EFFECTS OF ANODAL TRANSCRANIAL DIRECT CURRENT STIMULATION OVER THE MOTOR CORTEX ON RESPONSE PROCESSING.

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B Psychology (Hons)

Submitted in total fulfilment of the requirements for the degree of Doctor of Philosophy (Psychology).

Faculty of Science and Information Technology

University of Newcastle, Australia

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Statement of Originality

The thesis contains no material which has been accepted for the award of any other degree or diploma in any university or other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text. I give consent to the final version of my thesis being made available worldwide when deposited in the University’s Digital Repository, subject to the provisions of the Copyright Act 1968.

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I hereby certify that the work embodied in this thesis contains a published paper/s/scholarly work of which I am a joint author. I have included as part of the thesis a written statement, endorsed by my supervisor, attesting to my contribution to the joint publication/s/scholarly work.

Alexander C. Conley
Publications

Publications arising from this thesis


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Conference presentations arising from this thesis


Statement of Contribution
I attest that Research Higher Degree candidate Alexander C. Conley made the following contributions to each of the papers that are submitted as part of his PhD thesis. Papers are listed below in the order they appear in this thesis followed by an outline of co-author contribution.

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A.C. Conley collected the data, ran the data analysis and took the lead in writing the manuscript. J. Marquez assisted in the preparation of the manuscript. M.W. Parsons assisted in the preparation of the manuscript. W.R. Fulham assisted with data analysis and the preparation of the manuscript. A. Heathcote assisted with data analysis and the preparation of the manuscript. F. Karayanidis contributed to the research design and the preparation of the manuscript.


A.C. Conley collected the data, ran the data analysis and took the lead in writing the manuscript. W.R. Fulham assisted with data analysis and the preparation of the manuscript. J. Marquez collected the data and assisted with the preparation of the manuscript. M.W. Parsons assisted with the preparation of the manuscript. F. Karayanidis contributed to the research design and the preparation of the manuscript.


A.C. Conley collected the data, ran the data analysis and took the lead in writing the manuscript. J. Marquez collected the data and assisted with the preparation of the manuscript. W.R. Fulham assisted with data analysis and the preparation of the manuscript. M.W. Parsons assisted with the preparation of the manuscript. F. Karayanidis contributed to the research design and the preparation of the manuscript.
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Abstract

Anodal transcranial direct current stimulation (tDCS) is the non-invasive application of a stimulating current which has been proposed as a possible intervention technique for a number of pathologies that affect the motor system, including Parkinson’s disease and stroke. When applied over the motor cortices, anodal tDCS has been shown to elicit long lasting changes to motor excitability. Application of anodal tDCS over the motor cortex has also been shown to improve performance on functional motor tasks. However, there is little knowledge of how anodal tDCS produces these changes. This thesis investigates whether anodal tDCS over the motor cortex influences responding, and if so how are response processes changed. This is explored across functional motor performance, as well as behavioural and electrophysiological performance on a cued go/nogo task with informative and uninformative cues. These experimental outcomes are assessed on three different subject groups, healthy young and older adults, as well as chronic stroke patients. The analysis of the results showed that while there was a small improvement of gross motor performance following anodal tDCS in healthy older adults, there was no beneficial effect of stimulation on either behavioural or electrophysiological data. This null effect was consistent across all three subject groups. Bayesian analyses confirmed that null effects models of the data were stronger fits compared to models which included an effect of stimulation. These results indicate that the application of anodal tDCS over the motor cortex does not impact response processes, which calls into question the efficacy of using anodal tDCS as a therapeutic intervention.
## Abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Meaning</th>
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<tbody>
<tr>
<td>ADM</td>
<td>Abductor digiti minimi</td>
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<tr>
<td>ANOVA</td>
<td>Analysis of variance</td>
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<tr>
<td>BDNF</td>
<td>Brain derived neurotrophic factor</td>
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<tr>
<td>BP</td>
<td>Berietschaftspotential</td>
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<tr>
<td>Ca&lt;sup&gt;+&lt;/sup&gt;</td>
<td>Calcium ions</td>
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<td>CNS</td>
<td>Central-Nervous System</td>
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<td>CNV</td>
<td>Contingent negative variation</td>
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<tr>
<td>CTI</td>
<td>Cue-target interval</td>
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<tr>
<td>CYC</td>
<td>Cycloserine</td>
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<td>DA</td>
<td>Dopamine</td>
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<tr>
<td>DLPFC</td>
<td>Dorsolateral prefrontal cortex</td>
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<td>ECT</td>
<td>Electro-cortical therapy</td>
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<td>EEG</td>
<td>Electroencephalography</td>
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<td>EMG</td>
<td>Electro-myography</td>
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<td>EOG</td>
<td>Electro-oculogram</td>
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<tr>
<td>ERP</td>
<td>Event-related potential</td>
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<tr>
<td>FDI</td>
<td>First dorsal interosseous</td>
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<tr>
<td>FMS</td>
<td>Fugl-Meyer Scale</td>
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<tr>
<td>GABA</td>
<td>γ-amino-butyric acid</td>
</tr>
<tr>
<td>HEOG</td>
<td>Horizontal electro-oculogram</td>
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<tr>
<td>JTT</td>
<td>Jebsen Taylor Hand Function Test</td>
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<td>LRP</td>
<td>Lateralised readiness potential</td>
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<tr>
<td>LTP</td>
<td>Long-term potentiation</td>
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<td>mA</td>
<td>Milliamp</td>
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<tr>
<td>M1</td>
<td>Primary motor cortex</td>
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<td>MEP</td>
<td>Motor-evoked potential</td>
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<td>MoCA</td>
<td>Montreal Cognitive Assessment</td>
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<td>MRS</td>
<td>Modified Rankin Scale</td>
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<tr>
<td>NMDA</td>
<td>N-methyl d-aspartate</td>
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<tr>
<td>RT</td>
<td>Reaction time</td>
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<tr>
<td>SMA</td>
<td>Supplementary motor area</td>
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<tr>
<td>SRTT</td>
<td>Serial reaction time task</td>
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<td>SSRT</td>
<td>Stop-signal reaction time</td>
</tr>
<tr>
<td>SST</td>
<td>Stop-signal task</td>
</tr>
<tr>
<td>TA</td>
<td>Tibialis anterior</td>
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<tr>
<td>tDCS</td>
<td>Transcranial direct current stimulation</td>
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<tr>
<td>TEP</td>
<td>TMS-evoked potential</td>
</tr>
<tr>
<td>TMS</td>
<td>Transcranial magnetic stimulation</td>
</tr>
<tr>
<td>µA</td>
<td>Microamp</td>
</tr>
<tr>
<td>µV</td>
<td>Microvolt</td>
</tr>
<tr>
<td>VEOG</td>
<td>Vertical electro-oculogram</td>
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Thesis Overview

Transcranial direct current stimulation or tDCS is a non-invasive brain stimulation technique which generates a weak electrical field between two electrodes placed on the scalp. Depending on the placement of the electrodes, this electrical field has been shown to depolarise or hyperpolarise underlying cortical areas. Research showing that tDCS can facilitate motor excitability has led to interest in tDCS as a possible clinical intervention technique for a number of pathologies including Parkinson’s disease and post-stroke rehabilitation. However, as interest in the technique has been increasing, so has the number of studies that show no improvement of performance following the application of tDCS. At present, there is still much we do not know about how tDCS affects cortical excitability, and how these changes impact motor performance.

The aim of this thesis is to investigate the effects of anodal tDCS over the motor cortex on response processes in both healthy younger and older adults, as well as in chronic stroke patients. Although research of the effects of transcranial direct current stimulation in both healthy and clinical samples is increasing, there is still much that is unknown about the mechanisms by which tDCS effects changes in the cortex. In this thesis, we specifically investigate the way in which motor cortex stimulation, which is the most targeted area of use for tDCS, affects specific response processes such as the preparation, selection or activation of a response. These mechanisms, while critical for the engagement of movement, have not yet been adequately investigated. Across four experiments, we assess the likelihood of beneficial changes in behavioural and electrophysiological performance following anodal tDCS over the motor cortex. To isolate these specific processes we have used a cued go/nogo paradigm with informative and non-informative cues. The use of these different cue types allowed the comparison of different levels of preparation, while the choice between two different target types on each trial allowed for assessment of response selection.
The structure of this thesis is as follows. Chapter 1 describes the properties of tDCS and then outlines the findings of previous experiments that have applied anodal tDCS over the motor cortex. These experiments are separated into a number of categories including those focusing on motor excitability, functional motor performance and also the performance on psychophysiological tasks following anodal tDCS over the motor cortex. There is then a description of the results of studies that have assessed the effectiveness of tDCS over the motor cortex in stroke patients. Following this Chapter 2 discusses the main electrophysiological waveforms (ERPs) that are associated with the response processes that are assessed in the experimental chapters. These ERPs are the contingent negative variation, which indexes response preparation, and the lateralised readiness potential which assesses both response selection and activation, depending on whether it is time-locked to the target or the response. There is also a description of the P300 peak, and its relationship with stimulus evaluation. This chapter ends with an investigation into the effects of the small number of studies that have assessed the impact of anodal tDCS on ERPs. This introduction is followed by four experimental chapters.

Chapter 3 examines whether anodal tDCS over the dominant or non-dominant motor cortex enhances the performance of healthy older adults on functional motor performance on the Jebsen Taylor Hand Function Test (JTT, Jebsen, Taylor, Trieschmann, Trotter & Howard, 1969) and grip force measures, and response speed on a cued go/nogo task. While anodal tDCS over the dominant motor cortex improved JTT performance, there was no impact of stimulation over the non-dominant motor cortex on the JTT. There was no effect of anodal tDCS of either motor cortex on both the grip strength tests, and the performance on the cued go/nogo paradigm.

Chapter 4 examines the behavioural performance of healthy younger adults following anodal tDCS over the dominant motor cortex using the cued go/nogo task. This chapter also
assesses whether there is an impact of the time between stimulation cessation and task completion on the effectiveness of anodal tDCS over the motor cortex. The effect of delay was assessed by comparing behavioural performance when stimulation was applied simultaneously, directly before or approximately 40 minutes before task completion. Analysis showed that there was no impact of anodal tDCS on behavioural performance of the young adults. Additionally, this pattern of results was consistent across all three timing conditions, indicating that there was no effect of the delay between stimulation cessation and task completion on the effectiveness of anodal tDCS. Bayesian analyses confirmed that null models were stronger fits for the data compared to models that included an effect of stimulation.

Chapter 5 examines the effects of anodal tDCS over the motor cortex on electrophysiological components associated with responding. Across two experiments we assess the effectiveness of anodal tDCS at enhancing response preparation, selection and activation in healthy younger (Experiment 1) and older adults (Experiment 2). The analyses of these two experiments display a failure of anodal tDCS over the motor cortex to enhance any response-related ERP waveform. Once again, Bayesian analyses confirm that models that include stimulation are weaker fits of the electrophysiological data compared to null effects models.

The last experimental chapter, Chapter 6, assesses whether there is an effect of tDCS on behavioural and electrophysiological performance of chronic stroke patients. The performance of the patients on the cued go/nogo was assessed following anodal stimulation of the affected motor cortex and also after cathodal stimulation of the unaffected motor cortex. Similar to the results in healthy subjects, neither anodal nor cathodal tDCS elicited changes to cued go/nogo performance that were significantly different from sham.

The final chapter, Chapter 7, is the general discussion of the results and the implications of the four experimental chapters. The consistent finding of null effects following
anodal stimulation over the motor cortex in all subject groups implies that anodal tDCS does not alter either behavioural performance or the processes that are involved in the generation of a response. Moreover, the results of the experimental chapters indicate that single session interventions utilising anodal tDCS over the motor cortex are not appropriate to elicit changes to response processes. In contrast future research should investigate whether multiple sessions, applied in conjunction with training, is able to produce enhancements to response processes.
Chapter 1: Effects of Transcranial Direct Current Stimulation over the Motor Cortex

1.1: Introduction to Transcranial Direct Current Stimulation

1.1.1: Description of Transcranial Direct Current Stimulation

Transcranial direct current stimulation (tDCS) is the non-invasive application of a weak electrical current to the surface of the neocortex. This current generates a diffuse electrical field between two or more rubber electrodes placed over the scalp (Utz, Dimova, Oppenlander & Kerkhoff, 2010). To maintain conduction and a constant current density across the electrodes, they are placed inside saline soaked sponges. The electrodes are typically placed so that one electrode acts as the stimulating electrode and the other acts as the reference, although this is not always the case. The reference electrode is usually placed over an area that is thought to be ‘inert’ in the specific context i.e., not relevant to the particular cognitive or motor processes of interest. For instance, for stimulation of the motor cortex, the reference electrode is often placed over the contralateral supraorbital region or the ipsilateral shoulder. The location of placement of the positive (anodal) or negative (cathodal) electrode determines the type of stimulation that is generated (Utz et al., 2010).

For research studying the effects of tDCS in humans, the current strength of the stimulation is typically below 5mA for safety reasons. The exact current strength used varies depending on the intended location of stimulation. For instance, as the hand motor area is on the surface of the cortex, studies looking at the effects of tDCS on hand actions typically use a 1mA current. In contrast, research looking at the effects of tDCS on foot activity uses a stronger current (e.g., 2mA) because the area of the motor cortex controlling foot movement is located deeper in the neocortex (Jeffery, Norton, Roy & Gorassini, 2007). More important
than the current strength is the current density, which is the strength of the current across the stimulating electrode. When applying a 1mA current through a 35cm$^2$ electrode, the current density will be $1/35^{th}$ in each cm$^2$, or 0.029mA/cm$^2$. This is the most common current density used in studies assessing the effects of tDCS of the motor cortex (e.g., first used by Nitsche & Paulus, 2000). Studies that use electrodes of a different size, typically alter the current strength so that the density stays at 0.029mA/cm$^2$.

When the tDCS current is switched on, some participants experience mild physiological sensations, such as a slight tingling or itching under the electrodes. This physiological sensation dissipates after a short period of time. Studies assessing the effectiveness of anodal tDCS usually compare the effects of the experimental or real stimulation condition to a control or sham stimulation condition (Utz et al., 2010). The sham condition usually consists of a very short ramp up of the current, no longer than 30 seconds, and then the current is switched off. This short period of stimulation gives the participant the experience of these physiological symptoms, without the longer-lasting effects produced by longer periods of stimulation. The presence of the physiological sensations in both the stimulation and sham conditions allows single-blinded administration of stimulation, as participants are unable to distinguish between active and sham stimulation sessions.

1.1.2: Animal Studies

A definitive effect of DC currents on cortical activity was first observed in animal studies. The effect of electrical stimulation of the neocortex on neuronal activity was found to depend on the polarity of stimulation and the depth of the neuron. In the cat brain, anodal stimulation increased whereas cathodal stimulation reduced the activity recorded from surface neurons (Creutzfeldt, Fromm, & Kapp, 1962). This effect was reversed for deeper neurons; anodal currents inhibited and cathodal currents increased activity of pyramidal tract neurons. When electrodes were placed over the surface of the somatosensory cortex of the rodent, short
periods of stimulation produced changes on the evoked potentials at the forepaw both during the stimulation period, and for a period after the current had been terminated (Bindman, Lippold & Redfearn, 1964). For instance, a stimulation period of at least 5 minutes produced an effect for up to 5 hours, regardless of polarity.

Anodal stimulation by ventrolateral thalamic stimulation produced greater depolarisation or increased firing rate of cells in the motor cortex, while cathodal stimulation increased hyperpolarisation, which decreased neuronal firing rate (see Figure 1.1). The rate of action potentials was associated with a more excitable motor cortex following anodal stimulation (Purpura & McMurty, 1964). Anodal stimulation of the cortex also enhanced the amplitude of the evoked potentials compared to the baseline recordings measured at the forepaw. In contrast, evoked potential amplitude was reduced following cathodal stimulation, compared to baseline recordings (Bindman, Lippold & Redfearn, 1964; Purpura & McMurty, 1964). These changes to motor output have been replicated in more recent work. For instance Cambiaghi et al. (2010) applied a current of 250µA for 10 minutes over the motor area and showed that motor evoked-potential (MEP) amplitude increased following anodal stimulation and decreased following cathodal stimulation. In summary, in animal studies, polarising currents have been shown to consistently produce changes in both neuronal firing rate and motor output.
Animal studies have also shown that stimulation of the neocortex produces changes to the physiology of the neurons. Islam, Aftabuddin, Moriwaki, Hattori & Hori (1995a) reported that when 3µA of anodal stimulation was applied for 30 minutes over the sensorimotor area of the rat brain, it produced an increase in calcium ions (Ca\(^+\)) in the grey matter. The increase in Ca\(^+\) remained significant at 24 and 72 hours post-stimulation. Using similar parameters, Islam et al. (1995b) reported that anodal stimulation increased the expression of the N-methyl-d-aspartate (NMDA) dependent protein c-FOS for at least 24 hours. This stimulation effect was abolished when animals were pre-administered the NMDA antagonist MK-801. Thus anodal stimulation of sensorimotor cortex appears to affect activation of NMDA. Given the association between NDMA and neuronal learning, the increase in NMDA expression found following anodal stimulation gave rise to the theory that stimulation may influence long-term potentiation (LTP), a short-term learning mechanism. More recent animal studies have examined mediators of this LTP effect.
Fritsch et al. (2010) examined the effectiveness of direct current stimulation coupled with low synaptic activation on selected mutant mice. The effectiveness of anodal direct current stimulation was greatly reduced in mice with deficiencies in brain-derived neurotrophic factor (BDNF), suggesting that BDNF may be involved in the activation of NMDA-receptor driven LTP. Animal models have suggested that tDCS may also affect cerebral blood pressure itself. Wachter et al. (2011) assessed the changes in cerebral blood flow in male rats for thirty minutes following either anodal or cathodal tDCS. Fifteen minutes of anodal tDCS increased cerebral blood flow, while cathodal tDCS for the same length of time decreased cerebral blood flow. This effect was seen as promising for possible clinical trials in stroke. The finding that application of weak electrical currents in animals could induce physiological changes related to LTP encouraged investigation into the properties of direct current stimulation in humans.

1.1.3: Proposed clinical benefits of tDCS

Research using tDCS has increased considerably over the past decade, with a recent PubMed search returning over 1700 articles. Much of this increase is due to growing interest in applying tDCS in a clinical or therapeutic setting. Indeed, tDCS has been proposed as a possible intervention for over a dozen major clinical conditions (for a review see: Tortella et al., 2015). Both anodal and cathodal tDCS have been applied over many different cortical areas, using a variety of montages. The most commonly stimulated areas are the dorsolateral prefrontal cortex and the sensorimotor cortex. tDCS over the dorsolateral prefrontal cortex has been investigated heavily for possible uses in the treatment of depression, as it has been proposed as a cheaper, less-invasive alternative to ECT (Brunoni et al., 2012). Application of tDCS over the motor cortices has garnered great interest for motor recovery and rehabilitation in clinical conditions such as Parkinson’s disease and stroke (Floel, 2014; Marquez, van Vliet, McElduff, Lagopoulos & Parsons, 2015). Broadly, application of tDCS to motor areas has produced more
consistent outcomes than tDCS over the dorsolateral prefrontal cortex (Jacobson, Koslowsky & Lavidor, 2012).

1.2: tDCS over the Motor Cortex in Humans

1.2.1: Effects on Motor Excitability - Initial Investigations/Breakthrough

Many studies in humans have examined the effects of tDCS over the primary motor cortex (M1) on motor excitability by measuring changes to the MEPs elicited by transcranial magnetic stimulation (TMS; e.g. Nitsche & Paulus, 2000). This methodology allows comparison with earlier animal work as it produces a measure of motor output. With this technique, the effect of a TMS pulse over the hand motor area on the electromyographic signal (EMG) from the corresponding hand muscles can be measured both during and following tDCS stimulation.

The application of tDCS over M1 in young adults has been shown to produce changes to the excitability of hand muscles during stimulation and extending beyond the cessation of direct current. TMS-elicited MEPs recorded at the right abductor digiti minimi muscle (ADM) increased in amplitude during anodal tDCS over M1 compared to sham. In contrast, application of cathodal tDCS decreased the amplitude of MEPs (Nitsche & Paulus, 2000). In this seminal study, Nitsche and Paulus assessed the relative effectiveness of a number of different electrode montages and stimulation parameters in producing tDCS-induced change in motor output. The largest effect on motor output was obtained with a montage that included the stimulating electrode over M1 and the reference electrode over the contralateral supraorbital region with 35cm$^2$ electrodes and a stimulation current of 1mA (see Figure 1.2). This montage has since become the standard for most studies on the effects of tDCS of the motor area.

1 This section describes mainly the online application of anodal tDCS. A more detailed examination of offline effects is found in section 1.2.4 of this chapter.
Consistent with the findings of animal studies, Nitsche and Paulus (2000) found that longer stimulation periods produced longer-lasting post-stimulation effects. Applying tDCS for a short period of time, such as 5 minutes, induced changes in the MEP responses elicited by TMS that lasted for another 5 minutes after the current was switched off. Sustained anodal tDCS over the dominant M1 for at least 13 minutes, increased MEP amplitude at the right ADM for more than one hour (Nitsche & Paulus, 2001). Cathodal tDCS over M1 for 9 minutes produced a decrease in MEP amplitude at the ADM for a similar length of time (Nitsche et al., 2003c). These longer duration excitability effects for both anodal and cathodal tDCS were replicated following a 10 minute stimulation session of the dominant M1 (Furubayashi et al., 2008) and have been reported on MEPs recorded from the leg as well as the hand (Jeffery et al., 2007). Following 10 minutes of 2mA anodal tDCS, the amplitude of evoked potentials recorded at the tibialis anterior (TA) were significantly larger compared to sham for at least one hour post-stimulation (Jeffery et al., 2007). Both the MEP amplitude and the duration of the effect following the cessation of tDCS replicated findings in animals (Bindman, Lippold & Redfearn, 1964; Purpura & McMurty, 1964). The fact that these post-stimulation effects
extended temporally with longer periods of stimulation indicates that tDCS over the motor cortex may be doing more than just shifting the membrane potential of interneurons (Nitsche & Paulus, 2001; Nitsche et al., 2003c).

1.2.2: Neural basis of tDCS effects - Pharmacological studies

A number of studies have examined the effects of tDCS on neurotransmitter activity at the synapse. This is done by administration of neurotransmitter agonists or antagonists (e.g., dopamine (DA) and NMDA) prior to administration of tDCS and examining effects on MEP amplitude. Generally, the effect of tDCS on MEP amplitude was abolished by the addition of either an NMDA antagonist or a synaptic channel blocker, indicating that tDCS has a strong effect on signal transmission at the neuronal level (Nitsche et al., 2009). The effect of anodal tDCS over the left M1 on MEP amplitude recorded from the right ADM was also eliminated following oral administration of sodium and calcium ion channel blockers, which cause interference of signal transmission at the synapse (Nitsche et al., 2003a). These pharmacological antagonists abolished both simultaneous and after-effects of stimulation. In separate studies, the after-effects of anodal tDCS were eradicated by the NMDA receptor antagonist dextromethorphan (Nitsche et al., 2003a) and the DA antagonist sulpiride (Nitsche et al., 2006). These results indicate that tDCS strongly affects DA and NMDA activity at the synaptic level in the motor cortex, and that both these neurotransmitters are responsible for maintaining the effect of anodal tDCS in the neocortex post-stimulation. Both DA and NMDA are important neurotransmitters in the brain and are heavily involved in synaptic plasticity and short-term learning. The fact that the increase in motor potential amplitude induced by anodal tDCS may be mediated by NMDA activity suggests that anodal stimulation may have LTP-like effects (Nitsche et al., 2003a).

The effects of neurotransmitter agonists on anodal tDCS have also been investigated. Oral administration of either D-Cycloserine (CYC), an NMDA agonist (Nitsche et al., 2004a), or
citalopram, a serotonin agonist (Nitsche et al., 2009), enhanced the post-stimulation effects of anodal tDCS. Specifically, the amplitude of TMS-elicited MEPs at the right ADM was larger following administration of citalopram and lasted longer following ingestion of CYC. Nitsche and colleagues attributed these effects to two different mechanisms: CYC by modulating NMDA receptors at the synapse (Nitsche et al., 2004a), and citalopram by facilitating calcium at the synapse (Nitsche et al., 2009).

