse MMN amplitudes alterations with anodal tDCS after an induced deficient state with cathodal tDCS was not successful. It is difficult to interpret these findings as changes in cortical excitability following stimulation peak around 20 minutes post-stimulation (if applied for 20 minutes) in sensory and cognitive domains, which is when anodal stimulation was administered, and therefore is difficult to confirm if anodal tDCS did or did not enhance pitch MMN from a deficient state, based on our design. Further studies investigating the duration and reversibility of tDCS effects on sensory and cognitive processing are needed.

In healthy participants and individuals with schizophrenia, pitch MMN was most consistently significantly modulated by tDCS. Duration MMN changes were only found in the tDCS drug study. Source localization studies have shown that MMN generators for each acoustic deviant feature have different cortical generators (Giard et al 1990; Garrido 2009; Paavilainen et al., 1991; Schairer et al. 2001), which may help explain consistent findings with pitch, but not duration deviants. MMN receives contribution from a bilateral supratemporal process at the auditory cortices and a predominantly right-hemispheric frontal process (Giard et al. 1990; Näätänen et al. 2007). Activity in the temporal-auditory sources is associated with the establishment of memory traces and comparison with stimulus-specific features (pre-perceptual deviance detection) and the specific location of the these sources slightly differs depending on the physical feature eliciting the MMN (Giard et al. 1990; Näätänen et al. 2007; Shalgi and

Deouell 2007). These differences in auditory sources may explain the varying effects of tDCS on established memory traces (depending on length and duration) and different deviant features (relatively easy or hard acoustic discrimination) found in the series of studies. Current findings suggest that tDCS seems to have a variable effect on the different generators, which agrees with other tDCS-MMN investigations which found differential effects of tDCS on distinct MMN deviants (Chen et al., 2014). In future tDCS-MMN investigations, the 5 deviant optimal MMN paradigm (Näätänen et al., 2004) could be used to further elucidate the effect of tDCS on different discrimination abilities.

In the initial pilot study, MMN was recorded from frontal and mastoid sites, reflecting contributions from temporal and frontal cortex generators (Näätänen et al., 2007). Anodal stimulation improved the MMN component at frontal regions, where the MMN amplitude is maximal. As mentioned, MMN is generated by two separated yet connected neural sources in the temporal and frontal cortex, the latter which is involved in a cognitive, comparator-based mechanism and the attention switching response to relevant stimuli (Giard et al., 1990; Garrido, 2009; Näätänen, 2007). Current results suggest that anodal tDCS improved the frontal comparator-based response under low discriminability conditions, however tDCS-induced effects on temporal MMN generators are likely to have contributed to frontal MMN improvements with anodal tDCS as stimulation of the AC was necessary for MMN modulation. Heimrath and colleagues (2014) also found that MMN was significantly enhanced when stimulating the left auditory cortex specifically. Anodal stimulation of the frontal cortex was not found to significantly increase MMN amplitudes in healthy participants or in patients with Sz, which agrees with other tDCS-MMN investigations which stimulated the prefrontal cortex (Chen et al., 2014; Knechtel et al. 2014; Weigl et al., 2016). Current findings suggest that tDCS-MMN

amplitude increases depend on stimulation in proximity of the temporal (auditory) cortex generators, which play a more critical role in the automaticity of acoustic deviance detection compared to the frontal generators, which are thought to be implicated in attentional orienting to deviance (Garrido 2009; Näätänen 2007; Näätänen & Kähkönen, 2009).

The MMN response is only observed following presentation of a deviant stimuli or rule violation that is mismatched with the current sensory memory trace, representing sensory memory updating or predictive coding of auditory stimuli, and is therefore separate from obligatory electrical brain responses to incoming stimuli, such as the N1 ERP component (Näätänen, 1995; Näätänen et al., 2005; Näätänen et al., 2011; Winkler, 2007). Significant MMN response to tDCS was separate from participants' N1 ERP response, as N1 was not found to be significantly modulated by tDCS in the current set of studies, suggesting that tDCS does not alter N1-indexed sensory registration, but rather the sensory memory updating and change/rule violation detection (Näätänen 1995; Näätänen et al. 2005; 2007; 2011). These findings agree with other studies which have shown that tDCS has a surprising temporal specificity, having significant effects on discrimination, error-related and feedback-related processing and memory-related processing, but there are few studies that support that tDCS effectively modulates early perceptual ERPs (Reinhart and Woodman, 2015). Näätänen's (2011) model of auditory processing attempts to explain how auditory information is processed. First sound stimulus is rapidly analyzed by the different feature detectors (i.e. standard and deviant characteristics) whose outputs are temporarily integrated. The integrated sensory information in the mechanisms of sensory memory that evolves in time provides the central sound representation that is consciously experienced, depending on the strength of the attention-call signal elicited by the features indexed by the N1 amplitude (Näätänen et al. 2011). If some discernible change in