Nitsche and colleagues also examined the effects on neural inhibition on changes caused by anodal tDCS (Nitsche et al., 2004b). As γ-amino-butyric acid (GABA) is the main inhibitory neuron in the brain (Connors, Malenka & Silva, 1988; Trepel & Racine, 2000), it was expected that the short-acting GABA agonist lorazepram would act similarly to the DA and NMDA antagonists, abolishing the post-stimulation effects. Contrary to expectations, the GABA agonist delayed the onset but did not abolish post-stimulation effects. This finding indicates that the effects of tDCS on neurotransmitter systems is varied and raises the question of whether the post-tDCS effects show dose-dependent effects or a consistent response. Fresnoza, Paulus, Nitsche and Kuo (2014) examined this issue using low, medium and high dosages of the DA precursor L-DOPA. Compared to placebo, D1 receptor activation following tDCS produced an inverted U-shaped dose-response. These findings suggest that there is an optimal activation threshold for tDCS on neurotransmitter activity. Indeed, these pharmacological studies support the notion that anodal tDCS in particular may be beneficial at a clinical level for such conditions as depression and post-trauma recovery.

1.2.3: tDCS Studies on Motor Excitability

The effects of tDCS over M1 on spinal cord excitability and inter-hemispheric inhibition have been examined in order to define response pathways affected by tDCS (Nitsche et al., 2005). The post-stimulation effects of tDCS on spinal cord excitability were investigated using TMS-evoked MEPs elicited at rest and during activity. Measures of resting or active motor threshold
were recorded by EMG at the right abductor digiti minimi in the hand (Nitsche et al., 2005), and also by the quadriceps H-reflex recorded at the tibialis anterior in the leg (Roche, Lackmy, Achache, Bussel, & Katz, 2012). In contrast to MEP amplitude changes, tDCS caused no change in either resting or active thresholds compared to sham. Indeed, Roche et al. (2012) found decreased facilitation of the H-reflex at the tibialis anterior following anodal tDCS compared to sham, suggesting that anodal tDCS can elicit facilitation of the lumbar spinal network.

In contrast to the many studies that report effects of anodal tDCS over M1 on motor excitability, a number of recent studies have reported findings that contradict the anodal excitation/cathodal inhibition hypothesis (Jacobson, Koslowsky & Lavidor, 2012). Antal, Terney, Poreisz and Paulus (2007) found that anodal tDCS administered before either a cognitive or a motor task resulted in decreased MEP amplitude compared to either sham or concurrent anodal tDCS and motor task completion. Cathodal tDCS produced similar effects. Batsikadze, Moliadze, Paulus, Kuo and Nitsche (2013) found that compared to sham, 2mA cathodal tDCS over M1 increased MEP amplitude whereas 1mA cathodal tDCS over M1 decreased MEP amplitude. These studies suggest that the effects of tDCS over M1 on motor excitability are variable and complex. Indeed, a recent study by Wiethoff, Hamada and Rothwell (2014) examined variability of response to both anodal and cathodal tDCS over M1 in a group of fifty-three young adults. They looked at the likelihood that participants would elicit MEPs in response to either one or both the stimulation polarities. Approximately half the participants showed no effect to either anodal or cathodal tDCS, whereas the other participants responded to both anodal and cathodal tDCS. Interestingly, for these participants, both anodal and cathodal tDCS enhanced the recorded MEPs compared to baseline. Indeed, recent studies have shown that there could also be differences in the ability of tDCS over M1 to enhance performance across different age groups. Fujiyama et al. (2014) has found that older adults have a delayed effect of anodal tDCS compared to younger adults, with older
adults taking 30min longer to reach the same level of excitability as young adults reach immediately post-tDCS. Clearly, much work is still needed in order to establish tDCS protocols that produce reliable excitation or inhibition effects.

Nevertheless, based on the early research showing a change in motor output and neurotransmitter activity following the application of tDCS, there has been a swell of interest in using this technique in rehabilitation. In particular, the increased activity following anodal tDCS is seen as very promising for future use in a number of fields, most notably in trauma recovery (Hummel & Cohen 2006). However, changes to TMS-induced motor output do not necessarily imply direct changes in functional motor capacity. Research into the effects of tDCS on motor function has sought to determine the effectiveness of the technique in the clinical setting.

1.2.4: Functional Performance Following Anodal tDCS

Understanding how anodal tDCS affects motor control is a necessary foundation for developing a broader conceptual framework of how best to utilise tDCS in motor rehabilitation. One line of work has examined the effects of anodal tDCS on gross motor control in young adults. One test frequently used is the Jebsen Taylor Hand Function Test (JTT, Jebsen, Taylor, Trieschmann, Trotter & Howard, 1969) which examines the speed of completing a number of daily life skills, such as writing, feeding and grasping. In young, right-handed adults, anodal tDCS of the right M1 increased the speed of JTT completion with the left, non-dominant hand (Boggio et al., 2006). However, performance did not improve for the dominant hand following anodal tDCS of the left M1. This was attributed to a ceiling effect for dominant hand function in young healthy participants, who are at their peak motor ability (Oliviero et al., 2006). In contrast, older adults did show improvement of dominant hand performance on the JTT following anodal tDCS over the dominant M1 (Hummel et al., 2010; Zimerman & Hummel 2010), suggesting that age-related neuromuscular decline affects
dominant hand motor function making it amenable to tDCS (Wu & Hallet, 2005). Cogiamanian, Marceglia, Ardolino, Barbieri and Priori (2007) assessed the impact of anodal tDCS on muscular strength and endurance. Compared to sham, anodal tDCS of the non-dominant M1 in young adults increased isometric endurance of the contralateral upper arm. The motor output of the biceps brachii also increased as evidenced by increased MEP amplitude. Krishnan, Ranganathan, Kantak, Dhaher and Rymer (2014) found similar effects on force of the biceps brachii following anodal tDCS. Increased maximal contractions of the biceps brachii were recorded following anodal tDCS compared to sham. Tanaka, Hanakawa, Honda and Watanabe (2009) showed that anodal tDCS over M1 elicited similar enhancement of the pinch force of the first and second toes on the left leg.

Despite the above promising evidence, the timing of stimulation required to produce the greatest effects on gross motor performance is not clear. Kim and Ko (2013) looked at the effect of anodal tDCS over M1 on a voluntary grip exercise. Online stimulation (i.e., stimulation applied while participants completed the grip task) resulted in a larger MEP effect compared to baseline than offline stimulation (i.e. tDCS and task conducted consecutively). Conversely, Thirugnanasambandam et al. (2011) found that voluntary motor contraction during anodal tDCS interfered with, and in some cases abolished, the stimulation effect. And in another study examining anodal tDCS in younger adults, Bortoletto, Pellicciari, Rodella and Miniussi (2015) showed that the type of practice strategy had a larger effect on overall task performance than tDCS. Examining the effects of anodal tDCS on the speed of thumb movements, the researchers used two different practice routines, fast movements and slower movements. The results showed that participants responded faster following anodal stimulation when they trained with the slower movements. In contrast, if participants trained on the faster movements, they performed worse following anodal tDCS compared to sham. Despite this
discrepancy, the finding that anodal tDCS increases gross motor speed and strength in healthy adults is important as it supports the potential of clinical applications for tDCS.

1.2.5: Application of tDCS over M1 in stroke patients

Possibly the greatest interest of all the proposed clinical applications of tDCS is for the rehabilitation of patients following a neurological trauma, such as stroke. This proposed use of tDCS in stroke rehabilitation first emerged from studies showing the effects of stimulation on neurotransmitters that are involved in long-term potentiation (Nitsche et al., 2003a). With evidence that anodal may enhance LTP in healthy controls, it was reasonable to infer that tDCS intervention may produce similar improvements in stroke patients. There were two different theories about how tDCS over M1 may be able to benefit stroke patients. The first idea was that anodal tDCS over the affected M1 may directly boost excitation to the damaged cortical areas. The other proposed that the application of cathodal tDCS over the unaffected M1 may dampen the unaffected sensorimotor area and this would in turn increase the excitation of the affected cortex.

Perhaps, unsurprisingly, the majority of studies examining the effects of tDCS over M1 in stroke patients have focused on finding improvements in functional motor tasks following a single stimulation session. This is likely due to the fact that in patients, functional improvements offer the most direct benefit. The first study to assess the effects of tDCS over M1 on stroke patients was Hummel and colleagues (2005) who assessed performance on the JTT. Both anodal tDCS over the affected motor cortex and cathodal tDCS over the non-affected cortex produced faster completion of JTT in patients. A number of subsequent studies assessed the effects of anodal tDCS on motor excitability in stroke patients using TMS-elicited MEPs at the hand and leg muscles. Suzuki and colleagues (2012) assessed the output of hand muscles at the first dorsal interosseous (FDI) in stroke patients compared to healthy young adults. As in controls, patients who received 10 minutes of anodal tDCS over M1 showed
increased MEP amplitude, which lasted for 10 minutes post stimulation. Madhavan, Weber and Stinear (2011) found a similar increase in MEP amplitude at the paretic ankle of patients following anodal tDCS compared to sham.

However, similar to the studies in controls, not all studies investigating the effects of tDCS over M1 in stroke patients have produced such positive results. Hesse et al. (2011) found that patients who received either anodal tDCS over the affected M1 or cathodal tDCS over the unaffected M1 did not improve on the Fugl-Meyer Motor Assessment (FMS, Fugl-Meyer, Jääskö, Leyman, Olsson & Steglind, 1974) compared to a sham condition. Likewise, Rossi, Sallustiom Di Legge, Stanzone and Koch (2013) found that there was no beneficial effect of anodal tDCS over the affected M1 on motor performance compared to sham. The researchers assessed performance on the FMS following the intervention and three months following completion of the study, with no differences in improvement observed between active and sham stimulation conditions. These mixed results across patient studies also support the need for greater understanding of the underlying mechanisms of tDCS. This need has led to examination of the effects of tDCS over M1 in psychophysiological experiments.

1.2.6: Psychophysiological Performance Following Anodal tDCS

A growing number of studies have examined the effects of tDCS on experimental tasks that are sensitive to response accuracy and speed, as well as learning effects. The first study to assess these properties was by Nitsche and colleagues (2003b), who assessed the effects of anodal and cathodal tDCS over the dominant M1 on response speed using a serial reaction-time task (SRTT). This task involves the completion of a series of practiced or randomised finger-tapping sequences. Guided by a visual prompt to select the appropriate finger to respond, participants had to complete a sequence of 12 finger strokes as quickly and accurately as possible.

Compared to either cathodal or sham, anodal tDCS decreased overall completion time, showing a beneficial effect on performance. Using a similar task, Vines, Nair and Schlaug (2006, 2008)
assessed the effects of anodal and cathodal tDCS over the dominant and non-dominant cortex. However, in contrast to Nitsche et al. (2003b), they measured response accuracy rather than response speed. The results displayed that, compared to sham; anodal tDCS of either dominant or non-dominant M1 increased the number of accurately completed sequences for the contralateral hand, but reduced accuracy for the ipsilateral hand. In contrast, cathodal tDCS of dominant M1 improved accuracy only for the ipsilateral (non-stimulated) left hand. The authors concluded that tDCS may influence transcallosal inhibition in the neocortex by altering the excitability of the stimulated M1 compared to the unstimulated M1 (Vines, Nair & Schlaug, 2008). Zimerman et al. (2013) reported that young and older adults showed a similar pattern of improvement on the SRTT following anodal tDCS over the dominant M1. However, the decrease in performance following cathodal tDCS over the ipsilateral M1 was much stronger for older as compared to younger adults (Zimerman, Heise, Gerloff, Cohen & Hummel, 2014). The researchers stated that this finding was consistent with evidence that older adults recruit bilateral cortical activation for tasks that are largely ipsilateral in young adults (Cabeza, 2002). Thus, the ipsilateral M1 is more involved in motor performance in older adults compared to younger adults.

Other studies have assessed the effects of multiple sessions of anodal tDCS over M1 on longer term skill acquisition. Young adults received consecutive daily stimulation sessions of either sham or anodal tDCS across a five day period, in conjunction with training on a visually directed pinch grip task (Reis et al., 2009; Schambra et al., 2011). This task involved using a visual prompt to apply a level of force to a pinch dynamometer. When the optimal force was applied, the trial would move onto the next trial. Skill acquisition was assessed as the level of change in both speed and accuracy across the five days. Anodal tDCS over dominant M1 produced greater improvement in skill acquisition at day 5 compared to sham (Reis et al., 2009). However, in a follow-up by Schambra and colleagues (2011), when anodal tDCS was
applied over the non-dominant M1, there was no improvement in skill acquisition rate compared to the sham condition. Another study also found no improvement at the follow-up session. Using a typing-task which consisted of repeated patterns of keystrokes on both hands, Gomes-Osman and Field-Fote (2013) showed that consecutive sessions of anodal tDCS over a 5-day training period improved performance compared to sham. However, this effect was not retained a week later. A study by Zimerman and colleagues (2013) assessing SRTT performance showed that these differences could be age-related. While both younger and older adults improved in SRTT performance following anodal tDCS over M1, these improvements were only retained 24 hours later for the older adults. In response to these discrepancies between studies, Saucedo Marquez, Zhang, Swinnen, Meesan & Wenderoth (2013) assessed whether the skill learning effect was task-dependent, by comparing the effect of anodal tDCS of the M1 over three consecutive days on the serial reaction time task and the visually-directed pinch task. Indeed, the effects of anodal tDCS differed between the two tasks: the serial reaction time task showed greater cumulative enhancement in performance whereas the pinch grip task did not. The authors concluded that the effect of anodal tDCS on performance and skill acquisition varies across different tasks. This possible task-dependent nature of improvements has been assessed more extensively in recent studies examining psychophysiological response processes.

These recent studies have looked at the effects of tDCS on well-known experimental paradigms that target specific cognitive and motor processes. In contrast to much of the earlier work on motor excitability output, these studies have produced discrepant findings regarding a beneficial effect of tDCS. Indeed, a number of these studies call into question early findings. Leite, Carvalho, Fregni and Gonçalves (2011) assessed the effects of tDCS on set-switching performance. Set-switching measures the ability to flexibly shift between different task-sets or contextual rules (for a review of task-switching literature see, Jamadar, Thienel &
The researchers examined the effects of anodal and cathodal tDCS over both the dominant M1 and the left dorsolateral prefrontal cortex (DLPFC) while participants switched between two visual cued tasks. On the cognitive task, participants were prompted by a visual cue to respond to either different shapes (square and triangle) or colours (blue and red), and to ignore distractor stimuli (hexagon or green). A trial consisted of two stimuli being presented in sequence; a repeat or shift trial was determined by whether the two stimuli were congruent or incongruent. The motor task consisted of the repetition of pre-learned motor sequences initiated by a visual prompt. Anodal tDCS of either the dominant M1 or the left DLPFC decreased RT on the cognitive task compared to sham. However, on the motor task, there was no difference in RT between anodal tDCS and sham, or an effect of stimulation on response accuracy. The authors concluded that anodal tDCS over either M1 or DLPFC may improve context updating processes.

Inconsistent effects have also been reported recently on 2-choice reaction tasks. In young adults, Pellicciari, Brignani and Miniussi (2013) found that improved performance on a choice RT task following anodal tDCS over the left M1 was due to practice, not stimulation. Specifically, following cessation of stimulation, participants’ response times decreased substantially, regardless of whether they had received active or sham intervention. Lindenberg, Nachtigall, Meinzer, Sieg and Floel (2013) assessed the effect of anodal tDCS over M1 on choice RT in healthy older adults. Using a three-choice go/nogo task, they found no choice RT difference between the active and sham stimulation conditions.

A few studies have used the stop-signal task (SST) to assess the effect of anodal tDCS on response inhibition. The SST involves the auditory or visual presentation of a stop stimulus shortly after the onset of the go stimulus that is mapped to a response. As the participant has already begun to prepare or execute their response before the onset of the stop signal, it allows the assessment of a participant’s ability to inhibit a prepotent response. By
manipulating the delay between go and stop onsets, it is possible to determine the individual’s inhibition threshold. As the supplementary motor area (SMA) has been shown to be involved in response inhibition, the three studies that have assessed the effects of anodal tDCS on the SST have looked specifically at the effects of stimulation over the SMA and the pre-SMA. These studies have found inconsistent effects of tDCS on response inhibition. Kwon & Kwon (2013) found that anodal tDCS over M1 or pre-SMA reduced stop-signal reaction time (SSRT) which is indicative of improved response inhibition. Hsu et al. (2011) found no effect of anodal tDCS over the pre-SMA on SSRT, but that participants had fewer errors on stop trials following anodal tDCS compared to cathodal tDCS or sham. Hadyuk-Costa, Drummond and Carlsen (2013) found that anodal tDCS over the SMA not only did not improve response speed, but also increased false alarm rate on stop trials.

In summary, these findings are not consistent with earlier work by Nitsche et al. (2003b) and Vines et al. (2008) who found faster and more accurate responding following anodal tDCS of M1. Overall, compared to the studies that measure MEPs, the effects of tDCS on performance on experimental tasks are very inconsistent. However, recent work has also questioned the consistency of the effects of tDCS over M1. In a recent meta-analysis, Jacobson, Koslowsky and Lavidor (2012) showed that the anodal excitation/ cathodal inhibition pattern of effects was not found in a third of the studies examined. A more recent systematic review by Horvath, Forte and Carter (2015a) challenged the validity of tDCS work further, pointing to the lack of consistency of tDCS effects outside the area of MEP modulation. These are disconcerting conclusions, especially given the broad interest in respect to clinical applications of tDCS. They highlight the fact that, before anodal tDCS is administered clinically; we need to define the mechanisms by which tDCS affects sensory, cognitive and motor processes. One technique which may be useful to identify these mechanisms is
electroencephalography (EEG), and in particular the analysis of event-related potentials (ERPs) recorded during task performance.
Chapter 2: Electroencephalography and Event-Related Potentials

Electroencephalography (EEG) records cortical electrical activity from electrodes placed over the scalp. The recorded electrical signals are derived from the summation of signals from a large number of neurons from the upper layers of the cerebrum (Murakami & Okada, 2006). EEG recordings have high temporal resolution, detecting millisecond changes in electrical activity. This makes the EEG signal ideal for examining the temporal dynamics of cortical processes that contribute to an overt behaviour. In psychophysiological experiments, one of the more common techniques used to examine changes to these cortical processes is to examine event-related potentials.

Event-related potentials (ERPs) isolate the electrical signature associated with different sensory, cognitive and motor processes by averaging EEG activity over a short interval time-locked to the triggering event (i.e., cue, target or response). By time-locking the waveforms to a particular task-based event, the waveforms convolve to highlight the changes in electrical activity that happen in the lead-up and in response to that particular event (for an overview see Luck, 2012). By manipulating task properties, researchers have been able to examine link changes to specific ERPs to distinct sensory, cognitive and response processes. ERPs have been used to identify a number of different types of cortical processes from very simple perceptual responses to different stimulus properties to higher order cognitive processes indicating the updating of rules or task-sets between trials. ERPs have also been able to identify differences in electrical morphology between healthy participants and subjects suffering from a number of different medical or psychopathologies. This chapter is specifically focusing on a number of ERPs waveforms that are related to motor preparation, stimulus processing, response selection and response generation. These components are all activated in the cued go/nogo paradigm that is used in the current thesis to investigate the mechanisms by which anodal tDCS affects motor performance.
2.1: Contingent Negative Variation

The contingent negative variation (CNV) is a slow negative shift that is associated with the preparation of a motor response (Walter, Cooper, Aldridge, McCallum & Winter, 1964; Kononwicz & Penney, 2016). It is typically elicited in S1-S2 paradigms, where the cue (S1) validly predicts the time of presentation of a target that will require a response (S2). As shown in Figure 2.1, around 500ms following cue onset, a gradual negative deflection begins and continues to increase up to the presentation of the target. The CNV typically consists of two subcomponents. The early CNV is a short, quickly rising deflection that follows from the preceding positive peak. The late CNV shows a longer more gradual increase in negativity leading up to target onset. These components will now be discussed in more detail.

![Figure 2.1](image)

Figure 2.1: Event-related potential derived from experimental data from our lab. This ERP is adapted from the cued go/nogo task which is used in the experimental chapters of this document (Chapters 3-6). At around 500ms following the onset of the cue, the CNV waveform begins. This deflection becomes more pronounced in the lead-up to target onset, as a result of greater anticipation or preparation of the upcoming target. Following the target onset the P300 is elicited, the latency and amplitude dependent on task properties.
2.1.1: Components of the CNV

The amplitude of the CNV waveform is associated with response speed, with larger CNV amplitudes resulting in faster responding compared to waveforms with reduced negativity (Falkenstein, Hoormann, Hohnsbiern & Kleinsorge, 2003). The amplitude of the CNV is affected by the contextual information given by cue and the expectancy that this generates in anticipation of the target presentation (Brunia, 1988). These two additive processes have been broadly mapped to the early and late CNV components. Topographically, the early CNV is maximal over the frontocentral negativity and can temporally overlap with the cue-related parietal positivity, while the later waveform is more centrally concentrated with a slower build up prior to target onset (Leuthold, Sommer & Ulrich, 2004; Los & Heslenfeld, 2005). The early CNV is associated with orientation to the contextual information contained in the cue (Rohrbaugh, Syndulko & Lindsley, 1976). It is sensitive to the strength of the contingency between S1 and S2, with cues that are neutral or invalid producing lower negativity and slower RT than valid, meaningful cues (Rockstroh, Elbert, Canavan, Lutzenberger, Birbaumer, 1989; Leuthold, Sommer & Ulrich, 2004; Brunia, van Boxtel & Bocker, 2012). The late CNV wave is related to motor preparation, i.e., using the information in the cue to selectively prepare for the upcoming target (Leuthold, Sommer & Ulrich, 2004). Structurally, the CNV is thought to result from excitatory postsynaptic potentials at pyramidal neurons in the primary and secondary motor areas (Brunia, van Boxtel & Bocker, 2012). The early section of the waveform is associated with the recall of stimulus context in the SMA and pre-SMA, whereas the later section of the waveform is associated with task-dependent preparation at M1 (Leuthold, Sommer & Ulrich, 2004; Leuthold & Jentzsch, 2009).

2.1.2: Task Properties and the CNV

The amplitude of the late CNV is influenced primarily by variations in the experimental paradigm that affect either event preparation or temporal preparation (Leuthold, Sommer &
Event preparation refers to preparation in the context of uncertainty about the type of response that will be required. Event preparation is determined by the amount of target information provided by the cue, as well as the strength of the relationship between the cue and the target (Rockstroh et al., 1989, Leuthold, Sommer & Ulrich, 2004). Cues can provide either partial or full information about the response required to the upcoming target, thereby determining the level of preparation that can be effected (Wild-Wall, Sangals, Sommer & Leuthold, 2003). Fully informative cues that predict the upcoming response result in larger early and late CNV amplitudes compared to partially informative cues or neutral cues (Wild-Wall et al., 2003; Leuthold, Sommer & Ulrich, 2004; Los & Heslenfeld, 2005; Leuthold & Jentzsch, 2009). In addition, the inclusion of distractor cues reduces the amplitude of the late CNV compared to control trials (Travis & Teece, 1998).

Temporal preparation refers to the level of certainty about the timing of the response. Temporal preparation is determined by the length of the interval between the cue and the target (cue-target interval: CTI) and affects amplitude of the late CNV wave as well as the speed and accuracy of responding. Indeed, trials with short cue-target intervals (CTIs, 400ms) produce slower RT and more errors compared to trials with long CTIs (1200ms, Los & Heslenfeld, 2005). Trillenberg, Verleger, Wascher, Wauschkuhn & Wessel (2000) found that CTIs greater than 1300ms can produce the two separate CNV subcomponents, but at shorter CTIs, the two components tend to be overlap (Rockstroh et al., 1989). Trillenberg and colleagues also found that CNV amplitude is larger at longer compared to shorter CTIs (1200 vs 1950 and 2600ms). They concluded that the longer CTI allowed for greater motor preparation. Falkenstein et al. (2003) examined whether there is a lower CTI threshold for CNV development, by comparing CNV amplitude for four CTIs: 300, 600, 900 and 1200ms. The late CNV waveform peaked prior to target onset and had a similar peak amplitude for the three longer CTIs. However, for the 300ms CTI, the CNV peaked after target onset, around 400ms.
post-cue. For the CTI of 600ms, the negative shift did not emerge until 350-400ms post-cue. This indicates that a CTI of around 400ms is needed to elicit a CNV waveform, and thus to engage in effortful motor preparation. In sum, the CNV provides a reliable electrophysiological measure of context and response preparation processes.

2.2: Lateralised Readiness Potential

The bereitschaftspotential or readiness potential (BP) is a sharp negative potential that is elicited immediately prior to a voluntary motor response over the contralateral motor cortex (Deecke, Scheid & Kornhuber, 1969; Deecke, Grozinger, Kornhuber, 1976). The BP is characterised as a sharp negative increase that occurs roughly 400ms prior to the initiation of voluntarily movements (Shibasaki & Hallet, 2006). The lateralised readiness potential (LRP) is extracted by averaging activity at electrodes over the left and right motor cortex for right and left-hand responses, respectively, thereby removing response-unrelated activity (Coles, Gratton & Donchin, 1988). The LRP is an index of the motor processes generated primarily in the contralateral M1 for hand movements (Coles, 1989). The LRP waveform can be extracted from EEG signal time-locked to the onset of either the stimulus or the response (Smulders & Miller, 2012). These two waveforms focus on different processes that will now be discussed.
2.2.1: Stimulus Locked Lateralised Readiness Potential

The stimulus-locked LRP (sLRP) measures the duration of premotoric processes leading up to response selection (Figure 2.2; (Osman, Moore & Ulrich, 1995; Masaki et al., 2004). The waveform encompasses a time interval between the presentation of the target and the onset of the negative-going deflection. The onset latency of the sLRP is regarded as the end point of premotoric processes or the point at which a response is selected (Smulders & Miller, 2012). Thus trials with earlier sLRP latencies are associated with easier, more efficient response selection processes compared to trials with later sLRP latencies. In addition, the onset of the sLRP is highly correlated with mean response time (Muller-Gethmann, Ulrich & Rinkenauer, 2003). The sLRP is affected by response conflict following the presentation of neutral or incongruent cues and targets. Using an Eriksen Flanker task, Gratton, Coles, Sirevaag, Eriksen and Donchin (1988) assessed the effect of response conflict on the LRP. The flanker task (Eriksen & Eriksen, 1974) involves the presentation of a target stimulus mapped to a lateralised response (such as < for a left hand response). In different conditions, the target is surrounded
on either side by flankers that are either congruent (<<<<), neutral (------) or incongruent (>>>>) to the central target stimulus. The flanking distractor stimuli on incongruent trials cause greater response conflict relative to the neutral or congruent trials, leading to slower responding. These incongruent trials elicit a small positive deflection before the waveform reverts to the strong negative sLRP deflection (Gratton et al., 1988). This early positive deflection or “dip” is associated with the initial preparation of an incorrect response followed by activation of the correct response (De Jong, Coles, Logan & Gratton, 1990). This positive dip has also been elicited by partially informative cues compared to fully informative cues (Eimer, 1995; Leuthold, Sommer & Ulrich, 1996).

![Response locked LRP waveform](image)

Figure 2.3: Response-locked LRP waveform derived from data from our lab.

### 2.2.2: Response Locked Lateralised Readiness Potential

The response locked LRP waveform (rLRP) is a measure of the duration of response execution processes, or the interval between the selection of the appropriate response and the overt physical response (Figure 2.3; Schroter & Leuthold, 2008). The rLRP waveform has been shown
to reflect effector-specific programming in M1 and premotor cortex (Wild-Wall et al., 2003, Masaki, Wild-Wall, Sangals & Sommer, 2004). Similar to the sLRP, the duration of the rLRP waveform is important. Longer durations between rLRP onset and the overt response are said to show less efficient response programming compared to waveforms with shorter durations (Smulders & Miller, 2012). In psychophysiological tasks, the onset of the rLRP varies as a function of task properties that increase or reduce response-level interference. With increasing levels of response difficulty, for instance from a single key response to a specific sequence, the duration of the rLRP waveform also increases (Smulders, Kok, Kenemans & Bashore, 1995). Similarly, increasing the number of possible responses also increases the duration of the rLRP waveform (Muller-Gethmann, Ulrich & Rinkenauer, 2003; Masaki et al., 2004). The level of response information provided in the cue can influence on the rLRP, with increasing information reducing the duration of the rLRP (Osman, Moore, & Ulrich, 1995; Muller-Gethmann, Rinkenauer, Stahl, & Ulrich, 2000).

2.3: The P300

The P300 is a large centroparietal positive deflection which peaks usually around 300ms after stimulus onset, but can peak as late as 800ms post-stimulus, depending on task requirements (Picton, 1992). The P300 is elicited in response to task-relevant stimuli and is sensitive to stimulus evaluation processes (Poilch & Kok, 1995). Specifically, the P300 is thought to index of contextual updating (Polich, 2003). The amplitude of the P300 is sensitive to a number of factors, including resource allocation, memory load and target probability (see Polich, 2012, for a review). P300 latency is thought to represent the speed of classification of the stimulus or event (Kutas, McCarthy & Donchin, 1977). As such, the latency of the P300 is therefore highly correlated with response speed, with longer latencies resulting in slower RT (Polich, 2007).

The amplitude and the latency of the P300 peak are both affected by a number of task properties. The amplitude of the P300 is affected by two additive factors, the probability of the
stimulus, and the difficulty of the task (Picton, 1992). Peak P300 amplitude generally increases with decreasing probability of task-relevant stimuli, but does not change in relation to distractors or irrelevant stimuli (Courchesne, Hillyard & Courchesne, 1977). Additionally, the amplitude of P300 peak is generally smaller and the onset latency is later for more difficult tasks compared to simpler tasks. These effects are thought to indicate the increased processing load needed to effectively evaluate the stimuli (Kahneman, 1973). The additive relationship of these factors indicates that rare, difficult stimuli will require greater processing compared to targets that are either easier or more common. The latency of the P300 is similarly affected by task difficulty, with harder tasks producing later peaks compared to easier tasks. The latency of the P300 is also modulated by the familiarity of the target, with shorter latencies to familiar as compared to unfamiliar stimuli (Johnson, Pfefferbaum & Kopell, 1985).

2.4: tDCS effects on ERPs

Few studies have examined the effects of tDCS on ERPs, though the majority of these have applied tDCS over the DLPFC not over M1. These studies have focused on facial processing (Kongthong, Minami & Nakauchi, 2013; Lafontaine, Theoret, Gosselin & Lippe, 2013), auditory processing (Knetchel et al., 2014), and inhibitory control (Lapenta, Minati, Fregni & Boggio, 2014). The effects of tDCS over DLPFC on ERP components have been mixed. Using a visual go/nogo task, Lapenta and colleagues found that anodal tDCS over the DLPFC decreased N2 amplitude and increased P3a amplitude to nogo targets. The N2 is a component associated with response conflict (for a review see, Folstein & van Petten, 2008), while the P3a is frontal positivity that occurs after 300ms in response to novelty stimuli (Polich, 2007). In response to nogo stimuli, the P3a is thought to reflect late motor inhibition (Gajewski & Falkenstein, 2013). These effects of anodal tDCS on ERPs were concluded to indicate that stimulation reduced response conflict following the presentation of a nogo target (Lapenta et al., 2014). Knetchel and colleagues (2014) found that anodal tDCS over the DLPFC increased N1 amplitude to
standards compared to targets on an auditory oddball task. However, there was no effect of stimulation on other ERPs waveforms including the P300. To date, there has only been one study that has assessed changes to the morphology of ERPs following anodal tDCS over the sensorimotor cortex (Pellicciari, Brignani & Miniussi, 2013). Pellicciari and colleagues used TMS to generate the ERPs, known as TEPs or TMS-evoked potentials. They found that, following anodal tDCS, TEP amplitudes increased in the ipsilateral hemisphere at frontocentral sites. However, the researchers did not examine how anodal tDCS over M1 affects the processes leading up to the muscular activity or the overt response to a presented stimulus. The rationale of this thesis is to answer these two key questions.

2.6: Conclusion – Rationale for papers

Despite an abundance of research into the properties of tDCS as a possible rehabilitation technique, there are still many gaps in our understanding of how it alters cortical activity. The focus of much of the previous research has been on measures that assess the effectiveness of anodal tDCS on the end point of the motor system activity. The earliest studies applying tDCS over the motor cortex assessed the response of the muscle using MEPs elicited by TMS (Nitsche & Paulus, 2000, 2001). Other studies have looked solely at the completion times of movements (Boggio et al., 2006). However the understanding of how anodal tDCS over M1 impacts the processes leading up to the overt movement are still very limited. This is unfortunate, as this knowledge is important for understanding how anodal tDCS can be used in clinical settings. The use of ERPs presents opportunities to uncover these effects, due to their high temporal resolution. As such it should be easy to isolate any changes in activity that occurs following anodal tDCS over M1 compared to sham.

To examine these ERPs discussed above, and also the associated response processes that they index, an experimental task should be used that can isolate and distinguish between each of the major processes. A conflict paradigm, such as a cued go/nogo task, is ideal to
compartmentalise these potentials (see Figure 2.4). As it is an S1-S2 paradigm, differential response preparation can be clearly measured by analysing the CNV during the CTI. The N200 and P300 peaks derived post-target onset can assess the determination of the stimulus processing and response inhibition processes. Additionally, by time-locking the analysis to the target or response, both target and response-locked LRPs can be extracted to examine response selection and activation. Therefore, the use of this task should be able to identify a beneficial effect of anodal tDCS over M1 on these ERP waveforms, which are directly relatable to these specific response processes.

This thesis is examining the effects of anodal tDCS over M1 on both behavioural responses and ERPs across three different subject groups. We assess the effects across healthy younger and older adults, as well as in chronic stroke patients. As such, we can compare whether the effect of anodal tDCS over M1 on response processes changes with both healthy ageing as well as with cortical trauma. This is an important component of this body of work, as older adults are rarely examined in tDCS literature. Rather, much of the prior literature has tried to directly extrapolate from the findings in younger adult groups to older clinical groups such as Parkinson’s disease or chronic stroke patients. As there are major cortical and neuromuscular changes that occur with healthy ageing that are not related to either disease or trauma (Wu & Hallet, 2005), this may not be the most appropriate comparison. Rather, examining the way in which anodal tDCS affects age-matched controls is a more appropriate reference point for a clinical sample. In this thesis, I first aim to understand the impact of anodal tDCS over M1 on response processes across healthy ageing, and then move on to examining a clinical sample. Therefore the first three experimental chapters focus on examining functional, behavioural and electrophysiological data across both stimulated and unstimulated hands in young and old adults, in order to comprehensively investigate how anodal tDCS affects response processes, as well as whether the effects of tDCS vary with age.
The first experimental chapter will focus on whether anodal tDCS enhances functional and behavioural performance of the dominant or non-dominant cortices in older adults. The results of the first experiment inform the direction of the second experimental chapter which expands on the behavioural changes following anodal tDCS, by assessing in younger adults whether enhancements by anodal tDCS are guided by the timing of stimulation and task performance. Following the examination of functional and behavioural performance, the third experimental chapter looks specifically at the response-related ERP waveforms to distinguish how anodal tDCS influences the processes leading up to the overt response. This detailed examination of the effects of tDCS over the motor cortex in the healthy older adults will provide a strong baseline against which to compare the effect in chronic stroke patients. As stated above, the use of response-related ERPs will allow us to identify the ways in which anodal tDCS enhances motor performance.
Figure 2.4: Cued go/nogo task used in all experimental chapters. The time course for (A) go and nogo trials in directional cue condition, and (B) go trials in non-directional cue condition. This paradigm is also later shown in Chapters 5 & 6.
Chapter 3: Anodal direct current stimulation in the healthy aged: effects determined by the hemisphere stimulated.²

3.1: Introduction

Transcranial direct current stimulation (tDCS) has been highlighted as a non-invasive method of modulating brain function. It has been consistently shown in healthy young adults that cortical activity can be temporarily altered by applying a weak continuous current between two electrodes positioned on the scalp. The effects depend on the position and polarity of the electrodes; specifically brain activity is increased by anodal stimulation and decreased by cathodal stimulation. The published beneficial effects are diverse and include improved: visuo-motor performance (Antal et al., 2004), implicit learning (Nitsche et al., 2003b; Kincses et al., 2004; Kang & Paik, 2011), procedural learning (Tecchio et al., 2010; Stagg et al., 2011), working memory (Zaehle, Sandmann et al. 2011), reaction time (Nitsche et al., 2003b), fine motor skills (Vines, Nair & Schlaug, 2006; Vines, Cerruti & Schlaug, 2008; Reis, 2009), functional performance (Boggio et al. 2006), and muscle endurance (Cogiamanian et al. 2007). Because it is portable, relatively inexpensive, and safe, there is a growing interest in utilizing tDCS in the management of several disease conditions which produce cognitive and movement dysfunction.

There is a paucity of research evaluating the effects of tDCS in the aged. The need for further research in this population is two-fold. Firstly, ageing is associated with an increased prevalence of disease conditions such as Stroke, Parkinson’s Disease and Alzheimer’s Disease.

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Extrapolating results from studies in young adults to patients with disease conditions prevalent in aged populations may not be valid given that both cortical structure and function change with age (Spreng, Wojtowicz & Grady, 2010). Ageing leads to alterations in the excitability of the motor cortex (Oliviero et al. 2006) which may impact on the effects of cortical stimulation. Furthermore, the comparison of movement related outcomes between different age groups may be invalid as studies have shown that the kinematics of limb movement is altered with age such that movement patterns become more rigid and reaction times are increased (Bennett & Castiello, 1995). Secondly, healthy aging is associated with a successive decline in cognitive and motor abilities which impair independent functioning (Burke & Barnes, 2006). It has been speculated (Zimerman & Hummel, 2010) that non-invasive brain stimulation may be able to ameliorate the decline in this population with obvious potential social and financial benefits.

To our present knowledge, only two clinical studies have examined the effects of anodal stimulation in the healthy aged. Hummel et al. (2010) and Zimerman et al. (2013) examined the effects of anodal tDCS applied over the motor cortex of older adults and demonstrated that upper limb functional performance could be improved in a manner consistent with the findings of younger patients. While these results are promising, they are limited in terms of generalisation as they only assessed the effects of dominant cortex stimulation on dominant hand function. As anodal stimulation is thought to increase excitation of the underlying cortex it is feasible that it may simultaneously decrease contralateral excitation via transcallosal inhibition, thus potentially impairing ipsilateral hand function. Similarly, behavioural effects of the cathode over the contralateral prefrontal cortex cannot be ruled out (Zimerman et al, 2013). Thus these preliminary positive findings warrant replication and more extensive study.
In this study, we used a double blind randomised controlled design to examine whether anodal stimulation of either hemisphere leads to improved performance of the contralateral hand and/or altered function of the ipsilateral hand. In addition, we examined movement preparation and selection using a cued go/nogo task while recording both behavioural and electroencephalography (EEG) data. Electrophysiologically, motor preparation is indexed by the contingent negative variation (CNV) component, indicating the level of readiness to respond to a predicted target (Leuthold, Sommer & Ulrich, 2004) and has been linked to the level of excitation in the supplementary motor cortex (Luck, 2005). Hence we can examine the effect of anodal tDCS on movement preparation by examining response times and CNV amplitude to prepared and unprepared responses following active and sham stimulation.

3.2: Methods

3.2.1: Subjects

Subjects were recruited from the Hunter Medical Research Institute volunteer register. We included 34 right handed subjects over the age of 40 years with normal physical and neurological functioning. The time in life when brain ageing begins is undefined, however genetic studies suggest measureable decline after the age of 40 years (Lu et al., 2004). Left handed subjects were excluded as laterality in the motor hand function tests might not be present in these subjects (Ozcan et al., 2004). Hand dominance was determined using the modified Edinburgh Handedness Inventory (Dragovic, 2004). Other exclusion criteria were: reduced cognitive functioning (i.e. a score of 24 or less on the Montreal Cognitive Assessment scale (Nasreddine et al., 2005), reported history of neurological disease or muscular dysfunction, psychiatric illness, use of CNS-acting medication, pregnancy, metal implants in the cranium or upper torso, unstable medical conditions, or skin lesions on the scalp.
3.2.2: Study design

Participants were allocated via computer generated randomization on a 1:1 ratio to one of two treatment orders: sham/tDCS or tDCS sham. They were then randomized to receive the intervention to either their dominant or non-dominant hemisphere. During each session, the assessment of function and strength was conducted prior to and immediately after the intervention. These assessments included the Jebsen Taylor Hand Function Test (JTT) - a validated timed test of seven functional tasks such as manipulating objects, writing, turning pages etc. (Jebsen et al., 1969) followed by key grip and pinch grip strength - maximal strength as measured by dynamometer.

Response processes were assessed using a cued go/nogo paradigm during which electrophysiological (EEG) data were recorded. This task included separate blocks of directional and non-directional cue blocks. All trials began with a small fixation cross which was followed after 500ms by the cue onset. The cue-target interval was 1500ms and the target stayed on the screen for 1000ms. The cue consisted of two white arrows pointing in opposite directions (<> for non-directional trials, and validly predicted the timing of target onset. The target was two green directional arrows in bold (<< or >>) that indicated the response hand. For directional trials, the cue consisted of two white arrows (>> or <<) that validly predicted the direction of the target arrows and therefore the required response. For 70% of trials the target was the predicted directional green arrows, identical to those in the non-directional cue condition. On the remaining 30% of trials the target was a red cross (x) indicating that the prepared response must be withheld (e.g. nogo trial). Participants were instructed to prepare a motor response with the hand indicated by the cue but wait until the target to emit the prepared response (go target) or withhold the response (nogo target). Participants completed three brief practice blocks prior to the intervention, and the task consisted of three blocks of the directional cues and two blocks of non-directional cues. The total duration of this testing
was 38 minutes and it occurred directly after the administration of the post-stimulation functional measures.

Both assessors and subjects were blinded to the type of intervention (sham/ anodal tDCS) which was applied in a cross-over sequence with a fixed washout period of three weeks. At the conclusion of each session, participants were asked to complete a questionnaire to indicate whether they believed they had received the active treatment or the sham condition and to document any adverse effects.

3.2.3: tDCS

Anodal tDCS was delivered using a commercially available, programmable, direct current stimulator (neuroConn DC-stimulator). Two saline-soaked, rectangular electrodes (35cm$^2$) were placed on the scalp with the anode positioned in the region over the primary motor cortex (centred on C3 for the dominant hemisphere and C4 for the non-dominant hemisphere) using the 10-20 electroencephalogram system. The correspondence of these surface areas to the primary motor cortices has been confirmed in neuroimaging studies (Herwig, Satrapi & Schonfeldt-Lecuona, 2003). The cathode was positioned on the contra lateral supraorbital region. This electrode arrangement is the most typically reported configuration for stimulating the cortical region which represents hand function (Floel & Cohen 2010; Hummel et al., 2010).

A current of 1mA was applied for 20 minutes. The stimulator was programmed to ramp up the current over several seconds to minimize discomfort. The participants were informed that they could expect to experience a tingling (but not unpleasant) sensation under the electrodes which would rapidly dissipate such that there was little or no physical perception of stimulation after approximately 2 minutes. The set up for the sham condition was identical with the stimulator programmed to turn off after the initial 30 seconds. This has previously been shown to be an effective sham condition which is indistinguishable from the true intervention (Hummel et al., 2005; Gandiga, Hummel & Cohen, 2006; Nitsche et al., 2008).
As several studies have demonstrated that the physiological state of the subject during stimulation can impede the effects of tDCS (Antal et al., 2007; Quartarone et al., 2004), subjects were instructed to sit quietly during the stimulation to avoid interference from cognitive or physical activity.

### 3.2.4: Data analysis

Demographic and disease characteristics of participants were compared between the intervention and control groups at baseline using Chi-square tests or Fisher’s exact test for characteristics with a small number of participants in some cells of cross-tabulations. The main functional outcome measure was the difference between a subject’s total score on the JTT before and after treatment for each stimulation condition. We also analysed the subscores of fine motor tasks (items 1 to 4) and gross motor tasks (items 5 to 7) on the JTT and both grip measures. The mean and 95% confidence intervals are reported for each intervention group (sham, tDCS) at each time point. The five motor function measures (total JTT score, gross and fine motor subscales of the JTT, and the two pinch-grip measures) were analysed using a four-way mixed-design analysis of variance (ANOVA), with one between subjects factor: Hemisphere of intervention (dominant, non-dominant) and three within subjects factors: Stimulation condition (anodal tDCS, sham), Hand (left, right) and Time (pre-, post-intervention). It is important to note that in these analyses an effect of anodal tDCS is represented in a significant stimulation x time interaction, i.e. greater improvement in responding from pre-intervention to post-intervention scores for active as compared to sham stimulation. Behavioural go/nogo task data were also analysed using a four-way mixed-design ANOVA with Hemisphere, Stimulation, Hand and Cue (directional, non-directional). To control for the effect of age on any significant effects we also re-ran these analyses including participants’ age as a covariate. Note that in these analyses, an effect of anodal tDCS is
represented in a significant stimulation main effect or interaction with other factors, as there was no pre-intervention assessment on the go/nogo task.

The EEG was continuously sampled at 2048 Hz/channel reference free using a BioSemi ActiView II system. Activity was recorded using a standard 64-channel montage as well as left and right mastoids, the supra-orbital and infra-orbital electrodes of each eye, and the two lateral orbital electrodes. Subjects were seated in front of a computer screen in a customised chair with a push button in each of the armrests. Continuous EEG files were re-referenced to average mastoids, and filtered at 0.02-30Hz. A 50Hz notch filter was used to remove line noise. EEG data were processed and analysed using EEG Display 6.3.12 (Fulham, 2012). EEG epochs were extracted from 500ms before cue onset to 1000ms after target onset and were over a 200ms interval prior to onset of the fixation cue. Mean amplitude of the late CNV was measured at the vertex (Cz) over 1300-1500ms after cue onset and was analysed using the same four-way mixed models ANOVA as the behavioural data.

3.3: Results

3.3.1 Participant characteristics

Demographic and clinical characteristics assessed included age, gender and cognition (MoCA). Average age was 61 years (range 41-86) with 19 males and 15 females. Age and gender were evenly distributed between the groups defined by the side of the cortex stimulated ($t = 0.61, P = 0.54; \chi^2 = 1.94, P = 0.16$). All MoCA scores were within normal limits (mean = 27.9, range 24-30) therefore no subjects were excluded from the analyses (Table 3.1). At baseline, all measures were consistent with age matched normative data (Jebsen et al., 1969).
Table 3.1: Demographic and Baseline Characteristics

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender</td>
<td>Males: 19 (56%)</td>
</tr>
<tr>
<td>Age</td>
<td>61.4 ± 12.2</td>
</tr>
<tr>
<td>MoCA</td>
<td>27.9 ± 2.0</td>
</tr>
<tr>
<td>JTT dominant hand</td>
<td>43.2 ± 7.7</td>
</tr>
<tr>
<td>JTT non-dominant hand</td>
<td>67.2 ± 13.5</td>
</tr>
<tr>
<td>Key grip dominant hand</td>
<td>18.2 ± 5.6</td>
</tr>
<tr>
<td>Key grip non-dominant hand</td>
<td>17.7 ± 4.7</td>
</tr>
<tr>
<td>Tip grip dominant hand</td>
<td>14.1 ± 4.1</td>
</tr>
<tr>
<td>Tip grip non-dominant hand</td>
<td>13.8 ± 4.0</td>
</tr>
</tbody>
</table>

Figures reported as mean ± standard deviations. JTT = Jebsen Taylor Test recorded in seconds, grip strength recorded as pounds per centimetre of pressure

3.3.2: Functional Motor Measures

**Total JTT:** As shown in Figure 3.1 (left), response time did not differ as a function of Hemisphere of intervention ($p>0.1$). JTT was completed faster with the right than with the left ($F(1, 32) = 455.09, p<0.001$). It was also completed faster post-intervention compared to pre-intervention ($F(1, 32) = 26.38, p<0.001$), indicating a significant practice effect. The significant interaction between Hand and Time ($F(1, 32) = 18.7, p<0.001$) indicates a greater improvement with practice for the left hand.