stimulation occurs, this results in the updating of auditory representation in sensory memory, which elicits the AC MMN component, which in turn activates the frontal cortex MMN generators, representing attention-call to auditory change. Executive mechanisms use sensory memory data to set up the attentional trace for to-be-attended input for further processing or response (Näätänen et al. 2011). It is suggested that anodal tDCS improves acoustic discrimination, which in turn increases MMN amplitudes, whereas cathodal tDCS decreases change detection mechanisms, and therefore reduces MMN amplitudes. Current findings show that tDCS only affected the amplitude of the MMN peak, reflecting the strength of the process, and not the latency, which reflects the timing or speed of processing (Luck, 2011).

6.5. tDCS-MMN effects in individuals with Sz

When we investigated the presence of AHs on tDCS-MMN effects, Sz patients with hallucinations had smaller MMNs (compared to those who did not experience hallucinations), which agrees with studies that have found that MMN and presence of AHs were correlated (Fisher et al., 2011; 2012; Youn et al., 2003) and a study by Fisher and colleagues (2008) that found significant MMN differences between symptom groups. When looking at the effects of tDCS, temporal stimulation significantly increased MMN amplitudes in patients with AHs. AHs are associated with abnormal activity in the left temporal-parietal junction and abnormal fronto-temporal connectivity (Hoffman & Hampson, 2011; Sommer et al., 2012), overlapping areas responsible for MMN generation. Moreover, fronto-temporal tDCS has been shown to effectively reduce AH severity (Andrade, 2013; Brunelin et al., 2012; Ferrucci et al., 2014; Mondino et al., 2015a; 2015b; Nawani et al., 2014). MMN differences in Sz patient groups may stem from auditory cortex defects in the hallucination group (Michie et al., 2002). It is suggested that this subset of patients may have had NMDA-receptor mediated disruption in fronto-temporal

function or connectivity associated with impaired MMN generation (Javitt et al., 1996; 2000), which allowed more benefit from temporal tDCS. Although we cannot support this suggestion within the current study, the findings of this thesis do indicate that significant anodal tDCS effects in the subset of Sz patients with AHs may be due to their low baseline MMNs, which may pre-dispose them to best respond to anodal tDCS based on our pilot study results.

As MMN is correlated with functional impairment, negative symptoms and duration of illness (Javitt et al., 2000; Kawokubo et al., 2007; Umbricht and Krjles, 2005), it was presumed that these chronic patients may show low baseline MMNs due to the clinical severity of these individuals which may have pre-disposed these participants to respond to tDCS, irrespective of AH presence, as was the case in the low MMN healthy control proxy group. Future studies may consider interindividual differences, such as hallucination presence or severity, and other factors such as baseline response, when developing treatment protocols for Sz patients. The complex relationship between tDCS, cortical excitability and cognitive or clinical symptoms, including AHs, is still fairly novel and warrants further investigation but the current findings demonstrate a neuromodulation technique capable of modulating MMN response, a proposed biomarker or endophenotype of Sz disorder, that can help to correct impaired neural mechanisms that contribute to patient dysfunction in everyday functioning. tDCS as an adjunctive treatment for sensory processing deficits in Sz may be advantageous as it has limited adverse effects. The pilot studies in the present thesis are the first step towards clinical application, which would require more research into the duration or repetitions required (e.g. daily or weekly tDCS sessions) to have lasting beneficial effects.

6.6. tDCS-NMDA mechanisms

In the pharmacological study, DMO, an uncompetitive NMDA antagonist, blocked the enhancing effect of anodal tDCS on MMN amplitudes observed during the placebo condition and in earlier pilot studies. These results suggest that tDCS-MMN changes were at least partially moderated by NMDAR activity. The current findings are the first to be investigated with the auditory cortex but they are similar to other studies which have investigated the effect of NMDA antagonism on tDCS-related motor evoked potential changes over motor regions (Liebetanz et al., 2002; Nitsche et al., 2002; 2003a; 2004; Stagg and Nitsche, 2011) and a magnetic resonance spectroscopy (MRS) study which found significantly higher combined glutamate and glutamine levels beneath the stimulating electrode (Clark et al., 2011). Specifically, tDCS induced post-synaptic polarization associated with long-term potentiation (LTP) is caused by altered pre-synaptic input due to changed firing rates which leads to enhanced NMDA receptor-efficiency resulting in an increase of the intracellular Ca^{2+} level. Anodal after-effects post-stimulation are suggested to induce LTP due to enhanced firing rate, and cathodal tDCS reduces firing rate followed by long-term depression (LTD; Libebetanz et al., 2002; Nitsche et al., 2002; 2003a; Stagg and Nitsche, 2011). It is suggested that dextromethorphan was effective as a channel blocker which prevented glutamatergic neurotransmission that allow for anodal tDCS after-effects.