There was a significant interaction between Stimulation and Time ($F(1, 32) = 4.31, p=0.046$), indicating more improvement following anodal tDCS compared to sham. This improvement was significantly greater for the left compared to the right hand (Stimulation x Time x Hand: $F$...
(1, 32) = 7.9, \(p=0.008\). As shown in Figure 1 (left), this left hand advantage was evident regardless of whether stimulation was over the left or the right hemisphere. This is supported by the absence of any significant Hemisphere main effect or interaction. Age significantly affected total JTT score (\(F (1, 31) = 6.3, \ p=0.017\)), but did not significantly mediate the size of the Stimulation x Time effect.

**Figure 3.1:** Effects of (A) Dominant and (B) non dominant hemisphere stimulation on functional performance. Time (seconds) to complete total 7 items of Jebsen Taylor Hand Junction test (total JTT), fine motor items of JTT and gross motor items of JTT, pre and post stimulation.

**Fine motor JTT:** Figure 3.1 (centre) shows that fine motor JTT scores produced results compatible with those of the total JTT score. As above, responding was faster for right than left hand responses (\(F (1, 32) = 407, \ p<0.001\)) and post-intervention compared to pre-intervention (\(F (1, 32) = 20.1, \ p<0.001\)). The improvement from pre- to post-intervention was again greater for left than for right hand responses (\(F (1, 32) = 17.8, \ p<0.001\)). There was a significant main
effect of Stimulation ($F(1, 32) = 5.34, p=0.027$) and an interaction between Stimulation and Hand ($F(1, 32) = 6.47, p=0.016$). Although there was no Stimulation x Time interaction, the data in Figure 1 (centre) suggest that, like total JTT, stimulation improved performance for the left hand. Again, there was no effect of Hemisphere of stimulation.

**Gross motor JTT:** As shown in Figure 3.1 (right), gross JTT was faster for right than left hand ($F(1, 32) = 20.8, p<0.001$), post-intervention compared to pre-intervention ($F(1, 32) = 5.5, p=0.026$), and this practice effect was greater for left than right hand responses ($F(1, 32) = 4.6, p=0.04$). However, there was no effect of Stimulation or Hemisphere.

**Grip measures:** Grip measure scores are shown in Figure 3.2. There was no stimulation x time interaction on either measure.

**Figure 3.2:** Effects of (A) dominant and (B) non dominant hemisphere stimulation on grip strength. Force (pounds per centimetre of pressure, lbs) exerted using key grip and tip pinch grip, pre and post stimulation.
3.3.3: Go/Nogo Task Behavioural Results

Both dominant and non-dominant hemisphere stimulation groups responded faster to directional compared to non-directional cues ($F(1, 32) = 153.7$, $p<0.001$) consistent with use of cues to prepare a motor response. As evident in Figure 3.3, anodal tDCS stimulation did not reduce reaction time ($p>0.2$). In fact, for the dominant hemisphere stimulation group, stimulation appears to have increased reaction time, especially for directional cues. This is shown in the significant interaction between stimulation, cue and hemisphere group ($F(1, 32) = 6.99$, $p=0.013$).

![Figure 3.3: Reaction time (sham – active stimulation). Difference in reaction times between sham and active conditions (time in milliseconds) in response to directional and non-directional cues.](image)

_Sample Figure 3.3: Reaction time (sham – active stimulation). Difference in reaction times between sham and active conditions (time in milliseconds) in response to directional and non-directional cues._
3.3.4: Electrophysiological Results

The electrophysiological data of two participants were removed from the analysis: one because of a high level of artefact and the other because of a technical problem resulting in loss of data. Therefore, ERP analyses were completed on the remaining 32 participants. CNV amplitude was larger for directional than non-directional cues \( (F(1, 30) = 8.96, p=0.005) \), indicating successful preparation in anticipation of target onset. Consistent with no behavioural effect of anodal tDCS on reaction time, anodal tDCS did not affect CNV amplitude \( (F<1) \).

3.3.5: Participant tolerance

Participants reported mild and temporary sensory effects which were equivalent for the sham and stimulation sessions. There were no adverse reactions and no drop outs from the study.

3.4: Discussion

3.4.1: Major findings

The principal finding of this study was that a single session of anodal tDCS over the motor cortex of healthy aged subjects resulted in improved functional performance of fine motor tasks of the non-dominant hand irrespective of whether it was the dominant or non-dominant cortex which was stimulated. As anticipated, the dominant hand responded faster in all tasks however its performance did not improve with anodal tDCS. Electrophysiologically, participants elicited larger CNV amplitudes for directional compared to non-directional cues however there was no beneficial effect of anodal tDCS on reaction times or response preparation on the go/no go task.

We anticipated improved performance of the contralateral hand with anodal stimulation. This was not observed with the dominant hand/cortex. This asymmetry in response to cortical stimulation has previously been observed in young subjects (Boggio et al.,
and may reflect asymmetrical use of the hemispheres whereby the reduced dexterity and use of the non-dominant hand leads to relatively decreased cortical excitability of the non-dominant motor cortex (De Gennaro et al., 2004). The lack of effects in the dominant hand may represent a ceiling effect given that the dominant hemisphere is already optimally activated therefore increasing the excitability of this region with tDCS would infer no additional benefit on function (Zimerman & Hummel, 2010). This is supported by the findings of Furuya et al. (2014) who found that tDCS improved skilled finger movements in novice subjects but not in trained pianists indicating that functional changes in the motor cortex are dependent on the level of the expertise required for the task. Similarly it may reflect a ceiling effect of the assessment task itself which was relatively simple. In contrast there was statistically significant improvement in non-dominant hand function. TMS studies have shown that the non-dominant cortex has a higher motor threshold suggesting tDCS may represent an effective way to lower the threshold, increase excitability and therefore hand performance (De Gennaro et al., 2004).

Our findings are in conflict with previous work in older adults which reported improved performance of the dominant hand with dominant hemisphere stimulation. This may be due to our sample being on average 9 years younger and potentially less impaired thus having less scope for measureable improvement than the participants of the Hummel et al. (2010) study; or due to the more complex nature of the task used by Zimerman et al. (2013) where a finger tapping sequence was used. Our study supports the notion that there is a degree of task specificity in the effects of tDCS (Hummel et al., 2010) such that the benefits were more pronounced on the fine motor tasks of the JTT and not the gross motor tasks, and there was no measurable change in the measures of grip strength.

Task specificity of the effects of tDCS may in part explain the disparity between the functional task results and performance on the go/nogo task. While on the functional tasks,
stimulation produced some improvement in non-dominant hand performance, on the cued go/nogo task, there was no evidence of a positive effect of stimulation. In fact, dominant hemisphere stimulation resulted in slower reaction time compared to sham. Although improved function is the ultimate goal of stimulation, functional performance is the cumulative effect of many processes and is only an indirect and non-specific measure of motor-related cortical excitability. In contrast, tasks such as the cued go/nogo task presented here can be used to dissect motor performance into its underlying processes, and examine the level at which stimulation affects motor output. Here we report two levels: the final outcome (RT) and the earliest evidence of motor preparation (CNV). The CNV indicates the level of readiness to respond to a validly predicted target and has been linked to level of excitation in the supplementary motor area and primary motor cortex (Luck, 2005). On analysis of final outcome (RT) and motor preparation (CNV), the current findings indicate that, despite some evidence of non-specific enhancement of non-dominant hand response speed with both dominant and non-dominant cortex stimulation, neither stimulation condition improved motor preparation or response speed. This may also be due to the timing of the stimulation in relation to the timing of EEG recordings which commenced approximately 40 minutes after the stimulation due to the time required for the functional assessments and EEG set up. Therefore any excitability effects may have returned to baseline in this time, or the functional assessments may have negated the effects of the stimulation. Thirugnanasambandam et al. (2011) demonstrated that the effects of anodal tDCS were reduced when stimulation was followed by an isometric muscle contraction which was sustained for two minutes. Our assessment of grip strength may have produced the same negating effect however as the EEG task required different neuronal circuits to the grip task, and the effects of tDCS are thought to be network specific, (Abraham et al., 2001) this can only be speculated. Similarly, there is debate in the literature whether tDCS and task performance should occur sequentially or concurrently. Some authors report that behavioural facilitation only occurred when tDCS was
applied during the task execution (Guleyupoglu et al., 2013; Stagg et al., 2011) yet others state that tDCS must be applied prior to the task (Fertonani et al., 2014; Vallar & Bolognini, 2011). The effect of timing on the application of tDCS and the measurement of the response clearly needs further examination.

Improvement in non-dominant hand performance with anodal tDCS of the dominant hemisphere was not anticipated. Due to transcallosal inhibition, it would be reasonable to expect that anodal stimulation may lead to decreased excitability of the contralateral cortex and result in a detrimental effect on performance of the ipsilateral hand. The fact that the reverse occurred with respect to dominant cortex stimulation suggests that the ipsilateral motor cortex may, in certain instances, be relevant for motor performance in the non-dominant hand. This may especially be the case in older adults, as functional neuroimaging studies have demonstrated that the ageing brain shows more diffuse activation with less lateralisation during unilateral functional movement than in the young brain (Cabeza et al., 1997). Hence it is possible that participants recruited additional networks from the dominant hemisphere to compensate for age-related functional impairment and that tDCS has the capacity to augment this in older adults.

3.4.2: Limitations

We aimed to evaluate the effects of anodal stimulation of the primary motor cortex. However motor skill acquisition is a complex process involving multiple brain areas including prefrontal structures. The anodal montage used, whereby both electrodes are placed on the scalp, may have produced unwanted effects under the reference electrode. That is, anodal tDCS of the motor cortex occurs concurrently with cathodal stimulation of the frontopolar cortex potentially causing widespread excitability changes (Lang et al., 2005). Furthermore, we used relatively large electrodes (35cm²) which cover not only the primary motor cortex but also the adjacent cortices reducing the focality of the stimulation. In particularly, stimulation of the
premotor cortex cannot be excluded however to date the effects of stimulation in this region are few and inconsistent (Pavlova et al., 2014). Although this is the most commonly adopted electrode montage, future studies using an extracephalic reference or smaller anode electrode may overcome this concern.

3.4.3: Clinical implications

Previous studies have neglected to measure the bilateral upper limb effects of tDCS and therefore overlooked the potential importance of the ipsilateral descending pathways for movement performance. Current stroke research studies apply cathodal stimulation (not anodal) to the intact hemisphere to decrease excitability of this region in order to decrease transcallosal inhibition to the lesioned hemisphere. If we infer from our results that differences in the performance of the dominant and non-dominant hand reflect to some degree the differences between the paretic and non-paretic hands of stroke patients, our results would advocate the use of anodal stimulation to the intact hemisphere. This would seem particularly pertinent in the case of severe cortical stroke whereby the ipsilesional tracts may be the only intact descending pathway from the cortex. A neurophysiological model of ipsilateral limb control in stroke has recently been proposed (Bradnam, Stinear & Byblow, 2013) and warrants further investigation.

3.4.4: Conclusion

A large body of tDCS research has focused on healthy young adults. This is a fundamental limitation as the main recipients of tDCS in the clinical setting are likely to be much older. There are considerable discrepancies regarding the effects of anodal tDCS on motor performance. This may be due to the nature of the task, the outcome measured, or multiple physical and anatomical differences between subjects. This study is unique in the breadth of examination to include both hemispheres and both upper limbs and demonstrated that the two hemispheres respond differently to anodal stimulation. This has established the
foundations for subsequent comparisons between healthy aged subjects and patients with prevalent disease conditions such as stroke.
Chapter 4: Anodal tDCS over the motor cortex on prepared and unprepared responses in young adults.³

4.1: Introduction

There is a growing body of literature on the potential therapeutic effects of anodal transcranial direct current stimulation (tDCS) across a wide range of physical and mental pathologies. However, despite many reports of functional benefits in areas as diverse as motor recovery post-stroke and depressive symptomology, little is known about the mechanisms by which tDCS produces these improvements. In this study, we examined the effect of anodal tDCS over the motor cortex (M1) on prepared and unprepared motor responses in healthy young adults using a go/no-go paradigm.

Transcranial direct current stimulation (tDCS) is the generation of a weak electrical current through the neocortex via scalp electrodes (Nitsche & Paulus, 2000, Utz et al., 2010). Sustained application of a mild electrical current has been shown to generate changes at the synaptic level that persist following cessation of stimulation (Nitsche & Paulus, 2000). The nature of these ‘after-effects’ depends on the polarity of stimulation. tDCS delivers subthreshold stimulation that affects neuronal firing rate by manipulating the balance of ions inside and outside the neural membrane. Cathodal stimulation produces hyperpolarization whereas anodal stimulation produces depolarization of the resting membrane potential (Nitsche et al., 2003c). Typically, studies compare a 5-30 minute period of active stimulation against a sham condition, which consists of a brief 30 second ramp-up of current followed by a

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rest period of the same duration as the active stimulation (Utz et al., 2010). As sensory effects are generally limited to the early phase of stimulation, this sham stimulation condition tends to be indistinguishable from the active stimulation condition (Utz et al., 2010).

4.1.1: Effects of tDCS on M1

Most studies examining the effects of anodal tDCS on motor processes have looked at one of two outcome measures: output of excitability or functional motor tasks. The effect of anodal tDCS over M1 on motor output of hand and leg muscles has been examined in young adults by measuring changes in the amplitude of the motor evoked potential (MEP) elicited by transcranial magnetic stimulation (TMS). Specifically, when applied to the motor cortex, TMS elicits a MEP that is recorded at the effector muscle using electromyography (EMG). Nitsche & Paulus (2000) reported changes in TMS-elicited MEP amplitude both during and after the cessation of tDCS. Following five minutes of anodal tDCS, the amplitude of the MEP that was elicited by TMS was significantly greater than baseline and remained increased for a further five minutes. Longer stimulation periods produced more sustained changes to MEPs. For instance, thirteen minutes of anodal tDCS over the dominant (left) M1 increased TMS-elicited MEP amplitude at the right abductor digiti minimi muscle (ADM) for more than one hour (Nitsche & Paulus, 2001). Similar effects of anodal tDCS on motor excitability output have been shown at the tibialis anterior muscle (TA) in the leg (Jeffery et al., 2007). The mechanisms behind these effects of anodal tDCS have been examined using pharmacological agents. Post-stimulation effects on MEPs were abolished following NMDA blockers, indicating that changes in excitability from tDCS may enhance a long-term potentiation (LTP)-like mechanism (Nitsche et al., 2003a).

The effects of anodal tDCS on gross and fine motor skills have also been examined, especially as these have direct clinical implications. Gross motor tasks regularly used in clinical studies include the Jebsen Taylor Hand Function Test (JTT; Jebsen et al., 1969) and grip force
measures. Following anodal tDCS over the dominant M1, young adults showed faster JTT completion (Boggio et al., 2006) and increased isometric force (Cogiamanian et al., 2007) with the non-dominant hand compared to sham. This paradoxical improvement in non-dominant hand performance, in the absence of an effect on dominant hand performance, has been attributed to a ceiling effect as the dominant (right) hand of young adults is likely to be at peak excitability (Zimerman & Hummel, 2010).

The effect of anodal tDCS on fine motor tasks has been assessed using accuracy of motor sequence completion. Anodal tDCS over the dominant M1 in right-handed adults produced more accurate responses to learned number sequences (Nitsche et al., 2003b, Vines, Cerruti & Schlaug, 2008, Vines, Nair & Schlaug, 2008). Bihemispheric anodal tDCS increased response accuracy to randomly presented letter sequences for both left and right hands (Gomes-Osman & Field-Fote, 2013). This improved response accuracy following anodal tDCS over M1 in young adults has been attributed to increased efficiency of motor pathways. However, as response accuracy only captures the end point of a series of cognitive and motor processes, it is not a direct measure of motor pathway efficiency. Moreover, motor sequence tasks do not differentiate between cognitive and motor processes contributing to final motor output.

4.1.2: Present Study

In this study, we examined the effects of anodal tDCS over the motor cortex on behavioural performance. As the motor cortex is directly involved in the generation of movement, facilitation as a result of anodal tDCS should lead to improved performance, and especially faster reaction time (RT). We used a cued go/no-go paradigm in order to examine whether anodal tDCS over the left M1 in healthy young adults would differentially affect performance of prepared and unprepared motor responses. A double-blind randomised controlled design was used, with anodal tDCS and sham sessions separated by a three-week
washout period. Based on previous findings of increased motor excitability and improved motor sequence and functional motor task performance following stimulation (see Nitsche et al. (2008), for a review), we hypothesised that anodal tDCS would improve performance (i.e. increase response speed and reduce error rate) compared to the sham condition.

4.2: Experiment 1

In Experiment 1, active tDCS stimulation of the dominant (left) motor cortex was expected to improve performance compared to sham stimulation, and the effect was expected to be greater for the directional than non-directional cue conditions, indicating a direct effect of anodal tDCS over M1 on motor preparation.

4.2.1: Methods

4.2.1.1: Participants

Twenty-four participants (9 males, mean age ± standard error: 21.3 ± 2.5yrs) were recruited from a 1st year research volunteer group at the University of Newcastle. Participants were screened for suitability for direct current stimulation (i.e., excluding epilepsy, major heart condition, neurological disease, metal implants). All participants were right handed as measured by the Edinburgh Handedness Inventory (Oldfield, 1971). Order of active and sham stimulation conditions was randomised between subjects. Twelve participants (4 males, 20.8 ± 0.5yrs) received sham in the first session and 12 participants (5 males, 21.8 ± 0.9yrs) received active tDCS in the first session.

4.2.1.2: Ethics Statement

This study protocol was approved by the University of Newcastle’s Human Research Ethics Committee (H-2013-0115), and complied with the Declaration of Helsinki. All participants gave written informed consent before beginning participation in this study. This consent form was approved by the University of Newcastle’s Human Research Ethics Committee.
4.2.1.3: Transcranial direct current stimulation settings

Anodal tDCS stimulation was delivered by a battery-driven constant-current stimulator (neuroConn GmbH, Germany) and involved the application of a 1mA current for 20 minutes using two rectangular rubber electrodes (35cm²) soaked in saline. The current density of the electrodes was 28.6µA/cm². The anode was placed over the left primary motor cortex (M1), while the cathode was placed over the supraorbital region of the contralateral hemisphere. The location of the hand area over M1 was determined as the C3 electrode according to the international 10/20 system, as used in (Bachmann et al., 2010). This montage has been shown to be effective at increasing the excitability of the dominant motor cortex (Nitsche & Paulus, 2000, 2001).

Sham stimulation involved the application of a 1mA current for 40s (10s ramp up and 30s stimulation) followed by 20min delay to match the duration of the active stimulation session. Both experimenter and participant were blind to the order of stimulation. An experimenter who was not involved in testing determined a pseudo-random sequence of active/sham stimulation orders, so as to have equivalent numbers in each order. Participants were sequentially allocated to one of these. The two sessions were scheduled at least three weeks apart to avoid any carry over effects of tDCS stimulation.

4.2.1.4: Cued go/no-go paradigm

Each trial began with a fixation cross (500ms). This was replaced by a cue (S1) which validly predicted the onset of the target (S2) after a fixed cue-target interval (CTI=1500ms). The target remained visible for 1000ms during which interval a response was emitted. The interval between target onset and the onset of the next cue was jittered with a mean of 2000ms (random sequence, 1500 - 2500ms).

Directional and non-directional cues were presented in different blocks that were delivered in a randomised order. On non-directional cue blocks, the cue consisted of two black
arrowheads that pointed in different directions (i.e., <->) and validly predicted the timing of target onset but not the response required. The target was two green directional arrowheads (i.e., <<, >>) that specified whether to make a left-hand or a right-hand response with the respective index-finger using buttons attached to the arm rests. During the CTI, participants could prepare to process the target, but were not aware whether they would need to prepare a left-hand or a right-hand response. On directional cue blocks, the cue was two black arrowheads that validly predicted the required response. The targets were the same as for non-directional cues. On 30% of the trials, the target was a ‘no-go’ stimulus (i.e., a red X) indicating that a response must be withheld. So, on these informative cue blocks, participants could use the cues to prepare a left-hand or a right-hand response, but had to await target onset to check whether the response must be withheld. Participants completed five blocks of 80 trials: two with non-directional and three with directional cues. Prior to testing on each session, participants completed one practice block of thirty trials on each cue type.

4.2.1.5: Procedure

In each session, participants received a 20 minute period of either sham or anodal stimulation. This was followed by completion of the Grooved Pegboard Test (Schmidt et al., 2000), the Digit Span test (Weschler, 1997), the Trail Making Test (Tombaugh, 2004) and two practice blocks of the cued go/no-go paradigm. The grooved pegboard task examines whether anodal tDCS over M1 improves fine motor control in addition to response preparation. To examine that anodal tDCS applied over the motor cortex affects non-motor processes, we included brief tests of working memory, set-shifting, digit span and trail making following stimulation intervention. Participants were then prepared for EEG recording and completed the cued go/no-go paradigm. Results of the EEG results will be reported in detail in Chapter 5. The go/nogo task began approximately 40min after termination of stimulation. At the completion of each session, participants were given a short questionnaire assessing their comfort during the tDCS
intervention and whether they believed they had received active or sham stimulation in that session.

4.2.1.6: Statistical Analysis

Response speed on go trials was analysed using a four-way mixed-design GLM with Order (sham first, active first) as a between-groups condition and three repeated measures conditions: Stimulation (active, sham), Cue (directional, non-directional) and Response Hand (left, right). Standard error of the mean was calculated using the procedure developed by Morey (2008). Data from Digit Span, Trail Making and Grooved Pegboard tests were analysed using a three-way mixed-design GLM with Order, Stimulation and Digit Sequence (forward, backward), Trail Type (A,B), or Response Hand (left, right), respectively.

4.2.2: Results

Some participants reported experiencing mild discomfort during the initial phases of stimulation in both active and sham sessions. The most common sensation was a mild itching or prickling sensation under the electrodes, but no participant requested to discontinue testing. Chi-square analysis showed that participants did not accurately predict whether they had received active or sham ($\chi^2(1) = 1.5$, $p=0.22$).

Table 4.1 show means for the Digit Span, Trail Making and Grooved Pegboard tasks. On the Digit Span test, there was no effect of stimulation or interaction between stimulation and other factors. On the Trail Making Test, performance was faster on Trails A than Trails B ($F (1, 21) = 66.45$, $p<0.001$). On the Grooved Pegboard test, responding was faster with the right than the left hand ($F (1, 21) = 14.5$, $p=0.001$). Both tests showed no main effect of stimulation (both $F (1, 21) < 1$). Additionally, they both produced a significant interaction between stimulation and order ($F (1, 21) = 7.58$, $p=0.012$; $F (1, 21) = 21.63$, $p <0.001$), indicating that responding was faster on the second session, irrespective of stimulation condition.
Table 4.1: Results for Digit Span, Trail Making and Pegboard tests in Experiment 1.

<table>
<thead>
<tr>
<th>Test</th>
<th>Active Mean</th>
<th>Sham Mean</th>
<th>t-Statistic</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Digit Span</td>
<td>11.1</td>
<td>11.2</td>
<td>-0.448</td>
<td>0.66</td>
</tr>
<tr>
<td>Trail Making*</td>
<td>28.28</td>
<td>31.99</td>
<td>-0.693</td>
<td>0.5</td>
</tr>
<tr>
<td>Pegboard</td>
<td>62.93</td>
<td>62.97</td>
<td>-0.034</td>
<td>0.97</td>
</tr>
</tbody>
</table>

*Difference score between Trails A and Trails B

Figure 4.1 shows reaction times for directional and non-directional cue conditions under active and sham stimulation. As error rate on go trials (2.23%) and false alarm rate for no-go trials (2.03%) were very low, they are not statistically analysed. Response time was faster for directional than for non-directional cues ($F(1, 22) = 132.6, p<0.001$; 388 vs 460 ms) and for right than left hand responses ($F(1, 22) = 22.6, p<0.001$). This right hand advantage was larger for non-directional cues (Cue*Hand: $F(1, 22) = 9.4, p=0.006$). As shown in Figure 4.1, stimulation had no effect on RT ($423$ vs $425$ ms, $F<1$), and did not interact with other factors (Stimulation*Cue: $F<1$; Stimulation*Hand: $F(1, 22) = 1.76, p=0.2$). There was also no main effect of order ($F<1$) or interaction between order and other factors (Stimulation*Order: $F<1$; Cue*Order: $F<1$; Hand*Order: $F(1, 22) = 1.75, p=0.2$).
4.2.3: Discussion

On the cued go/no-go task, participants showed the expected advantage for directional versus non-directional cues and for right vs. left hand responses. Error and false alarm rates were low for both cue conditions. These results confirm that participants were attending to the task, using cues to prepare their response and waiting for the target before responding on directional cue blocks. Contrary to our predictions, there was no evidence for a beneficial effect of anodal tDCS over M1 on response speed for either directional or non-directional cues.
(Figure 4.1). We conclude that response preparation and response selection as assessed by a cued go/no-go task are not affected by anodal tDCS over dominant M1 in young adults.

The finding that, in young adults, dominant hand performance is not enhanced by anodal tDCS over the dominant M1 is consistent with the JTT findings by Boggio et al. (2006). However, Boggio et al. (2006) did find improvement of non-dominant hand performance, and concluded that, in healthy young adults, stimulation improves JTT performance with the less efficient non-dominant. The current data do not support this conclusion, as tDCS did not improve responding with either the dominant or the non-dominant hand. Indeed, the marginal interaction between stimulation condition and response hand ($p=0.13$) was driven by participants being slightly slower with their dominant hand following stimulation compared to sham.

The absence of a significant effect is unlikely to result from our choice of stimulation parameters, as they were selected to match optimal parameters defined by Nitsche & Paulus (2000). However, the long delay between the cessation of stimulation and the start of the cued go/nogo task may have contributed to the absence of a stimulation effect. In this experiment, the go/nogo task did not commence until approximately 40 minutes after stimulation, because participants first completed the fine motor and working memory tasks, practiced the experimental task and were set up for EEG recording. Therefore it is possible that the effects of stimulation may have been attenuated before the onset of testing. Note, however, that this is unlikely, as performance on the grooved pegboard task, which requires fine motor control and was performed directly following stimulation, was also not affected by anodal tDCS. Nevertheless, in order to examine whether the delay between stimulation and test may be responsible for the absence of an effect of anodal tDCS on response speed on the cued go/nogo task, we completed two further experiments that manipulated the delay between stimulation and testing.
4.3: Experiment 2

In Experiment 2, the test session commenced immediately after stimulation. To minimise the interval between stimulation and task onset, we did not include the Grooved Pegboard, Digit Span and Trail Making tasks, and recorded only behavioural data on the cued go/no-go paradigm.

4.3.1: Methods

Eight right-handed participants (3 male, 22.3±3.4yrs) completed the cued go/no-go directly after active and sham stimulation conditions. Except as specified, all task, stimulation and analyses parameters were identical to those used in Experiment 1. Participants completed task practice before receiving the stimulation intervention, to reduce any delay between stimulation and test.

4.3.2: Results and Discussion

Participant reported similar physiological sensations as in Experiment 1. Error and false alarm rates were again very low (2.2% and 3.2% respectively) and were not statistically analysed. Figure 4.2 shows that mean RT was faster for directional compared to non-directional cues ($F(1, 6) = 193, p<0.001; 309$ vs $385$ ms) and for right than left hands ($F(1, 6) = 8.4, p=0.027$). This right hand advantage was larger on blocks with non-directional compared to directional cues ($F(1, 6) = 14.1, p=0.009$). Despite there being no delay between stimulation and task onset, there was again no effect of anodal tDCS on RT ($F(1, 6) = 1.18, p=0.32$), or any interaction between stimulation and any other factor ($F<1$). While the sample used in this study was smaller than that in Experiment 1, the pattern of results was highly consistent. Thus, Experiment 2 supports the conclusion that anodal tDCS over the motor cortex has no effect on response speed on a cued go/nogo task.
4.4: Experiment 3

While most studies assess the effects of tDCS on subsequent behaviour, others use online stimulation i.e., assess performance during the course of stimulation (Nitsche et al., 2003b, Hsu et al., 2011, Kwon & Kwon, 2013). As Experiments 1 and 2 showed no effects of tDCS on the cued go/nogo task when it was completed after stimulation, in Experiment 3, we assessed
whether the expected improvement of RT on prepared and unprepared motor responses may be induced by online tDCS.

4.4.1: Methods
Twenty right-handed participants (7 males, 21.2±2.9yrs) completed active and sham stimulation conditions in a pseudorandom sequence. All task parameters were identical to Experiment 2, with the exception that the cued go/no-go task commenced immediately after the onset of stimulation.

4.4.2: Results
As in previous experiments, participants reported mild itching sensations. Error rates were again very low (2.3% incorrect, 2.1% false alarms). As shown in Figure 4.3, RT was faster for directional than non-directional cues ($F(1, 18) = 109.7$, $p<0.001$; 357 vs 430ms), and the right hand RT advantage was greater for non-directional cues ($F(1, 18) = 8.175$, $p=0.01$). Once again, there was no main effect of anodal tDCS ($F(1, 18) = 0.6$, $p=0.8$).

There was, however, a main effect of stimulation order, as RT was faster for participants who received active stimulation first than participants who received active second ($F(1, 18) = 9.57$, $p=0.006$; 364 vs 423 ms). There was also a three-way interaction between stimulation, response hand and stimulation order ($F(1, 18) = 6.99$, $p=0.017$). This effect emerged because left-hand responses following active tDCS were slower for participants who received stimulation second compared to those who received stimulation first ($F(1, 18) = 8.18$, $p=0.017$).
As in Experiments 1 and 2, participants responded faster to directional than non-directional cues and showed a right hand advantage, especially for non-directional cues. Despite receiving active tDCS simultaneously with task performance, again responding was not faster under active than sham stimulation conditions. Therefore, across the three experiments, we show a highly consistent finding that, in young adults, anodal tDCS over left motor cortex does not
improve response speed with either the dominant or the non-dominant hand. Importantly, Experiment 3 shows that this null effect cannot be accounted for by the length of the delay between stimulation and test.

Again, however, our result is not consistent with some previous studies. For example, Nitsche et al. (2003b) showed that the improvement in response speed was greater with online than offline application of anodal tDCS over M1. They concluded that online application of anodal tDCS produces additive enhancement of response processes. However, this is not a consistent finding. For example, Miyaguchi et al. (2013) found worse performance following online anodal tDCS compared to offline tDCS.

4.5: Bayesian analyses across the three experiments

All three behavioural experiments failed to reject the null hypothesis that anodal tDCS has no effect on motor performance in a cued go/no-go task. However, in frequentist statistics, failure to reject the null hypothesis does not allow us to conclusively affirm that anodal tDCS has no effect on motor performance on this task. In order to test the strength of this null hypothesis, we took a Bayesian approach using the default-prior method for linear models (Rouder & Morey, 2012) to repeat the factorial ANOVA analyses, including a between-subjects factor of timing of stimulation (delayed, immediate, simultaneous). This analysis produces Bayes factors, which equal the ratio of the evidence for one vs. another model, assuming the models are equally likely a-priori, and quantify the factor by which the data should change prior beliefs about the relative merits of each model. A Bayes factor greater than 10 is often considered as providing strong evidence for a model (Jeffreys, 1961, Kass & Rafferty, 1995). We performed the analysis using the BayesFactor package in R (Morey & Rouder, 2013) calculating Bayes factors for all hierarchical ANOVA models (i.e., higher order terms are only included with their constituents). We report Bayes factors assuming the default setting for the fixed-effect prior (r=0.5).
The analysis showed that the best model included a main effect of cue (directional vs.
non-directional), hand (left vs. right), stimulation order (active first vs. sham first), and timing
(delayed, immediate, simultaneous), as well as an interaction between order and timing of
stimulation. The between group effect of timing emerged because participants in Experiment 2
had overall faster RT than the other two groups, especially when receiving active stimulation in
the 1st session. The absence of a main effect of stimulation or an interaction between
stimulation and timing shows no behavioural benefit of anodal tDCS, regardless of when it was
received in respect to the cued go/no-go task. The Bayes factor for this model relative to the
best model that included one or more effects of stimulation (a single main effect) was 11.1. In
other words, a null effect of stimulation is favoured by a factor of around eleven, indicating
strong evidence that anodal tDCS has no effect on RT in young adults. Therefore, Bayesian
analysis of data pooled across the three experiments supported the hypothesis that anodal
tDCS over the dominant motor cortex is statistically unlikely to improve response speed in
young adults.

4.6: General Discussion

In this study, we investigated the effects of anodal tDCS on motor responding in a cued go/no-
go task in healthy young adults. All participants showed good task performance with very low
error and false alarm rates. In addition, as expected, they responded faster for directional
(prepared) than non-directional (unprepared) cues and with their right (dominant) than their
left hand. Anodal tDCS was applied over the dominant (right) hemisphere and order of
stimulation (active or sham first) was randomised, with both participant and experimenter
being blind to the stimulation condition. A long 3-week washout period was used to avoid any
contamination between stimulation and sham. We chose stimulation parameters previously
shown to be effective in facilitating motor output and gross motor performance (Nitsche &
Paulus, 2000).
Contrary to our original hypothesis, anodal tDCS of the dominant motor cortex did not significantly improve performance relative to sham stimulation on either prepared (directional) or unprepared (non-directional) responses performed with either the contralateral (stimulated) or the ipsilateral (non-stimulated hand). Given the unexpected absence of a significant stimulation effect in Experiment 1, we asked whether the relatively long delay (40 min) between stimulation and the onset of the cued go/nogo task may have resulted in an attenuation of the effect of stimulation. To test the possibility that the stimulation-test delay was responsible for the absence of a stimulation effect, we conducted two more experiments in which stimulation was applied immediately before task onset (Experiment 2) or during task completion (Experiment 3). Both experiments produced results consistent with Experiment 1; although participants performed the task well, performance was not enhanced by anodal tDCS over the dominant M1. Bayesian analysis confirmed the strength of the null hypothesis for stimulation. Across the three experiments, the most appropriate model did not include a positive effect of anodal tDCS on performance. Indeed, the strongest model that included stimulation was eleven times less powerful than the strongest model without stimulation. We therefore conclude that anodal tDCS over the motor cortex has no effect on speed of responding on a cued go/no-go task in healthy young adults.

This conclusion is at odds with prior evidence of enhanced TMS-induced MEP following anodal stimulation of dominant M1 in young adults (Nitsche & Paulus, 2000) and improved motor performance on functional tasks of fine and gross motor skills, such as the JTT (Boggio et al., 2006). These early findings have set the framework regarding possible beneficial effects of anodal tDCS over the motor cortex in clinical rehabilitation. However, more recent research using anodal tDCS over M1 has not produced consistently positive findings. For instance, while a number of studies report that anodal tDCS over M1 enhances accuracy and response speed on finger-tapping sequence tasks (Vines, Cerruti & Schlaug, 2008, Vines, Nair & Schlaug, 2008,
Gomes-Osman & Field-Fote, 2013), Leite et al. (2011) found no effect of anodal tDCS over M1 on response accuracy or RT using a motor sequence task.

Inconsistent effects have also been reported in studies that examine the effects of anodal tDCS over M1 on response inhibition in young adults using the stop-signal task (SST). Kwon & Kwon (2013) found that anodal tDCS over M1 improved response inhibition (i.e., reduced stop-signal reaction time, SSRT), while Hsu et al. (2011) reported no effect on SSRT following stimulation over either M1 or pre-SMA. Effects of anodal tDCS on response accuracy in the stop-signal task have also been inconsistent. Hsu et al. (2011) showed a reduction in false alarm rate following pre-SMA stimulation, whereas Hadyuk-Costa, Drummond & Carlsen (2013) showed increased false alarm rate after anodal tDCS over M1. Anodal tDCS over M1 in healthy older adults also showed no beneficial effects on a choice RT task (Lindenberg et al., 2013). Pellicciari, Brignani & Miniussi (2013) found that anodal tDCS over M1 did not improve response speed on a speeded detection task, over and above the effect of sham.

These discrepancies may be partly related to differences in task properties. Bortoletto et al. (2015) found that simultaneous application of anodal tDCS over the motor cortex reduced performance compared to sham on a fast motor task, but not a slow motor task. Indeed, in a recent review, Miniussi, Harris & Ruzzoli (2013) concluded that, in novel tasks, rather than facilitating processing efficiency, anodal tDCS may produce more cortical noise and interfere with task processing. This could explain the difference between findings with sequenced tapping tasks (Vines, Cerruti & Schlaug, 2008, Vines, Nair & Schlaug, 2008, Gomes-Osman & Field-Fote, 2013) and tasks with more complex demands.

Alternatively, the absence of an effect of tDCS on response time in the cued go/nogo task may be related to the stimulation parameters used. The specific montage used here (i.e., active electrode over left motor region and reference over right supraorbital region) was selected as it has been shown to stimulate the motor cortex (Nitsche et al., 2003b). However,
given the poor focal nature of tDCS, it is likely that stimulation spread across a greater area on
the surface of the neocortex (Miranda et al., 2013). Moreover, the placement of the reference
electrode over the contralateral supraorbital region may have attenuated the effect of the
anode by decreasing activation of the right orbitofrontal area. Nevertheless, the fact that these
stimulation parameters have been previously shown to positively affect motor task
performance indicates that this is unlikely to account for the lack of stimulation effects in this
study.

An alternative explanation for the lack of a stimulation effect may be that our healthy
young adults were performing at peak and had no room to improve response speed. This is
supported by the low error rate. However, participants showed clear response time
differences between prepared and unprepared responses, as well as between left and right
hands. Even if prepared right hand responses were at ceiling, stimulation could have improved
unprepared and/or left hand responses. Moreover, positive effects of anodal tDCS have been
reported in young adults even with simple motor tasks, such as with the JTT (Boggio et al.,
2006) or force measurement (Cogiamanian et al., 2007).

In conclusion, the present study shows that anodal tDCS over the dominant motor
cortex in young people did not improve speed of responding to either directional or non-
directional cues in a cued go/nogo task. This effect was replicated in three experiments which
varied the timing between stimulation and test. Recently, Pellicciari, Brignani and Miniussi
(2013) suggested that anodal tDCS may modulate the efficiency of cortico-motor pathways,
instead of eliciting direct excitation. It is therefore possible that, although there is no effect on
overt behavioural outcome, tDCS may produce subtle differences in cognitive and motor
processes that contribute to response selection and activation. Event-related potentials (ERP)
allow the measurement of electrophysiological components associated with cognitive and
motor processes at different temporal windows and may help address this question.
5.1: Introduction

Research into the potential merits of anodal transcranial direct current stimulation (tDCS) for therapeutic interventions in both motor (e.g., stroke) and psychological (e.g., depression) conditions is increasing, reflecting a desire to gain a greater understanding of the method by which tDCS elicits change in the neocortex. In this paper, we utilize the high temporal resolution of event-related brain potentials (ERPs) to identify the mechanisms by which anodal tDCS over the motor cortex may affect response processes in healthy young and older adults.

Transcranial direct current stimulation (tDCS) involves the application of a weak current across the surface of the cortex via scalp electrodes (Nitsche & Paulus, 2000; Utz et al., 2010). When applied over the motor cortex, this current generates changes to motor output (Nitsche & Paulus, 2000). The nature of these changes is dependent on the positioning of stimulation and reference electrodes. Positive or anodal tDCS over the primary motor cortex (M1) increases the amplitude of motor-evoked potentials (MEPs) elicited by transcranial magnetic stimulation (TMS) pulses, whereas negative or cathodal tDCS reduces MEP amplitude (Nitsche & Paulus, 2001, Nitsche et al., 2003b, Utz et al., 2010). Functionally, the application of anodal tDCS over M1 has been shown to improve performance on motor control tasks. For

instance, after receiving anodal tDCS over the M1, both young and old adults exhibited faster completion of the Jebsen Taylor Hand Function Test (JTT, Jebsen et al., 1969) which assesses performance of a number of functional upper limb movements (Boggio et al., 2006, Hummel et al., 2010). Improvements have also been shown on a range of cued movement tasks. Anodal tDCS over the dominant M1 resulted in faster and more accurate responses on sequential tapping tasks (Nitsche et al., 2003a, Vines et al., 2006, 2008). Healthy young adults also showed improved skill acquisition on a visually-directed pinch task following consecutive daily sessions of anodal tDCS over the M1 (Reis et al., 2009, Schambra et al., 2011). This improved speed and/or accuracy of motor performance following the application of anodal tDCS over the M1 in healthy adults is thought to be consistent with improved efficiency of motor pathways (Jacobson et al., 2012). Such findings have motivated the use of anodal tDCS over M1 as a rehabilitation tool in pathologies characterised by motor dysfunction (for a review see Floel, 2014). Encouraging findings show that the application of anodal tDCS over M1 may restore some motor functioning in patients suffering from Parkinson’s disease (Fregni et al., 2006), dystonia (Benninger et al., 2011) and following a severe neurological trauma such as a stroke (O’Shea et al., 2014).

However, this rush to endorse anodal tDCS as a neurological intervention may be premature. A number of recent studies have failed to find a beneficial effect of anodal tDCS over M1 on performance in either young or old adults. On choice reaction time tasks, studies have shown no performance improvement following anodal tDCS compared to sham in either young adults (Pellicciari et al., 2013) or old adults (Lindenberg et al., 2013). Using a cued go/nogo task, Conley et al. (2015) found no impact of anodal tDCS over dominant M1 on response speed for either the dominant or the non-dominant hand in healthy young adults. That these null findings have all been evidenced using attention-driven response paradigms indicates that anodal tDCS may fail to enhance communication between the prefrontal cortex
and the primary and secondary motor areas. Investigation into the mechanisms by which anodal tDCS over M1 affects motor processes is therefore essential to establish the efficacy of anodal tDCS as a potential therapeutic intervention tool.

It is also important to examine the effectiveness of anodal tDCS on these response processes in healthy older adults. It is well known that healthy ageing is associated with gradual alterations to both cortical structure and functioning (Buckner, 2004, Raz et al., 2005, Seidler et al., 2010) as well as a reduction in processing speed (Salthouse, 2000). This decline in processing speed is associated with decreased behavioural performance and changes in ERP waveforms in older compared to young adults (Polich, 1997, Sterr & Dean, 2008, Ren et al., 2013). As most clinical neurological disorders that are likely to benefit from motor cortex tDCS interventions emerge in older adults, it is imperative to investigate the effects of stimulation in older adults, as they provide a much more appropriate baseline for clinical studies than do young adults.

As conventional behavioural measures, such as mean response times (RT) and error rates, represent the endpoint of decision making, they do not offer direct insight into the temporal evolution of attentional and motor processes that lead up to a response. The excellent temporal resolution of event-related potentials (ERPs) offers the capability to measure these processes and may therefore identify effects of anodal tDCS even in the absence of an overt behavioural effect. The few studies that have examined the effects of tDCS on ERPs have not investigated motor processes (Kongthong et al., 2013, Lafontaine et al., 2013, Lapenta et al., 2014). The only study to examine changes to ERP morphology following anodal tDCS over M1 measured TMS-elicited ERP rather than task-driven ERP components associated with stimulus and response processing (Pellicciari et al., 2013). Thus, it is still unclear whether tDCS over M1 affects the morphology or timing of ERP components associated with response processes.
A number of ERP components are associated with motor processes. The contingent negative variation (CNV) is a slow negative deflection that indexes processes associated with preparation of a motor response (Rockstroh et al., 1989). It typically emerges after a warning stimulus (cue) heralds the occurrence of an imperative stimulus (target) to which the participant must respond (Walter et al., 1964, Leuthold et al., 2004). Cues that provide valid information about the response required to the upcoming target generate a larger CNV compared to neutral cues (Leuthold & Schroter, 2011). CNV amplitude is indicative of level of motor preparation, with larger CNV being associated with faster responding. The CNV is associated with increased activation in both the M1 and the supplementary motor area (SMA, Gomez et al., 2003). Response selection and activation processes are indexed by the lateralised readiness potential (LRP). The LRP is a large negative deflection that indicates greater activation over the motor cortex of the hand associated with a correct response (Coles, 1989). When time-locked to the onset of the target (tLRP), it represents pre-motoric processes leading up to response selection. When time-locked to the onset of the response (rLRP), it represents motor processes leading from response selection to response execution (Masaki et al., 2004). The LRP is associated with activation at M1 and SMA, consistent with a role in motor planning and execution (Praamstra et al., 1996).

ERPs can be used to differentiate between motor and non-motor effects of anodal tDCS stimulation. This is particularly important because, although anodal tDCS over M1 is intended to stimulate the primary motor cortex, stimulation may spread to other cortical areas depending on electrode size and location of the reference electrode (Miranda et al., 2013). In order to show specific effects of tDCS stimulation on motor processes, it is necessary to show that it specifically affects ERP components associated with response processes (e.g., CNV, LRP) and not ERP components associated with sensory and attention processes, such as the target-locked P300 (Picton, 1992, Linden, 2005, Polich, 2007). The P300 is a parietal positive peak that
peaks at least 300ms after the presentation of a task-relevant stimulus. P300 amplitude varies with task difficulty and target information, and its peak latency represents completion of stimulus evaluation. Functionally, the P300 is associated with activation in different cortical areas depending on the stimulus modality. Auditory stimuli elicit increased cortical activation in the inferior temporal cortex, whereas visual stimuli increase cortical activity at the posterior parietal cortex (Bledowski et al., 2004, Linden, 2005). These three ERP components can be thus used to measure attentional and motor processes that contribute to the timing and accuracy of a motor response.

In this study, we examined whether anodal tDCS over the dominant or the non-dominant M1 produces selective changes to motor processes, as evidenced by response-related ERP components during a cued go/nogo task. The effects of anodal tDCS over M1 were examined in two experiments: one in healthy young and the other in healthy older adults. The cued go/nogo paradigm was used to elicit both motor and non-motor ERP components in order to test whether effects of anodal tDCS over M1 were specific to motor processes (Figure 1). This paradigm was selected because it manipulates the timing of response preparation processes by altering the contextual information given by the visual cue. Lapenta et al. (2014) examined the effects of anodal tDCS on ERPs elicited on a go/nogo task, but stimulation was applied over the dorsolateral prefrontal cortex (DLPFC), rather than the motor cortex.

In the cued go/nogo task used here, some blocks used directional cues (Figure 1A) that provided valid information about whether the target would require a left or a right hand response, allowing preparation of the response required after target onset. Other blocks used non-directional cues (Figure 1B) that provided valid information about the timing of the upcoming target, but not its direction (Figure 1B). Thus participants could anticipate target onset but not prepare a left or right motor response. During the CTI, directional cues were expected to elicit a larger CNV than non-directional cues, indicating the anticipatory
preparation of the motor response. The efficiency of target processing can be assessed in the peak amplitude of the P300 (Kutas et al., 1977). Directional cues were expected to elicit a smaller P300 component compared to non-directional cues. As noted above, the target-locked LRP (tLRP) indexes response selection and the response-locked LRP (rLRP) is linked to response activation. Directional cues were expected to elicit an earlier tLRP and shorter duration of the rLRP compared to the non-directional cues, as greater preparation requires less effort to select and execute the appropriate response.