Furthermore, MMN alterations were independently moderated by the NMDA antagonist as MMN amplitudes were significantly reduced after sham stimulation (i.e. no stimulation) with DMO administration, which was not seen with placebo**.** These significant MMN reductions present with NMDA antagonism replicate findings of other NMDA-MMN investigations, such as the use of ketamine protocols (Rosburg and Kreitschmann-Andermahr 2015; Umbricht et al.

2002) and data from animal work (Javitt et al. 1996; Tikhonravov et al. 2008), which have shown significant modulation of MMN with glutamatergic agents. The present tDCS-MMN findings are likely reflective of MMN's high reliance on the glutamatergic neurotransmitter system and cortical NMDA receptors, particularly for after-effects post-stimulation, however a variety of neurotransmitters have been implicated in tDCS modulatory effects including dopamine, acetylcholine, serotonin and GABA (Liebetanz et al., 2002; Nitsche et al., 2002; 2003a; 2004; Kuo et al., 2007; Stagg and Nitsche, 2011; Medeiros, 2012). As tDCS induces glutamatergic plasticity, the technique has increasingly been used to investigate the therapeutic effects in neuropsychiatric disorders with dysfunctional glutamate systems (Kuo, Paulus and Nitsche, 2014), which is the basis for our pilot study in Sz, in which an NMDA dysfunction is theorized to play a central role (Javitt, 2000; 2007; 2010).

6.7. tDCS and Working Memory

The results of our study with anodal tDCS are largely supported by the literature which has shown enhancement of memory-based processing with tDCS. Frontal anodal stimulation improves WM performance on the N-back memory task, as seen in other studies when tDCS was administered over the prefrontal cortex (Fregni et al. 2005; Hill et al. 2015; Keeser et al. 2011; Teo et al. 2011). The tDCS-memory performance improvements in the pharmacological study were only seen in the active drug condition, when sensory discrimination was attenuated due to NMDA antagonist effects. As WM accuracy in healthy participants was very high, it is possible ceiling effects prevented anodal tDCS from further increasing performance during placebo administration. The drug-related reduction in sensory processing after DMO administration may have allowed for the enhancing effects of anodal tDCS on memory performance. This finding

agrees with decades of research which has found that the NMDAR system is implicated in many cognitive processes including working memory (Rezvani, 2006).

Findings from the clinical Sz study also showed an increase in performance accuracy and response speed on the 2-back WM task after frontal stimulation. Current findings did not show WM improvements after temporal tDCS or with 'sham' stimulation. This is to be expected as WM enhancement is dependent on frontal executive processes (Wood & Grafman, 2003), which would require active stimulation of frontal brain areas, as opposed to temporal regions. These positive results agree with other studies that have investigated tDCS-WM effects in both healthy volunteers and individuals diagnosed with neuropsychiatric disorders (Boggio et al., 2006; 2008; Brunoni & Vanderhasselt, 2014; Ferrucci et al., 2008; Fregni et al., 2005; 2006; Hoy et al., 2014; Vercammen et al., 2011). In the present study, faster response time on the WM task was correlated with a greater frontal tDCS-related MMN change. This finding suggests a relationship between frontal tDCS-MMN amplitudes changes and WM performance, which agrees with previously established associations between MMN and WM deficits (Javitt et al., 1995). tDCS has been found to be associated with improved accurate reaction times (Hoy et al., 2014; Teo et al. 2011) and the current finding suggests that tDCS-related MMN-indexed pitch processing changes, which are assumed to rely on sensory memory context updating (Näätänen, Kujala, & Winkler, 2011), may be related to speed of processing during the memory task.