5.2: Experiment 1

In Conley et al. (2015), we showed that anodal tDCS stimulation over the dominant M1 in young adults had no effect on behavioural response speed during a cued go/nogo task delivered during, immediately after or shortly after stimulation. Here, we examine whether this stimulation may have had an effect on ERP components representing response processes that led up to the motor response and that were not captured by response time.

Specifically, anodal tDCS over M1 could be expected to improve response preparation, resulting in larger CNV compared to the sham condition. It could facilitate response selection or response activation, reducing tLRP onset latency or rLRP duration, respectively. Finally, given evidence that anodal tDCS over M1 may have corollary effects on adjoining frontal and parietal areas (Miranda et al., 2013) involved in stimulus evaluation and context updating, it could impact amplitude and/or latency of the target-locked P300 component in either direction – either improving or reducing efficiency of attentional processes. As anodal tDCS was delivered over the dominant M1, any effects were expected to be greater over the dominant hemisphere.
5.2.1: Method

5.2.1.1: Participants
Twenty-four healthy young adults completed active and sham stimulation sessions\(^5\). One participant was removed from the analyses due to excessive artefact in their electrophysiological recording, so the remaining analysis was performed on twenty-three participants (9 males, mean age \(21.2 \pm 2.5\) yrs). All participants were right handed as measured by the Edinburgh Handedness Inventory (Oldfield, 1971). All participants were screened for non-suitability for direct current stimulation, including epilepsy, major heart condition or any neurological implants.

The protocol was approved by the Hunter New England Human Research Ethics Committee (H-2013-0115), and was in accordance with the Declaration of Helsinki. All participants provided written informed consent prior to commencing the experiment.

5.2.1.2: Transcranial Direct Current Stimulation Settings
Anodal tDCS stimulation was delivered by a battery-driven constant-current stimulator (neuroConn GmbH, Germany) and involved the application of a 1mA current continuously for 20 minutes (with 10s ramp-up/down at the beginning and end of the intervention) using two rectangular rubber electrodes (35cm\(^2\)) soaked in saline. The current density of the electrodes was 28.6\(\mu\)A/cm\(^2\). The anode was placed over the left M1, while the cathode was placed over the supraorbital region of the contralateral hemisphere. Electrode placement on the scalp over the hand area of M1 was determined using the scheme for placement of the C3 EEG electrode according to the International 10/20 system, as used in Bachmann et al. (2010). This montage has previously been shown to be effective at increasing the excitability of the dominant M1.

\(^5\) The behavioural results were presented in Experiment 1 of Conley et al. (2015). Here we report the behavioural data very briefly, to allow comparison with data from older adults in Experiment 2.
The sham stimulation condition involved the application of a 1mA current for 50s (10s ramp up and 40s application) followed by 20min delay to match the duration of the active stimulation session. Stimulation conditions (active or sham) were assigned a code by one experimenter. Another experimenter who was blind to the correspondence between codes and stimulation condition entered the code during the experimental session. Thus neither participant nor the experimenter running the session was aware of the stimulation condition applied. Order of active and sham stimulation conditions was counterbalanced between subjects. Sessions were scheduled at least three weeks apart to avoid any carryover effects of stimulation.

5.2.1.3: Cued Go/Nogo Paradigm

The task consisted of a S1-S2 trial sequence, where the cue (S1) validly predicted the onset of the target (S2) after a fixed cue-target interval (CTI=1500ms, Figure 5.1). Each trial began with a fixation cross (500ms) that was replaced by the cue (1500ms) which was, in turn, replaced by the target. Directional and non-directional cues were presented in separate randomised blocks. On non-directional cue blocks (Figure 1B), the cue consisted of two white arrows pointing in different directions (i.e., < >), and validly predicted the timing of target onset. The target was two green directional arrows (i.e., <<, >>) that specified a compatible left or right hand response. On directional cue blocks, the cue consisted of the same two white arrows, but now they pointed in the same direction (i.e., >> or <<) validly predicting the green target. However, on 30% of trials, the target was a ‘nogo’ stimulus (i.e., a red X) indicating that a response must be withheld. So, on informative cue blocks, participants could use the cues to prepare a left or right hand response, but had to await target onset to check whether the response must be withheld. On both directional and non-directional cue blocks, the target remained visible for 1000ms and the subsequent target-cue interval was jittered (mean 2000ms, random sequence, 1500 - 2500ms). Participants completed five blocks of 80 trials.
(two blocks of non-directional and three blocks of directional cue conditions) and were instructed about the significance of non-informative and informative cues. Prior to testing on each session, participants completed two practice blocks (30 trials/block): one for each cue type.
A. Directional Cue

Go Trial

No-Go Trial

B. Non-Directional Cue

Go Trial

Figure 5.1: Cued go/nogo task. The time course for (A) go and nogo trials in directional cue condition, and (B) go trials in non-directional cue condition.
5.2.1.4: Procedure

In the first session, participants provided informed consent and completed a medical screening form and the Edinburgh Handedness Inventory. Prior to the administration of anodal tDCS or sham, participants completed the Grooved Pegboard Test (Schmidt et al., 2000) with left and right hands. The stimulation electrodes were then applied and participants received twenty minutes of either active or sham stimulation. Following stimulation, participants repeated the Grooved Pegboard Test and completed the Digit Span test (forward, backward, ascending), the Trail Making Test (Tombaugh, 2003) and practice on the experimental task. The results of these tests are discussed in Conley et al. (2015). After EEG was set up, participants completed the experimental blocks of the cued go/nogo paradigm. EEG testing commenced approximately 40min after termination of stimulation. At the completion of this session, participants were given a short questionnaire assessing their subjective comfort during the intervention, and were asked whether they thought they had received anodal tDCS or sham. Participants returned three weeks later to complete the second session, in which they received the other stimulation intervention.

5.2.1.5: EEG Recording and Processing

Electrophysiological data was continuously sampled from 64 scalp electrodes at 2048 Hz/channel reference free using a Biosemi ActiView II system. Vertical and horizontal electro-oculogram (EOG) was recorded from the lateral, supra-orbital and infra-orbital electrodes of each eye. Continuous EEG files were referenced offline to average mastoids and filtered using a 0.02-30Hz bandpass filter and a 50Hz notch filter to remove line noise. EEG data were processed and analysed using EEGDisplay 6.3.12 (Fulham, 2012).

Target-locked ERP waveforms were derived from 3000ms epochs extracted from 300ms prior to fixation onset to 800ms after target onset. Separate waveforms were derived for go trials under each cue condition, for each hand and stimulation condition. CNV amplitude
was measured at Cz as the mean amplitude over 1300-1500ms post-cue (i.e., 200ms prior to target onset) using a 200ms baseline preceding the onset of the fixation point. Peak amplitude of the target-locked P300 was measured at Pz over 200-500ms after target onset, relative to a 200ms pretarget baseline in order to take variability in CNV into account.

Target-locked (tLRP) and response-locked (rLRP) LRPs were extracted from the C3/C4 electrode pair using the averaging method explained by Coles (1989):

\[
LRP = \left[ \frac{Mean(C4 - C3)_{LHR esponses} + Mean(C3 - C4)_{RHR esponses}}{2} \right]
\]

Both target- and response-locked waveforms were filtered using a 30Hz zero-phase, low pass filter to reduce high frequency noise. tLRP waves were baselined across 200ms prior to the onset of the target, whereas rLRP waves were baselined between 500 and 700ms prior to the overt response. Onset latencies were extracted using 25% fractional area latency, which used the mean amplitude across the defined window as the threshold. The windows used were between 100 to 600ms post-target onset for tLRPs and over 300ms to 100ms before the response for rLRPs.

5.2.1.6: Data Analyses

Both the response times and the mean ERPs for go trials were analysed using a repeated measures generalised linear model with three within-subjects factors: Stimulation (active, sham), Cue (directional, non-directional) and Response Hand (left, right). As accuracy was very high, these scores were not analysed statistically. LRP analysis included only the Stimulation and Cue factors.
5.2.2: Results

5.2.2.1: Behavioural Results

Mean response times for young adults are displayed in Table 5.1 (top). As shown in Figure 5.2A, young adults responded faster to directional than to non-directional cues ($F (1, 22) = 149.86, p<0.001$) and with their right than their left hand ($F (1, 22) = 20.63, p<0.001$). The right hand advantage was greater for non-directional cues ($F (1, 22) = 10.17, p=0.004$). There was no effect of anodal tDCS on response speed (tDCS: 425.7±11.7 ms, Sham: 426.2±11.7 ms; $F (1, 22) < 1$).

Table 5.1: Mean response time (RT, milliseconds) for young and old adults for each cue and hand following anodal tDCS and sham.

<table>
<thead>
<tr>
<th>Group/Stimulation</th>
<th>Directional Left</th>
<th>Directional Right</th>
<th>Non-Directional Left</th>
<th>Non-Directional Right</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Young Adult</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Active</td>
<td>392.8 (14.0)</td>
<td>388.0 (12.7)</td>
<td>470.5 (11.1)</td>
<td>451.6 (10.5)</td>
</tr>
<tr>
<td>Sham</td>
<td>396.1 (13.8)</td>
<td>388.1 (12.0)</td>
<td>473.3 (13.8)</td>
<td>447.2 (11.0)</td>
</tr>
<tr>
<td><strong>Old Adult</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dominant</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Active</td>
<td>500.5 (14.4)</td>
<td>499.0 (14.8)</td>
<td>570.2 (16.2)</td>
<td>565.1 (16.4)</td>
</tr>
<tr>
<td>Sham</td>
<td>477.3 (14.3)</td>
<td>474.6 (12.9)</td>
<td>558.1 (15.9)</td>
<td>549.1 (16.3)</td>
</tr>
<tr>
<td>Non-Dominant</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Active</td>
<td>497.8 (16.5)</td>
<td>483.3 (16.9)</td>
<td>584.1 (18.5)</td>
<td>576.7 (18.8)</td>
</tr>
<tr>
<td>Sham</td>
<td>497.7 (16.3)</td>
<td>489.6 (14.7)</td>
<td>578.7 (18.2)</td>
<td>563.5 (18.7)</td>
</tr>
</tbody>
</table>
Figure 5.2: Mean response time (RT) following anodal tDCS (red) and sham (black) for (A) Young adults over dominant hemisphere, and (B) Old adults over dominant hemisphere and non-dominant hemisphere. Significant main effects are represented by asterisks (***p < 0.001).
5.2.2.2: Electrophysiological Results

Mean amplitudes for CNV and P300 ERP components for young adults are displayed in Tables 5.2 (top) & 5.3 (top), respectively. Peak P300 latencies are displayed for young adults in Table 5.4 (top). Figure 5.3A shows cue-locked ERP waveforms at Cz and Pz electrodes following anodal tDCS and sham stimulation. The CNV emerged around 500ms post-cue onset, peaking just before target onset. CNV amplitude was larger for directional than non-directional cues (-4.9 vs -2.8µV; F (1, 22) = 18.9, p<0.001) but did not vary with response hand (F<1). As shown in Figure 5.3A, stimulation did not affect CNV amplitude or interact with cue or response hand (all F<1).

Table 5.2: Mean contingent negative variation (CNV) amplitude at Cz (microvolts) for young and old adults for each cue and hand following anodal tDCS and sham.

<table>
<thead>
<tr>
<th>Group/Stimulation</th>
<th>Directional Left</th>
<th>Directional Right</th>
<th>Non-Directional Left</th>
<th>Non-Directional Right</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Young Adult</strong></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Active</td>
<td>-5.0 (0.9)</td>
<td>-5.0 (0.9)</td>
<td>-3.1 (0.8)</td>
<td>-2.8 (0.7)</td>
</tr>
<tr>
<td>Sham</td>
<td>-5.3 (0.97)</td>
<td>-4.2 (1.0)</td>
<td>-2.9 (0.5)</td>
<td>-2.6 (1.0)</td>
</tr>
<tr>
<td><strong>Old Adult</strong></td>
<td></td>
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<tr>
<td><strong>Dominant</strong></td>
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</tr>
<tr>
<td>Active</td>
<td>-5.4 (1.1)</td>
<td>-4.0 (1.0)</td>
<td>-4.2 (0.9)</td>
<td>-3.9 (1.0)</td>
</tr>
<tr>
<td>Sham</td>
<td>-5.5 (0.97)</td>
<td>-6.1 (0.97)</td>
<td>-3.9 (1.3)</td>
<td>-4.1 (0.9)</td>
</tr>
<tr>
<td><strong>Non-Dominant</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Active</td>
<td>-7.4 (1.2)</td>
<td>-6.4 (1.2)</td>
<td>-4.5 (1.0)</td>
<td>-4.5 (1.2)</td>
</tr>
<tr>
<td>Sham</td>
<td>-5.8 (1.1)</td>
<td>-6.8 (1.1)</td>
<td>-4.9 (1.4)</td>
<td>-4.3 (1.1)</td>
</tr>
</tbody>
</table>
A large P300 emerged parietally following target onset. P300 amplitude was smaller for directional than non-directional cues (10.9 vs 16.2 µV; F (1, 22) = 36.4, p<0.001) and marginally for left than right hand responses (F (1, 22) = 5.2, p=0.03). There was no effect of cue or response hand on P300 latency (both p>0.3). There was no effect of stimulation on P300 amplitude or latency (all p>0.05).

Figure 5.3: ERP waveforms for directional (blue) and non-directional (red) conditions at Cz (left) and Pz (right) following active (i.e., anodal tDCS) and sham stimulation for (A) young and (B) old adults.
Mean tLRP and rLRP onset latencies for young adults are displayed in Table 5.5 (top).

As shown in Figure 5.4A (left), tLRP emerged earlier for directional than for non-directional cues, with the latter showing an early positive dip (cue: 271 vs. 313ms; F (1, 22) = 38.0, p<0.001). rLRP had a later onset for directional than non-directional cues (Figure 4A, right; -109ms vs. -131ms; F (1, 22) = 25.4, p<0.001). Stimulation did not significantly affect the onset latency of either tLRP or rLRP (all F<1).

Table 5.3: Peak P300 amplitude at Pz (microvolts) for young and old adults for each cue and hand following anodal tDCS and sham.

<table>
<thead>
<tr>
<th>Group/Stimulation</th>
<th>Directional Left</th>
<th>Directional Right</th>
<th>Non-Directional Left</th>
<th>Non-Directional Right</th>
</tr>
</thead>
<tbody>
<tr>
<td>Young Adult</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Active</td>
<td>13.8 (1.0)</td>
<td>15.1 (1.1)</td>
<td>17.9 (1.1)</td>
<td>18.9 (0.9)</td>
</tr>
<tr>
<td>Sham</td>
<td>14.6 (1.1)</td>
<td>15.0 (1.1)</td>
<td>17.7 (1.0)</td>
<td>17.9 (1.1)</td>
</tr>
<tr>
<td>Old Adult</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dominant</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Active</td>
<td>15.0 (1.2)</td>
<td>15.0 (1.2)</td>
<td>19.0 (1.4)</td>
<td>17.6 (1.4)</td>
</tr>
<tr>
<td>Sham</td>
<td>15.9 (1.1)</td>
<td>15.1 (1.1)</td>
<td>19.3 (1.3)</td>
<td>18.3 (1.3)</td>
</tr>
<tr>
<td>Non-Dominant</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Active</td>
<td>13.9 (1.4)</td>
<td>14.5 (1.4)</td>
<td>17.2 (1.6)</td>
<td>15.9 (1.6)</td>
</tr>
<tr>
<td>Sham</td>
<td>13.9 (1.2)</td>
<td>13.8 (1.3)</td>
<td>15.5 (1.5)</td>
<td>14.9 (1.5)</td>
</tr>
</tbody>
</table>
5.2.3: Discussion

Overall, behavioural and electrophysiological findings show that participants completed the task as expected, preparing their response to directional cues and waiting for target onset before responding for both cue types. The CNV was larger for directional cues that allowed response preparation, whereas the P300 was larger for non-directional cues that required greater post-target processing. LRPCs also indicated that prepared responses showed earlier response selection (tLRP) and faster response activation (rLRP, Wild-Wall et al., 2003).

Interestingly, despite the simple nature of the task, response selection for the non-directional cues showed a large ‘dip’ in the target-locked LRP, suggesting at least partial preparation of both responses in the CTI. This is likely to account at least partly for the RT delay for directional vs non-directional cue blocks.

Despite the fact that the task showed strong behavioural and ERP effects in the expected direction, there was no evidence of any effect of anodal tDCS over M1 on any of the measures. As the sample consisted of healthy young adults, and stimulation was applied to M1 corresponding to their dominant hand, it is possible that the lack of any effect of anodal tDCS is due to a ceiling effect that precluded any further improvement (Wu & Hallet, 2005).

5.3: Experiment 2

In Experiment 2, we examined whether there are beneficial effects of anodal tDCS to the dominant or non-dominant motor cortex on response processes in older healthy adults. Ageing is associated with reduced processing speed (Ren et al., 2013), as well as changes to the morphology of ERP waveforms associated with cognitive and response processes (Cespon et al., 2013). Compared to young adults, old adults tend to show slower stimulus evaluation, as evidenced by increased P300 latency across the lifespan (see Polich, 1996 for a review). Old adults also show slower response selection (tLRP) and response activation (rLRP) processes compared to young adults (Yordanova et al., 2004, Kolev et al., 2006). Additionally, differences
in CNV activation between younger and older adults suggest changes in response preparation processes (Falkenstein et al., 2002, Sterr & Dean, 2004, Golob et al., 2005).

As positive effects of anodal tDCS are more likely to emerge when motor processes are less efficient at baseline, we examined the effects of anodal tDCS on both dominant and non-dominant motor cortices. In the present experiment, we expected that old adults would show improved motor performance on the cued go/nogo task and associated ERP components after anodal tDCS over the M1, and that the tDCS effect would be greater when applied over the non-dominant hemisphere. For both dominant and non-dominant hemisphere stimulation, the effect should be greater for the contralateral than the ipsilateral hand.

5.3.1: Method

5.3.1.1: Participants

Thirty-nine right-handed healthy older adults\(^6\) completed testing under anodal tDCS and sham stimulation in separate sessions. Due to excessive EEG artefact, two participants were removed from further analyses, resulting in a final sample of thirty-seven participants (19 males, mean age 59.9 ± 10.9yrs). Participants were screened and assessed for handedness, as reported in Experiment 1. Participants also completed the Montreal Cognitive Assessment (MoCA, McLennan et al., 2011) to screen against dementia (27.33 ± 0.31). Participants were randomly assigned to stimulation condition: twenty-one participants (12 males, mean age 58.8 ± 9.9yrs) received anodal tDCS over their dominant motor area, whereas the remaining sixteen participants (7 males, mean age 61.2 ± 12.2yrs) received active tDCS over their non-dominant motor area. Participants in both groups were randomly assigned to stimulation order as

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\(^6\) Data from most of these participants contributed to Marquez et al. (2015) which focused on clinical measures of motor performance (e.g., JTT and grip tasks).
described in Experiment 1. This study was approved by the University of Newcastle’s Human Research Ethics (H-2010-1339).

5.3.1.2: Design & Procedure

The parameters of the tDCS stimulation, the cued go/nogo paradigm and EEG recording were identical to those reported in Experiment 1, except as indicated below.

These older participants completed two tests of motor functioning often used clinically in stroke assessment, the Jebsen Taylor Hand Function Test (JTT (Jebsen et al., 1969) and pinch grip tests (Hinson & Gench, 1989) both prior to and following tDCS intervention. In the cued go/nogo paradigm, the target-cue interval between trials was extended to accommodate slower response times in older adults (mean 3000ms, random sequence, 2500 - 3500ms). The statistical analyses of both the behavioural and the electrophysiological data included the between subjects factor: Stimulation Hemisphere (dominant vs non-dominant). Target-locked P300 amplitude was estimated across a 250-650 ms interval.

5.3.2: Results

5.3.2.1: Behavioural Results

Results of the JTT and the pinch-grip tasks are presented in Marquez et al (2015). For the cued go/nogo task, error rate for go trials and false alarm rate for nogo trials were very low and not statistically analysed (1.61% and 0.1%, respectively). As shown in Figure 5.2(B), response time was faster for directional than for non-directional cues ($F(1, 35) = 144.04, p<0.001; 490$ vs $568.2ms$). There was no main effect of response hand ($p>0.05$) or hemisphere ($F<1$).

There was also no main effect of stimulation (tDCS: $534.6\pm11$, Sham: $523.6\pm10$; $F(1, 35) = 2.12, p>0.1$). However, there was a three-way interaction between stimulation, cue and hemisphere ($F (1, 35) = 5.2, p=0.03$). However, simple effects analyses within dominant and non-dominant hemisphere group separately resulted in no main effect of stimulation or
stimulation by cue interaction (both $p>0.1$). As shown in Figure 5.2B, stimulation over the dominant hemisphere showed a tendency for tDCS to increase (rather than decrease) RT. Stimulation over the non-dominant hemisphere showed a similar tendency for non-directional cues, but a small trend for faster RT under stimulation than sham for directional cues.

5.3.2.1: Electrophysiological Results

**CNV**: Cue-locked ERP waveforms for healthy older adults are shown in Figure 5.3B. Both groups developed a centrally-maximal CNV, that was larger for directional than non-directional cues (-5.9 vs -4.3 μV; $F(1, 35) = 12.8, p<0.001$). There was no effect of response hand or interaction between cue and response hand on CNV amplitude (both $F<1$).

Figure 5.3B shows that tDCS over the dominant hemisphere appears to have reduced the effect of cue type on CNV amplitude. However, statistical analyses showed that stimulation had no main effect or interaction with response hand or hemisphere (all $p>0.05$). Moreover, the direction of the effect is opposite to our prediction that stimulation would *increase* response preparation and hence result in greater CNV difference between directional and non-directional cues.
Table 5.4: P300 peak latencies (milliseconds) at Pz for young and old adults for each cue and hand following anodal tDCS and sham.

<table>
<thead>
<tr>
<th>Group/Stimulation</th>
<th>Directional Left</th>
<th>Directional Right</th>
<th>Non-Directional Left</th>
<th>Non-Directional Right</th>
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</thead>
<tbody>
<tr>
<td><strong>Young Adult</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Active</td>
<td>342.4 (16.9)</td>
<td>337.45 (18.5)</td>
<td>344.2 (10.6)</td>
<td>357.4 (6.9)</td>
</tr>
<tr>
<td>Sham</td>
<td>317.1 (14.3)</td>
<td>336.7 (15.9)</td>
<td>345.0 (14.3)</td>
<td>344.8 (10.4)</td>
</tr>
<tr>
<td><strong>Old Adult</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dominant</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Active</td>
<td>465.6 (22.1)</td>
<td>470.0 (22.6)</td>
<td>442.4 (12.7)</td>
<td>448.2 (12.5)</td>
</tr>
<tr>
<td>Sham</td>
<td>469.5 (24.6)</td>
<td>432.7 (22.9)</td>
<td>452.9 (11.3)</td>
<td>470.0 (12.7)</td>
</tr>
<tr>
<td>Non-Dominant</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Active</td>
<td>420.4 (25.3)</td>
<td>429.3 (25.9)</td>
<td>447.7 (14.5)</td>
<td>465.0 (14.3)</td>
</tr>
<tr>
<td>Sham</td>
<td>424.4 (28.2)</td>
<td>431.7 (26.3)</td>
<td>468.6 (13.0)</td>
<td>454.4 (14.6)</td>
</tr>
</tbody>
</table>

**P300:** The target-locked P300 was larger for non-directional cues compared to directional cues (14.6 vs 17.2 µV; $F(1, 35) = 35.6, p<0.001$; Figure 3B). There was a main effect of response hand, which showed significantly larger amplitudes for left compared to right hand responses ($F(1, 35) = 4.3, p=0.047$). There was no main effect of stimulation on P300 amplitude or any interaction with other factors (all $p>0.7$). P300 latency was not significantly affected by either cue or response hand (both $p>0.1$). While there was no main effect of stimulation ($F(1, 35) <1, p<0.8$), the 4-way interaction between hemisphere, stimulation, response hand and cue was significant ($F(1, 35) = 4.6, p=0.04$). However this interaction also did not survive correction in simple analyses within each group.
Figure 5.4: LRP waveforms for directional (blue) and non-directional (red) conditions in target-locked (left) and response-locked (right) LRP waveforms following active (i.e., anodal tDCS) and sham for (A) young and (B) old adults.

**LRP:** The target-locked and response-locked LRPs for the old adults (Figure 5.4B) showed a pattern similar to that in young adults. tLRP emerged earlier and rLRP had a shorter duration for directional than non-directional cues (340.1 vs 381.3ms; $F(1, 35) = 35.36, p<0.001$; -118.6 vs -163.5ms; $F(1, 35) = 34.6, p<0.001$, respectively). There was no main effect of stimulation or interaction between stimulation and other factors for either tLRP or rLRP (both $p>0.2$).
Table 5.5: Mean onset latencies (milliseconds) for target-locked (tLRP) and response-locked (rLRP) for young and old for each cue and hand following anodal tDCS and sham.

<table>
<thead>
<tr>
<th>Group/Stimulation</th>
<th>Directional Left</th>
<th>Directional Right</th>
<th>Non-Directional Left</th>
<th>Non-Directional Right</th>
</tr>
</thead>
<tbody>
<tr>
<td>Young Adult</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Active</td>
<td>273.8 (10.3)</td>
<td>312.9 (7.4)</td>
<td>-107.7 (8.5)</td>
<td>-134.0 (9.1)</td>
</tr>
<tr>
<td>Sham</td>
<td>268.1 (10.0)</td>
<td>313.3 (6.9)</td>
<td>-109.2 (9.1)</td>
<td>-128.6 (7.8)</td>
</tr>
<tr>
<td>Old Adult</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dominant</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Active</td>
<td>335.1 (13.9)</td>
<td>376.9 (6.8)</td>
<td>-130.6 (12.5)</td>
<td>-166.2 (10.8)</td>
</tr>
<tr>
<td>Sham</td>
<td>348.1 (11.1)</td>
<td>371.9 (7.7)</td>
<td>-106.0 (7.6)</td>
<td>-159.0 (9.9)</td>
</tr>
<tr>
<td>Non-Dominant</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Active</td>
<td>335.4 (15.9)</td>
<td>387.4 (7.8)</td>
<td>-115.8 (14.3)</td>
<td>-165.1 (12.4)</td>
</tr>
<tr>
<td>Sham</td>
<td>341.7 (12.7)</td>
<td>389.1 (8.8)</td>
<td>-122.0 (8.8)</td>
<td>-163.6 (11.4)</td>
</tr>
</tbody>
</table>

5.3.3: Discussion

Both behavioural and ERP measures showed a similar pattern to that seen in young adults, with faster responding, larger CNV, smaller P300, earlier tLRP onset and later rLRP onset for directional than non-directional cues. Old adults were noticeably slower in response times and P300 latencies than young adults (Table 5.1 & 5.4), consistent with a disruption of motor processes with increasing age. Nevertheless, again, we found no effect of anodal tDCS over M1 on behavioural performance, ERP or LRP waveforms that would be consistent with enhancement of motor processes.

5.4: Bayesian Analysis

Across both experiments, we found no evidence that anodal tDCS over M1 has a beneficial effect on either behavioural performance or the morphology of response-related ERPs. However, frequentist statistics do not allow us to conclusively assert that anodal tDCS over M1 has no effect on either response speed or motor-related ERP components. To assess the
strength of the evidence in favour of a beneficial effect of tDCS vs. the null effects model, we performed Bayesian model selection analysis separately for each experiment on the factorial analyses of variance for each of the major ERP components as well as for response speed for older adults. As in Conley et al. (2015), we used the default-prior method for linear models as defined by Rouder and colleagues (2012) to create Bayes factors for each possible model. Bayesian analysis was performed using the BayesFactor package in R (Morey & Rouder, 2013), assuming the default setting for the fixed-effect prior ($r=0.5$).

For Experiment 1, Bayesian analysis for response time was reported in Conley et al. (2015) and showed that the null effects model was 11 times more likely to fit the data than the strongest model including stimulation. The strongest model for each ERP component (CNV and P300 amplitude, target and response-locked LRP latencies) included only cue as a factor. For each ERP component, the strongest model that included stimulation as a factor had Bayes factors that were at least 4 times smaller than the null effects model. Consistent with the RT results for the young adults reported in Conley et al. (2015), there is evidence for no beneficial effect of anodal tDCS over M1 on motor-related ERPs in healthy younger adults.

In Experiment 2, Bayesian analysis of response times showed that the most likely model to predict the data had an effect of cue factor only. The strongest model that included stimulation was around two and a half times less likely to predict than the null effects model. As seen in Table 2, any effect of anodal tDCS stimulation on RT tended to be in the opposite direction than predicted (i.e., a slowing effect). The strongest model to predict both CNV and P300 amplitude included both hemisphere and cue factors. The strongest models that included stimulation condition had Bayes factors that were seven and six times smaller than the null effects model for CNV and P300, respectively. The strongest model to predict each LRP component consisted of cue condition only, and the strongest models that included stimulation were five and six times weaker than the null effects models for the rLRP and tLRP,
respectively. Thus, consistent with the findings in young adults, healthy old adults also showed no beneficial effect of anodal tDCS over M1 on ERPs.

5.5: General Discussion

This study investigated the effects of anodal tDCS over M1 on ERPs related to response processes in healthy young and old adults. Despite clear evidence that the task produced the expected behavioural and ERP effects, there was no evidence for a beneficial effect of anodal tDCS over the dominant M1 (young and old groups) or the non-dominant M1 (old group) on either response time or motor-related ERP waveforms. In fact, Bayesian analysis showed support for no effect of anodal tDCS over the M1; the null effect of stimulation model was at least twice as likely to explain the data as the strongest model including stimulation as a factor.

It is possible that the absence of a significant effect of anodal stimulation over the M1 may be due to a number of specific parameters in this study. For instance, the absence of an effect of stimulation on RT may be due to a ceiling effect, as both younger and older adults showed very high performance (i.e., above 90% accuracy). However, this interpretation is unlikely to explain the absence of any effect on ERP components that represent response preparation, selection and activation processes, as these represent the effectiveness of the underlying motor processes, rather than the decision process itself. This is especially true for older adults who showed typical ageing effects on ERP and given the well documented decline in cognitive and neuromuscular functioning in healthy ageing (Raz et al., 2005, Wu & Hallet, 2005). We therefore conclude that anodal tDCS over M1 does not affect motor ERPs in either healthy younger or older adults.

Another factor that may have contributed to the null results is the delay between application of stimulation and onset of testing, which arose because of the need to set up the EEG recording. It could be argued that the 30-40min delay may have abolished any effect of anodal tDCS over M1 on behaviour or ERPs. However, this is unlikely. Firstly, Conley et al.
(2015) found no effect on behavioural performance in young adults, even when the task commenced immediately after stimulation or was completed concurrently with stimulation. Previous studies suggest that the long stimulation session used here (20min) should elicit sustained post-stimulation effects lasting a minimum of one hour (Monte-Silva et al., 2010). Indeed, studies that have observed enhanced ERPs following anodal tDCS over DLPFC (Kongthong et al., 2013, Lafontaine et al., 2013, Lapenta et al., 2014) have found sustained effects following even briefer stimulation sessions (e.g., 13-20min). One of these studies applied tDCS concurrently with the EEG recording (Lafontaine et al., 2013). However, the other studies set up EEG recordings after tDCS and would have had similar delays between the cessation of stimulation and task performance (19 and 128 channel EEG systems for Kongthong et al. and Lapenta et al., respectively). Additionally in Lapenta et al., participants also completed another assessment between tDCS and EEG setup. We conclude that it is unlikely that the delay between tDCS intervention cessation and task performance can account for the lack of stimulation effects.

A final potential contributor to the null effect may be the specific stimulation parameters. We chose stimulation parameters that are commonly used in studies applying anodal tDCS over M1 and that have been shown to enhance both motor excitability (Nitsche & Paulus, 2001) and gross motor performance (Boggio et al., 2006). Recent computational models of anodal tDCS over M1 using the same stimulation parameters show an elicited electrical field that spread across most of the frontocentral areas of the cortex (Miranda et al., 2013). This indicates that the current should spread over areas that are directly involved in the response processes required by the cued go/nogo task (Praamstra et al., 1996, Gomez et al., 2003). Additionally, previous research has produced effects on ERPs following anodal tDCS using 1mA currents (Kongthong et al. 2013). Therefore, stimulation parameters are unlikely to account for null effects.
Finally, the absence of improvement in performance following anodal tDCS over M1 is consistent with a number of recent studies (Bortoletto et al., 2015, Montenegro et al., 2015). Over the last five years, an increasing number of studies have failed to show facilitation of performance following anodal tDCS over the M1, consistent with the increased interest in reporting null as well as positive results. Null effects have been observed in motor function (Wiethoff et al., 2014, Montenegro et al., 2015) and visuomotor tasks (Ambrus et al., 2016), in both healthy young (Pellicciari et al., 2013) and older adults (Lindenberg et al., 2013). Indeed, a recent meta-analysis found that, with the exception of TMS studies of motor output, there is little consistent evidence of facilitation of performance following anodal tDCS over M1 (Horvath, Forte & Carter, 2015). The present study provides additional evidence for null effects following anodal tDCS over M1, by showing that electrophysiological measures associated with motor preparation (CNV), response selection (tLRP) and response execution (rLRP) are not affected by anodal tDCS over the M1 in either young or old adults.
Chapter 6: Effects of transcranial direct current stimulation over the sensorimotor areas on response processes in chronic stroke patients.\(^7\)

6.1: Introduction

Transcranial direct current stimulation (tDCS) has been proposed as a potential rehabilitation technique for a number of clinical populations; including depression and schizophrenia (see Tortella et al., 2015, for a review). However, the most promising of these applications is the use of tDCS over the motor cortices in patients following a stroke. Stroke is one of the most common causes of death and disability (Murray & Lopez, 1997), with the cortical damage resulting in major complications to the daily functioning of many survivors (Donnan et al., 2008). Post-stroke motor rehabilitation can be very effective at restoring movement. However its effectiveness can vary depending on the severity of the stroke. A systematic review conducted by Hendriks, van Limbeek, Geurts and Zwarts (2002) found that although 65% of hospitalised stroke victims showed some degree of motor recovery, less than 15% of patients who experienced paralysis showed significant recovery of motor function.

The rationale for the use of tDCS over the motor areas following stroke is that stimulation may boost the effect of rehabilitation strategies and thus promote greater functional recovery (Johansson, 2011; Floel, 2014). The application of anodal tDCS over M1 has been shown to produce long-lasting enhancement of motor excitability output (Nitsche & Paulus, 2001), as well as motor function improvements on both gross motor (Boggio et al., 2006) and fine motor tasks (Nitsche et al., 2003b). Thus among both clinicians and researchers, \(^7\)

there is great potential for using tDCS to assist in the rehabilitation of stroke victims. There are
two ways in which tDCS has been hypothesised to enhance functionality of the affected
sensorimotor area (Bradham, Stinear & Byblow, 2013). The first involves applying anodal tDCS
over the affected M1 to directly increase excitability of this damaged area. The second involves
applying cathodal tDCS over the unaffected M1 to inhibit its activity and hence indirectly
increase excitability of the affected M1 through a reorganisation of interhemispheric
excitation.

Previous research into the effects of tDCS over M1 in stroke patients has examined
either motor excitability changes as measured by TMS-evoked motor evoked-potential (MEP)
amplitude or functional motor performance (for a review see Bastani & Jaberzadeh, 2012). The
application of anodal tDCS over the affected M1 in stroke patients has been shown to increase
MEP amplitude compared to sham, as also seen in healthy controls. This effect has been
observed at MEPs recorded at the hand (Edwards et al., 2009) as well as at the foot
has been measured using tasks like the Jebsen Taylor Hand Function Test (JTT, Jebsen et al.,
1969), a test of motor control using daily life skill tests, and measures of pinch or grip strength.
A number of studies have found improvements in performance on both the JTT and grip
strength in stroke patients following anodal tDCS over the affected M1 (Fregni et al., 2005;
Hummel et al., 2005, 2006), cathodal tDCS over the unaffected M1 (Fregni et al., 2005), as well
as bihemispheric tDCS (Bolognini et al., 2011; Lefebvre et al., 2014). These improvements in
performance have been taken as support for the use of tDCS over M1 in clinical intervention
programs.

However not all studies have shown such positive results. A review by Lüdemann-
Podubecká, Bösl, Rothhardt and Verheyden (2014) found that, many of the studies that
showed motor improvements following tDCS over the sensorimotor areas in post-stroke
patients, actually reported performance changes that failed to reach statistical significance. Specifically, nearly two-thirds of the studies applying anodal tDCS, and over half of those using cathodal tDCS failed to show statistically significant improvements in performance compared to sham. This is an alarming finding, especially given recent evidence in healthy controls for large variability in the effect of tDSC intervention (Wiethoff, Hamada & Rothwell, 2014). Indeed, a recent systematic review of tDSC in stroke concluded that there is no conclusive evidence that tDSC over M1 is beneficial for stroke patients (Marquez et al., 2013).

The mechanisms by which tDSC effects change in controls and patients is not well understood. In controls, a number of studies do not show any benefit of anodal tDSC on response speed in cued response tasks (Lindenberg et al., 2013, Pellicciari, Brignani & Miniussi, 2013). Indeed, in Chapter 5 (Conley, Fulham, Marquez, Parsons & Karayanidis, 2016), we assessed the effect of anodal tDSC over M1 on a cued go/nogo task in healthy younger and older adults. Assessing both behaviour and response-related ERP waveforms, we found no beneficial effect of anodal tDSC over M1 on performance in either age group. However, previous research using cued response paradigms in conjunction with tDSC over M1 in stroke patients has shown positive results. A recent study by O’Shea and colleagues (2014) found that both anodal tDSC over the affected M1 and cathodal tDSC over the unaffected M1 significantly improved response speed in chronic stroke patients compared to sham. This effect was more pronounced following anodal tDSC. As such, even if there are no beneficial effects of tDSC on response processes in healthy controls, it is possible that stroke patients may still benefit this intervention.

In the present study, we examine whether anodal and/or cathodal tDSC over the sensorimotor cortex improves behavioural or electrophysiological performance on a cued go/nogo task in chronic stroke patients. Using a repeated measures design, three stimulation interventions are compared: anodal tDSC over the affected motor area, cathodal tDSC over the
unaffected motor area and sham stimulation. In each session, participants completed functional motor tasks and the cued go/nogo task. The results on the functional tasks are discussed in detail in Marquez, Conley, Karayanidis, Lagopoulos and Parsons (in preparation). Briefly, anodal stimulation over the affected M1 produced no significant effect compared to sham on the JTT or the grip tasks. Cathodal tDCS over the unaffected M1 improved affected hand performance on the JTT compared to the sham condition; however, this improvement was only present for patients with more severe disabilities. While this finding is encouraging, in the same participants, cathodal tDCS also resulted in decreased isometric motor performance as assessed by grip strength, indicating that any positive effects of cathodal tDCS are not found uniformly across all areas of motor functioning. In this present study, we examine behavioural performance and ERP measures of response processes in a subset of these stroke patients using the same cued go/nogo task as in the earlier studies (Ch. 3-5). These data were collected in the same session as the functional data reported by Marquez et al. (in preparation). To assess the effects of anodal and cathodal tDCS on motor preparation to informative and non-informative cues the contingent negative variation (CNV) prior to target onset will be assessed. To assess the effects of tDCS on stimulus processing, the P300 peak following the onset of the target will be extracted over the parietal cortex where it is maximal (Picton, 1992). Based on the findings of the systematic review by Marquez and colleagues (2013), we expect that the application of cathodal tDCS over the unaffected M1 will be more likely to produce improvements in performance compared to sham and to anodal tDCS over the affected M1. We expect that these improvements will be shown primarily in larger CNV amplitudes, and faster response times following cathodal tDCS compared to sham.
6.2: Methods

6.2.1: Patients

Twenty-three chronic stroke patients completed all three experimental sessions. Three patients were excluded due to high artefact with their EEG recordings, which left a sample of twenty (9 female, mean age 63.4±2.5yrs). Patients involved in this study all had suffered damage to either their dominant or non-dominant sensorimotor area. All patients completed the experimental sessions at least six months post-stroke. Patients were screened for exclusion criteria that would indicate non-suitability for direct current stimulation, including epilepsy, a major heart condition or any neurological implants. Descriptive statistics are provided in Table 6.1. The protocol was approved by the Hunter New England Human Research Ethics Committee (H-2010-1339) and was in accordance with the Declaration of Helsinki. Most patients were right handed as measured by the Edinburgh Handedness Inventory (Oldfield, 1971). The level of disability was determined by the Modified Rankin Scale (MRS, Rankin, 1957). The order of the anodal, cathodal and sham sessions was randomised between patients.

Table 6.1: Descriptive statistics of chronic stroke patients. Abbreviations: standard error of the mean (SEM), Montreal Cognitive Assessment (MoCA), Modified Rankin Scale (MRS).

<table>
<thead>
<tr>
<th>Measure</th>
<th>Mean ± SEM</th>
<th>Count</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>63.4±2.5</td>
<td></td>
</tr>
<tr>
<td>Sex(Male/Female)</td>
<td></td>
<td>11/9</td>
</tr>
<tr>
<td>Handedness(Left/Right)</td>
<td></td>
<td>5/15</td>
</tr>
<tr>
<td>Lesioned Hemisphere (Left/Right)</td>
<td></td>
<td>14/6</td>
</tr>
<tr>
<td>Affected Hemisphere (Dominant/Non-dominant Hand)</td>
<td></td>
<td>12/8</td>
</tr>
<tr>
<td>MoCA Score</td>
<td>25.2±0.7</td>
<td></td>
</tr>
<tr>
<td>MRS Level (1/2/3)</td>
<td></td>
<td>14/4/2</td>
</tr>
<tr>
<td>Months Post-Stroke</td>
<td>75.5±9.7</td>
<td></td>
</tr>
</tbody>
</table>
6.2.2: Design

The procedure, task (Figure 6.1) and stimulation parameters for this study were the same as in Chapter 5 (Conley et al., 2016), except where noted below.

**TDCS Settings:** For anodal stimulation, the anode was placed over the affected primary motor cortex (M1), while the cathode was placed over the supraorbital region of the contralateral hemisphere. For cathodal stimulation, the cathode was placed over the unaffected M1, and the anode was placed over the contralateral supraorbital region. The location of hand area of M1 was determined as the C3 or C4 electrodes according to the international 10/20 system.

**Procedure:** All patients completed the first experimental session without tDCS intervention, in which they were administered the Montreal Cognitive Assessment (MoCA, Nasreddine et al., 2005), as well as the Jebsen Taylor Hand Function Test (JTT, Jebsen et al., 1969) and pinch grip tests (Mathiowetz et al., 1985). The next three experimental sessions began with the practice blocks of the cued go/nogo task followed by the 20min tDCS intervention with anodal, cathodal and sham stimulation presented in randomised order across sessions. Patients then completed the post-stimulation runs of the two functional motor tasks, before completing the cued go/nogo task. The analysis of the functional motor tasks is discussed in Marquez et al. (In preparation). The three intervention sessions were scheduled at least two weeks apart to avoid any carry over effects of the cortical stimulation.
Figure 6.1: Cued go/nogo task with informative and uninformative cues. The time course for directional go and nogo trials (A), as well as non-directional trials (B) is plotted from fixation to cue to target, and then to the beginning of the next trial.
6.2.3: EEG and Data Analysis

The EEG recording and statistical analysis procedures are identical to those in Chapter 5 (Conley et al., 2016), except where explained below. Unlike in Chapter 5, lateralised readiness potentials (LRPs) were not assessed as it was not appropriate to average the EEG signal from affected and unaffected sensorimotor cortices.

For the data analyses, both the response times and the mean ERPs for go trials were analysed using a repeated measures generalised linear model with three within-subjects factors: Stimulation (anodal, cathodal, sham), Cue (directional, non-directional) and Response Hand (affected, unaffected). Main effects of stimulation were assessed by simple contrasts between each stimulation condition and sham. Significant interactions involving stimulation were assessed by examining simple effects and then by simple contrasts between each stimulation condition and sham.

6.3: Results

6.3.1: Behavioural

Accuracy on the task was very high. Across all experimental sessions, mean error rate was below 5% of trials (Anodal: 3.4, Cathodal: 3.9, Sham: 3.7%). As such, the analysis was performed on response times only. Figure 6.2 shows the mean response times (RT) on the cued go/nogo task following each stimulation condition. As expected, patients responded faster to directional cues than non-directional cues (623 vs 698.3ms; $F(1, 19) = 60$, $p<0.001$). RT did not differ between affected and unaffected hand ($p>0.2$). There was no effect of stimulation on RT as shown in Figure 2 by the differed coloured bars (Anodal: 652ms, Cathodal: 668ms, Sham: 661ms; $F(2, 38) = 0.77$, $p=0.47$). Although there was a marginally significant interaction between stimulation condition, cue type and response hand ($F(2, 38) = 2.9$, $p=0.066$), when we examined the effect of stimulation on each cue type separately, there was
no main effect of stimulation, nor an interaction between stimulation and response hand (all $p>0.09$).

![Figure 6.2: Mean response time for chronic stroke patients following anodal tDCS (red), cathodal tDCS (blue) and sham (grey). Response times for each cue and response hand are displayed.](image)

### 6.3.2: Electrophysiological Results

Figure 6.3 displays the ERPs of patients at Cz and Pz following each stimulation condition.

During the cue-target interval, directional cues elicited a significantly larger CNV at Cz (Figure 3 left panel) than non-directional cues (-4.9 vs -2.8 μV, $F (1, 19) = 29.7$, $p<0.001$). There was a significant effect of response hand, a larger CNV for the affected hand compared to the unaffected hand (-4.3 vs -3.4 μV, $F (1, 19) = 8.7$, $p<0.01$). There was however no effect of tDCS on CNV amplitude (Anodal: -4.1, Cathodal: -3.8, Sham: -3.6 μV; $F (2, 38) = 0.7$, $p=0.5$).
After target onset, there was a large P300 evident especially for non-informative cues (Figure 6.3 right panel). There was a main effect of cue type on P300 amplitude with larger P300 amplitudes to non-directional compared to directional cues (7.9 vs 13.3 µV; \( F(1, 19) = 145.9, p<0.001 \)). However, there was no significant effect of stimulation condition on P300 amplitude (Anodal: 10.5, Cathodal: 10.6, Sham: 10.9 µV; \( F(2, 38) = 0.4, p=0.9 \)), or latency (Anodal: 457.7, Cathodal: 463.2, Sham: 446.7ms; \( F(2, 38) = 0.29, p=0.7 \)). There were also no other significant main effects or interactions (all \( p>0.08 \)).

![Figure 6.3: Grand average ERP waveforms at Cz and Pz for chronic stroke patients following anodal tDCS (a), cathodal tDCS (b), and sham (c). Waveforms are displayed for the directional (blue) and non-directional (red) cues.](image)
6.4: Discussion

The present study investigated the effects of anodal and cathodal tDCS over the affected and unaffected M1 of chronic stroke patients on behavioural and electrophysiological performance. The patients performed the task well, responding faster for directional compared to non-directional trials. Analysis of the ERP results showed that patients were able to prepare more for the directional cue trials, as indexed by a larger CNV waveform. After target onset, patients displayed larger P300 amplitudes to targets on non-directional trials. This pattern of result is consistent with the results seen in both healthy younger and older adults (Conley et al., 2016, Chapter 5). Across both behavioural and electrophysiological measures, there was no beneficial effect of either anodal or cathodal tDCS compared to sham. This finding is also consistent to previous results with this paradigm in healthy younger and older adults (Conley et al., 2015, 2016; Chapters 4 & 5).