6.8. Limitations

The primary limitation of the current set of studies was the small sample size of participants for each study. Many tDCS studies are conducted with a sample of 12 (Chen et al., 2014a; 2014b; Kang et al., 2009; Vines et al., 2006; Zaehle et al., 2011), however our analyses examined stratified groups of low- and high-baseline MMN individuals and it is likely that these

pilot studies were statistically underpowered. Our analysis did show significant effects of tDCS, even with low power and as previous studies have shown MMN modulations to be baseline-dependent (Knott et al., 2014b), we could not leave out this factor in our investigations with healthy participants and strongly suggest assessing baseline-dependency effects in future studies. In the patient sample, significant tDCS were only shown in a subsample of chronic Sz patients who experienced hallucinations, a group which demonstrated the smallest MMN amplitudes, similar to the low MMN healthy proxy group. Patients also remained on their current course of atypical anti-psychotics, including clozapine. It is possible that antipsychotic medication may have altered cortical excitability in our sample, but the interaction effects of antipsychotics on tDCS are currently unclear or mixed (Bruoni et al., 2012; Horton et al. 2011; Korostenskaja et al., 2005; Umbricht et al., 1998). It is suggested that future studies investigate the effect of duration and severity of the illness on beneficial tDCS effects, as well as medication status.

Concerning pharmacological investigations, only a single dose of dextromethorphan hydrobromide (50 mL) was assessed, which was chosen based on previous studies which have found significant tDCS effect's on motor-evoked response, which used an equivalent dose of DMO (Liebetanz et al. 2002; Nitsche, Fricke et al. 2003). The 50 mL dose was sufficient to produce significant attenuations of tDCS effects on MMN at peak drug absorption; however, although sedation effects and vertigo were reported with both the active drug and with placebo, symptoms were more significant for DMO than placebo administration and these could have affected current results. Granting MMN amplitude is relatively immune to state, and is still intact in drowsy, sleeping or certain comatose individuals (Morlet and Fischer 2014), future studies could consider controlling for sedation effects by comparing DMO effects with a comparator drug such as an anxiolytic agent. Future studies could also consider assessing the effect of

different doses, or different NMDA antagonist and agonists on tDCS-sensory discrimination effects. It is also suggested to investigate the effect of other neurotransmitters on tDCS-auditory processing as catecholamines, serotonin, acetylcholine and GABA, among other neurotransmitters, have been implicated in tDCS modulatory effects.

Another limitation is the use of sham stimulation as a control condition. Sham parameters were chosen based on reports that perceived sensations on the skin, such as tingling (during the 30 seconds of sham tDCS) re-create the same sensations experienced with anodal stimulation and produce no after-effects (Gandiga et al., 2006). The results of the adverse events questionnaire confirmed the effectiveness of blinding and no significant changes were seen with sham stimulation. Finally, the inability to localize specific cortical sources in this study remains a limitation. It has been suggested that individualized current modeling and computational neurostimulation be used to interpret results, taking into account anatomical variation and the dynamics of polarity and orientation (De Berker, Bikson and Bestmann, 2013). It is also suggested to improve and demonstrate the focality of tDCS like Kuo and colleagues (2013) with the use of high definition electrode arrays. However, tDCS does have peak current density over the targeted region (Neuling et al. 2012) and by adjusting the current density (current strength/ electrode size), the efficacy of tDCS can be optimized to reach targeted brain regions (Nitsche et al. 2008). tDCS parameters used in previous studies for cognitive improvement were employed in this study with regard to electrode size, placement and current intensity (Nitsche et al. 2007; Heimrath et al. 2016) and although we limited our investigation to one intensity setting for a set stimulation duration (2 mA for 20 minutes), our results do indicate modulation of both sensory discrimination and WM performance with active stimulation. Future studies should continue to

use recommended protocols for clinical and research use of tDCS, such as those suggested in the regulatory considerations from a tDCS expert panel (Fregni et al., 2014).

6.9. Conclusion

Notwithstanding limitations, the current studies have provided crucial novel information about tDCS effects on MMN-indexed auditory discrimination and WM performance in healthy participants and individuals with Sz. The current set of results demonstrated that temporal anodal tDCS improved MMN-indexed auditory discrimination, particularly in individuals with relatively low sensory discriminability, and demonstrated reduced MMN amplitudes with cathodal tDCS, particularly in individuals with relatively high discriminability. This thesis underscores the importance of the use of neurophysiological markers based on baseline response for the preclinical assessment of tDCS. Moreover, the pharmacological study demonstrated the first evidence of NMDA modulation of tDCS' effects on MMN-indexed auditory discrimination. Enhancement of working memory function with frontal anodal tDCS was also replicated in the current thesis. Future studies are needed to investigate basic research in healthy mechanisms, including source localization for tDCS-MMN effects, and to continue to characterize tDCS protocols for optimal increases in sensory and cognitive functioning for the potential clinical implications for patients with central auditory processing deficits associated with dysfunctional glutamatergic transmission.

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