These findings are not consistent with the small number of other studies that have examined the effects of anodal tDCS over the affected M1 in stroke patients using either behavioural tasks or EEG. Anodal tDCS over the affected M1 was found to improve behavioural performance on a choice RT task compared to sham or cathodal tDCS over the unaffected M1 (Stagg et al., 2012; O'Shea et al., 2014). This behavioural improvement was accompanied by greater cortical activation in the affected sensorimotor region as shown by fMRI. The application of anodal tDCS over the affected M1 was also found to reduce the latency of both the N200 and P300 on an auditory oddball task (D’agata et al., 2016). This reduction was concluded to reflect improved cognitive and attentional processes of patients following stimulation compared to sham. Another study that has used EEG looked at the effectiveness of anodal tDCS over affected M1 on motor imagery (Kasashima et al., 2012). Time-frequency analysis of the activity while viewing images of finger movements showed an increase in alpha desynchronisation following anodal tDCS compared to sham.
However, the results of the present study are consistent with some of the recent papers assessing functional improvements in stroke patients following tDCS over M1. Rossi and colleagues (2013) assessed the effectiveness of a week-long tDCS intervention on functional recovery in stroke patients. They found that, directly following the fifth session as well as 90 days later, there was no effect of anodal tDCS over the affected M1 on motor functioning. van Asseldonk and Boonstra (2016) showed that anodal tDCS over the affected motor area had no benefit on affected leg function. Additionally, Lüdemann-Podubecká and colleagues (2014) noted that most of the positive effects reported by studies assessing the effects of tDCS in stroke are in fact positive trends that were not statistically significant. This may be partly due to the small sample size in many of these studies (Bastani & Jaberzadeh, 2012), but may also partly result from confirmation bias.

A possible reason for the pattern of results observed in this study is that the majority of the subjects were high functioning patients. Indeed, none were identified as having severe disabilities, and over two-thirds of the sample scored the lowest level of disability on the MRS (Rankin, 1957). It is possible that the patients were not impaired enough for tDCS intervention to be effective. However, there is prior evidence that tDCS is more effective at producing beneficial effects in mildly, rather than severely disabled patients (Bradnam et al., 2012). Additionally, all patients completed the study at least 6 months following the initial trauma. The beneficial effects of tDCS have been greater in chronic stroke patients than in either acute or subacute groups (see Marquez et al., 2013 for a review). Therefore, neither severity of disability nor chronicity of illness are likely to account for the present null findings of stimulation.

In conclusion, the present study shows that neither anodal tDCS over the affected hemisphere, nor cathodal tDCS over the unaffected hemisphere improved the performance of chronic stroke patients on a cued go/nogo task. These findings are consistent with our
previous work using this task in healthy younger and older adults (Conley et al., 2015, 2016). However, it should be noted that these findings are only from single intervention session of either anodal or cathodal tDCS. A recent systematic review of studies using multiple sessions of anodal tDCS over M1 showed positive effects on motor learning (Kang, Summers & Cauraugh, 2016, but see Rossi et al., 2013). It is possible that with repeated intervention and training sessions, an enhancement of behavioural and/or electrophysiological performance would have been observed.
Chapter 7: General Discussion

7.1: Overview of findings

Transcranial direct current stimulation (tDCS) has been investigated in both clinical and normative samples for over 15 years. Over this period, there has been growing interest in using tDCS as an intervention for a number of specific medical and psychological pathologies (see Nitsche et al., 2008; Tortella et al., 2015 for more information). Much of this interest has been specifically related to the application of anodal tDCS over the sensorimotor cortex, where stimulation has been seen as a possible low-cost addition to rehabilitation programs for patients suffering from Parkinson’s disease or recovering from stroke (see Johansson, 2010 for a review). This interest has been driven mainly by a number of studies highlighting the effectiveness of anodal tDCS over M1 at enhancing motor excitability measured by TMS-elicited MEPs. However, despite over a decade of research, there is still much that we do not know about the way in which stimulation over M1 is producing these changes. Additionally, more recently, there are increasing doubt about the reported effectiveness of anodal tDCS; as studies are emerging that fail to find significant beneficial effects. The aim of this thesis has been to investigate whether anodal tDCS improves motor performance. The primary focus was whether anodal tDCS changes either response speed or response-related ERP components elicited during a conflict task in healthy adults and chronic stroke patients. Across the four experimental chapters, we examine the effectiveness of anodal tDCS over M1 on both functional motor skills and a response selection task. This thesis goes beyond the previous research into tDCS, by examining how specific response processes are affected by tDCS intervention.

Chapter 3 (Marquez et al., 2015) examined the effects of anodal tDCS over the dominant or the non-dominant M1 on functional and behavioural performance in healthy
older adults. In this study, performance on the JTT, pinch grip measures, and behavioural responses on a cued go/nogo task were assessed following anodal tDCS or sham. Anodal tDCS improved completion time for total and fine motor JTT subscores compared to sham. This improvement was only present following anodal tDCS over the dominant M1. There was no effect of anodal tDCS on gross motor JTT subtests, pinch grip measures or behavioural performance.

Chapter 4 (Conley et al., 2015) investigated the effects of anodal tDCS over the dominant M1 on cued go/nogo performance in healthy young adults. In three experiments, we examined whether the effectiveness of anodal tDCS on task performance varied across different delays between stimulation and test. Specifically, anodal tDCS or sham was applied concurrently with task performance, directly before task performance, or approximately 40 min before task performance. There was no effect of stimulation on cued go/nogo performance in any of the three conditions. Bayesian analyses confirmed that a null effects model provided a better explanation of the data than a model with an effect of stimulation.

In Chapter 5 (Conley et al., 2016), we measured the effects of anodal tDCS over M1 on ERP waveforms related to specific response processes in healthy young and old adults. The CNV was used to measure response preparation, while the target-locked and response-locked LRP s assessed response selection and response activation mechanisms, respectively. We also assessed the effects of anodal tDCS over M1 on stimulus evaluation, which is indexed by the P300 peak (Picton, 1992). In both young adults (experiment 1) and old adults (experiment 2), anodal tDCS had no effect on ERP waveforms, consistent with the behavioural results on the cued go/nogo task. Once again Bayesian analyses confirmed that models that included an effect of stimulation were weaker than the null effects model. Finally, in Chapter 6 (Conley, Marquez, Fulham, Parsons & Karayanidis, in preparation), we assessed the effects of anodal over the affected M1 and cathodal tDCS over the non-affected M1 in chronic stroke patients.
Across three sessions, the patients received anodal, cathodal and sham intervention before completing the cued go/nogo task. As seen in healthy adults, there was no effect of either anodal or cathodal stimulation on behavioural or electrophysiological measures.

In summary, despite some evidence for a small enhancement of functional performance following anodal tDCS over M1 in older adults (Marquez et al., 2015), there was an overwhelming lack of positive effects on behavioural and electrophysiological performance in young adults, old adults or chronic stroke survivors. The implications of these findings for the field of neuromodulation will be discussed below.

7.2: Implications for effectiveness of tDCS of motor cortex

The findings reported here show a consistent pattern of no impact of anodal tDCS over M1 on response processing. This pattern was consistent across healthy adults of varying age as well as in the presence of cortical trauma caused by a stroke. The failure to find a significant effect of stimulation over M1 on performance, using the same stimulation parameters previously used to facilitate performance on serial reaction time tasks (Nitsche et al., 2003b), and the same tDCS duration as research that has shown motor function performance (Boggio et al., 2006); is consistent with emerging concerns within the neuromodulation literature (Horvath, Forte & Carter, 2015a) which have been gaining momentum across the last five years.

While the majority of early studies investigating the effects of anodal tDCS over M1 reported overwhelmingly positive results, over the past few years a number of studies have tempered the strength of this conclusion. As discussed in detail in Chapter 1, experiments using a range of performance measures have either failed to find an improvement of performance or have found large inter-individual variability in response to tDCS intervention. The findings presented in this thesis support this recent shift in the literature. Rather than identifying a specific motor process that is enhanced by anodal tDCS over M1, the results
across Chapters 4-6 have shown a consistent absence of facilitation. This calls into question the ability of anodal tDCS to reliably facilitate motor performance.

Unlike the results of behavioural or gross motor tasks, response processes measured by ERPs show the effectiveness of the cortical response in the lead-up to the overt movement. An understanding of how these processes are affected by different perceptual and contextual properties is crucial to our understanding of how we engage with the world around us (discussed more in Chapter 2). Therefore the understanding of how tDCS affects these processes is critical for the overall understanding of how tDCS works as a neuromodulatory technique. Using ERPs to identify the specific effects of stimulation on response-related cortical processes would allow us to design protocols that combine stimulation with motor training to improve that response mechanism. Yet across three studies, the present thesis questions whether tDCS has any effect on response processes and associated motor performance. This consistent finding questions the value of tDCS in improving motor performance.

This question regarding the reliability of any effect of anodal tDCS over M1 on motor performance is becoming a critical issue in the field. At present, the most consistent of the beneficial effects post-tDCS has been the enhancement of TMS-elicited MEPs. In contrast, the strength of results from other experimental setups has been called into question (see Horvath, Forte & Carter, 2015a, for a more in-depth analysis). Additionally, recent research has revealed large individual variability in responsiveness to tDCS. For instance, as discussed in Chapter 1, Wiethoff and colleagues (2014) reported that around half of the participants in their sample of 56 subjects failed to show any beneficial effect of either anodal or cathodal tDCS on TMS-elicited MEPs. They concluded that the effects of tDCS are highly variable, questioning the consistency of findings reported in much of the earlier literature. Chew, Ho & Loo (2015) found significant inter- and intra-individual variability in the effects of different current strengths of
anodal tDCS over M1 on TMS-elicited MEPs. More than half of the participants did not show excitation at any current strength, while others showed enhanced MEPs following stimulation at weaker, but not stronger currents. Another recent study by Horvath, Vogrin, Carter, Cook and Forte (2016) found that recorded MEPs from the same participants were highly variable across multiple testing sessions. Across nine consecutive testing sessions, participants were equally likely to exhibit with excitatory and inhibitory effects following anodal tDCS over M1.

Additionally, a review by Labruna and colleagues (2015) also supported the existence of large inter-individual variability in responsiveness to tDCS. Across three studies, the level of sensitivity to TMS was directly related to the effects of anodal tDCS over M1. Participants who responded better to TMS, defined as the ability to produce MEPs with an amplitude of 1mV or more from a magnetic pulse, were more likely to also show facilitation from anodal tDCS. In contrast, participants who were less receptive to TMS pulses showed almost no effect of tDCS.

Even recent articles that support the facilitation by anodal tDCS acknowledge that a number of issues can mitigate its effectiveness. A recent systematic review examining the effects of anodal tDCS on cognitive and motor functions in older adults provides a clear example (Summers, Kang & Caraugh 2016). While Summers et al. concluded that anodal tDCS may enhance cognitive and motor performance; they concede that the large inter-individual differences found in some studies raise a serious caveat to the effectiveness of tDCS interventions. The researchers specifically highlight that individual differences in physiology, such as skull thickness, may have a major impact in determining how likely it is that an individual will respond to tDCS intervention, i.e., thicker skulls may limit the ability of a current to reach the cortex.

In summary, the field is increasingly acknowledging that there are a number of potential limitations to the effectiveness of tDCS in enhancing motor performance. The findings of the current thesis contribute to this scepticism by consistently showing no evidence
of tDCS-induced modulation of response processes in healthy adults or stroke survivors. Hence, it is becoming increasingly clear that there are substantial questions about whether, when and how anodal tDCS may enhance motor performance, and what are the optimal conditions, or individual parameters, that may produce a positive response to the intervention.

7.3: Implications for anodal tDCS in ageing and clinical samples

A key implication from the present studies is that there is no impact from a single intervention session of tDCS on either behavioural or response processes in healthy older or chronic stroke patients. While Chapter 3 (Marquez et al., 2015) showed an improvement in functional motor performance on the JTT following anodal tDCS of the dominant M1 compared to sham in healthy older adults, this effect was not large. The only significant stimulation effect was found on performance on the total JTT. However, the effect size was quite small (i.e., \( \eta_p^2 < 0.2 \)), suggesting that it may be the result of a Type 1 error.

The implication that a single intervention session of tDCS is not effective in producing an enhancement of performance in stroke patients is consistent with a number of recent articles. Two systematic reviews by Marquez and colleagues (2013), and Elsner, Kugler, Pohl and Merholz (2013) assessed the results of studies using both single and multiple intervention sessions. Both articles found that neither anodal nor cathodal tDCS over M1 improved motor outcomes in stroke patients. In contrast, a more recent systematic review by Kang, Summers and Caraugh (2016) examined studies that assessed the effect of multiple tDCS intervention sessions on motor learning. They found that multiple intervention sessions were more likely to produce facilitation of motor learning than single sessions. Taken in light of the results of the experimental studies in this thesis, it appears safe to conclude that there is no evidence to support an effect of single session of tDCS over M1 in patients. Whether and under what conditions multiple sessions may induce facilitation of motor performance, remains an important question for future research.
7.4: Methodological considerations

The consistent finding of no beneficial effect of tDCS over the motor cortex on behavioural or ERP measures of motor processes questions the effectiveness of tDCS as an intervention for motor deficits. However, it is important to examine whether there are any particular methodological aspects of this series of studies that may taper the strength of this conclusion. Below, we examine potential methodological issues associated with the task itself, and also with the stimulation parameters applied over M1.

7.4.1: Effectiveness of the go/nogo task

The cued go/nogo task with informative and non-informative cues was utilised as it can differentiate between a number of response processes. Cued response time paradigms (often called S1-S2 paradigms) have been used to elicit ERP components associated with response preparation, selection and activation processes (Walter et al., 1964, Sanders, 1975). The comparison between informative and non-informative cue types allowed us to examine whether tDCS impacts response preparation processes under fully or partially prepared conditions. Both informative and non-informative cue blocks required response selection. Informative cues required selection between executing (go trials) and withholding (no-go trials) a prepared motor response. Non-informative cues required selection between an unprepared left or right hand response. The very high accuracy rates may suggest that the task was too easy, resulting in ceiling effects that could not be improved further by stimulation. However, there are a number of points that argue against this conclusion.

Firstly, across all three studies (Chapters 3-6), the informative (i.e., directional cues) which allowed full response preparation produced larger CNV amplitude, earlier tLRP latency and faster response time compared to non-directional cues. As expected, participants in the healthy groups respond faster with their dominant right hand, whereas there was no difference in RT between hands in the stroke patients. The task also demonstrated a clear
behavioural difference between the three subject groups, with young adults responding faster than older adults, and older adults responding faster than the patient group. This pattern of findings indicates that indeed there was differential activation of motor processes across conditions even in the most efficient young group, suggesting that the task produced the expected improvements across conditions. Therefore, there is no evidence to suggest that this task is unsuitable to evoke performance changes in subjects. Rather, the evidence suggests that stimulation did not enhance the response processes that are involved in correctly executing the task.

Indeed, the fact that the participants performed accurately does not preclude the capacity to improve performance. If anodal tDCS enhanced performance, then this improvement could have been manifest in decreased response times, and/or more efficient response preparation, selective and execution processes compared to sham. Moreover, if this facilitation was dependent on level of performance, we would expect it to be greater in the older adults and stroke patients, whose performance was poorer than that of young adults. In that case, the difference between young and older adults, and older adults and patients in behavioural and/or electrophysiological performance should have been reduced following anodal tDCS compared to sham. The failure of tDCS to produce any improvement in behaviour or motor ERPs in any of the three groups strongly supports our conclusion that it is ineffective in modulating response processes elicited in the cued go/nogo paradigm.

7.4.2: Suitability of the stimulation parameters

All experiments in this thesis used the same stimulation parameters which were chosen on the basis of being the most consistent montage to produce enhanced performance over M1 (see Methods in Chapters 3-6 for details). While research applying tDCS over M1 has used a variety of different stimulation parameters, the parameters used here have been reliably shown to produce significant modulation of motor excitability as measured by MEPs.
While the current montage is the one most consistently used to stimulate the motor cortex, other studies have reported significant effects with other configurations. For instance, another common configuration uses bilateral motor cortex stimulation, with the anode and cathode electrodes placed over the sensorimotor areas in each hemisphere (Vines, Cerruti & Schlaug, 2008). However, this configuration has also resulted in discrepant outcomes. Nitsche and Paulus (2000) showed that it produced no significant effect of anodal tDCS on motor excitability. Similarly, some researchers argue that the size of electrodes can affect the effect of tDCS on performance (Nitsche et al., 2007). These researchers argue that smaller electrodes are more focal and better at enhancing performance than larger electrodes (Dmochowski, Bikson & Parra, 2012). However, a recent meta-analysis by Ho and colleagues (2016) did not support this argument, observing that larger electrodes consistently produced larger enhancements in MEP amplitudes compared to smaller electrodes.

Finally, it is possible that the current strength used in this study was not strong enough to reach the appropriate cortical regions. There is some evidence that higher current strength may increase the chance of producing a beneficial effect on performance (Miyaguchi et al., 2013). Indeed, the theory is that at higher current strengths, anodal tDCS may initially activate inhibitory pathways, before shifting to enhance the depolarisation of neurons (Moliadze et al., 2012). However a number of recent studies have shown large individual variability across all levels of stimulation strength (Wiethoff, Hamada & Rothwell, 2014, Chew et al., 2015), so there is not a convincing argument that increased strength of stimulation may have produced positive results. In summary, there is no conclusive evidence that a different set of stimulation parameters may have increased the chances of performance facilitation.

7.5: Spread of electrical field of anodal tDCS interventions

Compared to other neuromodulation methods, such as TMS, non-invasive brain stimulation by tDCS is not a very focal technique. Rather than targeting a specific area, the current spreads
across the cortical areas that are situated between the two electrodes (Miranda, Mekonnen, Salvador & Ruffini, 2013). Computational models of the electrical field of stimulation indicate that the electrode montage used here generates in a large electrical field which spreads across the frontal half of the cortex (Miranda et al., 2013; Rahman et al., 2013). Interestingly, these models have shown that the site of peak activation across the electrical field is situated midway between the two electrodes. Hence for the current montage, this midpoint is in the PFC, which is anterior to M1. This means that, contrary to our intention, the sensorimotor cortex may not be the area that received the strongest magnitude of current flow with this stimulation configuration. Hence, both the current study and previous studies applying anodal tDCS with this montage may have missed the peak site for motor cortex facilitation. However, it is important to note that the secondary motor cortices (SMA and premotor cortex) are located anterior to M1, and are likely to have received high current flow from the generated field. These areas are highly interconnected with M1 (Graziano & Aflalo, 2007) and are involved in response processes such as inhibition and preparation. Moreover, while the intended site of stimulation may not have received the greatest volume of electrical activity, it is likely that there was still substantial current flow in this area (Miranda et al., 2013; Ho et al., 2016). The PFC itself is also heavily involved in conflict processes (Ridderinkof, van der Wildenberg, Segalowitz & Carter, 2004; Kerns, 2006). Therefore, the fact that the peak area was located anterior and medial of the stimulation site could have been expected to impact processes related to preparation and selection of responses.

Computational models of stimulation reveal another factor that is not well acknowledged in the tDCS literature. The current generated between the anode and the cathode electrodes could be producing both excitation and inhibition in different cortical areas that cancel each other out, thereby resulting in a “zero-sum model” (Brem, Fried, Horvath, Robertson & Pascual-Leone, 2014, Fertonani & Miniussi, 2016). As tDCS produces a diffuse
field, it is possible that activity in some areas increases and in other areas reduces as a result of stimulation. This model predicts that if these two cortical areas are both parts of the same functional network needed to complete a task, then the result will be a zero-sum change on performance (Summers et al., 2016). In the context of the studies presented in this thesis, this could mean that the lack of an effect on performance may not be due to a failure to elicit facilitation per se, but rather a failure to produce a greater net effect of facilitation. Identification of the specific areas that may have been facilitated or inhibited is beyond the scope of this thesis. However, the zero-sum model of stimulation should be investigated more thoroughly in future research.

7.6: Is there evidence that tDCS is more effective at enhancing cognitive compared to motor function?

While most studies have focussed on the effects of tDCS over M1, there has also been increasing interest in the effects of tDCS over the dorsolateral prefrontal cortex (DLPFC) on a number of different cognitive processes. These studies have used a range of different psychophysiological tasks to assess processes such as working memory (Martin, Liu, Alonzo, Green & Loo, 2014), visual discrimination (Lafontaine et al., 2013), and response inhibition (Lapenta et al., 2013).

When applied over the left DLPFC, anodal tDCS has been shown to enhance performance on conflict-driven working memory tasks like the n-back task (Martin et al., 2014) as well as auditory discrimination of oddball tasks (Knechtel et al., 2014). In contrast, Lapenta and colleagues (2014) found that anodal tDCS over the right DLPFC reduced N2 amplitude and increased P3a amplitude to nogo stimuli, but had no effect on go stimuli in a go/nogo task.

However, other studies show discrepant results, questioning the reliability of the effects of tDCS over the DLPFC. Ambrus and colleagues (2011) showed that anodal tDCS over
either the left or the right DLPFC did not improve performance on a visual categorisation task. Similarly, anodal tDCS over either DLPFC recently failed to improve recognition memory performance compared to sham, in healthy young adults (Smirni, Turriziani, Mangano, Cipolotti & Oliviero, 2015). In addition, two recent reviews have shown that there are a number of inconsistencies with the cognitive changes reported following tDCS over the DLFPC. A meta-analysis by Jacobson and colleagues (2012) showed that the pattern of results in studies investigating the effects of tDCS on cognitive processes was much less reliable than the evidence for tDCS facilitating motor processes. More striking perhaps were the results of Horvath and colleagues (2015b), who found no statistically significant effect of tDCS interventions over the DLPFC on tasks assessing executive function, language or memory. This evidence indicates that the application of tDCS over the DLPFC is no more consistent at producing beneficial effects than stimulation over the sensorimotor area.

A point to consider is that the majority of these results are from studies that, like the experiments reported here, assessed performance following a single tDCS session. In contrast, there is evidence that repeated sessions of anodal tDCS over the DLPFC can enhance the effects of cognitive training (Filmer, Dux & Mattingley, 2014). A recent study from Filmer, Varghese, Hawkins, Mattingley and Dux (2016) showed that consecutive daily sessions of anodal tDCS over the left DLPFC improved response time compared to cathodal tDCS or sham on an auditory decision making task and a visual search task. The authors concluded that the application of tDCS may improve the transference of training gains, by improving the ability of the brain to acquire and process information. It is possible that multiple intervention sessions could consistently enhance performance of cognitive functions.

7.7: Future directions and final comments

The aim of this thesis was to identify the effects of anodal tDCS over M1 on motor processes, and specifically whether anodal tDCS improved response processes, such as preparation,
selection and execution, in both healthy controls and stroke patients. However, we consistently found no beneficial effect of anodal tDCS over M1 on either behavioural or electrophysiological data. Bayesian analyses supported the null-effects model over models that included stimulation. The lack of facilitation in cued go/nogo performance following tDCS over M1 was observed across all three subject groups. The sole benefit of anodal tDCS over M1 was seen in one measure of functional motor performance in healthy older adults (see Chapter 3). However, even this effect size was very small, possibly indicating a type 1 error. In summary, this thesis has shown that a single session of anodal tDCS over M1 is not sufficient to produce changes in behavioural or electrophysiological performance on a conflict task.

In conclusion, the findings of this thesis call into question the ability of anodal tDCS over M1 to elicit beneficial effects on motor performance. However, it may be premature to abandon the possibility that tDCS may prove to be a viable therapeutic treatment option. For instance, research into multiple intervention sessions seems to be producing promising results (Filmer, Dux & Mattingley, 2014). Recent research applying anodal tDCS over the left DLPFC across a number of sessions in healthy adults has been able to improve performance on auditory and visual discrimination tasks compared to sham (Filmer et al., 2016). Additionally, the use of multiple tDCS interventions over the motor cortex in stroke patients has also shown promise in enhancing motor learning (for a review see Kang, Summers, & Cauraugh, 2016). These results indicate that a more appropriate use of tDCS may be in assisting with training regimes, rather than as a stand-alone modulation technique. Future research should endeavour to uncover this efficacy of using anodal tDCS in this setting.
Chapter 8: References


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